EFMC
International Symposium on Medicinal Chemistry

Ljubljana, Slovenia September 2-6, 2018

BOOK OF ABSTRACTS

Organised by:

Sekcija za Farmacevtsko Kemijo
Slovenskega Farmacevtskega Društva
Section for Medicinal Chemistry
of the Slovenian Pharmaceutical Society

Slovensko Farmacevtsko Društvo
SLOVENIAN PHARMACEUTICAL SOCIETY

On behalf of:

EFMC
European Federation
for Medicinal Chemistry

Ljubljana, Slovenia
September 2-6, 2018
www.efmc-ismc.org

EFMC
International Symposium on Medicinal Chemistry

Basel, CH September 6-10, 2020

SEE YOU IN BASEL IN 2020 !

Basel, Switzerland
September 6-10, 2020

Basel Congress Center
www.efmc-ismc.org

EFMC

Organised by the Division of Medicinal Chemistry & Chemical Biology (DMCCB) of the Swiss Chemical Society (SCS), on behalf of the European Federation for Medicinal Chemistry (EFMC).

TABLE OF CONTENT

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

PLENARY LECTURES

ZIKA VIRUS: AN OLD VIRUS WITH A NEW FACE

Tatjana Avšič Županc

University of Ljubljana, Faculty of Medicine, Institute of Microbiology and Immunology, Ljubljana, Slovenia

Zika virus (ZIKV) is a mosquito-borne flavivirus that represented a public health emergency during the recent epidemic. This obscure virus was limited to sporadic cases in Africa and South East Asia until the outbreaks in the Pacific in 2007 and 2013, and during the recent emergence of Zika virus in Brazil in 2015, when it rapidly spread throughout the Americas. Most ZIKV infections are subclinical or characterized by mild self-limiting symptoms including fever with a rash, conjunctivitis and arthralgia. However, neurological complications, by triggering Guillain-Barré syndrome in adults, and neurodevelopmental abnormalities, including microcephaly in babies born to infected mothers, known as congenital Zika syndrome (CZS), have released remarkable advances in understanding the transmission, spread and adverse outcomes of infection. In addition to mosquito vectors, sexual transmission of ZIKV was established and diagnostic studies have confirmed viral RNA in semen, and vaginal secretions of symptomatic patients up to 6 months following the onset of symptoms. Besides, ZIKV is most closely related to the four serotypes of Dengue (DENV) and the sequence similarity between ZIKV and DENV poses unique issues for diagnosis and vaccination, and has implications for disease pathogenesis due to antibody cross-reactivity.Currently, neither a specific antiviral drug nor a vaccine is available for treating or preventing ZIKV infection. However, there are several promising drug targets encoded by the virus or present in host cells.Vaccine development is an active and challenging area of research, but concerns for ZIKV vaccine development include immune-mediated enhancement (ADE) of DENV infection and Guillain–Barré syndrome due to the possible induction of autoreactive antibodies and/or T cells. Thus, current preventive strategies rely on decreasing infected bites, particularly in pregnant women, and on providing up-to-date recommendations to reduce the risk of non-vector transmission of Zika virus.

IT'S A SMALL MOLECULE WORLD: MEDICINAL CHEMISTRY CHALLENGES AND OPPORTUNITIES FOR THE NEXT DECADE

Bayard Huck

Merck Healthcare, Merck KGaA, Darmstadt, Germany

The drug discovery industry is constantly shifting and changing. Medicinal Chemists must be nimble in their quest as drug hunters to identify new medicines of the future. This talk will elaborate the challenges and opportunities of medicinal chemistry that will accelerate progress to benefit patients.

SYNTHETIC GLYCOCONJUGATE VACCINES AGAINST BACTERIAL INFECTIONS

Peter H. Seeberger

Max-Planck Institut for Colloids and Interfaces , Am Mühlenberg 1, 14476 Potsdam, Germany

Most pathogens, including bacteria, fungi, viruses and protozoa, carry unique sugars on their surface. Currently, several glycoconjugate vaccines against bacteria are successfully marketed. Since many pathogens cannot be cultured and the isolation of pure oligosaccharides is difficult, synthetic oligosaccharide antigens are an attractive alternative. In this plenary lecture I will describe a medicinal chemistry approach to the development of semi- and fully synthetic glycoconjugate vaccines against severe bacterial infections, including resistant hospital microorganisms. This approach is fueled by oligosaccharides prepared by automated glycan assembly[1,2] that has been commercialized.[3]Quality control of synthetic oligosaccharides is ensured by ion mobility mass spectrometry (IM-MS).[4]

Vaccine programs aimed at protection from a series of *Streptococcus pneumoniae*serotypes,[5]*Clostridium difficile* [6]and *Klebsiella pneumoniae*[7]have progressed to the late preclinical stages and are now advanced to the clinic by *Vaxxilon AG.*

Synthetic oligosaccharides serve as basis for tools such as glycan microarrays and for the production of monoclonal antibodies.

References

1) Plante, O.J.; Palmacci, E.R.; Seeberger, P.H.; Science2001, 291, 1523.
2) Seeberger, P.H.; Acc. Chem. Res. 2015, 48, 1450.
3) Hahm, H.S.; Schlegel, M.K.; Hurevich, M.; ... Seeberger, P.H.; ProcNat Acad Sci USA,2017, 114, E3385; www.glycouniverse.de
4) Hofmann, J.; Hahm, H.S.; Seeberger, P.H.; Pagel, K.; Nature2015, 526, 241.
5) Parameswarappa, S.G.; Reppe, K.; Geissner, A.; .... Seeberger, P.H.; Cell Chem. Bio.2016,23, 1407; Schumann, B.; Hahm, H.S.; …. Seeberger, P.H.; Science Transl. Med. 2017, 9, eaaf5347; Seeberger, P.H.; Pereira, C.L.; Govindan, S.; Beilstein J. Org. Chem. 2017, 13, 164. Lisboa, M.P.; Khan, N.; Martin, C.; Xua, F.-F.; Reppe, K.; Geissner, A.; Witzenrath, M.; Govindan, S.; Pereira, C.L.; Seeberger, P.H.; PNAS2017,114, 11063.
6) Martin, C.E.; Broecker, F.; Oberli, M.A.; …..Seeberger, P.H.; J. Am.Chem. Soc.2013, 135, 9713; Broecker, F.; Hanske, J.;…. Seeberger, P.H.; Nature Comm. 2016, 7, 11224; Broecker, F.; Martin, C.E.; Anish, C.; Seeberger, P.H.; Cell Chem. Bio.2016,23, 1014.
7) Seeberger, P.H.; Pereira, C.L.; Khan, N.; Xiao, G.; et al. Angew.Chem.Int.Ed.2017,56, 13973.

THE THERAPEUTIC CHALLENGE OF THE NEW ERA: DEVELOPING AND MAKING AVAILABLE LIFE CHANGING TREATMENT TO PATIENTS WITH RARE DISEASES

Diego Ardigó

R&D Rare Diseases Unit Head, Corporate Drug Development, Chiesi Farmaceutici S.p.A., Via Palermo, 26 A, 43122 PARMA, Italy

Rare diseases individually affect a small percentage of the population and in the most extreme cases only a handful of patients worldwide. However, it is estimated the existence of more than 7000 of such conditions and, taken together, they collectively affect more than 5% of the worldwide population. These conditions are often orphan of effective disease-modifying treatments and their epidemiology is so limited that would not support the return of investment in developing new treatments needed by pharma companies to make new therapies available. Orphan drug legislations and regulatory/ financial incentives are now established in many geographies to support profit and no-profit organizations in the development of new treatments for these conditions. Nevertheless, the field of rare disease remains highly challenging in terms of developing, licensing, and bringing to patients new drugs. In addition to the technical complexities due to the limited biological and medical knowledge available on many of these diseases, their genetic nature and pathophysiology, and the small size of trial populations, new challenges have emerged in terms of economic sustainability and technologies needed to treat some of them (e.g. advanced therapies). The presentation will provide a technical, medical, regulatory, and economic picture of the present of rare disease drug development and commercialization from the perspective of a pharmaceutical industry and will highlight the existing challenges together with a perspective on the future of the field.

SUGARS & PROTEINS: GLYCOMIMETICS TO TARGET INFECTIOUS DISEASE

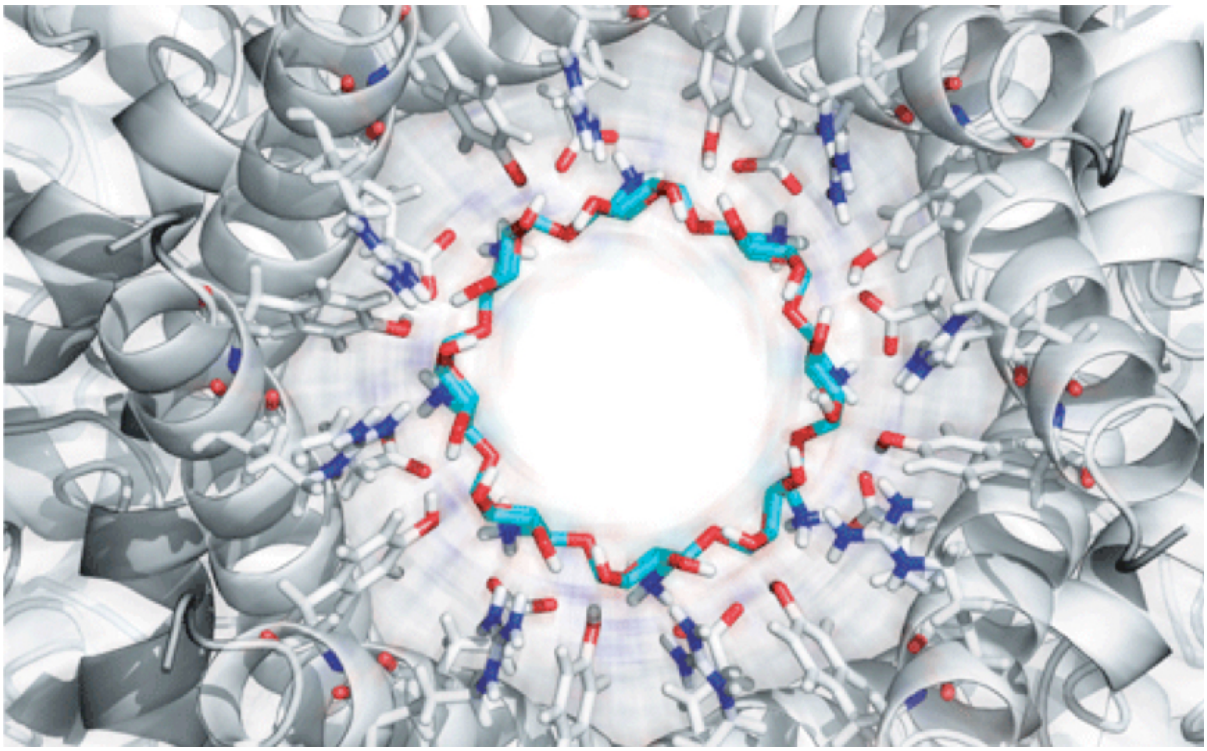
Benjamin Davis

Oxford University, Chemistry Research Laboratory, 12 Mansfield Road, OX1 3TA Oxford, United Kingdom

Our work studies the interplay of biomolecules – proteins, sugars and their modifications.

Strategies to both diagnose, monitor and treat pathogens have never been more urgently needed. The, often unique glycobiology of pathogens reveals not only fascinating mechanistic biology but also suggests potentially selective strategies for targeting and intervention in associated disease.[1-5]

This lecture will cover emerging areas in our group in the chemical glycobiology of pathogens, particularly bacterial pathogens, and the use of this knowledge to develop novel, potentially medically-relevant strategies.



References

1) Nature Chemical Biology 2011, 7, 228
2) Nature Chemistry 2012, 4, 539
3) Nature Chemistry, 2013, 5, 651
4) Nature Chemistry, 2016, 8, 242
5) Nature Chemistry, 2016, 8, 461

DEVELOPMENT OF NON-NUCLEOSIDIC COMPOUNDS AGAINST DNA VIRUSES OF THE HERPES GROUP. THE ERA AFTER NUCLEOSIDES: LETERMOVIR AND PRITELIVIR

Helga Rübsamen-Schaeff

AiCuris Anti-infective Cures GmbH, Wuppertal, Germany

While viruses like HIV or Hepatitis C have seen very active research for antiviral drugs, allowing to turn the HIV-infection from a death sentence into a manageable disease and to cure Hepatitis C, there was only very little activity in the search for novel drugs against viruses of the herpes group. Most existing drugs are polymerase-inhibitors and the majority of them are nucleoside analogues, with the known shortcomings of this compound class.

In an attempt to generate novel drugs against herpes viruses with increased potency and/or tolerability, we chose to address different viral targets with novel chemical compound classes. Pritelivir, a primase-helicase inhibitor was discovered as a highly potent drug against Herpes Simplex Virus and Letermovir, a quinazoline targeting the viral terminase, was generated against the Human Cytomegalovirus. In a clinical phase II study, Pritelivir has shown superiority over the present “gold standard” Valtrex. In a phase III study, Letermovir was shown to protect stem cell transplanted patients from HCMV-reactivation leading to an increased survival rate. Letermovir has meanwhile been licensed in several countries and was introduced into the market as Prevymis™. The research strategies leading to the choice of these novel viral targets and to the optimization of the compounds will be discussed.

SMART CHEMICAL PROBES: FROM BROMODOMAIN LIGANDS TO NATURAL PRODUCTS

Cristina Nevado (1), Andrea Unzue (1), Amedeo Caflisch (2), Aymeric Dolbois (1), Riccardo Cribiu (1), Maria Maluzynska-Hoffman (1), Tim Knehans (2), Pawel Sledz (2), Laurent Batiste (2), Vlad Pascanu (1), Manuela Brutsch (1), Xuan Wang (1), Dimitrios Spiliotopoulos (2)

1) Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH 8057, Zurich
2) Department of Biochemistry. University of Zurich, Winterthurerstrasse 190, CH 8057, Zurich

To expand chemical space and ensure synthetic accessibility is of upmost importance for the discovery of lab-designed binders for novel protein classes as well as for the development of compounds against hard-to-drug proteins. Here, we will present AutoCouple, a de novo approach to computational ligand design focused on the diversity-oriented generation of chemical entities via virtual couplings. In a benchmark application, chemically diverse compounds with low-nanomolar potency for the CBP bromodomain and extremely high selectivity against the BRD4(1) bromodomain were achieved by the synthesis of about 50 derivatives of the original fragment.1,2,3

On the other hand, natural products continue to be a prolific source of bioactive compounds. However, in most cases, their exact cellular targets remain unknown. Here we will present a computational-target-derivatization combined approach to unravel the mode of action of iriomoteolide-3a, a 15-membered macrolide recently isolated from Amphidinium sp. Our results showcase iriomoteolides as novel and easily tunable chemical probes for the in vitro study of actin dynamics in the context of cell motility processes including cell invasion and division.4,5

References

1) Unzue, A.; Xu, M.; Dong, J.; Wiedmer, L.; Spiliotopoulos, D.; Caf-lisch, A.; Nevado, C. J. Med. Chem. 2015, 59, 1350.
2) Unzue, A.; Zhao, H.; Lolli, G.; Dong, J.; Zhu, J.; Zechner, M.; Dol-bois, A.; Caflisch, A.; Nevado, C.; J. Med. Chem. 2016, 59, 3087.
3) Batiste, L.; Unzue, A.; Dolbois, A.; Hassler, F.; Wang, X.; Spiliotopoulos, D.; Nevado, C.; Caflisch, C. ACS Central Science, 2018, 4, 180.
4) Cribiu, R.; Jager, C.; Nevado, C. Angew Chem Int Ed Engl, 2009, 48, 8780.
5) Unzue, A.; Cribiú, R.; Hoffman,M. M.; Knehans, T.; Lafleur, K.; Caflisch, A.; Nevado, C. Chem. Sci. 2018, 9, 3793.

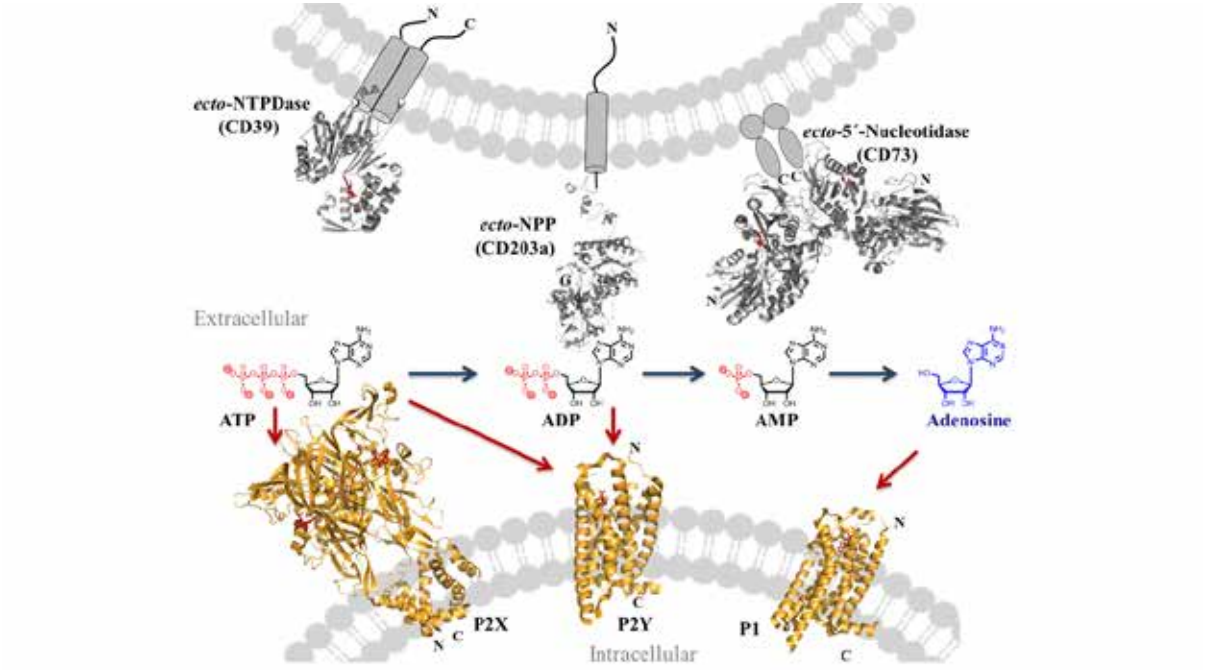
AWARD & PRIZE LECTURES

TOOLS AND DRUGS FOR PURINE TARGETS – IMPORTANT PLAYERS IN INFLAMMATION AND CANCER

Christa E. Müller

PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry 1, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany, christa.mueller@uni-bonn.de

Purine nucleosides and nucleotides are important extracellular signaling molecules that activate cell membrane receptors. Adenosine (or P1) receptors and P2Y nucleotide receptors are G protein-coupled receptors (GPCRs), while P2X nucleotide receptors are ligand-gated ion channels. The concentrations of extracellular nucleosides and nucleotides are tightly regulated by ecto-nucleotidases including ecto-nucleoside triphosphate diphosphohydrolases (ecto-NTPDases, CD39), ecto-nucleotide pyrophosphatases (ecto-NPPs, CD203a) and ecto-5'-nucleotidase (CD73).



Nucleoside and nucleotide signalling plays a major role in all parts of the body especially under pathological conditions, e.g. in inflammation, pain, immune reactions and cancer. While nucleotides such as ATP are pro-inflammatory and increase pain sensation, adenosine is strongly immunosuppressant and involved in the immune escape of cancer cells. In addition, GPCRs activated by the nucleobase adenine have been identified and designated P0 receptors. Our group has focused (i) on the development and characterization of assays, tool compounds and drugs for P0, P1 and P2 receptors and ectonucleotidases, and (ii) on studies directed towards gaining structural information regarding protein-ligand interactions. Moreover, we have explored a series of orphan GPCRs related to purine-activated receptors. Recently, we have been developing tools for directly studying and inhibiting G proteins.

HOW BEST TO DISCOVER BIOACTIVE SMALL MOLECULES?

Adam Nelson

School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK

Our knowledge of the biological relevance of chemical space is based, to a large extent, on its historical exploration by synthesis (and biosynthesis). However, chemists' exploration of chemical space has been uneven and unsystematic: the known organic chemistry 'universe' is dominated by a small number of scaffolds that are found in a large number of small molecules. Developing synthetic approaches that allow broad tracts of chemical space to be explored has proved extremely challenging.

This presentation will describe synthetic approaches that can underpin the discovery of novel bioactive small molecules. Synthetic approaches that allow the systematic variation of ligand scaffold will be described, including an approach that yielded natural product-like molecules with unprecedented skeletal diversity. Finally, a novel approach to bioactive small molecule discovery will be described - activity-directed synthesis - in which bioactive small molecules emerge in parallel with an associated synthetic route.

ACTIVITY-BASED PROTEOMICS - PROTEIN AND LIGAND DISCOVERY ON A GLOBAL SCALE

Benjamin Cravatt

Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037

Advances in DNA sequencing have radically accelerated our understanding of the genetic basis of human disease. However, many of human genes encode proteins that remain uncharacterized and lack selective small-molecule probes. The functional annotation of these proteins should enrich our knowledge of the biochemical pathways that support human physiology and disease, as well as lead to the discovery of new therapeutic targets. To address these problems, we have introduced chemical proteomic technologies that globally profile the functional state of proteins in native biological systems. Prominent among these methods is activity-based protein profiling (ABPP), which utilizes chemical probes to map the activity state of large numbers of proteins in parallel. In this lecture, I will describe the application of ABPP to discover and functionally annotate proteins in mammalian physiology and disease. I will also discuss the generation and implementation of advanced ABPP platforms for proteome-wide ligand discovery and how the integration of these global 'ligandability' maps with emergent human genetic information can expand the druggable fraction of the human proteome for basic and translational research objectives.

THE CHALLENGES OF RESTORING PROGRAMMED CELL DEATH THROUGH MCL1 INHIBITION

Márton Csékei

Servier Research Institute of Medicinal Chemistry Záhony u. 7., 1031 Budapest, Hungary, marton.csekei@servier.com

Tumour cells that harbour genetic mutations and are recognized as abnormal should be naturally eliminated but they maintain their existence by a combination of multiple activities – also known as the hallmarks of cancer [1]. One of these hallmarks is the evasion of apoptosis, the programmed cell death. The restoration of the apoptotic cascade in tumor cells has long been recognized as a promising way to treat cancer but the major members of this protein family, BCL2, MCL1, and BCL-xL have remained elusive targets decades long for drug discovery. Helped by our better understanding of these targets and increased expertise in inhibiting protein-protein interactions the decade long efforts of the pharmaceutical industry has recently been rewarded by the identification of potent and selective inhibitors for some family members [2,3].

The presentation overviews the challenges we faced in our discovery program and the solutions that helped our progress. A particular emphasis will be given to the earlier stages of the project where establishing reliable and relevant assays, structural biology tools, and a robust SAR to drive medicinal chemistry had to be tackled in parallel. The lessons learned during this period will also be summarised since they could help fellow medicinal chemists in tackling new complex targets.

References

1) D. Hanahan, R.A. Weinberg Cell, 2011, 144, 646.
2) A. Ashkenazi, W.J. Fairbrother, J.D. Leverson, A.J. Souers Nature Reviews in Drug Discovery, 2017, 16, 273.
3) A. Kotschy, Z. Szlávik, J. Murray et al. Nature, 2016, 538, 477.

CARBOHYDRATE-BINDING PROTEINS AS TARGETS FOR ANTI-INFECTIVES AND DIAGNOSTICS: ESKAPE PATHOGEN PSEUDOMONAS AERUGINOSA AND ITS LECTINS

Alexander Titz

Helmholtz-Institut für Pharmazeutische Forschung Saarland, Saarbrücken, Germany

Pseudomonas aeruginosa causes a substantial number of nosocomial infections and is the leading cause of death of cystic fibrosis patients. This Gram-negative bacterium is highly resistant against antibiotics and further protects itself by forming a biofilm. Moreover, a high genomic variability among clinical isolates complicates therapy.

Its lectin LecB, a carbohydrate-binding protein, is a virulence factor and necessary for adhesion and biofilm formation.[1] We analyzed the sequence of LecB variants in a library of clinical bacterial isolates and demonstrate that it can serve as a marker for strain family classification. LecB from the highly virulent model strain PA14 presents 13% sequence divergence with LecB from the well characterized PAO1 strain. Despite several amino acid variations at the carbohydrate binding site, glycan array analysis showed a comparable binding specificity for both variants.[2]

Based on the crystal structures of the lectin with its glycan ligands, we dissected the contributions of individual functional groups to protein binding in a biophysics-guided approach. This knowledge was then used for the development of small and drug-like glycan-based molecules as LecB inhibitors as future anti-biofilm compounds in chronic *P. aeruginosa* infections.[3-7] Multiparameter optimization yielded potent anti-biofilm compounds for both strain types and oral availability in mice.[8]

Thus, the different LecB sequences serve as marker for strain classification, but due to comparable ligand selectivity, LecB is a highly promising target for anti-virulence therapies, addressing members from both *P. aeruginosa* families, PAO1 and PA14.

In contrast, LecA binds galactosides with much lower affinity hampering therapeutic intervention at this target. Therefore, we have developed the first covalent inhibitor of a lectin and employed this LecA-specific irreversible inhibitor for LecA-dependent biofilm imagining of *P. aeruginosa*.[9]

References

1) Wagner, S.; Sommer, R.; Hinsberger, S.; Lu, C.; Hartmann, R.W.; Empting, M.; Titz, A. J. Med. Chem. 2016, 5929-5969.
2) Sommer, R.; Wagner, S.; Varrot, A.; Nycholat, C.; Khaledi, A.; Häussler, S.; Paulson, J.; Imberty, A.; Titz, A. Chem. Sci. 2016, 7, 4990-5001.
3) Sommer, R.; Hauck, D.; Varrot, A.; Wagner, S.; Prestel, A.; Möller, H.M.; Imberty, A.; Titz, A. ChemistryOpen 2015, 4, 756-767.
4) Hofmann, A.; Sommer, R.; Hauck, D.; Stifel, J.; Göttker-Schnetmann, I.; Titz, A. Carbohydr. Res. 2015, 412, 34-42.
5) Sommer, R.; Exner, T.E.; Titz, A. PLoS ONE 2014, 9(11): e112822.
6) Hauck, D.; Joachim, I.; Frommeyer, B.; Varrot, A.; Philipp, B.; Möller, H.M.; Imberty, A.; Exner, T.E.; Titz, A. ACS Chem. Biol. 2013, 8(8), 1775-1784.
7) Beshr, G.; Sommer, R.; Hauck, D.; Siebert, D.C.B.; Hofmann, A.; Imberty, A.; Titz, A. Med. Chem. Commun. 2016, 7, 519-530.
8) Sommer, R.; Wagner, S.; Rox, K.; Varrot, A.; Hauck, D.; Wamhoff, E.-C.; Schreiber, J.; Ryckmans, T.; Brunner, T.; Rademacher, C.; Hartmann, R. W.; Brönstrup, M.; Imberty, A.; Titz, A. J. Am. Chem. Soc. 2018, 140(7), 2537-2545.
9) Wagner, S.; Hauck, D.; Hoffmann, M.; Sommer, R.; Joachim, I.; Müller, R.; Imberty, A.; Varrot, A.; Titz, A. Angew. Chem. Int. Ed. Engl. 2017, 56, 16559-16564.

DISCOVERY OF CFTR MODULATORS FOR THE TREATMENT OF CYSTIC FIBROSIS

Peter Grootenhuis

Vertex Pharmaceuticals Incorporated, San Diego, USA

Cystic Fibrosis (CF) is an autosomal recessive disorder affecting ~70,000 patients worldwide. CF is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) protein that result from mutations in the CFTR gene. Defects in the CFTR protein lead to reduced chloride transport resulting in thick, sticky mucus that causes abnormalities in multiple organs. In the lungs, this excess mucus can lead to progressive loss of lung function and premature death. Several HTS campaigns were performed to identify CFTR modulator hits that are able to increase CFTR function. Extensive optimization efforts eventually resulted in the identification of three CFTR modulator drugs. Ivacaftor, a CFTR potentiator, increases CFTR channel gating while lumacaftor and tezacaftor, known as a CFTR correctors, increase processing and trafficking of mutant CFTR to the cell surface. A perspective will be provided on recent developments in CFTR modulator therapies.

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

INVITED LECTURES & ORAL COMMUNICATIONS

ARTIFICIAL INTELLIGENCE. NOT JUST ANOTHER NAME FOR IN SILICO DESIGN

David Leahy

The Discovery Bus Ltd, Oakridge Centre, Gibhill Farm, SK10 5SE, UK

It is tempting to see the recent wave of interest in Artificial Intelligence (AI) as the hype of a new business cycle. After all, much of what is labelled AI are technologies such as machine learning that our industry has used for decades and which we know as “in silico design’. The technologies are widely available and in the hands of an expert working alongside a drug discovery team, they can have a significant impact on drug discovery projects, particularly in the early lead discovery phase.

Nevertheless, these provide incremental improvements. Better toolkits extracting better insight from bigger datasets, faster. In silico design, not AI.

AI is a co-worker not a toolkit. It doesn’t have a user interface. AI is software that makes decisions and which operates autonomously. It is composed of multiple software ‘agents’ that run tasks out of sight and control of the ‘user’. It has know-how, tacit knowledge and experience. It makes decisions based on clear goals and the decisions are the big ones, such as

- “Which compound should we make next?”,
- “which compounds get sent for which assays”,
- “which project should we invest in?"

The premise of the talk is that like many other industries, drug discovery has the potential for an AI revolution. It isn’t here yet, but the technology is proven and working piecemeal in multiple domains. AI builds on the richness of tools and technologies of *in silico* design, but it is the autonomy of action that differentiates AI.

The talk builds on this premise by defining a ‘Turing Test’ for medicinal chemistry. It reviews and explains the components of an AI system that could satisfy the test using examples from drug discovery wherever possible.

These elements include belief networks for refining tacit knowledge coupled with new learning and inference methods. It also covers decision making systems in the context of drug discovery.

The talk is a forward looking and opinionated definition of AI, what makes it different from in silico design and the practical steps towards a functional autonomous decision making system for drug discovery.

RE-ENERGISING SMALL MOLECULE DRUG DISCOVERY

Willem van Hoorn

Exscientia Ltd, 36 St Giles', Oxford, OX1 3LD, UK

The optimisation trajectory of hit to lead to candidate is the most expensive part of drug discovery. Exscientia's Centaur drug discovery platform promises to bring that cost down significantly by combining the strengths of AI compound design and human strategic thinking. A high level overview of the technology is presented and results are shown from a prospective proof of concept study as well as a successful collaboration that resulted in the delivery of a clinical candidate in less than a year.

http://drug.design

Computer-Aided Synthesis Planning

Marwin Segler

BENEVOLENTAI, London, United Kingdom

Computer-aided retrosynthesis, also known as computer aided synthesis planning (CASP), is one of the oldest and legendary research topics on the intersection of artificial intelligence and chemistry [1,2]. CASP would be a highly valuable tool to find better synthetic routes and to determine the synthesizability of virtual de-novo designed compounds. However, despite several waves of research, CASP was never widely accepted by chemists, because the systems were slow, and the results were considered to be of unsatisfactory quality [3,4,5].

In this talk, recent findings on retrosynthesis using deep learning and modern search algorithms [6,7] are presented. First, we show that deep neural networks can be trained on very large reaction datasets to predict and rank the most suitable (automatically extracted) transforms to apply to a molecule [6]. This way of training also allows the machine to learn the tolerated and conflicting functional groups of a transform implicitly [6]. In earlier approaches, this information had to be entered manually by experts. Second, to perform search, we employ Monte Carlo Tree Search (MCTS). MCTS allows to efficiently treat problems with very large branching factors, and does not rely strongly on hand-designed search heuristics, which makes it very well suited for retrosynthesis [7].

In comparison to the established search techniques, our approach solves twice as many molecules and is almost two orders of magnitudes faster [7]. Furthermore, we conducted double blind tests to assess the quality of the results. Here, for the first time, organic chemists could not distinguish between real routes taken from the literature and predicted routes [7].

References

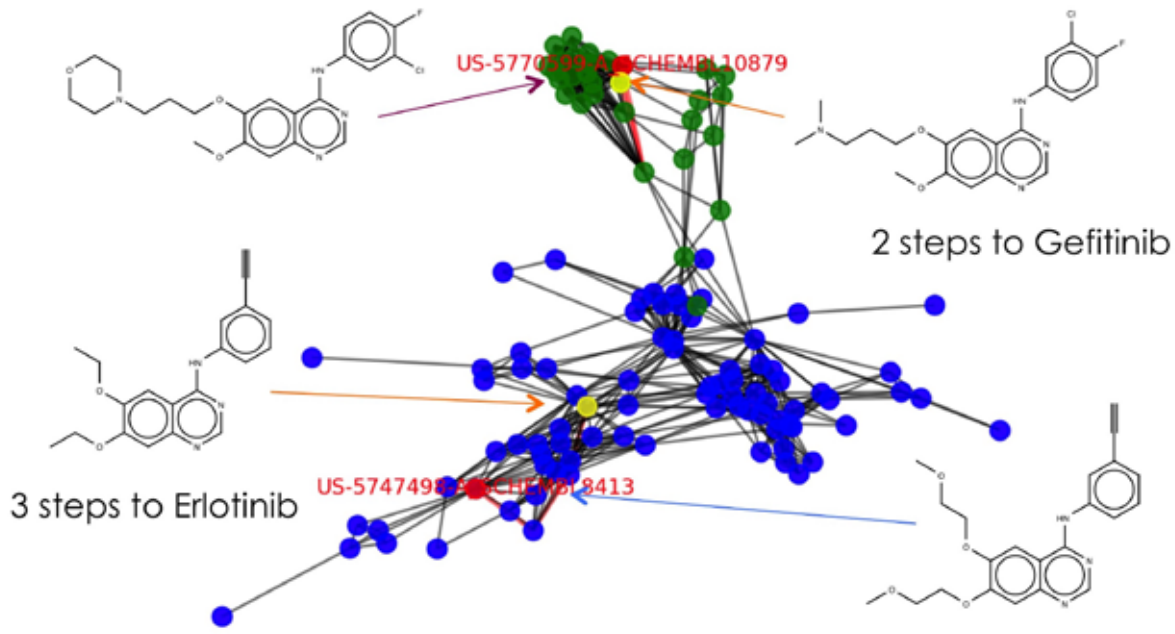
1) G. Vleduts, Information Storage and Retrieval, 1963, 117
2) E.J. Corey, W.T. Wipke. Science, 1969, 166, 178
3) W.D. Ihlenfeldt, J. Gasteiger, Angew. Chem. Int. Ed., 1996, 34, 2613
4) S. Szymkuc et al., Angew. Chem. Int. Ed., 2016, 55, 5904
5) A. Cook et al., W. Interd. Rev. Comp. Mol. Sci., 2012, 79
6) M. Segler, M. P. Waller, Chem. Eur. J. 2017, 23, 5966
7) M. Segler, M. Preuß, M. P. Waller, Nature 2018, 555, 604

POTENCY AND PATENTS, NEW ARENAS FOR MATCHED MOLECULAR PAIR ANALYSIS IN THE AI WORLD

Alexander Dossetter (1), Edward Griffen (1), Shane Montague (1), Andrew Leach (2)

1) MedChemica Ltd, Biohub, Alderley Park, Macclesfield, Chesire SK10 4TG United Kingdom
2) Liverpool John Moores University, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF , United Kingdom

Of the many approaches computational chemistry has brought to drug hunting, medicinal chemists often find matched molecular pair analysis (MMPA) and QSAR to be two of the most useful.[1] The key strength of MMPA is in its interpretability, in contrast to other machine learning methods.[2,3,4] Here we show how MMPA and QSAR may be combined to understand potency SAR while maintaining highly interpretable models. We further extend the paradigm by showing how network analysis can be applied to potency data sets to identify pivotal compounds and even in cases such as patents where the biological information is missing, useful inferences can be made. Insights from these analyses and outputs can be applied to current projects to provide new directions and with new results, models can be regenerated to close the loop and achieve the desired goal of learning machines that augment expert medicinal chemists.



Network graph showing the relationship between two patents of clinically used kinase inhibitors [Each spot is a compound and each line a matched pair relationship]

References

1) Dossetter AG, Griffen EJ, Leach AG. Matched Molecular Pair Analysis in drug discovery. Drug Discov Today 2013, 18, 721. doi:10.1016/j.drudis.2013.03.003.
2) Kramer C, Ting A, Zheng H, Hert J, Schindler T, Stahl M, et al. Learning Medicinal Chemistry Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Rules from Cross-Company Matched Molecular Pairs Analysis (MMPA): Miniperspective. J Med Chem 2017. doi:10.1021/acs.jmedchem.7b00935.
3) Lukac I, Zarnecka J, Griffen EJ, Dossetter AG, St-Gallay SA, Enoch SJ, et al. Turbocharging Matched Molecular Pair Analysis: Optimizing the Identification and Analysis of Pairs. J Chem Inf Model 2017, 57, 2424. doi:10.1021/acs.jcim.7b00335.
4) Lukac I, Zarnecka J, Griffen EJ, Dossetter AG, St-Gallay SA, Enoch SJ, et al. Turbocharging Matched Molecular Pair Analysis: Optimizing the Identification and Analysis of Pairs. J Chem Inf Model 2017, 57, 2424. doi:10.1021/acs.jcim.7b00335.

SMALL MOLECULE IMMUNE CHECKPOINT ANTAGONISTS FOR CANCER THERAPY

Murali Ramachandra

AURIGENE DISCOVERY TECHNOLOGIES LIMITED, 39-40, KIADB Industrial Area, Electronic City, Phase II, Hosur Road, Bangalore – 560100, INDIA

Activation of anti-tumor immune response by specific inhibition of immune checkpoint pathways using monoclonal antibodies have now become of the mainstay in cancer therapy as evidenced by their widespread use in an expanding list of indications. Although these antibodies show impressive durable clinical activity, they suffer from the shortcomings including response only in a subset of cancer patients, need to administer by intravenous injection and immune-related adverse events (irAEs) due to the breaking of immune self-tolerance. Therefore, there is a strong rationale to consider non-antibody based approaches for immune checkpoint protein inhibition towards achieving the desirable response in the clinic.

Small molecule-based therapeutic approaches offer the potential to address the shortcomings of antibody-based checkpoint inhibitors. Because of their significantly smaller size, oral dosing providing convenience to patients would be possible. A greater response rate is likely due to higher tumor distribution and the possibility of simultaneous targeting of similar proteins in a manner analogous to small-molecule kinase inhibitors targeting more than one target vs. exquisite selectivity of an antibody to a kinase target. Because of the smaller size, potentially these agents can recognize binding pockets conserved among proteins of the same protein family. Due to their shorter pharmacokinetic profile, small-molecule agents may allow better management of irAEs and could be better options for use in combination with other agents. Additionally, in view of the significantly lower costs associated with drug manufacturing and drug administration (oral vs intravenous infusion), small molecule antagonists may make the treatment more affordable.

Our efforts in the last few years at Aurigene have resulted in the discovery of a series of small molecule agents targeting either individual checkpoint protein or dually targeting two non-redundant checkpoint proteins with pockets of sequence similarity. We have focused on targeting immune checkpoint proteins that suppress predominantly T-cell responses (such as PD-L1, VISTA and TIM3) as well as those that limit innate immune responses (such as CD47-SIRPα). Our rational design approach takes advantage of synthesizing loop-strand sequences from the interphase, determining critical pharmacophore required for functional antagonism and further optimizing the pharmacophore on a non-peptidic template.

The most advanced compound, CA-170, which dually targets PD-L1 and VISTA, is currently in Phase 2 clinical development. CA-170 has shown excellent oral bioavailability, tolerability, immune pharmacodynamic effects in both tumor and circulation in Phase I studies. Other advanced agents that are completing IND-enabling studies include CA-327, a dual antagonist of PD-L1 and TIM3, and AUR-103, an antagonist of CD47-SIRPα signaling.

TANKYRASE INHIBITOR DEVELOPMENT: EVIDENCE FOR POTENTIAL IN CANCER IMMUNE THERAPY

Anita Wegert (1), Jo Waaler (5), Ruben Leenders (1), Sven Sowa (2), Upendra Rao Anumala (3), Piotr Nieczypor (1), Marc Nazaré (3,4), Lari Lehtiö (2), Stefan Krauss (5)

1) Mercachem bv, Kerkenbos 1013, 6546 BB Nijmegen, Netherlands
2) Faculty of Biochemistry and Molecular Medicine, Biocenter Oulu, University of Oulu, Finland
3) Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Campus Berlin-Buch, 13125 Berlin, Germany
4) Berlin Institute of Health (BIH), Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany
5) Hybrid Technology Hub - Centre of Excellence, University of Oslo and Oslo University Hospital, Norway

WNT/β-catenin signaling regulates key cellular functions including proliferation, differentiation, migration, apoptosis, stem cell renewal and immune system modulation. Abberrant WNT/ β-catenin signaling is found in multiple cancers. In particular, the recently described role of the WNT/β-catenin pathway in regulating immune cell infiltration in the tumor micro-environment suggests an impact of the pathway on immunotherapy [1]. Hence, WNT-directed therapeutic intervention represents an area of significant developmental focus.

The Poly-ADP-ribosylases tankyrase 1 and 2 are cental biotargets in the WNT/β-catenin signaling pathway, regulating the turnover of the protein complex that controls β-catenin stability and in adition impacting the hippo signaling pathway. Several small molecules have been identified that inhibit tankyrases 1 and 2 [2], and we have earlier show efficacy of tankyrase inhibitors in WNT dependent adenoma and tumor models [3, 4].

Here we describe the successful discovery of a selective tankyrase inhibitor from a hit stage to a late lead stage with potential as a preclinical candidate [5, 6]. In addition, we show proof of concept for our tankyrase inhibitor as a immune modulatory agent.

References

1) Pai et al. J Hematol Oncol. 2017 May 5;10(1):101
2) Ferri et al. Eur J Med Chem. 2017 Dec 15;142:506-522
3) Waaler J, et al. Cancer Res. 2012 Jun 1;72(11):2822-32
4) Lau T, et al. Cancer Res. 2013 May 15;73(10):3132-44
5) Voronkov et al. J Med Chem. 2013 Apr 11;56(7):3012-23
6) Anumala et al. J Med Chem. 2017 Dec 28;60(24):10013-10025

SMALL MOLECULES INHIBITING PD1-PDL1 IMMUNE CHECKPOINT

Shabnam Shaabani (1), Tryfon Zarganes-Tzitzikas (2), Constantinos Neochoritis (2), Niels van Oosterwijk (2), Amalia Dolga (3), Krzysztof M. Zak (4), Przemyslaw Grudnik (4), Katarzyna Magiera (4), Philip Elsinga (5), Grzegorz Dubin (4), Tad Holak (4), Alexander Doemling (1)

1) Department of Drug Design, University of Groningen, Groningen, Netherlands
2) SMIO Pharma, Groningen, Netherlands
3) Department of Physiology, University of Groningen, Groningen, Netherlands
4) Jaggielonian University, Krakow, Poland
5) Faculty of Medical Sciences, UMCG, Groningen, Netherlands

Immune checkpoint blockade (ICB) of programmed cell death-1 receptor (PD1) and its ligand (PDL1) restores T-cell activation in many systems and thus it is a rarely occurring therapeutic breakthrough in cancer therapy. Currently, interfering PD1-PDL1 axis with monoclonal antibodies (mAbs) has provided unprecedented results in cancer treatment. However, mAbs are expensive to produce and their high molecular weight leads to poor tissue and tumor penetration. Therefore, search for non-mAbs including small molecules is needed.

We have recently solved the co-crystal structure of human PD1 interaction with PDL1.[1] This protein-protein interaction (PPI) is largely flat and featureless and by all commonly used predictive technologies/software difficult to drug. Based on the knowledge of the 3D structures of the human PPI and the availability of multiple tools in our laboratories, we have discovered several novel classes of potent PD1-PDL1 inhibitors including small molecules and artificial macrocycles using structure based drug design, fragment screening and screening of directed multicomponent reaction libraries. Here, we will analyze the PPI and a co-crystal structure of a cyclic peptide potently binding to PDL1.[2] Interestingly, these peptides are capable of potently antagonizing PD-L1 signaling and similar to antibodies, can restore the function of T-cells. Next, we will discuss the cocrystal structure of small molecules with PDL1 and their biological activity.[3,4] Our inhibitors are directed against PD1 and PDL1 and have been characterized for their potency to prevent T-cell exhaustion. We found comparable activity to currently marketed antibodies in T-cell activation assay. Aside cancer we also investigate their usage in neuroinflammatory (Alzheimer's) and infectious diseases, which are both characterized by PD1-PDL1 overexpression in relevant cell types, a hallmark of T-cell exhaustion.

References

1) Zak, K. M. et al. Structure of the complex of human programmed death 1, PD-1, and its ligand PD-L1, Structure 2015, 23, 2341-2348.
2) Magiera-Mularz, K. et al. Bioactive macrocyclic inhibitors of the PD-1/PD-L1 immune checkpoint, Angew. Chem. Int. Ed. 2017, 56, 13732-13735.
3) Zak, K. M. et al. Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1), Oncotarget 2016, 7, 30323-30335.
4) Guzik, K. et al. Small-molecule inhibitors of the programmed cell death-1/programmed death-ligand 1 (PD-1/PD-L1) interaction via transiently induced protein states and dimerization of PD-L1, J. Med. Chem. 2017, 60, 5857-5867.

NEW SMALL-MOLECULE IMMUNE CHECKPOINT INHIBITORS: A STEP FORWARD IN CANCER IMMUNOTHERAPY

Rita C. Acúrcio (1), Jorge A. R. Salvador (2), Helena F. Florindo (1), Rita C. Guedes (1)

1) iMed.ULisboa, Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal
2) Center for Neurosciences and Cell Biology, Universidade de Coimbra, Coimbra, Portugal

Immunotherapy is currently a powerful strategy in cancer therapy with very exciting outcomes. In particular, modulation of immune checkpoint receptors have gain special attention. These immune regulators limit proliferation and activity of T cells and other immune cells enrolled in these signaling pathways. Under normal conditions, they are essential in modulation of immune responses; however, they are also one of the major mechanisms used by tumors to evade immune system recognition and destruction. To date, several immune checkpoint receptors have been identified and used as therapeutics in oncology, as programmed cell death protein 1 (PD-1). When engaged by one of its ligands (PD ligand 1 (PD-L1) and PD ligand 2) PD-1 limits autoimmunity. PD-1 ligands are upregulated in many human cancers and their blockade could lead to activation of T cells and therefore enforce tumor recognition. In fact, PD-1/PD-L1 pathway is one of the most successful pathways in the context of clinical cancer immunotherapy with several approved drugs. These successful therapies rely on the use of antibodies. However, despite their outstanding success, they still have numerous disadvantages as severe immune-related adverse events.

Recently, small-molecule modulators have emerged as safer therapeutic alternative. However, limited efforts have been directed toward immune checkpoint receptors. Our study is focus on the discovery of small-molecule inhibitors targeting PD-L1 in order to block PD-1/PD-L1 interaction and therefore overcome antibody therapy disadvantages. Limited structural information of PD-L1 led us to a detailed structural characterization based on *in silico* studies (molecular docking). After assessing structural features (e.g. flexibility and binding pocket) and following a computer assisted drug discovery approach we accomplished a structure based virtual screening campaign. Potential PD-L1 inhibitors were selected and their activity have been tested by Homogeneous Time Resolved Fluorescence (HTRF) assay. We were able to identify new small-molecule PD-L1 inhibitors that are currently being tested *in vitro*. Therefore, immune checkpoint blockade using small molecules represent a step forward in cancer immunotherapy.

Acknowledgements: We thank the Fundação para a Ciência e a Tecnologia for financial support PD/BD/128238/2016, UID/DTP/04138/2013, SAICTPAC/0019/2015 and ENMed/0051/2016

BREAKING THE LIMITS IN ANALYZING CARBOHYDRATE RECOGNITION BY NMR

Jesus Jimenez-Barbero

CIC bioGUNE, Bizkaia Technology Park, Derio, Spain

Molecular recognition by specific targets is at the heart of the life processes. In recent years, it has been shown that the interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization, embryogenesis, and tissue maturation, to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, the determination of the structural and conformational factors and the physicochemical features that govern the molecular recognition of these molecules is of paramount importance.

Particular attention will be paid to the application of state-of-the-art NMR methods both from the ligand and receptor's perspective to the study of molecular recognition processes between a variety of polypeptides of biomedical interest and carbohydrate-based molecules, drugs and inhibitors. NMR methods include not only the typical chemical shift perturbation analysis, Saturation Transfer Difference, and trNOESY experiments, but also novel 19F- and paramagnetic-based NMR methodologies that have permitted to access to information on large glycans, breaking the limits of the application of NMR in this field.The dissection of the key features that regulate molecular recognition processes between glycans and their receptors, especially lectins related to immune response and viral infections will be presented. The final aim is to dissect and to quantitatively evaluate the relative importance of polar (hydrogen bonding, electrostatic interactions) and non polar (van der Waals, CH-π) forces in these molecular recognition processes

References

1) Ardá A & Jiménez-Barbero J., Chem Comm, 2018, DOI: 10.1039/C8CC01444B
2) Canales A et al., Angew Chem Int Ed Engl. 2017;56(47):14987-91.
3) Unione L, et al., ACS Chem Biol. 2016;11(8):2149-57
4) Coelho H, et al., J Am Chem Soc. 2015;137(39):12438-41.
5) Canales Á, et al., J Am Chem Soc. 2014;136(22):8011-7.
6) Canales Á, et al., Angew Chem Int Ed Engl. 2013;52(51):13789-93

TARGETING OF CANCER SPECIFIC GLYCOPEPTIDE EPITOPES

Hans H. Wandall

Department of Cellular and Molecular Medicine, Center for Glycomics, University of Copenhagen, Copenhagen, Denmark

Aberrant glycosylation is a key feature of carcinogenesis involved in several hallmarks of cancer. Due to their strategic presentation on the surface of cancer cells, aberrant glycans serve as important targets for cancer immunotherapy. While most work has been investigating the importance of cancer-associated changes in glycosaminoglycans, N-linked glycosylation, and glycosphingolipids, we still have limited information on O-linked glycosylation. It is, however, clear that O-glycans are truncated in many cancers and that such truncated O-glycans are involved in several cancerous events, including increased growth and invasive potential. Importantly, not only the length of the O-glycans is relevant, but also the location of the individual O-glycans within a protein has implications for protein function and cancer growth. Combining genetic engineering and mass spectrometry, we have performed a systematic analysis of native-O-glycosylation using lectin affinity chromatography coupled to liquid chromatography mass spectrometry (LC-MS)/MS, and determined the precise location of O-glycans in multiple cell lines as well as human plasma, platelets, and endothelial cells. Collectively, our data illustrate the global properties of native O-glycosylation and provide a source of cancer-specific O-glycopeptide targets for immunotherapy. The importance of such O-glycopeptide epitopes in cancer therapy is demonstrated by the development of high affinity Tn-MUC1-specific monoclonal antibodies used to generate engineered CAR T Cells selectively targeting cancer cells across multiple cancer histotypes. The results provide support for targeting cancer-specific truncated O-glycans with immunotherapeutic measures.

A MULTIDISCIPLINARY STRATEGY TO SYNTHETIC CARBOHYDRATE-BASED CONJUGATES FOR VACCINATION AGAINST SHIGELLA: FROM CONCEPT TO FIRST-IN-HUMAN STUDY

Laurence Mulard (1,2)

1) Institut Pasteur, Chemistry of Biomolecules Unit, 28 rue du Dr Roux, 75 724 Paris Cedex 15, France
2) CNRS UMR3523, Institut Pasteur, 75015, France

Shigellosis, or bacillary dysentery, caused by the enteroinvasive bacteria *Shigella*, remains one of the top diarrheal diseases in children under five.[1] Species/serotype diversity and geographical distribution strongly support the need for a multivalent vaccine against *S. flexneri*. Epidemiological data suggest that protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the bacterial lipopolysaccharides (LPS). In the search for a highly immunogenic *Shigella* vaccine able to generate protective immunity in young children, we have engaged into the development of immunogens consisting of synthetic fragments of the putative O-Ags covalently linked *via* single point attachment to carrier proteins as a possible alternative to detoxified *Shigella* LPS-protein conjugates.

A multidisciplinary strategy interfacing medicinal chemistry and structure-based vaccinology was implemented. It consists firstly in the identification of sets of "protective" epitopes by use of a diversity of well-defined synthetic oligosaccharides representing fragments of the O-Ag of interest. Protein conjugates of the most promising oligosaccharides are then evaluated for their immunogenicity in mice. SF2a-TT15, a tetanus toxoid (TT) conjugate encompassing a synthetic hapten corresponding to three basic repeating units of the O-Ag from *S. flexneri* 2a (SF2a), the most prevalent *Shigella* serotype, was designed accordingly.[2] In preclinical studies, SF2a-TT15 has been shown to induce anti-LPS bactericidal antibodies. A GMP batch was produced and a first-in-human, single-blinded, observer-masked randomized, dose escalation, placebo-controlled study was conducted to assess safety and immunogenicity in healthy adult volunteers.[3]

With the first rationally designed synthetic oligosaccharide conjugate vaccine candidate in hand for the most prevalent *Shigella* serotype, this presentation provides an overview of our strategy for a broad coverage *Shigella* vaccine. Emphasis is on hapten selection, glycovaccine design and production of a GMP batch. Safety and immunogenicity data following first use in human are exposed and the next steps towards establishing efficacy in human are discussed. Moreover, the presentation reports progress on a synthetic carbohydrate-based vaccine designed to provide broad coverage against *S. flexneri*.

References

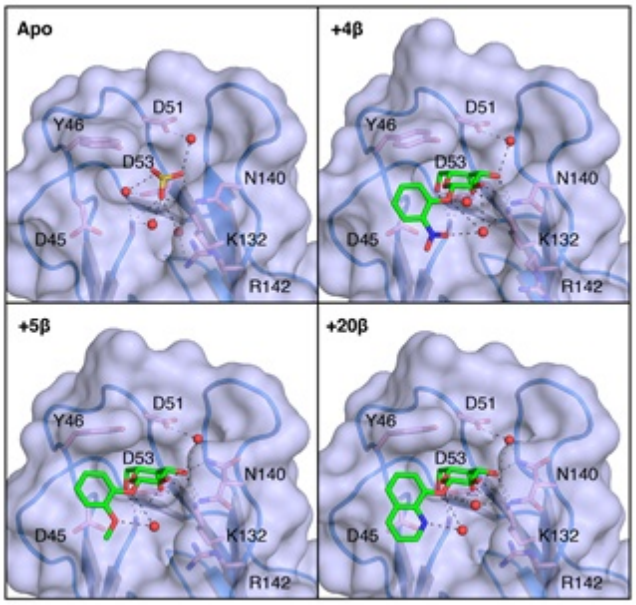
1) J. Liu, et al., Lancet 2016, 388, 1291-1301.
2) R. van der Put, et al., Bioconjugate Chem 2016, 27, 883-892.
3) https://clinicaltrials.gov/ct2/show/NCT02797236
4) Acknowledgements. This work was supported by the European Commission Seventh Framework Program (FP7/2007-2013) under Grant agreement No. 261472-STOPENTERICS and the Institut Pasteur Vaccinology Program (GPF Vaccinologie) under Grant agreement FlexBiVac.

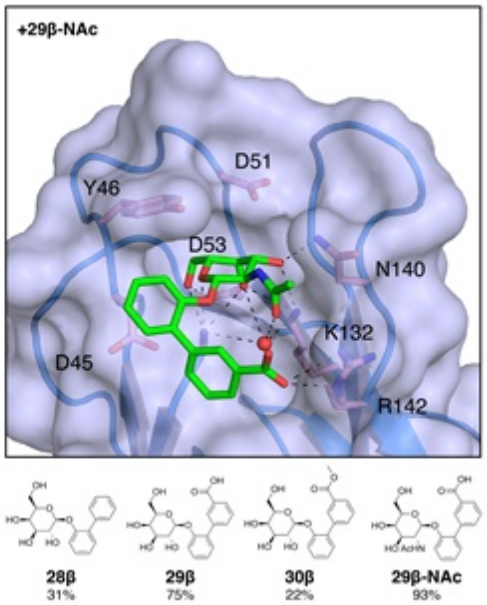
GlYCOSIDE ANTAGONISTS OF BACTERIAL LECTINS: NEW TREATMENT OPTIONS FOR RECURRENT AND ANTIBIOTIC RESISTANT UTI

James Janetka (1), Amarendar Maddirala (1), Ryan Chugani (1), Vasilios Kalas (2), Michael Hibbing (2), Jerry Pinkner (2), Scott Hultgren (2)

1) Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, Saint Louis, Missouri USA
2) Department of Molecular Microbiology, Washington University School of Medicine, Saint Louis, Missouri USA

The global rise in multi-drug resistant bacteria underscores the urgent need for new therapeutics to prevent and treat urinary tract infection (UTI). F9 pili are tipped with the FmlH adhesin, which is important for persistence of uropathogenic *Escherichia coli* (UPEC) in both the bladder and kidney during chronic UTI, one of the most common infections worldwide. Here, we describe the structure-guided drug design of high-affinity galactoside and N-acetylgalactosaminoside inhibitors of the FmlH bacterial adhesin. Through an interdisciplinary approach that blended medicinal chemistry, X-ray crystallography, virtual and biochemical screening, bio-layer interferometry, immunofluorescence, and mouse models of UTI, we have developed novel aryl galactoside and N-acetylgalactosaminosides that specifically binds FmlH with nanomolar affinity and have demonstrated one lead compound as an effective treatment for chronic UTIs and is synergistic when dosed in combination with a FimH mannoside inhibitor. When coupled with our past work on developing potent orally bioavailable mannoside FimH inhibitors for bladder infection (cystitis), this work on potent galactoside FmlH inhibitors important in kidney infection (pyelonephritis) further augments and solidifies the overwhelming therapeutic value of leveraging a deep understanding of structure-function-virulence relationships of bacterial adhesins for the development of anti-virulence strategies that disrupt host-pathogen interactions for treatment of infectious disease. These antibiotic-sparing approaches are effective in treating antibiotic-resistant forms of bacteria and have high potential to significantly reduce and even eliminate resistant microbes.





"MICRO-PHARMACOKINETICS": HOW LOCAL DRUG CONCENTRATION INFLUENCES OBSERVED BINDING KINETICS

Steven J Charlton

Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK

The affinity and kinetics of a drug binding to its target receptor are almost exclusively calculated using equations that assume the interacting molecules are homogeneously distributed in a solvent, with the concentration of drug available to bind target being equal to that in the bulk aqueous phase. While this assumption applies well to soluble enzymes, it is less satisfactory for membrane-associated targets (e.g. GPCRs) where the protein is embedded in a phospholipid bilayer. This is because the inclusion of phospholipid adds an additional amphiphilic compartment into which drugs may partition, depending on their physicochemical properties. In addition, the physical barriers associated with some physiological compartments (e.g. synapses) may restrict drug diffusion away from the receptor-compartment, further promoting drug "rebinding". This talk will introduce the concept of drug-membrane interactions, explore the consequences on observed receptor kinetics and outline our recent efforts to measure local drug concentrations at a sub-cellular level. It will then give a more clinical perspective, describing how local drug rebinding may be an important contributor to the extrapyramidal side effects of antipsychotic dopamine D2 receptor antagonists. Finally it will argue that receptor binding kinetics can not be fully understood without also considering the local drug concentration, stating the case for establishing "micro" PK/PD relationships for drugs against membrane targets.

ALLOSTERIC MODULATION OF THE MGLU2 RECEPTOR: FROM STRUCTURE-KINETIC RELATIONSHIPS TO IN VIVO EFFICACY

Laura H. Heitman

Division of Drug Discovery and Safety, LACDR, Leiden University, Einsteinweg 55, 2333CC Leiden, The Netherlands

Allosteric modulation of the metabotropic glutamate receptor 2 ($mGlu_2$), a class C G protein-coupled receptor (GPCR), is considered a promising approach for treatment of various psychiatric and neurological disorders, like schizophrenia. In recent years it has been emphasized that the concept of receptor binding kinetics can enhance the predictive value of in vitro experiments towards the clinic. Therefore, we aimed to evaluate this concept for the $mGlu_2$ receptor, by studying a library of positive allosteric modulators (PAMs) for this receptor.

Based on the results of a structurally diverse selection of both novel and reference $mGlu_2$ PAMs, a novel series of 7-aryl-1,2,4-triazolo[4,3-a]pyridines was selected. Full characterization of affinity and kinetics enabled evaluation of structure-affinity relationships (SAR) and structure-kinetics relationships (SKR). The $mGlu_2$ PAMs showed various kinetic profiles; values for the association rate constant k_{on} ranged over three orders of magnitude, whereas k_{off} and residence time (RT) values were within a smaller 10-fold range. Further analysis revealed that $k_{on}$was linearly correlated to affinity, while this was not the case for RT. Evaluation of the shortest and longest RT compound in the whole cell label-free xCELLigence® assay revealed that the longest RT compound displayed a functional effect that was not easily washed out. Lastly, the effects of the longest RT compound on sleep-wake states were determined, as a measure for central activity and target engagement. This indicated that its long RT translated into sustained inhibition of rapid eye movement (REM) in vivo.

In conclusion, we have shown that affinity-only driven selection results in $mGlu_2$ PAMs with high values for k_{on} , but not necessarily with a long RT. In addition, a long RT seems to be required for in vivo efficacy of $mGlu_2$ PAMs. This study further emphasizes the need to study target binding kinetics in early drug discovery.

TARGET BINDING KINETICS AND ITS RELEVANCE IN THE IN VIVO CONTEXT

Wilbert EA de Witte, Piet Hein van der Graaf, Meindert Danhof, Elizabeth CM de Lange

Division of Systems Biomedicine and Pharmacology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

For any drug that is administered to patients or that is being developed, is essential that the time course of its effects can be predicted to ensure rational drug therapy and drug development. After its administration, the time course of the effect of a drug can be influenced by all processes that constitute the complex system of the human body [1].

Our research aimed to elucidate how drug-target binding kinetics, in conjunction with plasma pharmacokinetics, tissue distribution kinetics, endogenous ligands competition, kinetics of signal transduction, target turnover, and homeostatic feedback mechanisms, determine the in vivo time course of drug action.

This presentation will deal with examples [2,3] that indicate under what conditions a low dissociation rate constant (k_{off}) value may result in

- prolongation of target occupancy
- selectivity for the therapeutic target compared to a secondary target
- more effective blocking of endogenous signaling

It is concluded that the in vivo context is important for the contribution of drug-target binding kinetics relative to other processes that govern the in vivo time course of drug action.

References

1) De Lange ECM, van der Brink W, Yamamoto Y, de Witte W, Wong YC. Novel CNS drug discovery and development approach: model-based integration to predict neuro-pharmacokinetics and pharmacodynamics. Expert Opin Drug Discov. 2017 Dec;12(12):1207-1218. doi: 10.1080/17460441.2017.1380623
2) Vlot AHC, Witte WEA, Danhof M, van der Graaf PH, van Westen GJP, de Lange ECM. Target and tissue selectivity prediction by integrated mechanistic pharmacokinetic-target binding and quantitative structure activity modelling AAPSJ. 2017 Dec 4;20(1):11. doi: 10.1208/s12248-017-0172-7
3) De Witte WEA, Vauquelin G, van der Graaf PH, de Lange EC. The influence of drug distribution and drug-target binding on target occupancy: The rate-limiting step approximation. Eur J Pharm Sci. 2017 May 12. pii: S0928-0987(17)30252-X. doi: 10.1016/j.ejps.2017.05.024

LARGE-SCALE ANALYSIS OF KINASE INHIBITORS' TARGET BINDING KINETICS AND ITS IMPLICATIONS FOR DRUG DISCOVERY

Victoria Georgi, Felix Schiele, Benedict-Tilman Berger, Andreas Steffen, Paula M. Zapata, Hans Briem, Stephan Menz, Cornelia Preusse, James D. Vasta, Matthew B. Robers, Michael Brands, Stefan Knapp, Amaury Fernández-Montalván

In recent years the importance of binding kinetics for target-based drug discovery was intensely discussed. Drug-target association and dissociation rates (k_{on}, k_{off}) are proposed as better predictors for clinical performance than steady-state affinity per se ($K_D = k_{off} / k_{on}$). For the analysis of this idea, comprehensive datasets are needed.

Here we present a large-scale binding kinetic characterization of a wide spectrum of 270 kinase inhibitors against 40 clinically relevant kinases.

We address the question whether and when target selectivity can be differently assessed from the equilibrium- and kinetic perspectives – in the cellular and in vivo context. Moreover, we demonstrate how in vivo target occupancy could be adjusted by utilizing the interplay of pharmacokinetics and the dynamics of drug-target interaction. Finally, the large dataset allows analyzing structure-kinetic relationships separately from the effect of structural features on affinity.

Our results contribute to realize the potential of binding kinetics for drug discovery and provide a rational basis for the design of kinetic rate constants.

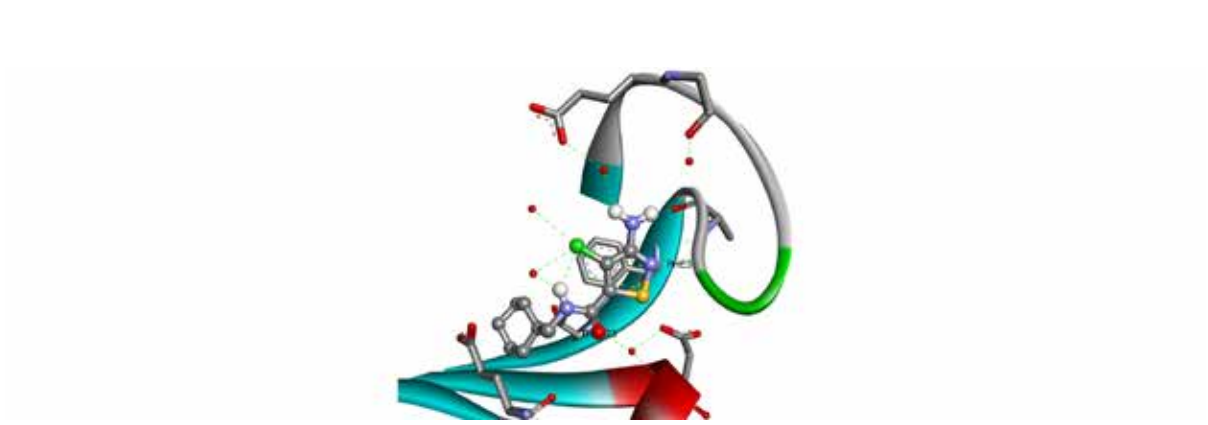
A NEW TARGET IN FUNGAL PROTEIN BIOSYNTHESIS: SHARED LEARNINGS FOR AGCHEM AND MEDCHEM

Bernd Essigmann (1), Jörg Freigang (3), Pierre Genix (1), Mathieu Gourgues (1), Yoann Huet (1), Philippe Kennel (4), Bernd Laber (2), Gudrun Lange (2), Viriginie Lempereur (1), Marc Mosrin (3), Jacopo Negroni (1), Stéphane Peyrard (1), Jörg Tiebes (2), Jullien Rey (2), François Villalba (1), David Bernier (1)

1) Bayer S.A.S. - R&D - Crop Science Division ; Centre de Recherche de La Dargoire, 14 impasse Pierre Baizet ; F-69009 Lyon, France
2) Bayer AG - R&D - Crop Science Division ; Industriepark Höchst ; D-65926 Frankfurt, Germany
3) Bayer AG - R&D - Crop Science Division ; Alfred-Nobel-Strasse 50 ; D-40789 Monheim, Germany
4) Bayer S.A.S. - R&D - Crop Science Division ; Centre de Recherche de Sophia Antipolis, 355, rue Dostoïevski - BP 153 ; F-06903 Sophia Antipolis Cedex, France

Many factors (such as increasing world population by near-to-constant arable area, changing food habits, increasing demand for alternative energies such as biofuels, and climatic changes) will impact the delicate balance between agricultural supply and need in the near future. To face these challenges, Bayer CropScience is committed to provide innovative solutions to improve and safeguard crop yields, while maintaining the highest standards in safety.

After a short introduction, an example from our internal research will be used to show how innovative small molecules chemistry in the field of isothiazoles led to the discovery of a new fungicidal family. This class exhibits efficacy against a broad spectrum of plant diseases, both in cell-test and in-planta. We will show that this class has a novel mode of action in protein biosynthesis. The stepwise elucidation of this mode of action will be presented, leading to the identification of an unprecedented target in the field of synthetic agricultural fungicides. The chemical exploration around our initial starting point will be presented, followed by preliminary SAR data and our hypotheses concerning conformational effects. X-ray data will be described, as well as learnings on interspecies selectivity and safety.



Finally, the interest of these compounds as chemical probes of this underexplored target, within an established family of protein-biosynthesis enzymes of general interest in life sciences, will be discussed.

NATURAL PRODUCTS AS LEADS IN AGROCHEMISTRY

Joachim Rheinheimer

Fungicide Chemistry Ludwigshafen, Global Research Crop Protection, BASF SE

Some natural products have been widely employed for crop protection purposes. Few are produced by fermentation while others have served as lead structures. Many commercial synthetic pyrethroids derived from plant origin have been developed as insecticides. Likewise, strobilurines originating from fungi have given rise to many successful fungicides applied in major crops.

However, the number of natural products showing biological activity against organisms relevant for plant protection is very large as compared to the limited number of substance classes which have been derived. For a long time, this has been a subject for controversial discussions. Until recently this has been the reason that discovery projects in natural products have been terminated in several agrochemical companies. Now a renewed interest can be observed.

Due to the widespread application of these molecules there is a substantial risk of resistance development. Indeed, some cases of resistance have been reported in the literature. An understanding of the general principles influencing resistance development is important in order to devise strategies to ensure a long performance lifetime of each specific product. For this reason, an understanding of the chemical and biological effects involved is crucial in order to create new molecules less affected by resistance development.

Evolution has constructed present natural products over a very long period of time. Some are presumed to exist for at least tens of millions of years. It is discussed how nature itself may have dealt with the resistance problem. From this, important ideas can be derived for active ingredient research.

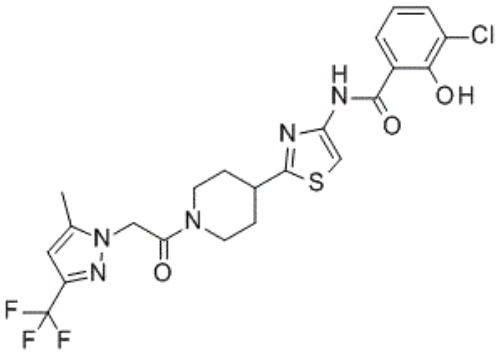
New, more precise, and cheaper methods for establishing protein structure and dynamics are now joining advanced modelling possibilities. This makes molecular design more rational. Also, this can add new value to existing substance classes. Once resistance occurs, these may not necessarily be lost forever. Instead, precise analyses of the proteins and inhibitors involved can show a way to overcome this challenge in some cases.

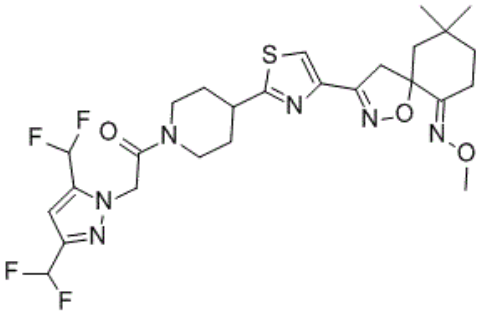
SYNTHESIS AND FUNGICIDAL ACTIVITY OF A NEW FAMILY OF OXYSTEROL BINDING PROTEIN INHIBITORS

Laura Quaranta, Clemens Lamberth, Sarah Sulzer, Martin Pouliot

Syngenta Crop Protection, Schaffhauserstrasse, CH-4332 Stein, Switzerland

Inhibitors targeting oxysterol binding protein have shown excellent fungicidal activity against plant diseases caused by oomycete pathogens. Oxathiapiprolin, discovered by DuPont researchers, have been the first compound of this class to reach the market and is commercialized by both DuPont and Syngenta under the trade names Zorvec™ and Orondis™ respectively. This talk will focus on the research done in Syngenta in this area. The synthesis of various subclasses of inhibitors will be presented, together with their antifungal activity. Amongst them bicyclic and spirocyclic isoxazolines have shown very high potency against downy mildew and late blight, while N-thiazol-4-yl-salicylamide class showed a unique spectrum including damping-off disease caused by *Pythium ultimum.*





TARGETING NON ESSENTIAL BACTERIAL TARGETS AS A NOVEL ROUTE TO COUNTERACT BACTERIAL RESISTANCE

Giannamaria Annunziato (1), Joana Magalhaes (1), Marco Pieroni (1), Sara Filisi (2), Andrea Mozzarelli (1), Barbara Campanini (1), Nina Franko (1), Clotilde Silvia Cabassi (2), Gabriele Costantino (1)

1) Dipartimento di Scienze degli Alimenti e del Farmaco- Università di Parma
2) Dipartimento di Scienze Mediche Veterinarie - Università di Parma

A growing number of Gram negative bacteria is becoming resistant to the available antibiotic treatments. Infections caused by these resistant strains fail to respond to the current treatment, therefore leading to prolonged illness, higher healthcare costs, and a greater risk of death. Therefore, the discovery and development of novel approaches to counteract the emergence of resistant pathogens is a crucial challenge.

Although not vital for bacteria survival, cysteine biosynthesis is an important way to establish antibiotic resistance in bacteria and its inhibition interferes heavily with the ability of pathogens to withstand oxidative stress, infect the host and persist as long- term infections. Moreover, since mammals lack this biosynthetic pathway, inhibition of cysteine biosynthesis could represent a selective target for antibacterial intervention. The last step of cysteine biosynthesis is catalyzed by *O*-acetylserine sulfhydrylase (OASS), that is found in two isoforms: *O*-acetylserine sulfhydrylase A (OASS-A) and *O*-phosphoserine sulfhydrylase B (OASS-B).

Based on the structure of the C-terminal portion of Serine Acetyl Transferase (SAT), the physiological inhibitor of these enzymes, we have recently reported the synthesis and the docking studies of a series of small molecules bearing a cyclopropancarboxylic acid scaffold, that showed a promising activity toward OASS-A. With the aim of improving the activity toward OASS-A, and considering that inhibition of OASS-B is as well important to prevent the biosynthesis of cysteine, we have been driven by molecular docking and Saturation Transfer Difference (STD) studies to design more potent OASS inhibitors. We were please to notice that, along with a potency toward OASS-A in the low nanomolar range, some of the novel inhibitor synthesized were found to possess the highest activity toward OASS-B reported so far.

The compound **1** with the highest affinity toward both OASS isoform was tested against *Salmonella typhimurium* strain in combination with other antibiotics showing a high potential to act as coadiuvant therapy.

RECENT ADVANCES IN BIFUNCTIONAL DEGRADER MOLECULES (E.G. SNIPER) FOR TARGETED PROTEIN DEGRADATION VIA THE UBIQUITIN PROTEASOME SYSTEM; STATUS AND OUTLOOK

Mikihiko Naito

Division of Molecular Target and Gene Therapy Products, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

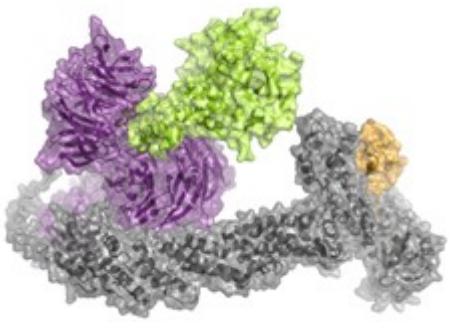
Inducing protein degradation by small molecules is a novel strategy for drug development. Recently, bifunctional degrader molecules named PROTACs (Proteolysis Targeting Chimeras) and SNIPERs (Specific and Nongenetic IAP-dependent Protein Erasers) are developed to induce proteasomal degradation of target proteins. These molecules are composed of two ligand moieties connected by a linker, one ligand for a target protein and the other for an E3 ubiquitin ligase, which is designed to cross-link these proteins in cells, thereby inducing the ubiquitylation and proteasomal degradation of the target protein.

Currently, we have successfully developed several SNIPERs against ERa, BCR-ABL, BRD4 and PDE4 by conjugation of a high affinity IAP ligand with 4-hydroxy tamoxifen, dasatinib, JQ1 and a PDE inhibitor, respectively, and these SNIPERs induce effective degradation of the respective target proteins at nano-molar concentrations. Consistent with the degradation, SNIPER(ER)-87, one of the most potent SNIPER against ERa shows an activity to suppress estrogen-dependent gene expression and proliferation of ERa-dependent breast cancer cells in vitro. In addition, SNIPER(ER)-87 shows an activity to induce degradation of ERa in tumor xenografts in mice, and inhibits tumor growth in vivo. I will overview the recent advances in bifunctional degraders.

TARGETING 'UNDRUGGABLE' TRANSCRIPTION AND TRANSLATION FACTORS FOR DEGRADATION WITH LOW MOLECULAR WEIGHT CEREBLON MODULATORS

Philip Chamberlain

Celgene, 10300 Campus Point Drive Suite 100, San Diego, California



Celgene has clinical approvals for lenalidomide in a variety of indications including myeloma and myelodysplastic syndrome. The molecular target for lenalidomide as well as the analogs pomalidomide and thalidomide, has been shown to be the protein cereblon [1]. Cereblon is part of the CRL4-CRBN E3 ubiquitin ligase complex, which catalyzes the transfer of ubiquitin to mark target proteins for degradation. Cereblon modulating drugs bind to the surface of cereblon to form a 'hotspot' for protein-protein interactions triggering the recruitment of proteins to the CRL4 E3 ubiquitin ligase complex where they can be ubiquitinated and subsequently degraded [2]. Through this mechanism, small molecule cereblon modulators cause the degradation of the zinc finger transcription factors Ikaros and Aiolos [3-5]. Lenalidomide was further shown to cause the degradation of the protein kinase CK1a, for clinical activity in a subset of patients with myelodysplastic syndrome with the del(5q) chromosomal lesion [6].

A search for cereblon modulators that degrade new proteins lead to the discovery of CC-885, which exhibits potent antiproliferative activity against a panel of tumor cell lines [7]. The potent anti-cancer activity of CC-885 is caused by the degradation of the protein translation factor G1 to S phase transition 1 (GSPT1). A crystal structure of cereblon in complex with the ligase adapter protein DDB1, as well as CC-885 and GSPT1, reveals that GSPT1 interacts with both CC-885 and the surface of cereblon. The principal molecular feature on GSPT1 that binds to cereblon is a beta-hairpin incorporating a glycine residue. Homology modeling indicated that a similar molecular feature mediates Ikaros recruitment, even though there is no common structural fold or sequence homology. This molecular feature, or 'degron', can be found in other proteins including families such as zinc finger transcription factors that had previously been considered undruggable. The implications of these finding in for future drug discovery will be discussed.

References

1) Ito, T., et al., Identification of a primary target of thalidomide teratogenicity. Science, 2010. 327(5971): p. 1345-50.
2) Chamberlain, P.P., et al., Structure of the human Cereblon-DDB1-lenalidomide complex reveals basis for responsiveness to thalidomide analogs. Nat Struct Mol Biol, 2014. 21(9): p. 803-9.
3) Gandhi, A.K., et al., Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). Br J Haematol, 2014. 164(6): p. 811-21.
4) Kronke, J., et al., Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science, 2014. 343(6168): p. 301-5.
5) Lu, G., et al., The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science, 2014. 343(6168): p. 305-9.
6) Kronke, J., et al., Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. Nature, 2015. 523(7559): p. 183-8.
7) Matyskiela, M.E., et al., A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. Nature, 2016. 535(7611): p. 252-7.

THE ZINC-DEGROME

Quinlan Sievers (1), Georg Petzold (2), Benjamin Ebert (1), Nicolas Thoma (2)

1) Broad Institute of Harvard and MIT, Cambridge, USA. Brigham and Women's Hospital, Division of Hematology, Harvard Medical School, Boston, USA.
Dana Farber Cancer Institute, Department of Medical Oncology, Boston, USA
2) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

The small molecule drugs thalidomide, lenalidomide, and pomalidomide induce the ubiquitination and proteasomal degradation of Ikaros (IKZF1) and Aiolos (IKZF3) by mediating their interaction with Cereblon (CRBN), the substrate receptor of the CRL4CRBN ubiquitin ligase. Here we screened the human Cys2-His2 (C2H2) zinc finger (ZF) proteome for degradation by CRL4CRBN in the presence of thalidomide analogues, identifying 11 ZF targets. Structural and functional characterization of the C2H2 zinc finger degron demonstrates how diverse ZF domains bind the drug-CRBN interface. Computational ZF docking, in conjunction with biochemical analysis, predicts that at least 50 zinc-fingers bind the drug-CRBN complexin vitro, a larger number than previously anticipated. These results provide strategies to degrade other zinc finger transcription factors.

DRUGGING THE FBW7 E3 LIGASE WITH A COMBINED COMPUTATIONAL AND BIOPHYSICAL APPROACH

Miriam Martínez-Cartró (1), Salvatore Scaffidi (1), Xavier Barril (1,2), Carles Galdeano (1)

1) Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Biomedicina (IBUB), Universitat de Barcelona. Av. Joan XXIII 27-31, 08028, Barcelona, Spain
2) Catalan Institution for Reasearch and Advanced Studies (ICREA), Passeig Lluís Companys, 23, 08010 Barcelona, Spain

E3 ubiquitin ligases (of which >700 are known in humans) confer substrate specificity to the protein ubiquitination pathway, making this unconventional enzyme class very attractive targets for specific and less toxic therapeutic intervention, reducing the promiscuity that can be related with other UPS components (i.e. proteasome inhibitors). Being a clear opportunity, the development of small-molecules against E3 ligases has been rewarded with very limited success.[1] However, the explosion of the protein degradation as a therapeutic strategy in recent years (i.e. PROTACS) have situated this protein class in the spotlight. Nevertheless, there is a significant mismatch between the number of E3 ligases and the number of drugs in clinical trials or approved, and the important questions remain, which is the best strategy to find new ligands that target E3 ligases? Could we develop allosteric ligands that target E3 ligases?

Herein, we have developed a multidisciplinary computational and biophysical approach to identify ligands that target E3 ligases, and specifically the Fbw7 E3 ligase. Fbw7 is one of the most commonly deregulated UPS protein in human cancers, which targets a range of substrates for degradation, including some key human oncoproteins including cyclin-E, MYC, Notch and Junk.[2] However, so far, no potent small molecule directly targeting the Fbw7 complex has been reported. As primary computational screening, we have performed a druggability assesment using MDmix,[3] followed by a docking-based virtual screening of the found ligandable *hot-spots*. Finally, a DUck filter has been applied.[4] The resulting potential *hits* have been tested by Surface Plasmon Resonance (SPR), followed by ligand-based NMR to confirm binding. Our approach has allowed us to identify ligands able to bind at the low micromolar level to the Fbw7 protein. Work is on-going to elucidate the binding mode and the potential mechanism of action of these new ligands.

References

1) Galdeano, C., Drugging the undruggable: targeting challenging E3 ligases for personalized medicine (2017) Fut. Med. Chem. 9, 347–350.
2) Hao, B., Oehlmann, S., Sowa, M. E., Harper, J. W. & Pavletich, N. P. Structure of a Fbw7-Skp1-Cyclin E Complex: Multisite-Phosphorylated Substrate Recognition by SCF Ubiquitin Ligases (2007) Mol. Cell 26, 131–143 .
3) Álvarez-García, D. & Barril, X., Molecular Simulations with Solvent Competition Quantify Water Displaceability and Provide Accurate Interaction Maps of Protein Binding Sites (2014) J. Med. Chem. 57, 8530–8539.
4) Ruiz-Carmona, S. et al., Dynamic undocking and the quasi-bound state as tools for drug discovery (2016) Nat. Chem. 9, 2–7.

DISCOVERY OF RG7916, A SELECTIVE SMN2 SPLICING MODIFIER FOR THE TREATMENT OF SPINAL MUSCULAR ATROPHY

Hasane Ratni

F. Hoffmann-La Roche Ltd., pRED, Pharma Research & Early Development, Roche Innovation Center Basel, Grenzacherstrasse 124, 4070 Basel, Switzerland

RNA splice modifiers are a new class of small molecule therapeutics. In terms of specificity and safety they represent profound challenges for medicinal chemistry. We have been working to develop orally-administrated, systemically-distributed small molecules to increase levels of functional SMN protein via the alternative splicing of the survival motor neuron 2 (*SMN2*) pre-mRNA for the treatment of SMA. SMA is a severe, progressive, inherited disease that leads to loss of motor function and ambulation, and reduces life expectancy. We will present the discovery of RG7916, its chemical structure, in vivo profile, the genome-wide splice site and gene expression analysis to assess its selectivity and its unique mode of action. This compound is currently undergoing testing in pivotal clinical trials in type 1, 2, and 3 SMA patients.

This work demonstrated that it is possible to design safe, selective and efficacious small molecule splicing modifiers, with a potential widespread implications in the research and development of several additional RNA-targeting therapies.

DISCOVERY OF LOU064, A COVALENT BTK INHIBITOR WITH BEST IN CLASS SELECTIVITY

Robert Pulz, Daniela Angst, Christian Beerli, Marc Bigaud, Janet Dawson, Francois Gessier, Sascha Gutmann, Rene Hersperger, Stefanie Harlfinger, Alexandra Hinniger, Philipp Janser, Guido Koch, Amanda Littlewood-Evans, Una McKeever, Laszlo Revesz, Clemens Scheufler, Anna Vulpetti, Rudolf Waelchli, Gisbert Weckbecker, Alfred Zimmerlin, Bruno Cenni

Novartis Institutes for BioMedical Research, Novartis Campus, Basel, Switzerland

Bruton's Tyrosine Kinase (BTK) is a cytoplasmic tyrosine kinase and a member of the TEC kinase family. It is selectively expressed in a subset of immune cells, including macrophages, mast cells, platelets and B cells. BTK is a key regulator of B cell antigen receptor signalling in B cells and of Fc receptor signalling in mast cells and macrophages. Based on a strong genetic and pharmacological validation, it is likely that a BTK inhibitor will have a positive impact on autoimmune diseases which are caused by autoreactive B cells and immune-complex driven inflammation.

We report the design, characterization and medicinal chemistry optimization of a series of highly selective covalent BTK inhibitors. The efforts resulted in the identification of the clinical candidate LOU064 as a highly potent inhibitor with a best in class selectivity profile. The preclinical profiling of LOU064 will be presented as well.

DISCOVERY OF A NOVEL ORAL NO- AND HEME-INDEPENDENT sGC ACTIVATOR BAY 1101042

Michael G. Hahn

Bayer AG, Pharmaceuticals, Medicinal Chemistry Wuppertal, Germany, michael.hahn1@bayer.com

Soluble guanylate cyclase (sGC) is a signal-transduction enzyme activated by nitric oxide (NO) and plays a key role in a variety of physiological processes such as vasodilatation, antiaggregation, antiproliferation and neuronal signaling. Impairments of the NO-sGC-signaling pathway have been implicated in the pathogenesis of various cardiovascular and other diseases. Current therapies that involve the use of organic nitrates and other NO donors have limitations, including non-specific interactions of NO with various biomolecules and lack of response and the development of tolerance. Consequently, innovative approaches are needed to realize the full potential of the NO/sGC/cGMP -signaling pathway.

Recently, two novel drug classes which activate the soluble guanylate cyclase (sGC) have been discovered. The sGC stimulators (heme-dependent) and sGC activators (heme-independent). sGC stimulators share a dual mode of action, they stimulate sGC directly and enhance sensitivity of sGC to low levels of bioavailable NO. In contrast, sGC activators are able to activate the pathologically changed heme-free sGC. The discovery of sGC activators offered the prime opportunity to design drugs for selective binding to the oxidized, heme free sGC generated by the influence of oxidative stress causally involved in many cardiovascular diseases (1).

The most advanced compound in this class was Cinaciguat (2). However, this compound was not suitable for oral administration. More recently, BAY 1101042 a second-generation sGC activator for oral administration has been discovered. The discovery of BAY 1101042 and the structural activity relationship within this class will be presented.

References

1) Evgenov O.V., Pacher P., Schmidt P.M., Haskó G., Schmidt H.H.H.W, Stasch JP. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. Nature Reviews Drug Discovery 2006, 5: 755-768.
2) Follmann M.; Griebenow N.; Hahn M. G.; Hartung J.; Mais F.-J.; Mittendorf J.; Schaefer M.; Schirok H. Stasch J.-P., Stoll F.; Straub A. Angew. Chem. Int. Ed. 2013, 52, 9442-9462: The Chemistry and Biology of Soluble Guanylate Cyclase Stimulators and Activators.

FIRST TIME DISCLOSURE OF BI 409306, A FIRST IN CLASS PDE9 INHIBITOR FOR THE TREATMENT OF CNS DISEASES

Riccardo Giovannini*, Holger Rosenbrock, Gerhard Schänzle*****

Boehringer-Ingelheim Pharma GmbH & Co. KG, 88397 Biberach, Germany;
Dept. of Medicinal Chemistry, **Dept. of CNS Diseases Research,*Dept. of Development DMPK.*

Inhibition of specific phosphodiesterases (PDEs) in the brain has gained in recent years attention as a potential new approach for the treatment of several CNS diseases. Among the PDEs family, PDE9A, highly expressed in the forebrain and medial temporal lobe structures, is specific for hydrolyzing cyclic guanosine monophosphate (cGMP), an important second messenger in glutamatergic neurons related to the NMDA receptor signaling. Hypofunction of the NMDA receptors, leading to synaptic stabilization & plasticity deficits, is believed to be the underlying cause of a number of CNS pathologies. Inhibition of PDE9, leading to an increase of cGMP levels has the potential to post-synaptically enhance the NMDA receptor related cGMP signalling and synaptic plasticity (Fig. 1), therefore representing a valuable therapeutic opportunity. The disclosure of the structure and the key features of the PDE9 inhibitor clinical candidate BI 409306 will be the focus of the presentation.

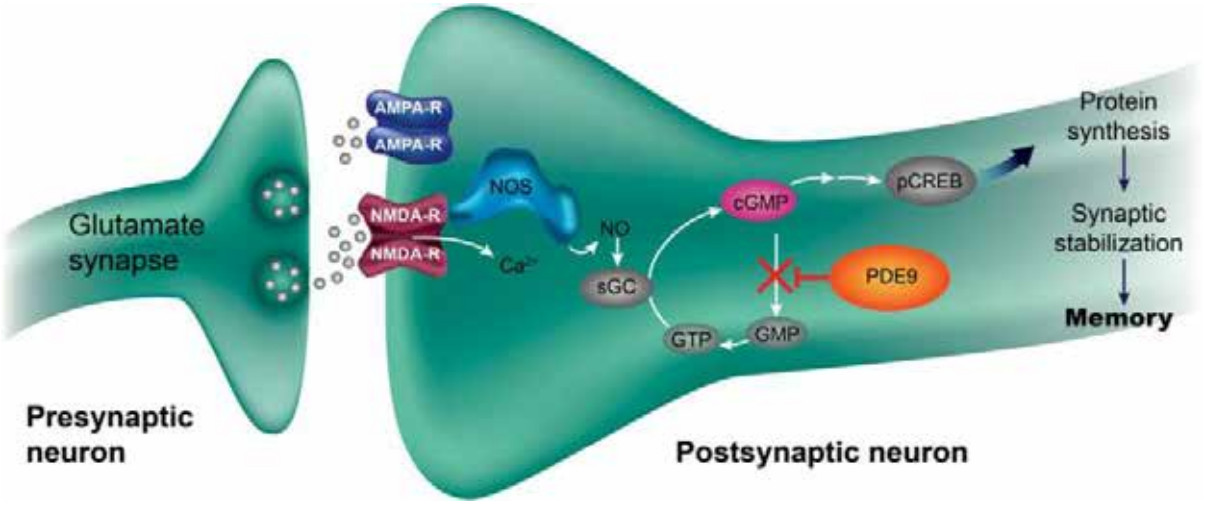


Fig. 1: Putative mechanism of action of PDE9 inhibition for strengthening synaptic plasticity (adapted from Moschetti et al., 2016, Br. J. Clin. Pharmacol. 82:1315-1324)

NEW MODALITIES FOR COMPLEX AND UNPRECEDENTED BIOLOGICAL TARGETS

Eric Valeur

Medicinal Chemistry, Cardiovascular, Renal & Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

The target landscape in all therapeutic areas in drug discovery is experiencing a broad metamorphosis. The need to move away from symptom treatment and focus on disease-modifying approaches is calling for novel biology, which is often associated with a range of targets of high complexity and frequently unprecedented. Progress in the understanding of cell biology and of mechanisms involved in the regulation of protein levels is also further extending the target scope, as illustrated with noncoding RNAs, and therefore represents a further reservoir of potential drug targets.

To address these targets and develop our biology understanding, chemical probes are required for both target-centric and phenotypic approaches. With this prospect in mind, small molecules play a major role, considering the breadth of modulators available for a wide range of targets. However, many targets originating from genomics or from the study of biological pathways are orphan from ligands and are not the prime applicable space for small molecules, typically due to their large surface area. Protein-protein interactions, and in particular transcription factors, remain a major challenge, despite some isolated examples of success. In this respect, other chemical modalities are better suited. These so-called 'New Modalities' cover many chemical classes including a new generation of usually hyper-modified peptides, macrocycles, a renaissance of natural products, and nucleic acid-based molecules. In addition, chemical modalities can be combined and linked to generate further New Modalities, as exemplified by the proteolysis targeting chimera (PROTAC).

These various modalities modulate proteins at different levels, including at the transcriptional and translational level. With this prospect in mind, the opportunity to place the selection of the mode-of-action (MOA) at the center of any drug discovery project will be highlighted, taking into consideration the many challenges faced with the identification and development of 'New Modalities' including hit finding, cell penetration and tissue access, to mention a few. Within the vast repertoire of modalities now accessible to medicinal chemists to develop probes and therapeutics, the presentation will highlight how some modalities such as modified peptides can afford unprecedented probes to discover novel biology. For example, a novel strategy consists in screening genetically encoded cyclic peptide libraries directly in bacterial cells, linking inhibition of a target to cell survival. With this approach a tool peptide against IDOL, an E3 ligase involved in the degradation of the LDL receptor and a regulator of blood cholesterol levels, could be identified and enabled the discovery of novel biological cross-talks around IDOL.

The specific delivery of antisense oligonucleotides, through conjugation to a homing peptide will also be presented. In this approach, a peptidic ligand to the GLP-1 receptor was leveraged, to enable the unprecedented productive uptake of oligonucleotides to pancreatic beta-cells in vivo.

Other examples of modalities, such a modified mRNA, will also be covered, with the goal to demonstrate how medicinal chemists can leverage and expand their skills to other modalities and explore their creativity to solve challenging biological questions, and select the 'right' modalities in drug discovery projects.

CELL PERMEABILITY WITH BEYOND 'RULE OF 5' MODALITIES – DO WE UNDERSTAND HOW THIS WORKS?

Nicholas Terrett

European Chemistry Lead, MSD Research GmbH, Ringstrasse 27, 6010 Kriens, Switzerland

The pharmaceutical and biotech industries are increasingly working with drug-discovery targets of a complexity that demands modalities extending beyond conventional 'Rule of 5' drug space. New technologies such as DNA-encoded chemistry, mRNA display, phage display and other library-generating and screening approaches are broadening the range of hit modalities that are commonly explored. However, larger molecules come with their own challenges in incorporating drug-like properties including solubility, stability and cell-membrane penetration.

Unlike 'Rule of 5' compliant molecules, where the drug discovery community has developed predictive rules for enhancing cell penetrant properties, larger modalities do not yet appear to follow any consistent and predictive rules. Part of the challenge lies in the diversity of routes by which larger molecules gain access to the cell cytosol. It seems highly probable that many beyond 'Rule of 5' molecules can gain cell entry by passive permeability. This achievement is remarkable when it is considered that larger molecules have to tread a fine line between having sufficient polar surface area (PSA) to gain adequate aqueous solubility and also being able to minimize PSA to pass through the lipophilic cell membrane. It has recently been demonstrated that this balance can only readily be attained if the molecule possesses some level of conformational flexibility, so that PSA can be tuned to match the local environment. Even with such 'chameleonic' physical properties, there appears to be an absolute physical size limit for passive permeability.

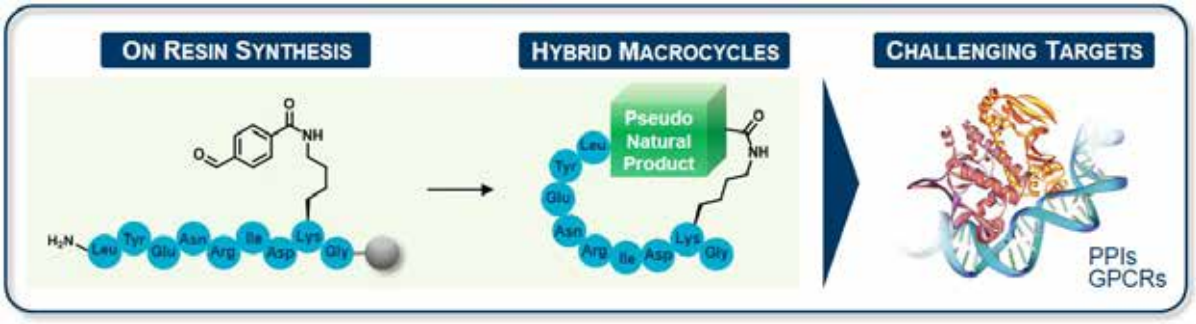
And yet larger molecules - including peptides - do enter cells, but this is generally achieved by mechanisms other than passive permeability. There seem to be numerous nuanced ways by which larger molecules in beyond 'Rule of 5' space enter cells, and peptides in particular can be engineered to take advantage of endosomal entry, in which a molecule is enveloped by phospholipids to gain cell entry. The intracellular endosomes then have to break up to release their cargo into the cytosol. Although our understanding is still at a primitive stage, a mix of cationic and lipophilic amino acid sidechains appears to enhance endocytosis.

This presentation aims to provide an overview of our understanding of how we can instill cell penetrant properties into beyond 'Rule of 5' molecules. Furthermore, it will be argued that structural modification with the aim of increasing cell penetration can only be undertaken if we understand the mechanism of cell entry.

NOVEL HYBRID MACROCYCLIC MODALITIES FOR STRUCTURE-BASED PROTEIN MIMETICS

Stéphanie M. Guéret (1,2), Marco Potowski (3), Sasikala Thavam (3), Rodrigo Carbajo Martinez (4), Anita Dellsén (5), Niklas HP Larsson (5), Göran Dahl (5), Malin Lemurell (2), Eric Valeur (2), Herbert Waldmann (3)

1) AstraZeneca-Max Planck Institute Satellite Unit, Dortmund, Germany
2) Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden
3) Abteilung Chemische Biologie, Max Planck Institute für Molekulare Physiologie, Dortmund, Germany
4) Oncology, IMED Biotech Unit, AstraZeneca, Chesterford Research Park, United Kingdom
5) Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden



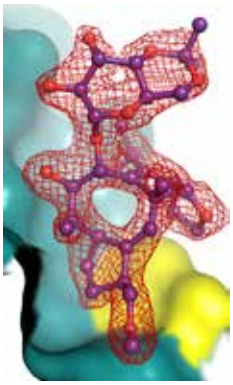
In the hunt for new drug candidates, macrocyclic scaffolds have raised interest to address biological targets containing large binding surfaces and protein-protein interactions (PPIs) mediated by unstructured omega-loops. However, the synthesis of peptidic macrocycles is currently restricted to classical macrocyclization methods such as lactam, disulfide, olefin and triazole bridges. Moreover, their high synthetic cost and lengthy synthetic routes limit their diversity and availability in the screening libraries. Herein, we report de novo combination of pseudo natural products and peptides to yield unprecedented hybrid macrocycles that inherit the biological relevance of the peptide inspired from a protein loop epitope and the unique stereogenic natural product character, while differing from the cyclic peptidomimetics currently available (e.g. Peptidream, PepScan, Bicycles). Our strategy makes use of an intramolecular imine cyclization followed by a late stage diastereoselective cycloaddition on resin to afford pseudo natural product – peptide hybrid macrocycles. Rapid structural variation of both the Csp3 moiety and the peptidic unit is enabled by leveraging the power of solid phase synthesis. The cycloaddition selectivity and absolute configuration of the major diastereoisomer was established using NMR and computational techniques. Incorporation of multiple peptide sequences inspired from the Agouti-Related Protein into our hybrid macrocycles revealed novel chemotypes that bind the melanocortin receptors with adjustable selectivity profile and partial agonistic activity for the human melanocortin receptor 1. Furthermore, a focused library of hybrid macrocycles bearing the DINNN epitope has been investigated to disrupt the SPSB2-iNOS protein-protein interaction. To summarize, the efficient integration of pseudo natural products into peptide epitopes yielded exotic new modalities to address GPCRs and PPIs.

SMALL-MOLECULE STABILIZATION OF PROTEIN-PROTEIN INTERACTIONS BY NATURAL PRODUCTS, SUPRAMOLECULAR LIGANDS, FRAGMENTS AND MACROCYCLES

Christian Ottmann

Laboratory of Chemical Biology, Department of Biomedical Engineering, Institute for Complex Molecular Systems, Eindhoven University of Technology, The Netherlands

Targeted pharmacological modulation of Protein-Protein Interactions (PPIs) is a promising strategy in Chemical Biology and Drug Development. However, in the vast majority of cases this concept has been realized only for inhibition of PPIs despite the fact that in many biomedical contexts stabilization of PPIs would be desirable [1]. The natural product fusicoccin A (FC-A) is stabilizing the binding of 14-3-3 adapter proteins to the plant H^+-ATPase PMA serving as proof-of-principle molecule for the possibility to address the widespread interactome of 14-3-3 proteins [2, 3]. In humans, these proteins interact with partner proteins implicated for example in cancer (Raf, p53, YAP/TAZ, Cdc25C), neurodegenerative diseases (Tau, α-Synuclein, LRRK2) or cystic fibrosis. We have used a fusicoccin-derivative (FC-THF) that stabilizes the interaction of 14-3-3 with the K^+ channel TASK-3 [4]. A similar concept can be applied for the enhancement of CFTR plasma membrane localization, an important aspect for the treatment of cystic fibrosis [5]. In a possible new strategy for cancer therapy we have shown how the fusicoccin class of natural products can stabilize the inhibitory interaction of 14-3-3 proteins with the estrogen receptor (ER), the protein kinase C-RAF, and the adapter protein Gab2 [6,7,8]. Together with the demonstration that 14-3-3 PPI stabilizers can be identified by screening conventional compound libraries [9,10] and the growing number of synthetic stabilizers reported by the community these studies support the concept of small-molecule PPI stabilization for biomedical research [11]. Very recently we also used supramolecular ligands to stabilize the interaction of 14-3-3 with Cdc25C [12] and used 14-3-3 as a bivalent assembly platform [13]. In addition, we have also contributed to inhibition of 14-3-3 PPIs, targeting for example the pathogenicity protein ExoS from *Pseudomonas aeruginosa* [14, 15] or Tau, implicated in Alzheimer's Disease [16, 17].



References

1) Thiel, P. et al. Angew. Chem. Int. Ed. 51 (2012), 2012-2018.
2) Ottmann, C. et al. Mol. Cell, 25 (2007), 427-440.
3) Skwarczynska, M. et al. PNAS 110 (2013), E377-86.
4) Anders, C. et al. Chemistry & Biology 20 (2013), 583–593.
5) Stevers, L. et al. PNAS 113 (2016), E1152-61
6) De Vries-van Leeuwen, I.J. et al. PNAS 110 (2013), 8894-9.
7) Molzan, M. et al. ACS Chem. Biol. 8 (2013), 1869-75.
8) Bier, D. et al. ChemMedChem. 11 (2016), 911-8.
9) Rose, R. et al. Angew. Chem. Int. Ed. 49 (2010), 4129-4132.
10) Richter, A. et al. Chemistry 18 (2012), 6520-6527.
11) Andrei, S.A. et al. Expert Opin. Drug Discov. 12 (2017), 925-94.
12)] Bier, D. et al. JACS 139 (2017), 16256-16263.
13) de Vink, P.J. et al. Angew. Chem. Int. Ed. 56 (2017), 8998-9002.
14) Bier, D. et al. Nature Chemistry 5 (2013), 234-9.
15) Glas, A. et al. Angew. Chem. Int. Ed. 53, (2014), 2489-93.
16) Joo, Y. et al. FASEB J. (2015), 29:4133-44.
17) Milroy, L.G. et al. Angew. Chem. Int. Ed. 54 (2015), 15720-4.

UNDERSTANDING AGGREGATION INHIBITION OF ALPHA-SYNUCLEIN AND TAU BY SMALL MOLECULES

Markus Zweckstetter

German Center for Neurodegenerative Diseases (DZNE), Von-Siebold-Str. 3a, 37075 Göttingen, Germany

Neurodegenerative diseases share related pathological processes characterized by the generation of proteinaceous deposits exhibiting excessive β-sheet structures. Growing evidence has implicated the aggregates in the onset, progression, and clinical symptoms of these disorders. The two most common neurodegenerative diseases are Alzheimer's Disease (AD) and Parkinson's Disease (PD). AD is characterized by the progressive accumulation of extracellular senile plaques consisting of β-amyloid polypeptide and intracellular neurofibrillary tangles consisting of Tau protein. PD is characterized pathologically by the detection of Lewy bodies, intraneuronal aggregates formed by misfolded species of the presynaptic protein α-synuclein. So far, only symptomatic treatment is available for AD and PD.

Because of the connection of the misfolding and aggregation of α-synuclein and Tau protein to a range of neurodegenerative disorders, which are collectively referred to as synucleinopathies and tauopathies, respectively, there is great interest in developing approaches that interfere with α-synuclein/Tau misfolding and aggregation. Indeed, a number of studies have reported small molecules that interfere with amyloid fibril formation of Tau and α-synuclein. Little is known however about the mechanisms of inhibition and the nature of the generated α-synuclein/Tau species. This lack of knowledge is based in part on the intrinsic properties of α-synuclein and Tau – that is the highly dynamic, intrinsically disordered nature of these two proteins, which precludes the use of X-ray crystallography or cryo-electron microscopy for the analysis of protein/small molecule complexes.

In my presentation I will discuss the use of NMR spectroscopy in combination with other biophysical tools and molecular dynamics simulations for a better understanding of the molecular mechanisms underlying the inhibition of aggregation of α-synuclein and Tau by small molecules.

DISCOVERY OF PEPTIDOMIMETICS TARGETING PROTEIN-PROTEIN INTERACTIONS OF ALPHA-SYNUCLEIN

Wolfgang Wrasidlo (1), Igor Tsigelny (1), Diana Price (2), Eliezer Masliah (1)

1) University of California,San Diego, USA, 9500 Gilman Drive, La Jolla, CA, 92093-0747
2) Neuropore Inc., 10835 Road to the cure, San Diego, CA, 92121

Alpha-synuclein, a typical intrinsically disordered protein can aggregate into toxic conformations which are present in sporadic and familial parkinson disease.

The ratioanl for the synthesis of peptidomimetics interacting directly with alpha-synuclein was as follows:

The structure of a-syn on membrane surfaces in the transition region of the helical and random coil between amino acid residues 92 and 102 contains a segment, KKDQLQK which in MD simulation studies was shown to be involved in protein- protein interactions. A structural analysis of this segment identified it to contain a beta-turn. Beta-turns are known as recognition motifs for protein- protein interactions leading to oligomerization phenomena. Thus we hypothesized that small molecules mimicking this structural motif would compete against this protein interaction and inhibit the propagation of monomer to toxic oligomers and aggregation of a-syn.

The design of these bicyclic peptidomimetics was based on this hypothesis. In preliminary studies we synthesized the linear peptide. and showed that it was capable of inhibiting the oligomerization of a-syn. We then designed and synthesized bicyclic analogs incorporating a scaffold with a privileged beta-turn motif to increase 1. proteolytic stability and thus increasing the biological half life and oral availability, 2. avoiding cell permeability issues and 3. reducing conformational flexibility which can limit affinity and selectivity to the target. NPT100-18, a prototype mimetic was selected as a candidate for extensive in vitro and in vivo testing. The results showed that NPT100-18 interacted directly with with a-syn, inhibited the formation of oligomeric species both in vitro and in vivo, enhanced axonal transport in iPSC based models of synucleopathies and improved motoric function in A-syn transgenic mice.

TARGETING THE MONOMERIC INTRINSICALLY DISORDERED STRUCTURAL STATE OF TAU AND ALPHA-SYNUCLEIN BY SMALL MOLECULES AS A POTENTIAL THERAPEUTIC STRATEGY FOR ALZHEIMER'S AND PARKINSON'S DISEASE

Gergely Tóth (1,2)

1) Cantabio Pharmaceuticals Inc., Sunnyvale, United States
2) MTA-TTK-NAP B - Drug Discovery Research Group – Neurodegenerative Diseases, Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

The misfolding of intrinsically disordered proteins (IDPs) such as tau and a-synuclein (aSyn) has been associated with the on-set and progression of Alzheimer's (AD) and Parkinson's (PD) diseases. A potential strategy to alleviate the aggregation of IDPs is to maintain their native functional state by small molecule binding. However, the targeting of the native state of IDPs by small molecules has been challenging due to their heterogeneous conformational ensembles.

To tackle this challenge, we initially investigated the structural basis of small molecule drugability of native monomeric Tau and aSyn. Two publicly available monomeric conformational ensembles of a shorter Tau construct K18 were analyzed using *in silico* structure based fragment mapping, which identified similar number of hot spots and small molecule binding sites on monomeric Tau ensembles as on tertiary folded proteins of similar size (Kiss *et. al.* ACS Chem. Neurosci. 2018, *in press*). Similarly, a structural ensemble of aSyn constructed using experimental NMR data and molecular dynamics simulations was analyzed, which identified diverse set of potential small molecule binding sites, some of which were present at an interface involving relatively long-range tertiary contacts (Toth *et. al.,* Plos One, 2014, 9(2):e87133).

Next, we applied two distinct high-throughput chemical microarray surface plasmon resonance imaging screen to detect the binding between small molecules and monomeric full-length Tau and aSyn. The screens identified novel set of drug-like fragment and lead-like compounds that bound to either Tau or aSyn. We verified that the majority of hit compounds from the Tau screen reduced the aggregation of different Tau constructs in vitro and in N2a cells (Pickhardt *et. al.,* Current Alzheimer Research, 2015 12, 814). Oral administration of selected hit compounds to Drosophila melanogaster, over-expressing full-length wild-type human Tau in their motorneurons, protected them from Tau-induced locomotive impairment by significantly increasing the climbing ability of the flies compared to controls.

These results demonstrate that Tau and aSyn are viable receptors of drug-like small molecules. Several of the identified novel aggregation inhibitors of Tau are drug-like small molecules that are suitable starting scaffolds for hit to lead optimization and for efficacy studies in relevant *in vivo*models of AD. Overall, these results support the potential and practical feasibility of the therapeutic strategy to target the monomeric state of IDPs by small molecules to reduce their misfolding and eliminate the formation of potential toxic oligomers. The drug discovery approach presented can be applied to other IDPs linked to other misfolding diseases.

BRAIN-PENETRANT AUTOPHAGY MODULATORS FOR TREATING NEURODEGENERATIVE DISEASES

Steve Andrews

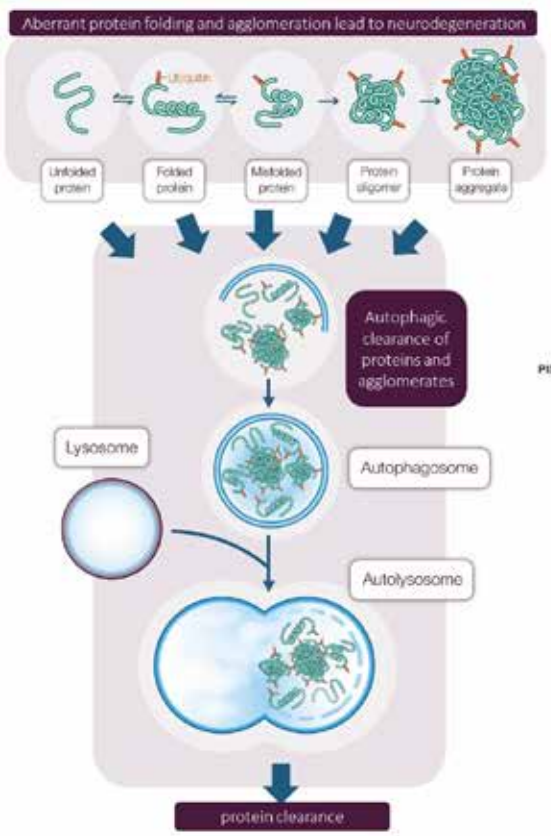
Alzheimer's Research UK Drug Discovery Institute, University of Cambridge, UK

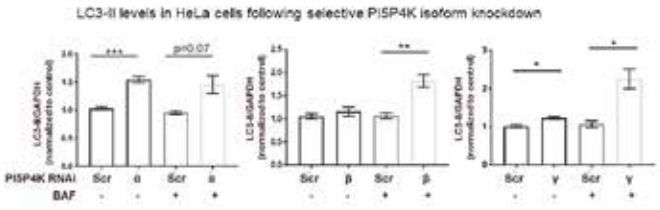
Results will be presented on Cambridge Drug Discovery Institute's (DDI's) small-molecule autophagy enhancers which were discovered through fragment and virtual screening against PI5P4 kinases which have previously been shown to play a role in autophagy (Vicinanza *et al.* doi:10.1016/j.molcel.2014.12.007). Optimised lead compounds show favourable rodent pharmacokinetics (high oral bioavailability and brain penetration) as well as efficacy in models of autophagy upregulation and protein clearance.

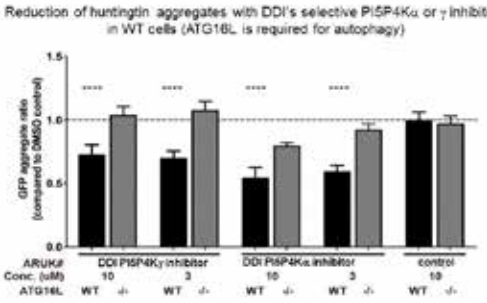
Globally, the number of people living with dementia is expected to increase from 50m in 2017 to 152m in 2050. Protein misfolding and aggregation are characteristics of the neurodegenerative diseases which cause dementia, including Alzheimer's, Parkinson's and Huntington's diseases, frontotemporal dementia and amyotrophic lateral sclerosis. Each disease has distinct brain pathology and causative protein(s), such as amyloid beta, hyperphosphorylated tau, alpha-synuclein, poly-Q huntingtin, TDP-43, FUS and SOD1.

Regulatory mechanisms of proteostasis such as autophagy are potential targets for disease-modifying therapies. Autophagy (literally meaning 'self-eating') is a degradation pathway that digests and recycles cell nutrients. The 2016 Nobel Prize in Physiology or Medicine was awarded for the elucidation of this mechanism in which autophagosomes engulf cellular components as large as organelles then fuse with lysosomes which contain the degradation machinery to 'eat' the contents (Menzies *et al*. doi:10.1038/nrn3961).

Cambridge DDI has identified selective inhibitors of PI5P4 kinase isoforms. siRNA knock down or small molecule inhibition of these kinases results in an increase in autophagy (e.g. increase in LC3-II levels). Furthermore, the small molecule PI5P4K inhibitors can reduce the levels of disease-causing poly-Q huntingtin aggregates in cells. Further validation for this mechanism has been provided by showing a lack of aggregate clearance in cells which have been genetically-modified (ATG16L -/-) to prevent autophagy.







INTRACELLULAR DNA SENSING IN HEALTH AND DISEASE

Andrea Ablasser

Global Health Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

Aberrant activation of innate immune pathways is associated with a spectrum of diseases. Progress into the molecular mechanisms of innate immune pathways has led to the promise of targeted therapeutic approaches, however the development of drugs that specifically act on molecules of interest remains challenging. In this talk I will present our approach to define highly potent and selective small-molecule antagonists of stimulator of interferon genes (STING), a central signaling component of the innate DNA sensor cyclic GMP-AMP synthase (cGAS). Mechanistically, the discovered compounds covalently target a conserved cysteine residue 91 and, thereby, block the activation-induced palmitoylation of STING. The identified compounds and their derivatives reduce STING-mediated inflammatory cytokine production both in human and in mouse cells. Furthermore, I will share data that document the efficacy of the compounds in attenuating pathological features of autoinflammatory disease in mice. In summary, this work uncovers an unanticipated mechanism to pharmacologically inhibit STING and demonstrates the potential of anti-STING therapies for the treatment of autoinflammatory disease.

MODIFICATION OF CYCLIC DINUCLEOTIDES TO ENHANCE MODULATION OF THE INNATE IMMUNE RESPONSE

Diane M Coe

Allergic Inflammation DPU, Respiratory TAU, Medicines Research Centre, Gunnels Wood Road, Stevenage, United Kingdom SG1 2NY

The innate immune system utilizes a number of pattern-recognition receptors (PRRs) to detect pathogens and initiate host responses such as the production of type I interferons and pro-inflammatory cytokines. Recent advances in the understanding of nucleic acid sensing has demonstrated that a cytoplasmic enzyme, cyclic GMP-AMP synthase (cGAS), produces a novel cyclic di-nucleotide (CDN), 2'3'-cGAMP, characterised by mixed 2'5' and 3'5' phosphodiester linkages on detection of cytoplasmic DNA. 2'3'-cGAMP acts as a secondary messenger, binding to and activating the protein STING (Stimulator of Interferon genes) and initiating a signalling cascade via the protein kinases, IkB (IKK) and TANK binding kinase 1 (TBK1).

As small molecules capable of stimulating an innate immune response, CDNs and derivatives thereof, have attracted considerable interest as immunotherapeutics and vaccine adjuvants. This presentation will report on novel carbocyclic and 2'-fluoro analogues of 2'3'-cGAMP and 2',3'-CDA. STING binding and X-ray crystal structure data, whole blood stability and the results of evaluation in vivo in a murine model via intratumoral administration will be presented.

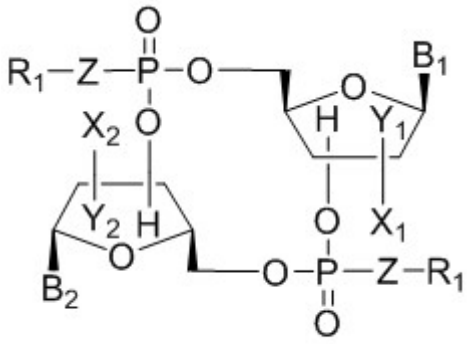
USE OF CYCLIC DINUCLEOTIDES (CDNs) TO INDUCE STIMULATOR OF INTERFERON GENES (STING)-DEPENDENT ANTITUMOR IMMUNITY

Cédric BOULARAN, Thierry LIOUX, Fabienne VERNEJOUL, Laure DUCASSE, Anne Sophie BAUDRU, Marc Antoine MAUNY, Alain LAMOUREUX, Eric PEROUZEL, Michèle TIRABY

InvivoGen, 5 rue Jean Rodier, 31400 Toulouse, France

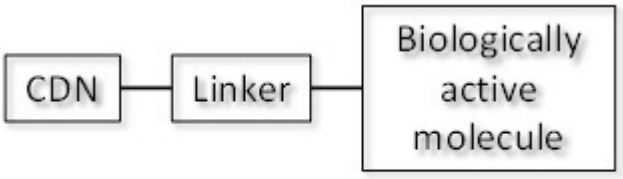
Resistance to immune checkpoint inhibitors (ICI) treatment can be attributed to insufficient infiltration into the tumor microenvironment and/or priming of T cell, lack of suitable neo-antigens presentation and/or impairment of interferon signaling. The administration of cyclic dinucleotides (CDNs) allows the activation of Stimulator of Interferon Genes (STING), a key player in immunity, and represents a clinical opportunity to overcome these limitations by activating both the innate and adaptive immune systems. CDNs contribution to anti-tumor immunity has been reported in different cancer histotypes, and relies mainly on type 1 interferon immunostimulatory effects.

We will describe the design, synthesis, *in vitro* biological activity and antitumor properties of three generations of STING agonists. The first generation includes compounds with a structure derived from the endogenous mammalian STING ligand, 2',3'-cGAMP. The second generation contains CDNs optimized for potency featuring 2'-Fluoro and/or phosphorothioate modifications (Formula I).



Formula I

The molecules of third generation are functionalized CDNs with the potency of second generation CDNs and an additional biological activity (Formula II).



Formula II

We will discuss the comparative advantages of the three CDN generations at the pharmacological and preclinical levels. We will present the *in vivo* data of our two lead compounds used alone or in combination with ICI in multiple aggressive tumor models, including B16 melanoma and CT26 colon carcinoma.

IN VIVO ANTI-VIRAL AND -TUMOR EFFECT OF 3'3'-cAIMP STING ACTIVATION

Martin K. Thomsen

Department of Clinical Medicine, Aarhus University, Denmark

Activation of the STING pathway is currently being explored in cancer immune therapy. The first STING agonist tested was DMXAA, but failed in clinical trial, since it does not bind human STING. The second generation of STING agonists are cyclic di-nucleotides (CDNs), either the naturally produced 2'3' cyclic GMP-AMP or derivatives, and many of these has proven to have high affinity for all common human variations of STING. In our work, we have focused on 3'3'-cyclic 3'3'-cAIMP (Invivogen) for analysis of antiviral and anticancer activity in mice.

Administration of 3'3'-cAIMP to mice induce a strong IFN response with upregulation of ISG and minor activation of other immune pathways such as NFkB. Mice were treated with 3'3'-cAIMP either before or during genital HSV2 infection and progression of disease and antiviral action was evaluated. Mice treated after infection had a reduced virus load and increased survival, whereas mice treated prior to infection were completely protected from infection. Furthermore, local administration had a major impact on virus load, but displayed a minimum systemic immune activation. Administration of 3'3'-cAIMP to the vaginal mucus layer induced a strong ISG signature in the epithelial layer indicating a direct action of the CDN in this anatomical compartment. Overall, 3'3'-cAIMP administration decreased virus load and induced limited immunopathology.

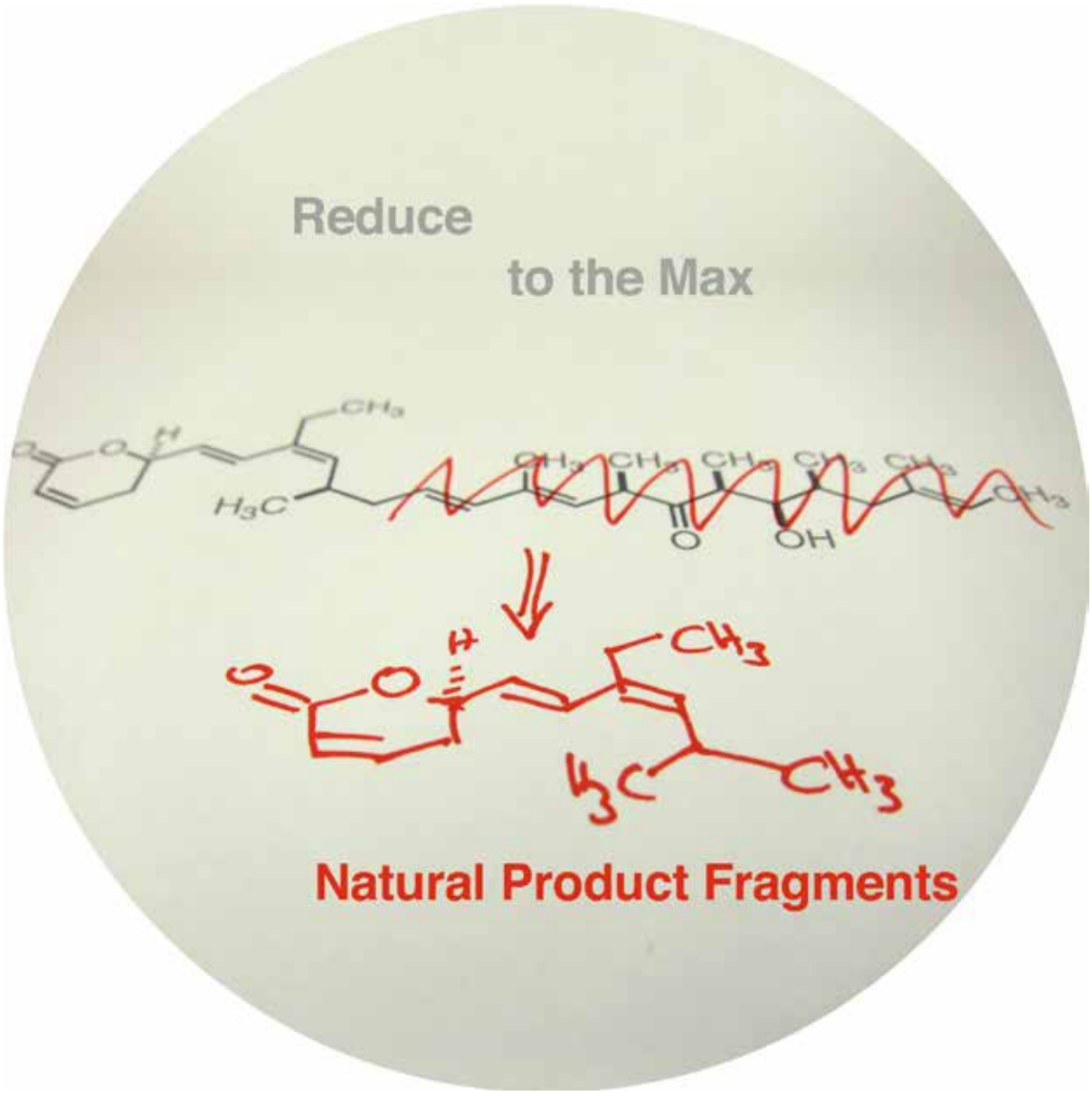
It has emerged that application of CDN to xenograft tumors can be used in cancer immune therapy. Here CDN have been administrated intramural, which has resulted in tumor reduction and activation of CD8 positive t-cells in experimental models. To address the effect of CDN on a heterogenic tumor by systemic delivery we induced liver tumors in the mice by chemical application. Tumor-bearing mice were treated for a month with 3'3'-cAIMP and left untreated for another month. Tumor load was followed by MR scanning over the period. A proximal 50% of the tumors respond to the treatment with reduces tumor volume or total tumor regression. These data show that 3'3'-cAIMP has an antitumor effect to a subset of heterogenic liver tumors.

CAPTURING BIOLOGICAL ACTIVITY IN NATURAL PRODUCT FRAGMENTS BY CHEMICAL SYNTHESIS

Karl Gademann

Department of Chemistry, University of Zurich, CH-8057 Zürich, Switzerland

Natural products have had an immense influence on science and have directly led to the introduction of many drugs. Organic chemistry, and its unique ability to tailor natural products through synthesis, provides an extraordinary approach to unlock the full potential of natural products. In this presentation, an approach based on natural product derived fragments is presented that can successfully address some of the current challenges in drug discovery. These fragments often display significantly reduced molecular weights, reduced structural complexity, a reduced number of synthetic steps, while retaining or even improving key biological parameters such as potency or selectivity. Examples from various stages of the drug development process up to the clinic are presented, as well as from our own research. All these concepts have the potential to identify the next generation of drug candidates inspired by natural products.



References

1) Review: E. A. Crane, K. Gademann, Angew. Chem. Int. Ed. 2017, 55, 3882. (Open Access)

STUCK IN A RUT WITH OLD CHEMISTRY

Jonas Boström

Medicinal Chemistry, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg, Pepparedsleden 1, SE-431 83, Sweden

Drug design is still mainly about getting compounds into assays. This has affected what medicinal chemists make. By analyzing which reactions were used in the past (30 years ago), with the one that are used in today's medicinal chemistry labs we observe a stagnation in the field of synthetic methodologies [1]. There is a mind-set that we default into reactions that we know. This is understandable – it is easy to get stuck in rut with things you know work reliably [2]. However, it is not entirely fair to give synthetic chemists a difficult time about a lack of adventurous spirit if we cannot provide accurate enough predictions to convince them to try something new. A lot of exciting innovation is indeed going on [3], and thoughts about how to bring new synthetic chemistry into the drug discovery labs will be discussed.

References

1) Brown, D.G. and Boström, J. Analysis of past and present synthetic methodologies on medicinal chemistry: Where have all the new reactions gone?, 2016, J. Med. Chem. 59, 4443–4458.
2) Boström, J., Brown, D.G. Stuck in a Rut with old chemistry. Drug Discov. Today, 2016 21, 701-703.
3) Boström, J., Brown, D.G., Young, R.J., Keserű, G.M. Expanding the medicinal chemistry synthetic toolbox, Nature Reviews Drug Discovery, accepted.

NEW CHEMICAL LIBRARIES IN EXPLORATION OF CHEMICAL SPACE

Jurij Svete

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

Various heterocyclic systems are important scaffolds for the preparation of compound libraries for medicinal and pharmaceutical applications. Due to biological activity of many of their derivatives, pyrazoles and their annulated analogues are attractive target structures. In the course of our studies aimed at preparation of novel chemical entities, we were focused in particular on pyrazole-based scaffolds with N–N structural motif. A structure-based survey on planning and setting target structures, development of synthetic methods, and executing the syntheses of combinatorial libraries of the representative compounds will be presented. The whole project started based on the preparation of pyrazole analogues of histamine and resulted in the synthesis of a series of novel compound types. For example, libraries of novel [1,5-*a*]pyrimidine derivatives were synthesized [1, 2] and synthetic methods for the preparation of the first representatives of the previously unknown saturated analogues were developed [3, 4]. All these synthetic methods enable preparation of target compounds in high yield and purity upon simple workup [1–4]. Syntheses of novel 3D-rich systems will also be presented.

References

1) Ahmetaj, S.; Velikanje, N.; Grošelj, U.; Prek. B.; Golobič, A.; Kočar, D.; Dahmann, G.; Stanovnik, B.; Svete, J. Mol. Divers. 2013, 17, 731-743.
2) Drev, M.; Grošelj, U.; Mevec, Š.; Pušavec, E.; Štrekelj, J.; Golobič, A.; Dahmann, G.; Stanovnik, B.; Svete, J. Tetrahedron 2014, 70, 8267-8279.
3) Lombar, K.; Grošelj, U.; Dahmann, G.; Stanovnik, B.; Svete, J. Synthesis 2015, 47, 497-506.
4) Mirnik, J.; Grošelj, U.; Novak, A.; Dahmann, G.; Golobič, A.; Kasunič, M.; Stanovnik, B.; Svete, J. Synthesis 2013, 45, 3404-3412.

INTEGRATED SYSTEM FOR THE EXPEDITED GENERATION AND CHARACTERIZATION OF DRUG-LIKE LIBRARIES FOR HIT-TO-LEAD EXPLORATIONS

Antimo Gioiello (1), Bruno Cerra (1), Andrea Carotti (1), Roccaldo Sardella (1), Alessandro Di Michele (2), Antonio Macchiarulo (1), Daniela Passeri (3), Roberto Pellicciari (3)

1) Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo, 1, 06123 Perugia, Italy
2) Department of Physic and Geology, University of Perugia, Via del Liceo, 1, 06123 Perugia, Italy
3) TES Pharma, Corso Vannucci, 47, 06121 Perugia, Italy

The discovery of new lead compounds relies on the iterative generation of structure-activity and structure-property relationship data. Typically, the process is compartmentalized and includes molecular design, chemical synthesis, physicochemical assay, and biological screening whose data analysis drives the next learning cycle. Using traditional approaches a significant time delay may occur from design hypothesis to results leading to slow and expensive hit-to-lead explorations, and attrition during early drug discovery.

Recent advances in flow technologies, computational methods, and biological testing have shown the potential to accelerate hit discovery and optimization thus improving the mapping of the biological and chemical space of druggable targets.[1]

In this communication, our recent efforts aimed at simplifying and expediting early stages of drug discovery are reported. In particular, we describe our ongoing work directed towards the development of an integrated platform for the building and characterization of multicomponent drug-like libraries. Flow synthesizers are coupled with continuous downstream operations, predictive software, computational analysis, and analytical devices to easily generate compounds collections readily available for biological screenings. The prototype system has been applied to the synthesis, purification, characterization and evaluation of chiral tetracyclic tetrahydroquinolines enabling the discovery of a novel class of Pregnane X Receptor (PXR) modulators.[2]

References

1) (a) Schneider, G. Nature Rev Drug Discov. 2017; (b) Gioiello, A. Chemistry Today 2017, 35, 8.
2) Banerjee M, Robbins D, Chen T. Drug Discov Today 2015, 20, 618.

MULTI-FUNCTIONAL TREATMENTS FOR MULTI-FACTORIAL NEURODEGENERATIVE DISORDERS: THE CHALLENGE OF ALZHEIMER'S DISEASE

Mark John Millan

CTI Neuropsychiatry, IDR Servier, Croissy sur Seine, Paris, France

Alzheimer's disease (AD) represents a huge socio-medical challenge. Currently-available drugs offer only modest symptomatic benefit and no agent blocks progression of the disorder. Cholinesterase inhibitors like Donezepil, and the NMDA receptor (R) modulator, Memantine, are directed at a single molecular target. Further, the most broadly evaluated agents, antibodies against b-amyloid and inhibitors of proteolytic generation from APP, possess a unitary MOA, and have not yet proven clinically active.

One might ask whether these, or any other highly-specific MOA, will do the business inasmuch as idiopathic AD is multi-factorial. It is characterised by a bewildering suite of risk/causal factors, cognitive and psychiatric symptoms, and pathophysiological changes, with heterogeneity amongst patients translating into differential responsiveness to treatments. Further, clinical diagnosis usually takes place well after the emergence of cellular pathology, perhaps too late for disease-modifying (DM) treatment. Improvements in biomarkers, like b-amyloid imaging and circulating miRNAs, may allow more precocious intervention. However, tolerance/cost will need to be very favourable for pre-symptomatic compliance/prescription, and the question remains of treatment with *what*. The present talk suggests that interventions possessing *multiple* MOAs justify particular attention.

Life-style changes from Mediterranean diet to exercise to improved sleep might be considered multi-functional approaches with potential benefits on, for example, cerebral bioenergetics and neuroinflammation. Their adoption in recent years may be related to the *decreasing* incidence (percentagewise) of AD. Further, optimized lifestyle could be a foundation for testing new medication or, at the very least, energy balance and activity levels should be individually-monitored in participants from the outset.

Intriguingly, improved sleep enhances extracellular clearance of neurotoxic proteins, and a promising line or "R&D" is the enhancement of intracellular protein clearance *via* the autophagic-lysosomal network (ALN). In a sense, this *is*, functionally-speaking, a multi-modal approach since the ALN clears not just b-amyloid but also other classes of neurotoxic protein as well as damaged mitochondria Moreover, drugs like Curcurmin, Resveratrol and Methylene Blue favour ALN clearance while possessing additional MOAs, like anti-aggregant properties. The final verdict on such agents is awaited but they are certainly not a panacea, so it is important to pursue new multi-target. In this light, the present talk exemplifies the type of approach that may be undertaken towards the development of more effective, multi-functional interventions for symptomatic and DM control of AD.

First, as regards the better control of symptoms, a broader focus than purely neurocognitive defects would be advisable, and employing improved readouts *vs* those conventionally used to date. Many classes of GPCR control not just "Learning and Memory", but also social cognition as well as other psychiatric domains affected in AD. One example is 5-HT6Rs, which have not proven sufficiently active in the clinic, but which could be combined with other classes of GPCR like dopamine D3R antagonists. Both should synergistically promote cortically-integrated social cognition and neurocognition by suppressing mTOR over-activation and reinforcing cholinergic and glutamatergic signalling. Further 5-HT6R blockade may afford complementary anxio-depressive properties, while D3R antagonism might moderate the occurrence of psychotic episodes.

Second, misprocessed, misfolded and oligomerised tau is a partner in crime with b-amyloid, since it spreads through the brain in parallel with cognitive decline, while higher-order forms block the proteosome. Its targeting is under evaluation. However, an attractive approach would be to attack pathological forms and oligomerisation of both b-amyloid *and* tau while avoiding interference with normally-functioning protein. One strategy, dubbed the "Common Conformational Motif," is being pioneered by Treventis*. It is rooted in transthyretin-inspired, *in silico* models of epitope commonalities between b-amyloid *and* tau in order to construct "surrogate crystal structures" that orient the screening, characterisation and validation of small, multi-target molecules that interfere with the misfolding and oligomerisation of both proteins1. Cell-free and *in vivo* models support dual activity of a range of chemical structures, and functional efficacy in animal models for AD is being evaluated1.

Finding broadly-effective DM and symptomatic treatments for AD will not be easy. In addition to early intervention and exploitation on a favourable life-style background, interventions with multiple MOAs that counter several pathological processes driving the disorder may have the greatest chance of success.

**Treventis is partnering with Servier, and Mark Reed at Treventis is thanked for provision of this information.*

References

1) Weaver, DF et al, Design and optimization of an anti-protein misfolding agent for AD, SfN, 479.22, 2017.

BEYOND SINGLE-TARGET ACTIVITIES: USING POLYPHARMACOLOGY AND SYSTEMS READOUTS FOR COMPOUND SELECTION AND MODE-OF-ACTION ANALYSIS

Andreas Bender

Centre for Molecular Informatics, Department of Chemistry, University of Cambridge

While the selection of compounds based on single-target activities assumed to confer efficacy has been performed extensively in the recent past, it has now become more clear that this single-target based approach is only reliably applicable in certain situations (such as where inhibition of a particular signalling cascade is clearly responsible for a given effect, or in certain situations in the infectious disease area). In more complex situations, however, it appears that the modulation of multiple targets seems to be necessary to achieve efficacy, and that also systems-based readouts (such as those based on transcriptomics data) hold relevant information for both compound efficacy and toxicity.

In this presentation, we will discuss several case studies from different disease areas, such as cancer and infectious diseases, where the modulation of multiple targets seems to have an advantage over targeting only single proteins. The modulation of those proteins can be achieved either via single compounds, or compound combinations, and the selection of compounds can either be performed on the protein target level, or the systems (transcriptomics readout) level. Examples for all of those cases will be included in this presentation, also illustrating that mode-of-action analysis and compound selection are two sides of the same coin and hence intrinsically related, and showing that both efficacy- and toxicity-related signals can be identified in systems readouts, such as gene expression data.

IN SILICO POLYPHARMACOLOGY

Giovanni Bottegoni

Heptares Therapeutics Ltd, BioPark, Broadwater Road, AL7 3AX, Welwyn Garden City, United Kingdom,
giovanni.bottegoni@heptares.com

Until recently, computer-assisted polypharmacology was an appealing idea, but only occasionally applied.[1] However, in recent years, multiple studies contributed to demonstrating that advanced computational methods can be efficiently rewired for rationally designing compounds endowed with activity at multiple targets. This appears particularly relevant in CNS-related conditions, which are often associated to a multi-layered aetiology. The application of advanced ligand- and structure-based virtual screening methods, the increasing role of molecular dynamics,[2] and the way these protocols can be efficiently coupled to biophysical techniques will be discussed. Selected examples of how computational design translated into actionable insights for synthesizing multi-target directed ligands will be reported. Last, an outlook on the current trends and most recent developments in the field will be provided.

References

1) Bottegoni G, Favia AD, Recanatini M, Cavalli A., 2012 Drug Discovery Today 17(1-2) 23:34
2) De Vivo M, Masetti M, Bottegoni G, Cavalli A. 2016 J Med Chem 59(9) 4035:4061

METABOTROPIC GLUTAMATE RECEPTOR TYPE 2 POSITIVE ALLOSTERIC MODULATORS (MGLU2 RECEPTOR PAMS) AS A TRANSFORMATIONAL EPILEPSY TREATMENT

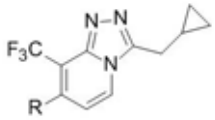
Jose Cid (1), Andrés A. Trabanco (1), Marc Ceusters (2), Hilde Lavreysen (2), Roy Twyman (3)

1) Neuroscience Medicinal Chemistry. Janssen Research and Development, Toledo, Spain
2) Janssen Research and Development, Beerse, Belgium
3) Janssen Research and Development, Titusville, FL, USA

Epilepsy is one of the most common neurological diseases affecting approximately 50 million people worldwide (1). Under medical care, about 70% of patients become seizure-free, however, approximately 30% of patients has 'intractable seizures' that do not respond to medication or have unmanageable side effects (2). Levetiracetam (LEV) is the primary agent for treating epilepsy. LEV has proven effective in the treatment of epilepsy(3), although its clinical use can be hindered by dose-limiting side effects (30-50% discontinue despite having some benefit (4). Newer antiepileptic drugs (AEDs) that can effectively modify the development of epilepsy by targeting the underlying epileptogenic process are desirable.

Positive allosteric modulators (PAMs) of $mGlu_2$ receptors have emerged recently as promising novel therapeutic approaches for the treatment of several CNS disorders, including epilepsy (5). Activation of the $mGlu_2$ receptor results in reduced glutamate release and decreases excitability (6,7). Prior to seizure activity, increases in extracellular glutamate are measured in human hippocampus and the increase is sustained during epileptogenic activity (8), thus lending support to the idea that a reduction in glutamate levels may be of benefit in the treatment of epilepsy.

Herein we report the structure-activity relationship of a series of $mGlu_2$ PAM triazolopyridines (9,10) with anticonvulsant efficacy. Moreover, in combination with LEV, a strong and specific pharmacodynamic synergy was observed, shifting the potency of LEV without worsening of tolerability(5,11).This combination has the potential to reduce the dose of LEV required to produce full efficacy, thereby, potentially reducing its side effects. Thus, $mGlu_2$ PAMs have the potential to be first in class, first add-on drug to the leading LEV.



triazolopyrimidine series

References

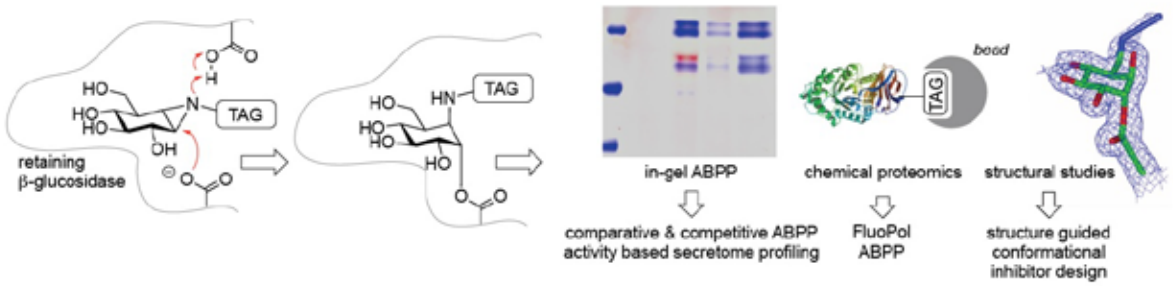
1) Banerjee PN et al. The descriptive epidemiology of epilepsy, a review. Epilepsy Res. 2009, 85, 31–45.
2) Fisher RS et al. Operational classification of seizure types by the international league against epilepsy: position paper of the ILAE commission for classification and terminology. Epilepsia, 2017, 58, 522–530.
3) Abou-Khalil B. Levetiracetam in the treatment of epilepsy. Neuropsychiatr Dis Treat, 2008, 4, 507–523
4) Dinkelacker V et al. Aggressive behavior of epilepsy patients in the course of levetiracetam add-on therapy: report of 33 mild to severe cases. Epilepsy Behav, 2003, 4, 537–547.
5) Cameron S. Metcalf Efficacy of mGlu2-positive allosteric modulators alone and in combination with levetiracetam in the mouse 6 Hz model of psychomotor seizures Epilepsia, 2017, 58, 484–493,
6) Caulder EH et al. Activation of group 2 metabotropic glutamate receptors reduces behavioral and electrographic correlates of pilocarpine induced status epilepticus. Epilepsy Res 2014, 108, 171–181
7) Barton ME et al. Comparison of the effect of glutamate receptor modulators in the 6 Hz and maximal electroshock seizure models. Epilepsy Res, 2003; 56, 17–26.
8) During M J et al Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet (London, England), 1993, 341, 1607-10
9) Cid J et al. Discovery of 3-Cyclopropylmethyl-7-(4-phenylpiperidin-1-yl)-8-trifluoromethyl[1,2,4]triazolo[4,3-a]pyridine (JNJ-42153605): A Positive Allosteric Modulator of the Metabotropic Glutamate 2 Receptor. J. Med. Chem. 2012, 55, 8770–8789
10) Cid J et al. Discovery of 8-Trifluoromethyl-3-cyclopropylmethyl-7-[(4-(2,4-difluorophenyl)-1-piperazinyl)methyl]-1,2,4-triazolo[4,3-a]pyridine (JNJ-46356479), a Selective and Orally Bioavailable mGlu2 Receptor Positive Allosteric Modulator (PAM). J. Med. Chem. 2016, 59, 8495–8507.
11) Metcalf CS et al. Potent and selective pharmacodynamic synergy between the metabotropic glutamate receptor subtype 2–positive allosteric modulator JNJ-46356479 and levetiracetam in the mouse 6-Hz (44-mA) model. Epilepsia. 2018, 59, 724–735.

ACTIVITY-BASED GLYCOSIDASE PROFILING IN BIOMEDICINE AND BIOTECHNOLOGY

Herman S. Overkleeft

Leiden Institute of Chemistry, Leiden University, The Netherlands
h.s.overkleeft@chem.leidenuniv.nl

Activity-based protein profiling (ABPP) is a rapidly emerging field in chemical biology research. Enzymes that employ a mechanism in processing their substrate that involves formation of a covalent enzymeintermediate adduct can be blocked by mechanism-based suicide inhibitors: compounds that react within the enzyme active site to form a covalent and irreversible adduct. Introduction of a reporter moiety ('TAG' in the below picture) yields an activity-based probe (ABP) through which enzyme activities can be discovered (comparative ABPP) and the efficacy enzyme inhibitors in complex biological systems analyzed (competitive ABPP).



Our work on ABPP development focuses on retaining glycosidases: hydrolytic enzymes able to cleave interglycosidic linkages and that do so through the formation of covalent enzyme-substrate intermediates. Configurational and functional analogues of the natural product and mechanism-based retaining betaglucosidase inhibitor, cyclophellitol, prove to be highly versatile tools to study retaining glycosidases of various nature and origin in relation to human health and disease, but also in the field of biotechnology. In this lecture the current state in the design, synthesis and application of synthetic cyclophellitol derivatives in studying retaining glycosidases will be presented. Discussed subjects will include 1) diagnosis of human lysosomal exoglycosidases in relation to lysosomal storage disorders; 2) glycosylation of cyclophellitol derivatives top arrive at retaining endoglycosidase ABPs and 3) application of glycosidase ABPs in the functional profiling of fungal secretomes for the discovery of glycosidases for biotechnology application.

References

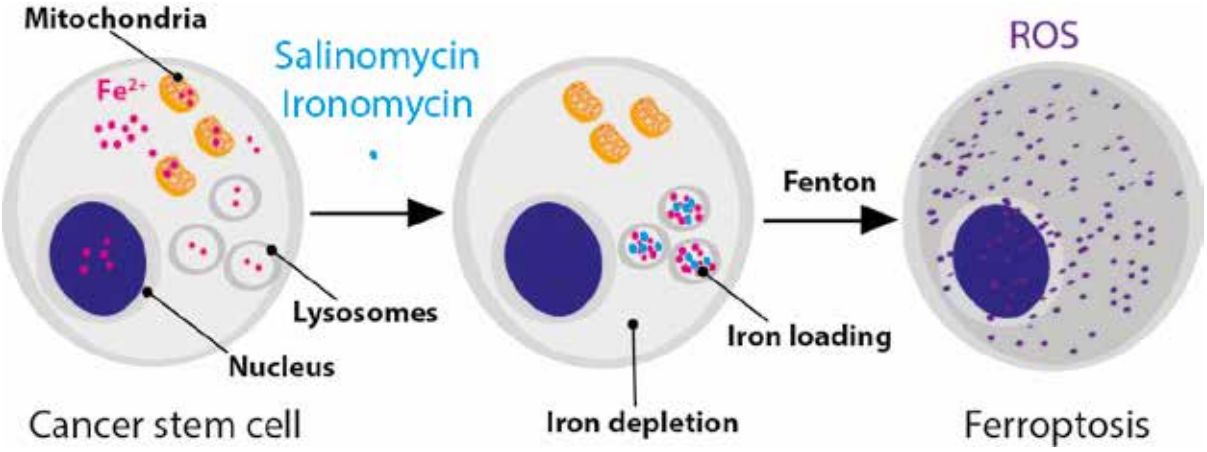
1) M. D. Witte, W. W. Kallemeijn, J. Aten, K.-Y. Li, A. Strijland, W. E. Donker-Koopman, B. Blijlevens, G. Kramer, A. M. C. H. van den Nieuwendijk, B. I. Florea, B. Hooibrink, C. E. M. Hollak, R. Ottenhoff, R. G. Boot, G. A. van der Marel, H. S. Overkleeft and J. M. F. G. Aerts, Ultrasensitive in situ visualization of active glucocerebrosidase molecules, Nat. Chem. Biol. 2010, 6, 907-913.
2) J. Jiang, C.-L. Kuo, L. Wu, C. Franke, W. W. Kallemeijn, B. I. Florea, E. van Meel, G. A. van der Marel, J. D. C. Codée, R. G. Boot, G. J. Davies, H. S. Overkleeft and J. M. F. G. Aerts, Detection of active mammalian GH31 alpha-glucosidases in health and disease using in-class, broad-spectrum activity-based probes, ACS Cent. Sci. 2016, 2, 351-358.

AN IRON HAND OVER CANCER STEM CELLS

Tatiana Cañeque, Sebastian Müller, Fabien Sindikubwabo, Raphaël Rodriguez

Institut Curie, 26 rue d'Ulm, 75005 Paris

Cancer stem cells have been shown to be refractory to conventional therapeutic agents, can promote metastasis and have been linked to cancer relapse[1,2,3]. Salinomycin can selectively kill cancer stem cells[4]. We have shown that salinomycin derivatives accumulate in lysosomes and sequester iron in this organelle. As a result, accumulation of iron leads to the production of reactive oxygen species and lysosomal membrane permeabilization, which in turn promotes cell death by ferroptosis[5,6]. This investigation has revealed the prevalence of iron homeostasis in cancer stem cells and paved the way towards the development of next generation therapeutics[5,6]. It demonstrates that the chemical reactivity of iron can be reprogrammed by means of small molecule intervention and raises a putative role of this metal in the maintenance of a mesenchymal state of cancer cells[5,6].



References

1) M. N. Nieto, R. Y.-J. Huang, R. A. Jackson, J. P. Thiery, Cell, 2016, 166, 21.
2) W. L. Tam, R. A. Weinberg, Nat. Med., 2013, 19, 1438.
3) D. R. Pattabiraman, R. A. Weinberg, Nat. Rev. Drug Discov., 2014, 13, 497.
4) P. B. Gupta, T. T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R. A. Weinberg, E. S. Lander, Cell, 2009, 138, 645.
5) T. T. Mai, A. Hamaï, A. Hienzsch, T. Cañeque, S. Müller, J. Wicinski, O. Cabaud, C. Leroy, A. David, V. Acevedo, A. Ryo, C. Ginestier, D. Birnbaum, E. Charafe-Jauffret, P. Codogno, M. Mehrpour, R. Rodriguez, Nat. Chem., 2017, 9, 1025.
6) A. Hamaï, T. Cañeque, S. Müller, T. T. Mai, A. Hienzsch, C. Ginestier, E. Charafe-Jauffret, P. Codogno, M. Mehrpour, R. Rodriguez* Autophagy, 2017, 13, 1465.

CHARTING THE STRUCTURE-RESISTANCE LANDSCAPE OF NOVEL ANTIBIOTICS

Tamás Révész, Ákos Nyerges, Gábor Draskovits, Petra Szili, Dávid Balogh, Csaba Pál

Csaba Pál Laboratory, Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62. Szeged, Hungary , http://group.szbk.u-szeged.hu/sysbiol/EvGEn

Resistance threats the success of clinical therapies and challenge pharmaceutical industry to constantly develop better antimicrobials. Meanwhile, the bioactivity of a newly launched antibiotic may be quickly compromised by the rapid rise of resistance against the given compound.

Our aim is to investigate the relationship between the chemical structure of antibiotics and the emerging resistance mechanisms. To reach this aim we combine *in vivo* mutational effect analysis (AMAT) with *in silico* molecular modeling. AMAT (Accelerated Mutagenesis of Antibiotic Targets) is a novel targeted mutagenesis technology from our lab and it allows us to map resistance mutation with an extreme resolution at the antibiotic target directly in clinical pathogens. With computational modelling, AMAT is capable to chart drug-protein interactions and provide novel insights into the alterations at the drug target that causes resistance.

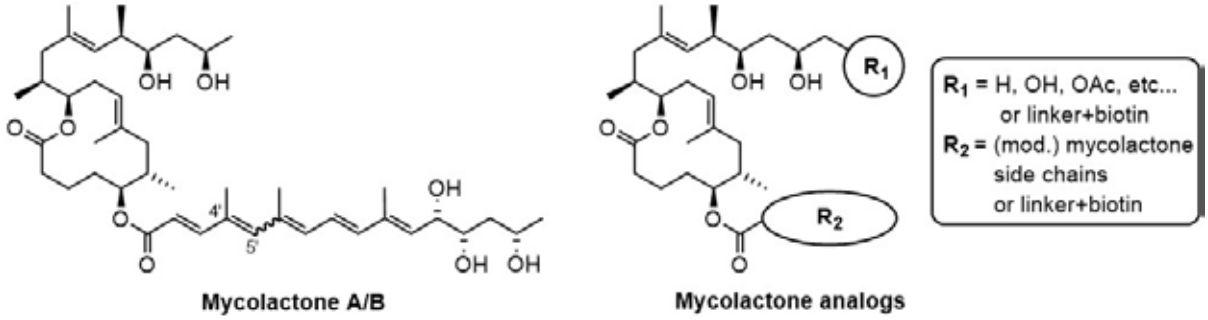
The combination of these techniques enables us to investigate the structure-resistance landscape of antibiotics and facilitate the understanding of molecular mechanisms behind the target-mediated resistance. In the long term, it can pave the way to develop new antibiotic compounds with increased resistance tolerance.

BURULI ULCER AND THE MTOR PATHWAY: TOTAL SYNTHESIS, STRUCTURE–ACTIVITY AND TARGET ELUCIDATION STUDIES OF MYCOLACTONES

Matthias Gehringer (1,3), Raphael Bieri (2), Philipp Gersbach (3), Nicole Scherr (2), Marie-Thérèse Ruf (2), Patrick Mäder (3), Gerd Pluschke (3), Karl-Heinz Altmann

1) Institute of Pharmaceutical Sciences, University of Tuebingen, Auf der Morgenstelle 8, 72076 Tuebingen, Germany
2) Department of Molecular Immunology, Swiss Tropical and Public Health Institute, Socinstr. 57, 4051 Basel, Switzerland
3) Institute of Pharmaceutical Sciences, ETH Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

Mycolactones are a group of complex macrolactones with very interesting cytotoxic, immunosuppressive and analgesic properties. As the exotoxins of the human pathogen *Mycobacterium ulcerans*, mycolactones are central to the pathogenesis of the neglected disease Buruli ulcer, a severe and chronic medical condition characterized by extensive necrotic skin ulcers. Mycolactone A/B, the most biologically active member of the mycolactone family of polyketides naturally occurs as a 2:3 mixture of the *cis*- and the *trans*-isomer at the $\Delta^{4',5'}$ double bond of the lower pentaenoate side chain.[1] However, despite extensive research in several academic laboratories, it is not yet clear which of these isomers represents the major contributor to bioactivity. Moreover, the molecular mechanisms of mycolactones action are heavily debated but none of the targets proposed in the previous literature was rigorously validated.



Intrigued by the biological activities of mycolactones, we synthesized a variety of analogs by total synthesis.[2] These compounds featuring modifications at the lower side chain (R_2) and the upper core extension (R_1) were used to derive essential structure–activity relationships. With the aim of identifying novel druggable targets, biotinylated mycolactones were prepared for target deconvolution studies. By using these tagged probes in conjunction with qPCR, RNAi and immunoblotting, we identified the mechanistic Target of Rapamycin (mTOR) signaling pathway as the key driver of mycolactone action.[3] We showed that mycolactone A/B targets the 12 kDa FK506-binding protein (FKBP12) and interferes with the assembly of the mTORC2 multiprotein signaling complex thereby preventing the activation of the downstream protein kinase Akt. The resulting dephosphorylation of the Akt-targeted transcription factor Forkhead box O3 (FoxO3) triggers the expression of the pro-apoptotic Bcl-2-like protein 11 (Bim) driving cells into apoptosis. Bim knockout protected cells from mycolactone toxicity *in vitro* and prevented the Buruli ulcer phenotype in *M. ulcerans*-infected mice confirming our results *in vivo*. Very recently, we prepared rigidified mycolactone analogs for elucidating the influence of the $\Delta^{4',5'}$-*cis*/*trans* isomerism on bioactivity. The synthesis and the SAR of these analogs will also be presented.

References

1) Chany, A.-C. et al., Nat. Prod. Rep. 2013, 30, 1527–1567.
2) Gehringer, M. and Altmann, K.-H., Beilstein J. Org. Chem. 2017, 13, 1596–1660.
3) Bieri, R. et al., ACS Chem. Biol. 2017, 12, 1297–1307.

SULFUR- AND SILICON-BASED FLUORINATION REAGENTS FOR MEDICINAL CHEMISTRY

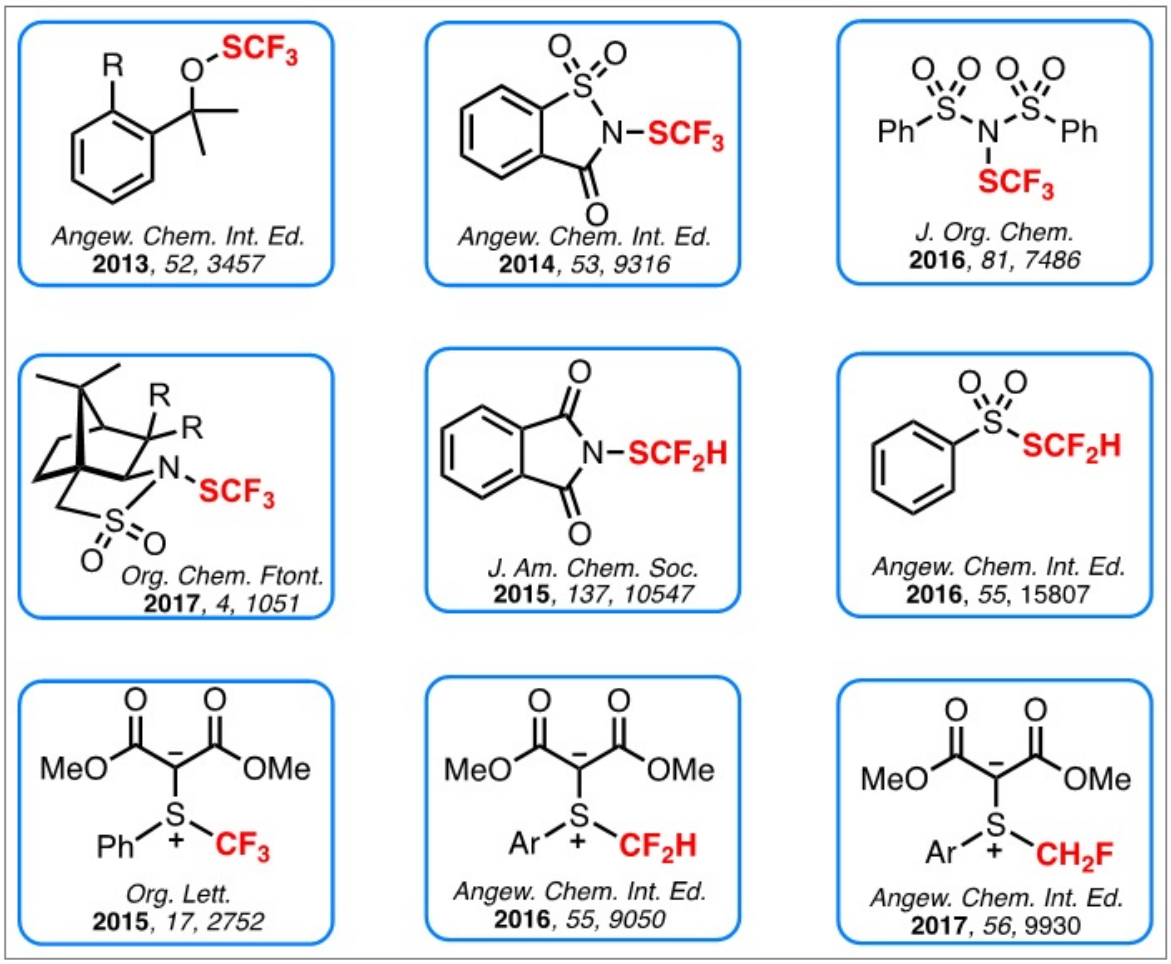
Qilong Shen

Shanghai Institute of Organic Chemistry, CAS, Shanghai 200032, China
shenql@sioc.ac.cn

Due to the well-known “fluorine effect” of the fluorine atom and the fluorinated groups on the chemical, physical and biological properties of a given molecule, incorporation of a fluorine atom or a fluoroalkyl group into has become a routine practise in the development of drugs or agrochemicals. Consequently, development of efficient methods that could late-stage introduction of fluorine or fluorinated groups of the drug molecules have been of intense current interests.

Among the rapidly increasing and powerful fluoroalkylating methods, direct fluoroalkylation of a nucleophile with an electrophilic fluoroalkylating reagent arguably represents one of the most versatile and actively studied methods for the preparation of fluoroalkylated compounds. Accordingly, several classes of electrophilic fluoroalkylating reagents has been developed by the groups of Yagupolskii, Umemoto,Togni, Shibata,Prakash, among others, thus providing a strong driving force for the discovery of the new fluoroalkylation methodologies.

Even though some of these reagents have been commercialized, further broad applications of these electrophilic fluoroalkylating reagents were largely hampered by their relatively complicated synthetic procedures. In the past eight years, we have discovered several electrophilic fluoroalkylating reagents including electrophilic trifluoromethylthiolating reagents, difluoromethylthiolating reagent and new trifluoromethylating, difluoromethylating, monofluoromethylating reagents based on sulfonium ylide skeleton. These reagents can be easily synthesized and reacted with a variety of nucleophiles, thus providing a toolbox for incorporation of fluoroalkyl groups for new drug discovery.

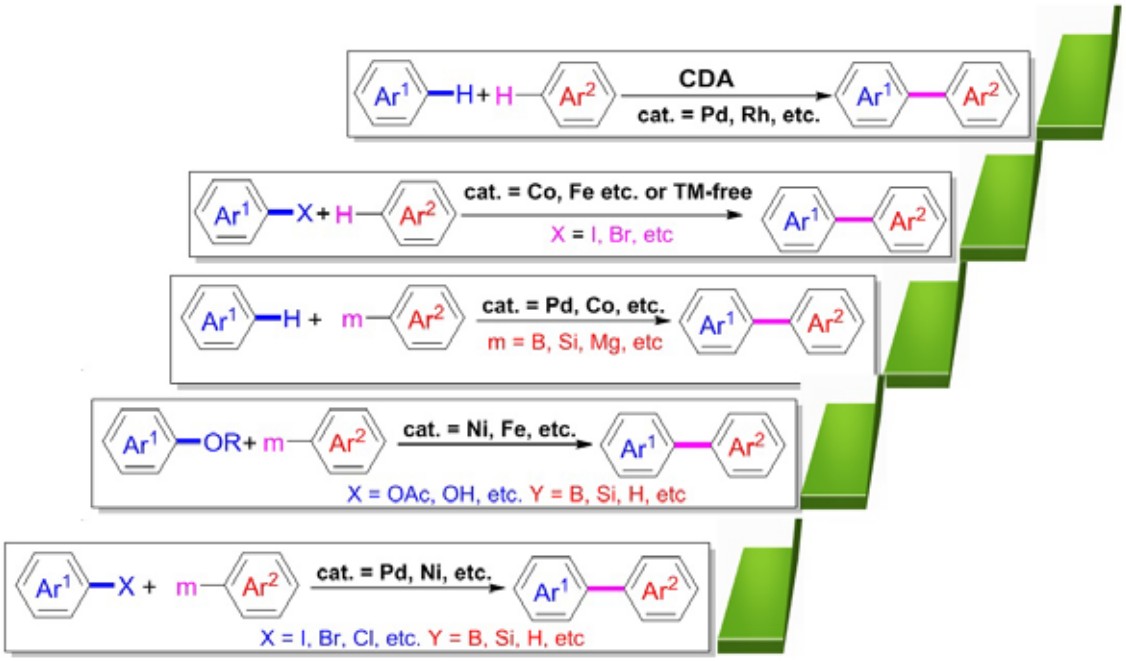


UPGRADING CROSS COUPLING TOWARD BIARYL SCAFFOLDS

Zhang-Jie Shi

Department of Chemistry, Fudan University, Shanghai 200433, China
zjshi@fudan.edu.cn

Conventional cross coupling is one of the most powerful methods to construct carbon-carbon bonds starting from organohalides and organometallic reagents, catalyzed by late transition-metal catalysts in general.[1] With our and others' efforts, the electrophilic partner can be taken place of by O-based electrophiles.[2] C-H bonds could also applied as coupling partners, coupled with various organometallic reagents, as well as another molecule of C-H bonds.[3] To avoid the utilization of late and heavy transition-metal catalysts, the earth-abundant transition-metal and even metal free catalytic systems were built up to proceed the cross coupling between organohalides and arenes.[4] These studies may lead the evolution of cross coupling in an environmentally benign manner.



References

1) de Meijere, A., Diederich, F., Metal-Catalyzed Cross-Coupling. Wiley-VCH: Weinheim: 2004.
2) Su, B., Ca, Z.-C., Sh, Z.-J. Acc. Chem. Res. 2015, 48, 886.
3) Yu, D.-G., Li, B.-J., Shi, Z.-J. Tetrahedron, 2012, 68, 5130.
4) Sun, C.-L., Li, H., Yu, D.-G., Yu, M., Zhou, X., Lu, X.-Y., Huang, K., Zheng, S.-F., Li, B.-J., Shi, Z.-J. Nat. Chem. 2010, 2, 1044.

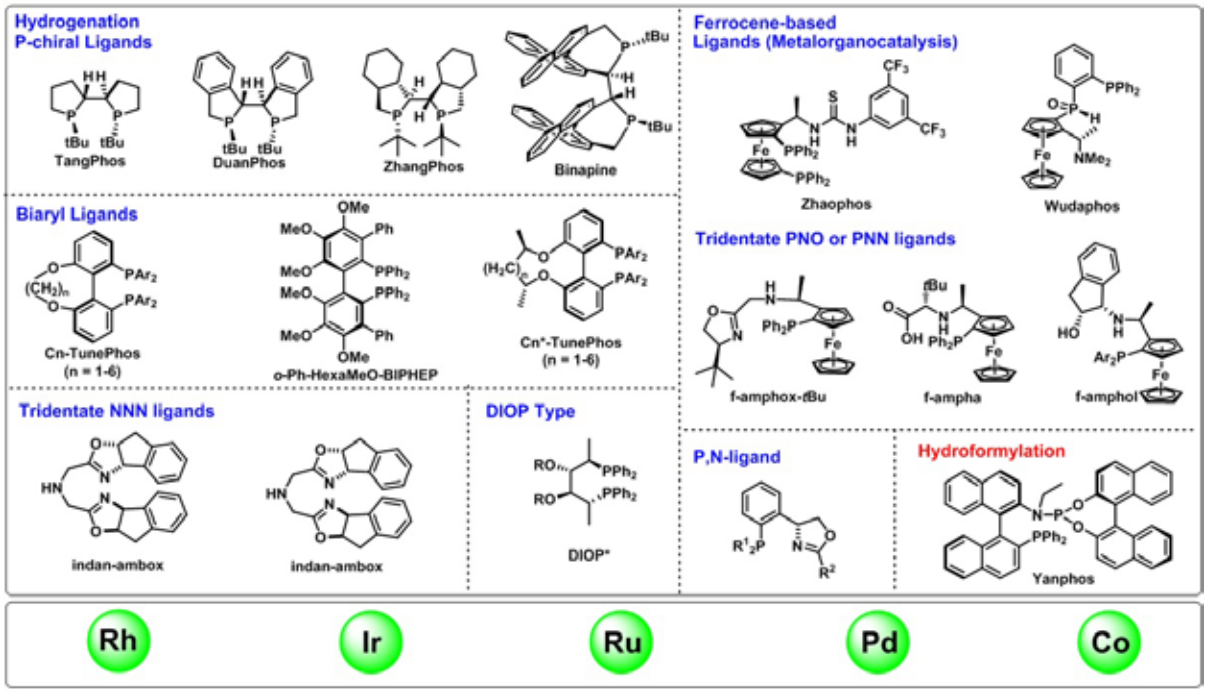
PRACTICAL ASYMMETRIC HYDROGENATION

Xumu Zhang

Department of Chemistry, Southern University of Science and Technology

Dr. William Knowles, in his 2001 Nobel Lecture, describes his 1960s and 70s work in developing asymmetric hydrogenation catalysts. Now, 45 years later after the first commercial application of asymmetric catalysis, although major advances have been made (e.g.; Professor Noyori's Nobel prize winning work in asymmetric hydrogenation), significant challenges remain. This presentation describes innovation in asymmetric hydrogenation catalysis from both an academic and industrial perspective. Having invented a catalyst that addresses an unmet need in asymmetric hydrogenation, many challenges remain before the catalyst provides an economic return. The knowledge gained and shortcomings recognized during scale-up and commercialization can lead to greatly improved 'next generation' catalysts.

This presentation highlights recent advances in our labs and the commercialization of many chiral phosphine ligands by Chiral Quest, Inc. The broad array of our chiral catalyst toolbox and their numerous applications for a variety of functional group hydrogenations will be reviewed. The emphasis will be on the practical application of asymmetric hydrogenation to make chiral pharmaceutical in ton scale.



ASYMMETRIC SYNTHESIS OF STATIN API AS THE HYPOLIPIDEMIC AGENTS: THE EVOLUTION FROM THE CHEMICAL KINETIC RESOLUTION TO THE ASYMMETRIC CATALYTIC TECHNOLOGY (AN ODESSY)

Fener Chen

FUDAN UNIVERSITY, China

This lecture could be divided in to the following four main sections.

1. Introduction of the research background: The treatment of hyperlipidemia and the development of statin drugs.

2. The technical shortcomings of the traditional synthesis technology strategy of statin APIs.

3. The synthetic new strategy of statin drugs *via* the chemical kinetic resolution methods.

4. The new asymmetric synthesis strategy statin drugs *via* the chiral catalysis technology using the selective catalyst.

OBETICHOLIC ACID, LEADING IN THE NASH RACE. HISTORY AND PERSPECTIVES

Roberto Pellicciari

TES Pharma, Perugia, Italy

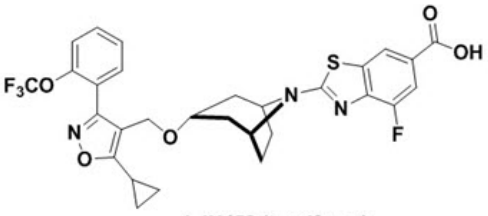
Nonalcoholic fatty liver disease (NAFDL) is an increasingly recognized liver disease caused by fat accumulation affecting around 30% of the world population and 70-80% of individuals who are obese and diabetics. The progression from fatty liver/steatosis to nonalcoholic steatohepatitis (NASH) increases the risks for fibrosis, and/or cirrhosis. NASH, projected to become the most common indication for liver transplant in the next decade, is also a risk factor for type 2 diabetes and end stage kidney disease There are currently no medications approved for the treatment of NASH. In recent years, many drug candidates acting on various pathophysiological NASH processes have entered clinical development. Among the targets, several nuclear receptors, such as FXR and PPARs have shown to have therapeutic potential for the treatment of NASH. The Farnesoid X receptor (FXR), primarily expressed in the liver, gut and liver, plays a key role in bile acids, cholesterol and glucose homeostasis and has been shown to have anti-inflammatory and anti-fibrogenic properties thus representing a suitable therapeutic option for NASH patients. Of all the steroidal and non-steroidal FXR agonists the most clinically advanced is Obeticholic acid (OCA) , a semisynthetic bile acid derivative approved in May 2016 by the US FDA for the treatment of Primary Biliary Cholangitis (PBC). OCA is currently the only FDA-designated Breaktrough Therapy in development for NASH and with the Phase3 trials REGENERATE and REVERSE underway is on track to be the first approved NASH therapy. In this talk I will summarize the history of OCA, its current state of development and its role in the emerging opinion that therapeutic approaches for treating NASH will not be single drug therapies.

DISCOVERY OF LJN452 (TROPIFEXOR), A HIGHLY POTENT, NON-BILE ACID FXR AGONIST FOR THE TREATMENT OF CHOLESTATIC LIVER DISEASES AND NASH

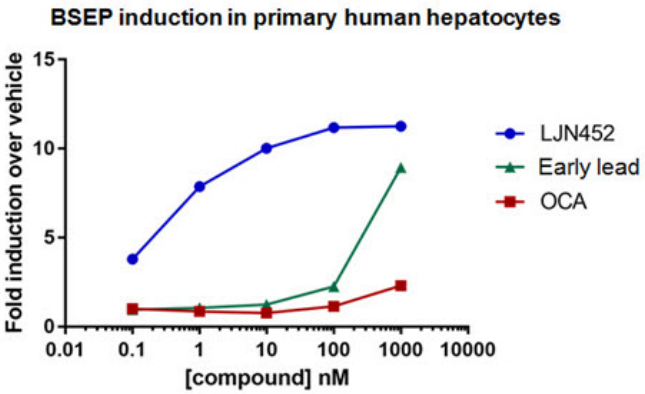
David Tully

Novartis Institutes for Biomedical Research, 5300 Chiron Way, Emeryville, CA 94608-2916

The farnesoid X receptor (FXR) is a nuclear receptor that serves as the master regulator of bile acid metabolism and signalling. Activation of FXR inhibits bile acid synthesis and increases bile acid conjugation, transport, and excretion, thereby protecting the liver from the harmful effects of bile accumulation. There has been considerable interest in FXR as a therapeutic target for the treatment of cholestatic liver diseases and non-alcoholic steatohepatitis (NASH). This presentation highlights the discovery of LJN452 (tropifexor), which is a novel, highly potent, non-bile acid FXR agonist currently being evaluated in phase 2 human clinical trials in patients with NASH and primary biliary cholangitis (PBC).



LJN452 (tropifexor)
FXR HTRF EC_{50} = 0.20 nM (92%)
FXR BSEP-luc EC_{50} = 0.26 nM (89%)



References

1) Tully, D. C., et al. J. Med. Chem. 2017, 60 (24), 9960–9973.

THE FIRST CLASS OF ORALLY AVAILABLE MONO-SACCHARIDE GALECTIN-3 INHIBITORS FOR TREATMENT OF FIBROSIS (NASH) AND CANCER

Fredrik Zetterberg

Galecto Biotech, Medicinaregatan 8 A, S-413 46 Gothenburg, Sweden

Galectin-3 is a β-D-galactopyranoside specific lectin which is involved in the pathology of fibrosis and cancer. We recently finalized a successful phase I/IIa study with our galectin-3 inhibitor, TD139, which is being developed as an inhaled treatment of Idiopatic pulmonary fibrosis (IPF). Since genetic depletion or inhibition of galectin-3 reduces fibrosis in other organs such as liver, kidney and heart, there is a need for a systemically available galectin-3 inhibitor. We report here how we developed the first orally available high affinity (nM) galectin-3 inhibitors and the effects of these in PD models of fibrosis and cancer.

Designing small high affinity lectin inhibitors with a natural saccharide as a starting point is a major challenge. In general mono- and disaccharides bind with an affinity in the low μM-mM range due to that the lectin binding sites are shallow and polar. Inhibitors with high polarity also in general have limited oral bioavailability, which indeed is the case for the disaccharide TD139. By introduction of non-natural aromatic substituents to the 1 and 3-position of α-D-galactopyranoside the polar surface area was reduced and lipophilicity increased to result in compounds with PK properties suitable for oral administration. Then high affinity compounds could be achieved by the use of specific interactions, such as fluorine-amide, phenyl-arginine, sulfur- π and halogen bonds.

Further, we have shown that these compounds reduce development of fibrosis in a CCl4 mouse model and that they also reduce tumor growth and metastasis in a model of Lewis Lung Carcinoma. We are currently in the process of taking candidates of this class to man for development of new treatments of fibrosis (NASH) and cancer.

DEVELOPMENT OF SMALL-MOLECULE INHIBITORS OF ADIPOSE TRIGLYCERIDE LIPASE (ATGL)

A. Migglautsch (1), C. J. Doler (1), M. Schweiger (2), N. Mayer (1), E. Fuchs (1), G. Grabner (2), M. Romauch (2), R. Zechner (2), R. Zimmermann (2), R. Breinbauer (1)

1) Institute of Organic Chemistry, Graz University of Technology
2) Institute of Molecular Biosciences, University of Graz

Adipose Triglyceride Lipase (ATGL) is the first and rate-limiting enzyme in the catalytic cascade of lipolysis.[1] Hence, ATGL is primarily responsible for the mobilization of fatty acids (FAs) from cellular triglyceride stores [2] and in consequence the level of circulating FAs.[3] As high levels of serum FAs are closely linked to the development of non-alcoholic fatty liver disease (NAFLD) and insulin resistance, which further progresses to liver steatosis and type II diabetes, respectively, ATGL represents an interesting pharmacological target. This is strongly supported by the results of ATGL knock out studies in mice, which show an increase in insulin sensitivity.[3, 4]

Recently, we described the first potent inhibitor of murine ATGL, Atglistatin® (IC_{50} = 0.7 µM). Treatment with Atglistatin effectively reduces FA mobilization *in vitro* and *in vivo*, which leads to a tremendous increase of insulin sensitivity and resistance against the development of NAFLD in mice fed a high fat diet. Still, mice showed no loss in muscle weight or accumulation of TGs in ectopic tissue such as skeletal muscle, or heart in contrast to ATGL-k.o. mice.[4]

The structure of Atglistatin has been developed from the hit compound (shown in Figure 1) in an intense optimization process and is designed to overcome toxicity and solubility issues while increasing potency. It can be produced in a three-step-synthesis. However, Atglistatin inhibits only murine ATGL. To overcome this issue, we are currently working on further optimization of the lead structure to produce a 2nd generation inhibitor.

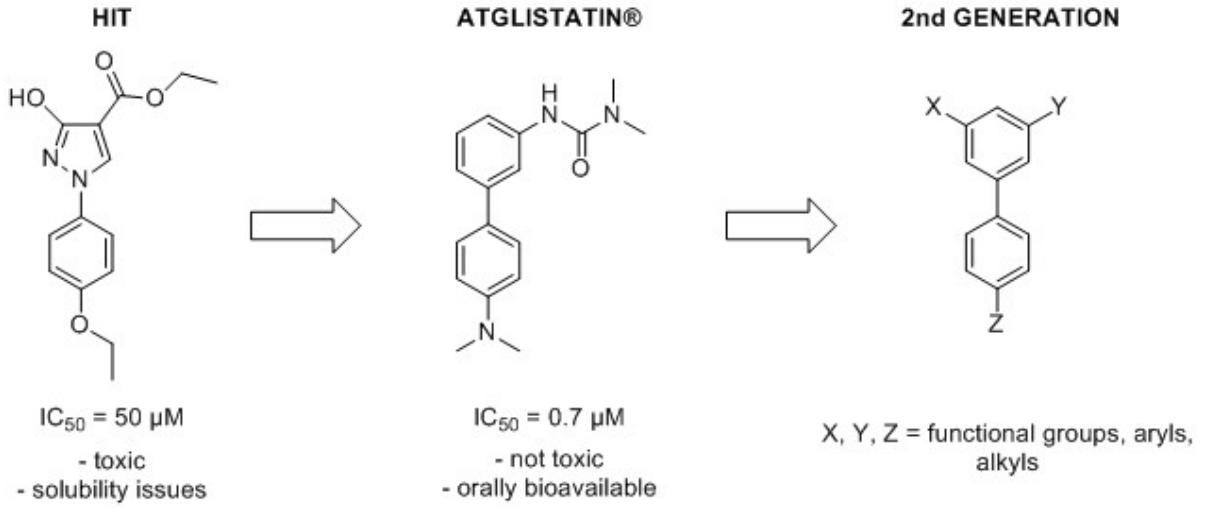


Figure 1: Development of Atglistatin®

References

1) A. Lass, R. Zimmermann, M. Oberer, R. Zechner, Prog. Lip. Res. 2011, 11, 14-27.
2) N. Mayer, M. Schweiger, M. Romauch, G. Grabner, T. Eichmann, E. Fuchs, J. Ivkovic, C. Heier, I. Mrak, A. Lass, G. Höfler, C. Fledelius, R. Zechner, R. Zimmermann, R. Breinbauer, Nat. Chem. Biol. 2013, 9, 785-787.
3) N. Mayer, M. Schweiger, M. C. Melcher, C. Fledelius, R. Zechner, R. Zimmermann, R. Breinbauer, Bioorg. Med. Chem. 2015, 23, 2904-1916.
4) M. Schweiger, M. Rommauch, G. F. Grabner, R. Schreiber, T. O. Eichmann, S. Yamada, S. Hütter, O. Knittelfelder, C. Diwoky, C. Doler, N. Mayer, W. De Cecco, R. Breinbauer, R. Zimmermann, R. Zechner, Nature Commun. 2017, 8, 14859.

EGFR TRIPLE MUTANT L858R_T790M_ C797S RECENT SET-BACKS AND NEW HOPE IN FIGHTING MUTANT NON-SMALL CELL LUNG CANCER

Marcel Günther (3), Jonas Lategahn (1), Michael Juchum (3), Eva Döring (3), Marina Keul (1), Julian Engel (1), Hannah Tumbrink (1), Daniel Rauh (1), Heiner Fiebig (2), Gerhard Kelter (2), Stefan Laufer (3)

1) Technische Universität Dortmund, Fakultät für Chemie und Chemische Biologie Otto-Hahn-Strasse 4a, 44227 Dortmund, Germany
2) Cell Biology & Compound Screening Oncotest GmbH, Am Flughafen 12–14, 79108 Freiburg, Germany
3) Eberhard Karls Universität Tíbingen, Institute of Pharmaceutical Sciences, Pharmaceutical and Medicinal Chemistry, Auf der Morgenstelle 8, 72076 Tíbingen, Germany

The discovery of EGFR L858R and del19 activating mutations in non-small cell lung cancer patients intensified the change of thinking towards personalized tumor therapy. “Oncogene addiction” to the EGFR signalling pathway paved the way for the development of the small molecules erlotinib and gefitinib as mutant selective, first generation tyrosine kinase inhibitors. Initial good results were overshadowed by imminent resistance development mainly via the gatekeeper point mutation T790M.Rational efforts in drug design finally led to irreversible third generation, mutant selective EGFR inhibitors with promising results in patient with acquired T790M mutations that became resistant to first generation TKIs. Recently, a third point mutation C797S was discovered in the cancer tissue of patients. This particular mutation renders irreversible bond formation with the cysteine impossible. Thus this acquired mutation leads to resistance to the actual gold standard osimertinib (FDA-approved 2015).

Beside the development of potent allosteric inhibitors, a target hopping approach from pyridinylimidazole-based p38 MAPK inhibitors to EGFR inhibitors led to trisubstituted imidazoles as structural novel class of EGFR-inhibitors. The approach yielded very potent reversible and irreversible inhibitors of the EGFR L858R, L858R/T790M and L858R/T790M/C797S mutants with submicromolar IC50s. These compounds show apart from a covalent binding mode to the double mutant additional noncovalent binding properties at the triple mutant. Furthermore, high cellular as well as wild type sparing activity (comparable to osimertinib) in L858R/T790M mutant cancer cell lines, good kinome selectivity profile and metabolic stability could be achieved. Example compound shows IC50 (EGFR-L858R/T790M) = < 0.5 nM and EGFRL858R/T790M/C797S down to 6 nM. Cellular EC50 value reaches down to 6 nM in a double mutant L858R/T790M cell line. In sum, this new class of EGFR inhibitors together with this rational approach to inhibit EGFR L858R/T790M/C797S may stimulate the development of either improved trisubstituted imidazoles as candidates or probes.In addition the design approach might be transfered to other structural classes of EGFR inhibitors.

References

1) Angew. Chem. Int. Ed. 2016, 55, 10890 –10894,
2) J. Med. Chem. 2017, 60, 4636–4656 and J. Med. Chem. 2017, 60 (13), 5613-5637

DESIGN AND RATIONALE FOR EXQUISITE SELECTIVITY OF PRECLINICAL AND CLINICAL KINASE INHIBITORS

Laurent Schio

Sanofi, Vitry/Seine, France

It is estimated that 5% of the human genome is dedicated to protein phosphorylation; a common biological process involved in normal cellular pathways and cascades. However kinases activity has been reported to be deregulated in a broad range of diseases (Parkinson, inflammation, cancers…) which has triggered over the last two decades a massive interest in identifying and developing selective kinase inhibitors.

Cancer treatment is definitively the most important therapeutic area where kinase inhibitors have found their application and about 30 synthetic small molecular drugs have received marketing approval, on top of a handful of monoclonal antibodies. The development of the targeted therapy paradigm in Oncology as well as the clinical demand for much better tolerated treatments have conducted research towards the discovery of selective to exquisite kinase inhibitors.

Few years ago, a chemogenetic study[1] based on kinase sequence similarity and structure Structure-Activity relationships (SARs) analysis underlined 16 privileged residues in the active site that are recurrently involved in kinase protein stabilization and ligand binding. Interestingly, this analysis pointed out the importance of residues which play a role in kinase conformation regulation (active vs inactive) and which can serve as levers for selectivity optimization of inhibitors.

It was originally thought that DFG-out conformation related inhibitors would be more selective than those targeting active conformations as this inactive conformation has not been observed by X-ray studies across the all kinome. This selectivity trend has not been eventually confirmed but αC helix-out conformation related inhibitors (e.g. MEK and HER inhibitors) revealed exquisite selectivity profiles but potentially at the expense of potency against oncogenic mutants.

We will describe in this communication what were the medicinal chemistry and in silico drug design strategies undertaken in our group to identify and develop selective kinase inhibitors for three oncology projects,leveraging interactions with specific protein residues, active and inactive conformations and stable/unstable water molecules in the ATP cleft. [2-5]

References

1) Martin E, Mukherjee P, J. Chem. Inf. Model., 2012, 52:156-170
2) Pasquier B, El-Ahmad Y, Filoche-Rommé B et al, J. Med. Chem., 2015, 58: 376−400
3) Carry JC, Clerc F, Minoux H et al, J. Med. Chem., 2015, 58: 362−375
4) Certal V, Carry JC, Frank Halley F et al, J. Med. Chem., 2014, 57: 903−920
5) Robinson D, Bertrand T, Carry JC et al, J. Chem. Inf. Model., 2016, 56: 886−894

CHEMICAL PROBES FOR NEW THERAPEUTIC CONCEPT DISCOVERY

Peter Ettmayer (1), Andreas Bergner (1), Harald Engelhardt (1), Andreas Gollner (1), Renate Schnitzer (1), Florian Montel (2)

1) Boehringer Ingelheim RCV GmbH & Co KG
2) Boehringer Ingelheim, Biberach

Protein kinases are highly tractable targets for drug discovery. However, the biological function and therapeutic potential of 80% of the 500+ protein kinases in the human kinome remain poorly studied. There is a strong need for more selective molecular probes to aid the discovery of new therapeutic concepts. Boehringer-Ingelheim recently launched opnMe, an open innovation portal providing access to Boehringer Ingelheim's molecule library for open sharing and collaboration to the benefit of drug discovery. Most of the well-characterized pre-clinical chemical and biological compounds can be ordered for free without entering into intellectual property negotiations. This contribution will focus on the discovery of Boehringer-Ingelheim´s CDK8 inhibitor that is available on opnMe and illustrates how kinase selectivity considerations were utilized for hit finding as well as for mining the corporate database for the donation of 106 compounds for the kinase chemogenomic set (KCGS). This Structural Genomic Consortium (SGC) initated publicly available collection of (fully annotated)selective kinase inhibitors, which covers more than half the human kinome, will aid the prioritization of chemical probes for understudied kinases.

https://opnme.com/home

David H. Drewry et al., Progress towards a public chemogenomic set for protein kinases and a call for contributions, PLoS ONE 12(8): e0181585

DISCOVERY OF THE TYK2 SELECTIVE INHIBITOR PF-6826647 FOR THE TREATMENT OF CROHN'S DISEASE, AND OTHER AUTOIMMUNE CONDITIONS

Brian S Gerstenberger (1), Mary Ellen Banker (2), Matthew F Brown (2), James D Clark (3), Dowty Martin (1), Fensome Andrew (1), Matthew M Hayward (2), Martin Hegen (3), Brett D Hollingshead (4), John D Knafels (2), David W Lin (2), Tsung H Lin (4), Dafydd Owen (1), Eddine Saiah (1), Raman Sharma (2), Felix Vajdos (2), Fabien Vincent (2), Wright Stephen (2), Li H Xing (1), Xiaojing Yang (2), Xin Yang (2), Liying Zhang (1)

1) Medicine Design, Pfizer Inc, 1 Portland Street, Cambridge, MA 02139
2) Medicine Design, Pfizer Inc, Eastern Point Road, Groton, CT 06340
3) Inflammation and Immunology, Pfizer Inc, 1 Portland St, Cambridge, MA 02139
4) Drug Safety and Metabolism, Pfizer Inc, 1 Portland Street, Cambridge, MA 02139

Tyrosine kinase 2 (TYK2) is one of the four members of the Janus (JAK) family of kinases, which also includes JAK1, JAK2 and JAK3. JAK kinase hetero-, and to a lesser extent homo, pairs play a key role in signal transduction of cytokines which signal through the JAK- Signal and Transduction of Transcription (STAT) pathway. TYK2 is important in IL-23 and IL-12 signaling where it pairs with JAK2, and Type I interferon signaling where it pairs with JAK1. Genome-wide association studies have associated TYK2 loss of function loci with a number of auto-immune diseases including Crohn's disease, ulcerative colitis, psoriasis, systemic lupus erythematosus, and, rheumatoid arthritis. The pharmacological profile of a selective TYK2 kinase inhibitor provides an opportunity to address a number of auto-immune diseases with a differentiated profile.

In this presentation we describe the discovery of an ATP competitive pyrazolopyrazinyl series of selective TYK2 inhibitors. The target profile and balanced selectivity against JAK2, was established through understanding of PK/PD relationships developed from our clinical experience. Through a structurally enabled program a scaffold hopping effort lead to several Type 1 kinase hinge designs and a preferred lead template. Further potency optimization involved leveraging P-loop engagement and simulated water thermodynamics. ADME properties in general in the program were favorable, however issues with non-P450 clearance pathways were identified, and a successful solution will be described. This effort led to the identification of PF-06826647 a potent and selective inhibitor of TYK2 which is currently in Phase 1 clinical studies.

DEVELOPMENT OF POTENT, SELECTIVE, CNS PENETRANT SMALL MOLECULE INHIBITORS OF NOTUM TO POTENTIATE WNT SIGNALING FOR THE MAINTAINANCE OF SYNAPTIC FUNCTION IN ALZHEIMER'S DISEASE

William Mahy (1), Sarah Jolly (1), Yuguang Zhao (2), Nicky Willis (1), Hannah Woodward (1), Benjamin Atkinson (1), David Steadman (1), Elliott Bayle (1), James Sipthorp (1), Fiona Jeganathan (1), Artur Costa (1), Stefan Constantinou (1), Georgie Lines (1), Magda Bictash (1), Jamie Bilsland (1,3), Reinis Ruza (2), Luca Vecchia (2), Laura Schuhmacher (4,5), Patricia Salinas (4), J.P. Vincent (5), E. Yvonne Jones (2), Paul Whiting (1,3), Paul V. Fish (1,5)

1) ARUK UCL Drug Discovery Institute, London UK
2) University of Oxford, Oxford UK
3) Dementia Research Institute at UCL, London UK
4) UCL, London UK
5) The Crick Institute, London UK

The Wnt signaling pathway has been shown to regulate crucial aspects of cell fate determination, organogenesis, cell migration and polarity.[1] Importantly, compromised Wnt signaling has been implicated in the perturbation of synaptic integrity and function in Alzheimer's disease (AD).[2]

Palmitoleoylation of Wnt proteins is required for efficient binding to Frizzled receptors and the subsequent signal transduction. The carboxylesterase Notum has been shown to act as a key negative regulator of the Wnt signaling pathway in Drosophila by specifically mediating the depalmitoleoylation of Wnt proteins.[3,4] Notum is expressed in the mammalian central nervous system (CNS): Notum is upregulated at mRNA level in whole brain lysates in AD model (APP-PS1 mice) and upregulated in human AD patient brain samples. We are currently investigating the role of Notum in modulating Wnt signaling in the CNS. We propose that inhibition of Notum could prolong Wnt signaling, with potential beneficial effects to neuronal health in AD.

To identify Notum inhibitors,[5] a fragment library screening approach was performed using crystal soaking X-ray crystallography. A robust primary fluorescence assay has been developed for the characterisation of Notum inhibitors, along with a secondary, more biologically relevant, native substrate assay.

A number of novel fragment hits were identified as Notum inhibitors with micromolar affinity (1 uM to >1 mM). A rational, structure based drug design (SBDD) process was used to generate highly potent (<10 nM) inhibitors of Notum with good aqueous solubility, in vitro metabolic stability, cell permeability and CNS penetration in vivo. Our key goal is to develop a 'fit for purpose' Notum inhibitor to determine the role of this enzyme in modulating Wnt signaling in the mammalian CNS, and its potential as a therapeutic for AD. In addition, we will present our empirical-based learnings on the modulation of heterocycle acidic pKa to achieve satisfactory CNS penetration.

References

1) Organogenesis 2008, 4, 68-75
2) Neuron 2014, 84, 63–77.
3) Nature 2015, 519, 187-192.
4) Dev Cell. 2015, 32, 719–730.
5) For CNS restricted Notum inhibitors, see Bioorg. Med. Chem. Lett. 2016, 26, 1525-1528.

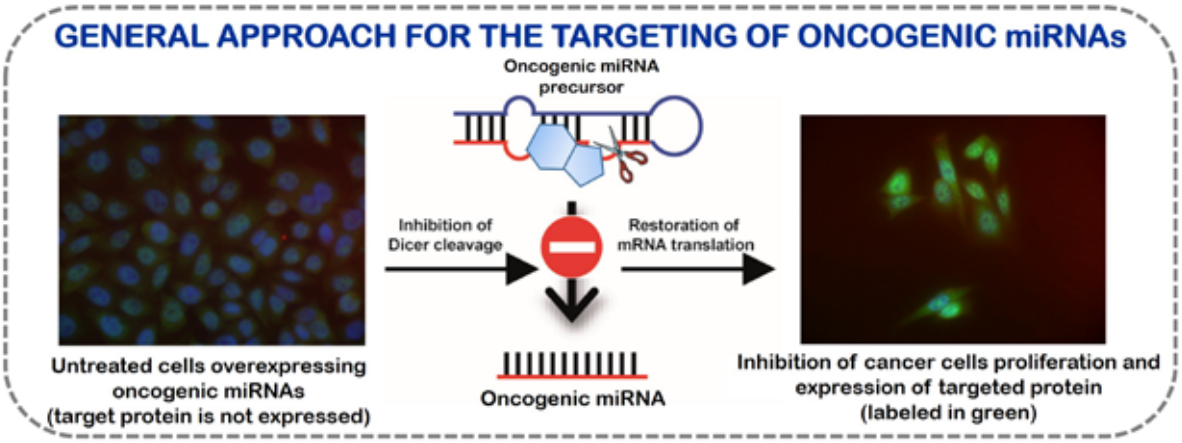
SYNTHETIC SMALL-MOLECULE RNA LIGANDS: SCOPE AND THERAPEUTIC APPLICATIONS

Cathy Staedel (1), Duc Duy Vo (2), Thi Phuong Anh Tran (2), Audrey Di Giorgio (2), Fabien Darfeuille (1), Maria Duca (2)

1) ARNA Laboratory, University of Bordeaux, CNRS UMR5320, INSERM U1212, 146 rue Léo Saignat, 33076 Bordeaux, France
2) Institute of Chemistry of Nice (ICN), Université Côte d'Azur, CNRS UMR7272, Parc Valrose 06100 Nice, France

MicroRNAs (miRNAs) are a recently discovered category of small RNA molecules that regulate gene expression at the post-transcriptional level. Accumulating evidence indicates that miRNAs are aberrantly expressed in a variety of human cancers, thus being oncogenic and that the inhibition of oncogenic miRNAs (defined as the blocking of miRNAs' production or function) would find application in the therapy of different types of cancer in which these miRNAs are implicated (1).

Our work aims at the development of small-molecule drugs targeting specific oncogenic miRNAs production as illustrated in *Figure 1A* (2). Toward this aim, we perform both the synthesis of new RNA ligands (*Figure 1B*) and the screening of compounds libraries (*Figure 1C*). Both approaches are based on a high throughput *in vitro* assays and demonstrated to be successful in identifying compounds able to interfere with the biogenesis of oncogenic miRNAs in a selective manner at the intracellular level. Thanks to these works, we demonstrated that it is possible to inhibit miRNAs production using synthetic small molecules and that this kind of approach could be applied in future anticancer therapies. Noteworthy, these RNA ligands could find extremely important applications as chemical biology tools for the improvement of our understanding of miRNAs biological pathways.



References

1) Ling H., Fabbri M., Calin, G.A. Nature Rev. Drug. Disc. 2013 12, 847; Velagapudi S.P., Vummidi B.R., Disney M.D. Curr. Op. Chem. Biol. 2015 24, 97.
2) Staedel, C., Tran, T.P.A., Giraud, J., Darfeuille, F., Di Giorgio, A., Tourasse, N.J., Salin, F., Uriac, P., Duca, M. Scientific Reports 2018 8, 1667; Vo, D.D., Duca, M. Methods Mol. Biol. 2017 1517, 137; Di Giorgio, A., Tran, T.P.A., Duca, M. Future Med. Chem. 2016 8, 803; Vo, D.D., Tran, T.P.A. Staedel, C., Benhida, R., Darfeuille, F., Di Giorgio, A., Duca, M. Chem. Eur. J. 2016 22, 5350; Tran T.P.A., Vo D.D., Di Giorgio A., Duca M. Bioorg. Med. Chem. 2015 23, 5344; Vo D.D., Staedel C., Zehnacker L., Benhida R., Darfeuille F., Duca M. ACS Chem. Biol. 2014 9, 711.

DISCOVERY OF FIRST-IN CLASS, SELECTIVE AND NONCOVALENT SMALL MOLECULE INHIBITORS OF DNMT1

Allan Jordan

Cancer Research UK Manchester Institute, University of Manchester, Alderley Park, Manchester, U.K., SK10 4TG

Aberrant DNA hypermethylation within gene promoter regions and subsequent gene silencing are near universal hallmarks of human cancer. Upon DNA replication, these methylation profiles are copied onto the newly-synthesized DNA strand by DNA methytransferase 1 (DNMT1), ensuring heritability of the epigenetic profile upon cell division.

Reversal of DNA methyl marks by a hypomethylating agent such as decitabine, delivers clinical benefit for the treatment of cancers such as acute myeloid leukemia. However, such agents have considerable drawbacks, including IV administration, poor PK properties, lack of selectivity and a mechanism that requires incorporation into replicating DNA, all of which limit their therapeutic benefit. This indirect, irreversible inhibition of the entire DNMT family (DNMT1, 3a and 3b), and subsequent DNA damage, induces significant dose-limiting toxicity, preventing sufficient target engagement required for maximal demethylation and limiting therapeutic utility. As a result, the past few decades have seen considerable interest in the development of potent, selective DNMT1 inhibitors. However, these attempts have been fraught with difficulty and have delivered little, if any, success.

Through an innovative industry / not-for-profit collaborative drug discovery program, we have successfully delivered agents which overcome many of these limitations. An extensive high-throughput screen and robust screening cascade development revealed just a single molecule which was found to be non-DNA incorporating and highly selective for DNMT1 over DNMT3a or DNMT3b. Structure-activity relationship (SAR) optimization of the series led to the discovery of potent tool compounds that induced robust decreases in global DNA methylation in cancer cells, induced transcriptional activation of many silenced genes, and inhibited cancer cell growth. In vivo investigations with these agents demonstrated appreciable exposure, decreased DNA methylation and a dose-dependent decrease in tumor growth with regression at well-tolerated doses, without the toxicity observed with decitabine.

This presentation will describe our work in this area, detailing the challenges faced along the way and sharing our learning as to how these were overcome.

ASTX660, THE FIRST FRAGMENT-DERIVED IAP ANTAGONIST IN THE CLINIC

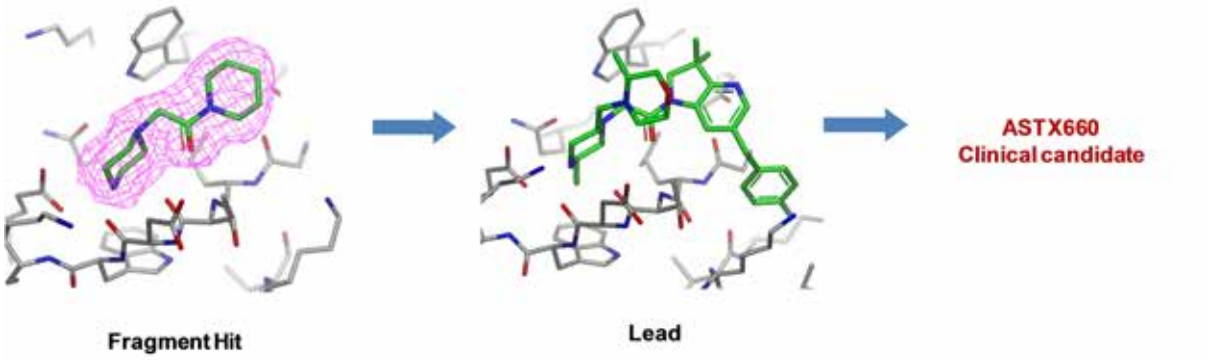
Steven Howard

on behalf of the IAP project team at Astex Pharmaceuticals, Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, CB4 0QA, Cambridge, UK, Steven.howard@astx.com

The Inhibitor of Apoptosis Proteins (IAPs) are key regulators of anti-apoptotic and pro-survival signalling pathways. Overexpression of IAPs occurs in various cancers and has been associated with tumour progression and resistance to treatment.

Astex has successfully applied fragment based drug discovery (FBDD) to develop clinical candidate ASTX660, a non-peptidic, potent antagonist of both cellular inhibitor of apoptosis and X-linked inhibitor of apoptosis proteins (cIAP and XIAP respectively). This profile provides ASTX660 with a different pharmacological profile compared to previously reported, alanine-based, peptidomimetic antagonists.

Using our fragment screening approach, Pyramid™, low molecular weight, non-peptidic fragments hits were identified which bind with millimolar affinities to both cIAP1 and XIAP. Structure based hit optimisation, guided by X-ray crystallography, together with computational studies and NMR solution conformational analysis, led to the identification of potent (nM) lead molecules. Subsequent lead optimization focused on reducing off-target activity and improving pharmacokinetic properties. This resulted in ASTX660, a potent non-peptidic IAP antagonist which is structurally distinct from all previously reported IAP antagonists in the clinic. ASTX660 is currently being tested in a phase I/II clinical trial (NCT02503423) and we propose that its unique profile may offer improved efficacy over more cIAP selective antagonists. Chemical structures of all compounds (incl ASTX660) will be shown.

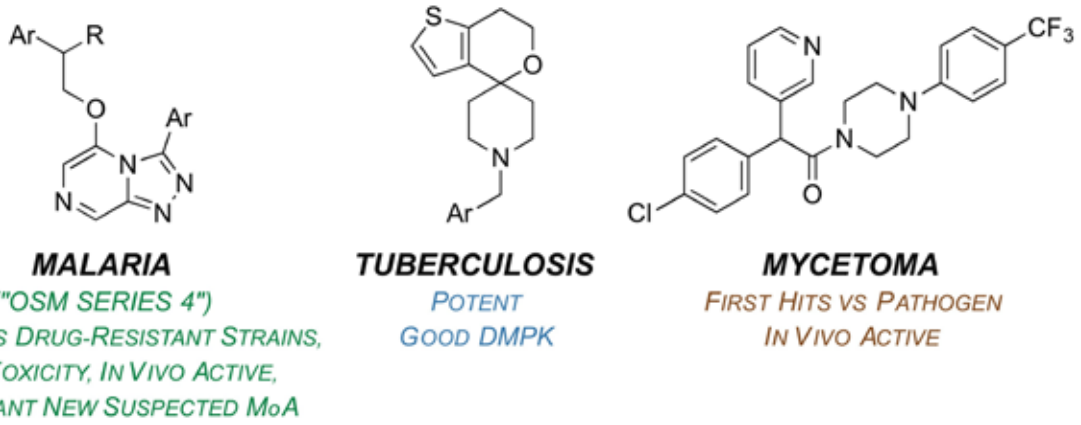


ALL BUGS ARE SHALLOW: OPEN SOURCE DRUG DISCOVERY

Matthew Todd

School of Chemistry, The University of Sydney, Sydney NSW 2006, Australia.
Email: matthew.todd@sydney.edu.au; @mattoddchem

The open sharing of research is being increasingly recognised as a driver of innovation in biomedical research. Many funding agencies now mandate the open sharing of project-related data, and most pharmaceutical companies are engaged in substantial "open innovation" programs. We have demonstrated the logical extension of such ideas to "open source" drug discovery in which all data and ideas are shared in real time, anyone can participate and no patent protection is sought. The pilot project involved the synthesis of an enantiopure version of the world's most widely used anthelmintic, praziquantel.[1] With the Medicines for Malaria Venture (MMV) we have more recently demonstrated successful execution of hit-to-lead projects in the Open Source Malaria Consortium, to which scientists have contributed from the private and public sectors, ranging from academics to school students.[2] Highly promising series are now being explored for the potential treatment of tuberculosis and mycetoma, the latter as part of the newly-announced MycetOS consortium with the Drugs for Neglected Diseases Initiative (DNDi).[3] We have recently argued for the expansion of such an approach to full-scale drug development as a competing model for the traditional pharmaceutical industry,[4] and this will be outlined.



References

1) Open Science is a Research Accelerator, M. Woelfle, P. Olliaro and M. H. Todd, Nature Chem. 2011, 3, 745–748 (10.1038/nchem.1149).
2) Open Source Drug Discovery: Highly Potent Antimalarial Compounds Derived from the Tres Cantos Arylpyrroles, A. E. Williamson, et al. ACS Cent. Sci. 2016, 2, 687–701 (10.1021/acscentsci.6b00086).
3) Project website at https://github.com/OpenSourceMycetoma, see also the preprint available at https://www.biorxiv.org/content/early/2018/02/02/258905.
4) An Open Source Pharma Roadmap, M. Balasegaram, P. Kolb, J. McKew, J. Menon, P. Olliaro, T. Sablinski, Z. Thomas, M. H. Todd, E. Torreele and J. Wilbanks, PLoS Med. 2017, 14(4), e1002276 (DOI: 10.1371/journal.pmed.1002276).

TOWARDS THE DEVELOPMENT OF NOVEL INHIBITORS FOR CHIKUNGUNYA VIRUS INFECTION: APPROACHES IN STRUCTURE-ACTIVITY-METABOLISM RELATIONSHIP (SAMR) STUDIES

Kuan Chieh Ching (1), Quy T.N. Tran (1), Lisa F.P. Ng (2), Christina L.L. Chai (1)

1) Department of Pharmacy, Faculty of Science, National University of Singapore, Block S4A, Level 3, 18 Science Drive 4, Singapore 117543
*2) Singapore Immunology Network, A*STAR, 8A Biomedical Grove, Immunos Building, Level 4, Singapore 13864*

The re-emergence, on the global scale, of lesser known arbovirus infectious diseases such as Zika and Chikungunya virus (CHIKV) infections has taken the world by surprise. The lack of approved anti-virals for the treatment of these diseases has meant that the most effective method for the prevention of infection is through controlling the spread of virus borne vectors. Thus there is an urgent need to investigate and develop anti-viral ''cures'' for these diseases, which would retard and prevent the spread of the viruses. Our studies are specifically focused on Chikungunya virus infection in view of the rapid spread of this virus in South East Asia. In this paper, we report our approaches to the optimization of lead compounds **1a** and **1b** which have promising anti-viral activities but very short metabolic half-lives. Through our studies, we identified compounds such as **2c** and **2d** that inhibit CHIKV replication in the low micromolar range. In a preliminary pharmacokinetic study using human liver microsomes, compound **2c** showed significant improvement in in vitro metabolic stability, with a half-life of 28 min. These results represent a substantial advancement in the early preclinical development of a new class of novel antiviral drugs against Chikungunya infection.

1a: R = H
1b: R=Br

2c: R = H
2d: R=Br

IDENTIFICATION OF NEW ANTIMALARIAL GSK607: AN EXAMPLE OF ADAPTIVE AND DIFFERENTIATED EARLY DRUG DEVELOPMENT

Esther Fernandez

GlaxoSmithKline, Diseases of the Developing World (DDW), Malaria DPU
Severo Ochoa 2, 28760 Tres Cantos, Spain

Malaria remains a major global health problem. In 2016 alone, 216 million cases of malaria were reported, and more than 400,000 deaths occurred. Since 2010, emerging resistance to current front-line ACTs (Artemisinin Combination Therapies) has been detected in endemic countries. Therefore, there is an urgency for new therapies based on novel modes of action, able to relieve symptoms as fast as the artemisinins and/or block malaria transmission. During the past few years, the antimalarial community has focused their efforts on phenotypic screening as a pragmatic approach to identify new hits.

Quinazolindione series was identified as a very promising family with dual activity (both schizonticidal and gametocytocidal) from our published phenotypic set of antimalarial hits (TCAMS). Initial weaknesses of the series were modest *in vitro* and *in vivo* potency as well as poor pharmacokinetic profile (low oral bioavailability and high clearance). After a Lead Optimisation program, GSK607 was identified having excellent *in vitro* and *in vivo* potency as well a very good developability. In addition, *Pf*ATP4 pathway implicated in regulation of *P. falciparum* sodium homeostasis was identified as the potential target. However, a key problem encountered during the preclinical evaluation of GSK607 was its inconsistent PK profile across preclinical species (mouse, rat and dog) which prevented reliable prediction of PK parameters in humans and precluded a well-founded assessment of the potential for clinical development of the compound. Therefore, an open label microdose FTIH study was conducted in order to assess the human pharmacokinetics of GSK607.

A detailed description of the Medicinal Chemistry identification and development of GSK607 as well as its progression to a human microdose study will be provided in the talk.

MECHANISMS OF HIV-1 NUCLEOCAPSID PROTEIN INHIBITION BY SMALL MOLECULES TARGETING RNA

Alice Sosic (1), Francesco Frecentese (2), Caterina Carraro (1), Giuseppe Caliendo (2), Vincenzo Santagada (2), Richard Goettlich (3), Dan Fabris (4), Barbara Gatto (1)

1) Dipartimento di Scienze del Farmaco, Università di Padova, via Marzolo 5, 35131 Padova, Italy
2) Dipartimento di Farmacia, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131 Napoli, Italy
3) Institute of Organic Chemistry, Justus Liebig University Giessen, Germany
4) The RNA Institute and Department of Chemistry, State University of New York, 1400 Washington Avenue, Albany, New York 12222, United States

Human Immunodeficiency Virus (HIV) infection remains of major public health importance in Europe as well as in the developing countries. Most of the commonly available drugs, although potent and selective, experienced clinical failures and severe side effects. Alternative antiretroviral drugs and novel therapeutic strategies are thus urgently needed to overcome the emergence of resistance to existing drugs.

The HIV-1 nucleocapsid (NC) protein is a nucleic acid chaperone playing a pivotal role in essential steps of the viral life cycle and represents an excellent molecular target for drug development. Even if different classes of molecules have been proposed as anti-NC agents, drug-candidates interfering with NC functions are still missing in the therapeutic arsenal against HIV.

By searching for new NC inhibitors, we identified 2,6-dipeptidyl-anthraquinones as a promising class of nucleic acid-binding compounds able to interfere with NC activities. Exploring the structure-activity relationship of related series of 2,6-dipeptydil-anthraquinones differing in the aminoacyl linkers, we identified the key structural requirements necessary for the development of potent in vitro NC inhibitors and demonstrated their molecular mechanisms of NC inhibition. Seeking to increase the potency of this class of compounds, we have explored the effects of chirality in the linker connecting the planar nucleus to the basic side chains. We showed that the non-natural linker configuration imparted unexpected RNA targeting properties to the 2,6-peptidyl-anthraquinones and significantly enhanced their NC inhibition in vitro in the micro molar range.

The success in this particular research field motivated us to continue our research in this direction and we recently proposed a new strategy to impair NC-mediated processes, consisting in the employment of bis-3-chloropiperidines (BICEPS) as RNA cross-linking agents. Binding modes have been widely investigated by electrospray ionization mass spectrometry (ESI-MS) analysis and we unambiguously elucidated at the molecular level the reaction of BICEPS with RNA. BICEPS covalently bind to viral RNA sequences substrates of NC protein and are able to freeze their tridimensional conformations, thus impairing the NC protein activities *in vitro*.

UNRAVELLING THE MYSTERIES OF THE SPHINX: NOVEL TARGETS AND SMALL-MOLECULE THERAPEUTICS FROM THE SPHINGOLIPID SYNTHESIS AND SIGNALING PATHWAY

Bernie Flynn

Monash Institute of Pharmaceutical Science, Monash University, 381 Royal Pde, Parkville, VIC, 3052

Sphingolipids were first identified over 140 years ago and were named after the mythological Sphinx in light of their enigmatic nature. Much of this enigma still remains, for despite their intimate involvement in a range of pathophysiological processes few effective drug agents have been identified. Our group is seeking to address this through a novel approach to phenotypic drug discovery using focused libraries (Fig 1).[1] By exploiting the consensus SAR of different drug-like modulators of the various enzymes and receptors to which sphingolipids bind we are generating a focussed library of non-lipid sphingolipid mimetics (NLSM). In-parallel screening of this library in phenotypic assays and in available target assays is used to discover novel bioactives. SAR-correlations between target and phenotypic effects are used to identify the responsible target(s). Lipidomic analysis and cell signalling studies further confirm the target and/or can help identify other potential targets for which we do not currently have an assay. This process has helped us identify new lead structures with anticancer and antifibrotic activity, these include both selective and multi-targeting modulators of sphingolipid synthesis and signaling.

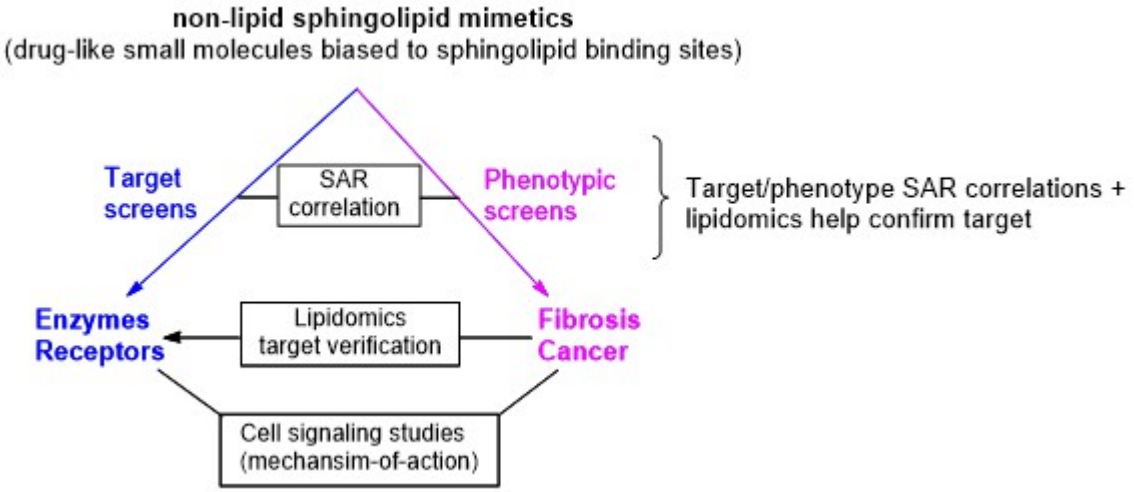


Figure 1: Phenotypic drug discovery based on non-lipid sphingolipid mimetics

References

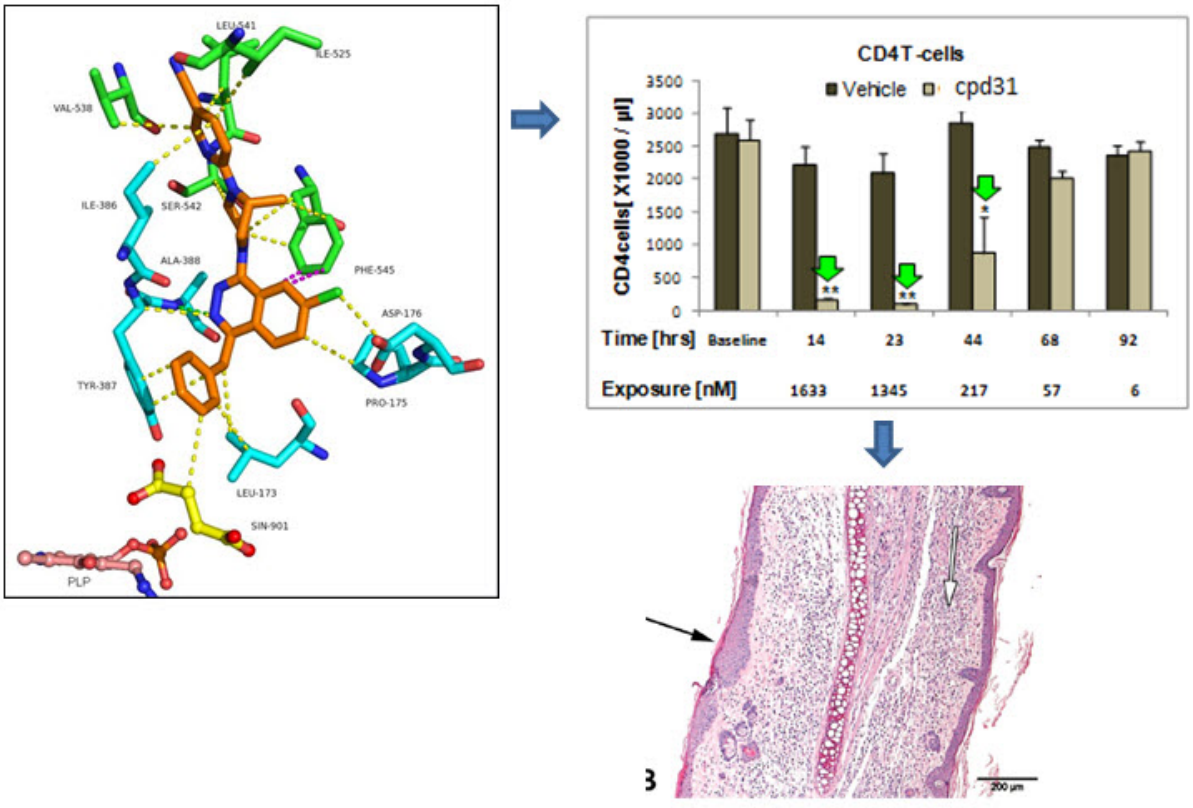
1) Aurelio, L.; Scullino, C. V.; Pitman, M. R.; Sexton, A.; Oliver, V.; Davies, L.; Rebello, R. J.; Furic, L.; Creek, D. J.; Pitson, S. M.; Flynn, B. L. J. Med. Chem. 2016, 59, 965-984.

ACTIVE SITE INHIBITORS OF SPHINGOSINE 1-PHOSPHATE LYASE - EXPLORING NOVEL BIOLOGY WITH TOOL COMPOUNDS

Sven Weiler (1), Nadine Braendlin (2), Christian Beerli (2), Christian Bergsdorf (2), Anna Schubart (2), Honnappa Srinivas (2), Andreas Billich (2), Jens Schümann (2), Luca Arista (2), René Beerli (2), Erika Loetscher (2), Thomas Knöpfel (2), Angela Mackay (2), Thomas Troxler (2), Anna Vulpetti (2), Alessandro Piaia (2), Armelle Grevot (2), David Ledieu (2), Pierre Moulin (2), Berndt Oberhauser (2)

1) Basilea Pharmaceutica International Ltd.; Basel, CH-4005, Switzerland
2) Novartis Institutes for BioMedical Research, Basel, CH-4002, Switzerland

Sphingosine 1-phosphate lyase (S1PL) catalyzes the irreversible retro-aldol reaction of S1P to 2-hexadecanal and phosphoethanolamine, with that tightly controlling intracellular S1P concentrations. The S1P gradient between various tissues promotes migration of T cells from secondary lymphoid organs into the lymphatic and blood circulation and an invasion of pathogenic T cells into the CNS. Disturbing this gradient has been a successful approach to treat Multiple Sclerosis with FTY720 (Gilenya) being approved by the FDA in 2010. FTY720 works as a functional S1P-receptor antagonist. In theory an inhibitor of S1PL should lead to the same positive effect, potentially with a different side effect profile. Earlier studies with inducible S1PL knockout mice looked promising and the objective was to verify those findings with an S1PL inhibitor as pharmacological tool and to establish an alternative treatment modality for MS. Functional inhibitors were known at the outset of our program; however those compounds do not directly bind to S1PL. We have therefore decided to search for active site inhibitors of S1PL. A High Throughput Screen with shortened human S1PL (1-61) yielded several hit classes. Structural biology helped to guide medicinal chemistry and to explain SAR. One hit class (phthalazines) looked particularly promising and here we describe the optimization of an early hit towards the first orally active direct S1PL inhibitor with efficacy in an experimental animal model of Multiple Sclerosis. This tool compound, together with the results from ko animals gave valuable insights not only to explore efficacy but also to understand toxicology findings seen in compound treated and ko animals.



CONTROLLING SPHINGOSINE-1-PHOSPHATE LEVELS AS A THERAPEUTIC STRATEGY

Webster Santos

900 West Campus Drive, Blacksburg, VA 24061, USA

Sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that acts as a ligand for five G-protein coupled receptors (S1P1-5) whose downstream effects are implicated in a variety of important pathologies. The role of S1P in lymphocyte trafficking is firmly established; indeed, an S1P1 receptor agonist has been approved by the FDA as an immunosuppressive therapy for multiple sclerosis. The synthesis of S1P is catalyzed by sphingosine kinase (SphK) isoforms 1 and 2, and hence, inhibitors of this phosphorylation step are pivotal in understanding the physiological functions of SphKs. In this presentation, we will discuss the development of in vivo probes to understand S1P-sphingosine kinase relationship function. Our studies demonstrate that SphK1 and 2 selective inhibitors decrease S1P levels in cultured cells. However, in vivo administration of SphK1 inhibitor depressed blood S1P levels while SphK2 inhibitor increased levels of S1P. The increased accumulation of S1P in the blood of SphK2 inhibitor treated mice appears to result from decreased clearance of S1P from blood; thus, SphK2 has a function beyond simply generating S1P in cells. Taken together, these compounds provide an in vivo chemical toolkit to interrogate the effect of increasing or decreasing S1P levels, and whether such a maneuver can have implications in disease states.

CYCLIC DINUCLEOTIDE STING AGONISTS AS ANTI-TUMOR AGENTS

B. Wesley Trotter

Merck & Co., Inc., Boston, MA, USA

Pharmacological activation of innate immune, danger-sensing pathways has recently emerged as a promising strategy for enhancing cancer immunotherapy. In particular, agonists of the Stimulator of Interferon Genes (STING) protein have demonstrated robust efficacy in syngeneic mouse tumor models via apparent generation of innate and adaptive anti-tumor immune responses as well as tumor-specific immune memory. The identification of an endogenous cyclic dinucleotide (CDN) agonist of human STING, 2',3'-cGAMP, has spurred interest in discovery of novel CDNs suitable for clinical investigation in cancer patients.

This presentation will detail efforts at MSD to elucidate structure-activity relationships in a synthetically complex cyclic dinucleotide structural class. Optimization of total synthesis routes to novel CDNs along with implementation of new synthetic strategies has enabled a survey of previously unexplored CDN scaffold modifications. Computational and biostructural methods have been applied to influence design of novel STING agonists. This work has resulted in the invention of a variety of potent, selective STING agonists.

Characterization of select molecules in mouse tumor models will be presented, including evidence of impressive anti-tumor effects in both single-agent treatment and combination treatment with an anti-PD1 antibody. In addition, we have demonstrated evidence for induction of anti-tumor immune memory in mouse models and have generated translational data in human tumor samples supporting advancement of STING agonists to the clinic.

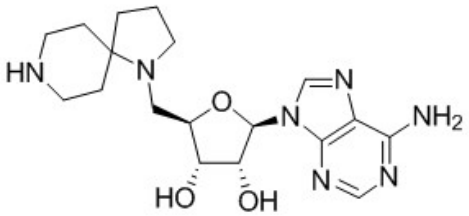
DISCOVERY OF JNJ-64619178 AS A POTENT AND SELECTIVE PRMT5 INHIBITOR FOR THE TREATMENT OF LUNG AND HEMATOLOGIC CANCERS

Jan Willem Thuring (1), Tongfei Wu (1), Dirk Brehmer (2), Weimei Sun (1), Vineet Pande (1), Geert Mannens (1), Petra Vinken (1), Lijs Beke (2), Didier Berthelot (1), Lieven Meerpoel (1), Marcel Viellevoye (1), Gaston Diels (1), Wim Schepens (1), Brian Shook (1), Edward C. Lawson (1), An Boeckx (2), Marc Parade (2), Tinne Verhulst (2), Hillary Millar (2), Ron Gilissen (1), Erika van Heerden (2), Sylvestre Dossou (1), Carol Yanovich (1), James P. Edwards (1), Matthew Lorenzi (2), Sylvie Laquerre (2)

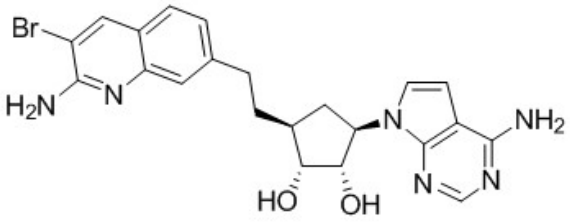
1) Discovery Sciences, Janssen Pharmaceutica Research & Development
2) Oncology Lung Disease Area Stronghold Drug Discovery, Janssen Pharmaceutica Research & Development

Protein arginine methyltransferase 5 (PRMT5) is an enzyme that can symmetrically di-methylate the guanidium moiety of arginine residues in histones and non-histone proteins by using S-adenosyl methionine (SAM) as the methyl donor. In complex with methylosome protein 50 (MEP50), PRMT5 regulates a plethora of cellular processes, such as epigenetics and splicing. Over-expression of PRMT5-MEP50 has been implicated in lung and hematologic cancers as well as in other diseases.[1]

In 2017, we reported JNJ-64619178 as a potent and selective PRMT5 inhibitor and a candidate for clinical development. We herein report some of the medicinal chemistry concepts that led to the discovery of JNJ-64619178.[2]



1



JNJ-64619178

To find inhibitors of the catalytic function of PRMT5 and related methyl transferases, we designed an exploratory library of SAM analogues as potential starting points for drug discovery efforts. From screening this library against PRMT5, compound **1** emerged as a selective and tractable SAM-competitive hit, which also inhibited the symmetric dimethylation of arginine in cells. However, this molecule and closely related analogues demonstrated poor oral bioavailability in rodents. Guided by X-ray crystallography, we designed new inhibitors to improve the enzymatic & cellular activity while also tuning the physicochemical properties to optimize for oral delivery. These efforts resulted in the discovery of JNJ-64619178, an orally bioavailable PRMT5 inhibitor that demonstrates robust tumor regression in a human small cell lung cancer mouse xenograft model.

References

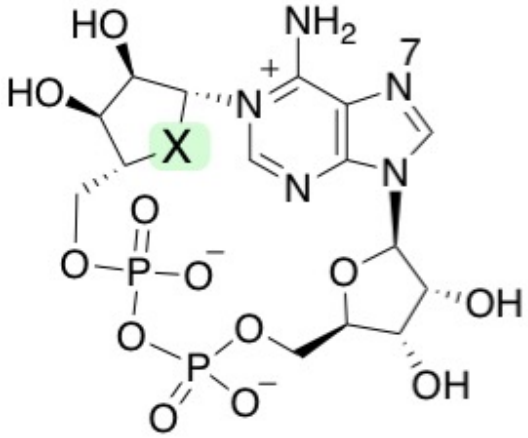
1) N. Stopa, J. E. Krebs, D. Shechter, Cellular and molecular life sciences : CMLS 2015, 72, 2041-2059
2) D. Brehmer, T. Wu, G. Mannens, L. Beke, P. Vinken, D. Gaffney, W. Sun, V. Pande, J.-W. Thuring, H. Millar, I. Poggesi, I. Somers, A. Boeckx, M. Parade, E. v. Heerde, T. Nys, C. Yanovich, B. Herkert, T. Verhulst, M. D. Jardin, L. Meerpoel, C. Moy, G. Diels, M. Viellevoye, W. Schepens, A. Poncelet, J. T. Linders, E. C. Lawson, J. P. Edwards, D. Chetty, S. Laquerre, M. V. Lorenzi, Cancer research 2017, 77, DDT02-04-DDT02-04

DESIGN AND SYNTHESIS OF CONGERNERS OF CYCLIC ADP-RIBOSE, A CA2+-MOBILIZING SECOND MESSENGER, TOWARD IDENTIFICATION OF THE TARGET PROTEIN

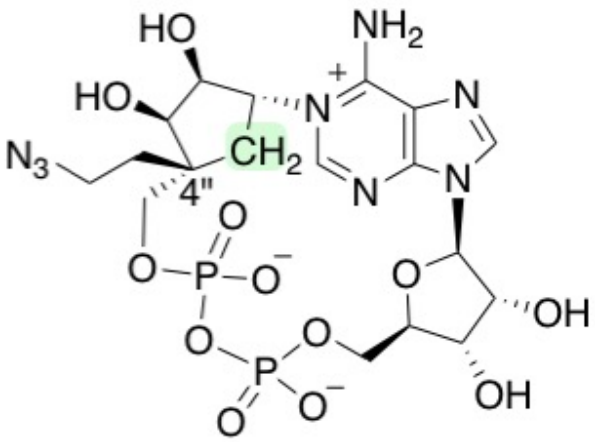
Satoshi Shuto

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Cyclic ADP-ribose (cADPR) is a chemically and biologically unstable Ca^{2+}-signaling mediator in with the characteristic 18-membered ring consisting of an adenine, two riboses and a pyrophosphate. We have worked on medicinal chemical study of cyclic ADP-ribose (cADPR) to develop stable agonists of cADPR, stable antagonists of cADPR, and biological probes for the identification of the proteins binding to cADPR. We have developed cADP-carbocyclic-ribose (cADPcR) and -4-thioribose (cADPtR) as satable equivalents of cADPR,[1,2] which, due to the stability, can be useful prototypes to design bilogical tools for investigating the cADPR-related signaling pathways. Using cADPcR as a prototype, N_3-cADPcR was developed as an effective precursor for synthesizing biological tools. I will present our recent results on cADPR-related studies.[3,4]



X = O: cADPR
X = CH_2: cADPcR
X = S: cADPtR



N_3-cADPcR

References

1) J. Am. Chem. Soc. 2005, 127, 8846-8855.
2) Angew. Chem. Int. Ed., 2013, 52, 6633-6637
3) J. Med. Chem. 2016, 59, 7282–7286.
4) J. Med. Chem. 2017, 60, 5868–5875.

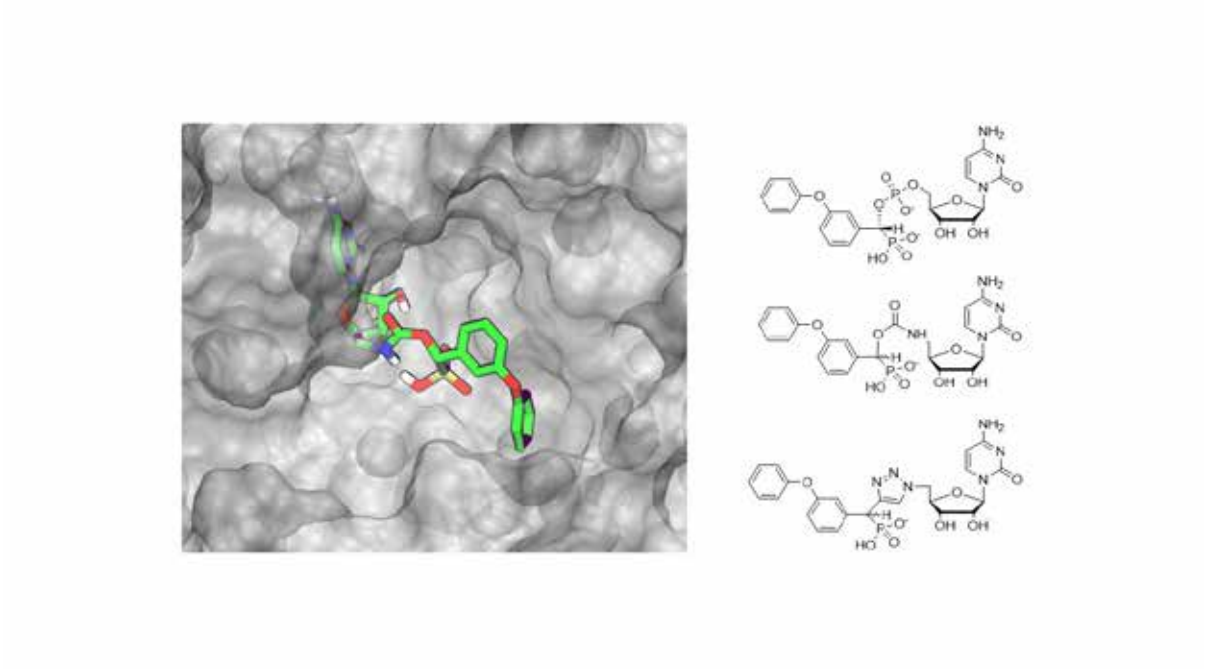
DEVELOPMENT OF NOVEL SIALYLTRANSFERASE INHIBITORS VIA COMPUTER AIDED DRUG DESIGN

Andrew Montgomery (1), Christopher Dobie (1), Laura Hallam (1), Remi Szabo (1), Haibo Yu (1,2), Danielle Skropeta (1,2)

1) School of Chemistry, University of Wollongong, NSW, Australia
2) Centre for Medical and Molecular Bioscience, University of Wollongong, Wollongong, NSW, Australia

Sialyltransferases (STs) catalyse the synthesis of sialylated glycoconjugates involved in cell-cell interactions. Overexpression of STs is observed in many different types of cancers and is thought to promote metastasis through altered sialylation patterns of the cell. A wide range of ST inhibitors have been developed based on the natural donor, CMP-Neu5Ac, as potential new antimetastatic agents[1]. To improve selectivity, pharmacokinetic properties and overall ease of synthesis of these inhibitors, we have investigated the replacement of the charged phosphodiester linker present in many ST inhibitors with a neutral isostere such as a carbamate or triazole, in a combined computational and experimental study.

Molecular docking, molecular dynamics simulations and binding free energy calculations have been undertaken with the human ST6Gal I crystal structure. These calculations have indicated that compounds containing either carbamate or triazole linkers can potentially bind to human ST6Gal I comparable to their phosphodiester-linked counterparts, suggesting that these linkers are suitable neutral isosteres[2,3]. These findings are surprising as the phosphodiester linker was previously believed to be important for binding. Further analyses has indicated that there is a strong enthalpy-entropy compensation contributing to these findings[4]. The synthetic component of this study, includes the preparation of required α-hydroxyphosphonate (αHP), alkyne and nucleoside synthons along with the successful coupling of these to generate the target compounds. Carbamate-linked targets were prepared by coupling αHP and nucleoside fragments using 4-nitrophenylchloroformate, while triazole-linked compounds were prepared from alkyne and nucleoside fragments using “click” chemistry. Overall this work has provided rationale for the replacement of the charged phosphodiester linker as well as readily scaleable synthetic pathways to access our target compounds. This facilitates further biological investigation of carbamate and triazole-linked ST inhibitors as potential new anti-metastatic agents.



References

1) R. Szabo and D. Skropeta, Medicinal Research Reviews, 37, 219-270 (2017).
2) A. Montgomery et al., Journal of Molecular Recognition, 29, 210-222 (2016).
3) A. P. Montgomery et al., Advances in Protein Chemistry and Structural Biology, Vol. 109, 25-76 (2017).
4) A. P. Montgomery et al., Scientific Reports, 7, 14428 (2017).

NONPEPTIDIC SELECTIVE INHIBITORS OF IMMUNOPROTEASOME

Izidor Sosič (1), Martina Gobec (1), Péter Ábrányi-Balogh (2), György M. Keserű (2), Irena Mlinarič Raščan (1), Stanislav Gobec (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
2) Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt 2, H-1117 Budapest, Hungary

The proteasome is an intracellular protease that represents a vital part of the ubiquitin-proteasome system. It degrades many proteins and has critical functions in several biological processes. The constitutive isoform (cCP) of the proteasome is expressed in all eukaryotic cells while its immunomodulatory isoform, the immunoproteasome (iCP) is mainly expressed in cells associated with the immune system. Notably, the expression of the iCP can be induced in non-immune tissues by pro-inflammatory cytokines. Dysregulation of the proteasomes is known to lead to the development of diverse diseases, such as malignancies, autoimmune and inflammatory diseases. The research shows that selective inhibition of the iCP has great potential as a novel approach for the treatment of inflammatory diseases and a wide range of autoimmune disorders [1]. So far, the known inhibitors of the iCP encompass mostly compounds of peptidic type that are prone to poor metabolic stability and low bioavailability [2].

In our research, we are focusing on the identification and development of non-peptidic compounds of both non-covalent and covalent nature that selectively inhibit the chymotrypsin-like (β5i) subunit of the iCP. Molecules of this type have several advantages; besides better stability it is also possible to cover greater chemical and property space, providing more medicinal chemistry options during their optimization. As our initial approach to develop non-peptidic inhibitors, we used virtual-screening and subsequent chemical optimization. Biochemical evaluation of reversibly and irreversibly acting compounds showed that these non-peptidic molecules selectively block the β5i subunit of the human iCP on cell lysates and on intact cells [3]. Our current efforts are devoted to further improvements of the described non-peptidic inhibitors of the iCP by using scaffold morphing and scaffold hopping approaches, as well as to discovering new non-peptidic scaffolds and electrophilic warheads via screening of libraries of both non-covalent fragments and electrophilic warheads.

References

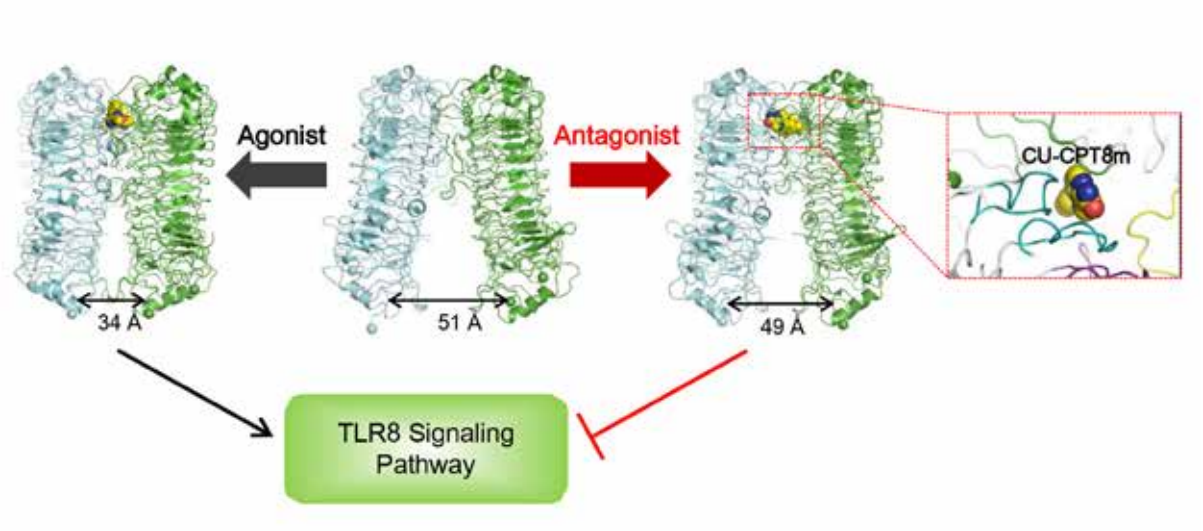
1) T. Muchamuel, et al. Nature Medicine 15, 781–787, (2009)
2) E. Ogorevc, et al. Expert Opinion on Therapeutic Patents, in press, (2018)
3) I. Sosič, et al. Angewandte Chemie International Edition 55, 5745–5748, (2016)

SMALL MOLECULE IMMUNOMODULATORS THAT TARGET TOLL-LIKE RECEPTORS

Hang Yin

Tsinghua University, Department of Chemistry,New Life Science Building 332, 100084 Beijing, China

Protein-protein and protein–nucleic acid interfaces have been regarded as “undruggable” despite their importance in many biological processes. The toll-like receptors (TLRs) provide exciting targets for a number of autoimmune diseases, infectious diseases, pain management, and cancers. Using multidisciplinary approaches, we have successfully developed novel exogenous small molecule probes that were shown to be competitive inhibitors or activators of various TLRs with high affinity and specificity. Some of the lead compounds are currently in the pipeline for further drug discovery.



References

1) Zhang, S.; Hu, Z.; Tanji, H.; Jiang, S.; Das, N.; Li, J.; Sakaniwa, K.; Jin, J.; Bian, Y.; Ohto, U.; Shimizu, T.; *Yin, H.; “Small Molecule Inhibition of TLR8 through Stabilization of Its Resting State”, Nat. Chem. Biol. 2018, 14, 58-64.
2) Das, N.; Dewan, V.; Grace, P. M.; Gunn, R. J.; Tamura, R.; Tzarum, N.; Watkins, L. R.; Wilson, I. A.; *Yin, H.; “HMGB-1 Activates Proinflammatory Signaling via TLR5 Leading to Allodynia”, Cell Rep. 2016, 17, 1128-1140.
3) Cheng, K.; Gao, M.; Godfroy, J. I. III; Brown, P. N.; Kastelowitz, N.; *Yin, H.; “Specific Activation of the TLR1-TLR2 Heterodimer by Small-molecule Agonists”, Sci. Adv. 2015, 1, e1400139.
4) Cheng, K.; Wang, X. H.; Zhang, S.; *Yin, H.; “Discovery of Small-Molecules that Inhibit the Protein Complex of Toll-Like Receptor 1 and 2”, Angew. Chem. Int. Ed. 2012, 51, 12246-12249.
5) Wang, X. H.; Loram, L. C.; Ramos, K.; de Jesus, A.; Thomas, J.; Cheng, K.; Reddy, A.; Somogyi, A. A.; Hutchinson, M. R.; Watkins, L. R.; *Yin, H.; “Morphine Activates Neuroinflammation in a Parallel Manner to Endotoxin”, Proc. Natl. Acad. Sci. U. S. A. 2012, 109, 6325-6330.

A DUAL MODULATOR OF FARNESOID X RECEPTOR AND SOLUBLE EPOXIDE HYDROLASE TO TREAT NON-ALCOHOLIC STEATOHEPATITIS

Daniel Merk

ETH Zurich, Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, Vladimir-Prelog-Weg 4, CH-8093 Zürich, Switzerland

Non-alcoholic steatohepatitis (NASH) arising from western diet and lifestyle evolves as serious health burden with alarming incidence.[1] Characterized by accumulation of fat in liver subsequently causing inflammation and fibrosis, NASH is strongly associated with the metabolic syndrome.[1] Although its high prevalence elicited intensive research for novel treatment options, there is still no satisfying therapy.[2] Several potential targets were identified for NASH treatment and promising clinical data has been reported for the farnesoid X receptor (FXR) agonist obeticholic acid.[3] The nuclear receptor FXR acts as cellular bile acid sensor whose activation amongst many other beneficial metabolic effects reduces liver fat content and fibrosis.[4] Moreover, inhibition of soluble epoxide hydrolase (sEH)[5,6] proved effective in treating NASH in vivo. As enzyme of the arachidonic acid cascade located in the CYP pathway, sEH degrades anti-inflammatory epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs) and is highly expressed in liver. Its inhibition hindering EET degradation causes anti-inflammatory effects.[6] Considering the multifactorial nature of NASH involving metabolic dysbalance and inflammatory processes, modulation of multiple targets might provide superior therapeutic efficacy and combining anti-steatotic/-fibrotic FXR activation with anti-inflammatory inhibition of sEH promises synergistic activity.

To exploit the concept of dual FXR/sEH modulation for NASH treatment, we have developed dual agents comprising FXR agonistic and sEH inhibitory potency. Initially, we merged known pharmacophores[7,8] for both targets to generate a lead exhibiting moderate FXR activation and sEH inhibition. Systematic exploration of the compound class' structure-activity relationship (SAR) on both targets allowed optimizing the dual potency on FXR and sEH to low nanomolar values. Using all gathered SAR information, we finally designed a dual modulator with well-balanced single-digit nanomolar potency on both targets.[9]

Extensive in vitro evaluation of the dual modulator revealed marked FXR target gene induction accompanied by robust inhibition of sEH activity in hepatocytes. The compound turned out non-toxic and extraordinarily selective amongst nuclear receptors as well as the membrane bile acid receptor TGR5. Moreover, compared to FXR agonists and sEH inhibitors alone, the dual modulator possessed superior anti-inflammatory activity. A pilot in vivo study confirmed this encouraging data with a favorable pharmacokinetic profile and engagement on both targets in vivo. In animal disease models of NASH, the dual modulator exhibited therapeutic efficacy by preventing hepatic fat accumulation and liver fibrosis with improved NAFLD activity score and a liver histology indistinguishable from healthy mice.

In summary, we have developed a first-in-class dual FXR agonist/sEH inhibitor with high potency and favorable in vitro profile as well as therapeutic efficacy in vivo. IND enabling studies are ongoing and our results encourage further exploration of dual FXR/sEH targeting for NASH treatment.

References

1) Rinella, M. E. JAMA 2015, 313(22), 2263–2273.
2) Gawrieh, S.; Chalasani, N. Semin. Liver Dis. 2015, 35(3), 338–348.
3) Neuschwander-Tetri, B. et al. Lancet 2014, 385(9972), 956–965.
4) Arab, J. et al. Hepatology 2016, 65(1):350-362.
5) Liu, Y. et al. PLoS One 2012, 7(6), e39165.
6) He, J. et al. J. Diabetes 2016, 8(3), 305–313.
7) Merk, D. et al. J. Med. Chem. 2014, 57(19), 8035–8055.
8) Blöcher, R. et al. J. Med. Chem. 2016, 59(1), 61–81.
9) Schmidt, J. et al. J. Med. Chem. 2017, 60(18), 7703–7724.

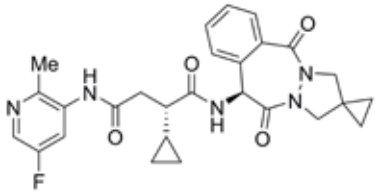
DISCOVERY OF SPL-707: A POTENT, SELECTIVE, AND ORALLY BIOAVAILABLE SPPL2a INHIBITOR

Juraj Velcicky (1), Ursula Bodendorf (1), Pascal Rigollier (1), Robert Epple (2), Daniel R. Beisner (2), Danilo Guerini (1), Philip Smith (1), Bo Liu (2), Roland Feifel (1), Peter Wipfli (1), Reiner Aichholz (1), Philippe Couttet (1), Ina Dix (1), Toni Widmer (3), Ben Wen (2), Trixi Brandl (1)

1) Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland
2) GNF, 10675 John Jay Hopkins Drive, San Diego, California 92121, United States
3) Chemical and Pharmaceutical Profiling, Novartis Pharma AG, CH-4002 Basel, Switzerland

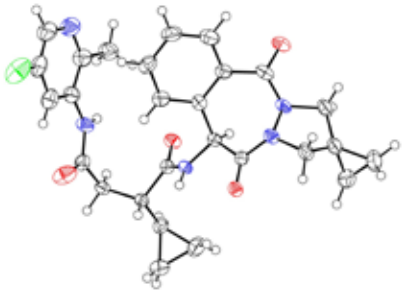
SPPL2a, Signal peptide peptidase-like 2a, a recently discovered[1] aspartyl protease has been shown to play an important role in the development and function of antigen presenting cells such as B lymphocytes and dendritic cells.[2] This enzyme is related to presenilin, the catalytic subunit of the γ-secretase complex. γ-Secretase inhibitor **LY-411,575** could be identified as a potent while unselective SPPL2a inhibitor with poor oral exposure in rodents which served as a starting point for further optimization. Since γ-secretase is involved in processing of Notch-1 leading to serious side effects such as severe intestinal toxicity or development of skin cancer, a main goal of our chemistry efforts was to gain selectivity against γ-secretase/Notch processing. Subsequent optimization of the pharmacokinetic parameters led to discovery of the first, potent, selective and orally bioavailable SPPL2a inhibitor **SPL-707**.[3]

SPL-707 significantly inhibits processing of the endogenous SPPL2a substrate (CD74/p8 fragment) in rodents at ≤10 mg/kg bid po. Oral dosing (11 days at ≥10 mg/kg bid) of **SPL-707**in mice recapitulated the phenotype seen in Sppl2a-deficient mice (reduced number of specific B cells and myeloid dendritic cells). These results show that selectivity within the family of intramembrane cleaving aspartyl proteases can be achieved and SPPL2a is an interesting novel pharmacological target with a potential for treatment of antigen driven autoimmune diseases.



SPL-707

IC_{50} (SPPL2a):	0.08 µM
IC_{50} (γ-secretase):	6.1 µM
IC_{50} (SPP):	3.7 µM
rat AUC po d.n.:	8787 nM.h



References

1) Friedmann, E.; Hauben, E.; Maylandt, K.; Schleeger, S.; Vreugde, S.; Lichtenthaler, S. F.; Kuhn, P.-H.; Stauffer, D.; Rovelli, G.; Martoglio, B. Nat. Cell Biol. 2006, 8, 843–848.
2) Mentrup, T.; Fluhrer, R.; Schröder, B. Eur. J. Cell Biol. 2017, 96, 372–382.
3) Velcicky, J.; Bodendorf, U.; Rigollier, P.; Epple, R.; Beisner, D. R.; Guerini, D.; Smith, P.; Liu, B.; Feifel, R.; Wipfli, P.; Aichholz, R.; Couttet, P.; Dix, I.; Widmer, T.; Wen, B.; Brandl, T. J. Med. Chem. 2018, 61, 865–880.

DESIGN, SYNTHESIS, AND MECHANISM OF BETA-GLUCOCEREBROSIDASE ACTIVATORS FOR GAUCHER'S AND PARKINSON'S DISEASES

Richard B. Silverman (1), Dimitri Krainc (2), Jianbin Zheng (1,2)

1) Department of Chemistry, Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, and Center for Developmental Therapeutics, Northwestern University, Evanston, Illinois 60208 USA
2) Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Gaucher's disease is the most common lysosomal storage disease, and is caused by a recessively inherited deficiency in β-glucocerebrosidase (GCase), which leads to accumulation of toxic lipid substrates. More recently, GCase mutations were also found as a major risk factor for Parkinson's disease and dementia with Lewy bodies. The accumulation in neurons of glucosylceramide, the substrate of GCase, promotes the formation of α-synuclein oligomers, which are considered toxic in Parkinson's disease. Previous work by others and us has shown that α-synuclein aggregation can be induced by reduction of GCase activity in cells or animal models. Many GCase mutations have been identified, which result in single amino acid substitutions of the enzyme. Most of these mutations, including the prevalent Gaucher N370S mutation, result in the enzyme still functional, although with very low residual GCase activity as a result of enzyme misfolding and proteasome-mediated breakdown. The most common successful treatment for Gaucher's disease is enzyme replacement therapy, by which wild-type GCase is infused into the patient every two weeks.

An emerging therapeutic approach involves the restoration of proper folding and lysosome delivery of degradation-prone mutant GCase using small molecules as pharmacological chaperones. This approach could be more effective if the chaperones also activated the enzyme, which might restore its lysosomal activity. In this lecture, I will discuss our development of two classes of activators of GCase, which stabilize wild-type and N370S mutant GCase, and increase its abundance and activity in patient-derived fibroblast cells and in media at the low pH of lysosomes. We also identified a family of potent inhibitors of GCase, which we were able to convert to activators by structural modification. In order to identify the GCase binding pocket of these activators, we developed a covalent modification strategy for attachment to various lysine residues, and obtained the crystal structure, which allowed us to identify the allosteric binding pocket. Covalent modification of GCase induced dimerization in one class of compounds, which was observed by native mass spectrometry, its crystal structure, size-exclusion chromatography with multi-angle light scattering detection, and negative staining transmission electron microscopy. The other class of compounds gave single lysine modification without dimerization. Upon covalent modification we observed enhanced activation of GCase that was more stable than wild-type enzyme at pH of lysosomes, and was taken up in patient-derived fibroblast cells better than wild-type enzyme. Covalently modified GCase has the potential to be utilized as an improved enzyme replacement therapy.

POSITIVE ALLOSTERIC MODULATORS OF THE GABA-B RECEPTOR

Sean C. Turner

Global Pharmaceutical Research and Development, AbbVie GmbH, Knollstrasse 50, Ludwigshafen, Germany

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and binds to several receptor subtypes, including the $GABA_B$ receptor. $GABA_B$ receptors are involved in many physiological processes, because they play important roles in neuronal excitability and synaptic neurotransmission.

The $GABA_B$ receptor agonist baclofen has been known for more than 40 years to reduce muscle rigidity and spasm. In the past two decades, evidence has been accumulating than modulation of $GABA_B$ receptor plays a role in addiction, anxiety, pain, migraine, gastro-esophageal reflux disease and overactive bladder.

$GABA_B$ receptor positive allosteric modulators (PAMs) have been investigated as potential therapeutics based on the rationale that because receptor activation by PAMs does not produce $GABA_B$ receptor desensitization, tolerance should be reduced, compared to a full agonist.

This presentation will chart the medicinal chemistry development of the field of $GABA_B$ PAMs and provide a case study of such a program conducted at AbbVie which identified two series - pyrazolo pyrimidines and isoxazolo pyridazinenones – which were suitable for lead optimization.

THE VALUE OF ORTHOGONAL TECHNIQUES FOR ELUCIDATING BINDING SITE(S) OF GPCR ALLOSTERIC MODULATORS: A CASE STUDY WITH POSITIVE ALLOSTERIC MODULATORS OF DOPAMINE RECEPTORS

Anne Valade

UCB Biopharma S.A., NewMedicines, Discovery Science, Braine-l'Alleud, Belgium

The G protein-coupled receptor (GPCR) superfamily is the largest group of plasma membrane receptors. According to recent estimates, around 350 GPCRs are of potential interest to treat many major diseases including CNS and inflammatory conditions. These receptors can be modulated via small molecules acting at the well-conserved orthosteric site or, more interestingly, interacting at an allosteric site. Targeting allosteric sites brings interesting alternative strategies to develop more efficacious and selective therapies. However, allosteric sites are generally less well conserved and therefore little is known around their mode of action.

In the absence of a crystal structure, elucidation of allosteric binding sites of GPCRs has proven very challenging, generally requiring extensive site-directed mutagenesis studies. We propose an alternative approach using two orthogonal techniques: Hydrogen-Deuterium Exchange MS (HDX-MS), to probe the allosteric binding site, and 'informed' site directed mutagenesis studies. This combined methodology has been successfully used to confirm the binding site of positive allosteric modulators of the D1 receptor.

AMPA RECEPTOR POSITIVE ALLOSTERIC MODULATORS BASED ON NEW SCAFFOLDS: DESIGN, SYNTHESIS, AND STUDIES

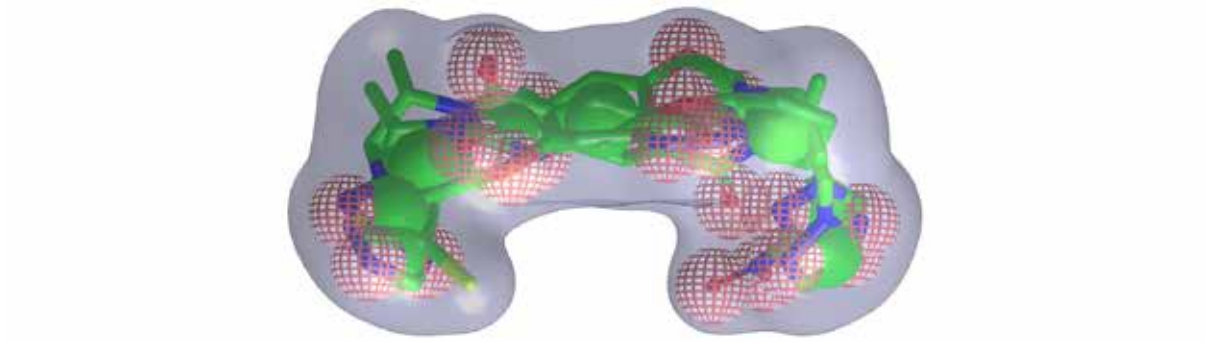
Vladimir A. Palyulin (1,2), Mstislav I. Lavrov (1,2), Dmitry S. Karlov (1,2), Eugene V. Radchenko (1,2), Elena B. Averina (1,2), Kseniya N. Sedenkova (1,2), Dmitry A. Vasilenko (1), Anna A. Nazarova (1), Nadezhda S. Temnyakova (1), Evgenia M. Bovina (1), Polina N. Veremeeva (1), Vladimir L. Zamoyski (2), Vladimir V. Grigoriev (1,2)

1) Department of Chemistry, Lomonosov Moscow State University, Moscow, 119991, Russia
2) Institute of Physiologically Active Compounds RAS, Severny Proezd 1, Chernogolovka, Moscow Region, 142432, Russia

Positive allosteric modulators (PAMs) of AMPA receptors (one of types of ionotropic glutamate receptors) have a significant influence on learning and memory consolidation. It is also shown in experiments that the ion currents caused by such modulators and further postsynaptic membrane depolarization launch the mechanism of gene expression responsible for the synthesis of NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor). Thus the drugs having this mechanism of action could be efficient for the treatment of neurodegenerative diseases.

In this report the techniques are considered for computer-aided design of AMPA receptor modulators based on new scaffolds as well as the approaches to their synthesis and the results of physiological activity studies. The molecular dynamics simulations for a series of AMPA receptor PAMs bound on the interface between two glutamate-binding domains have demonstrated a good correlation of the MM-GBSA and MM-PBSA binding energies with the experimental pEC_{50} values. The Molecular Field Topology Analysis (MFTA) QSAR method developed by us was quite helpful in the modeling of ligand selectivity and multi-target activity in terms of local properties such as the atomic charges, group van der Waals radii, and local lipophilicity. In addition, the 3D QSAR and pharmacophore models of the AMPA receptor PAMs have been constructed. The virtual screening of large compound libraries using the above mentioned models as the filters as well as the *de novo* design of the structures fitting the PAM binding site and based on new scaffolds allowed us to find a series of novel highly potent positive allosteric modulators of AMPA receptors. Several compounds are on the final stages of preclinical studies. The modulators found have a unique combination of properties including picomolar activity and very low toxicity.

This work was supported by the Russian Science Foundation (grant No. 17-15-01455).



References

1) Lavrov M.I., Grigor'ev V.V., Bachurin S.O., Palyulin V.A., Zefirov N.S., Dokl. Biochem. Biophys., 2015, 464, 322.
2) Radchenko E.V., Karlov D.S., Lavrov M.I., Palyulin V.A., Mendeleev Commun., 2017, 27, 623.

PET MOLECULAR IMAGING - AN OVERVIEW

Antony Gee

School of Biomedical Engineering and Imaging Sciences, King's College London, UK. email: antony.gee@kcl.ac.uk

Positron Emission Tomography (PET) has become a powerful tool for decision making in mordern therapeutic R&D.

The technique allows the regional tissue pharmcokinetics of tracer doses of labelled compounds to be followed and quantified in the living human body.

The technique can be used to answer questions such as:

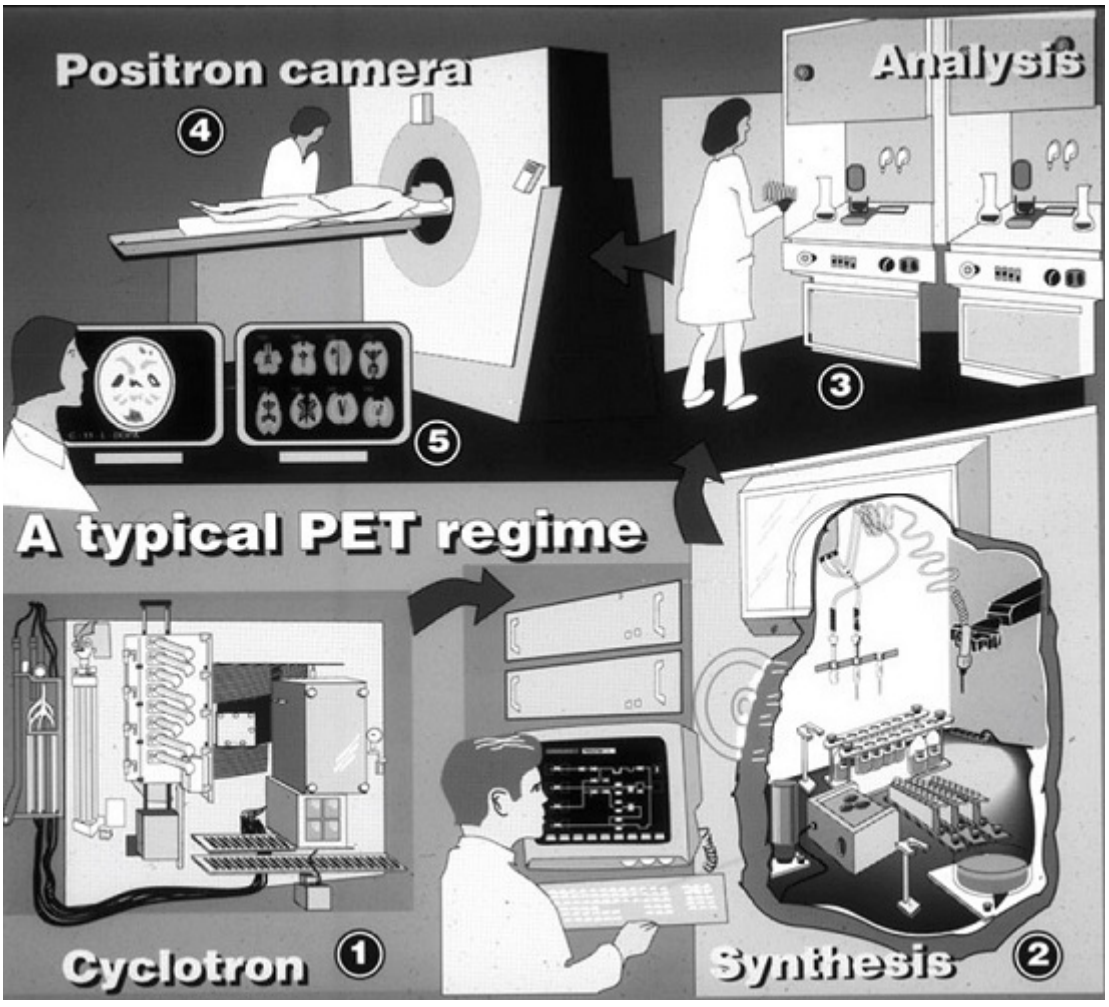
1) What is the biodistribution of a labelled drug candidate in humans e.g. brain concentration, tumour concentration

2) What is the drug-target engagement (e.g. % target tissue receptor occupancy) for a given dose of drug

3) What is the half-life of drug-target engagement

4) what are the early downstram phamacodynamic responses of drug action (e.g. second messenger response, rate of proein synthesis, glucose metabolism).

PET relies on the synthesis of molecules labelled with cyclotron-produced positron-emitting radionuclides, such as Carbon-11, Fluorine-18 and Zirconium-89. An overview of the PET technique will be given in addition to examples of how this can be used for enhancing decision making in a drug discovery and development setting.



PET IN NEUROSCIENCE DRUG DISCOVERY AND DEVELOPMENT

Magnus Schou (1,2)

1) PET Science Centre, Precision Medicine and Genomics, IMED Biotech Unit, AstraZeneca, Karolinska Institutet, S-171 76 Stockholm, Sweden
2) Department of Clinical Neuroscience, Karolinska Institutet, S-17176 Stockholm, Sweden

Because of the invaluable information provided by translational molecular imaging, Positron Emmision Tomography (PET) is today an integrated part of neuroscience drug discovery and development. This talk will focus on the three major applications of PET in drug discovery and development, namely: i) *PET microdosing studies* - in which radiolabeled drug candidates are traced in vivo to quantify drug exposure in the target organ of interest, ii) *target occupancy studies* - that aim to establish relationships between drug exposure in plasma and target occupancy in the organ of interest, and iii) *biomarker studies,* in which an imaging biomarker of disease pathology is used for patient segmentation and to evaluate treatment efficacy. Examples from all three applications will be presented, together with the major challenges associated with each application.

PET FOR ONCOLOGY DRUG DISCOVERY AND DEVELOPMENT

Albert D. Windhorst

VU University Medical Center, Departments of Radiology & Nuclear Medicine, 1081 HV Amsterdam, The Netherlands

Positron Emission Tomography (PET) with 2-[^{18}F]fluoro-deoxyglucose([^{18}F]FDG) has become routine practice in modern oncology diagnosis and treatment monitoring and has shown its high clinical value. However, also drawbacks of [^{18}F]FDG have become apparent. While [^{18}F]FDG has a broad spectrum of applications, the need for more specific PET tracers is growing, particularly in relation to drug development and clinical care. Modern drugs in oncology act at very specific targets, e.g. an antigen presented on tumor cell membrane surface or at kinases that harbor a defined mutation. As a result less side effects are observed with these so-called targeted drugs. However, the target of the drug is often not present in all patients. This creates difficulties in phase II/III clinical trials in which randomized double blind trials are the gold standard. If only a sub-population of the subjects in the clinical trial have the required target, these type of studies are destined to fail since the clinical outcome is measured over the whole group.

To assess the clinical effect of targeted drugs pre-selection of eligible patients should be applied. This requires a technique that is capable of identifying those patients that have tumors expressing the target. This could be achieved by immunohistochemistry on tissue biopsies. While this technique can be executed at low cost, its main drawback is that it is not representative for the whole tumor from where the biopsy is taken, since tumors are heterogeneous and a false negative outcome could occur. Moreover, metastases are often not biopsied and could show large biochemical differences compared to the primary tumor and some tumors cannot be biopsied due to their location

PET would provide the means for whole body assessment of the expression of the target of interest, at the primary tumor as well as metastases. It can give information about: i) uptake in critical healthy organs to anticipate toxicity; ii) the interpatient variation in pharmacokinetics and tumor targeting and iii) the mechanism of action of the drug.[1,2] This would require a PET tracer that binds to the target and to meet this demand, various tracers have been developed in recent years and applied successfully in clinical research. A selection of these will be discussed in this contribution. Focus will be on radiosynthesis and application tyrosine kinase inhibitors[3,4], ^{89}Zr labeled monoclonal antibodies and a case study with [^{11}C]docetaxel[5].

References

1) Y.W.S. Jauw, C.W. Menke-van der Houven van Oordt, O.S. Hoekstra, N.H. Hendrikse, D.J. Vugts, J.M. Zijlstra, M.C. Huisman, G.A.M.S. van Dongen, Frontiers in Pharmacology, 2016, doi: 10.3389/fphar.2016.00131 and
2) G.A.M.S. van Dongen, M.C. Huisman, R. Boellaard, N.H. Hendrikse, A.D. Windhorst, G.W.M. Visser, C.F.M. Molthoff, D.J. Vugts, Q.J. Nucl. Med. Mol. Imag. 2015;59:18-38.
3) P. Slobbe, A.J. Poot, A.D. Windhorst, G.A.M.S. van Dongen, PET imaging with small-molecule tyrosine kinase inhibitors: TKI-PET. Drug Disc Today, 2012;17(20/21):1175-1187.
4) I. Bahce, E.F Smit, M. Lubberink, A.A.M van der Veldt, M. Yaqub, A.D. Windhorst, R.C. Schuit, E.F.B.J.M Thunnissen, D.A.M Heideman, P.E Postmus, A.A Lammertsma, N.H. Hendrikse, In vivo detection of epidermal growth factor receptor exon 19 deletions in non-small cell lung cancer patients using [11C]erlotinib. Clin. Canc. Res. 2013;19:183-193. and I. Bahce, M. Yaqub, H. Errami, R.C. Schuit, P. Schober, E. Thunnissen, A.D. Windhorst, A.A. Lammertsma, E.F. Smit, N.H. Hendrikse. Effects of erlotinib therapy on [11C]erlotinib uptake in EGFR mutated, advanced NSCLC. EJNMMI Res. 2016;6(1):10
5) A.A.M. van der Veldt, M. Lubberink, I. Bahce, M. Walraven, M.P. de Boer, H.N. Greuter, N.H. Hendrikse, J. Eriksson, A.D. Windhorst, P.E. Postmus, H.M. Verheul, E.H. Serné, A.A. Lammertsma, E.F. Smit, Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs, Cancer Cell 2012;21: 82–91. and A.A.M. van der Veldt, M. Lubberink, R.H.J. Mathijssen, W.J. Loos, G.J.M. Herder, H.N. Greuter, E.F.I. Comans, H.B. Rutten, J. Eriksson, A.D. Windhorst, N.H. Hendrikse, P.E. Postmus, E.F. Smit, A.A. Lammertsma. Towards prediction of efficacy of chemotherapy: a proof of concept study in lung cancer patients using [11C]docetaxel and positron emission tomography, Clin Cancer Res. 2013;19(15):4163-73.

DISCOVERY OF CANDIDATES FOR PET MOLECULAR IMAGING OF PATHOLOGICAL TDP-43 AGGREGATES IN FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

Vincent Darmency (1), Cedric Boudou (1), Tamara Seredenina (1), Tariq Afroz (1), Jacqueline Kocher (1), Heiko Kroth (1), Ajay Purohit (2), David Paterson (2), Laurent Martarello (2), Jan Stoehr (1), Andrea Pfeifer (1), Andreas Muhs (1)

1) AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne, Switzerland
2) Research & Early Discovery Imaging, Biomarkers, Biogen 225 Binney St, Cambridge, MA 02142, USA

TAR DNA-binding protein-43 (TDP-43) was identified more than a decade ago as the main component of ubiquitin-positive cytoplasmic inclusions present in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1]. However, the mechanism leading to loss of normal nuclear localization and cytoplasmic TDP-43 aggregation remains unclear. The lack of tools for accurate diagnosis and monitoring of disease progression have impeded the research and development of therapeutics for TDP-43 proteinopathies. Therefore, direct detection of pathological TDP-43 aggregates in patients with positron emission tomography (PET) imaging agents holds promise for disease early diagnosis and staging.

Our strategy is based on screening AC Immune's proprietary Morphomer^{TM} library by direct staining on post-mortem brain tissue from ALS and FTD patients, to directly prove target engagement. The confirmed hits are characterized for physico-chemical properties, ADME properties as well as selectivity over amyloid-beta and tau aggregates. In a subsequent step, compounds are tritium (^{3}H) labelled for affinity measurement by radiobinding assay. Molecules with favourable properties for CNS penetration were also profiled in *in vivo* pharmacokinetic studies in rodents. Further optimization by iterative design has allowed us to identify a set of small molecules that specifically bind to pathological TDP-43 and display suitable properties for further development as PET ligands.

References

1) Manuela Neumann, et al. Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. Science 314, 130 (2006).

FIRST DISCLOSURE OF THE CLINICAL CANDIDATE BAY-840, A POTENT AND SELECTIVE hBRADYKININ B1 ANTAGONIST FOR THE TREATMENT OF CHRONIC INFLAMMATORY DISEASES, GENERATED WITHIN THE BAYER-EVOTEC STRATEGIC ALLIANCE

Stefan Bäurle (1), Adam Davenport (2)

1) Bayer AG, Pharmaceuticals R&D, PH-RD-SMI-MCB-MC1, 13342 Berlin, Germany
2) Evotec (UK) Ltd., 114 Innovation Drive, Milton Park, Abingdon, Oxfordshire, OX14 4RZ (UK)

The GPCR Bradykinin B1 receptor (BDKRB1) is only marginally expressed under pathogen-free conditions and is highly up-regulated during chronic inflammation. Together with its endogenous ligands, is instrumental in the maintenance of inflammation. Activation of the B1 receptor induces pain, is profibrotic, proinflammatory and antagonists exhibit highly efficacious pain relief and suppression of inflammation in multiple animal models. This robust efficacy is derived from a unique dual mechanism of action involving modulation of inflammation and pain both on a local and neuronal level. The B1 receptor offers an attractive profile for treatment inflammation related conditions and a low adverse effect risk is expected due to the lack of a physiological role of the target in pathogen-free conditions.

BAY-840 was identified as a highly potent, competitive human B1 receptor antagonist. We report on the identification of the lead structure from high-throughput screening, establishment of structure-activity relationships and DMPK optimization. The discovery of BAY-840 is a result of a successful strategic alliance between Bayer AG Pharmaceuticals and Evotec AG. The structure as well as the pharmacological and pharmacokinetic profile of the compound will be disclosed. BAY-840 is currently in preclinical development.

DISCOVERY OF GDC-0077: A HIGHLY SELECTIVE INHIBITOR AND DEGRADER OF MUTANT PI3K-ALPHA

Jamie Knight (1), Marie-Gabrielle Braun (2), Connie Chan (2), Saundra Clausen (2), Kyle Edgar (2), Charles Eigenbrot (2), Richard Elliott (1), Nick Endres (2), Lori Friedman (2), Keira Gerland (2), Xiao-Hu Gu (2), Pat Hamilton (2), Chong Han (2), Emily Hanan (2), Robert Heald (1), Rebecca Hong (2), Philip Jackson (1), Sean Kelly (2), Man-Ling Lee (2), Aijun Lu (2), Calum MacLeod (1), Aija McKenzie (2), Michelle Nannini (2), Raman Narukulla (1), Amanda Nguyen (2), Jodie Pang (2), Hans Purkey (2), Laurent Salphati (2), Deepak Sampath (2), Stephen Schmidt (2), Leah Schutt (2), Kyung Song (2), Steven Staben (2), Mark Ultsch (2), Jianfeng Xin (2), Kuen Yeap (1), Amy Young (2)

1) Charles River Discovery Research Services UK Ltd., 8-9 Spire Green Centre, Harlow, Essex, CM19 5TR, UK
2) Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA

The phosphatidylinositol 3-kinase (PI3K) signaling pathway is a major regulator of tumor cell growth, proliferation and survival. Dysregulation of the PI3K/Akt/mTOR signaling pathway through multiple mechanisms has been described in solid tumor malignancies, including activating and transforming “hotspot” mutations of *PIK3CA* that encodes the p110 alpha subunit of PI3K. Hotspot mutations of *PIK3CA*, in particular the kinase domain mutation H1047R, are highly prevalent in breast cancer. Herein we describe the optimisation of a series of novel benzoxazepin-oxazolidinone inhibitors of PI3K-alpha. Structure-based design was utilised to enhance isoform-specific interactions within the binding site, leading to potent inhibitors of PI3K-alpha with greater than 300-fold selectivity over the other Class I PI3K isoforms, PI3K-beta, -delta, and -gamma. Further optimisation of pharmacokinetic properties, including the use of physicochemical property models, led to the discovery of GDC-0077. In addition to ATP competitive inhibition, GDC-0077 induces the selective degradation of the mutant PI3K-alpha protein in a proteasome dependent fashion, resulting in the reduction of PI3K pathway biomarkers. In vivo, daily oral treatment with GDC-0077 induces tumor regressions in *PIK3CA* mutant breast cancer xenograft models. These results support the continued evaluation of GDC-0077, which has entered Phase I development, as a treatment for patients with *PIK3CA*-mutant cancer.

ANTI-HBV DRUG DISCOVERY ENABLED BY STRUCTURE-BASED DRUG DESIGN AND PHENOTYPIC SCREENING

Hong Shen

Roche Innovation Center Shanghai, 720 Cai Lun Road, Building 5, Shanghai, 201203, China

Chronic hepatits B virus (HBV) infection is a highly unmet medical need with 250 million chronic carriers worldwide. 15-40% of HBV chronic carriers will eventually develop clinical diseases, such as hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). The end stage of liver diseases caused by HBV infection claim the lives of approximately 700,000 patients annually. Unfortunately, the current standard of care cannot cure majority of the HBV patients. As such, multi-pronged therapeutic approaches have been attempted to increase the functional cure rate.

In this presentation, two anti-HBV drug discovery programs with distinct medicinal chemistry approaches will be introduced. In the first case, chemical and structural biology studies facilitated the understanding of the binding mode of HBV capsid inhibitors, which enabled medicinal chemists to precisely design molecules with improved properties while maintaining binding affinity. Ultimately, a novel clinical compound was obtained with a significantly improved target compound profile versus previously described molecules of the same structural scaffold. In the second example, an innovative phenotypic screening was utilized to successfully identify viral expression inhibitors that reduce the production of HBV surface antigen (HBsAg) from infected hepatocytes, which culminates in the discovery of a first-in-class clinical compound. Lessons regarding the advantages and challenges of phenotypic screening will be shared.

References

1) Ann. Rep. Med. Chem. 2013. 48, 265.
2) J. Med. Chem. 2016, 59, 7651.
3) Scientific Reports, 2017, 7, 42374.
4) J. Med. Chem. 2017, 60, 3352.

DISCOVERY OF AZD4573, A POTENT AND SELECTIVE INHIBITOR OF CDK9 THAT ENABLES TRANSIENT TARGET ENGAGEMENT FOR THE TREATMENT OF HAEMATOLOGICAL MALIGNANCIES

Bernard Barlaam (1), Chris De Savi (2), Lisa Drew (2), Andrew D. Ferguson (3), Douglas Ferguson (2), Chungang Gu (2), Janet Hawkins (1), Alexander W. Hird (2), Michelle L. Lamb (2), Nichole O'Connell (3), Kurt Pike (1), Theresa Proia (2), Maryann San Martin (2), Melissa M. Vasbinder (2), Jeff Varnes (2), Jianyan Wang (4), Wenlin Shao (2)

1) Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom
2) Oncology, IMED Biotech Unit, AstraZeneca, Boston, USA
3) Discovery Sciences, IMED Biotech Unit, AstraZeneca, Boston, USA
4) Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Boston, USA

Cyclin-dependent kinase 9 (CDK9) is a serine/threonine kinase that regulates elongation of transcription through phosphorylation of RNA polymerase II at serine 2 (p-Ser2-RNAPII). Transient inhibition of CDK9 results in reduced protein levels for genes that have short half-lives of transcripts and proteins, thus presenting a potential therapeutic opportunity in tumors dependent upon oncogenes fitting such criteria. One example is Mcl-1, an anti-apoptotic protein that plays a key role in cancer cell survival.

A potent and selective CDK9 inhibitor having appropriate physical properties and pharmacokinetics (intravenous administration and short $t_{1/2}$) would enable short yet tuneable target engagement, allowing high flexibility in order to optimize the efficacy / tolerability balance in the clinic. We previously reported the identification of AZ5576 from an amidopyridine series, as a potent, highly selective and orally bioavailable preclinical inhibitor of CDK9.

Here we report further optimization of this series with a focus on pharmacokinetic and physicochemical properties suitable for an intravenous agent with short target engagement. We discuss the Structure Activity Relationships (SAR) and Structure Property Relationships (SPR) in this series, specifically increasing human metabolic clearance (in order to achieve short half-life) and solubility whilst improving potency. This work led to the identification of AZD4573, a potent inhibitor of CDK9 (IC_{50} of < 0.004 μM) with fast-off binding kinetics ($t_{1/2}$ 16 min) and high selectivity versus other kinases, including other CDK family kinases. AZD4573 exhibits a short half-life in multiple preclinical species (less than one hour in rat, dog and monkey) and good solubility for intravenous administration. Short-term treatment with AZD4573 led to a rapid dose- and time-dependent decrease in cellular pSer2-RNAPII, resulting in activation of caspase 3 and cell apoptosis in a broad range of haematological cancer cell lines (e.g. caspase activation EC_{50} 0.0137 μM in an acute myeloid leukemia model MV4-11). Correspondingly, in vivo efficacy was demonstrated in xenograft models derived from multiple haematological tumours (e.g. regression at 15 mg/kg twice weekly in MV4-11 xenografts). These results support AZD4573 as a clinical candidate for the treatment of haematological malignancies. This presentation will represent the first full disclosure of the different Medicinal Chemistry strategies used in the discovery of AZD4573.

References

1) Cidado J et al, AZ5576, a novel, potent and selective CDK9 inhibitor, induces rapid cell death and achieves efficacy in multiple preclinical hematological models, AACR poster presentation, 3572 (2016)

THE DISCOVERY OF CNP520, AN AMINO-1,4-OXAZINE BACE INHIBITOR IN PREVENTION STUDIES

Rainer Machauer

Novartis Pharma AG, Novartis Institute for Biomedical Research, 4002-Basel, Switzerland

The pathological features of Alzheimer's disease (AD), amyloid plaques and fibrillary tangles are well characterized and the major components of the plaques identified as β-amyloid peptides (Aβ). It is widely accepted that the monomers or oligomers of Aβ are neurotoxic and initiate the cascade of events leading to neuronal degeneration. Aβ is generated from the β-amyloid precursor protein (APP) by the sequential proteolytic action of the β- and γ-secretase enzymes. Inhibition of the membrane-bound aspartyl protease β-secretase (or BACE-1, β-site amyloid precursor protein cleaving enzyme) is widely considered one of the most promising therapeutic approaches for AD. We hypothesized that such a BACE-1 inhibitor treatment needs to start before the onset of significant neurodegeneration, before or at early stage of Aβ deposition. Such a prevention treatment requires a compound with excellent safety and tolerability.

We present results of our small molecule efforts on BACE-1 inhibition, leading to CNP520. In particular, we addressed potency, brain penetration, selectivity, and metabolism challenges and designed CNP520 to meet the requirements for preventive treatment. Non-clinical pharmacokinetic and pharmacodynamic as well as safety data supported the clinical investigation of CNP520. Early clinical results including CNP520 safety, tolerability, and dose-dependent CSF Aβ reduction will be discussed.

Clinical Phase II/III studies are ongoing in a cognitively healthy population of enhanced risk to develop symptoms of AD, testing the concept of prevention treatment in AD (Generation Study 1 and Generation Study 2).

C-NATRUIRETIC PEPTIDE AGONISTS FOR CARDIOVASCULAR DISEASE

David Selwood (1), Samuel Myers (1), Dan Conole (1), Cristina Pérez-Ternero (2), Adrian Hobbs (2)

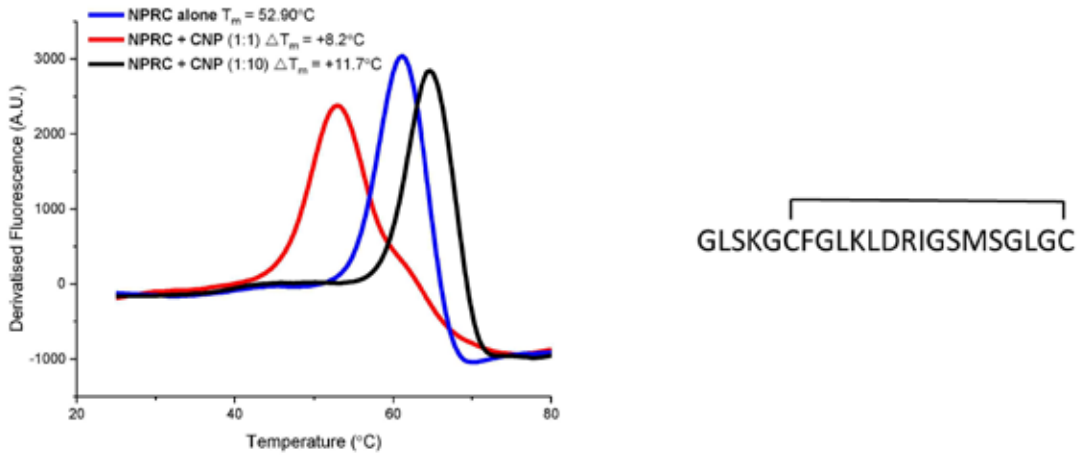
1) The Wolfson Institute for Biomedical Research, Division of Medicine, University College London. UK
2) William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

Cardiovascular disease is the leading cause of morbidity and mortality in the western world (17.7 million deaths per annum, World Health Organisation). Modern lifestyles, a combination of poor diet, lack of exercise, tobacco, and alcohol are thought to contribute to a rapid increase in numbers of people with cardiovascular disease and other conditions such as diabetes. Current treatments such as statins may reduce cardiovascular risk and the tissue damage caused by acute cardiovascular events but while the treatment of myocardial infarction is effective, heart failure remains a considerable problem. Heart failure, a loss of the heart's ability to pump efficiently, afflicts at least 40 million people worldwide and treatments are inadequate.

We have discovered an anti-atherogenic and anti-thrombotic signalling pathway in the cardiovascular system controlled by the C-type natriuretic peptide (CNP). This 22 mer cyclic peptide binds to the natriuretic peptide receptor-C (NPRC) on the cell surface and initiates signalling through a Gialpha protein-adenylate cyclase mediated mechanism. We have found this system to be pivotal in the regulation of vascular tone, endothelial and smooth muscle proliferation, and in the activation of leukocytes and platelets. In model studies, we have found that activation of the system can protect against atheroma and MI. CNP signalling through NPRC is also responsible for the cytoprotective effect of CNP.

This lecture will describe our programme leading to the identification of small molecule activators of the NPRC receptor, a challenging target for small molecule modulation. The detailed medicinal chemistry will be described. Biophysical assay development was key in being able to characterise the interaction of the molecules with this transmembrane spanning receptor. Surface plasmon resonance (Biacore),fluorescence polarization and thermal shift data will be shown together with the assessment of the molecules in functional (blood vessel) assays.

Funding: This work was funded by a translational award from the British Heart Foundation (TG/15/3/31692), Wellcome Trust grants (084449/Z/07/Z and 078496/Z/05/Z) and UCL Business PLC (PoC-12-007).



Derivatised (differentiated) FL NPR-C protein melt curves for increasing concentrations of agonist CNP

References

1) Moyes AJ, Khambata RS, Villar I, Bubb KJ, Baliga RS, Lumsden NG, Xiao F, Gane PJ, Rebstock AS, Worthington RJ, Simone MI, Mota F, Rivilla F, Vallejo S, Peiro C, Sanchez Ferrer CF, Djordjevic S, Caulfield MJ, MacAllister RJ, Selwood DL, Ahluwalia A, Hobbs AJ. Endothelial C-type natriuretic peptide maintains vascular homeostasis. J Clin Invest. 2014 Sep;124(9):4039-51.

THE DISCOVERY OF SEMAGLUTIDE - A JOURNEY FROM ALA SCAN TO STRUCTURAL DESIGN OF GLP-1 ANALOGUES

Jesper Lau

Global Research Technologies,, Novo Nordisk A/S, 2760 Maaloev, Denmark

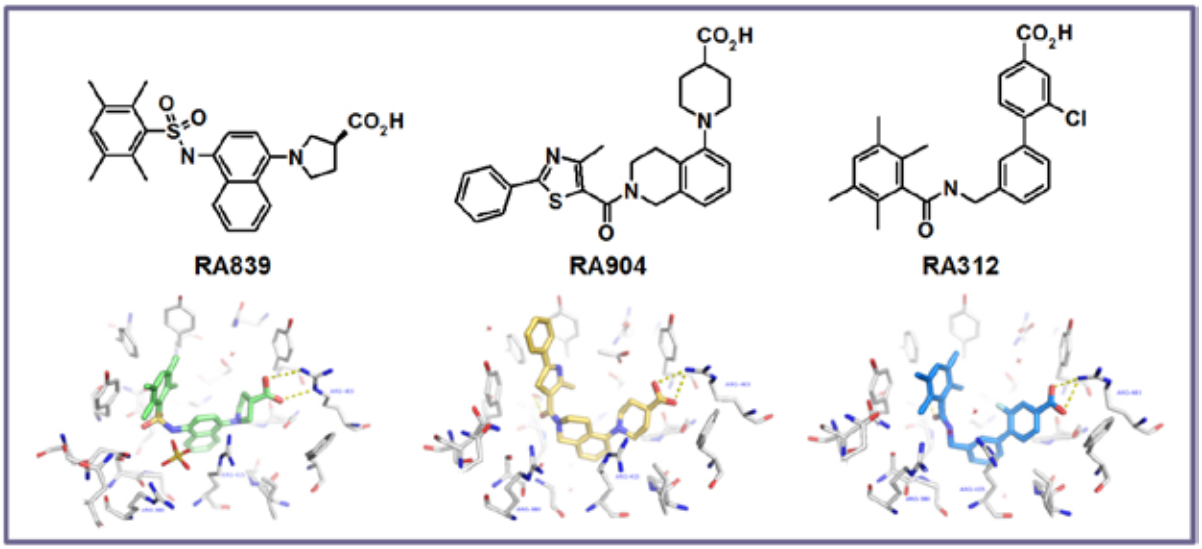
Since the early clinical findings of using glucagon like peptide-1 (GLP-1) to regulate blood glucose levels in the late 80´s there has been an increasing interest to discover and develop GLP-1 receptor agonists for treatment of type 2 diabetes. The increased non-clinical and clinical understanding of the mechanism of action of GLP-1 and the parallel technical development have now brought the scientific community to a very high level of understanding with structural insight of the interaction of peptides and small molecules with GPCR class B receptors. The discovery and development of semaglutide is a fantastic example on how technology and biologic understanding have developed in parallel ending with a superior peptide for treatment of diabetes.

DISCOVERY AND OPTIMIZATION OF NON-COVALENT, SELECTIVE, AND BIOAVAILABLE SMALL MOLECULE INHIBITORS OF THE KEAP1-NRF2 PATHWAY

Hauke Szillat, Angela Dudda, Christian K. Engel, Heiner Glombik, Stefan Güssregen, Aimo Kannt, Peter Monecke, Sven Ruf, Dieter Schmoll

Sanofi R&D, Industriepark Höchst, D-65926 Frankfurt, Germany

The KEAP1 - Nrf2 signaling pathway is a promising target in type 2 diabetes and other disease areas [1]. Our discovery and optimization of three novel non-covalent chemical series represented by compounds RA839, RA904, and RA312, will be presented.



Starting from high-throughput screening two hits were identified and optimized by systematic exploration of the structure-property relationships using structure-based design accompanied by transfer of information between series. RA904 and RA312 differentiate themselves from known Keap1-Nrf2 inhibitors in combining high potency, selectivity, and oral bioavailability with Nrf2-related antioxidative effects in cellular assays and target engagement in in vivo studies. These novel Keap1-Nrf2 inhibitors are attractive tool compounds to elucidate further the therapeutic potential of the inhibition of the protein-protein interaction (PPI) between Keap1 and Nrf2.

References

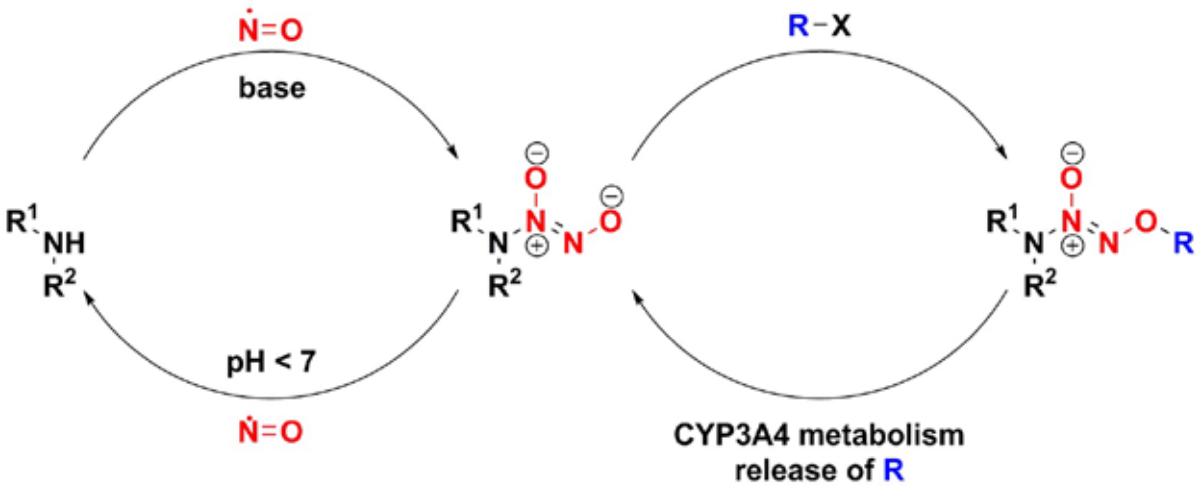
1) Drug discovery today: Technologies (2017), 24, 11-17.

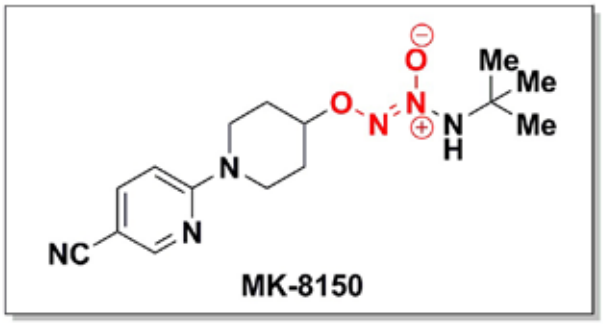
DISCOVERY AND CLINICAL EVALUATION OF MK-8150, A NOVEL NITRIC OXIDE DONOR WITH A UNIQUE MECHANISM OF NITRIC OXIDE RELEASE

Amjad Ali (1), Michael M.C. Lo (1), Brent Whitehead (1), Timothy Henderson (1), Lin Yan (1), Pei Huo (1), Doris Cully (2), Kaushik Mitra (3), Ying Li (3), R. Scott Hoerrner (4), Mark Weisel (4), Yong-Li Zhong (4), Liming Yang (5), Shu-Yu Sun (5)

1) Department of Discovery Chemistry, Merck Research Laboratories, 2000 Galloping Hill Rd., Kenilworth, NJ 07033
2) Department of Hypertension, Merck Research Laboratories, 2000 Galloping Hill Rd., Kenilworth, NJ 07033
3) Department of Drug Metabolism & Pharmacokinetics, Merck Research Laboratories, 2000 Galloping Hill Rd., Kenilworth, NJ 07033
4) Department of Process Chemistry, Merck Research Laboratories, 2000 Galloping Hill Rd., Kenilworth, NJ 07033
5) Department of In vivo Pharmacology, Merck Research Laboratories, 2000 Galloping Hill Rd., Kenilworth, NJ 07033

Nitric oxide donors are widely used to treat cardiovascular disease, but their major limitation is the development of tolerance, a multifactorial process to which the in vivo release of nitric oxide is thought to contribute. This presentation will describe the preclinical and clinical results of a translational drug development effort to create a next-generation nitric oxide donor. The SAR, pharmacokinetic properties and *in vivo* mechanism of NO release will be described in detail for a series of key leads, including MK-8150.





NOTES

POSTERS - TECHNOLOGIES

Artificial Intelligence Applications in Medicinal Chemistry

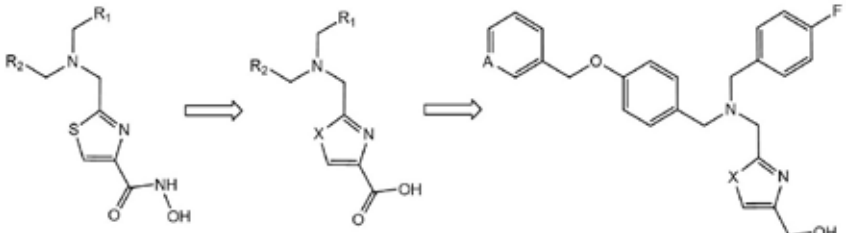
DEVELOPMENT OF MATRIX METALLOPROTEINASE-2 INHIBITORS FOR CARDIOPROTECTION

György Dormán (1,2), Péter Bencsik (3,4), Anikó Görbe (3,4), István Hajdú (2), Sándor Cseh (2), Péter Ferdinandy (3,5)

1) Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Szeged, Zrínyi u. 9. Szeged, H-6720, Hungary
2) TargetEx Ltd., Madách Imre utca 31/2., Dunakeszi, H-2120, Hungary
3) Pharmahungary Group, Hajnóczy u. 6, H-6722, Szeged, Hungary
4) Department of Biochemistry, Faculty of Medicine, University of Szeged, Dom ter 9. Szeged, H-6720, Hungary
5) Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér 4., Budapest, H-1089, Hungary

Our group has previously shown that moderate inhibition of matrix metalloproteinases-2 (MMP-2) is a powerful tool to attenuate acute cardiac ischemia/reperfusion injury (I/R) and to decrease myocardial infarct size.[1] Based on the above findings the objective of our ongoing research was shifted to develop novel inhibitors for MMP-2 for acute cardioprotection.

In pilot studies, novel substituted carboxylic acid derivatives were synthesized based on imidazole and thiazole scaffolds and tested in a screeening cascade for MMP inhibition. We found that the MMP-2 inhibiting effects of thiazole carboxylic acid-based compounds are superior in efficacy than the conventional hydroxamic acid derivatives of the same molecules.



R_1, R_2 = (subs.) aryl, aralkyl

X = S, NH
A = CH, N

Based on these results, a 568-membered focused library of imidazole and thiazole compounds was generated in silico and the library members were docked to the 3D model of MMP-2. Altogether 45 compounds showed a docking score >70, from which 30 were successfully synthesized. These compounds were in vitro screened first by a fluorescent assay employing MMP-2 catalytic domain and the hits were further investigated by gelatin zymography assay. MMP-2 was inhibited by 12 compounds below 100 μM, from which 8 showed 7-10-fold selectivity against MMP-1, which can be rationalized by the structural architectures of the compounds.[2]

Seven compounds were selected to assess cardio-cytoprotective efficacy, using neonatal rat cardiac myocytes subjected to simulated I/R injury and 6 compounds showed significant cytoprotecion. One compound significantly decreased infarct size at 1 μM in isolated rat hearts subjected to 30 min global ischemia and 120 min reperfusion. In summary, we identified a promising novel cytoprotective MMP-2 inhibitor lead candidate for the treatment of acute myocardial infarction.[3]

References

1) Bencsik, P., et al., Moderate inhibition of myocardial matrix metalloproteinase-2 by ilomastat is cardioprotective. Pharmacol Res, 2014. 80: p. 36-42.
2) Dorman, G., et al., Matrix metalloproteinase inhibitors: a critical appraisal of design principles and proposed therapeutic utility. Drugs, 2010. 70(8): p. 949-64.
3) Bencsik, P., et al., Development of matrix metalloproteinase-2 inhibitors for cardioprotection, Frontier Pharm. (accepted)

AI FOR CHEMISTRY OPTIMISATION: COMBINING MACHINE LEARNING AND DOMAIN KNOWLEDGE

Matthew Segall, Edmund Champness, Peter Hunt, Tamsin Mansley

Optibrium Ltd., Cambridge, United Kingdom

Artificial Intelligence involves the application of machine learning algorithms in the context of domain knowledge. In the case of compound design, this involves integration of information from multiple perspectives: understanding of structure-activity relationships (SAR), based on data from previously studied compounds; expertise from diverse fields to define the multi-parameter optimisation (MPO) objectives of a project; and knowledge of synthetic strategies that may be applicable to create the next rounds of compounds for investigation. All of these forms of knowledge can be captured and applied computationally: Machine learning methods can generate quantitative structure-activity relationship (QSAR) models to predict the properties of novel, virtual compounds; MPO methods capture the desired property criteria for a successful compound for a specific project and rigorously define an objective function to guide optimisation; and, evolutionary algorithms can be applied to explore optimisation strategies captured as structural transformations that reflect steps made in previous chemistry projects.

Here, we will describe these methods and illustrate how they can be seamlessly combined to rigorously explore new, relevant compound ideas and prioritise those most likely to achieve a project objective. This approach can help to stimulate the search for new optimisation strategies and explore a much broader range of compounds than could be achieved based on a single chemist's or even a project team's experience. Example applications include the optimisation of compounds with a desired polypharmacology or selectivity profile and exploration of lead hopping strategies to overcome pharmacokinetic issues, while maintaining target potency.

References

1) O. Obrezanova, M.D. Segall, J. Chem. Inf. Model. (2010) 50 (6), pp. 1053-1061
2) M.D. Segall, Curr. Pharm. Des. (2012) 18(9) pp. 1292-1310
3) M.D. Segall et al., J. Chem. Inf. Model. (2011) 51(11) pp. 2967-2976
4) I. Ujváry, J. Hayward, In N. Brown ed., Bioisosteres in Medicinal Chemistry (2012)

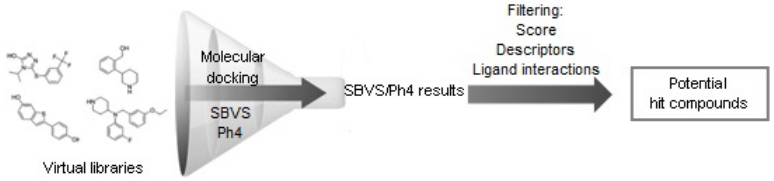
NEW MOLECULES TO INHIBIT THE 20S PROTEASOME IN ANTICANCER THERAPY: COMPUTER-AIDED DRUG DESIGN METHODOLOGIES AND BIOLOGICAL EVALUATION

Romina A Guedes (1,2), Carlota Leonardo Sousa (1), Andre Falcao (3), Eleonora Gallerani (4), Riccardo Gavioli (4), Andreia Neves Carvalho (1), Maria Joao Gama (1), Jorge AR Salvador (2,5), Rita C Guedes (1)

1) iMed.ULisboa, Faculty of Farmacy, University of Lisbon, Av. Professor Gama Pinto. 1649-003 Lisboa, Portugal
2) Center for Neurosciences and Cell Biology, University of Coimbra. Rua Larga – Faculdade de Medicina, Pólo I, 1º andar, 3004-504 Coimbra, Portugal
3) Department of Informatics, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisboa, Portugal
4) University of Ferrara. Department of life sciences and biotechnology. Via Fossato di Mortara 64A, quarto piano. 44121 - Ferrara, Italia
5) Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

The ubiquitin proteasome system is a nonlysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle and apoptosis but also in inflammatory and immune processes, carcinogenesis, among other clinical situations. Usually in protein homeostasis the defective proteins are ubiquitinated and are proteolysed into short peptides by the proteasome. Proteasome substrates include, for example, signalling molecules, tumour suppressors, cell cycle regulators and transcription factors. Proteasome inhibition results in an interruption of the degradation of these substrates, leading to activation of apoptotic pathways and, eventually, cell death. Rapidly growing cells, such as cancer cells, are particularly susceptible to proteasome inhibition mechanisms.[1][2]

This work relies on a computational-based drug discovery approach to find alternative new, selective (and more effective) small molecules as reversible proteasome inhibitors that can overcome the severe adverse drug reactions demonstrated by in use drugs. The efforts to discover new anticancer drugs described here combine different computer-aided drug design techniques (i.e. molecular docking, pharmacophore modeling, structure-based virtual screening and molecular descriptors calculation) in order to identify potential hit compounds (picture below). The selected compounds were tested in cell growth inhibition assays, being also performed inhibition assays for the chymotrypsin-like, trypsin-like and caspase-like activities of the proteasome using fluorogenic substrates.



*Acknowledgements:*Fundação para a Ciência e a Tecnologia (SFRH/BD/104441/2014, PTDC/QEQ-MED/7042/2014, UID/DTP/04138/2013, SAICTPAC/0019/2015). Salvador J.A.R thanks PT2020 (Programa Operacional do Centro 2020), and the financial support by FEDER (COMPETE 2020 Programme), project CENTRO-01-0247-FEDER-003269, drugs2CAD.

References

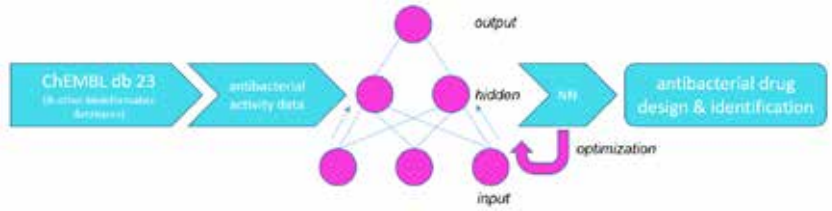
1) B. Ruggeri; S. Miknyoczki; B. Dorsey; A.-M.Hui. Adv Pharmacol.,57, 91-135 (2009)
2) E.M. Huber; M. Groll. Angew. Chem. Int. Ed. Engl, 51, 8708–20 (2012).

DEEP LEARNING APPLICATIONS IN THE DESIGN AND IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS

Marko Jukic, Stanislav Gobec

University of Ljubljana, Faculty of Pharmacy, Department of Medicinal Chemistry, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia

Contemporary medical practice is elevating the need for antibacterial drugs and with it, an imminent upsurge of bacterial resistance is observed. [1] In lieu of diminished effectiveness of antibacterials in *materia medica*, identification of novel antibacterial compounds serves as a crucial topic for research investment. [2] Deep learning as a selection of machine learning techniques can produce high level abstractions from large and heterogeneous data sets of high-dimensions and methodology is suitable for composite property predictions. [3] Last-mentioned input data set was collected from expanding ChEMBL v23 database where pruned libraries of antibacterial compounds were constructed. [4] Using Keras neural networks Python API and multiple bioinformatics software packages, molecular fingerprints and descriptors were calculated and served as input data for training, testing and optimization of deep neural network models. [5] Constructed models were able to identify compounds with or without antibacterial activity against Gram-positive (S. aureus) or Gram-negative (E. coli) bacteria with higher accuracy when compared to several alternative or classical QSAR approaches. We were also able to prospectively study the antibacterial properties of in-house databases and confirm the results with *in vitro* antimicrobial evaluation on relevant bacterial strains. Furthermore, we postulate the application of reported methodology for library enrichment and antibacterial compound design.



References

1) J. M. A. Blair, M. A. Webber, A. J. Baylay, D. O. Ogbolu, L. J. V. Piddock, Molecular mechanisms of antibiotic resistance, Nature Reviews Microbiology, 13 (2015), 42–51.
2) E. D. Brown, G. D. Wright, Antibacterial drug discovery in the resistance era, Nature, 529 (2016), 336–343.
3) Y. LeCun, Y. Bengio, G. Hinton, Deep Learning. Nature. 521 (2015), 436-444.
4) A. Gaulton, A. Hersey, M. Nowotka, A. P. Bento, J. Chambers, D. Mendez, P. Mutowo, F. Atkinskon, L. J. Bellis, E. Cibrián-Uhalte, M. Davies, N. Dedman, A. Karlsson, M. P. Magariños, J. P. Overington, G. Papadatos, I. Smit, A. R. Leach, The ChEMBL database in 2017, Nucleic acids research, 45 (2017), D945-D954.
5) F. Chollet, F. (2015). Keras. url:https://keras.io.

P005

DEEP LEARNING FOR LIGAND-BASED DE NOVO DESIGN IN LEAD OPTIMIZATION: A REAL LIFE CASE STUDY

Quentin Perron (2), Olivier Mirguet (1), Anne Rojas (1), Arnaud Gohier (1), Pierre Ducrot (1), Philippe Gloanec (1), Marie-Pierre Bourguignon (1), Patricia Sansilvestri (1), Hamza Tajmouati (2), Nicolas Do Huu (2), Yann Gaston-Mathé (2), Françoise Gellibert (1)

1) Institut De Recherche Servier, 125 Chemin de Ronde, 78290 Croissy, France
2) Iktos, 61 rue blomet 75015 Paris, France

Introduction:
Multi-Parameter Optimization (MPO) is a major challenge in New Chemical Entity (NCE) drug discovery projects, and the inability to identify molecules meeting all the criteria of lead optimization (LO) is an important cause of NCE project failure. Several ligand- and structure-based de novo design methods have been published over the past decades, some of which have proved useful multiobjective optimization (ref 1, 2). However, there is still need for improvement to better address the chemical feasibility of generated compounds as well as increasing the explored chemical space while tackling the MPO challenge.

Recently, promising results have been reported for deep learning generative models applied to de novo molecular design (ref 3), but until now, to our knowledge, no report has been made of the value of this new technology for addressing MPO in an actual drug discovery project.

Our objective in this study was to evaluate the potential of a ligand-based de novo design technology using deep learning generative models to accelerate the discovery of an optimized lead compound meeting all in vitro late stage LO criteria.

Materials and methods:
The project data set comprised 880 molecules tested on 11 biological assays, with variable rates of missing data: 1 activity criteria (phenotypic assay) (40%), 6 selectivity criteria (58%), 4 DMPK criteria (microsomal stability and permeability) (53%) assays. No compound was simultaneously meeting all predefined success criteria. 3 molecules were satisfying 9/11 objectives.

Single task QSAR models were developed based on the data set for all 11 objectives. Iktos molecule generator, a proprietary algorithm using deep learning generative models, was then used to design virtual molecules fulfilling all 11 objectives according to a multi-objective fitness function built from the predictive QSAR models.

From the virtual molecules proposed by the generator, 20 molecules were selected for synthesis, based on activity predictions, molecular diversity, and synthetic accessibility. They were then tested on all 11 assays and evaluated with regards to the predefined objectives. Performances were compared to the molecules previously tested.

Results:
150 virtual compounds predicted to meet all 11 objectives simultaneously were proposed by Iktos molecule generator. 11 compounds were synthesized and tested.

For most of the objectives, the new molecules outperformed the molecules of the initial dataset, including the 50 most recent ones. Average number of objectives hit was 9.5 for the new molecules vs. 5.9 previously. Hit rate was >90% for all selectivity and permeability targets and 65% for activity. Stability however was decreased with a 55% hit rate. More importantly, in the 11 new compounds, 1 met simultaneously all 11 objectives of the project, and 2 met 10/11 objectives and were just below the required threshold, within the margin of error of the assay, regarding the last objective.

Conclusions and perspectives:
To our knowledge, this is the first report of a successful application of deep learning for de novo design to solve an MPO issue in an actual drug discovery project, moreover on a large number of objectives. This is a demonstration of the potential of this technology to bring substantial improvements to medicinal chemistry. The use of such approach in the earlier phases (hit to lead, early LO) is under investigation.

References

1) Firth N.C. et al. MOARF, an Integrated Workflow for Multiobjective Optimization: Implementation, Synthesis, and Biological Evaluation. J. Chem. Inf. Model. 2015
2) Nicolaou, C. A., Brown, N. Multi-Objective Optimization Methods in Drug Design. Drug Disc. Today Technol. 2013
3) Chen, H. et al. The rise of deep learning in drug discovery Drug Disc. Today 2018

P006

ARTIFICIAL INTELLIGENCE IN MEDICINAL CHEMISTRY: A REAL AVENUE FOR SPEEDING UP NEURODRUGS DISCOVERY PROCESS

Víctor Sebastián Pérez (1), Maria Jimena Martinez (2), Fiorella Cravero (3), Carlos Requena (1), Carlos Roca (1), Mónica F. Diaz (3,4), Juan Antonio Páez (5), Ramón Gómez Arrayás (6,7), Javier Adrio (6,7), Carnen Gil (1), Ana Martínez (1), Ignacio Ponzoni (2,8), Nuria E. Campillo (1)

1) Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040, Madrid, Spain
2) Instituto de Ciencias e Ingeniería de la Computación (ICIC), UNS-CONICET, Bahía Blanca, Argentina
3) Planta Piloto de Ing Química (PLAPIQUI), UNS-CONICET, Co. Carrindanga km.7, CC 717, Bahía Blanca, Argentina
4) Departamento de Ingeniería Química, Universidad Nacional del Sur (UNS), Bahía Blanca, Argentina
5) Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006, Madrid, Spain
6) Depto. de Química Orgánica, (UAM). Cantoblanco, 28049, Madrid, Spain
7) Institute for Advanced Research in Chemical Sciences (IAdChem), UAM, 28049, Madrid, Spain
8) Departamento de Ciencias e Ingeniería de la Computación, Universidad Nacional del Sur (UNS)

Artificial Intelligence (AI) has recently become an essential part of the technology industry, solving many challenging problems in computer sciences. Also, the biopharmaceutical industry is looking toward AI to speed up drug discovery, cut R&D costs, decrease failure rates in drug trials and creates better medicines.

In this sense, machine learning (ML) approaches have emerged as very powerful tools that can be applied in several steps of the iterative drug discovery process[1,2] (Figure 1), such as Quantitative Structure Activity Relationship (QSAR) for the prediction of activity of large untested databases, discovery of hit compounds or synthesis prioritization for lead optimization. In order to reduce attrition rate in later stages of drug discovery and avoid compounds with undesirable properties, the development of QSPR models for the prediction of the pharmacokinetic and toxicological (ADMET) profile plays also a key role in lead optimization.[3]

For this purpose, in this work we have developed several QSXR models (X: activity, enantiomeric excess and ADME properties) to optimize drug discovery process in neurodrugs.

The first model was developed to identify inhibitors of BACE1. The work-set includes compounds with a representative chemical space and a wide variety of drug-like properties available from different databases. Models were obtained by the application of several ML methods, model hybridizing strategies, combinatorial analysis and visual analytics. A performance of 85% for corrected classified compounds and ROC value of 0.88 was obtained. Our approach contributes to achieve a QSAR model that can be a useful virtual screening method for prediction of BACE1 inhibitors with a wide applicability domain.

Once the hit is identified, hit optimization process is carried out using chemical synthesis where several ML methods can be developed to predict the outcome of the reaction.[4] Also, ADME properties are essential in lead optimization. One of the critical steps in QSXR modeling is the identification of the most informative molecular descriptors. For this purpose, two main general approaches can be used: feature selection and feature learning.[5] To address both issues, a performance comparative study of two state-of-art methods taking into account these two approaches was carried out using different databases at these stages of the drug discovery process. These databases include enantiomeric excess in the chemical synthesis and blood brain barrier or human intestinal absorption in physicochemical properties assessment. Regression and classification models were built for the three datasets using both approaches together with their potential hybridization to analyze which technique achieves a better performance to be further applied.[6]

References

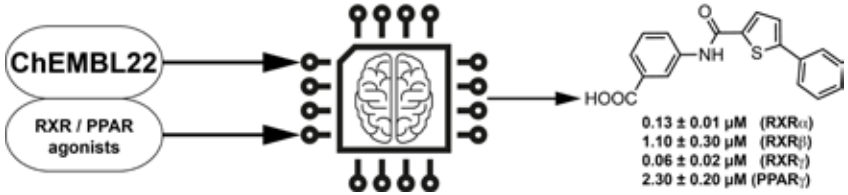
1) Nature 2017; 549(7673):445-447.
2) Nat Rev Drug Discovery 2018; (17):97–113.
3) Curr Top Med Chem 2006; 6(15):1579–1591.
4) ACS Cent. Sci 2017; 3 (5): 434–443.
5) J Chem Inf Model 2014; 54(3): 837-843.
6) Sci Rep 2017; 7(1): 2403: 1-19

PROSPECTIVE APPLICATIONS OF ARTIFICIAL INTELLIGENCE IN DE NOVO MOLECULAR DESIGN FOR DRUG DISCOVERY

Lukas Friedrich, Daniel Merk, Gisbert Schneider

Swiss Federal Institute of Technology (ETH), Department of Chemistry and Applied Biosciences, Vladimir-Prelog-Weg 4, 8093 Zurich, Switzerland

Modern instances of artificial intelligence (AI) (e.g., deep learning) and the availability of large chemical and biological datasets enable the development of innovative concepts in drug discovery and development.[1] We have applied a so-called "generative" deep learning model based on a deep recurrent neural network (RNN) containing long short-term memory (LSTM) for *de novo* molecular design.[2] This computational model was first trained to capture the grammar of SMILES representations of bioactive small molecules, and then used to automatically generate SMILES strings of new chemical entities (NCEs). By means of transfer learning, the model could be fine-tuned to create target-focused sets of molecules. In a pioneering prospective study,[3] the generative RNN was trained on bioactive molecules (540'000) from a public compound database (ChEMBL22) and further fine-tuned with a small set of 25 known agonists of retinoid X and peroxisome proliferator-activated receptors (RXR, PPAR). The *de novo* designs generated by this model were ranked computationally, and five top-ranked compounds were synthesized. Four out of five molecules showed nano- to micromolar potencies against the studied targets (RXR and/or PPAR) with distinct activity profiles. In further prospective studies, we applied generative AI models to the *de novo* design of bioactive natural product mimetics. The computer-generated NCEs resemble structure of the natural product and inherited the bioactivity profile of their template. Our results highlight generative AI as an innovative knowledge-driven approach to obtain pharmacologically relevant NCEs.[4]



References

1) E. Gawehn, J. A. Hiss, G. Schneider, Mol. Inf. 2016, 35, 3–14
2) A. Gupta, A. T. Müller, B. J. H. Huisman, J. A. Fuchs, P. Schneider, G. Schneider, Mol. Inf. 2018, 37, 1700111
3) D. Merk, L. Friedrich, F. Grisoni, G. Schneider, Mol. Inf. 2018, 37, 1700153.
4) G. Schneider, Nat. Rev. Drug Discov. 2018, 17, 97-113

NOTES

POSTERS - TECHNOLOGIES

Timing is Everything: Target Binding Kinetics and Pharmacokinetics

P010

ANALYSIS OF PROTEIN TUNNELS AND LIGAND BINDING TRAJECTORIES IN DRUG DESIGN

Piia Kokkonen (1,2), Jiri Filipovic (3), Ondrej Vavra (1), Adam Jurcik (4), Sergio Marques (1,2), Gaspar Pinto (2), Barbora Kozlikova (4), Jan Brezovsky (1), David Bednar (1,2), Jiri Damborsky (1,2)

1) Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Kamenice 5/A13, 625 00 Brno, Czech Republic
2) International Clinical Research Center, St. Anne's University Hospital Brno, Pekarska 53, 656 91 Brno, Czech Republic
3) Institute of Computer Science, Masaryk University, Botanicka 554/68a, 602 00 Brno, Czech Republic
4) Human Computer Interaction Laboratory, Faculty of Informatics, Masaryk University, Botanicka 68a, 602 00 Brno, Czech Republic

Protein tunnels and gates are attractive targets for drug design [1]. Tunnels are important for the transport of ligands, solvent and ions, and can be found in many enzymes, ion channels and membrane proteins. To study the protein tunnels using a user-friendly graphical interface we have developed Caver Analyst 2.0 [2]. Caver Analyst can be used to identify tunnels in both static structures as well as molecular dynamics trajectories. Studying tunnels in protein assemblies from molecular dynamics simulations offers possibilities to observe transient tunnels and their changes in time. To study the transport of ligands through the protein tunnels, we have developed CaverDock [3,4]. CaverDock is fast, robust and accurate tool which allows the screening of binding and unbinding processes for pharmacologically interesting compounds. It is based on a modified AutoDock Vina algorithm [5] and we have previously successfully tested it with many pharmaceutically interesting targets, such as cytochrome P450 17A1 and leukotriene A4 hydrolase/aminopeptidase [6]. CaverDock is efficient method for virtual screening of compounds: one simulation took on average less than an hour and >90% of the studied cases led to a successfully calculated binding/unbinding trajectory. Caver Analyst 2.0 and CaverDock 1.0 are available free of charge at https://www.caver.cz/ and https://loschmidt.chemi.muni.cz/caverdock/.

References

1) Marques, S.M., et al. 2016: Enzyme Tunnels and Gates as Relevant Targets in Drug Design. Medicinal Research Reviews 37: 1095-1139.
2) Jurcik, A., et al. 2018: Caver Analyst 2.0: Analysis and Visualization of Channels and Tunnels in Protein Structures and Molecular Dynamics Trajectories. Bioinformatics (under review).
3) Vavra, O., et al. 2018: CaverDock: A New Tool for Analysis of Ligand Binding and Unbinding Based on Molecular Docking. (in preparation)
4) Filipovic, J., et al. 2018: A Novel Method for Analysis of Ligand Binding and Unbinding Based on Molecular Docking (in preparation)
5) Trott, O. and Olson, A.J., 2010: AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization and Multithreading. Journal of Computational Chemistry 31: 455-461.
6) Pinto, G., et al. 2018: Screening of Binding and Unbinding of Inhibitors via Tunnels and Channels using Novel Software CaverDock. ChemMedChem (under review).

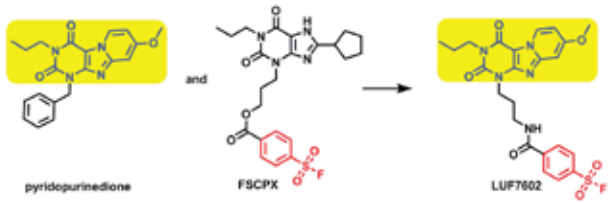
P011

A COVALENT ANTAGONIST FOR THE HUMAN ADENOSINE A3 RECEPTOR

Xue Yang, Jacobus P.D. van Veldhoven, Boaz J. Kuiper, Jelle Offringa, Eelke B. Lenselink, Laura H. Heitman, Daan van der Es , Ad P. IJzerman

Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research, Leiden University, Einsteinweg 55, 2333 CC Leiden, the Netherlands

The human A_3 adenosine receptor (hA_3R) plays an important role in both physiological and pathophysiologic conditions, such as cell proliferation, cell differentiation, neuroprotection, cardioprotection, and apoptosis.[1] In the past we have searched for potent and selective hA_3R antagonists, leading to a set of structurally diverse antagonist classes. In particular, tricyclic xanthine derivatives of 1H,3H-pyrido[2,1-*f*]purine-2,4-dione have been reported to exert high affinity and selectivity for hA_3R.[2,3] Building on these results, we report an analog, LUF7602, equipped with a reactive electrophilic fluorosulfonyl functionality, as a selective covalent antagonist of hA_3R.



In a radioligand binding assay, this ligand acted as a potent antagonist, with an apparent affinity for the hA_3R in the nanomolar range. Its apparent affinity increased with longer incubation time, suggesting an increasing level of irreversible binding over time. An *in* silico hA_3R-homology model was used to study the binding mode, indicating that a tyrosine residue $Y265^{7.36}$ was responsible for the covalent bond formation. Site-directed mutagenesis was performed to demonstrate that the amino acid residue was the unique anchor point of the covalent interaction. Subsequently, LUF7602 was tested in $[^{35}S]GTP\gamma S$ functional assays. Preincubation with LUF7602 caused a concomitant decline in the agonist's maximal response, indicating insurmountable antagonism, another proof of the covalent receptor labeling. In contrast, coincubation with this antagonist generated a parallel rightward shift of the agonist's concentration-effect curve with no alteration of the maximal effect, suggesting the insurmountable antagonism was competitive, due to an irreversible blockade to reduce the total receptor population available.

All these data contribute to a better understanding of the covalent interaction between LUF7602 with the receptor. This covalent antagonist may serve as a valuable molecular translational tool for further investigating the role of hA_3R in different pathophysiological conditions.

References

1) Borea, P. A.; Varani, K.; Vincenzi, F.; Baraldi, P. G.; Tabrizi, M. A.; Merighi, S.; Gessi, S. The A3 adenosine receptor: history and perspectives. Pharmacol. Rev. 2015, 67, 74-102.
2) Xia, L.; Burger, W. A. C.; van Veldhoven, J. P. D.; Kuiper, B. J.; van Duijl, T. T.; Lenselink, E. B.; Paasman, E.; Heitman, L. H.; IJzerman, A. P. Structure-affinity relationships and structure-kinetics relationships of pyrido[2,1-f]purine-2,4-dione derivatives as human adenosine A3 receptor antagonists. J. Med. Chem 2017, 60, 7555-7568.
3) Priego, E. M.; Kuenzel, J. V.; IJzerman, A. P.; Camarasa, M. J.; Perez-Perez, M. J. Pyrido[2,1-f]purine-2,4-dione derivatives as a novel class of highly potent human A3 adenosine receptor antagonists. J. Med. Chem 2002, 45, 3337-3344

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - TECHNOLOGIES

New Chemical Modalities in Medicinal Chemistry

P013

BORONIC ESTER MACROCYCLES AS NEW E.COLI TYPE I SIGNAL PEPTIDASE INHIBITORS. SYNTHESIS, BIOLOGICAL EVALUATION AND CONFORMATION OF MACROCYCLIZATION

Andrea Benediktsdottir (1), Mate Erdelyi (2), Edouard Zamaratski (1), Natalia Szalaj (1), Lu Lu (3), Sha Cao (4), Gustav Olanders (1), Sherry L. Mowbray (3), Peter Brandt (1), Anders Karlén (1)

1) Uppsala University, Department of Medicinal Chemistry, Box 574, 751 23 Uppsala Sweden.
2) Uppsala University, Department of Chemistry, BMC, Box 576, SE-751 23 Uppsala, Sweden
3) Uppsala University, Department of Cell and Molecular Biology, BMC, Box 596, SE-751 24 Uppsala, Sweden
4) Uppsala University, Department of Medical Biochemistry and Microbiology, BMC, Box 582, SE-751 23 Uppsala, Sweden

Bacterial type I signal peptidase, with its vital role in bacterial viability, is a promising antibacterial drug target. In the environment of steadily growing antimicrobial resistance, we developed novel macrocyclic oligopeptides capable of *E.coli* type I signal peptidase (*Ec*LepB) inhibition and exhibiting good antibacterial activity. We designed unique macrocyclic boronic esters based on previously published linear lipopeptidic *Ec*LepB inhibitors.[1] Macrocyclization was confirmed by mass spectrometric and NMR analyses of a polyalanine analogue, which has a simplified structure that enabled unambiguous and quick spectral analyses. ^{1}H NMR assignment was performed using a NOESY-TOCSY backbone walk, and further corroborated by the cross peaks observed in gCOSY, ^{1}H,^{13}C- and ^{1}H,^{15}N-gHSQC, and ^{1}H,^{13}C- and ^{1}H,^{15}N-gHMBC spectra. Among the synthesized macrocycles, we identified potent enzyme inhibitors in the low nanomolar range and with good antimicrobial activity. We also explored structural modifications influencing toxicity and hemolytic activity.

References

1) M. De Rosa, L. Lu, E. Zamaratski, N. Szałaj, S. Cao, H. Wadensten, L. Lenhammar, J. Gising, A.K. Roos, D.L. Huseby, R. Larsson, P.E. Andrén, D. Hughes, P. Brandt, S.L. Mowbray, A. Karlén, Design, synthesis and in vitro biological evaluation of oligopeptides targeting E. coli type I signal peptidase (LepB), Bioorg. Med. Chem. 25 (2017) 897–911. doi:10.1016/j.bmc.2016.12.003.

P014

NOVEL SMALL MOLECULE SHIP2 INHIBITORS FOR TREATMENT OF TYPE 2 DIABETES

Mika Berg (1), Jette-Britt Naams (2), Laura Hautala (2), Kristiina Wähälä (1,3), Sanna Lehtonen (2)

1) Department of Chemistry, University of Helsinki, Helsinki, Finland
2) Department of Pathology, University of Helsinki, Helsinki, Finland
3) Department of Biochemistry and Development Biology, University of Helsinki, Helsinki, Finland

One of the fastest growing major human health issues in the world is diabetes of which over 90 % represents type 2 diabetes mellitus (T2DM). Recently, diabetes has been classified into five refined sub-groups to be able to individualize the treatment of this metabolic disorder. Patients with T2DM suffering from severe insulin resistance, the inability of the body to fully respond to insulin, have the highest risk of diabetic kidney diseases. However, all T2DM patients have been prescribed similar diabetes treatment. The new classification of diabetes enables development of new effective and more individualized treatments.[1]

Lipid phosphatase SHIP2 (SH2 domain-containing inositol 5´-phoshatase 2) is a negative regulator of the insulin signalling pathway. It is upregulated in muscle, adipose and kidney tissue in experimental models of diabetes.[2]In our previous studies[3]we showed that metformin, the well-known anti-diabetic drug, increases the insulin sensitivity of peripheral tissues by inhibiting the catalytic activity of SHIP2. Our data indicate that SHIP2 is a potential therapeutic target for the treatment of insulin resistance in T2DM. To date, however, only few small molecule SHIP2 inhibitors have been identified, and they possess poor bioavailability and pharmacokinetic properties and none of them are in clinical use.[4]

We have designed and synthesized a molecular library of various diaryl compounds and their derivatives with a common core unit as new potential SHIP2 inhibitors. To demonstrate that these compounds act as SHIP2 inhibitors in vitro, we tested their efficiency to inhibit the catalytic activity of SHIP2 using both purified SHIP2 fusion protein and cultured cells. We also tested their ability to activate the insulin signalling pathway. These compounds are potent SHIP2 inhibitors and support the role of SHIP2 as an excellent target to treat insulin resistance. These SHIP2 inhibitors can be used as an avenue to identify and design novel molecules that can be used to develop new insulin sensitizers for future clinical trials.

References

1) Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., Vikman, P., Prasad, R. B., Mansour Aly, D., Almgren, P., Wessman, Y., Shaat, N., Spégel, P., Mulder, H., Lindholm, E., Melander, O., Hansson, O., Malmqvist, U., Lernmark, Å., Lahti, K., Forsén, T., Tuomi, T., Rosengren, A. H. and Groop, L. (2018), Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables, Lancet Diabetes Endocrinol., 6, 361-369.
2) Hyvönen, M. E., Saurus, P., Wasik, A., Heikkilä, E., Havana, M., Trokovic, R., Saleem, M., Holthöfer, H. and Lehtonen, S. (2010), Lipid phosphatase SHIP2 downregulates insulin signalling in podocytes, Mol. Cell. Endocrinol., 328, 70-79.
3) Polianskyte-Prause, Z., Tolvanen, T. A., Lindfors, S., Latvala, M., Wang, H., Dash, S. N., Berg, M., Dumont, V., Nisen, H., Mirtti, T., Groop, P-H., Wähälä, K., Tienari, J. and Lehtonen, S., Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity (submitted) 2018.
4) Viernes, D.R., Choi, L.B., Kerr, W.G. and Chisholm, J.D. (2014), Discovery and development of small molecule SHIP phosphatase modulators, Med. Res. Rev., 34, 795-824.

DESIGN OF NOVEL PEPTIDE DRUG CONJUGATE WARHEADS AS NOVEL POTENTIAL ANTICANCER AGENTS

Béla Bertók (1), György Dormán (1,2), Gábor Mező (3), László Kőhidai (4), Orsolya Láng (4), Csaba Magyar (5)

1) ComInnex Inc., H-1031 Budapest, Záhony utca 7., Hungary. bela.bertok@cominnex.com
2) Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Szeged, H-6720 Szeged Zrínyi u. 9., Hungary
3) Faculty of Science, Institute of Chemistry, Eötvös Lóránd University, H-1117 Budapest, Pázmány P. stny. 1/A., Hungary
4) Semmelweis University, H-1085 Budapest, Üllői út 26., Hungary
5) Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1117 Budapest, Magyar Tudósok Körútja 2., Hungary

Over the 10 years ComInnex specialized on developing high quality drug-like libraries (typically 100 – 300 members per library) in order to cover chemical space of potential biological targets effectively. The applied strategy focuses on non-flat 3-dimensional templates and libraries (Smart Diversity Approach™) that result in screening compounds with more favourable physicochemical properties, higher sp3/sp2 atom ratio, and novel 3-dimensional shapes with various functionalities.

Next to the Antibody-Drug Conjugates (ADCs) peptide based Small Molecule-Drug Conjugates (SMDCs) were developed for targeted tumour therapy. Peptide homing devices (e.g. hormone peptides) were conjugated with potential cytotoxic „warheads" through cleavable linkers [1]. In order to discover novel drugs our objective was to develop potential apoptosis agonist small molecules using our design concept. In the initial library generation process we focused on meeting the following requirements: lead-like properties, synthetic feasibility, novelty and diversity. Our goal was to develop cytotoxic compounds combined with the strategy of ensuring the selectivity via linking to appropriate targeting peptides.

In an initial compound set several hundred diverse compounds were synthesized. After stability assessment and strict quality control (purity: > 95 %) and LogD/LogS assessment 139 compounds containing linker connection functional groups were finally selected and submitted for cytotoxicity tests on PANC1 (pancreas tumour) cell line. For cytotoxicity assay real-time, impedance-based cell analysis was applied.

The identified hit compounds were subjected to a virtual target identification process based on the structural similarities to existing compounds acting on known biological targets. One of the novel compound groups showing activity are similar to XIAP (X-linked inhibitor of apoptosis) blockers, thus, as a result may activate the apoptosis machinery as well.

Based on these findings our attention turned to designing a focused XIAP inhibitor targeted compound library. The available crystal structure of XIAP allowed to generate 3D models in order to identify the major interactions of the hit compounds and allowing to design more effective XIAP antagonists.

In the present poster we report the preliminary results of the new combined concept and the 3D structure-based hit and virtual screening model.

This work is supported by Hungarian grant (National Research, Development and Innovation Office: National Competitiveness and Excellence Program, #NVKP16-1-2016-0036).

References

1) Mező G, Manea M., Expert Opin. Drug Deliv., 2010, 7, 79-96

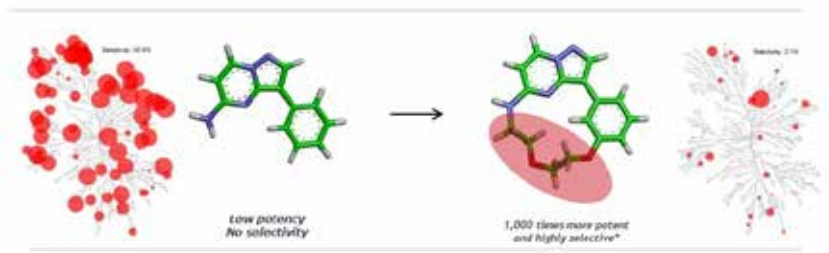
NANOCYCLIX®: NEXT GENERATION KINASE INHIBITORS FOR THE PROBE BASED DRUG DISCOVERY

Petra Blom, Pascal Benderitter, Nicolas Faucher, Marie-Hélène Fouchet, Fabrice Viviani, Alexis Denis, Jan Hoflack, Philippe Genne

Oncodesign, 20 rue Jean Mazen BP27627, 21076 Dijon

Macrocycles have been emerging as a valuable class of pharmacological agents over the past decade. A growing appreciation of potent and selective protein-ligand interactions, which are not easily addressed using small molecules, calls for the development of inhibitors that are more sophisticated than traditional open form, non-macrocyclic small molecules. Macrocyclic kinase inhibitors have reached advanced clinical testing and are having a significant impact in different disease areas such as oncology and inflammation. The number of reports of innovative macrocycles in preclinical research is continuously increasing in literature.

At Oncodesign, macrocyclisation is systematically applied within all projects leading to a Nanocyclix® platform. Restriction of the conformational freedom in small molecules can result in high affinity and selectivity for various classes of biological targets such as, but not only, kinases. Bioactive Nanocyclix® are designed with the goal of pre-organizing their three dimensional shape into a well-defined conformation. The rationale is to diminish entropic penalties in the course of a protein/ligand interaction, which occur between the proteins active pocket and the macrocyclic ligand. The conformational rigidity also results in a high degree of specificity, not only between target classes, but also in a very high degree of selectivity within a target class such as kinases and even within closely related sub kinase families (wild-type forms and/or mutants). This high degree of selectivity is based on three dimensional shape complementarity between the kinases active pocket and the Nanocyclix® ligand.



The Nanocyclix® chemistry technology comprises the generation of compounds through a macrocylization process which results in small, low MW kinase inhibitors with a unique binding mode and mode of action compared to the open form kinase inhibitors. Not only the Nanocyclix® technology is used in the search for therapeutically active agents, but also in the identification of novel, clinically relevant PET tracers.

In this presentation, we describe the Nanocyclix® platform, characteristics of the technology, applied medicinal chemistry approaches as well as examples in different projects.

P017

NOVEL BENZNIDAZOL ANALOGES AS TRYPANOCIDAL AGENTS

Marcelly C. S. Rezende (1,2), Debora I. Leite (1,2), Mariana A. A. Aleixo (5), Policarpo A. S. Junior (3), Maria de Nazare Correia Soeiro (4), Maria C. Nonato (5), Alcione S. de Carvalho (2), Núbia Boechat (2)

1) Universidade Federal do Rio de Janeiro, Centro de Ciencias da Saude, Instituto de Ciencias Biomedicas, Programa de Pos-graduacao em Farmacologia e Quimica Medicinal
2) Fundacao Oswaldo Cruz, Instituto de Tecnologia em Farmacos, Departamento de Sintese de Farmacos
3) Fundacao Oswaldo Cruz, Instituto Rene Rachou, Laboratorio de Parasitologia Celular e Molecular
4) Fundacao Oswaldo Cruz, IOC, Laboratorio de Biologia Celular
5) Universidade de Sao Paulo, Faculddade de Ciencias Farmaceuticas de Ribeirao Preto, Lab. Cristalografia de Proteinas

The migration patterns of people from South and Central America to North America, Asia and Europe have changed the outlook of Chagas disease (CD), an illness caused by infection of the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) [1-2]. The available drugs for treatment of CD, benznidazole (**Bnz**) and nifurtimox are toxic and not active on the chronic phase, and cases of resistant have been described. In Brazil, only **Bnz** is used for treatment of CD. Therefore, the search for new analogues of this drug could be an important approach. In this work, we report the synthesis, evaluation on the enzyme nitroreductase (TcNTR) and against *T. cruzi* amastigote and trypomastigote forms of novel *N*-acylhydrazone-2-nitroimidazoles (**1-9**) and 1,2,3-triazoles derivatives (**10-12**) as analogue of **Bnz** (Figure 1). The substitution of the amide group in **Bnz** by a 1,2,3-triazolic ring (**10-12**) could be justified because they present similar chemical properties, volume and planarity.

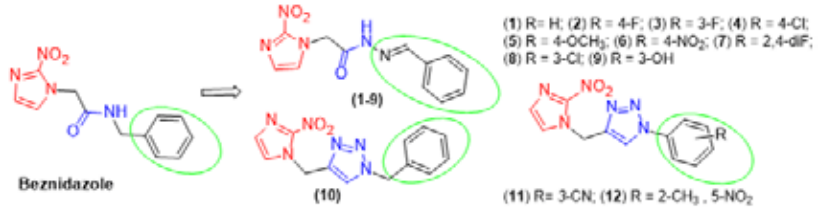


Figure 1. Design of 2-nitroimidazoles (**1-12**) analogue of Bnz.

All synthesized compounds were evaluated against *T. cruzi* amastigote and trypomastigote forms in a single assay (Table 1). To evaluate the mammalian cytotoxicity and to determine the selectivity index (SI), compounds were tested against murine fibroblasts (L929 cell line). The SI was calculated as the ratio of CC_{50} for L929 cells to IC_{50} for parasite. The assay against *T. cruzi* showed analogues **9** and **11** equipotent to **Bz**. Interestingly, the nitroreductase enzymatic (TcNTR) evaluation of analogs no indicated a good correlation with *T. cruzi* assay, thus identifying that the mechanism of action of the novel compounds could be otherwise. However, **2**, **3** and **7** analogues were excellent substrate of *TcNTR*.

Table 1: *In vitro* effect of some compounds on the growth of *T. cruzi* and on the *TcNTR* enzymatic assay (20 µg/mL TcNTR, 100 µMol/L NADH, 200 µMol/L of compound).

Compounds	*T. cruzi* (Tulahuen) IC_{50} (mM)	L929 cells CC_{50} (mM)	SI	Kobs	error	Activity (%)
Bzn	**3.8 ± 0.8**	**2381**	**626**	**0.063**	**0.001**	**100.00**
4	**17.1 ± 3.4**	**> 200**	**> 11.7**	**0.04**	**0.01**	**60**
5	**11.6 ± 4.8.2**	**483± 223.4**	**41.6**	**0.026**	**0.009**	**40**
6	**82.9 ± 16.3**	**> 800**	**> 9.6**	**0.03**	**0.01**	**50**
9	**5.4 ± 1.3**	**645.5 ± 6.4**	**119.5**	**0.060**	**0.0006**	**44**
11	**4.4 ± 1.7**	**182**	**42**	**-**	**-**	**-**

The authors thank the CNPq, CAPES and FAPERJ for financial support.

References

1) P. J. Hotez, E. Dumonteil, L. Woc-Colburn, et al., PLoS Negl. Trop. Dis. 2012, 6, e1498
2) WHO | Chagas disease (American trypanosomiasi.s). WHO 2017

P019

NOVEL, DIFFERENTIATED ANTIBODY-DRUG CONJUGATE WARHEADS FROM ANALYSIS OF THE NCI SCREENING DATABASE

Daniel Brough, Michael. J. Waring

Northern Institute for Cancer Research, School of Chemistry, Newcastle University, Newcastle upon Tyne, U.K

Antibody-drug conjugates (ADCs) combine the selective nature of targeted therapies with potent cytotoxic warheads utilising linker technology to deliver selectively the warhead to the target.[1] Proof of concept has already been observed with licensed ADCs on the market, e.g. Kadcyla and Adcetris, giving confidence to the use of ADCs in cancer treatment, with many ADCs in clinical testing. Many of these ADCs consist of cytotoxic drugs derived from complex natural products, consequently leading to synthetically long and complicated routes.[2] To address this problem, we have identified novel warheads, with the use of screening the NCI database, that are structurally simpler drugs with differing cellular activity profiles. This may also address resistance issues. Seven compounds were selected and two have been explored initially,-: nitroacridines and quinolones. Nitroacridines inherently target hypoxic cells and are known to intercalate with DNA, while quinolones are known tubulin binders.[3][4] Our work has focused on synthesis of these cytotoxic warheads as non-cleavable and cleavable drugs as well as a cleavable dipeptide linker with an aim to conjugate with antibodies and test across a cell panel. The parent compounds have been tested along with their non-cleavable counterparts in MCF-7 cell lines confirming cytotoxicity for the parent compounds only.

References

1) Casi. G. and Neri. D. J.Control.Release. 2012, 161, 422
2) Polakis, P. Pharmacol.Rev. 2016, 68, 3
3) Denny. W. A. J.Med.Chem. 33 1990, 33, 1288.
4) Li. L. J.Med.Chem. 1994, 37, 3400

NEW BIONET COMPOUNDS FOR CNS DISEASES

Nikolay T. Tzvetkov (1), Steve Brough (2)

1) NTZ Lab Ltd., Krasno selo 198, 1618 Sofia, Bulgaria
2) Key Organics Ltd, Highfield Road Industrial Estate, Camelford, Cornwall, PL32 9RA, UK

A new collection of nine CNS active compounds, which have been recently licensed, is now available in the BIONET collection. This screening collection with experimentally-determined bioactivity, lipophilicity (LogP/D7.4), aqueous solubility, GIT and BBB permeability, chemical stability and toxicity will address the need of new, robust, and multipotent small molecules for the treatment and diagnosis of CNS diseases, such as Parkinson´s disease, Alzheimer´s disease, dementia and/or other neurodegenerative diseases [1–3]. NMR and LC-MS analysis allowed the careful control of the compound quality. Importantly, the newly discovered and well-validated
molecules are enriched in heterocyclic scaffolds and specifically substituted phenyl moieties commonly found in CNS drug candidates, and spans chemical space that minimally overlaps with existing commercial collections. In addition, the compounds are easily accessible and offer the possibility of broad structural diversities in order to further explore the chemical space within further biological screening on relevant CNS targets [4]. This poster will summarize the design, synthesis, biological activity, and experimental ADME features of this potential next generation compounds that are available for further screening and elaboration for CNS disease treatment.

References

1) Tzvetkov, N. T. PCT Patent WO 2014/107771 A1, NTZ Lab Ltd., 2014.
2) Tzvetkov, N. T.; Stammler, H.-G.; Neumann, B.; Hristova, S.; Antonov, L.; Gastreich, M. Crystal structures, binding interactions, and ADME evaluation of brain penetrant N-substituted indazole-5-carboxamides as subnanomolar, selective monoamine oxidase B and dual MAO-A/B inhibitors. Eur. J. Med. Chem., 2017, 127, 470–492.
3) Tzvetkov, N. T.; Hinz, S.; Küppers, P.; Gastreich, M.; Müller, C. E. Indazole- and indole-5-carboxamides: Selective and reversible monoamine oxidase B inhibitors with subnanomolar potency. J. Med. Chem., 2014, 57, 6679–6703.
4) Tzvetkov, N. T. and Antonov, L. Subnanomolar indazole-5-carboxamides inhibitors of monoamine oxidase B (MAO-B) continued: indications of iron binding, experimental evidence for optimized solubility and brain penetration. J. Enz. Inh. Med. Chem., 2017, 32(1), 960–967.

IN VITRO INHIBITORY POTENTIAL OF NOVEL OESTRANE DERIVATIVES ON HUMAN ALDO-KETO REDUCTASE SUPERFAMILY MEMBERS AKR1C1, AKR1C2 AND AKR1C3

Tomaž Büdefeld (1), Ildikó Bacsa (2), Erzsébet Mernyák (2), Tea Lanišnik Rižner (1)

1) Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia
2) Department of Organic Chemistry, University of Szeged, Dóm tér 8., H-6720 Szeged, Hungary

Aldo-keto reductase (AKR) family 1 members 1-3 (AKR1C1-3) are NAD(P)H-dependent oxidoreductases catalysing the conversion of variety of biologically active compounds. As such, AKR1C1-3 represent key enzymes involved in the pathophysiology (e.g. altered metabolism of steroid hormones and/or prostaglandins) of steroid hormone-dependent diseases, such as prostate and breast cancer and endometriosis, as well as chemoresistance to anticancer drugs and thus remain paramount therapeutic targets. Here, we used an *in vitro* enzyme assay to characterise the inhibitory potential of 37 compounds, 13ß-oestrone (E1), 13α-oestrone and 17-deoxy-13α-oestrone derivatives against AKR1C1-3. Catalytic activity of AKR1C1-3 was measured spectrophotometrically by monitoring the oxidation of artificial substrate 1-acenaphthenol in the presence of NAD^+ at 340 nm. Halogenation (Cl, Br, I) of the 13α-oestrone core at the C2 and/or C4 position increased the inhibitory potential with a level of inhibition being dependent on the target enzyme, nature of the halogen and substitution pattern of the ring A. The most potent inhibitors belonging to this class of compounds were 2-iodo, 4-bromo (AKR1C1, IC_{50}=0.727 μM), 2,4-dichloro (AKR1C1, IC_{50}=2.802μM; AKR1C2, IC_{50}=4.593 μM; AKR1C3, IC_{50}=12.792 μM), 2,4-dibromo (AKR1C1, IC_{50}=5.305 μM; AKR1C2, IC_{50}=7.330μM) and 4-iodo derivatives (AKR1C3, IC_{50}=12.277 μM). Halogenation of the 13ß-oestrone core at the C2 and/or C4 position increased the inhibitory potential, where 2,4-dichloro E1 showed the most potent inhibitory activity against AKR1C1 and AKR1C3 (AKR1C1, IC_{50}=1.572μM; AKR1C3, IC_{50}=6.307 μM) but had no effect on AKR1C2 activity. 17-Deoxy-13α-oestrone derivatives preferentially inhibited AKR1C2 with the 4-chloro derivative showing high activity (AKR1C2, IC_{50}=0.898 μM).

3-Methyl or –benzyl ethers of the monohalogenated 13α-oestrone derivatives displayed substantially weaker inhibitory potential compared to the parent halogenated compounds. Oestrone-derived compounds containing an azide (-N_3) or *O*-allyl group at the C15 position exerted pronounced inhibition of AKR1C2 activity, with IC_{50} values being 3.801 μM and 0.485 μM respectively, and only moderately (56%-63% inhibition at 100 μM) inhibited AKR1C1 and AKR1C3 activity.

The present study showed that halogenation of the 13α-and 13ß-oestrane core at the C2 and/or C4 position and functionalisation of C15 enhanced *in vitro* inhibitory properties of oestrane-derived inhibitors against AKR1C1-3, thus providing further guidance for design and development of more potent and specific inhibitors of AKR1C1-3.

P022

NEW PYRAZOLOPYRIMIDINE-SULFONAMIDES AGAINST PLASMODIUM FALCIPARUM

Luiz Pinheiro (1), Flavia Silveira (1), Kamilla Rogerio (1), Leonardo Carvalho (2), Núbia Boechat (1)

1) Fundacao Oswaldo Cruz, Instituto de Tecnologia em Farmacos, Dept. de Sintese de Farmacos, Rio de Janeiro, Brazil.
2) Fundacao Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratorio de Pesquisa em Malaria, Rio de Janeiro, Brazil.

We have synthesized a new series of quinoline-sulfadoxine hybrids, planned by molecular hybridization between the quinoline ring and the benzenesulfonamide moiety present in chloroquine and sulfadoxine. Compound **I** exhibited selectivity index (SI) values (1102.2) and IC_{50} (0.09 mM) higher than chloroquine (834.74; 0.46 mM). When evaluated against *P. berghei* malaria, it was inhibited the parasitemia by 49% on day 5 after inoculation, contributing to the discovery of new prototype.[1]

In order to obtain new compounds with anti-*P. falciparum* activity, we used the compound **I** to design the new 1*H*-pyrazolo[3,4-*d*]pyrimidine-sulfonamide derivatives (**1-9**). The quinoline ring was replaced by the system 1*H*-pyrazolo[3,4-*d*]pyrimidine by ring isosterism. An *N*-(4-aminobutyl)benzenesulfonamide moiety was attached at the 4-position of the 1*H*-pyrazolo[3,4-*d*]pyrimidine ring (Figure 1).

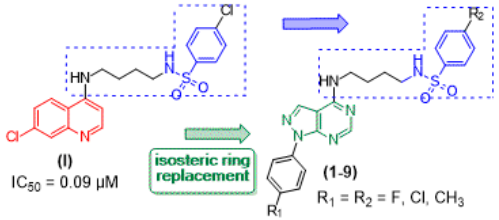
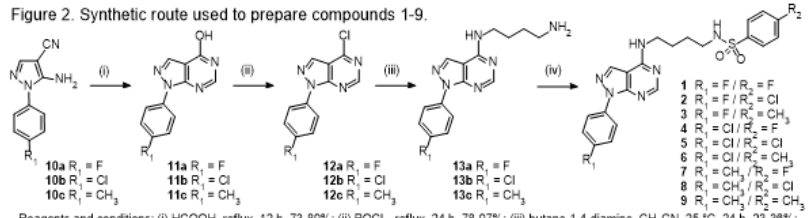


Figure 1. Rational approach to the design of compounds **1-9**.

The compounds **11a-c** could be prepared from the reaction of suitable 5-amino-pyrazoles (**10a-c**) and formic acid.[2] The derivatives **11a-c** were refluxed with $POCl_3$ to produce **12a-c**. The compounds **13a-c** were synthesized by the nucleophilic substitution reaction between **12a-c** and butane-1,4-diamine. The reaction[1] between **13a-c** and the appropriate sulfonyl chloride produced the target compounds **1-9** (Figure 2).

Figure 2. Synthetic route used to prepare compounds 1-9.



Reagents and conditions: (i) HCOOH, reflux, 12 h, 73-89%; (ii) $POCl_3$, reflux, 24 h, 78-97%; (iii) butane-1,4-diamine, CH_3CN, 25 °C, 24 h, 23-36%; (iv) appropriate sulfonyl chloride, DMF, TEA, 90 °C, 24 h, 25-79%.

Among the 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **1-9** synthesized none of these were toxic to BGM cells. The compound **3** (R_1 = F / R_2 = CH_3) presented SI value 62.90 and IC_{50} = 5.13 µM lower than the sulfadoxine drug control (SI = 20.70; IC_{50} = 15.00 µM), in the anti-HRPII assay. The chloroquine and the prototype **I** is still more potent than **1-9** derivatives. The pyrazolo[3,4-*d*]pyrimidine is promising for further studies of antimalarial.

The authors thank the CNPq, CAPES and FAPERJ for financial support.

References

1) Pinheiro, L. C. S.; et al, Bioorg. Med. Chem. 2015, 23, 5979-5984.
2) Soliman, A. M.; et al Synthetic Comm. 2012, 42, 2748-2762.

P023

TARGETED DELIVERY OF DUOCARMYCIN SA ANALOGUES VIA THE THOMSEN-FRIEDENREICH ANTIGEN

Oliver Cartwright, Mark Searcey, David Russell

School of Pharmacy, University Of East Anglia, Norwich Research Park, Norwich

CC-1065 and the duocarmycins, including duocarmycin SA (Fig 1), are natural products which have been shown to be ultrapotent antitumour antibiotics with IC_{50} values within the picomolar range. The biological effect of the agents occurs due to a sequence-selective alkylation of DNA, leading to a cascade of cellular events, resulting in apoptosis (Fig 1).[1]

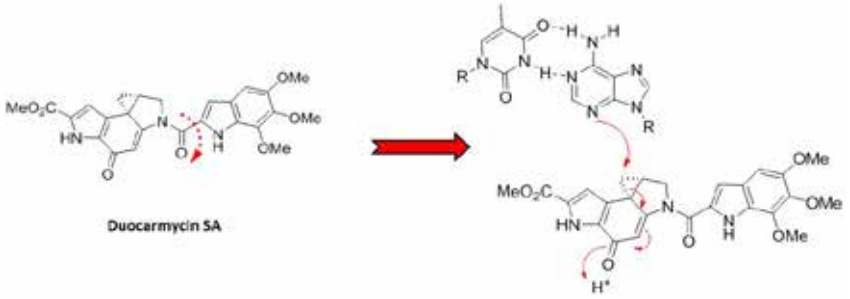


Figure 1: A DNA binding induced conformational change leads to disruption of the vinylogous amide and consequent activation of the cyclopropane ring. This leads to a S_N2 reaction involving attack by the N-3 position of adenine

The high potency and broad spectrum of antitumour activity of the CC-1065 and duocarmycin family has demonstrated the potential of these agents as clinical candidates. However, these agents have been found to be too toxic for systemic use and a lack of targeting has meant they have never fulfilled their potential as possible chemotherapeutic agents.

The Thomsen-Friedenreich antigen (TF) antigen presents an attractive moiety to achieve targeted delivery of agents to cancer cells. This is because this disaccharide has been shown to be overexpressed in 90% of primary human carcinomas but is cryptic within healthy cells. Previous work has demonstrated the potential of this antigen in achieving targeted delivery.[2]

We will discuss strategies employed to try and achieve the targeted delivery of duocarmycin based agents via the TF antigen with an overall aim of improving the therapeutic index of this family.

References

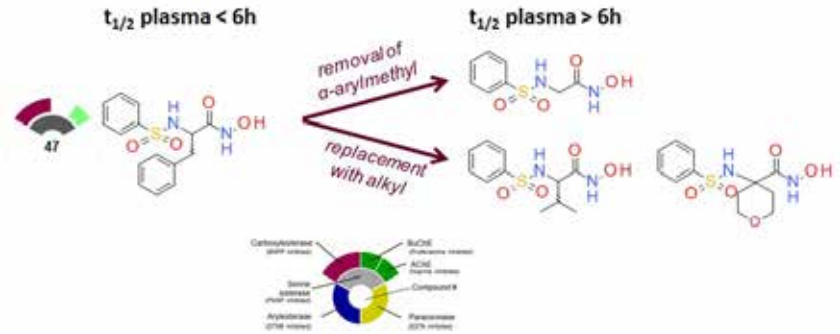
1) Boger, D. L., & Johnson, D. S. (1996). CC-1065 and the duocarmycins: Understanding their biological function through mechanistic studies.Angewandte Chemie International Edition in English, 35(13-14), 1438-1474.
2) Obaid, G., Chambrier, I., Cook, M. J., & Russell, D. A. (2012). Targeting the Oncofetal Thomsen–Friedenreich Disaccharide Using Jacalin-PEG Phthalocyanine Gold Nanoparticles for Photodynamic Cancer Therapy.Angewandte Chemie International Edition, 51(25), 6158-6162.

CONTROLLING PLASMA STABILITY OF HYDROXAMIC ACIDS: A MEDCHEM TOOLBOX

Paul Hermant (1), Piveteau Catherine (1), Alexandre Biela (1), Damien Bosc (1), Benoit Deprez (1), Rebecca Deprez-Poulain (1,2)

1) INSERM U1177 Drugs and Molecules for Living Systems; Drug Discovery unit; Pasteur Institute Lille, University of Lille 2. 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE
2) Institut Universitaire de France, IUF

Hydroxamic acids are outstanding zinc chelating groups that can be used to design potent and selective metalloenzyme inhibitors in various therapeutic areas. Some hydroxamic acids display a high plasma clearance resulting in poor in vivo activity, though they may be very potent compounds in vitro. We designed a 57-member library of hydroxamic acids to explore the structure-plasma stability relationships in these series and identify both which enzyme(s) and which pharmacophores are critical for plasma stability. Arylesterases and carboxylesterases were identified as the main metabolic enzymes for hydroxamic acids. Finally, we suggest structural features to be introduced or removed to improve stability. This work provides thus the first medicinal chemistry toolbox (experimental procedures and structural guidance) to assess and control the plasma stability of hydroxamic acids and realize their full potential as in vivo pharmacological probes and therapeutic agents. This study is particularly relevant to preclinical development as it allows to obtain compounds equally stable in human and rodent models.



References

1) Hermant P. et al. Controlling Plasma Stability of Hydroxamic Acids: A MedChem Toolbox. . J. Med. Chem. 2017, 60, 21, 9067-9089.
2) Deprez-Poulain, R., et al. Catalytic site inhibition of insulin-degrading enzyme by a small molecule induces glucose intolerance in mice. Nature Communications,2015, 6. ncomms9250

EXPANDING PARKIN TOOLBOX - NOVEL CHEMICAL PROBES TO EXPLORE PARKIN ACTIVATION

Sofia Domingos (1,2,3), Rita C. Guedes (1), Teresa Duarte (2), Lucília Saraiva (3), Rui Moreira (1)

1) Research Institute of Medicines (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Portugal
2) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Portugal
3) UCIBIO/REQUIMTE, Laboratório de Microbiologia, Faculdade de Farmácia da Universidade do Porto, Portugal

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder worldwide, affecting approximately 1.5% of the population above 60 years old and 4% of the population at the age of 80 [1].

Although PD is primarily a sporadic disorder of unclear aetiology, it is now clear that genetic factors contribute to the pathogenesis of the disease. For example, mutations in the *parkin* gene, which encodes Parkin protein, are a relatively frequent cause of autosomal recessive early-onset forms of PD [1].

Parkin is a ring-in-between-ring (RBR) E3 ubiquitin ligase, composed by six distinct domains. The catalytic module of PARKIN has a multidomain architecture consisting of RING1, IBR and RING2 domains (the latter harbouring the catalytic cysteine), and is responsible for the ubiquitination and consecutive proteasome degradation of a number of protein substrates [2,3].

The ubiquitination-proteasome system is fundamental to several cellular events and its malfunction induces impairment in mitophagy and accumulation of dysfunctional mitochondria, indicating that loss-of-function of Parkin protein may be a key to the neurodegeneration process and to the pathogenesis of PD. Therefore, restoring Parkin function using rationally designed peptides and small molecules has been emerging as a potential therapy for Parkin-linked PD.

However, medicinal chemistry approaches to regulate this pathway have always been hindered by the lack of suitable robust methodologies for screening endeavours [2,3].

To address this challenge, a series of activity-based probes for profiling Parkin activity is being developed. Concurrently, a yeast-based phenotypic assay [4] is being implemented and the biological activity of selected probes evaluated.

These novel chemical tools hold promise as innovative biomarkers for Parkin activation, providing the bases for Parkin high-throughput screening campaigns.

Acknowledgments

We thank the Fundação para a Ciência e Tecnologia for financial support of the iMed.ULisboa UID/DTP/04138/2013, UCIBIO/REQUIMTE UID/MULTI/04378/2013 and Sofia Domingos PhD grant PD/BD/114281/2016 from FCT PhD Programme in Medicines and Pharmaceutical Innovation (i3DU).

References

1) Elbaz A.; Carcaillon L.; Kab S.; Moisan F., Revue Neurologique 2016, 172, 14-26
2) Kumar A. et al., Nature Structural & Molecular Biology 2017, 24, 475-483
3) Truban D. et al., Journal of Parkinson's Disease 2017, 7, 13-29
4) Pereira C., Costa V., Martins L. M.; Saraiva L., Experimental Cell Research, 333, 73-79

ACCELERATING MULTIPLE MEDICINAL CHEMISTRY PROJECTS USING MATCHED MOLECULAR PAIR ANALYSIS FOR KNOWLEDGE BASED DESIGN – A REVIEW FROM THE PAST 8 YEARS OF USE AT THE FRONT LINE

Alexander Dossetter (1), Edward Griffen (1), Shane Montague (1), Andrew Leach (2)

1) MedChemica Ltd, Biohub, Alderley Park, Macclesfield, Chesire SK10 4TG United Kingdom
2) Liverpool John Moores University, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF , United Kingdom

The technical methods and results of Matched Molecular Pair Analysis (MMPA) applied from a small, individual assay scale through large pharma scale, to multiple pharma data sharing scale have been published and reviewed.[1,2,3,4] The drive behind these efforts has been to derive a medicinal chemistry knowledge base (i.e. definitive textbook) that can be applied to drug discovery projects. The aim is to greatly decrease the time in lead identification and optimization by the synthesis of fewer compounds. Given this context, how does this work on projects? How do the chemists make decisions? What are the results? The talk will answer these questions through project examples where MMPA has been applied and how this led to drug candidates. The projects disclosed are from multiple organisations and describe Cathepsin K inhibitors, Glucokinase Inhibitors, 11β-Hydroxysteroid Dehydrogenase Type I Inhibitors (11β-HSD1), Ghrelin inverse antagonists and Tubulin Polymerization inhibitors. An overview of MMPA will be presented and each project will be briefly described with a focus on how the chemists used MMPA to understand SAR and design compounds. The impact of project progress to CD will be quantified.

References

1) Dossetter AG, Griffen EJ, Leach AG. Matched Molecular Pair Analysis in drug discovery. Drug Discov Today 2013, 18, 724. doi:10.1016/j.drudis.2013.03.003.
2) Kramer C, Ting A, Zheng H, Hert J, Schindler T, Stahl M, et al. Learning Medicinal Chemistry Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Rules from Cross-Company Matched Molecular Pairs Analysis (MMPA): Miniperspective. J Med Chem 2017. doi:10.1021/acs.jmedchem.7b00935.
3) Kramer C, Ting A, Zheng H, Hert J, Schindler T, Stahl M, et al. Learning Medicinal Chemistry Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Rules from Cross-Company Matched Molecular Pairs Analysis (MMPA): Miniperspective. J Med Chem 2017. doi:10.1021/acs.jmedchem.7b00935.
4) Kramer C, Ting A, Zheng H, Hert J, Schindler T, Stahl M, et al. Learning Medicinal Chemistry Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Rules from Cross-Company Matched Molecular Pairs Analysis (MMPA): Miniperspective. J Med Chem 2017. doi:10.1021/acs.jmedchem.7b00935.

TARGETING RAS WITH MACROCYCLIC PEPTIDES

Gabriele Fumagalli (1,2), Andrew P. Thomas (2), Willem Nissink (2), Jonathan Tart (2), David R. Spring (1)

1) University of Cambridge, Chemistry Department, Cambridge, CB2 1EW, UK
2) Oncology, Innovative Medicines and Early Development, AstraZeneca, 310 Milton Road, Cambridge, CB4 0FZ, UK

KRas is a key GTPase involved in the proliferation of many cancers and therefore it has been the subject to many efforts to develop and deliver inhibitors.[1] Due to the high, pico-molar affinity for its natural ligands GTP and GDP, development of direct small molecule antagonists has been unsuccessful. As part of the search for novel binding sites and ligands for Ras, AstraZeneca scientists identified peptide ligands binding to a similar site to that recently reported by the Takeda group.[2] The most potent example contained a 16 amino acids sequence bearing a macrocycle at its core. Analysis of structures of peptide-RAS complexes derived by X-ray crystallography and computational modelling suggests that three core lipophilic interactions at the macrocycle level were key to the activity. The aim of the work presented here was to use this pharmacophore information to discover small macrocyclic peptides which could offer greater potential as cell permeable probes. Application of a diversity-oriented macrocyclic synthesis approach coupled with solid supported peptide synthesis allowed implementation of an effective synthesise-test-analyse-design strategy. We were able to quickly deliver a range of macrocycles, exploring different parts of the chemical space in the binding pocket. Exploration of different chemistries to achieve macrocyclisation, including click chemistry, metathesis, Heck and Glazer couplings, allowed us to build a diverse initial library of 63 macrocycles. *In vitro* testing and SAR analysis of this library identified the requirements for minimally active macrocycles. More systematic exploration of SAR has been carried out to further explore and define the key features for binding and to identify more potent macrocycles. The outcome of these strategies, a journey from molecules with potency of greater than 100 mM to low micromolar hits, will be reported.

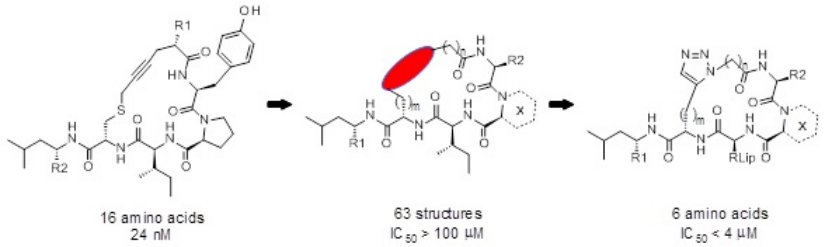


Figure 1: development of a novel family of macrocyclic peptides for Ras inhibition. From left to right: macrocyclic core of the initial 16 amino acids sequence; general structure of a member of our macrocyclic peptides library; low micromolar compounds.

References

1) Cox, A. D. & Der, C. J. Small GTPases 2010, 1, 2–27
2) Sogabe, S. et al. ACS Med. Chem. Lett. 2017, 8, 732−736

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL, POTENT AND IRREVERSIBLE XIAP INHIBITORS WITH SUPERIOR CELLULAR ACTIVITY IN REFRACTORY TUMORS

Luca Gambini, Carlo Baggio, Parima Udompholkul, Ahmed Salem, Maurizio Pellecchia

Division of Biomedical Sciences, School of Medicine, University of California, Riverside, 900 University Avenue, Riverside, California 92521, United States

The anti-apoptotic protein XIAP is a member of a larger family of proteins responsible for the development of cancer resistance to chemotherapy. Compounds designed to inhibit XIAP activity, often derived from the tetrapeptide sequence AVPI recognized by many IAPs, lack of true selectivity. Their activity against other two member of the IAPs family, cIAP1/cIAP2 may results in the activation of the TNF-α pathway and results in inflammation. Recently, an enthalpy screening performed in our laboratory identified Lys311 in the binding pocket of the BIR3 domain of XIAP as possible target to engineer increased potency and selectivity in AVPI-type ligands.[1] On these premises, we report a new generation of compounds capable of covalently interact with the BIR3 domain of XIAP exploiting the nucleophilic nature of the Lysine ε–amino group, an ideal substrate for aza-Micheal addition. We were able to show how these new molecules selectively reacts only with the Lys311 of XIAP, without cross reacting with the close neighbor Lys322, conserved across the multiple IAPs. We also found that by carefully tailoring the P4 position of the AVPI sequence, in combination with the covalent warhead in P2, complete selectivity for the BIR3 domain of XIAP in *in-vitro* experiments was attained. The obtained compounds were tested against leukemia and multiple myeloma cell lines in proliferation assays were they out-performed LCL-161, a non-selective IAPs inhibitor currently in Phase II. As a further proof of their therapeutics importance, they were also able to restore gemcitabine activity when tested against resistant pancreatic cancer cell lines. Further studies on the pharmacokinetics properties of these compounds are currently ongoing.

References

1) Enthalpy-Based Screening of Focused Combinatorial Libraries for the Identification of Potent and Selective Ligands. ACS Chem Biol. 2017 Dec 15;12(12):2981-2989. doi: 10.1021/acschembio.7b00717

DESIGN AND OPTIMIZATION OF A POTENT EPHA2-AGONISTIC PEPTIDE DIMER FOR SINGLE OR COMBINATION THERAPY

Luca Gambini, Parima Udompholkul, Ahmed Salem, Carlo Baggio, Maurizio Pellecchia

Division of Biomedical Sciences, School of Medicine, University of California, Riverside, 900 University Avenue, Riverside, California 92521, United States

EphA2 is a tyrosine kinase receptor overexpressed in several types of cancers and it is often correlated with poor prognosis. Development of novel, targeted anti-cancer therapies requires the design and optimization of selective tumor-targeting agents that are not only potent, but are also stable and amenable to conjugation with chemotherapeutic drugs. While short peptides represent potentially an excellent platform for these purposes, they are often degraded and eliminated too rapidly in in vivo. Our lab investigated novel tumor-homing agents, namely agonistic peptides of 10-13 amino-acids, targeting the EphA2 ligand-binding domain. As expected, since EphA2 activation requires clustering of its natural ligand, ephrins, we found that dimeric versions of these agents are very effective in inducing receptor dimerization and internalization. Moreover, our in vivo efficacy studies also showed that when our EphA2-tareting agent (123B9)2 was conjugated to paclitaxel (PTX), it was very effective in capturing and killing EphA2-expressing breast cancer cells in a metastatic mouse model [1]. However, these agents are still relatively weak in potency, with affinities in the double digit micromolar range for the EphA2. Hence, we further optimized 123B9, leading to the identification of 135B12, with affinity in the low micromolar range, which was used subsequently to produce a crystal structure of it in complex with EphA2. Modification of the 135B12 sequence, guided by the crystal structure, led to the synthesis of 135H11 (affinity in the nanomolar range), that is to date the most potent agonistic agent of its class. 135H12, the dimeric version of 135H11, was tested alone in EphA2-overexpressing cancer cell lines and showed, at low doses, a remarkable ability to (i) induce EphA2 degradation, (ii) decrease cancer cell proliferation, (iii) restore sensitivity to chemotherapeutic agents in resistant cell lines, and (iv) inhibit tumor cell migration. The extent of these effects is currently being investigated in mouse models to assess the efficacy of 135H12 as a single agent or in combination with other chemotherapeutic drugs.

References

1) Reduction of Circulating Cancer Cells and Metastases in Breast-Cancer Models by a Potent EphA2-Agonistic Peptide–Drug Conjugate J. Med. Chem., 2018, 61 (5), pp 2052–2061

DISCOVERY AND STRUCTURE-ACTIVITY RELATIONSHIPS OF POTENT PFKFB3 KINASE INHIBITORS

Charles-Henry Fabritius (1), Nicolas Boutard (1), Arkadiusz Białas (1), Aleksandra Sabiniarz (1), Paweł Guzik (1), Katarzyna Banaszak (1), Marcin Bień (1), Anna Buda (1), Ewelina Cieluch (1), Anna Cierpich (1), Łukasz Dudek (1), Hans-Michael Eggenweiler (2), Joanna Fogt (1), Andrzej Gondela (1), Krzysztof Jakubiec (1), Mirek Jurzak (3), Agata Kitlinska (1), Piotr Kowalczyk (1), Maciej Kujawa (1), Katarzyna Kwiecińska (1), Marcin Leś, Ralph Lindemann (4), Monika Maciuszek, Maciej Mikulski (1), Paulina Niedziejko (1), Alicja Obara (1), Henryk Pawlik (1), Tomasz Rzymski (1), Magdalena Sieprawska-Lupa (1), Marta Sowińska (1), Joanna Szeremeta-Spisak (1), Agata Stachowicz (1), Mateusz M. Tomczyk (1), Katarzyna Wiklik (1), Łukasz Włoszczak (1), Adrian Zarębski (1), Krzysztof Brzózka (1), Mateusz Nowak (1)

1) Selvita S.A., Bobrzyńskiego, 14, 30-348 Kraków, Poland
2) Discovery Pharmacology, Merck Biopharma, Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany
3) Translational Innovation Platform Oncology, Merck Biopharma, Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany
4) Medicinal Chemistry, Merck Biopharma, Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany

Glycolysis is a non-oxidative metabolic pathway in which glucose is degraded by cells to generate ATP (adenosine triphosphate), i.e. energy. While healthy cells are only favoring this pathway in hypoxia conditions, many cancer cells favour glycolysis to generate ATP, even in the presence of oxygen. Hence, the glycolytic rate can be up to 200 times greater in malignant rapidly-growing tumor cells than in healthy cells. This switch of energy metabolism in cancer cells to the process of “aerobic glycolysis” is known as the “Warburg Effect”.[1] Glycolysis is regulated by several enzymes, including phosphofructokinases, which catalyze several irreversible reactions in the course of this metabolic pathway. 6-phosphofructo-1-kinase (PFK-1), which converts fructose-6-phosphate (F6P) in fructose-1,6-bisphosphate (F1,6-BP), is considered to be the rate-limiting enzyme in the process of converting glucose into pyruvate, the precursor of anaerobic ATP production. PFK-1 is allosterically activated by fructose-2,6-bisphosphate (F2,6-BP) which is synthesized from F6P by phosphofructokinase-2 (PFK-2). Four isoforms of the PFK-2 family are known, namely PFKFB1, PFKFB2, PFKFB3, and PFKFB4. Many cancer types such as colon, prostate, pancreatic, breast, thyroid, leukemia, lung, ovarian tumors, exhibit an overexpression of the hypoxia-inducible form PFKFB3. [1] Thus, PFK-2 and in particular its PFKFB3 isoforms constitute promising targets for cancer therapy using small molecules inhibiting their action. In this optic, we report a novel series of compounds identified as potent PFKFB3 inhibitors. Docking as well as crystallographic studies pointed out binding to the ATP site of the kinase domain and allowed for structure driven potency optimization. Several compounds displayed low nanomolar activity on target as well as potent inhibition of cellular production of F-2,6-BP, and may prove useful as tool compounds to better understand the role of the PFKFB3 enzyme in cancer metabolism, cell cycle, apoptosis and angiogenesis, besides holding great potentials to find a lead compound for further development and optimization in our search for new anticancer drugs.

References

1) 1. D. G. Brooke et al., Biorganic & Medicinal Chemistry 22 (2014) 1029-1039; T. V. Pyrkov et al., ChemMedChem 2013, 8, 1322-1329

THE USE OF IRREVERSIBLE LIGANDS IN THE QUEST TO OBTAIN THE FIRST LIGAND-BOUND X-RAY STRUCTURES OF THE ADENOSINE A1 RECEPTOR

Manuela Jörg (1), Alisa Glukhova (2), Alaa Abdul-Ridha (2), David Thal (2), Anh Nguyen (2), Elizabeth Vecchio (2), Patrick Sexton (2), Paul White (2), Lauren May (2), Arthur Christopoulos (2), Peter Scammells (1)

1) Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria, 3052, Australia
2) Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria, 3052, Australia

Irreversible ligands have been proven to be useful pharmacological tools in the study of structural and functional features in drug receptor pharmacology of G protein-coupled receptors (GPCRs).[1] Recent advances in the field, which made it possible to obtain ligand-bound X-ray structures by co-crystallizing GPCRs with covalently bound probes, have been one of the major drivers behind the increased interest in the development of novel irreversible probes targeting GPCRs. Here, we will present our quest to solve the first X-ray structure of the adenosine A_1 receptor. This includes our efforts to obtain the first X-ray structure of the adenosine A_1 receptor, which was stabilized using DU-172, an irreversible antagonist (Figure 1).[2] Furthermore, we have successfully designed, synthesized and evaluated novel irreversible agonists of the adenosine A_1 receptor (Figure 2).[3] Four of these compounds, were shown to possess similar potency and efficacy to the reference high efficacy agonist, NECA, in an assay of ERK1/2 phosphorylation assay and two irreversible agonists demonstrated an ability to stabilize purified, detergent-solubilised adenosine A_1 receptors in a ThermoFluor assay to a significantly higher degree than NECA. Thus, these results offer an attractive starting point for a range of experiments including our quest to solve the first active-state X-ray structure of the adenosine A_1 receptor.

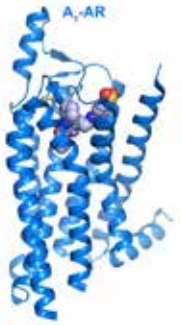


Figure 1. Ligand-bound X-ray structure of the adenosine A_1 receptor with DU-172

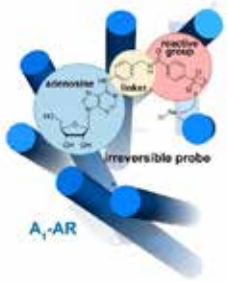


Figure 2. Novel irreversibly binding adenosine A_1 receptor agonist

References

1) M. Jörg, P.J. Scammells, Guidelines for the Synthesis of Small-Molecule Irreversible Probes Targeting G Protein-Coupled Receptors, ChemMedChem 2016, 11, 1488-1498.
2) A. Glukhova, D.M. Thal, A.T.N. Nguyen, E.A. Vecchio, M. Jörg, P.J. Scammells, L.T. May, P.M. Sexton, A. Christopoulos, Structure of the Adenosine A1 Receptor Reveals the Basis for Subtype Selectivity, Cell 2017, 168, 867-877.e13.
3) M. Jörg, A. Glukhova, A. Abdul-Ridha, E.A. Vecchio, A.T.N. Nguyen, P.M. Sexton, P.J. White, L.T. May, A. Christopoulos, P.J. Scammells, Novel Irreversible Agonists Acting at the A1 Adenosine Receptor, J. Med. Chem. 2016, 59, 11182-11194.

IMPACT OF THE STRUCTURES OF MACROCYCLIC MICHAEL ACCEPTORS DERIVED FROM SYRINGOLIN A ON COVALENT PROTEASOME INHIBITION

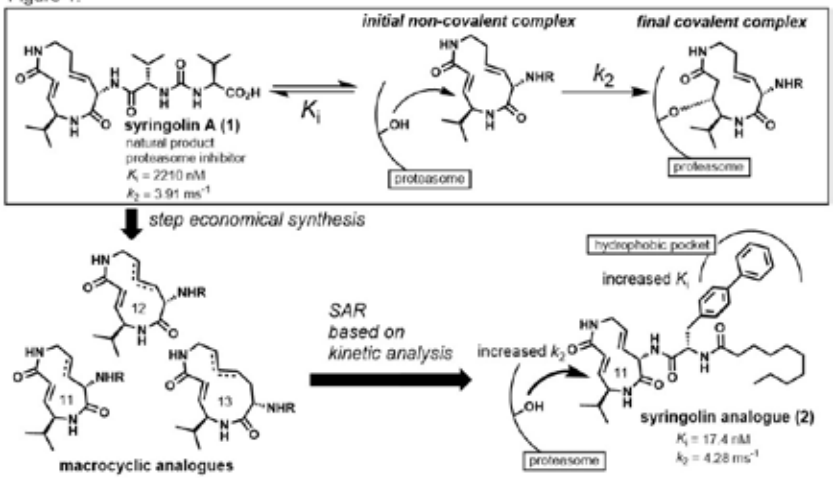
Shun Kitahata, Fumika Yakushiji, Satoshi Ichikawa

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan

Covalent inhibitors are compounds that form a covalent link with a functional group of the target enzyme or protein. Because the reactive functional group of the inhibitors may react with different enzymes and proteins, resulting in dangerous off-target effects, they have rarely been considered as starting points in molecularly targeted drug discovery programs. However, covalent inhibitors have recently been developed as targeted covalent drugs such as afatinib, neratinib, ibrutinib, etc., by suppressing reactivity to the other biomolecules.[1)]

The naturally occurring syringolin A (**1**) irreversibly inhibits proteasome by an oxa-Michael addition of the hydroxy group of the *N*-terminal threonine residue on the b5 subunit to the a, b-unsaturated carboxamide moiety embedded in the macrolactam.[2)] The process to form a covalent complex involves several steps (Figure 1). In the first step, a covalent inhibitor associates with its target protein *via* non-covalent interactions to form an inhibitor-protein complex, defined by the binding affinity K_i. A chemical reaction then takes place between the inhibitor and the protein to form a covalent complex, defined by the reaction rate k_2. Structure-based drug design using coordinates of a complex structure of a ligand and protein is a valuable approach, which allows us to rationally design inhibitors. However, this method is not always useful for designing covalent inhibitors because an X-ray crystal structure of covalent inhibitor/protein complexes is the reaction product and does not always reflect the association state. Therefore, detailed analysis of each step is necessary in the rational design of covalent inhibitors. We performed a systematic structure activity relationship (SAR) study and kinetic analysis of a series of syringolin analogues consisting of macrocycles with different ring sizes. Based on the obtained information, we developed a novel potent proteasome inhibitor (**2**). Details of the synthesis and its kinetic analysis of the analogues will be presented.

Figure 1.



References

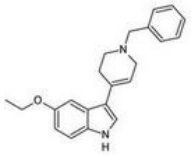
1) a) Singh, J. et al. Nature Rev. 2011, 10, 307-317. b) Bauer, R. A. Drug Discov. Today 2015, 20, 1061-1073.
2) a) Wäspi, U. et al. Mol. Plant-Microbe Interact. 1998, 11, 727-733. b) Groll, M. et al. Nature 2008, 452, 755-759.
3) a) Kitahata, S.; Ichikawa, S. et al. Org. Lett. 2016, 18, 2312-2315. b) Kitahata, S.; Yakushiji, F.; Ichikawa, S. Chem. Sci. 2017, 8, 6959-6963.

SYNTHESIS, STRUCTURAL AND THERMAL STUDIES OF D2AAK1_3 AS DOPAMINE D2 RECEPTOR ANTAGONIST

Magda Kondej (1), Agata Bartyzel (2), Monika Pitucha (3), Tomasz M. Wróbel (1), Andrea G. Silva (4), Dariusz Matosiuk (1), Marian Castro (4), Agnieszka A. Kaczor (1,5)

1) Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Laboratory, Faculty of Pharmacy with Division of Medical Analytics, Medical University, 4A Chodzki St., PL-20093 Lublin, Poland, E-mail: magda.kondej@onet.pl
2) Department of General and Coordination Chemistry, Maria Curie-Skłodowska University, Lublin, Poland
3) Independent Radiopharmacy Unit, Department of Organic Chemistry, Faculty of Pharmacy with Division of Medical Analytics, Medical University, 4A Chodzki St., PL-20093 Lublin, Poland
4) Department of Pharmacology, Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), Avda de Barcelona, E-15782 Santiago de Compostela, Spain
5) School of Pharmacy, University of Eastern Finland, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland

Compound D2AAK1_3 (see below) was designed as a modification of the lead structure D2AAK1 (an *in vivo* active multi-target compound with nanomolar affinity to a number of aminergic GPCRs)[1,2] and synthesized in the reaction of 5-ethoxyindole and 1-benzyl-4-piperidone in methanol/KOH. This compound has affinity to human dopamine D_2 receptor with K_i of 151 nM.



The aim of studies was structural and thermal characterization of the compound D2AAK1_3. In particular, X-ray studies, molecular docking and molecular dynamics as well as thermal analysis were performed [3].

The studied compound crystallizes in orthorhombic system, in chiral space group $P2_12_12_1$. The compound has a non-planar conformation. The dihedral angle between planes of benzyl group and indole moiety is 85.6(1) Å. The structure of compound is stabilized by a week N1-H1a···N2 hydrogen ($d_{D\cdots A}$ = 3.223(3) Å) bonds which leads to formation of one-dimensional chains running parallel to the [001] direction.

The studied compound was docked to the novel X-ray structure of the human dopamine D_2 receptor in the inactive state (PDB ID: 6CM4) and established the main contact between its protonatable nitrogen atom and Asp(3.32) of the receptor as expected for orthosteric ligand of aminergic GPCRs. The obtained binding pose was stable in molecular dynamics simulations.

Thermal stability of the compound was investigated using TG-DSC technique in air atmosphere. The studied compound is characterized by good thermally stability. During heating under oxidizing conditions, the first change has been recorded on the DSC curve as the endothermic peak (T_{peak} = 154 °C) and is associated with melting process. The enthalpy of fusion calculated from DSC is 26.42 kJ mol^{-1}. The combustion and thermal degradation of compound start over 200 °C and proceeds in three stages. The first step (204-389 °C) is characterized by a thermal decomposition of the greater part of the compound (54.41%) and probably is mainly associated with the defragmentation, release of volatile products and their combustion processes. The formed unstable products undergo further decomposition process which is not clearly marked on TG curve but it has been recorded on DTG curve. The last stage is observed in the temperature range 458-650 °C and corresponds to the complete destruction and combustion of the remaining parts of the compound. In order to better understand the mechanism of thermal decomposition of compounds the TG-FTIR analyses in air and nitrogen atmosphere were also performed.

References

1) Kaczor AA, Silva AG, Loza MI, Kolb P, Castro M, Poso A. ChemMedChem 2016;11(7):718-29.
2) Kaczor AA, Targowska-Duda KM, Budzyńska B, Biała G, Silva AG, Castro M. Neurochem Int. 2016;96:84-99.
3) Kondej M, Bartyzel A, Pitucha M, Wróbel TM, Silva AG, Matosiuk D, Castro M, Kaczor AA. J Anal Appl Pyrolysis, due to submitted

P035

DESIGN, SYNTHESIS AND ANTITUMOR ACTIVITY OF NOVEL QUINOLINE-BENZIMIDAZOLAMIDINE HYBRIDS

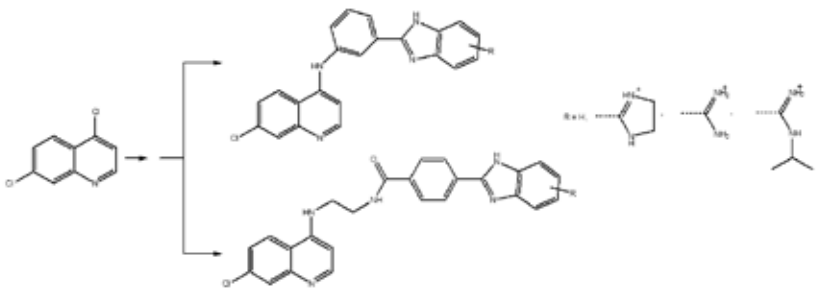
Luka Krstulović (1), Kristina Starčević (2), Teuta Opačak-Bernardi (3), Miroslav Bajić (1), Ljubica Glavaš-Obrovac (3)

1) Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, HR-10000 Zagreb, Croatia
2) Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, HR-10000 Zagreb, Croatia
3) Department of Medicinal Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, J. J. Strossmayer University of Osijek, J. Huttlera 4, HR-31000 Osijek, Croatia

Cancer is one of the most prominent health issues responsible for more than 1 million deaths per year in EU, costing annually more than billion euros. One of the emerging strategies for overcoming the drawbacks of the available anticancer therapeutics is the development of hybrid agents, composed of two distinct pharmocophores [1].

In continuing our work on the synthesis and evaluation of hybrid molecules[2] we have synthesized two series of new hybrids in which 7-chloro-4-aminoquinoline is linked with two different linkers to a benzimidazole amidine moiety. Moieties have been chosen, because both of them show anticancer activity. Several 7-chloro-4-aminoquinoline based antimalarial therapeutics are tested for their anticancer activity, two of them (chloroquine and hydroxychloroquine) are currently investigated in clinical trials for cancer therapy[3]. On the other hand benzimidazole moiety is found in many known pharmaceuticals, displaying besides anticancer a variety of biological effects, also positively charged amidine groups are known for their cellular and nuclear uptake.

Novel compounds were evaluated for their *in vitro* cytotoxic activity against normal epithelial (MDCK1), cervix adenocarcinoma (HeLa), colon adenocarcinoma (CaCo2), and leukemia (K562 and RaJi) cell lines using MTT assay.



References

1) S. Fortin and G. Berube, Expert Opin. Drug Discov. 8 (2013) 1029-1047.
2) L. Krstulović, I. Stolić, M. Jukić, T. Opačak-Bernardi, K. Starčević, M. Bajić, Lj. Glavaš-Obrovac, Eur. J. Med. Chem. 137 (2017) 196-210.
3) V. W. Rebecca and R. K. Amaravadi, Oncogene. 35 (2016) 1–11.

P036

1,3,4-THIADIAZOL-2-AMINE DERIVATIVES AS NOVEL UROTENSIN-II RECEPTOR ANTAGONIST

Chae Jo Lim, Kyu Yang Yi, Kwang-Seok Oh, Byung Ho Lee

Therapeutics & Biotechnology Division, Korea Research Institute of Chemical Technology, Yuseong-gu, Daejeon 34114, Republic of Korea

Heart failure is one of the leading causes of death due to various cardiovascular diseases, and its prevalence has become a serious global health problem. Since urotensin-II (U-II), a natural peptide ligand, is the most potent known vasoconstrictor, U-II and the urotensin-II receptor (UT) have recognized as one of the most potential therapeutic targets for treatment of cardiovascular diseases. U-II is a disulfide-linked cyclic neuropeptide that is expressed in various tissues and their function is regulated by UT or GPR14 as a G protein-coupled receptor. When U-II ligand binds to the UT, it affects control of a variety of physiological effects associated with a wide range of cardiovascular function such as hypertrophy, vasoconstriction, vasodilation, and cell proliferation through a complex signal transduction. Furthermore, a number of basic and clinical studies demonstrate that expression of UT is low or rarely found in normal myocardium, whereas plasma concentration of U-II and tissue expression of U-II and UT is found to display high levels in numerous cardio-renal diseases, including hypertension, heart failure, and atherosclerosis. Thus, these findings suggest that UT is considered to be a promising pharmacological target for treatment of heart failure and other cardiovascular diseases. Indeed, several UT antagonists have been found to have anti-hypertrophic effects in animal models.Despite great efforts devoted to the development of various pharmacophore derivatives of UT antagonists in several pharmaceutical companies, development of novel and potent UT antagonists is still required. In our continuing efforts, we utilized a virtual screening approach using Ligand Scout 3.0 (inte:ligand) to uncover new chemical scaffolds that could serve as UT antagonists. This study led to the identification of 1,3,4-thiadiazole ureas possessing the aryloxymethyl group at C-5 as hit compounds. And further SAR and optimization studies, probing the effects of aryloxymethyl group at C-5 on the 1,3,4-thiadiazol-2-amine moiety containing *N* -(3-chloro-4-(piperidin-4-yloxy)benzyl) group, led to the identification of the 3,4-dichloro analog, a highly potent UT antagonist with an IC_{50} value of 0.13 μM.

References

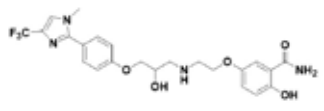
1) Lim, C. J.; Jang, J. Y.; Kim, S. H.; Lee, B. H.; Oh, K.-S.; Yi, K. Y. Bull. Korean Chem. Soc. 2015, 36, 2549
2) Kim, S.-k.; Goddard III, W. A.; Yi, K. Y.; Lee, B. H.; Lim, C. J.; Trzaskowski, B. ChemMedChem. 2014, 9, 1732.
3) Oh, K.-S.; Lee, S.; Lee, B. H. Assay Drug Dev. Technol. 2011, 9, 514.

EFFICACY-SELECTIVE BETA-2 ADRENOCEPTOR AGONISTS

Elliott Lumb (1), Jillian Baker (1), Peter Scammells (2), Michael Stocks (1)

1) University of Nottingham
2) Monash Institute of Pharmaceutical Sciences

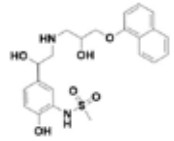
Approximately 1.2 million people in the UK are diagnosed with Chronic Obstructive Pulmonary Disease.[1] One of the main treatments for this disease are β_2-Adrenoceptor (AR) Agonists. Although there may be a plethora of this type of drug available, each have been shown to activate β_1ARs; Thus increasing heart rate, which can lead to complications for those with heart disease.[2] We investigated the high β_1 selectivity of the AR antagonist CGPA 20712A and identified the pharmacophores that determine the high β_1 selectivity and then carried out SAR. Following this we designed and synthesised a range of bivalent compounds, consisting of a β_2AR agonist linked to a β_1AR antagonist, such as (1). A number of these compounds have similar affinities at the β_1 and β_2 AR but are significantly more potent at the β_2AR.



CGP 20712A

β_1: pKd 8.70

β_2: pKd 5.88



(1)

β_1: pEC_{50} <5 pKd 6.66

β_2: pEC_{50} 6.96 pKd 7.14

References

1) Snell, N. Strachan, D. Hubbard, R. Gibson, J. Gruffydd-Jones, K. Jarrold, I. Epidemiology of chronic obstructive pulmonary disease (COPD) in the uk: findings from the british lung foundation's 'respiratory health of the nation' project. Thorax, 2016, 71(3), A20.
2) Baker, J. G. The selectivity of β-adrenoceptor agonists at human β1-, β2- and β3-adrenoceptors. British Journal of Pharmacology 2010, 160, 1048-1061.

COVALENT INHIBITION WITH A TERMINAL ALKYNE AS AN 'INERT' ELECTROPHILE

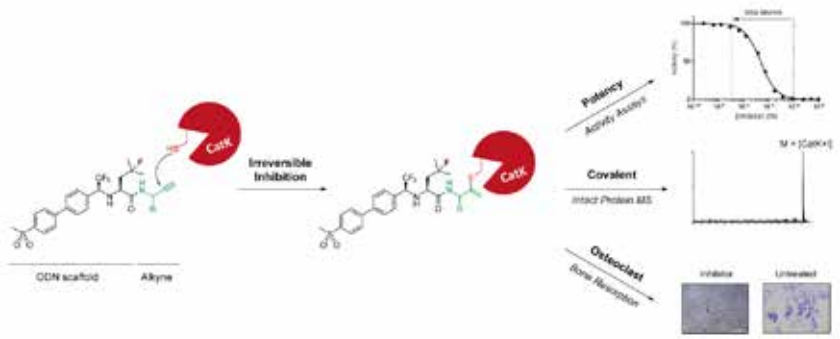
Elma Mons (1), Ineke Jansen (2), Bjorn van Doodewaerd (1), Stan van Boeckel (3), Boris Turk (4), Huib Ovaa (1)

1) Department of Chemical Immunology, Leiden University Medical Center, Leiden, The Netherlands
2) Department of Periodontology, Academic Center For Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands
3) Leiden Institute of Chemistry, Leiden University, Leiden, The Netherlands
4) Department of Biochemistry and Molecular and Structural Biology, J. Stefan Institute, Ljubljana, Slovenia

Irreversible covalent inhibitors were disfavored until the recent development and approval of kinase inhibitors Afatinib and Ibrutinib.[1] Utilizing an acrylamide as the electrophile, these inhibitors form an irreversible covalent bond with a non-conserved cysteine at their binding site. Protein activity can only be restored by *de novo* protein synthesis, resulting in a therapeutic effect that could last long after the inhibitor is cleared from the blood. Acrylamide moieties also form irreversible covalent bonds with non-targeted thiol residues, and the safety profile of irreversible inhibitors could be improved with the use of latent electrophiles such as terminal alkynes.

Terminal alkynes are generally considered 'inert' towards cellular components, and are therefore often used in bioorthogonal approaches as chemoselective 'Click' handles. However, in our group it was shown that a propargyl moiety on the C-terminus of Ubiquitin reacts in an activity-based manner with the catalytic cysteine residue in DUBs (DeUbiquitinating enzymes).[2] The lack of indiscriminate reactivity with thiol residues in non-targeted proteins or with excess thiols suggested a proximity-driven reactivity. Utilizing the alkyne moiety in a small molecule inhibitor could thus reduce adverse effects resulting from covalent off-target interactions.

We introduced propargyl derivatives onto the scaffold of Odanacatib (ODN), a selective inhibitor of Cathepsin K (CatK).[3] CatK is one of the most important cysteine proteases in bone degradation, and its aberrant activity has been implicated in diseases such as osteoporosis, osteoarthritis, bone metastasis and giant cell tumor of the bone. The alkyne moiety was positioned to be in close proximity of the catalytic cysteine residue on the active site, utilizing the alkyne moiety as a latent electrophile. Evaluation of the biochemical properties and inhibitory activity revealed that the compounds inhibit Cathepsin K activity with high selectivity compared to other Cathepsins. Inhibition of CatK activity was found to be irreversible, in activity assay on recombinant enzyme as well as in a functional bone resorption assay with human osteoclasts. Intact protein MS confirmed the formation of a covalent inhibitor-CatK complex. Further evaluation of the biological implications is ongoing.



References

1) J. Singh, R. C. Petter, T. A. Baillie, A. Whitty, Nat. Rev. Drug Discovery 2011, 10, 307.
2) R. Ekkebus, S. I. van Kasteren, Y. Kulathu, A. Scholten, I. Berlin, P. P. Geurink, A. de Jong, S. Goerdayal, J. Neefjes, A. J. R. Heck, D. Komander, H. Ovaa, J. Am. Chem. Soc. 2013, 135, 2867.
3) D. Brömme, F. Lecaille, Expert Opin. Invest. Drugs 2009, 18, 585.

P039

DESIGN, SYNTHESIS AND BIOLOGICAL CHARACTERIZATION OF FUNCTIONAL MOLECULAR PROBES FOR THE CREBBP BROMODOMAIN

Vlad Pascanu (1), Aymeric Dolbois (1), Xuan Wang (1,2), Manuela Brütsch (1), Amedeo Caflisch (2), Cristina Nevado (1)

1) Department of Chemistry – University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
2) Department of Biochemistry – University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

The ε-*N*-acetylation of lysine residues on histone tails is one of the most prevalent post-translational modifications. Bromodomains are protein modules (*ca.* 110 amino acids) that specifically recognize (read) these acetylated marks, mediating protein-protein interactions and their downstream biological function. Therefore, bromodomains are interesting targets for "reprogramming" the epigenome with the potential to access a previously unexplored therapeutic space.[1] Out of 61 different bromodomains identified in humans, BRD4(1) and the BET family have been the most investigated so far, leading to inhibitors already in phase II clinical trials.[2] In sharp contrast, the biological relevance of other bromodomains, like the CREBBP/EP300, remains unclear.

Originating from an *in silico* fragment–based approach, our group has successfully designed, synthesized and biologically characterized a series of acetylbenzene derivatives as low nanomolar CREBBP ligands.[3] They display unprecedented selectivity against the more accessible BRD4(1) bromodomain. Lead compounds from this campaign have been further derivatized into a series of functional probes using a newly developed linking strategy. Fluorescent probes, PROTACs and HyT probes have been synthesized and optimized in terms of solubility, cell permeability and metabolic stability. These valuable tool compounds will enable us to systematically modulate the activity of CREBBP and to study comparatively the differences between its inhibition and degradation in relevant *in vivo* models.

References

1) Muller, S.; Filippakopoulos, P.; Knapp, S. Expert Rev. Mol. Med. 2011, 13, e29.
2) Filippakopoulos, P.; Knapp, S. Nat. Rev. Drug Discov. 2014, 13, 337.
3) Batiste, L.; Unzue, A.; Dolbois, A.; Hassler, F.; Wang. X.; Deerain, N.; Zhu, J.; Spiliotopoulos, D.; Nevado, C.; Caflisch, A. ACS Cent. Sci. 2018, 4, 180.

P040

AS-10, A UNIQUE SE-ASPIRIN: PRE-CLINICAL EVIDENCES OF A POTENT AND SELECTIVE CANCER DRUG CANDIDATE

Daniel Plano (1,2), Deepkamal Karelia (2), Carmen Sanmartin (1), Junxuan Lu (2), Shantu Amin (2), Arun Kumar Sharma (2)

1) Universidad de Navarra, Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia y Nutrición, Campus Universitario, 31080, Pamplona, Spain. IdiSNA, Instituto de Investigación Sanitaria de Navarra
2) Department of Pharmacology, Penn State College of Medicine, Hershey, PA 17033, USA

Selenium (Se) is a micronutrient for humans with well-proven effects over the redox system. Low serum Se levels are associated with higher risk and poor prognosis of several cancers. For the last few years, mounting evidences have demonstrated that the incorporation of Se atom into organic frameworks is an attractive strategy in Medicinal Chemistry to design anticancer agents.

Continuing with our efforts to develop new Se containing small molecules [1], herein we present the synthesis, structure characterization and pre-clinical evaluation of our newly developed compound **AS-10** [2-((3-(2-acetoxybenzoyl)-1,3-selenazolidin-2-ylidene)carbamoyl)phenyl acetate].

AS-10 was selectively lethal to a variety of cancer cells as shown by NCI-60 Human Tumor Cell Lines Screen results. **AS-10** presents a mean growth percent value of -37.86% for all sixty cell lines, with melanoma, renal and central nervous system (CNS) cancers being dramatically sensitive. Also, interestingly, **AS-10** inhibited pancreatic cancer (PC) cells growth (IC_{50} 2.5-5.0 μM) for which no effective therapy currently exists. **AS-10** induced G1/G2 cell cycle arrest which was associated with increase of cell cycle inhibitory proteins p21 and p27, and induced apoptosis as evidenced by caspase 3/7 activity, PARP cleavage and Annexin V staining. **AS-10** also inhibited NF-κB DNA binding activity as well as NF-κB translocation to the nuclei upon stimulation by TNFα. Notably, **AS-10** potentiated cytotoxic activity of gemcitabine in PC cells. Furthermore, in LNCaP prostate cancer cells, **AS-10** decreased protein level of AR and its best known target PSA, and led to increased caspase-mediated apoptosis and expression of p53-DNA damage response proteins such as p21 and p-H2A.X. **AS-10** induced ROS in cancer cells as likely primary biochemical event. Finally, **AS-10** (47 mg/kg, *i.p*) inhibited subcutaneous colon tumor growth by ~70% without any apparent systemic toxicity.

Intellectual disclosure. All the information presented here is under protection: U.S. Utility Patent Application No. 15/457,587. *The Penn State Research Foundation*.

References

1) Plano D, Karelia DN, Pandey MK, Spallholz JE, Amin S, Sharma AK. J Med Chem. 2016, 59:1946-1959

SELECTIVE CHEMOPREVENTIVE EFFICACY OF P-XS-ASP TOWARDS SMOKE CARCINOGEN-INDUCED LUNG CANCER MODEL

Daniel Plano (1,2), Srinivasa Rao Ramisetti (2), Shang-Yuh Kim (2), César Aliaga (2), Timothy Cooper (2), Arthur Berg (2), Shantu Amin (2), Carmen Sanmartin (1), Junxuan Lu (2), Arun Kumar Sharma (2)

1) Universidad de Navarra, Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia y Nutrición, Campus Universitario, 31080, Pamplona, Spain. IdiSNA, Instituto de Investigación Sanitaria de Navarra
2) Department of Pharmacology; Penn State Cancer Institute, CH72; Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA

1,4-Phenylenebis(methylene)selenocyanate (*p*-XSC) has been shown to inhibit tobacco carcinogen NNK induced lung tumor development in several animal models. This had placed *p*-XSC on the National Cancer Institute´s (NCI) list of chemopreventive agents for clinical development, but there were systemic toxicity issues. *p*-XSC metabolizes through the formation of active bis-selenol (*p*-XSeH) along with the release of poisonous hydrogen cyanide (HCN). We recently developed *p*-XS-Asp, with a rationale that it would cleave *in vivo* to release the active *p*-XSeH and aspirin, thus making the compound less toxic and possibly more potent than *p*-XSC. Indeed, we previously presented (AACR Annual Meeting 2014) that *p*-XS-Asp inhibited NNK-induced lung tumorigenesis in A/J mice more effectively than *p*-XSC, and was also more tolerable. At doses of 15 ppm and 7.5 ppm Se, *p*-XS-Asp showed a significantly marked decrease in the percentage of lung cancer incidence *in vivo*

with only 50% and 87% of tumor incidence, as compared to *p*-XSC (79% and 100%), respectively. NNK-control showed an 100% tumor incidence. Likewise, the tumor multiplicity for *p*-XS-Asp group was 0.87 and 1.93 tumors/mouse as compared to the NNK-control (11.53) and *p*-XSC (1.66 and 4.10 tumors/mouse, respectively) at the two doses tested. Notably, blood chemistry and tissue analyses did not show systemic toxicity for the *p* -XS-Asp fed group.

We have now evaluated the underlying mechanisms of lung cancer preventive action of *p*-XS-Asp and its efficacy for inhibiting azoxymethane (AOM)- and dimethyl hydrazine (DMH)-induced Aberrant Crypt Foci (ACF) in Fischer F344 rats. At a dose of 7.5 ppm Se, *p*-XS-Asp was able to restore the expression of several genes (*MMP9*, *COX-2*, *Myc*, *SphK1* and *RELA*), that were over-expressed in the NNK group, to control or even lower levels. The *AKT1* gene expression was much lower in the lung tissue of *p*-XS-Asp treated mice at this dose compared to both negative and NNK control groups. Therefore, *p*-XS-Asp might be exerting its chemopreventive effect on NNK-induced lung tumorigenesis via inhibiting COX-2 mediated PI3K/Akt signaling pathway.

Interestingly, contrary to the striking inhibition of lung tumorigenesis, *p*-XS-Asp failed to significantly inhibit AOM- or DMH-induced formation of ACF in Fischer F344 rats. In both AOM and DMH models, aspirin (positive control) significantly reduced the number of ACF and large ACF per area (cm^2) by 37.4% and 33.8%, respectively. On the other hand, both *p*-XSC and *p*-XS-Asp showed no significant inhibitory effect on the formation of ACF and large ACF.

Taken together, our results have shown *p*-XS-Asp to selectively prevent the lung, but not colon, tumorigenesis, and thus is a promising candidate for further development as a lung cancer preventive agent. *p*-XS-Asp, thus is a promising candidate to future clinical evaluation as a lung cancer preventive agent, particularly in high-risk populations such as smokers.

SELENIZATION OF SMALL MOLECULES: FROM NORMAL- TO SUPER-MOLECULES TOWARDS CANCER

Daniel Plano (1,2), Carmen Sanmartin (1), Shantu Amin (2), Arun Kumar Sharma (2)

1) Universidad de Navarra, Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia y Nutrición, Campus Universitario, 31080, Pamplona, Spain. IdiSNA, Instituto de Investigación Sanitaria de Navarra
2) Department of Pharmacology; Penn State Cancer Institute, CH72; Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA

Selenium (Se) is a micronutrient for humans with well-proven effects over the redox system. Low serum Se levels are associated with higher risk and poor prognosis of several cancers. Recently, selenization of small molecules, meaning the introduction of a Se atom into an organic framework, has led to the development of several molecules with unique features. The chemical form by which Se is incorporated has demonstrated to be a crucial limiting factor to achieve the improvements on potency and selectivity of different small molecules against cancer.

Traditionally, incorporation of selenocyanate functionality has achieved several compounds with potent anticancer and/or chemopreventive activity *in vivo*, *i.e.* 1,4-phenylenebismethylene selenocyanate (*p*-XSC). More recently, modification of two non-steroideal anti-inflammatory drugs (NSAIDs), celecoxib and aspirin, yielded hybrid molecules with an outstanding increase in the antitumor effect of these Se-NSAIDs compared with the parent NSAIDs. Other functionalities used encompass methylseleno, diselenide and selenourea, *i.e.* PBISe and EI201, whom also present a significant increase in the potency.

Another modification worth mentioning is the inclusion of the Se atom in an ‘endo’ position of heterocyclic derivatives. This approach has achieved ebselen and AS-10, the former being under clinical development, that demonstrated potent and selective antitumor activity.

To conclude, we believe that selenization of small molecules is a relatively unexplored and very promising approach to confer exceptional characteristics to a plethora of skeletons towards cancer.

P043

ACYLSELENOUREA-DISELENIDE COMBINATION: POTENT AND SELECTIVE ANTITUMORAL AGENTS AND AUTOPHAGY INDUCTORS

Pablo Garnica (1), Ignacio Encío (2), Daniel Plano (1), Juan Antonio Palop (1), Carmen Sanmartin (1)

1) Universidad de Navarra, Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia y Nutrición, Campus Universitario, 31080, Pamplona, Spain. IdiSNA, Instituto de Investigación Sanitaria de Navarra
2) Department of Health Sciences, Public University of Navarra, Avda. Barañain s/n, E-31008 Pamplona, Spain

A series of sixteen new diselenide-selenourea conjugates have been designed following a fragment-based drug design strategy. All compounds have been characterized and required purity to perform biological evaluation has been confirmed. *In vitro* cytotoxicity potential has been evaluated against a panel of six cancer cell lines (MCF-7, PC-3, HT-29, HTB-54, CCRF-CEM and K-562) and two non-malignant derived cell lines (184B5 and BEAS-2B) in order to assess their potency and selectivity. Results revealed that MCF-7, CCRF-CEM and PC-3 were the most sensitive cell lines. Six of the tested compounds exhibited GI_{50} values under 10 μM in at least four cancer cell lines. Structure-wise those derivatives containing heterocyclic endings proved to be much more less selective than their carbocyclic homologs. Derivatives **2** and **7** were selected due to their high selectivity for breast adenocarcinoma cells and potency with GI_{50} values of 1.30 and 0.15 nM. Moreover, selectivity indexes were 12 and 121 times higher than those obtained for doxorubicin and were consequently selected to further study their mechanism of action. Preliminary mechanistic studies were carried out for those two hit compounds and proved that both derivatives arrest cell cycle in phase G2/M and that cell death is autophagy-mediated. This hypothesis was confirmed by the blockage of cell death with pre-treatment with wortmannin or chloroquine and the upregulation of the markers Beclin-1 and LC3B in MCF-7 cells. The potent antiproliferative activity in MCF-7 cell line in the nanomolar range with concomitant staggering selectivity index, highlights the potential of 3,5-dimethoxyphenyl analogue (**7**) as a new candidate to become a drug with clinical prospective against breast cancer.

P044

SMALL MOLECULES CONTAINING SELENIUM AS CHEMOTHERAPEUTIC AGENTS

Ana Carolina Ruberte (1), Carmen Sanmartin (1), Ignacio Encío (2), Daniel Plano (1)

1) Universidad de Navarra, Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia y Nutrición, Campus Universitario, 31080, Pamplona, Spain. IdiSNA, Instituto de Investigación Sanitaria de Navarra
2) Department of Health Sciences, Public University of Navarra, Avda. Barañain s/n, E-31008 Pamplona, Spain

Over the last years, several seleno-compounds such as ebselen (EBS) and PBISe have demonstrated to be promising compounds for therapy and prevention of diseases related with reactive oxygen species generation [1, 2]. For these reasons, we consider selenium might be an important tool in the development of new drugs.

Based on our experience in the development of innovative drugs as chemotherapeutic agents, we have designed and synthesized 37 novel seleno-compounds grouped in two series. The first one is formed by selenadiazoles, the position 5 being modulated with different amides. The second one comprises selenoureas with several substituents in *N* and *N'*. Cytotoxic activity for both series were determined in several cancer cells by MTT and the apoptotic status and cell cycle analysis of the cells were based on the TUNEL technique. Likewise, their radical scavenging activity was determined using the DPPH assay.

Three selenadiazole derivatives exhibited higher cytotoxic activity than EBS in solid tumors, along with higher selectivity indexes. The cytotoxic activity of the hit compound was remarkable in MCF-7 cells. Nevertheless, its cytostatic effect was independent of apoptosis induction or cell cycle modulation. The antioxidant capacity of four compounds was greater than EBS. On the contrary, selenourea derivatives possess potent cytotoxic activities in breast and prostate cells along with outstanding antioxidant effects. These derivatives present similar antioxidant capacity than ascorbic acid at high doses, some of them presenting higher antioxidant activity at low doses. Currently, the mechanisms implicated in both effects showed by these selenoureas are being characterized.

These series of compounds can serve as an excellent scaffold to achieve new and potent antioxidant compounds useful for several diseases, *i.e.* cancer, neurodegenerative, heart diseases and leishmaniasis, considering the high antioxidant activity and low toxicity showed by both series.

References

1) Lynch EJK. Semin Hear. 2009, 30:47-55
2) Chung CY, Madhunapantula SV, Desai D, Amin S, Robertson GP. Canver Prev Res (Phila). 2011, 4:935-948

SYNTHESIS OF SULFONIMIDAMIDE (SIA) BASED AMINO ACID BUILDING BLOCKS

Praveen Kumar Chinthakindi (1), Andrea Benediktsdottir (1), Yantao Chen (2), Per I Arvidsson (3), Edouard Zamaratski (1), Anja Sandström (1)

1) Department of Medicinal Chemistry, Drug Design and Discovery, Uppsala University, Sweden
2) Medicinal Chemistry, Cardiovascular and Metabolic Diseases, Innovative Medicines and Early Development, AstraZeneca R&D, Gothenburg, 431 83 Mölndal, Sweden
3) Science for Life Laboratory, Drug Discovery and Development Platform and Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Amide bond isosteres, unnatural amino acids and secondary structure mimetics are important building blocks useful in the development of pseudopeptides or peptidomimetics.[1] Development of such surrogates constitute an important research area in peptide drug discovery aiming to modulate physiochemical properties of peptides while keeping/enhancing their biological activity as well as selectivity against biological target.

Expanding on the recent tactical application of bioisosteres[2] i.e., sulfur−aza class of analogues,[3] Arvidsson group recently highlighted an emerging interest of sulfonimidamides (SIA) in contemporary drug design.[4] SIA is a chiral functional group that offers a wide range of advantages in drug design, like to construct high-quality compound libraries.[5] Sulfonimidamides (SIAs) have an extra "N" atom/handle in comparison with sulfonamide and imine N-substituents of sulfonimidamide are known to tune the physicochemical and biological properties. [6,7] For instance, enhanced lipophilicity and metabolic stability can be achieved. Also, single atom alterations are known to significantly improve the activity of the parent molecule.[8] The full potential of sulfonimidamides in regard to stability as well as biological activity needs to be further exploited in biologically relevant molecules.

Fig. 1 Synthesis of sulfonimidamide based amino acid building blocks

This poster summarizes our recent synthetic approaches to sulfonimidamide based amino acid building blocks (Fig. 1) and discusses the future opportunities in peptide chemistry utilizing solution phase and solid phase peptide synthesis.

References

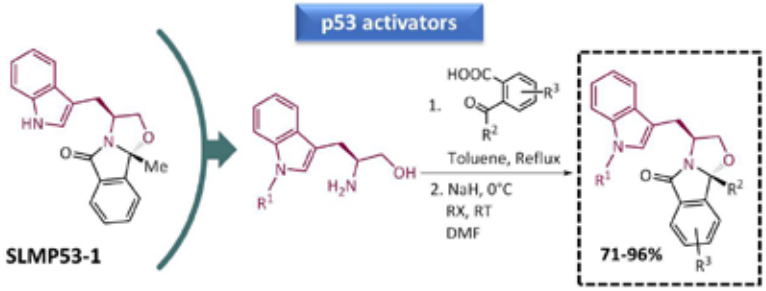
1) E. Valeur, S. M. Guéret, H. Adihou, R. Gopalakrishnan, M. Lemurell, H. Waldmann, T. N. Grossmann, A. T. Plowright, Angew. Chemie Int. Ed. 2017, 56, 10294–10323.
2) N. A. Meanwell, J. Med. Chem. 2011, 54, 2529–2591.
3) U. Lücking, Angew. Chemie Int. Ed. 2013, 52, 9399–9408.
4) P. K. Chinthakindi, T. Naicker, N. Thota, T. Govender, H. G. Kruger, P. I. Arvidsson, Angew. Chemie Int. Ed. 2017, 56, 4100–4109.
5) F. W. Goldberg, J. G. Kettle, T. Kogej, M. W. D. Perry, N. P. Tomkinson, Drug Discov. Today 2015, 20, 11–17.
6) L. D. Pennington, D. T. Moustakas, J. Med. Chem. 2017, 60, 3552–3579.
7) S. R. Borhade, R. Svensson, P. Brandt, P. Artursson, P. I. Arvidsson, A. Sandström, ChemMedChem 2015, 10, 455–460.
8) D. L. Boger, J. Org. Chem. 2017, 82, 11961–11980.

TRYPTOPHANOL-DERIVED OXAZOLOISOINDOLINONES: PROMISING SMALL MOLECULES FOR ANTICANCER THERAPY

Valentina Barcherini (1), Sara Gomes (2), Joana Soares (2), Margarida Espadinha (1), Joana Loureiro (2), Célia Gomes (3), Flávio Reis (3), Alexandra M. M. Antunes (4), Lucília Saraiva (2), Maria M. M. Santos (1)

1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal
2) LAQV/REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
3) IBILI, Faculdade de Medicina, Universidade de Coimbra, Portugal
4) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Reactivation of the tumor suppressor protein p53 is an attractive anticancer therapeutic strategy. One way to reactivate p53 is by inhibiting the p53-MDMs interactions. In this way, p53 can be liberated and act again as a tumor suppressor. Currently, nine small molecules that inhibit MDM2 have reached clinical trials. However, it is now considered that, for full p53 reactivation, dual inhibition of p53-MDM2 and p53-MDMX interactions is required. Previously, the preliminary screening of tryptophanol derivatives in yeast cell models to search for p53 activators led to the identification of the hit tryptophanol-derived oxazoloisoindolinone SLMP53-1. This small molecule showedp53-dependent anti-proliferative activity in human wild-type (wt) and mutant (mut) p53R280K-expressing tumor cells, and was selected for further optimization [1]. From this work, we developed compound DIMP53-1, a small molecule that inhibits the growth of wt/mut p53-expressing tumors, but not of p53-null tumors [2]. In this communication, we will present our optimization efforts on SLMP53-1. Synthesis, structure-activity relationship study, biological evaluation, and stability studies of a chemical library of enantiopure tryptophanol-derived oxazoloisoindolinones will be presented.



Acknowledgments: We thank funding from FCT (Fundação para a Ciência e a Tecnologia) through iMed.ULisboa (UID/DTP/04138/2013), LAQV/REQUIMTE (UID/MULTI/04378/2013), project PTDC/QUI-QOR/29664/2017, grant IF/00732/2013 (M.M.M. Santos), andfellowships PD/BI/135334/2017 (Valentina Barcherini), SFRH/BD/117931/2016 (Margarida Espadinha), SFRH/BD/96189/2013 (S. Gomes), and SFRH/BD/128673/2017 (J. B. Loureiro). We also thank the European Union and National Funds under the project (3599-PPCDT) PTDC/DTP-FTO/1981/2014– POCI-01-0145-FEDER-016581.

References

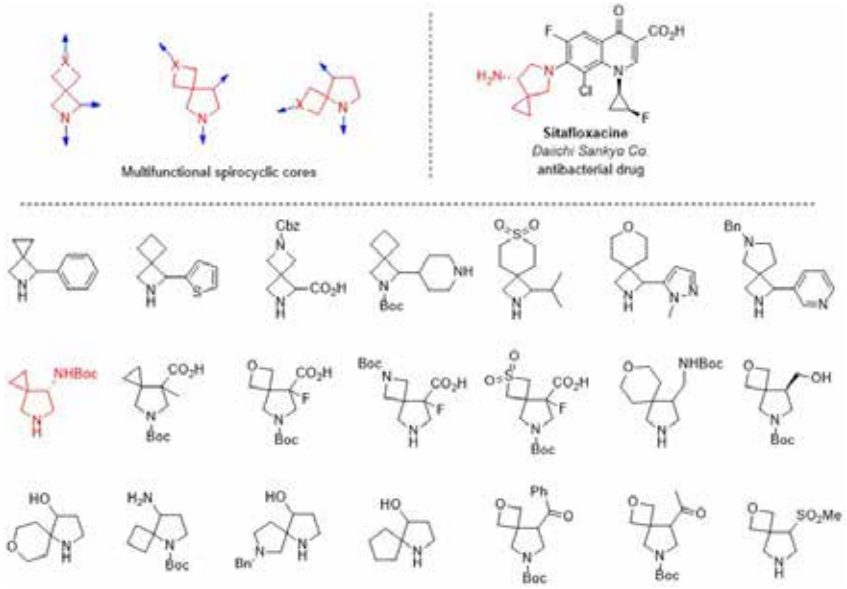
1) a) Soares J et al, Oncotarget 2016, 7, 4326; b) Saraiva L and Santos MMM et al, PCT/IB2014/062617, WO2014/207688 A1 (2014).
2) Soares J et al, Mol. Oncol. 2017, 11, 612.

RAPID ACCESS TO NOVEL MULTIFUNCTIONAL SPIROCYCLIC CORES FOR DRUG DISCOVERY

T. Savchenko, B. Chalyk, A. Kirichok, A. Chernykh, Pavel Mykhailiuk

Enamine Ltd, 78 Chervonotkatska Street, 02094 Kyiv, Ukraine

Trends in drug discovery are changing rapidly. During the past decade, terms "*Scaffold hopping*," "*Escape the Flatland*" and "*Conformational restriction*" have been introduced, and have already found huge practical application. Spiro compounds are especially interesting, because they are intrinsically both - 3D-shaped and conformationally restricted.[1]



In this work, we have rationally designed, synthesized and applied a library of novel multifunctional spirocyclic cores for drug discovery. Details of the synthesis and application of the obtained compounds will be discussed.[2-7]

References

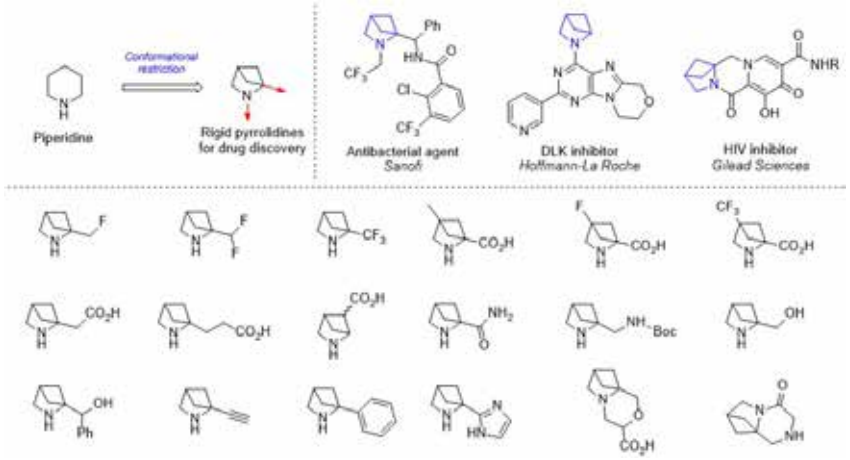
1) J. A. Burkhard et al. Angew. Chem. Int. Ed. 2010, 3524.
2) A. Kirichok et al. Chem. Eur. J. 2018, 21, 5444.
3) B. Chalyk et al. Chem. Eur. J. 2017, 23, 16782.
4) A. Kirichok et al. Angew. Chem. Int. Ed. 2017, 56, 8865.
5) A. Chernykh et al. Tetrahedron 2016, 1036.
6) A. Chernykh et al. J. Org. Chem. 2015, 3974.
7) T. Drugenko et al. Org. Lett. 2015, 3974.

CONFORMATIONALLY-RESTRICTED PYRROLIDINES FOR DRUG DISCOVERY

T. Savchenko, V. Levterov , P. Mykhailiuk

Enamine Ltd, 78 Chervonotkatska Street, 02094 Kyiv, Ukraine

"Conformational restriction" concept has already gained a considerable attention in medicinal chemistry.[1] Scientists are looking more and more now on 3D-shaped saturated building blocks.[2,3] In this context, intrinsically conformationally rigid bicyclic amines seem to be promising for drug discovery.



In this work, we have rationally designed, synthesized and applied a library of novel/previously scarcely available diverse bicyclic amines in medicinal chemistry.[4-6] The key synthesis step was photochemical [2+2]-cyclization. Details of the synthesis and application of the obtained compounds will be discussed.

References

1) F. Lovering et al. J. Med. Chem. 2009, 6752.
2) O. Wlochal et al. Org. Lett. 2014, 4094.
3) M. Westphal et al. ChemMedChem. 2015, 461.
4) A. N. Tkachenko et al. Org. Lett. 2009, 5674.
5) P. Mykhailiuk et al. J. Org. Chem. 2017, 8831.
6) V. Levterov et al. Manuscript under preparation.

FRAGMENT-BASED APPROACH APPLIED TO THE DISCOVERY OF PROTEIN-PROTEIN INTERACTION STABILISERS

Dario Valenti (1), João Neves (2), Alice Ballone (3), Madita Wolter (3), Federica Centorrino (3), Domenico Lentini Santo (4), Stanimira Hristeva (1), François-Xavier Cantrelle (2), Martin Corpet (1), Isabelle Landrieu (2), Tomáš Obšil (4), Dimitrios Tzalis (1), Christian Ottmann (3)

1) Medicinal Chemistry, Taros Chemicals GmbH & Co. KG, Emil-Figge-Straße 76a, 44227 Dortmund, Germany
2) NMR and Molecular Interactions, UMR 8576 CNRS, University of Lille, 59000 Lille, France
3) Department of Biomedical Engineering, Technische Universiteit Eindhoven, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands
4) Department of Physical and Macromolecular Chemistry, Charles University, Hlavova 2030, 12840 Prague 2, Czech Republic

Protein-protein interactions (PPIs) are constituents of numerous biological pathways and offer therapeutic intervention points into different pathologies such as cancer[1], inflammation[2], neurodegenerative[3] and metabolic diseases[4].
Since stabilisation of PPIs has not yet been explored in a systematic way, the TASPPI (TArgeted small-molecule Stabilisation of Protein-Protein Interactions) consortium[5] aims to identify chemical PPI stabilisers in order to develop new crucial therapeutic strategies in the treatment of the disease areas mentioned above.
The Taros fragment collection was selected as the compound source for developing small molecules able to stabilise the complexes of 14-3-3 protein and its partners. The collection is Ro3-compliant[6] and three-dimensionality and shape diversity have been emphasized as design parameters during the generation process of the library.
The physicochemical properties distribution of the fragment set (Figure 1, a-d) will be presented together with selected examples of novel structures originating from the proprietary collection of Taros.
The design was inspired by two main sources: (i) natural compounds and (ii) known scaffolds from drug discovery campaigns. Nicotine-like fragments (Figure 1, e) represent the perfect match between these two strategies that produced an interesting original Biocore[7] – the 1,3,5-trisubstitued triazole – and showcases a new concept in fragment design named “SAR by Biocores”. To date, the fragment collection comprises approx. 1.100 fragments and offers ample opportunities for expansion.
Fluorescence polarization, differential scanning fluorimetry, X-ray crystallography and NMR-based techniques have been applied by the consortium members during the primary screening and led to the identification of novel hits binding to different 14-3-3 complexes. These novel binders represent an important starting point for future medicinal chemistry-based fragment evolution campaigns.

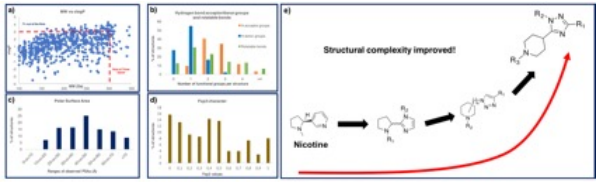


Figure 1. a-d) Physicochemical properties of the Taros fragment collection. **a)** MW and clogP correlation . **b)** Distribution of H-bond acceptors/donors and rotatable bonds. **c)** Polar Surface Area (PSA) distribution. **d)** Saturation index (fsp3) distribution (0 = completely flat, 1 = highly trhee-dimensional). **e)** Nicotine-like fragment structural evolution.

References

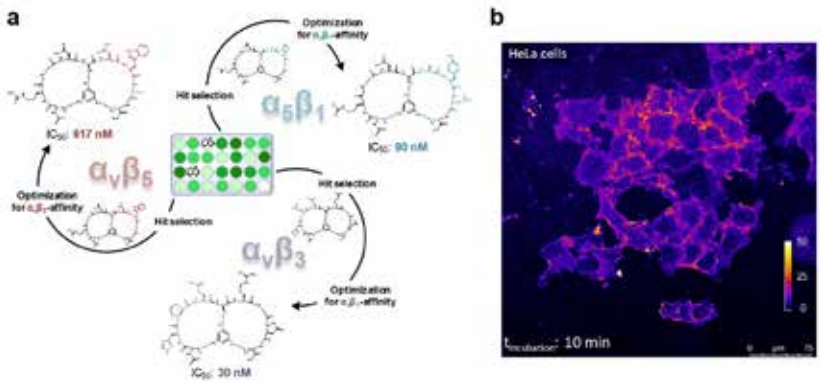
1) Waterman, M. J. F. et al. Nat. Genet. 19 (1998)
2) Aguilera, C. et al. J Cell Sci 119 (2006)
3) Hashiguchi, M. et al. J. Biol. Chem. 275 (2000)
4) Datta, S. R. et al. Mol. Cell 6 (2000)
5) TASPPI • Molecular glue for new drug development - http://wwww.tasppi.eu/
6) Congreve, M. et al. Drug Discov. Today 8 (2003)
7) Kombarov, R. et al. Mol. Divers. 14 (2010)

RGD BICYCLES: HIGH-AFFINITY LIGANDS FOR SELECTIVE INTEGRIN-TARGETING FOR CANCER THERAPY

D. Bernhagen (1), N. Gisbert Quilis (2), V. Jungbluth (2), J. Dostalek (2), F. Cipriani (3), K. Jalink (4), P. Timmerman (1,5)

1) Pepscan Therapeutics, Lelystad, The Netherlands
2) Biosensor Technologies, AIT Austrian Institute of Technology GmbH, Vienna, Austria
3) Technical Proteins Nanobiotechnology S.L., Valladolid, Spain
4) The Netherlands Cancer Institute, Amsterdam
5) Van ‘t Hoff Institute for Molecular Sciences, University of Amsterdam, Amsterdam, The Netherlands

Integrins, a group of 24 heterodimeric transmembrane proteins, mediate cell-cell and cell-extracellular matrix interactions via interaction with proteins such as fibronectin and vitronectin. Due to their involvement in cancer metastasis, in particular integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$, they are considered potential targets for cancer therapy. Bicyclic peptides recently attracted interest as a powerful platform for novel therapeutics because of their high binding affinities and proteolytic stability. We therefore screened hundreds of different bicyclic peptides for binding to integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$, which are overexpressed in various cancer cell lines, and gradually improved the affinities and selectivities. The best IC_{50} values were, for example, 30 nM for $\alpha_v\beta_3$ (GRGDS: 5 µM, knottin-RGD: 38 nM), and 90 nM for $\alpha_5\beta_1$ (GRGDS: >10 µM, knottin-RGD: 114 nM). We also studied integrin-binding on cells via confocal microscopy with Cy5-functionalized bicycles. Finally, cell behavior studies with peptide-functionalized soft 3D hydrogels and elastin-like recombinamers (ELRs) revealed superior cell adhesion and proliferation of the bicycles compared with conventional RGD-peptides.



a) Screening approach for high integrin-affinity bicyclic RGD-peptides; **b**) HeLa cells stained with a Cy5-functionalized integrin $\alpha_5\beta_1$-selective RGD-bicycle.

References

1) D. Bernhagen, L. De Laporte, P. Timmerman, Anal. Chem. 2017, 89, 5991–5997.
2) D. Bernhagen, N. Gisbert Quilis, V. Jungbluth, J. Dostalek, P. Timmerman, in preparation.
3) P. Li, P. P. Roller, Curr. Top. Med. Chem. 2002, 2, 325–341; V. Baeriswyl, C. Heinis, ChemMedChem 2013, 8, 377–384.
4) M. Barczyk, S. Carracedo, D. Gullberg, Cell Tissue Res. 2010, 339, 269–280; Y. Takada, X. Ye, S. Simon, Genome Biol. 2007, 8, 215.
5) R. H. Kimura, A. M. Levin, F. V Cochran, J. R. Cochran, Proteins 2009, 77, 359–369.

DISCOVRY OF N-ARYLSULFONYL INDOLE-2-CARBOXAMIDES AS NOVEL ANTI-DIABETIC AGENTS

Jianbo Bie, Shuainan Liu, Hualong Chen, Rongcui Li, Jie Zhou, Zhufang Shen, Bailing Xu

Institute of Materia Medica, Chinese Academy of Medical Sciences Peking Union Medical College, Beijing, 100050, China

Excessive hepatic glucose output is a key factor leading to fasting hyperglycemia and postprandial hyperglycemia in type 2 diabetes patients. Fructose-1, 6-bisphosphatase (FBPase), acting as a rate-limiting enzyme in gluconeogenesis pathway, plays an important role in the control of blood glucose. Furthermore, FBPase inhibitors have been verified to be capable of reducing hepatic glucose production and lowering blood glucose levels in animal models of diabetes. Therefore, Inhibition of FBPase may become a new strategy for the development of novel antidiabetic agents.

In our efforts to search for novel FBPase inhibitors, various novel indole derivatives were designed and synthesized, and their enzymatic inhibitory activities against FBPase were evaluated. As a result, a series of potent and structurally novel lead candidates were identified with IC_{50} values at 10^{-8} M level. Among them, the candidate BJB-2936 and its sodium salts were evaluated extensively in terms of pharmacodynamic and pharmacokinetic properties. Long-term administration of BJB 2936 and its sodium salts to diabetic animal models (KKA^{y} mice) resulted in significant glucose lowering and HbA1c reductions. The FBPase activity of liver in mice was inhibited in a dose dependent manner, and about 90% inhibition was achieved at an oral dose of 200 mg/kg. The pharmacokinetic parameters of BJB2936 and its sodium salts in rats were also investigated. It has been demonstrated that BJB2936 and its sodium salts were orally available.

In summary, a new class of structurally distinct N-arylsulfonyl indole-2-carboxamide was identified as FBPase inhibitors. The pronounced glucose lowering potency and the acceptable pharmacokinetic properties warrant this new class of FBPase inhibitors to be further developed as a novel therapeutic approach for the treatment of type 2 diabetes mellitus.

Acknowledgements

This work is supported by National Natural Science Foundation (No. 81502933), National Major Special Project on New Drug Innovation of China (2018ZX09711002-003-012) and CAMS Initiative for Innovative Medicine (CAMS-I2M-2-004)

References

1) Bie, J. B.; Liu, S. N.; Zhou, J.; Xu, B. L.; Shen, Z. F. Design, synthesis and biological evaluation of 7-nitro-1H-indole-2-carboxylic acid derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase. Bioorg. Med. Chem. 2014, 22, 1850-1862.
2) Bie, J. B.; Liu, S. N.; Li, Z. M.; Mu, Y. Z.; Xu, B. L.; Shen, Z. F. Discovery of novel indole derivatives as allosteric inhibitors of fructose-1,6-bisphosphatase. Eur. J. Med. Chem. 2015, 90, 394-405.

POSTERS - TECHNOLOGIES

Expanding Medicinal Chemists Synthetic Toolbox

DEVELOPMENT OF AN ENANTIOSPECIFIC SYNTHETIC ROUTE TO HSP CO-INDUCER ARIMOCLOMOL AND IT'S ANALOGUES

Benjamin N. Atkinson, Hannah Woodward, James Sipthorp, Paul V. Fish

Alzheimer's Research UK UCL Drug Discovery Institute, The Cruciform Building, Gower Street, London, WC1E 6BT

The Wnt signaling pathway has been shown to regulate crucial aspects of cell fate determination, organogenesis, cell migration and polarity.[1] Importantly, compromised Wnt signaling has been implicated in the perturbation of synaptic integrity and function in Alzheimer's disease (AD).[2]

Palmitoleoylation of Wnt proteins is required for efficient binding to Frizzled receptors and the subsequent signal transduction. The carboxylesterase Notum has been shown to act as a key negative regulator of the Wnt signaling pathway in Drosophila by specifically mediating the depalmitoleoylation of Wnt proteins.[3,4] Notum is expressed in the mammalian central nervous system (CNS): Notum is upregulated at mRNA level in whole brain lysates in AD model (APP-PS1 mice) and upregulated in human AD patient brain samples. We are currently investigating the role of Notum in modulating Wnt signaling in the CNS. We propose that inhibition of Notum could prolong Wnt signaling, with potential beneficial effects to neuronal health in AD.

To identify Notum inhibitors,[5] a fragment library screening approach was performed using crystal soaking X-ray crystallography. A robust primary fluorescence assay has been developed for the characterisation of Notum inhibitors, along with a secondary, more biologically relevant, native substrate assay.

A number of novel fragment hits were identified as Notum inhibitors with micromolar affinity (1 uM to >1 mM). A rational, structure based drug design (SBDD) process was used to generate highly potent (

References

1) Atkinson, B. N.; Woodward, H. L.; Sipthorp, J.; Fish, P. V., Org. Biomol. Chem. 2017, 15 (46), 9794-9799 and references therein.
2) Ürögdi, L.; Jegesné-Csákai, Z.; Gruber, L.; Ötvös, L.; Tóth, J.; Tömösközi, I.; Szakácsné-Schmidt, A.; Reider, F.; Schneiderné-Barlay, M. PCT Int. Appl, WO2001/79174. 2001.

PEPTIDE MACROCYCLES THAT PERMEATE MEMBRANE BARRIERS. COMBINING N-METHYLATION AND PRODRUG APPROACHES

Nicholas Barlow, Billy Williams-Noonan, David Chalmers, Philip Thompson, Ray Norton

Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University, Parkville, Vic., Australia

There is significant interest in developing peptide macrocycles as inhibitors of protein-protein interactions, which are generally considered to be difficult-to-drug targets. In recent years several groups[1-4] have focussed on the methylation of backbone amide nitrogen atoms as a means to enhance the passive permeability of peptide macrocycles. In particular, N-methylated cyclic hexapeptides have been studied extensively and the impact of N-methylation on lipophilicity and internal H-bonding has been shown to contribute to passive permeability.[2,4] While extraordinary enhancements in permeability are associated with the introduction of N-methyl groups in peptides such as cyclo(Leu-Leu-Leu-Leu-Pro-Tyr),[1] the inclusion of just a single polar or charged side chain dramatically reduces permeability, limiting the applicability of this approach in the development of permeable and biologically active peptide macrocycles.

Here we describe the use of a prodrug strategy in concert with N-methylation to temporarily mask polar surface area in order to confer permeability across biological membrane barriers. We designed and synthesised prodrug derivatives of Asp, Glu, Lys, Ser and His-containing cyclic peptides that masked hydrogen bond donors but were likely to be labile under physiological conditions. For each of the 6 polar amino acids investigated, we identified a prodrug group that could recover peptide permeability. While thorough optimisation and development of permeability groups for each polar amino acid is an ongoing focus for our group, these initial results highlight the utility of this approach. Furthermore, we have shown that, while N-methylation, and potentially internal hydrogen bonding, are important strategies for conferring membrane permeability to macrocycles, a prodrug strategy may broaden the range of peptide macrocycles that can be designed to penetrate biological barriers.

References

1) White, T. R. et al. On-resin N-methylation of cyclic peptides for discovery of orally bioavailable scaffolds. Nat. Chem. Biol. 7, 810-817 (2011).
2) Frost, J.R., Scully, C.C.G., Yudin, A.K. Oxadiazole grafts in peptide macrocycles. Nat. Chem. 8, 1105-1111 (2016).
3) Wang, C. K. et al. Rational design and synthesis of an orally bioavailable peptide guided by NMR amide temperature coefficients. Proc. Natl. Acad. Sci. U.S.A. 49, 17504-17509 (2014).
4) Hewitt, W. M. et al. Cell-Permeable Cyclic Peptides from Synthetic Libraries Inspired by Natural Product. J. Am. Chem. Soc. 137, 715-721 (2014).

BUILDING A DIVERSE AND EXPERIMENTALLY-CURATEDFRAGMENT LIBRARY

Andrew Lowerson (1), Steve Brough (1), Patrick McCarren (2), Steven Laplante (3), Michael Serrano-Wu (2)

1) Key Organics Ltd, Highfield Road Industrial Estate, Camelford, Cornwall, PL32 9RA, UK
2) Center for the Development of Therapeutics, Broad Institute, 415 Main Street, Cambridge, MA 02142, US
3) NMX Research and Solutions Inc., 500 Cartier Blvd., Suite 6000, Laval, Québec, Canada H7V 5B7

Fragment libraries are commonly assembled by Rule of 3 filtering followed by manual curation. However, the robust experimental data that ensures the proper physicochemical attributes needed for high-concentration screening is often lacking and replaced instead by *in silico* calculations of uncertain predictive value. A fragment collection with experimentally-determined aqueous solubility will address a major source of false positives and attrition in fragment screening libraries: **Aggregation, Stability, and Solubility**. ^{1}H NMR spectral data in aqueous buffer will further enable practitioners to rapidly build fragment pools and initiate screening.

Diversity selection methods in shape, scaffold, fingerprint, and predicted property space combined with industry-standard substructure filtering were used to select over 2,500 Key Organics compounds for experimental profiling. NMR and LCMS analysis allowed the careful selection of highly-soluble fragments with desirable physicochemical and stability characteristics. Importantly, the curated molecules are enriched in cyclic scaffolds commonly found in drug candidates, and spans chemical space that minimally overlaps with existing commercial collections. This poster will summarize the experimental and cheminformatic features of this next generation **Key Organics 'BIONET Premium Fragment Library'**

References

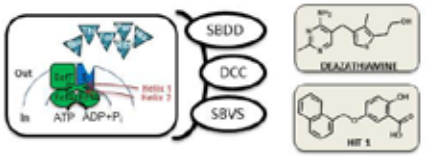
1) Bradley C. Pearce , Michael J. Sofia, Andrew C. Good, Dieter M. Drexler, and David A. Stock. An Empirical Process for the Design of High-Throughput Screening Deck Filters. Journal of Chemical Information and Modeling 2006, 46, 1060-1068.
2) Jonathan B. Baell and Georgina A. Holloway. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. J. Med. Chem., 2010, 53, 2719-2740.
3) David Lagorce, Olivier Sperandio, Hervé Galons, Maria A Miteva, and Bruno O Villoutreix. FAF-Drugs2: Free ADME/tox filtering tool to assist drug discovery and chemical biology projects. BMC Bioinformatics 2008, 9:396.
4) eroen Kazius, Ross McGuire, and Roberta Bursi. Derivation and Validation of Toxicophores for Mutagenicity Prediction. J. Med. Chem., 2005, 48, 312-320.
5) Robert F. Bruns and Ian A. Watson. Rules for identifying potentially reactive or promiscuous compounds. J. Med. Chem., 2012, 55, 9763-9772
6) Lisurek M, Rupp B, Wichard J, Neuenschwander M, von Kries JP, Frank R, Rademann J, Kühne R. Design of chemical libraries with potentially bioactive molecules applying a maximum common substructure concept. Mol Divers. 2010 May;14(2):401-8. doi: 10.1007/s11030-009-9187-z. Epub 2009 Aug 15
7) Richard D. Taylor, Malcolm MacCoss, and Alastair D. G. Lawson. Rings in Drugs. J. Med. Chem., 2014, 57 (14), pp 5845–5859.

EXPLORING HIT-IDENTIFICATION STRATEGIES FOR ECF TRANSPORTERS, A NOVEL ANTI-INFECTIVE TARGET

Diamanti Eleonora (1,2), Monjas Leticia (2), Swier Lolkje (3), Setyawati Inda (3), Bousis Spyridon (1,2), Herrmann Jennifer (4), Haupenthal Jörg (1), Müller Rolf (4), Slotboom Dirk (3), Hirsch Anna (1,2)

1) Drug Design and Optimization Department, Helmholtz Institute for Pharmaceutical Research Saarland, Germany
2) Stratingh Institute for Chemistry, University of Groningen, The Netherlands
3) Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands
4) Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland, Germany

The emergence of drug resistance against important pathogens poses an ever-growing health threat. The pipeline of novel drug candidates should be filled with molecules featuring an unprecedented mode of action and an unprecedented chemical structure. We address both challenges by using multiple hit-identification strategies targeting a novel and unexplored anti-infective drug target, called Energy-Coupling Factor (ECF) Transporter. The ECF module is an integral membrane protein involved in the uptake of essential micronutrients.[1]Hence, the inhibition of this transport should translate into a deficiency of vitamins in the bacterial cytosol. We embarked in a structure based drug design (SBDD) of thiamine analogue as binders of ThiT, while to explore the large and flexible substrate-binding pocket of the ThiT protein we used Dynamic Combinatorial Chemistry (DCC)[2,3]. A structure-based virtual screening (SBVS) provided us with the first allosteric inhibitors of the transporter for folate able to both reduce folate concentration in the cytosol and to reduce the bacterium growth. Additionally, the excellent drug-like properties of this chemical class of compounds triggered a medicinal chemistry campaign that turned out with the first inhibitors against the ECF transporters active against a plethora of pathogenic Gram-positive organism (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecium*).[4]



References

1) D. J. Slotboom. Nature Rev. Microbiol., 12, 79-87, (2014)
2) L. J. Y. M. Swier et al. ChemBioChem., 16, 79-87, (2015)
3) M. Mondal, A. K. H. Hirsch Chem. Soc. Rev., 44, 2455-2488, (2015)
4) E.Diamanti et al. Manuscript submitted

DRUG DISCOVERY AT THE SPEED OF SOUND

Alexander Domling (1), Shabnam Shaabani (1), Joe Olechno (2), Michael Kossenjans (3), Li Gao (1), Maryam Ahmadian Moghaddam (1), Ruixue Xue (1), Tryfonas Zarganes-Tzitzikas (4), Constantinos Neochoritis (4), Richard Ellson (2)

1) Department pf Drug Design, University of Groningen, A. Deusinglaan 1, 9700 AD Groningen, Netherlands
2) LABCYTE INC., 170 Rose Orchard Way, San Jose, California 95134, USA
3) AstraZeneca, R&D, Pepparedsleden 1, Mölndal, Sweden, SE-43183
4) Telesis BV, Hofstraat 22, 9712JC Groningen, Netherlands

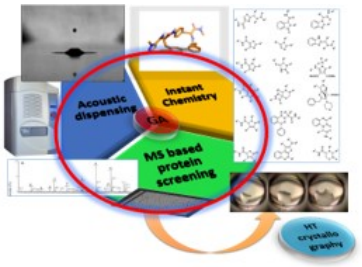
A blockbuster drug generates > $ 1 billion revenues per year. Each day not on the market corresponds to a loss of > $ 2.7 million. Multiple benchmark reports suggest development costs of drugs are skyrocketing while the introduction of novel drugs is decreasing or at best stagnating. Part of the problems can be attributed to the preclinical drug discovery and development involving expensive high throughput screening (HTS) and hit-to-lead campaigns using mostly traditional technologies.

Here we introduce a fundamentally novel approach towards preclinical drug discovery and development by blending Instant Chemistry, nL dispensing, acoustic-MS, uHTS and artificial intelligence.

Acoustic droplet ejection (ADE) technology allows for the fast, contact-less and accurate transfer of very small droplets (nL) from plate to plate of different high density formats. ADE has had a dramatic impact in different technology areas, including drug discovery, cancer research and genomic research and is used in many laboratories world-wide. However, ADE has never been used in miniaturization and acceleration of library synthesis for uHT to dramatically accelerate the preclinical drug discovery cycle.

One-pot multicomponent reactions (Instant Chemistry, MCRs) are suitable to create very large libraries of small molecules and macrocycles.[1-2] A prototype instrumentation platform is developed which allows for the parallel synthesis of hundreds of libraries of scaffolds on an unprecedented dense format. The platform is integrated with acoustic-MS for quality control and an efficient affinity-based mass-spectrometry screening platform using the same high density format. Artificial intelligence is developed to ensure never-seen-before fast cycle times for hit-2-lead progression.[3]

Here we will discuss the high throughput synthesis of muliple drug-like scaffolds, their corresponding analytics, as well as initial screening efforts against several protein protein interactions.



References

1) A. Domling, W. Wang, K. Wang, Chem. Rev. 2012, 112, 3083-3135.
2) R. Madharvachary et al., Angew. Chem. Intl. Ed. Engl. 2017, 56, 10725-10729.
3) L. Weber et al., Angew. Chem. Intl. Ed. Engl. 1995, 34, 1521-3773.

MEDICINAL CHEMISTRY TOOL BOX FOR RAPID ASSEMBLY OF PROTAC MOLECULES

Patrick Oude Alink, Marcel Scheepstra, Yannick Smits, Toine van den Bergh, Rob Winkel, Koen Hekking, Rutger Folmer

Mercachem, Kerkenbos 1013, 6546 BB Nijmegen, Netherlands

Proteolysis targeting chimeras (PROTACs) have recently received significant attention as a new modality for therapeutic intervention (recently reveiwed in [1]). The technology is based on hijacking E3 ligases to tag a protein of interest with ubiquitin for degradation by the proteasome. This involves the synthesis of a chimeric ligand in which a compound that binds to the protein target of interest is linked to a second molecule that binds an E3 ligase (usually either cereblon or VHL).

The linking of the two small molecule ligands is typically done through a polyethylene glycol (PEG) based linker, consisting of 3 to 6 glycol units. The optimal linker length as well as the level of lipophilicity need to be empirically determined using a relevant cellular assay (based on detection of protein amount, or a more functional assay).

PROTAC molecules are typically around 1 kDa in size, and not seldom link together two molecules with mediocre physico chemical properties (notably high LogP, low solubility). As a result, the synthesis, analysis and purification of these compounds can be more challenging than is the case for traditional small molecule ligands.

To address this challenge, we have developed a modular chemistry toolbox in which different linking strategies are combined with optimised analytical routines. This approach allows for the rapid assembly of a comprehensive set of different PROTAC molecules for any new target for which a ligand exists.

In our view, this approach can significantly speed up the development of potential PROTAC drugs, a process that can be very laborious with the currently described tools.

In addition, we are developing novel linkers that go beyond PEG, with the goal of fine-tuning the properties of the resulting bivalent compound.

An overview of our activities in this area will be presented.

References

1) Toure, M. & Crews, C.M. (2016) Small-Molecule PROTACS: New Approaches to Protein Degradation, Angew. Chemie Int. Ed. 55, 1966

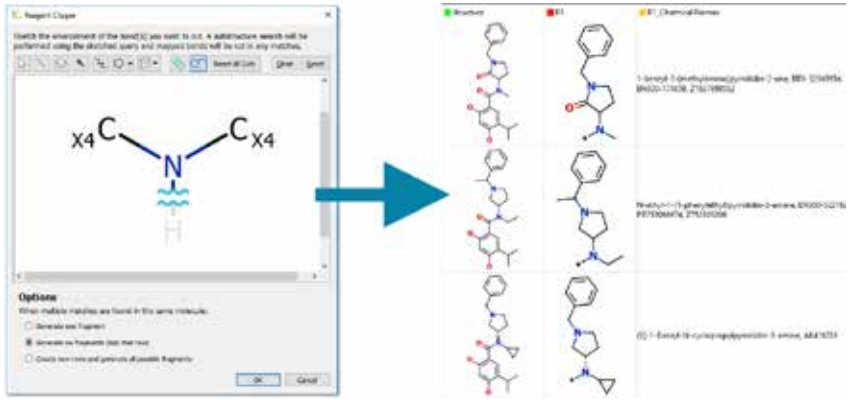
INTUITIVE WORKFLOW TO ENUMERATE AND EXPLORE LARGE VIRTUAL LIBRARIES

Aishling Cooke, James Chisholm, Edmund Champness, Peter Hunt, Matthew Segall, Tamsin Mansley

Optibrium Ltd., Cambridge, United Kingdom

Enumeration of a virtual library based on cores or scaffolds of interest helps to quickly explore potential substituents around hit or lead series and prioritise strategies that are most likely to yield high quality compounds. In this poster, we will describe a seamless workflow, beginning with a search of commercially available building blocks. These can then be 'clipped' to generate the corresponding R-groups for enumeration of virtual libraries, using a flexible and visual approach based on defining substitution points around a substructure search of the building blocks. This flexibility means that chemists are not restricted to a limited number of pre-defined patterns for reagent clipping and can adapt to many different reaction schemes, while the visual interface makes it intuitive and easy to use.

The resulting R-groups, corresponding to the available building blocks, can be incorporated into virtual libraries around scaffolds of interest. However, with an extensive list of R-groups, enumeration of a fully combinatorial library might generate a vast number of compounds, which may be too large to explore or even overwhelm the resources of a computer. Therefore, we will illustrate how the enumeration can be integrated with predictive modelling and multi-parameter optimisation, to prioritise and retain the compounds that are most likely to achieve the objectives of a project and avoid this 'combinatorial explosion'. The resulting compounds and corresponding building blocks guide the synthesis of focussed, high quality libraries targeting a project's optimisation objectives.

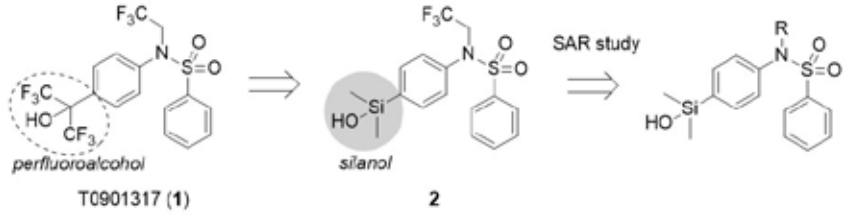


SILANOL AS A CHARACTERISTIC HYDROPHILIC PHARMACOPHORE --ALTERED ACTIVITY PROFILE OF A SILANOL ANALOGUE OF MULTI-TARGETING NUCLEAR RECEPTOR MODULATOR T0901317 --

Shinya Fujii, Hirozumi Toyama, Yuichi Hashimoto

Institute for Quantitative Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-0032, Japan

The alcoholic hydroxy group is one of the most important functional groups in biologically active compounds, and the use of novel substitutes for the alcoholic hydroxy group is a promising approach for structural development of drug candidates. Regarding development of alternatives to the alcoholic hydroxyl group, incorporation of heteroatoms is useful, and silanol has potential as an isosteric substructure of alcoholic hydroxy groups. To expand the utility of silanols, we focused on the similarity between silanol and perfluoroalcohol. Perfluoroalcohols show higher acidity and hydrophobicity than the corresponding hydrocarbon-based alcohols, owing to the differences of electronegativity and molecular volume between alkyl groups and the corresponding perfluoroalkyl groups. Silanols also show somewhat higher acidity and hydrophobicity than the corresponding alcohols because of the differences of electronic properties and molecular volume between silanols and the corresponding alcohols. The 1,1,1,3,3,3-hexafluoro-2-hydroxypropyl group, one of the perfluoroalcoholic functionalities, is a key structural motif of T0901317 (**1**), which is a benzenesulfonamide-based compound that exhibits agonistic activity toward multiple nuclear receptors, including LXRα and LXRβ, PXR, FXR, and inverse agonistic activity toward RORs. Previous researches revealed that the substituents around the hydroxy group have a significant influence on the activity and selectivity. So, we designed and synthesized silanol **2**, a silanol analog of **1**, and investigated the activity profile of **2**. The ligand potencies of the compounds toward hLXRs, hFXR, hPXR, and hRORs were evaluated by means of luciferase reporter gene assay in HEK293 cells. As a result, compound **2** exhibited significant agonistic activity toward PXR, modest partial agonistic activity toward FXR, and inverse agonistic activity toward RORs. Compound **2** exhibited neither agonistic nor antagonistic activity toward LXRs. The PXR agonistic activity of **2**is comparable to that of representative PXR agonist rifampicin. This result suggests that the silanol group could function as an isoster of the perfluoroalkyl group in the case of PXR ligands. We also investigated further modification and structural development of silanol derivatives. The detailed structure-activity relationship will be discussed.



References

1) Toyama H., et al, Bioorg. Med. Chem. Lett., 26, 1817–1820 (2016).

P068

FINDING NOVEL 14-3-3 PROTEIN-PROTEIN INTERACTION MODULATORS USING DYNAMIC COMBINATORIAL CHEMISTRY

Alwin M. Hartman (1), Anna K. H. Hirsch (2)

1) Chemical Biology 2 - Stratingh Institute for Chemistry,, Nijenborgh 7, 9747 AG Groningen, The Netherlands
2) Department of Drug Design and Optimization (DDOP), Helmholtz Institute for Pharmaceutical Research Saarland, Campus E8.1, D-66123 Saarbrücken, Germany, Tel: +49 681 98806 2100

Protein-Protein Interactions (PPIs) can be found in many biological processes. It is assumed that between 130,000 and 600,000 PPIs exist, some play a role in carcinomas others for example in cell-cycle regulation. The 14-3-3 protein family is known for its PPIs, as it is implicated in several diseases and biological processes. Proteins of this family do not have any enzymatic activity, however, they interact and regulate the activity of other proteins. Finding modulators which could stabilize or inhibit the PPIs, would constitute a tool to modulate these interactions and possibly interfere with undesired biological processes by targeting the corresponding PPIs. [1,2] Dynamic Combinatorial Chemistry (DCC) is a powerful tool to identify biologically active compounds. The strength of this technique is the amplification of the best binders by the target. [3] We pioneered, DCC for the identification of modulators of 14-3-3 proteins, representing its first application to a PPI. Biochemical evaluation of the amplified hits confirmed the activity and optimization of promising compounds is ongoing.

References

1) M. Skwarczynska, C. Ottmann, Protein-protein interactions as drug targets, Future Med. Chem. 7 (2015) 2195-2219,
2) A.M. Hartman, A.K.H. Hirsch, Molecular insight into specific 14-3-3 modulators: Inhibitors and stabilisers of protein–protein interactions of 14-3-3,Eur. J. Med. Chem. 136 (2017) 573-584
3) R. vd Vlag, A.K.H. Hirsch, Analytical Methods in Protein-Templated Dynamic Combinatorial Chemistry, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, DOI: 10.1016/B978-0-12-409547-2.12559-4

P069

EXPANDING THE MEDICINAL CHEMISTRY TOOLBOX

Jonas Boström (1), Dean Brown (2), Robert Young (3), György M. Keserű (4)

1) Medicinal Chemistry, CVRM IMED Biotech Unit, AstraZeneca, Gothenburg, Pepparedsleden 1, SE-431 83, Sweden
2) Discovery Sciences IMED Biotech Unit, AstraZeneca, 35 Gatehouse Drive, Waltham, Massachusetts 02451, USA
3) NCE-Medicinal Chemistry, GlaxoSmithKline R&D, Stevenage, Hertfordshire, SG1 2NY, UK
4) Medicinal Chemistry Research Group, Research Center for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2, H-1117 Budapest, Hungary

The key objectives of medicinal chemistry are to efficiently design and synthesize bioactive compounds that have the potential to become safe and efficacious drugs. Most medicinal chemistry programs rely on screening compound collections populated by a range of molecules derived from a set of known and robust chemistry reactions. Analysis of the role of synthetic organic chemistry in subsequent hit and lead optimization efforts suggests that just a few reactions dominate in the optimizations. Thus, the uptake of new synthetic methodologies in drug discovery is limited. Starting from the known limitations of reaction parameters, synthesis design tools, synthetic strategies and innovative chemistries, here we highlight opportunities for expansion of medicinal chemists' synthetic toolbox. More intense crosstalk between synthetic and medicinal chemists in industry and academia should enable enhanced impact of new methodologies in future drug discovery.

EXPLORING 3,794,923,5913,794,923,591 MOLECULES AVAILABLE ON DEMAND

Franca-M. Klingler (1), Marcus Gastreich (1), Yurii Moroz (2), Michael Bossert (2)

1) BioSolveIT GmbH, Sankt Augustin, Germany
2) Enamine Ltd, Kiev, Ukraine

Small molecules have been the major source of new drugs. However, the target space is limited and overused in-stock collections are less and less capable to provide new chemical entities (NCEs). In a joint venture, Enamine and BioSolveIT built the world's largest chemical space and made it ultra-fast searchable. The new product, called REALSpaceNavigator, comprising 650 million compounds, allows for efficient hit exploration, from finding previously unknown analogues to scaffold hopping. The technology supports fast similarity searching (about 2 min only) in the space and convenient analysis of the results - all behind your firewall to make sure your IP is protected. The chemical space encoded with more than 100 highly validated synthesis protocols and in-stock building blocks, provides an escape from availability bias of current stock screening collections towards IP free areas. Compounds selected from this space will be synthesized in 3 weeks with an exceptional success rate of 80% and above.

FROM FRAGMENT HITS TO MCR SMALL MOLECULES: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION

Markella Konstantinidou (1), Fandi Sutanto (1), Francesca Magari (2), Gerhard Klebe (2), Muhammet Y. Ünver (3), Varsha R. Jumde (3,4), Anna K. H. Hirsch (3,4), Alexander Dömling (1)

1) Group of Drug Design, Groningen Research Institute of Pharmacy, University of Groningen, A. Deusinglaan 1, 9713 AV, Groningen the Netherlands
2) Department of Pharmaceutical Chemistry, Philipps University Marburg, Marbacher Weg 6, 35032 Marburg, Germany
3) Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
4) Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) - Helmholtz Centre for Infection Research (HZI), Department of Drug Design and Optimization, 66123, Saarbrücken, Germany

Fragment-based drug design is a well-established strategy for the identification of lead compounds, both in industry and academia. The initial fragment hits are typically grown, linked or merged, in order to become drug-like compounds with improved affinity [1]. This approach has led to the development of successful drug candidates both on clinical trials and on the market [2]. The advantage of fragments is that they represent weak binders with the potential to be used in diverse synthetic routes. Noticeably, in a lot of cases they prove to be ideal starting materials for multi-component reaction chemistry (MCR). MCR chemistry allows the synthesis of complex scaffolds in a few steps, starting from commercially available building blocks [3,4]. In this work, we present how fragment hits can be used as starting materials for MCR chemistry in order to synthesize easily and effectively small molecules for biological targets. We have successfully applied this approach for the synthesis of inhibitors of an aspartyl protease and for the PPI of p53-MDM2. In the first case, an in-house protocol was developed to enumerate virtual MCR libraries, which were docked against our target. In the second case, the online software ANCHOR.QUERY [5] was used and led to effective scaffold hopping [6,7]. In both cases, compounds were selected for synthesis and then they were biologically evaluated. Co-crystal structures were obtained, supporting the design of the scaffolds. Currently, we are focusing on novel targets, including both enzymes and protein-protein interactions.

Acknowledgement: This project has received funding from the European Union's Framework Programme for Research and Innovation Horizon 2020 (2014 – 2020) under the Marie Skłodowska – Curie Grant Agreement No. 675555, Accelerated Early staGe Drug Discovery (AEGIS).

References

1) Erlanson DA, Introduction to fragment-based drug discovery. Top Curr Chem. 2012, 317, 1-32
2) Erlanson DA, Fesik SW, Hubbard RE, Jahnke W, Jhoti H. Twenty years on: the impact of fragments on drug discovery. Nat Rev Drug Discov. 2016, 15(9), 605-19
3) Dömling A, Wang W, Wang K. Chemistry and biology of multicomponent reactions. Chem. Rev. 2012, 112(6), 3083-3135
4) Zarganis – Tzitzikas T, Chandgude AL, Dömling, A. Multicomponent Reactions, Union of MCRs and Beyond. Chem. Rec. 2015, 15(5), 981-996
5) Koes DR, Dömling A, Camacho CJ. AnchorQuery: Rapid online virtual screening for small-molecule protein-protein interaction inhibitors. Protein Sci. 2018, 27(1), 229-232
6) Shaabani S, Neochoritis CG, Twarda-Clapa A, Musielak B, Holak TA, Dömling A. Scaffold hopping via ANCHOR.QUERY: β-lactams as potent p53-MDM2 antagonists, Medchemcomm. 2017, 8(5), 1046-1052
7) Surmiak E, Neochoritis CG, Musielak B, Twarda-Clapa A, Kurpiewska K, Dubin G, Camacho C, Holak TA, Dömling A. Rational design and synthesis of 1,5-disubstituted tetrazoles as potent inhibitors of the MDM2-p53 interaction, Eur J Med Chem 2017, 126, 384-407

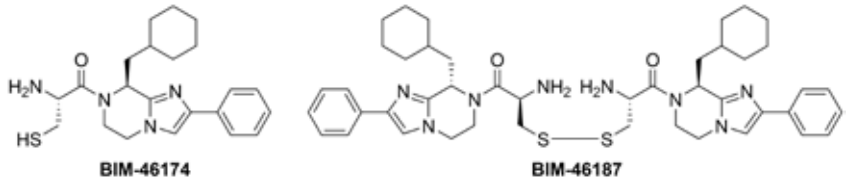
P072

TOWARDS G PROTEIN INHIBITION BY SMALL MOLECULES: PREPARATION OF BIM-46174 FRAGMENTS

Jim Küppers (1), Suvi Annala (2), Tobias Benkel (2), Evi Kostenis (2), Michael Gütschow (1)

1) Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany
2) Institute of Pharmaceutical Biology, University of Bonn, Nussallee 6, D-53115 Bonn, Germany

G protein-coupled receptors (GPCRs) comprise important therapeutic targets. Agonist binding to the receptor stabilizes its active conformation, which stimulates cytoplasmic heterotrimeric G proteins. The capability of acting as molecular switches and thus transmitting signals from the outside and modulating various intracellular effectors makes G proteins vitally important. Whereas GPCRs are major targets for the development of drugs, the G proteins have received less attention for medicinal chemists. Only a few G protein inhibitors are available, including natural products such as pertussis toxin, YM-254890, FR900359 and synthetic molecules such as NF449, BIM-46174 and BIM-46187. The two BIM molecules have been previously reported as pan-G protein inhibitors.[1] Recent investigations, however, revealed that BIM-46187 preferentially silences $G\alpha_q$ signalling in a cellular context-dependent manner. BIM-46187 traps $G\alpha_q$ in the empty pocket conformation by permitting GDP exit but preventing GTP entry.[2]



We are conducting structural reductions of the BIM molecules to monocyclic (**1, 2**) and bicyclic fragments (**3**) to gain first insights into the pharmacophore of BIM-type inhibitors. Synthetic attempts to change the bicyclic 5,6,7,8-tetrahydroimidazo[1,2-a]pyrazine core of BIM into monocyclic, piperazin-2-one containing fragments (**1, 2**) are reported. Furthermore, we synthesized a small library of bicyclic lactams (**3**) featuring fused pyrazine (m=0, n=1) and diazepine rings (m=n=1; m=0, n=2). The compounds will be subjected to pharmacological studies.

References

1) a) Ayoub, M. A.; Damian, M.; Gesprach, C.; Ferrandis, E.; Lavergne, O.; De Wever, O.; Banères, J.-L.; Pin, J.-P.; Prévost, G. P. J. Biol. Chem. 2009, 284, 29136-29145. b) Prévost, G. P.; Lonchampt, M. O.; Holbeck, S.; Attoub, S.; Zaharevitz, D.; Alley, M.; Wright, J;, Brezak, M. C.; Coulomb, H.; Savola, A.; Huchet, M.; Chaumeron, S.; Nguyen, Q. D.; Forgez, P.; Bruyneel, E.; Bracke, M.; Ferrandis, E.; Roubert, P.; Demarquay, D.; Gespach, C.; Kasprzyk, P. G. Cancer Res. 2006, 66, 9227-9234.
2) Schmitz, A.-L.; Schrage, R.; Gaffal, E.; Charpentier, T. H.; Wiest, J.; Hiltensperger, G.; Morschel, J.; Hennen, S.; Häußler, D.; Horn, V.; Wenzel, D.; Grundmann, M.; Büllesbach, K. M.; Schröder, R.; Brewitz, H. H.; Schmidt, J.; Gomeza, J.; Galés, C.; Fleischmann, B. K.; Tüting, T.; Imhof, D.; Tietze, D.; Gütschow, M.; Holzgrabe, U.; Sondek, J.; Harden, K.; Mohr, K; Kostenis, E. Chem. Biol. 2014, 21, 890-902.

P073

AN ARTIFICIAL INTELLIGENCE TECHNOLOGY FOR THE GENERATION OF SYNTHETICALLY-ENABLED SCAFFOLD AND LEAD ANALOGUE SPACE FOR MEDICINAL CHEMISTRY AND AI-DRIVEN DRUG DISCOVERY

Gergely Makara, Gabor Pocze, Laszlo Kovacs, Orsolya Demeter

ChemPass Ltd., 7 Zahony St, 1031 Budapest, Hungary

Throughout the world mankind is facing an ageing and growing population that requires more effective and safer medicines in all therapeutic areas. Despite significant advances in our understanding of the biological basis of diseases, pharmaceutical R&D is struggling to sustain the level of productivity and efficiency it reached in the second half of the 20th century. High rates of failure and the increasing cost of drug discovery as well as extended research and development timelines hinder the development of medicines. Due to these challenges there has been an increasing need for substantial innovations in the pharmaceutical industry.

It has been shown that if the selection of the synthetic targets in lead optimization cycles is supported by QSAR or deep learning methods, the number of compounds synthesized as well as the cycle time for each iteration can be significantly reduced. However, current AI-driven drug discovery techniques mostly select from human designed molecule subsets, existing compound databases, or computer-generated structure analogues that are synthetically not vetted. In case of the latter, if synthesis is not incorporated into the design, it can be reasonably feared that *laboratory synthesis time will grow and become a critical bottleneck*.

ChemPass has developed a rule-based artificial intelligence technology that can produce a large number of novel and synthetically-enabled lead analogues and scaffold hopping designs around lead structures. Since its introduction the cloud-based SynSpace software has been found by multiple organizations to generate more novel ideas around leads than medicinal chemist teams can do to support lead discovery projects.

We have also been developing an automated lead analysis toolbox and a synthesis-based library enumerator that in conjunction with SynSpace API can automatically carry out scaffold hopping and lead analogue idea generation and thereby offer large sets of novel and project specific lead-like structures to advanced AI platforms for selection.

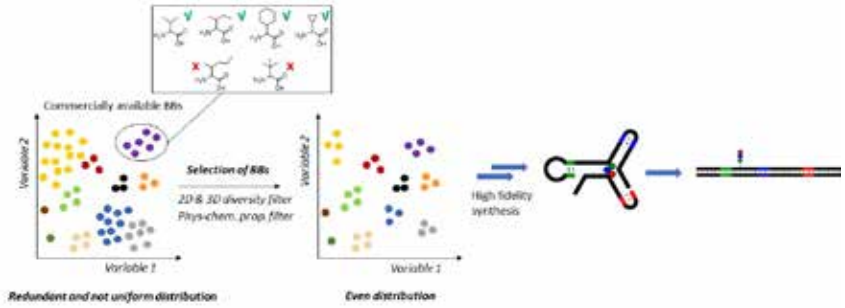
The ChemPass platforms have the biggest impact on a number of key parameters in drug discovery: cycle time, number of discovery cycles, the number of compounds to be synthesized and coverage of IP space. Improvements in these factors can be converted into higher sucess rates and major resource savings towards a more economical and productive candidate development phase.

ADVANCES IN DNA-ENCODED LIBRARY DESIGN AND SYNTHESIS

Iolanda Micco, Lars Petersen, Carlos Azevedo, Frank Sløk, Peter Blakskjær, Ole Kristensen, Charlotte Andersen, Nils Hansen

Vipergen ApS, Gammel Kongevej 23A, 1610 Copenhagen V, Denmark

In the last years, DNA Encoded Libraries (DEL) have captured the attention of many researchers and, with the first DEL-born candidate entering human clinical testing, they have exponentially raised their reputation in the scientific community. The impact in the drug discovery has been significant and many researchers have been exploring different ways to prepare DNA encoded libraries in a race to the largest library size. Now that the field has reached a good level of maturity and knowledge, we have the foundation to evaluate the DNA encoded library from different perspectives. Quantity doesn't encompass quality and researchers have learnt that higher numbers don't necessarily increase the chance to succeed. The real challenge is now how to get the largest library with the highest quality and three main key aspects become fundamental: fidelity of the process, access to the right chemical space for hit finding and tractability of the hits. For these reasons and as an indisputable source of library diversity, the design of the Building Blocks (BBs) is crucial.



In our laboratories we have developed an innovative synthetic methodology that allows chemical reactions between BBs in a stepwise procedure to form millions of trimeric molecules.[1] Coupling products are purified in each step ensuring high fidelity of the whole library assembly and consequently minimizing false positives or screening noises. A uniform selection of pharmacophore motifs at the BBs level and their spatial orientation is fundamental to have high balance between chemotypes (diversity) and their population density in the library. This reduces the negative effect of activity cliffs,[2] increasing the chance of finding key interactions for different classes of targets. Simultaneously, physicochemical descriptors of the BBs need to be an initial step of filtration to increase the probability for the final molecule of being developable. However, libraries will never be perfect and never be able to cover all the chemical space for all targets. For this reason, the ideal approach with a DNA-encoded library needs to be dynamic and based on the output of the library itself, allowing *in silico* filtrations and pointed modifications.

Several high potent ligands (best IC50 values down to 2 nM) that can bind different classes of targets have been identified in our laboratories.[3] The poster will be focused on how key aspects of the library design and synthesis have been addressed in our research lines, with a reference to a successful case study.

References

1) Hansen. (2015). Fidelity by design: Yoctoreactor and binder trap enrichment for small molecule DNA-encoded libraries and drug discovery. Current Opinion in Chemical Biology, 62-71
2) Stumpfe, D. (2012). Exploring Activity Cliffs in Medicinal Chemistry. Journal of Medicinal Chemistry, 2932–2942.
3) Hansen. (2016). Novel p38α MAP kinase inhibitors identified from yoctoReactor DNA-encoded small molecule library. Med. Chem. Commun., 1332-1339.

CRYSTALLOGRAPHIC SCREENING OF SP3-RICH FRAGMENT LIBRARY AS A NEW STRATEGY FOR FRAGMENT GROWING

Janis Mueller (1), Serghei Glinca (1), Stefan Merkl (1), Lars Haustedt (3), Gerhard Klebe (2)

1) CrystalsFirst GmbH, Marbacher Weg 6, 35037 Marburg, Germany
2) Philipps-University Marburg, Departement for Pharmaceutical Chemistry, Marbacher Weg 6, 35037 Marburg, Germany
3) AnalytiCon Discovery GmbH, Hermannswerder Haus 17, 14471 Potsdam

Fragment-based screening has become a powerful tool for the discovery of possible lead structures resulting in two approved drugs and more than 30 candidates in clinical trials over the course of the last two decades[1]. The advantage of such screenings is the effective exploration of possible chemical space through small organic molecules with MW usually below 250 Da. These fragments serve as probes that show possible hotspots in proteins and can be expanded or merged into lead-structures.

Most fragment-screening libraries have been designed by combinatorial chemistry and therefore exhibit a large number of sp2-carbons and aromatic rings. The enrichment of such chemotypes leads to a reduced scope of chemical space and chemical diversity in lead candidates. A strong sp2-driven chemistry might limit the success rate of drug discovery projects, since systematic studies found an increase of the sp3-carbon fraction, e. g. saturated rings, as the compound progresses from hit to the status of a drug candidate in clinical phases[2]. The initial Identification of diverse sp3-carbon-rich fragment hits enables fragment growing using well established organic chemistry. Instead of difficult incremental growing of sp2-carbon rich fragments via sp3-carbon-chemistry.

In the present study, we utilized CrystalsFirst's SmartSoak® technology and performed a crystallographic screen of 200 fragments derived from natural products comprising a high fraction of sp3-carbons. The preliminary results show an extraordinary hit rate over 30 % that allows a wide spectrum of follow-up compounds using analogues-by-catalogues approach or by classic organic chemistry.

References

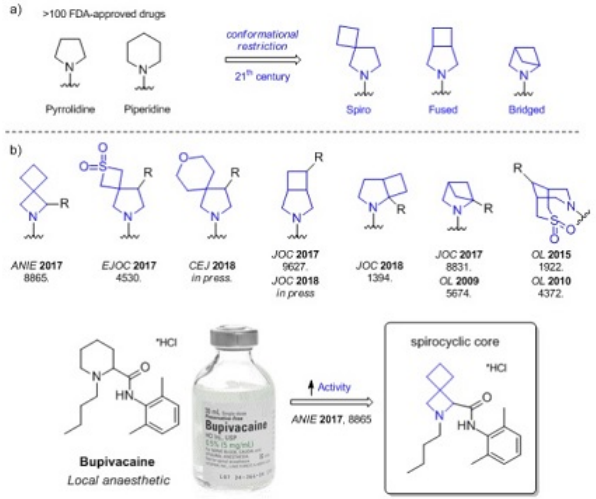
1) Erlanson et al., Nat. Rev. Drug Discov. 2016, 15 (9), 605–619.
2) Lovering et al., J. Med. Chem. 2009, 52 (21), 6752–6756.

SYNTHESIS OF NOVEL BICYCLIC AMINES FOR DRUG DESIGN

P. Mykhailiuk

Enamine, 78 Chervonotkatska Street, 01033 Kyiv, Ukraine

Designing novel bioactive molecules remains among the major challenges in modern drug discovery. Recent results emphasize the value of sp^3-rich compounds as highly potent yet underexplored molecular scaffolds.[1] Conformational rigidity and defined three-dimensional structure are among the key characteristics determining the overall physicochemical parameters of a drug candidate.[2,3] Not surprisingly, the smallest cycle – a cyclopropyl core – has been successfully exploited as a valuable structural motif vital for attaining the desired biological profiles in numerous approved and investigational drugs.[4] In this regard, two marketed drugs – sitafloxacin and saxagliptin – perfectly highlight the importance of spirocyclic and fused cyclopropanes in medicinal chemistry.



To further extend the scope of conformationally restricted compounds and advance their utility in drug discovery, we developed synthetic routes toward novel functional cyclopropyl scaffolds. Intermediate bromo-substituted spirocyclic and fused bicyclic compounds were successfully transformed into sulforylchlorides and boronic esters, versatile units for further modifications which can be used in drug discovery programmes.

References

1) A. Kirichok et al. Chem. Eur. J. 2018, in press (DOI: 10.1002/chem.201800193).
2) A. Kirichok, M. Kliachyna, I. Pishel, P. K. Mykhailiuk Angew. Chem. Int. Ed. 2017, 8865.
3) B. A. Chalyk, et al. Chem. Eur. J. 2018, 23, 16782.
4) B. A. Chalyk, et al. Eur. J. Org. Chem. 2017, 4530.
5) A. V. Denisenko, et al. J. Org. Chem. 2017, 9627.
6) T. Druzhenko, et al. J. Org. Chem. 2018, 1394.
7) E. Skalenko, et al. J. Org. Chem. 2018, in press (DOI: 10.1021/acs.joc.8b00077).
8) P. K. Mykhailiuk, V. Kubyshkin, T. Bach, N. Budisa J. Org. Chem. 2017, 8831.
9) T. Druzhenko, O. Denisenko, S. Zozulya, S. S. Shishkina, A. Tolmachev, P. K. Mykhailiuk Org. Lett. 2015, 1922.

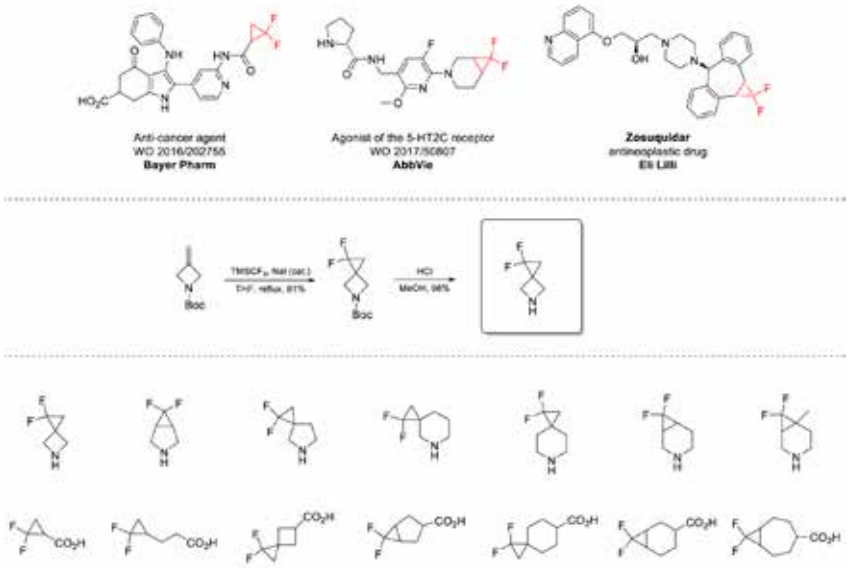
DIFLUOROCYCLOPROPANES FOR DRUG DISCOVERY

Pavel Mykhailiuk

Enamine, 78 Chervonotkatska Street, 01033 Kyiv, Ukraine

Up to 20% of all modern marketed drugs and even 30% of all agrochemicals are fluorine-containing organic compounds.[1] Difluorocyclopropane-containing compounds also gained popularity in drug discovery in recent years. In 2011, Prakash reported that the combination CF_3TMS/NaI efficiently converted the non-activated alkenes into the *gem*-difluorocyclopropanes.[2] Herein, we aim to use this procedure to convert the functionalized non-activated alkenes - amines, esters, nitriles, ethers and ketals - into the functionalized difluorocyclopropanes: novel building blocks for drug discovery.[3]

In this work, we have designed and synthesized difluorocyclopropanes for drug discovery. Details of the synthesis and application of the obtained compounds will be discussed.



References

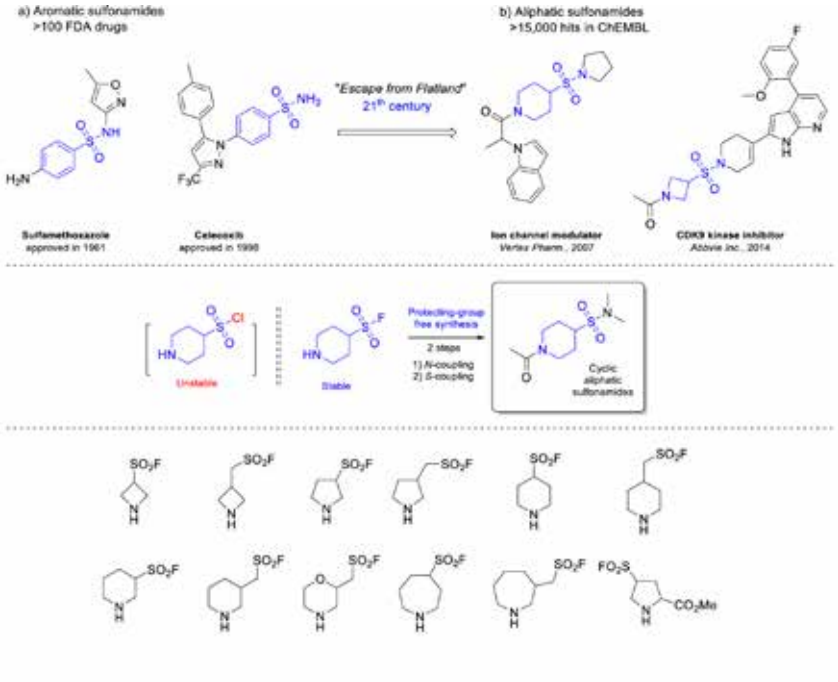
1) W. Hagmann. J. Med. Chem. 2008, 4360.
2) F. Wang et al. Angew. Chem. Int. Ed. 2011, 7153.
3) R. M. Bychek et al. Chem. Eur. J. 2018, in press (doi.org/10.1002/chem.201705708).

AMINOSULFONYL FLUORIDES – NOVEL SCAFFOLDS FOR PROTECTING-GROUP FREE SYNTHESIS OF SULFONAMIDES

Pavel Mykhailiuk

Enamine, 78 Chervonotkatska Street, 01033 Kyiv, Ukraine

Sulfonamide-containing drugs have revolutionized medicine. Since the introduction of Prontosil in the 1930s, sulfonamides have been extensively explored, as more than 100 FDA-approved drugs containing a sulfonamide have appeared on the market.[1] All these compounds, however, are aromatic sulfonamides. Aliphatic sulfonyl chlorides often decompose during storage and exhibit thermal liability.[2] On the other hand, sulfonyl fluorides are remarkably stable under mild acidic and basic conditions.[3] In this work, we have designed and synthesized cyclic saturated amino sulfonyl fluorides for drug discovery. The compounds were stable upon storage and could be used for the protecting-group free synthesis of sulfonamides.[4] Details of the synthesis and application of the obtained compounds will be discussed.



References

1) V. Law et al. Nucleic Acids Res. 2014, D1091.
2) S. Zhersh et al. Synthesis. 2011, 3669.
3) J. Dong et al. Angew. Chem. Int. Ed. 2014, 9430.
4) S. Zhersh et al. Manuscript under preparation.

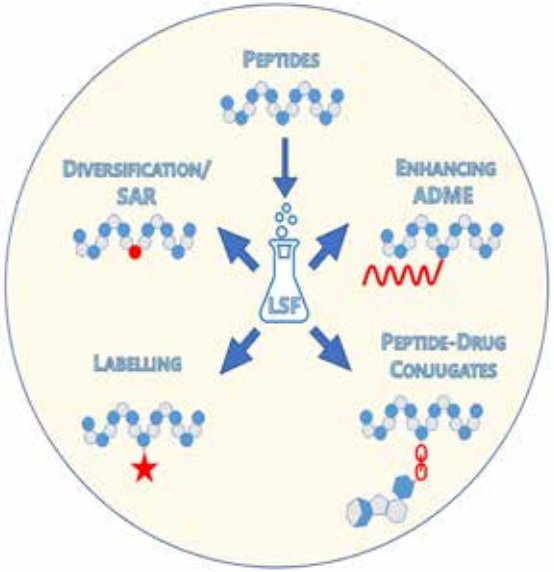
LATE-STAGE FUNCTIONALIZATION OF PEPTIDES: NOVEL SITE-SELECTIVE MODIFICATION & BIOCONJUGATION

Anaïs Noisier (1), Magnus Johansson (1), Laurent Knerr (1), William Drury (1), Eric Valeur (1), Ranganath Gopalakrishnan (2)

1) Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca Gothenburg, Pepparedsleden 1, SE-431 83 Mölndal, Sweden
2) AstraZeneca MPI Satellite Unit, Max Planck Institute für Molekulare Physiologie, Abteilung Chemische Biologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

The Late-Stage Functionalization (LSF) of peptides has emerged as a valuable strategy for the design of potent peptide-pharmaceuticals enabling rapid exploration of Structure-Activity Relationships (SAR).[1] Furthermore, LSF offers novel opportunities for the introduction of conjugation handles thus allowing for the generation of biological tools as well as peptide-drug conjugates.[2] However, commonly employed methods for the site-selective modification of complex unprotected peptides currently rely on the use of either non-natural amino acids or on the innate reactivity of a very limited number of natural residues (mainly Cysteine and Lysine).[3] Herein we report novel methods for the diversification of peptides under mild reaction conditions. Using this technology, versatile handles were introduced for the tagging and bioconjugation of peptide pharmaceuticals.

Figure 1:



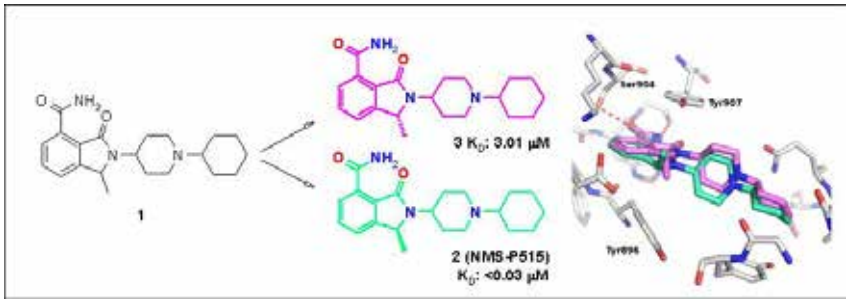
References

1) a) A. F. M. Noisier and M. A. Brimble, Chem. Rev. 2014, 114, 8775-8806; b) A. F. M. Noisier, M. J. Johansson, R. Gopalakrishnan, A. Dahlén, W. J. Drury III and E. Valeur, submitted
2) S. B. Gunnoo and A. Madder, Org. Biomol. Chem. 2016, 14, 8002-8013
3) O. Boutureira and G. J. L. Bernardes, Chem. Rev. 2015, 115, 2174-2195

DISCOVERY OF STEREOSPECIFIC PARP-1 INHIBITOR ISOINDOLINONE NMS-P515

Gianluca Papeo (1), Elena Casale (1), Marina Ciomei (1), Alessandra Cirla (1), Viviana Desperati (1), Daniele Donati (1), Eduard R. Felder (1), Arturo Galvani (1), Antonella Isacchi (1), Mikhail Y. Krasavin (2), Paolo Orsini (1), Helena Posteri (1), Federico Riccardi-Sirtori (1), Alessandra Scolaro (1), Alessia Montagnoli (1)

1) Oncology - Nerviano Medical Sciences (NMS), Viale Pasteur 10, 20014 Nerviano (MI) – Italy
2) Institute of Chemistry, St. Petersburg State University, 26 Universitetskyi Prospekt, Peterhof 198504 - Russian Federation



The genetic instability of certain tumor phenotypes arises from their deficiencies in fixing damaged DNA. These liabilities can be magnified by employing drugs that further hamper the cancer DNA Damage Response (DDR) machinery [1]. Archetype of this "synthetically lethal" approach, which represents one of the most exciting and visionary frontiers in cancer therapy, is the use of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors in cancer settings with compromised capabilities in repairing DNA double strand breaks, such as BRCA1/2 mutations carrying tumors. PARP-1 is the main component of a 17-membered family of proteins. It employs NAD^+ as the building block to assemble, *via* the release of nicotinamide, poly(ADP-ribose) chains onto several acceptor proteins including PARP-1 itself. This post-translational modification contributes in signaling the presence of DNA single- and double strand breaks, resulting in the recruitment of proteins involved in DNA repair. Nicotinamide mimics designed as inhibitors of PARP-1, initially developed to potentiate DNA-damaging agents, finally found their way to the clinic (e.g. Lynparza™, Rubraca™ and Zejula™) as monotherapy in those tumors that cannot withstand a DNA damage overload [2]. Aiming at discovering proprietary PARP-1 inhibitors, a High Throughput Screening (HTS) campaign using an innovative fluorescent polarization displacement assay [3] was undertaken at Nerviano Medical Sciences (NMS). This screening allowed the identification of the isoindolinones chemical class. A medicinal chemistry expansion of the series delivered **1**, a potent (PARP-1 K_D: D: >10 µM) inhibitor, with good cellular MoA (PAR assay, IC_{50}: 0.32 µM), excellent ADME profile and oral bioavailability. As **1** was a racemate, preparative-scale chiral HPLC separation of the corresponding enantiomers resulted in an exquisite stereospecific inhibition of PARP-1 *in vitro* [(*S*)- isomer **2** PARP-1 K_D: R)- isomer **3** PARP-1 K_D: 3.01 µM) and in cells [(*S*)- isomer PAR assay IC_{50}: 0.14 µM; (*R*)- isomer PAR assay IC_{50}: 11.2 µM). Co-crystal structures of both isomers with cPARP-1 allowed explaining the observed stereospecificity. After having ruled out potential loss of enantiopurity *in vitro* and *in vivo*, **2** (NMS-P515) was synthesized in an asymmetric fashion. NMS-P515 pharmacokinetic profile and its antitumor activity on a BRCA2-mutated pancreatic cancer model render the compound a suitable candidate to be further progressed in NMS PARP-1 inhibitors drug discovery program.

References

1) Brown, J. S.; O'Carrigan, B.; Jackson, S. P.; Yap, T. A.Cancer Discov. 2017, 7, 20-37.
2) Wang, Y.-Q.; Wang, P.-Y.; Wang, Y.-T.; Yang, G.-F.; Zhang, A.; Miao, Z.-H. J. Med. Chem. 2016, 59, 9575-9598.
3) Papeo, G.; Avanzi, N.; Bettoni, S.; Leone, A.; Paolucci, M.; Perego, R.; Quartieri F.; Riccardi-Sirtori, F.; Thieffine, S.; Montagnoli, A.; Lupi, R. J. Biomol. Screen. 2014, 19, 1212-1219.

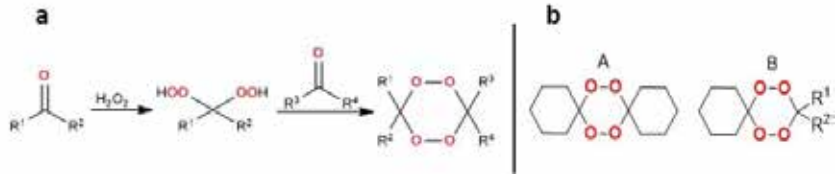
NON-SYMMETRIC 1,2,4,5-TETRAOXANES: SYNTHESIS WITH HYDROGEN PEROXIDE AND THEIR BIOLOGICAL ACTIVITY

Griša Prinčič (1), Jernej Iskra (1), Katarina Starkl Renar (1,2), Mohit K. Tiwari (3), Bharti Rajesh K. Shyamlal (3), Manas Mathur (4), Ajit K. Swami (4), Sandeep Chaudhary (3)

1) Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia
2) Department of Physical and Organic Chemistry, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana., Slovenia
3) Laboratory of Organic and Medicinal Chemistry, Department of Chemistry, Malaviya National Institute of Technology, Jawaharlal Nehru Marg, Jaipur-302017, India.
4) Department of Advance Molecular Microbiology, Seminal Applied Sciences Pvt. Ltd. Jaipur-302015, India

Until recent years hydrogen peroxide was mainly considered as a byproduct of oxidative metabolism. Over the past few years it has become accepted that hydrogen peroxide is also an important signaling molecule within the cell[1]. In chemistry, hydrogen peroxide is becoming increasingly important oxidant as environmentally friendlier alternative to classical oxidants (hypervalent iodine compounds, metals, …). The only byproduct after the reaction with H_2O_2 is water and is therefore often used as a reagent of choice in the field of »green chemistry«.

Reactions of hydrogen peroxide are, however, not limited only to oxidations. Its direct incorporation into organic scaffold leads to formation of organic peroxides with interesting properties and functionality. Biological activity of organic peroxide has, so far, been mainly associated with antimalarial properties of artemisinin and its derivatives. Recent research indicates that other biological activities such as antimicrobial and anticancer have not been given enough attention[2].1 ,2,4,5-tetraoxanes are a group of compounds with two peroxide moieties in six-cyclic ring and have proven antimalarial activity. They can be produced by acid-catalyzed condensation of a carbonyl compound and *gem*-dihydroperoxide in fluorinated alcohol through template catalysis (Figure a)[3]. *Gem* -dihydroperoxide is prepared by peroxidation of another carbonyl compound with H_2O_2.



The peroxidic ring in tetaoxane gives these compounds their biological activity. Interestingly, though, the type of structure on opposite sides of tetraoxane ring strongly determines biological activity; antimalarial activity was observed only on *di*spiro molecules, whereas spiro derivatives exhibited much lower, in some cases even negligible activity (Figure b).[4]

Our research has focused mainly on synthesis of non-symmetric 1,2,4,5-tetraoxanes by condensation of an appropriate substituted aldehyde (substituted benzaldehydes) or ketone and *gem*-dihydroperoxide with an acid catalyst in fluorinated alcohol as a solvent and template catalyst. Results of the synthesis and selectivity of formation of peroxide will be shown as well as new catalytic system that enables the use of neutral conditions in the formation of gem-dihydroperoxides and non-symmetric 1,2,4,5-tetraoxanes from ketones and aldehydes. This enabled us to synthesize a group of tetraoxanes with targeted properties (solubility, lipophilicity) that was screened for various biological activities (antimalarial, antibacterial, antifungal). Some of the derivatives have shown promising results, especially in antitubercular effectiveness in comparison to commercially available rifampicin drug.

References

1) Veal, E.; Day, A. Hydrogen Peroxide as a Signaling Molecule. Antioxid. Redox Signal. 2011, 15 (1), 147–151.
2) Vil, V. A.; Yaremenko, I. A.; Ilovaisky, A. I.; Terent'ev, A. O. Peroxides with Anthelmintic, Antiprotozoal, Fungicidal and Antiviral Bioactivity: Properties, Synthesis and Reactions. Molecules 2017, 22 (11).
3) Iskra, J.; Bonnet-Delpon, D.; Bégué, J. P. One-Pot Synthesis of Non-Symmetric Tetraoxanes with the H2O2/MTO/fluorous Alcohol System. Tetrahedron Lett. 2003, 44 (33), 6309–6312.
4) Terent'ev, A. O.; Borisov, D. A.; Vil, V. A.; Dembitsky, V. M. Synthesis of Five- and Six-Membered Cyclic Organic Peroxides: Key Transformations into Peroxide Ring-Retaining Products. Beilstein J. Org. Chem. 2014, 10, 34–114.

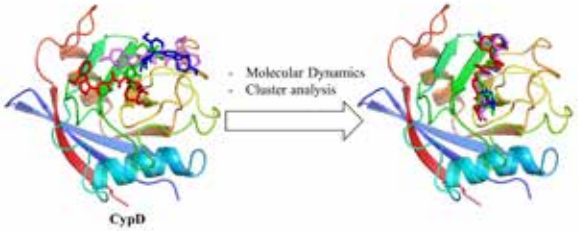
PREDICTING FRAGMENT BINDING BY MOLECULAR DYNAMICS : TOWARDS NEW INHIBITORS OF CYCLOPHILIN D

Carmine Marco MORGILLO (1), Vincent RODESCHINI (2), Didier ROCHE (2), Jean-Marc LANCELIN (1), Marine GILARDONE (2), Hugues LEMOINE (2), Catherine JORAND LEBRUN (3), Ulrich GRAEDLER (4), Xuliang JIAN (3), Theresa JOHNSON (3), Daniel SCHWARZ (4), Andreas MARX (4), Brigitta LEUTHNER (4)

1) Institut des Sciences Analytiques, Lyon, France
2) Edelris, Lyon, France
3) EMD Serono SRDI, Billerica, MA, USA
4) Merck Serono, Darmstadt, Germany

Fragment-based Drug Discovery has become a fantastic strategy to design target modulators and bring new drugs to the market. Although weak binders can be identified using well established biophysical methods like SPR, NMR or thermal shift assays, optimization to identify higher-affinity ligands is mainly guided by the information obtained from the fragment-protein X-Ray co-structure. For this reason, resource investment in medicinal chemistry is very often restricted to the fragment hits that succeed in providing good resolution crystals. It is therefore highly desirable to identify computational methods that can predict the binding modes of low affinity fragments from either the apo-protein or any available X-Ray structure of the protein.

We will report therein our results on the use of molecular dynamics (MD) simulations to investigate the specific binding modes of particular 3D-fragments towards the cyclophilin D target. The binding modes predicted by MD were found to be in excellent agreement with the experiments. This study suggests that MD can become a powerful tool in structure-based optimization of fragments to lead candidates.



VIRTUAL CHEMICAL SPACE WITH HIGH PREDICTABLE SYNTHETIC FEASIBILITY. IS IT ACHIEVABLE?

Dmitriy Volochnyuk (1,3), Sergey Ryabukhin (1,2), Oleksandr Grygorenko (1,2), Yurii Moroz (2)

1) Enamine Ltd., Chervonotkatska 78, Kyiv 02094, Ukraine
2) National Taras Shevchenko University of Kyiv, Volodymyrska Street, 60, Kyiv 01601, Ukraine
3) Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanska Street 5, Kyiv 02094, Ukraine

The current drug discovery especially against protein targets that are hard-to-drug is based on the exploration of new chemical space [1]. The major drawback of this approach is unpredictable success rate of the chemical synthesis of *in silico*/*cerebro* generated molecules. One of the approaches to the problem solution is disconnection of molecules following retrosynthetic rules, which produces readily available fragments that can be used later on to construct new libraries. The sets of appropriate synthesis trees as well as as well as the building block pool have been discussed previously [2]. Based on this methodology, the “validated virtual” chemical space were developed in majority of the Big Pharma companies for the time-pressure and cost effective compound library synthesis in MedChem projects [3]. However, these services are limited or inaccessible outside the companies for academic and small biotech users. A unique service, Enamine’s REAL database [4,5] is currently available on the market, providing 337 million of synthetically feasible compounds based on 122 validated chemical protocols / 68,200 in stock building blocks with >80% success rate.

In this talk, we describe the history and current state of this synthetic methodology which allows making the diverse compound libraries in a cost- and time-effective manner with high success rates. In particular, the reaction choice, validation and scope determination will be discussed. The in-house metrics for the building blocks involved into the existing synthetic trees for the enumeration will be disclosed for the first time. The purification platform as well as synthesis timing will be illustrated by representative case studies.

References

1) Peng, Z. Drug Discov. Today: Technol. 2013, 10, 387
2) Hartenfeller, M. et al. J. Chem. Inf. Model. 2011, 51, 3093
3) For example Pfizer Global Virtual Library (PGVL), Proximal Lilly Collection etc
4) Ryabukhin, S. V.; Chuprina, A. Chim. Oggi, 2007, 25, 58
5) Krotko, D.; Chuprina, A.; Shivanyuk, A.; Tolmachev, A. Chim. Oggi, 2010, 28, 16

P084

EXPANDING CHEMICAL SPACE VIA MICROBIAL BIOCATALYSIS LATE STAGE FUNCTIONALIZATION

Frank Scheffler (1), Richard Phipps (1), Headley Williams (1), Liam Evans (1), Jonathan Steele (1), Julia Shanu-Wilson (1), Eva Lenz (2), Scott Martin (2), Tom McGuire (2), Elisabetta Chiarparin (2), Sunil Sarda (2), Derek Barratt (2)

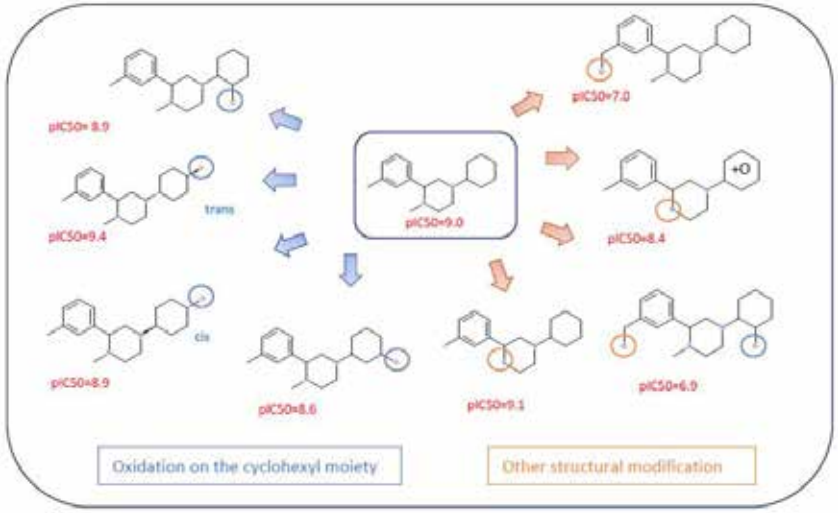
1) Hypha Discovery Ltd., 957-958 Buckingham Avenue, Slough, SL1 4NL
2) Innovative Medicines and Early Development, AstraZeneca, Cambridge Science Park, Milton Road, CB4 0WG

Introducing chemical diversity into a drug candidate late in the optimisation process has several applications including exploration of SAR (structure-activity relationships). Biocatalysis can provide access

to chemical space in a complementary manner to chemical synthesis, thereby broadening coverage of the SAR map to better understand how small changes in the molecular structure affect biological potency.

In this late stage functionalization project undertaken by Hypha and AstraZeneca, biotransformation of a small quantity of a drug lead was explored using a subset of microbes from Hypha's oxidative strain panel, resulting in the identification of eight active oxidised derivatives. Sufficient purified material was generated for structure elucidation by 2D NMR and subsequent pIC50 determination.

Several regio and stereoisomers were isolated as a result of oxidation on the cyclohexane moiety, together with desmethyl and benzylic hydroxylated derivatives, as well as combinations thereof. Hydroxylated derivatives were obtained that overlapped with those produced synthetically, in addition to novel "trickier to synthesise" compounds where hydroxylation was achieved in two distinct areas of the molecule. The study was valuable in revealing that different polar chemical space could be accessed in parallel which did not compromise potency, as part of a wider SAR map.



P085

THE WATER-SOLUBLE GLYCOFULLERENES FOR ANTI-CANCER THEREPIES

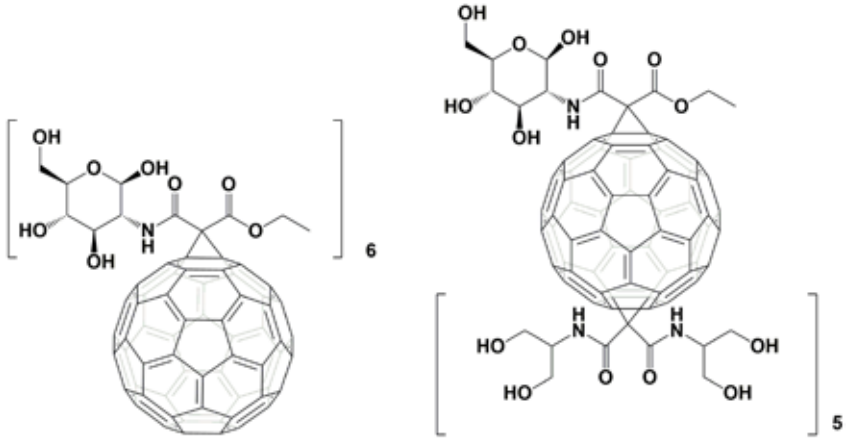
Maciej Serda (1,4), Matthew J. Ware (2), Jared Newton (2), Sanchit Sachdeva (2), Martyna Krzykawska-Serda (2,3), Katarzyna Malarz (1), Robert Musioł (1), Stuart J. Corr (2), Steven A. Curley (2), Lon J. Wilson (4)

1) Institute of Chemistry, University of Silesia in Katowice, 40-006, Katowice, Poland
2) Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX 77030, USA.
3) Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków 30-387, Poland
4) Department of Chemistry and Smalley-Curl Institute, Rice University, Houston, TX 77251, USA

The [60]fullerene molecule (C_{60}) and its derivatives are attractive constructs for biomedical applications. Glycoconjugated C_{60} derivatives are of particular interest as potential cancer targeting agents due to an upregulated metabolic glucose demand, especially in the case of pancreatic adenocarcinoma and its dense stroma, which is known to be driven by a subset of pancreatic stellate cells (PSCs). Herein we describe the synthesis and characterization of two D-glucosamine derivatives of [60]fullerene and their biological properties. The [60]fullerenes are inherently non-toxic up to concentrations of 10 mg/ml and are photoactive when illuminated with blue and green LED light, allowing its use as a photodynamic therapy agent.

It was observed that all fullerenes form two aggregate fraction 20-30 nm and 400-500 nm. Initial dark cytotoxicity studies on pancreatic cell lines PSCs and PANC-1 have been carried out using flow cytometry and propidium iodide (PI) apoptosis staining. It has been shown that all two glycofullerenes are non-toxic even in high concentrations (up to 10 mg/ml, incubation 3 and 24 hours). Moreover, synthesized [60]fullerene derivatives localizes preferentially in the nucleus of PSC cells, with some localization in the cell cytoplasm. Additionally, designed nanotherapeutics were tested for SRC kinase inhibition. Conducted experiments have shown that synthesized [60]fullerene derivatives selectively inhibited two kinases FYN A and LCK.

Figure 1 The structures of glycofullerene **1** and **2**.



1 **2**

Maciej Serda thanks National Science Center (Poland) for the support (grant UMO- 2016/23/D/NZ7/00912)

RAPID AND ACCESSIBLE IN SILICO MACROCYCLE DESIGN – APPLICATION TO BRD4

Martin Slater, Matthias Bauer, Giovanna Tedesco, Mark Mackey

Cresset, New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, SG8 0SS, UK

Macrocyclization of pharmaceutical compounds plays an increasing role in drug discovery. Macrocycles can provide several advantages such as favorable drug-like properties, increased selectivity and improved binding affinity.

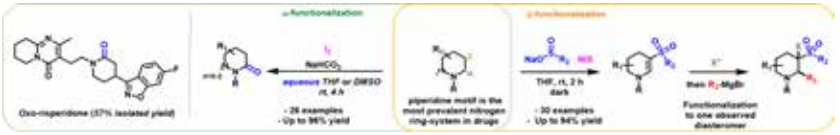
Macrocyclization of an existing lead series is not always easy. There are often multiple potential locations where the molecule could be cyclized, each with its own constraints in terms of synthetic feasibility, ideal linker length, required linker conformation, and pharmacophoric requirements from the active site. Challenging syntheses make it impractical to fully explore the possibilities in the lab.

Here we present a case study of designing macrocyclization strategies for reported BRD4 inhibitors with Spark, Cresset's bioisostere replacement and scaffold hopping tool. The Spark algorithms enable a rapid assessment of the ideal linker length and suggested chemistry for each cyclization option.

FROM LATE STAGE OXIDATION TO HETEROCYCLIC SYNTHESIS: NEW METHODOLOGY FOR DRUG DISCOVERY

Eric Talbot (1), Robert Griffiths (2,4), Wei Kong (2,3), Michael Willis (3), Glenn Burley (4)

1) Pharmaron, Hertford Road, EN11 9BU Hoddesdon, United Kingdom
2) GSK, Gunnels Wood Road, SG1 2NY, Stevenage, United Kingdom
3) Chemistry Research Laboratory,University of Oxford, Mansfield Road, OX1 3TA, Oxford, United Kingdom
4) Department of Pure and Applied Chemistry, University of Strathclyde,
295 Cathedral Street, G1 1XL, Glasgow, United Kingdom



Aliphatic azacycles are essential motifs in drug discovery, with 59% of unique small-molecule drugs approved by the FDA containing at least one nitrogen heterocycle.[1] Of these, the piperidine motif is the most prevalent nitrogen ring-system, highlighting the importance of this heterocycle in small-molecule drug discovery. Simple piperidines are readily available; hence, methods for the straightforward late-stage diversification of this ring-system, ideally exploiting C–H functionalization, are valuable tools for medicinal chemistry. In this presentation, we wished to report the successful realization of the a-oxidation[2] of aza-unsaturated rings using cheap and available iodine in mild conditions; as well as b-functionalization of piperidine ring to form cyclic enaminyl sulfones, and the use of the installed functionality as a unique nucleophile for wider functionalisation.[3]

References

1) E Vitaku, DT Smith, JT Njardarson, J. Med. Chem., 2014, 57, 10257–10274
2) RJ Griffiths, GA Burley, EPA Talbot Org. Lett., 2017, 19, 870-873
3) RJ Griffiths, WC Kong, GA Burley, M Willis, EPA Talbot Chem. Sci. 2018. doi: 10.1039/C7SC04900E

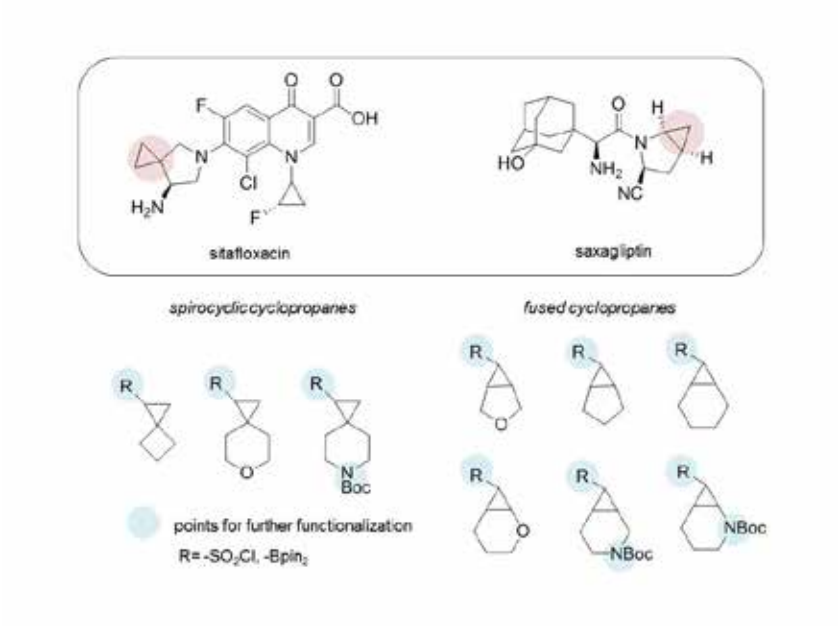
P088

TOWARDS UNEXPLORED REGIONS OF DRUG-LIKE CHEMICAL SPACE – NOVEL FUNCTIONAL SCAFFOLDS BASED ON SPIROCYCLIC AND FUSED CYCLOPROPANES

T. Savchenko, M. Vybornyi, R. Iminov, T. Savchiuk, Pavel Mykhailiuk

Enamine Ltd, 78 Chervonotkatska Street, 02094 Kyiv, Ukraine

Designing novel bioactive molecules remains among the major challenges in modern drug discovery. Recent results emphasize the value of sp^3-rich compounds as highly potent yet underexplored molecular scaffolds.[1] Conformational rigidity and defined three-dimensional structure are among the key characteristics determining the overall physicochemical parameters of a drug candidate.[2,3] Not surprisingly, the smallest cycle – a cyclopropyl core – has been successfully exploited as a valuable structural motif vital for attaining the desired biological profiles in numerous approved and investigational drugs.[4] In this regard, two marketed drugs – sitafloxacin and saxagliptin – perfectly highlight the importance of spirocyclic and fused cyclopropanes in medicinal chemistry.



To further extend the scope of conformationally restricted compounds and advance their utility in drug discovery, we developed synthetic routes toward novel functional cyclopropyl scaffolds. Intermediate bromo-substituted spirocyclic and fused bicyclic compounds were successfully transformed into sulforylchlorides and boronic esters, versatile units for further modifications which can be used in drug discovery programmes.

References

1) F. Lovering at al. J. Med. Chem. 2009, 52, 6752.
2) A. A. Kirichok et al. Angew. Chem. Int. Ed. 2017, 56, 8865
3) E. M. Carreira et al. Chem. Rev. 2014, 114, 8257.
4) T. T. Talele et al. J. Med. Chem. 2016, 59, 8712.

P089

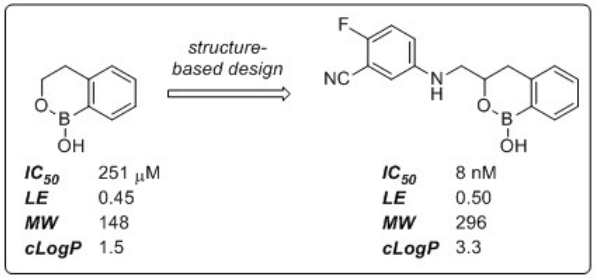
FRAGMENT PROPERTIES AND APPLICATION OF FBDD TO BORON-CONTAINING INHIBITORS OF LP-PLA2

David Twigg

Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, UK, CB4 0QA

The Astex fragment library is constantly evolving as information from fragment screening results is fed back into fragment library design. This poster will highlight some of the key properties of fragments and aspects such as molecular recognition, shape and synthesis. The focus will then turn to the use of boron-containing fragments in FBDD.

The unique properties of organoboron compounds allow for the formation of reversible covalent interactions with nucleophilic protein sidechains. This poster will highlight several examples of boron-containing fragments to illustrate the varied binding modes such ligands can adopt. An example of fragment-to-lead development will also be described from a collaboration between Astex and GSK against Lp-PLA_2. Elevated levels of this phospholipase have been linked to cardiovascular disease, dementia, diabetic macular edema and prostate cancer, making it an attractive target for inhibition. Organoboron fragments were identified via X-ray screening against Lp-PLA_2, and development of the weak hits using structure-based design provided a boron-containing lead series with good potency and excellent ligand efficiency for further optimisation.



NOVEL AND POTENT ORGANOPHOSPHOROUS UREASE INHIBITORS

Vassilios Ntatsopoulos (1), Stamatia Vassiliou (1), Katarzyna Macegoniuk (2), Artur Mucha (2), Lukasz Berlicki (2)

1) University of Athens, Department of Chemistry, Laboratory of Organic Chemistry, 15771, Panepistimiopolis Zografou, Athens, Greece
2) Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

Urease is a nickel-dependent metalloenzyme found in plants, some bacteria, and fungi. Bacterial enzyme is of special importance since it has been demonstrated as a potent virulence factor for some species. Especially it is central to Helicobacter pylori metabolism and virulence being necessary for its colonization of the gastric mucosa, and is a potent immunogen that elicits a vigorous immune response. Therefore, it is not surprising that efforts to design, synthesize and evaluate of new inhibitors of urease are and active field of medicinal chemistry.

Several potent Urease inhibitors were synthesized using Morita-Baylis-Hillman adducts. The novel compounds posses elongated side chains so that they are better accomodated to the enzyme active site.

Acknowledgement
The author thanks Special Account for Research Grants and National and Kapodistrian University of Athens for funding to attend the meeting.

References

1) Krajewska B, Ureases I. Functional, catalytic and kinetic properties: a review. J Mol Catal B 2009;59:9–21.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL LIPOYL HYBRIDS

Aikaterini Pagoni, Stamatia Vassiliou

University of Athens, Department of Chemistry, Laboratory of Organic Chemistry, 15771, Panepistimiopolis Zografou, Athens, Greece

Lipoic Acid (LA) is a natural disulfide compound present in almost all foods from animal and vegetable sources and plays an important role in pathological conditions characterized by oxidative stress such as: (i) scavenger of ROS, (ii) capacity to increase the level of reduced glutathioneand other antioxidant enzymes, (iii) downregulation of the inflammatory processes, (iv) scavenging of lipid peroxidation products, (v) redox active transition metal chelation, (vi) increase of ACh production by activation of choline acetyltransferase. On the basis of such activities, LA can exert beneficial effects in AD, possibly stabilizing cognitive functions.[1]

Several lipoyl-phenolic acid hybrids were synthesized and tested for their neuroprotective ativity. Caffeic acid, ferulic acid and 3,4-dihydroxyphenylacetic acid were tethered through a linker to lipoic acid.

Acknowledgement

The author thanks Special Account for Research Grants and National and Kapodistrian University of Athens for funding to attend the meeting.

References

1) Packer, L., Tritschler, H. J., and Wessel, K. (1997). Neuroprotection by the metabolic antioxidant alpha-lipoic acid. Free Radic. Biol. Med. 22, 359–378.

P092

NOVEL CATECHOLIC UREASE INHIBITORS

Aikaterini Pagoni (1), Theohari Daliani (1), Stamatia Vassiliou (1), Artur Mucha (2), Katarzyna Macegoniuk (2), Lukasz Berlicki (2)

1) University of Athens, Department of Chemistry, Laboratory of Organic Chemistry, Panepistimiopolis Zografou, 15701, Athens Greece
2) Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wrocław, Poland

In the history of therapeutics, covalent drugs occupy a very distinct category. While representing a significant fraction of the drugs on the market, very few have been deliberately designed to interact covalently with their biological target.

Several catechol-containing compounds were synthesiszed and tested as Urease inhibitors. The newly synthesized compounds exhibited strong irreversible inhibition supporting a recent study about covalently interacting with Urease[1].

Acknowledgement

The author thanks Special Account for Research Grants and National and Kapodistrian University of Athens for funding to attend the meeting.

References

1) Luca Mazzei, Michele Cianci, Francesco Musiani, Gábor Lente, Marta Palombo, Stefano Ciurli. Inactivation of urease by catechol: Kinetics and structure. Journal of Inorganic Biochemistry 2017, 166, 182–189.

P093

SELECTIVITY OF PYRAZOLOQUINOLINONE DERIVATIVES TOWARDS THE ALPHA+/BETA- INTERFACE OF THE GABAA RECEPTOR

Blanca A. Vega-Alanis (1), David C.B. Siebert (1), Xenia Simeone (2), Konstantina Bampali (2), Laurin Wimmer (1), Margot Ernst (2), Marko D. Mihovilovic (1)

1) Vienna University of Technology, Institute of Applied Synthetic Chemistry, Getreidemarkt 9/163-OC, 1060, Vienna, Austria
2) Medical University of Vienna, Center for Brain Research, Spitalgasse 4, 1090, Vienna, Austria

$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the central nervous system. These GABA-gated chloride channels are composed of five subunits that can belong to different subunit classes. Several pyrazoloquinolinone ligands have already been described as high affinity ligands of the benzodiazepine (Bz) binding site but also, they exert a positive modulatory effect at the alpha+beta- interfaces.[1,2] Previously, it was shown that some pyrazoloquinolinone derivatives showed preference towards beta1 containing receptors in terms of potency. Further studies in homology models and mutant receptors confirm that the amino acid located in position 41 of segment G in the beta1 and beta3 subunits strongly influences the potency and efficacy of the tested ligands.[3] In the present study, further pyrazoloquinolinone derivatives were studied and results showed that they possess improved functional selectivity. The results of this study are herein presented and the properties of these compounds will be further investigated.

References

1) Olsen RW, Sieghart W (2008). International union of pharmacology. LXX. Subtypes of gamma-aminobutyric acid (A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update Pharmacol Rev. 60(3): 243–260.
2) Varagic, Z. et al. (2013) Identification of novel positive allosteric modulators and null modulators at the GABAA receptor alpha+betainterface. British journal of pharmacology 169, 371-383.
3) Simeone X., Siebert DCB., et al. (2018). Molecular tools for GABAA receptors: High affinity ligands for β1-containing subtypes. Scientific Reports, 7(5674).

CYCLOBUTANE RING AS A CONFORMATIONAL RESTRICTION TOOL

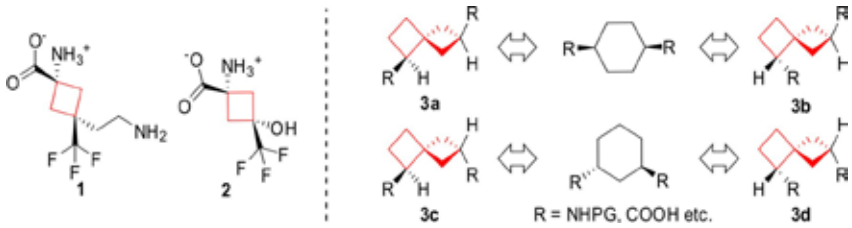
Dmytro Radchenko (1,2), Oleksandr Grygorenko (1,2), Igor Komarov (1,2), Ivan Kondratov (1,4), Dmitriy Volochnyuk (1,3)

1) Enamine Ltd. (www.enamine.net), Chervonotkatska Street 78, Kyiv 02094, Ukraine
2) Taras Shevchenko National University of Kyiv, Volodymyrska Street 60, Kyiv 01601, Ukraine
3) Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanska Street 5, Kyiv 02660, Ukraine
4) Institute of Bioorganic Chemistry & Petrochemistry, National Academy of Sciences of Ukraine, Murmanska Street 1, Kyiv 02660, Ukraine

Conformational restriction is a renowned approach to design of analogues, models and other practically useful compounds in many areas of science, first of all in bioorganic and medicinal chemistry. An important and desirable feature of this methodology is possibility of molecular structure modification without significant perturbation of the compound properties. In particular, DMPK-related physicochemical properties of conformationally restricted analogues such as molecular weight and liphophilicity can be a major concern. Small rings are among structural units which provide such possibility; in particular, cyclopropane-containing conformationally restricted analogues ("methanologues") have been widely used in drug discovery and adjacent areas of science. However, introducing cyclopropane into the parent molecules can significantly affect their electronic properties and hence chemical reactivity due to the partially unsaturated nature of this moiety. Cyclobutane ring does not have such drawbacks; on the other hand, it is small enough to satisfy the above-mentioned criteria as a conformational restriction tool. Nevertheless, the use of cyclobutanes in drug design and bioorganic chemistry was quite rare to date.

In this talk, we shall describe case studies from our recent research on cyclobutane-derived conformationally restricted compounds. In the first part, design and synthesis of cyclobutane-derived amino acids, as well as their incorporation into cell-penetrating peptides, will be described. In particular, fluorinated analogues of polar amino acids (such as lysine (**1**) and serine (**2**) analogues) which can be used as ^{19}F NMR labels will be discussed.

The second part of this presentation will include examples of using cyclobutane-derived scaffolds (such as spiro[3.3]heptane) in design of building blocks for medicinal chemistry. With the use of exit vector plot (EVP) tool, we will show that such building blocks (*e.g.* **3**) provide unusual but controllable spatial arrangements of the functional groups mounted onto the cyclobutane ring(s) and can be used as promising surrogates of common (hetero)aliphatic rings.



The presentation is based on both published (e. g. [1-4]) and unpublished results obtained in our groups in the last 10 yeas.

References

1) Chernykh, A. V. et al. J. Org. Chem. 2015, 80, 3974–3981
2) Feskov, I. V. et al. J. Org. Chem. 2017, 82, 12863–12868
3) Michurin, O. M. et al. Angew. Chem. Int. Ed. 2016, 55, 14595–14599
4) Tkachenko, A. N. et al. Angew. Chem. Int. Ed. 2013, 52, 1486-1489

COMMERCIALLY AVAILABLE CHEMICAL SPACE: DOES IT MEET MODERN REQUIREMENTS?

Dmitriy Volochnyuk (1,2), Sergey Ryabukhin (1), Yurii Moroz (1)

1) National Taras Shevchenko University of Kyiv, Volodymyrska Street, 60, Kyiv 01601, Ukraine
2) Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanska Street 5, Kyiv 02094, Ukraine

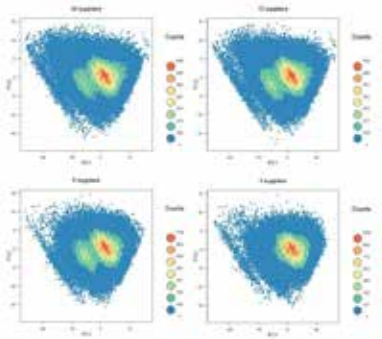
The content, size, and quality of compound collections used in HTS campaigns are fundamental to the success of the project; the most advanced screening technologies and the most physiologically relevant assays were though defeated by low quality of compound collections. The question, however, remains whether the available purchasable space allows to create a high-quality compound library for the HTS project that is comparable with selections from the Big Pharma repositories. While several analyses of the chemical space covered by vendor compound libraries (VCL) have been published recently [1] (including our studies [2,3]), the aforementioned question remains unanswered. The starting point of the study was generation of the chemical space covered by purchasable screening compounds using ZINC database of **16,902,208** unique structures including stereoisomers.

In this talk, we shall describe:

- a critical revision of the existing VCL from the user's standpoint and whether it competes with the available Big Pharma collections in supporting compound novelty, diversity, and quality;

- evaluation of possibility to easily create the high-quality compound library without involving cost-demanding compound management through a limited number of vendors. Such approach will include the vendor's selection;

- compound management optimization in the case of consolidated libraries from different vendors. To simplify compound management, we studied relationship between the quality of the selected sets and number of the suppliers.



Principal component analysis (PCA) for the compounds (Molecular Quantum Numbers were used as descriptors [4]) in the "perfect compound collection" built from 33, 12, 6 and 3 suppliers.

References

1) X. Lukas et. al J. Chem. Inf. Model., 2015, 55, 915.
2) A. Chuprina et. al J. Chem. Inf. Model., 2010, 50, 470.
3) T. Petrova et.al Med. Chem. Commun., 2012, 3, 571.
4) K. T. Nguyen et. al ChemMedChem, 2009, 4, 1803.

P096

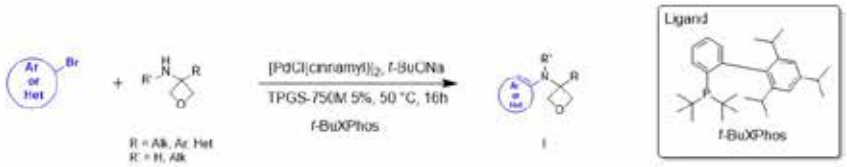
N-ARYLATION OF OXETANYLAMINE FOR THE PREPARTION OF N-ARYL-AMIDE ISOSTERES

Maud Bollenbach (1), William Lecroq (1), Patrick Wagner (1), Thomas Fessard (2), Martine Schmitt (1), Christophe Salome (2), Erick Carreira (2,3)

1) Laboratoire d'Innovation Thérapeutique , UMR7200 CNRS-UdS, 74 route du Rhin, 67401 Illkirch, France
2) SpiroChem AG, Rosental area, Mattenstrasse 24, 4058 Basel, Switzerland
3) Department of Chemistry and Applied Biosciences, Laboratorium fur Organische Chemie, Eidgenossische Technische Hochschule Zürich, Vladimir-Prelog-Weg 3, CH-8093 Zürich, Switzerland

Four-membered rings are witnessing significant prominence in medicinal chemistry discovery programs and various reports, that have documented the benefits accompanying their use in discovery candidates, are driving the increased visibility. The range of advantages includes structural novelty along with improved physicochemical and pharmaco-kinetic properties.The insertion of those strained systems remains challenging and poorly described in the literature. The development of new reactions to integrate it in more complex compounds is more and more needed.

A Pd cross-coupling approach for the synthesis of N-aryl-oxetanylamine has been developed. This method provides new building blocks potentially useful in medicinal chemistry as amide bioisosteres. The reactions are conducted in water employing the renewable feedstock surfactant TPGS-750-M (vitamin E) make these conditions adaptable for industrial production.



The N-arylated oxetanylamines were cleanly formed and could be isolated in good to excellent yields. Because of the mild reaction conditions, this reaction can tolerate a wide variety of functional groups. Moreover, many of the described products are inaccessible via other methods, and as all of them contain diverse functional groups allowing functionalization. Then, the building blocks can serve as potentially promising scaffolds for the design of drugs, pesticides, and advanced materials.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - TECHNOLOGIES

Development of New Synthetic Methodologies for Drug Discovery

IN SILICO PEPTIDE DIRECTED BINDING IDENTIFIES SELECTIVE MODULATORS OF THE P53/HDM2 AND HDMX PROTEIN-PROTEIN INTERACTIONS

Andrew M. Beekman, **Mark Searcey**

Schools of Chemistry and Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom

First line chemotherapy relies on DNA damaging compounds, commonly resulting in the activation of p53, inducing apoptosis. The side effects of DNA damaging compounds are extensive, and so activating p53 without DNA damage is desirable. Approximately 50% of cancers possess wild-type p53 which is inactivated, often by human double minute (*h*DM)2 and *h*DMX. Modulating the *h*DM2 p53 protein-protein interaction (PPI) is an effective way to target cancers. However, the scope of this strategy has been limited by *h*DMX. Despite the homologous nature of *h*DMX to *h*DM2 the proteins do not perform redundant roles. Our investigation of new methodology to target PPIs, termed peptide-directed binding,[1] exploited the dual inhibitor Ac-Phe-Met-Aib-Pmp-6-Cl-Trp-Glu-Ac_3c-Leu-NH_2 to develop small molecule probes to target *h*DM2 and *h* DMX (Fig 1).

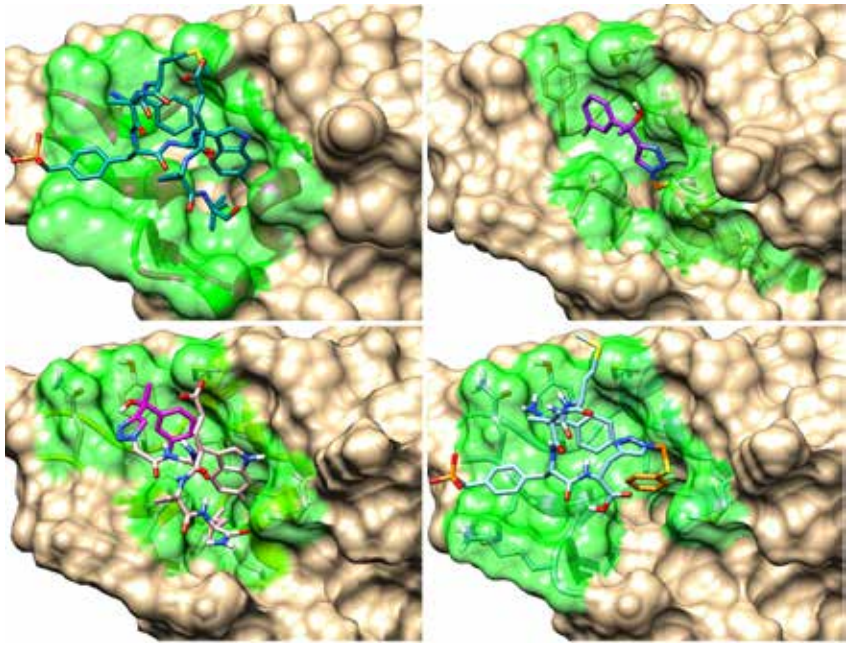


Figure 1. Schematic of in silico peptide directed binding identifying small molecule candidate.

We extend peptide directed binding to in silico methods allowing modelling to identify small molecules for synthesis. This represents a complementary method, improving the rapid and economic nature of this process.

References

1) A. M. Beekman, M. A. O'Connell, L. A. Howell, Angew. Chem. Int. Ed. 2017, 56, 10446.

DNA-ENCODED LIBRARIES - LIBRARY VALIDATION AND DEVELOPMENT OF SYNTHESIS METHODOLOGY

Andreas Brunschweiger, **Mateja Klika-Skopic**, **Verena Kunig**, **Marco Potowski**

TU Dortmund University, Department of Chemistry and Chemical Biology, Otto-Hahn-Str. 6, 44227 Dortmund, Germany

DNA-encoded small molecule libraries (DELs) are an established screening technology in drug research.[1] They enable efficient identification of binders for target proteins by selection and DNA sequencing. Encoded libraries are synthesized by combinatorial strategies with alternated organic preparative chemistry and encoding steps.[2] Currently, only a very small toolbox of organic preparative reactions is available for encoded synthesis due to the chemical instability of the DNA tag, and limiting the molecular diversity of libraries. We have developed a coding strategy, named TiDEC that utilizes a hexathymidine sequence “hexT” as a chemically very stable adapter oligonucleotide (figure 1).[3-5] It made several catalysts available for library synthesis in the initial step of DEL synthesis, among them acids, and transition metal ions. Following up, we have investigated the tolerance of different DNA sequences to reaction conditions and several catalysts towards development of novel DNA tagging strategies, to ultimately expand the chemical space of genetically tagged screening libraries.[5]

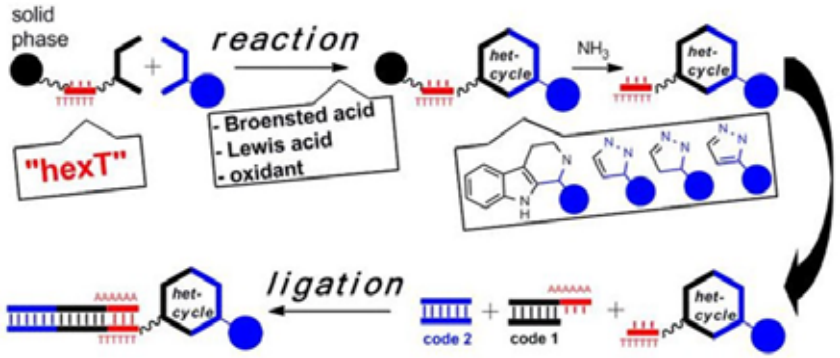


Figure 1: Starting DNA-encoded library synthesis from the hexT adapter oligonucleotide.

References

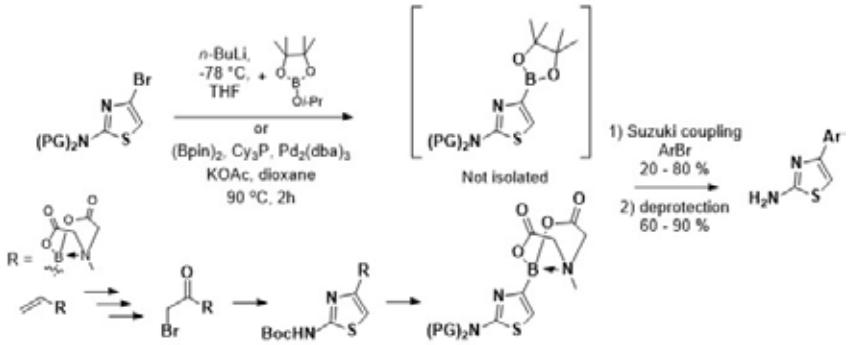
1) H. Salamon, ACS Chem. Biol. 2016, 11, 296-307.
2) M. Klika Škopić, Med. Chem. Commun. 2016, 7, 1957-1965.
3) M. Klika Škopić, Chem. Sci., 2017,8, 3356-3361.
4) A. Brunschweiger, 2015, EP15202448.5.
5) M. Klika Škopić, Org. Biomol. Chem., 2017,15, 8648-8654.

FLEXIBLE SYNTHESIS OF 4-SUBSTITUTED-2-AMINOTHIAZOLES

Benoit Carbain (1,2), Stepan Havel (1,2), Prashant Khirsariya (1,2), Kamil Paruch (1,2)

1) Department of Chemistry, CZ Openscreen, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
2) International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic

New synthetic routes for the preparation of 4-substituted-thiazol-2-amines, relevant intermediates in medicinal chemistry/drug discovery, are reported. The first method employs a 'reverse' Suzuki coupling of adequately protected 4-bromothiazol-2-amine, via the formation of the pinacol boronate ester (not isolated), with aryl halides. The second route utilizes the stable protected 4-MIDA boronate ester-2-aminothiazole that is used in subsequent Suzuki couplings with aryl halides.[1, 2]



References

1) Org. Lett., 2015, 17, 5594–5597
2) Tetrahedron, 2009, 65, 3130–3138

A FACILE AND EFFICIENT SYNTHESIS OF 1,6-DIAZECANES VIA INTERMOLECULAR DOUBLE AZA PRINS-TYPE CYCLIZATION

Hyun Mi Cho (1,2), Jae Kyun Lee (1), Sun-Joon Min (3), Jin Sung Tae (2), Yong Seo Cho (1)

1) Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science & Technology, Republic of Korea
2) Department of Chemistry, Yonsei University, Republic of Korea
3) Department of Chemical & Molecular Engineering/ Applied Chemistry, Hanyang University, Ansan, Republic of Korea

The Prins-type cyclization is one of the most useful reactions to the synthesis of heterocycles in natural compounds.[1] A lot of synthetic methods for 5,6-members nitrogen heterocycles have been reported.[2] However, the efficient synthetic methods for the 10-members nitrogen heterocycles have been reported few. The synthesis of these compounds is still remains a major synthetic challenge. Typically, the intramolecular 5-endo-trig cyclization would be kinetically disfavored according to Baldwin's rule.[3] Taking advantage of this, we developed the first efficient synthetic method of 10-members nitrogen heterocycles, 1,6- diazecanes, by an intermolecular double aza Prins-type cyclization[4] from (allenylmethyl)silane derivatives. The methodologies developed to obtain these compounds will be presented and discussed.

References

1) E. Arundale, L. A. Mikeska, Chem. Rev. 1952, 51(3), 505–555.
2) B. E. Maryanoff, H-C. Zhang, J. H. Cohen, I. J. Turchi, C. A. Maryanoff, Chem. Rev. 2004, 104, 1431-1628.
3) Jack E. Baldwin "Rules for Ring Closure". J. Chem. Soc., Chem. Commun. 1976, (18), 734–736.
4) P. R. Ullapu, S-J Min, S. N. Chavre, H. Choo, J. Lee, A. N. Pae, Y. Kim, M. H. Chang, and Y. S. Cho, Angew. Chem. Int. Ed. 2009, 48, 2196 –2200.

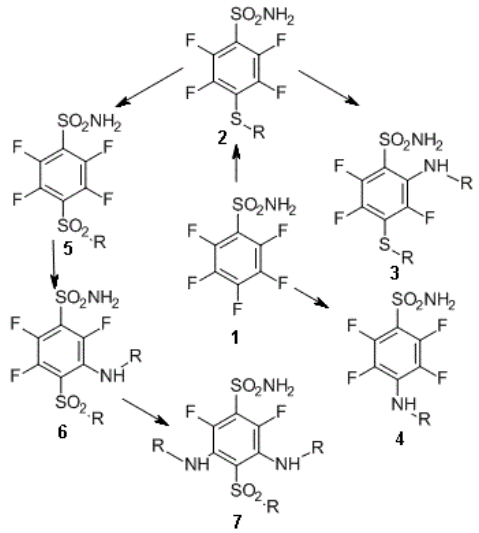
FUNCTIONALIZATION OF FLUORINATED BENZENESULFONAMIDES - AN APPROACH TOWARDS CARBONIC ANHYDRASE IX SELECTIVITY

Virginija Dudutienė (1), Asta Zubrienė (1), Justina Kazokaitė (1), Jānis Leitāns (2), Kaspars Tārs (2), Daumantas Matulis (1)

1) Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Vilnius, Lithuania
2) Latvian Biomedical Research and Study Center, Riga, Latvia

Carbonic anhydrases (CA) are zinc metalloenzymes, which catalyze the reversible hydration of carbon dioxide and regulate a broad range of physiological functions. There are 12 active CA isoforms in human which differ in cellular localization, distribution in organs and tissues, expression levels and kinetic properties. The increased activity or expression of different CA isoforms is often associated with various diseases. Isoform CA IX is implicated in cancer since its expression is nearly absent in healthy human but overexpression of CA IX in numerous hypoxic tumors is observed. Design of a selective and high-affinity inhibitor could be developed into an anticancer drug.

Here we investigate fluorinated benzenesulfonamides as CA inhibitors. The fluorine atoms contributed favorably to CA binding. Furthermore, the fluorinated benzenesulfonamides were subject to convenient nucleophilic aromatic substitution reactions which enabled the synthesis of a diversity of fluorinated compounds. A series of 4-substituted-2,3,5,6-tetrafluorobenezenesulfonamides (**2**, **4**, **5**), 2,4-substituted-3,5,6-trifluorobenzenesulfonamides (**3**), 3,4-substituted-2,5,6-trifluorobenzenesulfonamides (**6**), and 3,4,5-substituted-2,6-difluorobenzenesulfonamides (**7**) were synthesized[1,2,3]. Some of the fluorinated benzenesulfonamides bearing bulky hydrophobic groups at *ortho* and *meta* positions exhibited high selectivity and picomolar affinity for CA IX as confirmed by the binding assays. Crystallographic analysis showed the position of the compounds bound to CA IX and the effects in 2D and 3D cancer cell culture models of lead compounds showed compound anticancer activity.



References

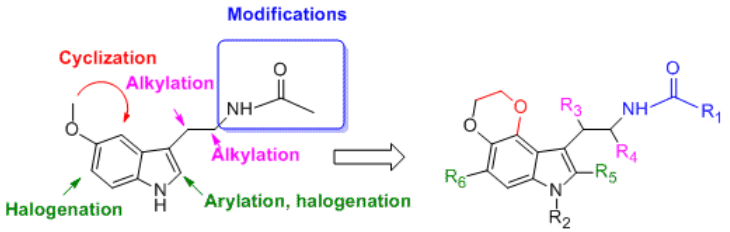
1) V.Dudutienė, et al. BMC, 2013, 21, 2093-2106.
2) V.Dudutienė, et al. JMedChem, 2014, 57, 9435-9446.
3) V.Dudutienė, et al. ChemMedChem, 2015, 10, 662-687.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW INDOLES DERIVATIVES AS POTENT MELATONINERGIC (MT1/MT2) AND SEROTONINERGIC (5-HT2C) DUAL LIGANDS

Romero Manel (1), Lizano Enric (1), Viñas Miquel (1), Vilaplana Marta (1), Rubio Jaime (3), Caignard Daniel-Henry (2), Pujol Maria Dolors (1)

1) Laboratori de Química Farmacèutica. Facultat de Fàrmacia i Ciencies de l'Alimentació. University of Barcelona. Campus Diagonal, Av. Joan XXIII, 27-31, 08028-Barcelona, Spain
2) Unité de Recherche Chimie Neurosciences, Institut de Recherches Servier, 78290 Croissy-sur-Seine, France
3) Laboratori de Química Fisica. Facultat de Química. University of Barcelona. Campus Diagonal, Carrer de Martí i Franquès, 1-11, 08028-Barcelona, Spain

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone produced in the brain region by the pineal gland during darkness, but also in the gastrointestinal tract, in retina and in skin from the essential amino acid tryptophan. Melatonin plays an important role in the regulation of seasonal and circadian fluctuations. Nowadays, melatonin is one of the most studied compounds by scientists due to both its physiological role and its therapeutic applications. It is also an antioxidant hormone with a particular role in the protection of nuclear and mitochondrial DNA. In recent years, many physiological properties of melatonin have been described resulting in much attention in the development of synthetic compounds possessing the indole ring. In this study, we developed a new scaffold that combines the indole and the 1,4-dioxan heterocycle. A small-molecule library of sixty analogue melatonin compounds were synthesized and in vitro biological activity was investigated. Most of the compounds showed significantly higher activity than melatonin.



The focus of this work was to optimize the most active compound, identify structure-activity relationships, and prepare compounds that are more efficacious. For all this, molecular modeling studies have been carried out.

Acknowledgments

We gratefully acknowledge support of this project by the Laboratories Servier (France).

References

1) Duroux, R.; Rami, M.; Landagaray, E.; Ettaoussi, M.; Caignard, D.-H.; Delagrange, P.; Melnyk, P.; Yous, S. Eur. J. Med. Chem. 2017, 141, 552-566.
2) Fuente Revenga, M.; Fernandez-Saez, N.; Herrera-Arozamena, C.; Morales-Garcia, J. A.; Alonso-Gil, S.; Perez-Castillo, A.; Caignard, D.-H.; Rivara, S.; Rodriguez-Franco, M. I. J. Med. Chem. 2015, 58, 4998-5014.

NEW SYNTHETIC APPROACH TOWARDS CEFTOBIPROLE ANALOGUES

Rok Frlan, Martina Hrast, Stanislav Gobec

University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana

Beta-lactam antibiotics play an important role in treatment of infectious diseases. Despite many versatile synthetic tools for the construction of this interesting scaffold, there is still a necessity for the development of new synthetic approaches. We have developed a novel and optimized synthetic route for the synthesis of *N* -substituted monobactam ring.

In phase one diverse protective groups were introduced onto amino group of starting dipeptides. Then in phase two various cyclization strategies were used to obtain the desired ring. A beta-lactam ring was obtained under the Mitsunobu reaction conditions exclusively. Although phthalimide and dibenzyl protective groups both enable us to obtain desired ring system, the latter was superior.

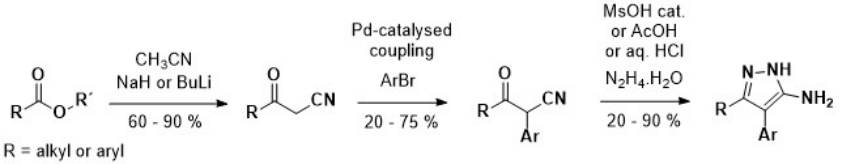
The methodology developed is uncomplicated and widely applicable for preparation of epimerically pure *N* -substituted monobactam compounds.

VERSATILE SYNTHESIS OF BUBSTITUTED 3-AMINOPYRAZOLES VIA PD-CATALYSED ALPHA-ARYLATION OF BETA-KETONITRILE

Stepan Havel (1,2), Benoit Carbain (1,2), Naresh Akavaram (1,2), Kamil Paruch (1,2)

1) Department of Chemistry, CZ Openscreen, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
2) International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic

A new strategy for the preparation of 4,5-substituted-3-aminopyrazoles, widely used intermediates in medicinal chemistry discovery, is reported. The synthetic methodology employs palladium-catalysed coupling of b-ketonitriles with arylbromides as the diversification step, followed by cyclisation with hydrazine which yields the desired aminopyrazoles. The b-ketonitriles are synthesized from readily available acetonitrile and esters.



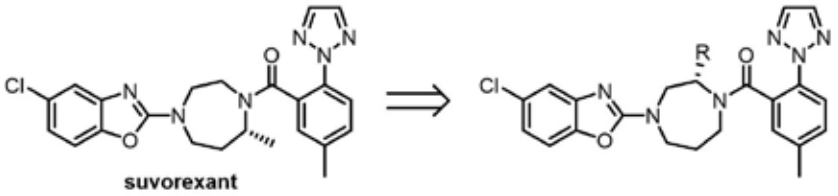
STRUCTURE-BASED DEVELOPMENT OF SELECTIVE OREXIN 1 RECEPTOR ANTAGONISTS DERIVED FROM SUVOREXANT

Jan Hellmann (1), Matthäus Drabek (2), Frank Kraus (1), Harald Hübner (1), Peter Kolb (2), Peter Gmeiner (1)

1) Department of Chemistry and Pharmacy, Medicinal Chemistry, Friedrich Alexander University, Nikolaus-Fiebiger-Str. 10, Erlangen, Germany
2) Department of Pharmaceutical Chemistry, Philipps-University Marburg, Marbacher Weg 6, Marburg, Germany

Orexins are neuropeptides that activate the rhodopsin-like G protein-coupled receptors OX1R and OX2R. The orexin system plays an important role in the regulation of the sleep-wake cycle and the regulation of feeding and emotions. The high resolution crystal structures of both receptor subtypes bound to the dual orexin receptor antagonist suvorexant provide valuable insights into the structural environment of the orthosteric binding sites.[1-2] Suvorexant is the only drug on the market targeting the orexin system and is prescribed for the treatment of insomnia.[3] There are only two non-conserved residues in the orthosteric binding site within 4 Å of the ligand. An alanine and a serine residue of the OX1R are substituted by threonine in the OX2R resulting in a slightly larger binding pocket of the OX1R compared to the OX2R's binding site. We wanted to exploit the available space in the OX1R's binding site to develop selective orexin 1 receptor antagonists based on the structure of suvorexant.

Hence, we established an enantioselective synthetic route starting from natural or artificial amino acids for suvorexant derivatives bearing an alkyl substituent at the central homopiperazine moiety. The substituents were expected to point towards one of the non-conserved residues resulting in a steric clash with the larger threonine side chain of the OX2R. We investigated various derivatives to induce subtype-selectivity towards the OX1R by docking experiments and synthesized the most promising candidates. All synthesized ligands were tested for their antagonist activity at both orexin receptor subtypes using a functional IP accumulation assay.



References

1) Yin, J. et al., Nature, 2015, 519 (7542), 247-250.
2) Yin, J. et al., Nat. Struct. Mol. Biol., 2016, 23 (4), 293-299.
3) Cox, C. D. et al., J. Med. Chem., 2010, 53 (14), 5320-5332.

MOESAIC: APPLICATION OF MATCHED MOLECULAR PAIRS TO SAR EXPLORATION

Andrew Henry

Chemical Computing Group, St Johns Innovation Centre, Cowley Road, Cambridge CB4 0WS, United Kingdom

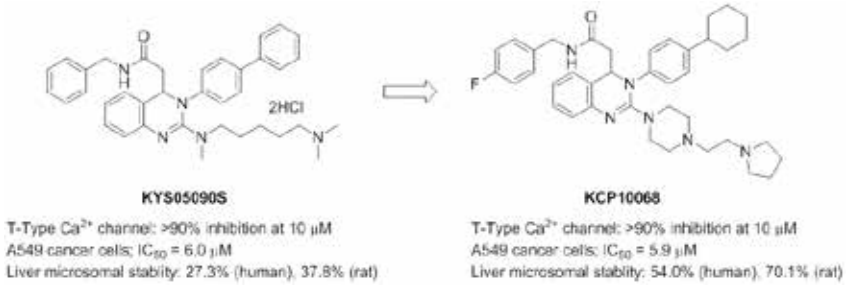
Managing and analyzing structure activity/property relationship data in medicinal chemistry projects is becoming ever more challenging, with larger data sets and parallel development of different structural series. Tools and methods for the efficient visualization, analysis and profiling of structures therefore remain of deep interest. Here, we will describe a new application, MOEsaic, which enhances typical medicinal chemistry workflows aimed at interrogating the SAR data through the use of interactive MMP analysis and R-group profiling, for guiding a campaign in its development.

DESIGN AND SYNTHESIS OF NOVEL 3,4-DIHYDROQUINAZOLINE DERIVATIVES AS BOTH ANTI-CANCER AGENT AND ANALGESIC AGENT

Changyoung Jang (1), Da Woon Jung (1), Ki Duck Ryu (1), Gerald W. Zamponi (2), Kyung-Tae Lee (3), Jae Yeol Lee (1)

1) Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 02447, Republic of Korea
2) Department of Physiology and Pharmacology, Hotchkiss Brain Institute and Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary T2N 4N1, Canada
3) Department of Life and Nanopharmaceutical Science, Kyung Hee University, Seoul 02447, Republic of Korea

As a bioisosteric strategy to overcome the poor metabolic stability of lead compound **KYS05090S**, a series of new fluoro-substituted 3,4-dihydroquinazoline derivatives was prepared and evaluated for T-type calcium channel ($Ca_V3.2$) blockade, cytotoxic effects and liver microsomal stability. Among them, compound **KCP10068F** containing 4-fluorobenzyl amide and 4-cyclohexylphenyl ring potently blocked $Ca_V3.2$ currents (>90% inhibition) at 10 μM concentration and also exhibited cytotoxic effect (IC_{50} = 5.9 μM) in A549 non-small cell lung cancer cells that was comparable to **KYS05090S**. Furthermore, **KCP10068F** showed approximately a 2-fold increase in liver metabolic stability in rat and human species compared to **KYS05090S**. Based on these overall results, **KCP10068F** may therefore represent a good backup compound for KYS05090S for further biological investigations as novel cytotoxic agent. In addition, **KCP10067F** was found to partially protect from inflammatory pain via a blockade of $Ca_V3.2$ channels.



SYNTHESIS OF 1,3-DIAMINE VIA AU(I)-CATALYZED INTRAMOLECULAR CYCLIZATION REACTION

Da Mi Kim, Jae-Sang Ryu

College of Pharmacy & Graduate School of Pharmaceutical Sciences, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-Gu, Seoul 03760, Republic of Korea

1,3-Diamine is an important structural motif in pharmaceuticals and natural products such as HIV-1 protease inhibitors and the marine alkaloids, batzelladines and manzacidin.[1-3] Also, 1,3-diamino fragment is a key core of chiral ligands and auxiliaries. Despite the importance of this structure, many existing synthetic methods suffer from poor efficiency and low yields. Thus, development of simple and efficacious methods are urgently necessary. In this study, we developed a synthetic protocol of various cyclic precursors of 1,3-diamines, via a straightforward Au(I)-catalyzed intramolecular cyclization reaction.[1] 5 mol% of gold catalysts smoothly mediated the reactions at room temperature and afforded cyclic precursors of 1,3-diamines in high yields with good diastereoselectivities. The ring-opening reaction of cyclic precursors led to the desired 1,3-diamines. Substrate synthesis and catalytic reactions will be presented.

References

1) X. Ji, H. Huang, Org. Biomol. Chem., 2016, 14, 10557–10566
2) A. S. Franklin, S. K. Ly, G. H. Mackin, L. E. Overman and A. J. Shaka, J.Org. Chem., 1998, 64, 1512
3) T. Hashimoto and K. Maruoka, Org. Biomol. Chem., 2008, 6, 829

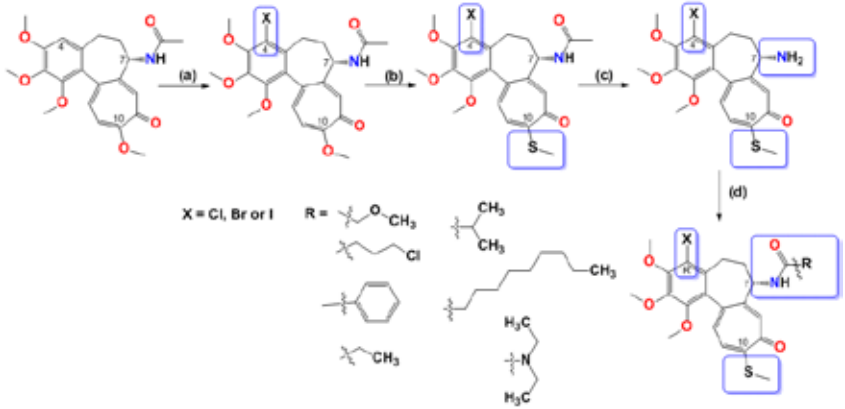
SYNTHESIS AND BIOLOGICAL EVALUATION OF TRIPLE-MODIFIED COLCHICINE DERIVATIVES AS POTENT TUBULIN-TARGETING ANTICANCER AGENTS

Greta Klejborowska (1), Ewa Maj (2), Joanna Wietrzyk (2), Mahshad Moshari (3), Jack A. Tuszynski (3), Adam Huczyński (1)

1) Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89b, 61-614 Poznań, Poland
2) Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocław, Poland
3) Department of Oncology, University of Alberta, Edmonton, Alberta T6G 1Z2, Canada

Colchicine (**1**), a well-known tropolone alkaloid isolated from *Colchicum autumnale,* is of particular interest due to its antimitotic properties. It has played an important role in studies of mitosis and the therapeutic potential of colchicine binding site has been considered for chemotherapy applications [1,2]. However, colchicine itself as well as many of its derivatives could not be used as anticancer drugs because of their high toxicity. Up to now many structure-activity relationship studies have been done to elucidate the structural features required for the tubulin binding [3–5].

Herein, we report the synthesis, spectroscopic analysis of a series of novel triple-modified in C-4, C-7, and C-10 positions colchicine derivatives, as well as evaluation of these derivatives as cytotoxic, tubulin-targeting agents.



Scheme 1. Synthesis of colchicine derivatives. Reagents and conditions: (a) NCS, acetonitrile, RT, NBS, acetonitrile, RT or NIS, AcOH, 70°C; (b) MeOH/H_2O, CH_3SNa, RT, (c) 2 M HCl, reflux, (d) Et_3N, DMAP, acyl chloride, CH_2Cl_2, RT.

The antiproliferative effect was tested *in vitro* on four human cancer cell lines and one normal murine embryonic fibroblast cell line (BALB/3T3). To better understand the interactions between the colchicine derivatives and tubulin, we also investigated potential binding modes of all studied compounds docked into colchicines binding site (CBS) of ßI tubulin.

Financial support by grant of the Polish National Science Center (NCN) – No. 2016/21/B/ST5/00111 is gratefully acknowledged.

References

1) A. Slobodnick, B. Shah, M. H. Pillinger, S. Krasnokutsky, Am. J. Med. 2015, 128, 461
2) C. Avendaño, J. C. Menéndez, Medicinal Chemistry of Anticancer Drugs, 2008
3) N. Yasobu, M. Kitajima, N. Kogure, Y. Shishido, T. Matsuzaki, M. Nagaoka, et al., ACS Med. Chem. Lett. 2011, 2, 348
4) A. Huczyński, J. Rutkowski, K. Popiel, E. Maj, J. Wietrzyk, J. Stefańska, et al., Eur. J. Med. Chem. 2014, 90, 296
5) A. Huczyński, U. Majcher, E. Maj, J. Wietrzyk, J. Janczak, M. Moshari, et al., Bioorg. Chem. 2016, 64, 103

PHENYLSULFONYL CYCLOPROPANE LACTONE AS A SOURCE OF LIGNAN-LIKE ANTILEISHMANIAL COMPOUNDS

Daniela Konrádová (1), David J.Y.D. Bon (2), Jiří Pospíšil (1,2)

1) Laboratory of Growth Regulators, Palacky University and Experimental Botany AS CR, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic
2) Department of Organic Chemistry, Faculty of Science, Palacky University, tř. 17. listopadu 1192/12, CZ-771 46 Olomouc, Czech Republic

Leishmaniasis is a parasitic disease occurring widely in Central and South America, Africa, South Asia and especially Middle-East area. World Health Organization estimates that Leishmaniosis affects between 700 000 to 1 million new cases every year and leads to the death of 20 000-30 000 patients. There are three clinical forms of this disease, which vary from disrupted skin lesion to damage of internal organs leading to death. Current treatments are less and less efficient (drug resistance), requires long hospitalization (4 to 12 weeks) and the treated patients often face to severe side effects of medications. In our group we expect that the development of new lignan-based pharmacophores might be an answer to desperate need of treatment of Leishmaniasis. Our hypothesis is based on the molecule known in the literature as Sanguinolignan – lignan of natural origin with proven leishmanicidal activity. In our contribution we wish to introduce our first efforts devoted to the lignan-inspired library-constitution. From the synthetic view point, our library use only one readily available key synthetic building block that is transformed in reagent-driven Diversity-Oriented synthesis in 2-3 steps in large collection of structurally highly diverse molecular scaffolds. The structural diversity of prepared molecules vary from fused tri- to tetracyclic molecules to tri- and tetracyclic spiro compounds with different ring sizes. Additionally, our first results obtained by biological evaluation of such chemical library will be presented.

LIBRARY OF MACROCYCLIC β-PEPTIDOMIMETIC LACTAMS: DESIGN AND SYNTHESIS

Volodymyr Kysil (1), Sergey Tkachenko (1), Haiji Xia (1), Elena Ryzshova (2), Olga Sviridova (2), Olga Shilova (2), Igor Rezekin (2)

1) ChemDiv, Inc., 6605 Nancy Ridge Drive, San Diego, CA 92121, USA
2) Chemical Diversity Research Institute, Rabochaya 2, Khimki, Moscow Region 114401, Russia

Despite the proven therapeutic potential of macrocyclic compounds, they have been under-explored and poorly exploited in drug discovery programs for some reasons. However, the most recent trends clearly show remarkably growing interest toward medium- and large-sized heterocycles, and this interest is not accidental since it is closely linked with the growing interest toward protein-protein interactions (PPI) as promising therapeutic targets and therefore small molecule PPI modulators. On the other hand, only few methodologies have been developed to date for the synthesis of medium- and large-sized molecules libraries.

Recently we reported synthesis of unique polyfunctional 10-12-membered lactams employing Bormann-Wasserman strategy. Herein we report further expansion of the strategy for the synthesis of novel functionally enriched, spiro- and fused scaffolds with incorporated (un)substituted b-alanine moiety. This structural feature has been used for design and synthesis of b-peptidomimetic library around these scaffolds.

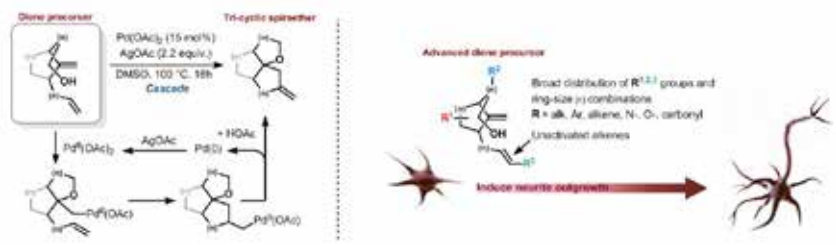
Further modifications of macrocyclic lactam scaffolds will be discussed.

METHYLENE - CYCLOALKYLACETATE (MCA) AS NOVEL NEUROTROPIC AGENTS

David Lankri, Dikla Haham, Adi Lahiani, Philip Lazarovici*, Dmitry Tsvelikhovsky*

School of Pharmacy, Institute for Drug Research, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel

Tricyclic spiroether structures can frequently be observed as scaffold segments of various biochemical compounds and drugs of natural origins. Examples of these structures have been identified among carbohydrates, terpenoids and alkaloids. Unfortunately, access to a large number of these target molecules and their structural analogues is either unknown or hindered by their multistep syntheses. We realized that most of the tricyclic spiranoid ethers might be derived from a simple and common collective precursor via a controlled intramolecular sequence of transformations. We discovered that monocyclic diene-alcohol precursor (see scheme) could serve as such building block for their synthesis via controlled Pd-catalyzed cascade cyclization reactions. We demonstrated, for the first time, a simple link between diene-alcohol cores and diverse medium-sized spiroether architectures. We have also noticed that precursors, employed as a platform for syntheses of spiroethers, possess the capacity to act as standalone cores of numerous natural products (such as dysidolide, halmic acid, angolensate and others). Our study, therefore, was inspired by the assumption that synthetic diene-alcohol scaffolds, which are small, rigid, and highly reminiscent of natural scaffolds, could serve as operational ligands for development of a neurotropic lead compound. Many diene-alcohol-based natural products have been firmly established to demonstrate pharmacological activities. Thus, we were motivated to apply our designed architectures to the discovery of novel neurotropic compounds using the pheochromocytoma (PC12) cell neuronal model. We investigated the neurotropic effect of a broad library of diene-alcohol and other related derivatives by comparison to NGF, a known neurotropic factor. Micrographs of the cells were collected by using a light microscope camera, and digitized photographs were analyzed for compound-induced neurotropic activity using an NIH image protocol. The results indicate that the alkene element, integrated within the cycloalkylacetate core, is indispensable for neurotropic activity. By employing this line of research, our ultimate aim is to single out a small molecules, bearing potential for treatment of brain disorders, caused by insufficient trophic support.



References

1) Lankri, D., Haham, D., Lahiani, A., Lazarovici, P., Tsvelikhovsky, D. ACS Chem. Neurosci., 2018, 9, 691-698.
2) Lankri, D., Mostinski, Y., Tsvelikhovsky, D. J. Org. Chem. 2017, 82, 9452-9463.
3) Albarghouti, G., Kotikalapudi, R., Lankri, D., Valerio, V., Tsvelikhovsky, D. Chem. Comm. 2016, 52, 3095-3098.

ONE-POT THREE-COMPONENT SYNTHESIS OF 1,4,5-TRISUBSTITUDE 1,2,3-TRIAZOLES

Soyun Lee, Jae-Sang Ryu

College of Pharmacy & Graduate School of Pharmaceutical Sciences,
Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul, 03760, Republic of Korea

Tricyclic spiroether structures can frequently be observed as scaffold segments of various biochemical compounds and drugs of natural origins. Examples of these structures have been identified among carbohydrates, terpenoids and alkaloids. Unfortunately, access to a large number of these target molecules and their structural analogues is either unknown or hindered by their multistep syntheses. We realized that most of the tricyclic spiranoid ethers might be derived from a simple and common collective precursor via a controlled intramolecular sequence of transformations. We discovered that monocyclic diene-alcohol precursor (see scheme) could serve as such building block for their synthesis via controlled Pd-catalyzed cascade cyclization reactions. We demonstrated, for the first time, a simple link between diene-alcohol cores and diverse medium-sized spiroether architectures. We have also noticed that precursors, employed as a platform for syntheses of spiroethers, possess the capacity to act as standalone cores of numerous natural products (such as dysidolide, halmic acid, angolensate and others). Our study, therefore, was inspired by the assumption that synthetic diene-alcohol scaffolds, which are small, rigid, and highly reminiscent of natural scaffolds, could serve as operational ligands for development of a neurotropic lead compound. Many diene-alcohol-based natural products have been firmly established to demonstrate pharmacological activities. Thus, we were motivated to apply our designed architectures to the discovery of novel neurotropic compounds using the pheochromocytoma (PC12) cell neuronal model. We investigated the neurotropic effect of a broad library of diene-alcohol and other related derivatives by comparison to NGF, a known neurotropic factor. Micrographs of the cells were collected by using a light microscope camera, and digitized photographs were analyzed for compound-induced neurotropic activity using an NIH image protocol. The results indicate that the alkene element, integrated within the cycloalkylacetate core, is indispensable for neurotropic activity. By employing this line of research, our ultimate aim is to single out a small molecules, bearing potential for treatment of brain disorders, caused by insufficient trophic support.

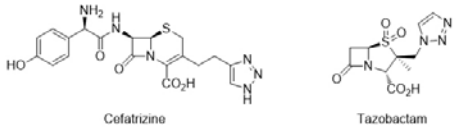


Figure 1. FDA-Approved 1,2,3-triazole Drugs

References

1) S. G. Agalave; S. R. Maujan; V. S. Pore, Chem. Asian J. 2011, 6, 2696.
2) D. Fournier; R. Hoogenboom; U. S. Schubert, Chem. Soc. Rev., 2007, 36, 1369.
3) J. F. Lutz, Angew. Chem. Int. Ed., 2008, 47, 2182.
4) O. S. Miljanic; W. R. Dichtel; I. Aprahamian; R. D. Rohde; H. D. Agnew; J. R. Heath; J. F. Stoddart, QSAR Comb. Sci., 2007, 26, 1165.
5) A. Nuzzi; A. Massi; A. Dondoni, QSAR Comb. Sci., 2007, 26, 1191.
6) S. Díez-González; A. Correa; L. Cavallo; S. P. Nolan, Chem. Eur. J. 2006, 12, 7558.

MILD TRANSFORMATION METHOD OF METHYLENE ACETALS TO BROMOFORMATES USING PhSTMS AND N-BROMOSUCCINIMIDE

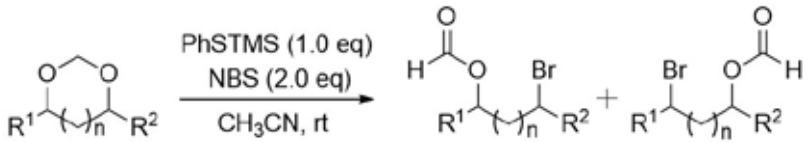
Tomohiro Maegawa (1), Misa Nogata (1), Yuuka Hirose (1), Akira Nakamura (1), Yasuyoshi Miki (1,2), Hiromichi Fujioka (3)

1) School of Pharmaceutical, Kindai University, Higashi-Osaka, Osaka 577-8502, Japan
2) Research Organization of Sciences and Technology, Research Center for Drug Discovery and Pharmaceutical Science, Ritsumeikan University, Nojihigashi, Kusatsu, Shiga 525-8577, Japan
3) Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565-0871, Japan

Protective groups are essential in organic syntheses and numerous such groups have been developed. Methylene acetal is one among the most popular protective group for diols and is stable under basic to medium acidic conditions. This stability, however, can make deprotection difficult, often requiring strongly acidic conditions.

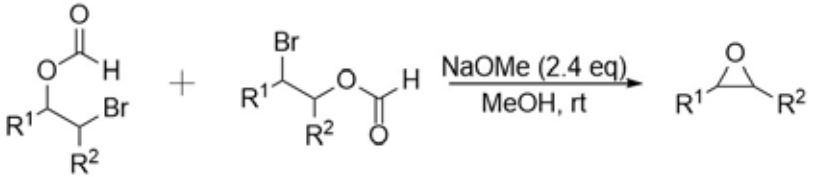
During the study of protection of diol to methylene acetal,[1] we found unexpected transformation reaction of methylene acetal under mild reaction conditions. Then we investigated the reaction conditions and we found that the combination of PhSTMS and *N*-bromosuccinimide (NBS) is the best conditions for the conversion from methylene acetal to bromoformates. (Scheme 1).[2] A variety of functional groups, including esters, ethers and halogens, were tolerated under the reaction conditions.

Scheme 1. Transformation of Methylene Acetals to Bromoformates



Further application of this reaction is the transformation of bromoformates to epoxides (Scheme 2). The treatment of bromoformates with NaOMe afforded the corresponding epoxides in good to high yields. In addition, a one-pot conversion of methylene acetal to epoxide was achieved.

Scheme 2. Conversion of bromeformates to Epoxides



References

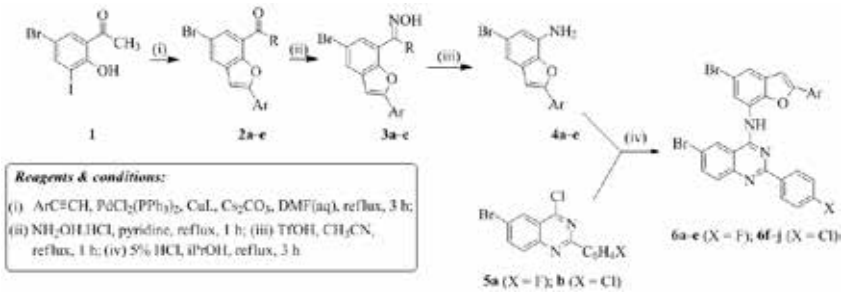
1) T. Maegawa, Y. Koutani, K. Otake, H. Fujioka, J. Org. Chem., 2013, 78, 3384.
2) T. Maegawa, M. Nogata, Y. Hirose, A. Nakamura, Y. Miki, H. Fujioka, J. Org. Chem., 2017, 82, 7608.

SYNTHESIS, CYTOTOXICITY AND MOLECULAR DOCKING OF THE BENZOFURAN–APPENDED 4-AMINOQUINAZOLINE HYBRIDS AS EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS

Marole Maluleka, Jack Mphahlele

Department of Chemistry, College of Science, Engineering and Technology, University of South Africa, Private Bag X06, Florida 1710, South Africa

Quinazolines and benzofurans have earned great interest in targeted therapies as antitumor drugs. Among the quinazoline analogues, the 4-aminoquinazolines are selective and effective inhibitors of the epidermal growth factor receptor tyrosine kinase (EGFR-TK) phosphorylation, which results from competitive binding at the ATP site.[1-3] The benzofuran–appended 4-aminoquinazoline **6a–h** were synthesized by merging the 7-aminobenzofurans 4 with the 4-chloroquinazolines **5a** and **5b** as represented in Scheme 1.



Scheme 1: Design of benzofuran-appended 4-aminoquinazolines using molecular hybridization

Compounds **6a–h** were evaluated for cytotoxicity in vitro against the A549, Caco-2) and C3A cell lines and for potential to induce apoptosis. Their capability to inhibit the EGFR-TK was evaluated experimentally complemented with molecular docking studies into the ATP binding site of the EGFR.

References

1) Arcadi, A.; Blesi, F. et al. Tetrahedron Lett. 2011, 52, 5149–5152.
2) Paumo, H.K.; Makhafola, T.J.; Mphahlele, M.J. Molecules 2016, 21, 1366–1383.
3) Mphahlele, M.J.; Paumo, H.K.; Choong, Y.S. Pharmaceuticals 2017, 10, 87–104.

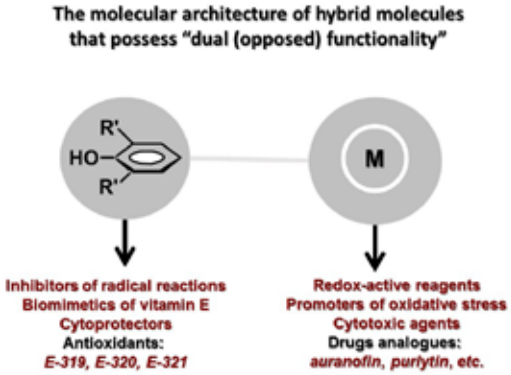
ORGANIC AND ORGANOMETALLIC DERIVATIVES OF α-TOCOPHEROL MIMETICS AS PROMISING CANDIDATES FOR SELECTIVE ANTICANCER AGENTS

Elena Milaeva, Dmitry Shpakovsky, Yulia Gracheva, Taisia Antonenko, Elena Kharitonashvili

Moscow State Lomonosov University, Department of Medicinal Chemistry & Fine Organic Synthesis, Lenin Hill 1-3, Moscow, 119991, Russia

The presentation will focus on a novel approach to design hybrid organic and organometallic physiollogically active compounds based on computer-aided design, new synthetic approaches and extensive biological screenings.

This study is focused on the construction of hybrid compounds with dual modes of action possessing 2,6-dialkylphenol group as a α-tocopherol mimetic and metal center. The hybrid compounds might show either prooxidative activity or antioxidative activity. The presence of metal atom allows extensive modification including coordination to the targeted specific groups to control and tune toxicity-activity profiles.



The synthesis and anti/prooxidant activity and cytotoxicity studies of novel hybrid compounds are presented and discussed.

The biological activity has been studied in *in vitro, ex vivo, in vivo* experiments in lipid peroxidation and mitochondria-associated processes, by using neurons, liver homogenates and in enzymatic reactions (*xanthine oxidase*, *lipoxygenase, glutathione reductase, thioredoxin reductase*).

Thus, we can conclude that the combination of two physiologically active moieties in a complex molecule is a promising approach to find the novel hybrid therapeutic agents with opposed biological mode of action.

References

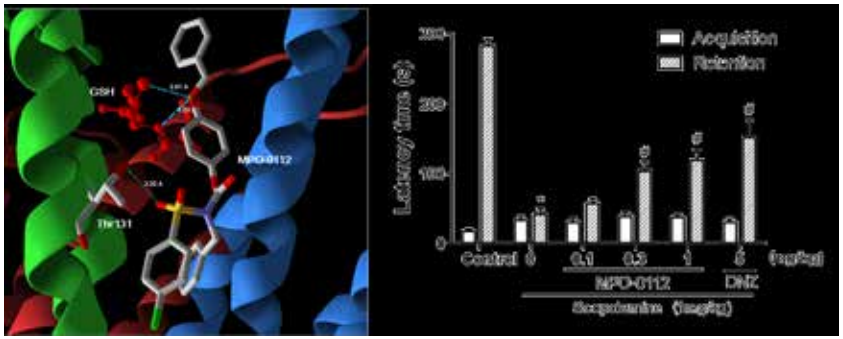
1) E.R. Milaeva & co-authors. Metallomics, 2018, 10, 406. Pure & Appl.Chem., 2017, 89, 1065. Polyhedron, 2017, 127, 512.Inorg. Chim.Acta, 2017, 455, 276. J. Organomet. Chem., 2017, 839, 60. Polyhedron, 2017, 127, 512. J. Catal., 2016, 341, 104. Bioorg.Med.Chem., 2016, 24, 712. J. Organomet. Chem., 2015, 782, 96. Dalton Trans., 2015, 44, 3614. Dalton Trans., 2014, 43, 6880. Dalton Trans., 2013, 42, 19, 6817. Med. Chem. Res., 2012, 21, 3523. Dalton Trans., 2012, 41, 14568. Curr. Topics Med. Chem., 2011, 11, 2703.

NOVEL PHENYLSULFONAMIDE DERIVATIVES AS INHIBITORS OF PGE2 SYNTHESIS AMELIORATE THE COGNITIVE IMPAIRMENT IN MICE INDUCED BY SCOPOLAMINE

Yoonhyoung Moon (1), Sun Young Kim (1), Hong Bin Yoon (1), Jae Kyun Lee (2), Kyung-Tae Lee (3), Jae Yeol Lee (1)

1) Research Institute for Basic Sciences and Department of Chemistry, College of Sciences, Kyung Hee University, Seoul 02447, Republic of Korea
2) Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Cheongyang, Seoul 02792, Republic of Korea
3) Department of Life and Nanopharmaceutical Science, Kyung Hee University, Seoul 02447, Republic of Korea

Our previous research showed that a novel series of phenylsulfonyl hydrazide derivatives reduced LPS-induced PGE_2 levels in RAW 264.7 macrophage cells via an inhibition of mPGES-1 enzyme. However, two regioisomers of phenylsulfonyl hydrazide exhibiting a wide range of biological activities were formed depending on the reaction conditions. In order to avoid this synthetic problem, a series of new benzenesulfonyl amides as analogues of phenylsulfonyl hydrazides was synthesized and biologically evaluated in vitro. As a result, **MPO-0112** strongly suppressed LPS-induced PGE_2 production (IC_{50}: 0.34 mM) with excellent selectivity over COX-enzymes (COX-1 and 2) and also inhibited mPGES-1 enzyme (IC_{50}: 7.37 mM) comparable to those of **MPO-0063**. According to the recent studies on the close correlation between up-regulation of mPGES-1 and Alzheimer's disease, we investigated whether **MPO-0112** can ameliorate scopolamine-induced memory impairment using the passive avoidance test. The memory impairment-ameliorating effect of **MPO-0112** (1.0 mg/kg, p.o.) was effective comparable to that of donepezil (5 mg/kg, p.o.) as a positive control. In addition, **MPO-0112** exhibited a favorable in vitro CYP profile, which is suggestive of no potential drug–drug interactions. Therefore, these overall results suggest that **MPO-0112** as selective mPGES-1 inhibitor may be a novel therapeutic agent for diseases associated with cognitive deficits, such as Alzheimer's disease.

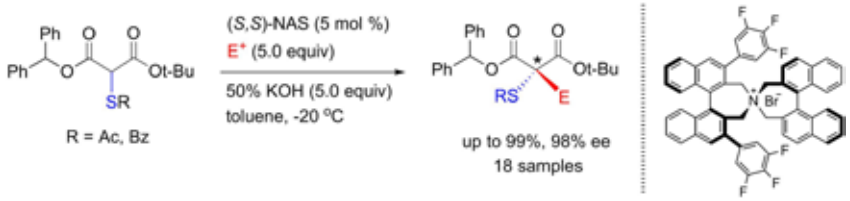


CONSTRUCTION OF CHIRAL α-THIO-QUATERNARY STEREOGENIC CENTERS VIA PHASE-TRANSFER CATALYZED ENANTIOSELECTIVE α-ALKYLATION OF α-ACYLTHIOMALONATES

Eunhye Park, Min Woo Ha, Byunggil Yang, Hyeung-geun Park

Research Institute of Pharmaceutical Sciences and College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Organosulfur compounds play important roles in biological system. Two of natural amino acids contain sulfur residues and their functional groups mediate various biological process. Also there are a lot of commercially available organosulfur compounds as best-selling drugs. As the need of variable sulfur containing compounds for the development of new drug is getting increased, the development of asymmetric synthetic methods of chiral organosulfur compounds with chirality residing at the carbon in connection with sulfur have been gradually important. So we planned to develop a new efficient method for the synthesis of chiral tertiary α-thio-malonates via the enantioselective phase-transfer catalytic α-alkylation. As a result, α-acylthiomalonates were developed as substrate and successfully applied to the phase-transfer catalytic α-alkylation in the presence of (*S*,*S*)-3,4,5-trifluorophenyl-NAS bromide to afford the corresponding α-acylthio-α-alkylmalonates in high chemical yields (up to 99%) and optical yields (up to 98% ee). In terms of diversity, the advantage of our α-alkylation method compared to the direct electrophilic α-sulfenylation is that a variety of chiral compounds can be easily prepared simply by changing the alkylating reagent in high enantioselectivity. To the best of our knowledge, this is the first report to accomplish enantioselective catalytic synthesis of quaternary α-thio-α-alkylmalonates.

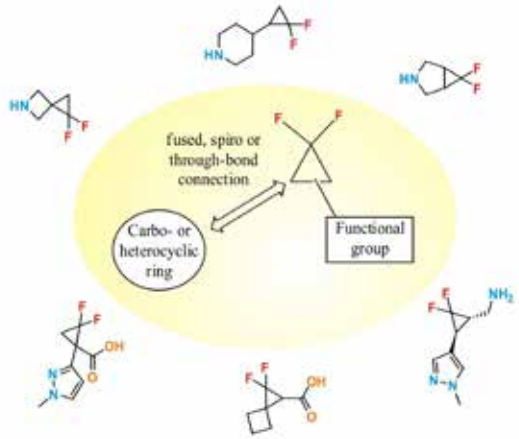


RUPPERT-PRAKASH REAGENT AS A DIFLUOROCARBENE SYNTHETIC EQUIVALENT FOR THE SYNTHESIS OF MEDCHEM-RELEVANT BUILDING BLOCKS

Pavel Nosik (1,2), Sergey Ryabukhin (1,2), Oleksandr Grygorenko (1,2), Dmitriy Volochnyuk (2,3)

1) National Taras Shevchenko University of Kyiv, Volodymyrska Street, 60, Kyiv 01601, Ukraine
2) Enamine Ltd, Chervonotkatska 78, Kyiv 02094, Ukraine
3) Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanska Street 5, Kyiv 02094, Ukraine

Difluorocyclopropanes have gained much attention as privileged structural motifs in medicinal chemistry since they combine two structural features widely recognized as attractive for early drug discovery, while fulfilling the recent lead-likeness criteria such as Ro2 for building blocks. Although numerous reagents for the construction of the difluorocyclopropane moiety were discovered in the last 50 years, until recently, these building blocks were considered as relatively hardly accessible. In 2011, Prakash, Olah and co-workers described the first use of the Ruppert–Prakash reagent (CF_3SiMe_3) – readily available and inexpensive starting material – for the difluorocyclopropanation of double bonds. In our work, we have expanded this methodology for the preparation of difluorocyclopropanes possessing functional groups suitable for the transformations most often used in medicinal chemistry (*i.e.* amines, carboxylic acids, ketones, hetaryl-substited compounds etc.). Moreover, we have established structure – reactivity relationship for the widest scope of substrates and showed that the process is governed mainly by electronic and to lesser extent – by steric factors, which can be explained by partially nonsynchronous transition state during addition of difluorocarbene (carbenoid) to the double bond [1]. Development of the slow addition protocol allowed extending applicability of the CF_3SiMe_3– NaI system towards substrates which have been previously referred to as unsuitable for this difluorocyclopropanation method (such as acrylic and cinnamic acids).



References

1) Nosik P. S. et al Adv. Synth. Catal. 2017, 359, 3126–3136

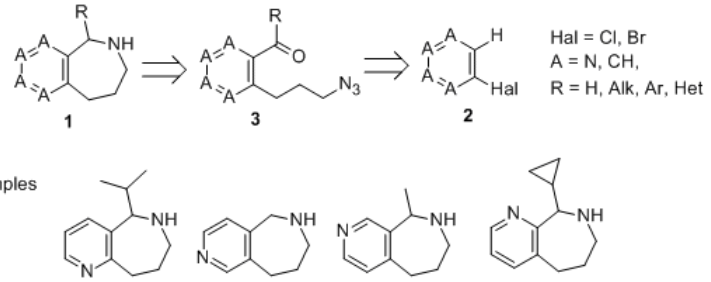
AN EFFICIENT APPROACH TO NOVEL TETRAHYDROPYRIDOAZEPINES. EXPANSION OF AZEPINES' DRUG-LIKE CHEMICAL SPACE.

Sergey Ryabukhin, Dmitriy Volochnyuk, Oleksandr Grygorenko, Andrii Subota

Enamine Ltd., Chervonotkatska Street 78, Kyiv 02094, Ukraine

The quest for lead-oriented synthesis proposed by medicinal chemistry in early 2010s have prompted for design and study of low-molecular-weight, hydrophilic, conformationally restricted and sp^3-enriched molecular scaffolds. Fused azepanes are promising chemotypes which comply with these criteria and in most cases possess sufficient novelty; moreover, azepane is in the top 100 most frequently used ring systems in small molecule drugs. 6,7,8,9-Tetrahydro-5*H*-pyrido[3,2-*c*]azepines (**1**), which contain fused azepane and pyridine rings, were evaluated as cannabinoid (CB2) receptor modulators (**2**), H_1-antihistamines (**3**), serotonin ($5HT_{2c}$) receptor agonists (**4**) and other biologically active compounds.

In this work, we report an alternative approach to novel and known, but hard-to-reach, tetrahydropyridoazepines of general formula **1**. Our methodology based on modification of chloro- or bromosubstituted pyridines **2**through appropriate ketoazides 3 to final azepines in 6-8 steps. (Scheme 1).



In conclusion, the developed methods for the preparation of tetrahydropyridoazepines included 6-8 steps and gave the title products in 7.7% overall yield and up to 10-20 g scales.

References

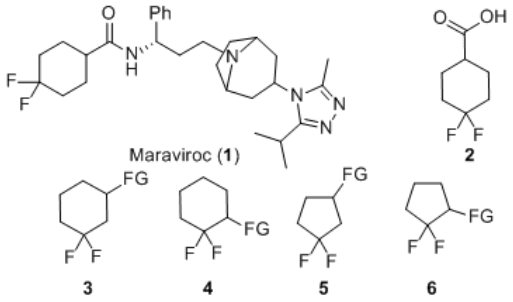
1) Subota, A. I.; Grygorenko, O. O.; Valter, Y. B.; Tairov, M. A.; Artamonov, O. S.; Volochnyuk, D. M.; Ryabukhin, S. V. Synlett 2015, 26, 408–411.
2) Subota, A. I.; Artamonov, O. S.; Gorlova A. O.; Volochnyuk, D. M.; Grygorenko, O. O. Tet. Lett. 2017, 58(20), 1989

SYNTHESIS OF GEM-DIFLUOROCYCLOPENTANE/HEXANE BUILDING BLOCKS – USEFUL REAGENT FOR DRUG DISCOVERY

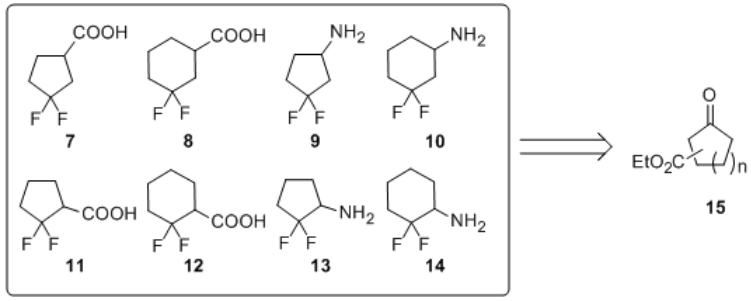
Sergey Ryabukhin, Dmitriy Volochnyuk, Oleksandr Grygorenko, Kostiantyn Melnykov, Dmitriy Sibgatulin

Enamine Ltd., Chervonotkatska Street 78, Kyiv 02094, Ukraine.

Fluorinated cycloalkane building blocks are important structural motifs which become increasingly important in various areas, and most of all in drug discovery and agrochemistry. One of the most prominent examples of this concept is related to development of Maraviroc (**1**), an antiretroviral drug approved by FDA in 2007. In this case, using 4,4-diflurocyclohexanecarboxylic acid (**2**) as a building block for the modification of optimized substance resulted in the compound with unique antiviral profile and lack of affinity for the hERG channel. It is not surprising therefore that compound **2** and other building blocks bearing 4,4-difluorocyclohexyl moiety were widely used in medicinal chemistry since then. On the contrary, isomeric and homologous *gem* -difluorocycloalkanes **3–6** were much less explored to date. Derivatives of these building blocks were evaluated as potent and selective cathepsin inhibitors, cholesteryl ester transfer protein (CETP) inhibitors, antibacterial agents, muscarinic M_3 receptor antagonists, bradykinin B_1 receptor antagonists, and opioid receptor-like 1 (ORL1) antagonists.



We describe a practical approach to *gem*-difluorocyclopentane/hexane building blocks **7–14** starting from appropriate ketoesters type **15** in 10-100g scale.



References

1) Melnykov, K.P.; Nosik, P.S.; Kurpil, B.B.; Sibgatulin, D.A.; Volochnyuk, D.M.; Ryabukhin, S.V.; Grygorenko, O.O. J. Fluor. Chem., 2017, 199, 60-66.

GOLD(I)-CATALYZED SYNTHESIS OF 4-ISOXAZOLINES AND ITS SYNTHETIC APPLICATION

Jae-Sang Ryu, Da Mi Kim

College of Pharmacy & Graduate School of Pharmaceutical Sciences,
Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul, 03760, Republic of Korea

4-Isoxazoline[1] (2,3-dihydroisoxazole) is one of the most versatile synthetic intermediates for the preparation of interesting natural products and pharmaceuticals, and is also frequently found in various biologically active compounds.[2] Despite its importance in chemistry and biology, only a few methods for the synthesis of 4-isoxazoline have been developed so far, and there still remains a need for novel efficient synthetic routes for the preparation of highly functionalized diverse 4-isoxazolines with high regioselectivity under mild conditions. In this presentation, we will discuss catalytic intramolecular cyclizations of propargylic *N*-hydroxylamines leading to 4-isoxazolines. The reactions proceed at room temperature in the presence of 5 mol% $(PPh_3)AuCl$ /AgOTf or in 5 mol% $(PPh_3)AuNTf_2$, and rapidly afford 4-isoxazoline derivatives in good to excellent yields.[3] This method was successfully applied to the stereoselective synthesis of a ceramide transporter protein (CERT)-dependent ceramide-trafficking inhibitor, (1*R*,3*S*)-HPA-12 (**Figure 1**).[4]

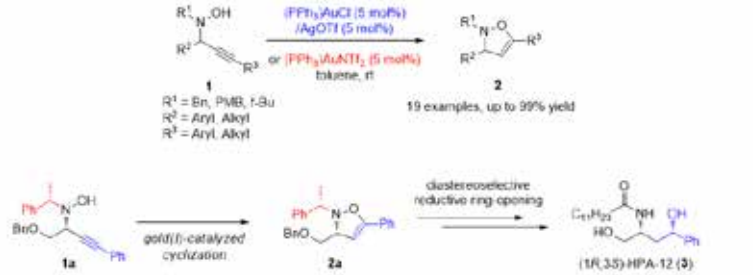


Figure 1. Au(I)-Catalyzed synthesis of 4-isoxazolines and its synthetic application.

References

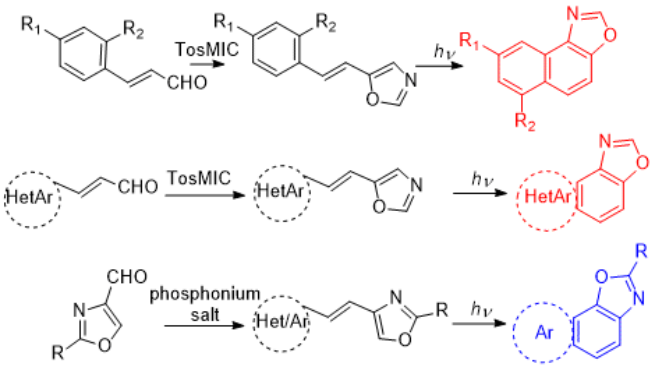
1) (a) T. M. V. D. Pinho e Melo, Eur. J. Org. Chem. 2010, 3363–3376; (b) J. P. Freeman, Chem. Rev. 1983, 83, 241–261.
2) P. N. Carlsen; T. J. Mann; A. H. Hoveyda; A. J. Frontier, Angew. Chem., Int. Ed. 2014, 53, 9334–9338.
3) B. Chandrasekhar; S. Ahn; J.-S. Ryu, J. Org. Chem. 2016, 81, 6740–6749.
4) B. Chandrasekhar; S. Ahn; J.-S. Ryu, Synthesis 2017, 49, 1569–1574.

BIOLOGICAL, ANTIOXIDANT AND CHOLINESTERASE INHIBITION ACTIVITY STUDIES ON NAPHTHO[1,2-d]/[2,1-d]OXAZOLES

Ivana Šagud, Irena Škorić

Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

Numerous 4/5-styryloxazoles were prepared and transformed into [1,2/2,1-*d*]-naphtooxazoles [1,2] by reaction of photochemical cyclization. Naphtooxazoles are hard to synthesize by ground chemistry methods and we have described a first photochemical synthesis of these compounds [1].



All of the precursors as well as the final products are described by spectroscopic methods. After these compounds were synthesized they were further tested for biological activity, antioxidant properties [2] as well as metabolic stability [3]. Results of the bioassays as well the results for the antioxidant properties will be presented on the poster. They also proved to be potent acetyl- and butyryl-cholinesterase inhibitors and these results will also be given and interpreted on the poster.

References

1) I. Šagud, F. Faraguna, Ž. Marinić, M. Šindler-Kulyk, J. Org. Chem. 76 (2011) 2904-2908.
2) Unpublished results
3) Poster presentation from Vanja Kelava at 5th EFMC Young Medicinal Chemist Symposium (EFMC-YMCS 2018)

DESIGN AND SYNTHESIS OF POTENTIAL ALLOSTERIC INHIBITORS OF TISSUE TRANSGLUTAMINASE

Ngoc Thi Anh Nguyen, Daniel Rathbone

School of Life & Health Sciences, Aston University, Birmingham, UK, B4 7ET

Tissue transglutaminase (TG2), the most ubiquitous member among the human transglutaminase enzyme family, is responsible for various modifications to proteins, particularly cross-linking of proteins into large molecular weight polymers that are more resistant to degradation. Under stress, adventitious cross-linking due to overexpression of TG2 has been implicated in numerous diseases such as celiac disease, fibrosis, neurodegenerative disorders and cancer. Therefore, TG2 is an ideal target for the development of potent, selective inhibitors with acceptable toxicity. Among TG2 inhibitors, irreversible inhibitors have been the most widely developed but their further progress in clinical trials is prevented due to potential toxicity. Our research has focused on the development of a new series of allosteric TG2 inhibitors containing no reactive functionality based on the structure of a lead allosteric inhibitor LDN-27219 [1, 2]. Computational modelling techniques including protein-ligand docking and molecular dynamic simulations have been used to identify the presumed allosteric site and then to design small molecules with optimal fit into the site. A series of potential allosteric inhibitors have been successfully synthesised, characterised and is about to be screened in vitro against TG2.

References

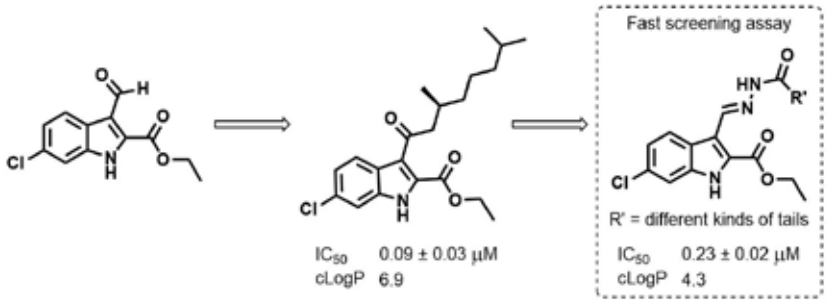
1) Duval E, Case A, Stein RL, Cuny GD. Structure-activity relationship study of novel tissue transglutaminase inhibitors. Bioorg Med Chem Lett. 2005;15(7):1885-9.
2) Case A, Stein RL. Kinetic analysis of the interaction of tissue transglutaminase with a nonpeptidic slow-binding inhibitor. Biochemistry. 2007;46(4):1106-15.

ACYLHYDRAZONES AS POTENT INHIBITORS OF HUMAN 15-LIPOXYGENASE-1

Ramon van der Vlag (1), Hao Guo (2), Nikolaos Eleftheriadis (2), Vladislav V. Gopko (1), Leticia Monjas (1), Frank J. Dekker (2), Anna K. H. Hirsch (1,3)

1) Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
2) Department of Pharmaceutical Gene Modulation, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands
3) Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Department of Drug Design and Optimization, Campus Building E8, 166123 Saarbrücken, Germany

Human 15-lipoxygenase-1 (15-LOX-1) is implicated in several inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD), asthma and chronic bronchitis as well as in various CNS diseases, such as Parkinson's and Alzheimer's disease.[1] In a previous study, a substituted indole emerged as a potent inhibitor, which is hampered by its lipophilicity.[2] To address this issue, we used an acylhydrazone-based combinatorial library in order to screen a variety of hydrazides in combination with the initial indole aldehyde hit. The acylhydrazone products are potent 15-LOX-1 inhibitors and are predicted to have less lipophilic character. Screening of such a combinatorial library with a sensitive assay represents a powerful tool to identify new inhibitors for unstable and/or precious protein targets.



References

1) Eleftheriadis, N.; Thee, S. A.; Zwinderman, M. R. H.; Leus, N. G. J.; Dekker, F. J. Activity-Based Probes for 15-Lipoxygenase-1. Angew. Chem. Int. Ed. 2016, 55, 12300–12305
2) Eleftheriadis, N.; Neochoritis, C. G.; Leus, N. G. J.; van der Wouden, P. E.; Dömling, A.; Dekker, F. J. Rational Development of a Potent 15-Lipoxygenase-1 Inhibitor with in Vitro and Ex Vivo Anti-Inflammatory Properties. J. Med. Chem. 2015, 58, 7850–7862

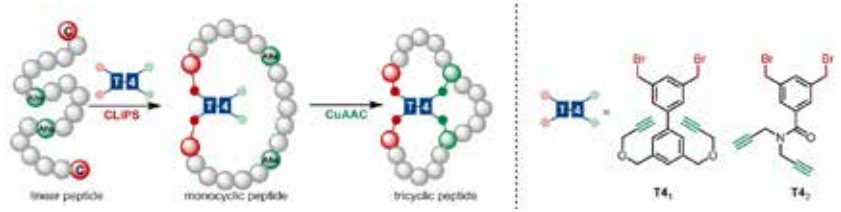
TRICYCLIC PEPTIDES VIA TEMPLATED TANDEM CLIPS/CUAAC CYCLIZATIONS

G.J.J. Richelle (1,2), H. Hiemstra (2), J.H. van Maarseveen (2), P. Timmerman (1,2)

1) Pepscan Therapeutics, Lelystad, The Netherlands
2) Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, The Netherlands

Multicyclic peptides provide a very attractive molecular format for the design of novel therapeutics.[1] Therefore, novel routes for synthesis and HTS-screening of this fascinating class of compounds are desperately needed. A decade ago, we launched a novel one-pot scaffold-assisted peptide-cyclization technology platform, termed "CLIPS", to generate a new class of bicyclic peptides[2] able to act as potent inhibitors of hitherto undruggable therapeutics targets.[3]

Following this, we now present a next-generation technology that combines both the CLIPS and CuAAC technology in order to create a novel class of isomerically pure tricyclic peptides.[4] We present four different CLIPS/CuAAC scaffolds and show their behaviour in the one-pot synthesis of tricyclic peptides.



Scheme 1: Tandem CLIPS/CuAAC reactions of a linear peptide onto T4 scaffolds to generate tricyclic peptides

References

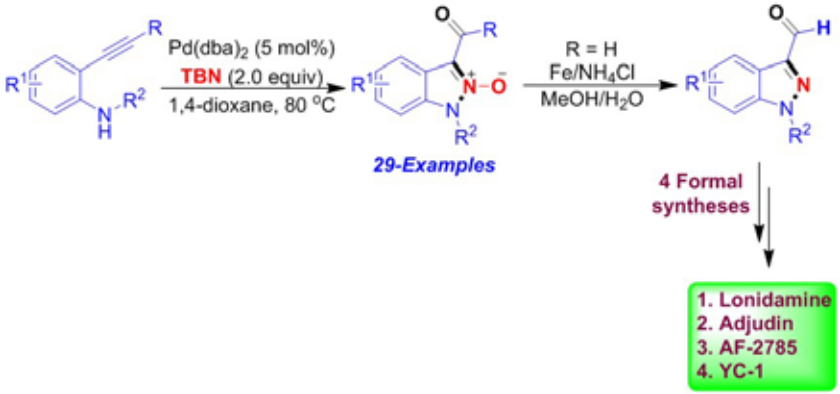
1) D. J. Craik, D. P. Fairlie, S. Liras, D. Price, Chem. Biol. Drug Des. 2013, 81, 136–147.
2) P. Timmerman, J. Beld, W. C. Puijk, R. H. Meloen, ChemBioChem 2005, 6, 821–824.
3) C. Heinis, T. Rutherford, S. Freund, G. Winter, Nat. Chem. Biol. 2009, 5, 502–507.
4) G. J. J. Richelle, S. Ori, H. Hiemstra, J. H. van Maarseveen, P. Timmerman, Angew. Chem. Int. Ed. 2018, 57, 501–505

BIS(DIBENZYLIDDEMEACETONE)PALLADIUM(0)/TERT-BUTYL NITRITE-CATALYZED CYCLIZATION OF O-ALKYNYLANILINES WITH TERT-BUTYL NITRITE: SYNTHESIS AND APPLICATIONS OF INDAZOLE-2-OXIDES

Gopal Chandru Senadi, Jeh-Jeng Wang

Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

Azaheterocyclic compounds have widespread applications in biology, chemistry and materials science. In particular, *N*-oxides of these molecules are ubiquitous structural motifs found in alkaloids and bioactive compounds and have several chemical applications. The classical approach to synthesize *N*-oxides is from the corresponding azaheterocyclic molecules with oxidants, such as peroxy acids. However, this method has certain limitations, such as the unselective over-oxidation of other *N*-atoms present in the molecules, the excessive amount of strong oxidant needed and the requirement of previously prepared parent heterocycles. Herein, we present an efficient method for the synthesis of 1-benzyl/arylindazole 2-oxides via a bis(dibenzylideneacetone) palladium(0) [Pd(dba)$_2$]/*tert*-butyl nitrite (TBN)-catalyzed reaction of *o*-alkynylaniline derivatives with TBN. The overall transformation involves the formation of three new bonds via *N*-nitrosation (N–NO), 5-*exo*-dig cyclization (C– N) and oxidation (C=O). The notable features are the mild reaction conditions, broad substrate scope and dual role of TBN as an NO source and redox co-catalyst. This strategy was implemented for the synthesis of indazole-3-carbaldehyde derivatives and the formal syntheses of pharmaceutically active YC-1, an anticancer agent (lonidamine), and the male contraceptive experimental drugs AF2785 and adjudin (AF-2364).[1]



References

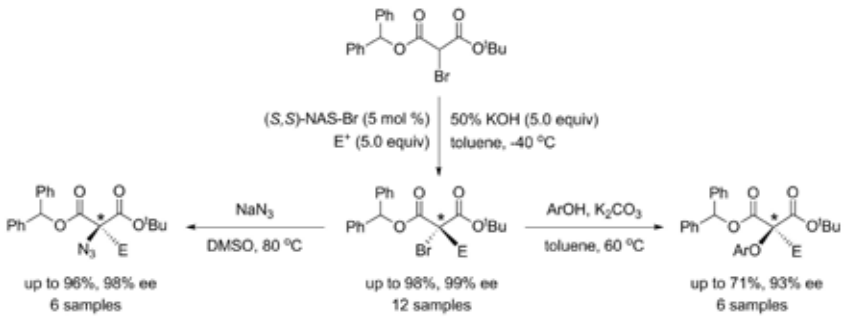
1) Senadi, G. C.; Wang, J. –Q.; Gore, B. S.; Wang, J. –J. Adv. Synth. Catal. 2018, 360, 491-501.

ENANTIOSELECTIVE SYNTHESIS OF CHIRAL α-AZIDO-α-ALKYLMALONATE AND α-ARYLOXY-α-ALKYLMALONATE VIA PHASE-TRANSFER CATALYZED α-ALKYLATION OF α-BROMOMALONATES, FOLLOWED BY SN2 SUBSTITUTION

Byunggil Yang, Doyoung Kim, Eunhye Park, Hyeung-geun Park

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

The synthesis of optically active nitrogen- or oxygen-containing organic compounds is very important in medicinal chemistry due to their versatile biological activities and pharmaceutical applications. The enantioselective synthetic methods for the α-amino-α-alkylmalonates and α-hydroxy-α-alkylmalonates have not been extensively studied. So we planned to develop a new method for the synthesis of chiral tertiary α-azido-malonates and α-aryloxy-malonates via the enantioselective phase-transfer catalytic α-alkylation. As a result, an efficient enantioselective synthetic methods for α-azido-α-alkylmalonates and α-aryloxy-α-alkylmalonates were successfully developed via the S_N2 substitution of azide and aryloxide to chiral α-bromo-α-alkylmalonates, prepared by asymmetric phase-transfer catalytic α-alkylation of diphenylmethyl tert-butyl α-bromomalonate. The α-alkylation of diphenylmethyl tert-butyl α-bromomalonate under phase-transfer catalytic conditions (50% KOH, toluene, –40 °C) in the presence of (*S*,*S*)-3,4,5-trifluorophenyl-NAS bromide afforded the corresponding α-bromo-α-alkylmalonates in high chemical yields (up to 99%) and high optical yields (up to 97% ee), which could be readily converted to α-azido-α-alkylmalonates (up to 99%, 98% ee) and α-aryloxy-α-alkylmalonates (up to 71%, 93% ee) by S_N2 substitution with sodium azide and aryloxides, respectively. The synthetic potential of this methodology was demonstrated via the synthesis of various versatile chiral intermediates.



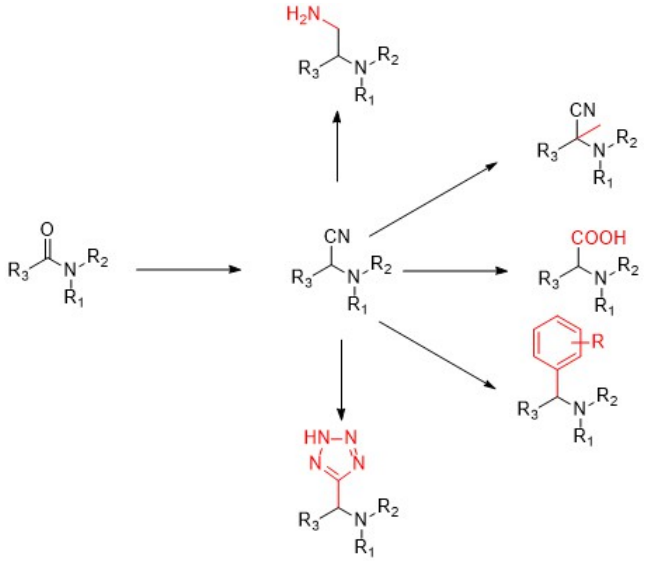
SYNTHESIS OF NEW STRAINED BUILDING BLOCKS VIA IRIDIUM CATALYSIS

Philippe Steinsoult (1), Patrick Wagner (1), Clement Janin (2), Thomas Fessard (2), Martine Schmitt (1), Christophe Salome (2)

1) Laboratoire d'Innovation Thérapeutique , UMR7200 CNRS-UdS, 74 route du Rhin, 67401 Illkirch, France
2) SpiroChem AG, Rosental area, Mattenstrasse 24, 4058 Basel, Switzerland

The synthesis of new building blocks is witnessing significant prominence in medicinal chemistry discovery programs and allows to anticipate the needs in the term of the crafting of those building blocks. The needs of new 3-D structure derivatives are always very important and development of new strategies is always highly researched.

Recently, Dixon *et al* described the use of an iridium catalyst (Vaska's catalyst) to introduce a cyanide group instead of the carbonyl group of amide leading, in 1 step, to an a-aminonitrile.[1] This moiety is very interesting in term of scope since it could lead to further derivatizations.



Scheme 1. Iridium-Catalyzed Reductive Strecker Reaction Amide and potential derivatizations.

After optimization of the protocol, the α-amino nitriles were successfully obtained on strained cycles (bicycloalcane) and spirocycles. The protocol is simple to perform and broad in scope.

References

1) Angel L. Fuentes de Arriba , Elena Lenci , Mahendra Sonawane, Odilon Formery, and Darren J. Dixon Angew. Chem. Int. Ed. 2017, 56, 1 – 6.

POSTERS - TECHNOLOGIES

Recent Developments in Nucleoside Medicinal Chemistry

MONOFUNCTIONAL 3-CHLOROPIPERIDINES TARGETING GUANOSINE RESIDUES: SYNTHESIS, ADDUCTS FORMATION AND CYTOTOXIC PROPERTIES

Caterina Carraro (1), Alexander Francke (2), Franziska Kohl (1), Alice Sosic (1), Valentina Gandin (1), Richard Goettlich (2), Barbara Gatto (1)

1) Dipartimento di Scienze del farmaco, Università degli Studi di Padova, Padova, Italy
2) Institut für Organische Chemie, Justus Liebig Universität, Gießen, Germany

For the hardly predictable mechanisms of tumor onset and the extremely variegated responses to state-of-art treatments, cancer represents one of the most arduous and challenging diseases of our time. Despite the successes of targeted therapy in treating cancer, the high cost of treatment with the new biologics are a limit to their use, both in the developed and developing countries, and alternative and inexpensive drugs are needed. In this direction, our studies started focusing on the synthesis and development of a new class of piperidine-based analogues of nitrogen mustards formally derived from the antibiotic and antineoplastic compound 593A. A first generation of bis-alkylating derivatives demonstrated the improved DNA alkylating properties of these compounds compared to the well-known nitrogen mustard chlorambucil, currently used in therapy [1-3].

With the aim to explore the chemical space of these alkylators, a new set of monofunctional chloropiperidines has been synthesized through a fast and affordable route providing high yields and purity. These molecules, whose common structure is shown in Figure 1, are characterized by a common chloropiperidine ring with alternative substituents at the nitrogen atom (aliphatic or aromatic) and possible endocyclic methylation. We investigated the mechanism and potency of DNA alkylation *in vitro* through electrophoretic techniques; additional experiments were performed to dissect the kinetics of reactions and to characterize the formation of adducts with specific DNA bases (Gs). Moreover, compounds were also tested for their interference with the activity of the human Topoisomerase II. Molecules were finally tested *ex vivo* on cancer cell lines in order to determine their cytotoxicity profile and to analyze the effect on the genomic DNA material. Results showed that nanomolar concentrations of these new monofunctional agents exhibit a very promising cytotoxic effect on a panel of tumor cell lines.

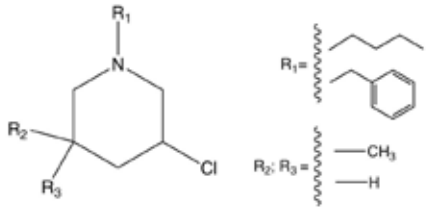


Figure 1. Chemical structure of the analyzed monofunctional chloropiperidines.

References

1) Zuravka, I., et al., Synthesis and DNA cleavage activity of Bis-3-chloropiperidines as alkylating agents. ChemMedChem, 2014. 9(9): p. 2178-85.
2) Zuravka, I., et al., Bis-3-chloropiperidines containing bridging lysine linkers: Influence of side chain structure on DNA alkylating activity. Bioorg Med Chem, 2015. 23(6): p. 1241-50.
3) Sosic, A., et al., Direct and Topoisomerase II Mediated DNA Damage by Bis-3-chloropiperidines: The Importance of Being an Earnest G. ChemMedChem, 2017.

INHIBITORS OF HUMAN ST8SIA AS NOVEL ANTI-METASTATIC AGENTS

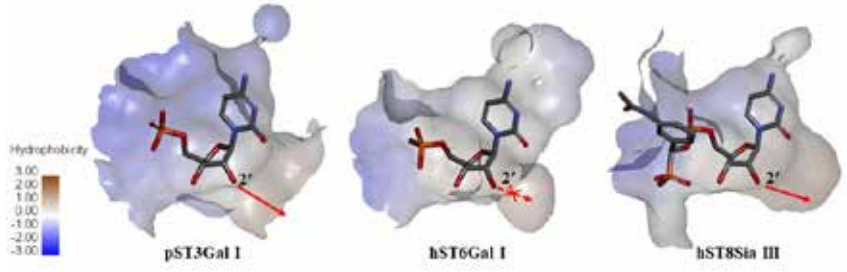
Christopher Dobie (1), Andrew Montgomery (1), Rémi Szabo (1), Rita Gerardy-Schahn (2), Susan Bellis (3), Haibo Yu (1,4), Danielle Skropeta (1,4,5)

1) University of Wollongong, Wollongong, Australia
2) Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany
3) Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, USA
4) Centre for Medical & Molecular Bioscience, University of Wollongong, Wollongong, Australia
5) Illawarra Health & Medical Research Institute, University of Wollongong, Wollongong, Australia

Upregulation of sialyltransferases (STs), the enzymes responsible for the addition of sialic acid to growing glycoconjugate chains, and the resultant hypersialylation of tumour cell surfaces is an established hallmark of many cancers including lung, breast, ovarian, pancreatic and prostate cancer [1]. The critical role of ST enzymes in tumour cell growth and metastasis, as well as links to multi-drug and radiation resistance, have seen STs emerge as a target for potential antimetastatic cancer treatments. There is also evidence showing down-regulation of some STs in neurological disorders such as Alzheimer's, schizophrenia, autism, and others – highlighting the need for selective inhibition. While multiple examples of potent ST inhibitors are seen in the literature, several challenges remain before they can proceed to the clinic including improving potency and selectivity, as well as addressing pharmacokinetic issues and synthetic accessibility. Herein, we present computational and synthetic studies towards a new generation of ST inhibitors, based on 1,2,3-triazole-linked compounds.

Computational modelling has also been undertaken using available structures of human STs to gauge potential selectivity for ST8 (altered expression in melanoma and prostate cancer, as well as in neurological disorders) over other ST3 and ST6 subtypes. These studied have revealed structural differences between substrate binding sites in ST subtypes whereupon variation of the nucleoside fragment could enhance selectivity [2].

To synthesise 1,2,3-triazole-linked inhibitors, a click reaction between an α-azidophosphonate and 5′-alkynyl uridine was utilised. Biological testing has been undertaken against various human ST subtypes with promising data observed. Results of the computational modelling, synthesis and biological evaluation of these novel ST inhibitors as potential anti-metastatic agents will be presented.



References

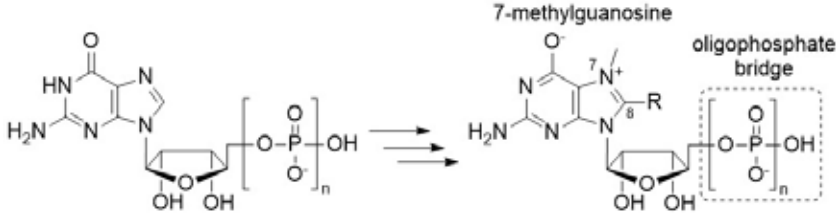
1) Szabo, R.; Skropeta, D., Advancement of Sialyltransferase Inhibitors: Therapeutic Challenges and Opportunities. Med Res Rev 2017, 37, 219-270 DOI: 10.1002/med.21407.
2) Dobie, C.; Montgomery, A. P.; Szabo, R.; Skropeta, D.; Yu, H., Computer-aided design of human sialyltransferase inhibitors of hST8Sia III. J Mol Recognit 2018, 31 (2), e2684-n/a DOI: 10.1002/jmr.2684.

SYNTHESIS AND CHARACTERISATION OF 7-METHYLGUANOSINE OLIGOPHOSPHATE ANALOGS MODIFIED IN C8 POSITION – POTENTIAL INHIBITORS OF PROTEINS INVOLVED IN MESSENGER RNA METABOLISM

Adam Mamot (1), Marcelina Bednarczyk (1,2), Mateusz Kozarski (1,2), Dorota Kubacka (2), Blazej Wojtczak (1), Joanna Kowalska (2), Jacek Jemielity (1)

1) University of Warsaw, Centre of New Technologies, Laboratory of Bioorganic Chemistry, Banacha 2c, 02-097 Warsaw (Poland)
2) University of Warsaw, Faculty of Physics, Institute of Experimental Physics, Division of Biophysics, Pasteura 5, 02-093 Warsaw (Poland)

The structure of 7-methylguanosine takes part in several different metabolic processes, that are crucial for functioning of eukaryotic cells. Due to its biological importance, several analogs od 7-methylguanosine are found in biological, biochemical and medicinal applications.[1,2] The aim of our work was to create selective inhibitors of different 7-methylguanosine-dependent processes. Proteins, that recognize 7-metylguanosine moiety share several similarities in mechanism of substrate binding. On one hand, this allows for an easy identification of structural features, that facilitate interactions in the binding sites.[3] On the other hand, those similarities leave little place for modifications, that would allow for a very selective inhibition. We developed a synthetic strategy, involving Suzuki cross-coupling reaction, that allowed for a quick and efficient synthesis of a library of 7-methylguanosine oligophosphates analogs modified in C8 position. Next, we performed screening studies of our compounds with selected proteins, that take part in mRNA metabolism: eIF4E, DcpS and cNIIIB.[4,5] Our studies show, that tested proteins exhibit different tolerance towards some of our compounds. The results suggest, that applied methodology can lead to a better understanding of the mechanisms of 7-methylguanosine binding and compounds with a therapeutic potential.



This project was financed by Foundation for Polish Science (TEAM/2016-2/13).

References

1) M. Ziemniak et al. Future Med. Chem. 2013, 5(10), 1141
2) A. Mamot et al., Angew. Chem. Int. Ed. 2017, 56, 15628
3) M. Warminski, P. J. Sikorski et al., Top. Curr. Chem. 2017, 375:16
4) M. Kozarski et al. Bioorg. Med. Chem. 2018, 26(1), 191
5) R. Kasprzyk et al. Org. & Biomol. Chem. 2016, 14, 3863

CYTOKININ NUCLEOSIDES AS SELECTIVE INHIBITORS OF HUMAN ENTEROVIRUS 71 REPLICATION

Vladimir Oslovsky (1), Mikhail Drenichev (1), Nikolay Kurochkin (1), Liang Sun (2), Carmen Mirabelli (2), Johan Neyts (2), Pieter Leyssen (2), Sergey Mikhailov (1)

1) Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str. 32, 119991 Moscow, Russian Federation
2) KU Leuven - University of Leuven, Department of Microbiology and Immunology, Laboratory for Virology and Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium

N^6-Substituted adenosines (cytokinin nucleosides) are an important group of biologically active natural compounds with broad spectrum of biological activities such as cytokinin, anticancer, antiviral, antiprotozoal and some others [1]. Recently, we demonstrated that naturally occurring plant cytokinin nucleosides 6-benzylaminopurine riboside (BAPR) and N^6-isopentenyladenosine exhibited potent antiviral effect on human enterovirus EV71, but were rather cytotoxic [2,3]. Thus, we selected BAPR as a promising compound for further optimization to identify more potent and selective compounds. We demonstrated that a number of BAPR analogs with different structure of the linker between the amino group of adenine heterocycle and the phenyl ring exhibited a pronounced antienteroviral activity [3]. The SAR study clearly showed that the antiviral activity is greatly dependent on the size of the linker and that a linker with a length of 2 or 3 carbon atoms provides the most potent activity. Furthermore, the compounds with double and triple bonds in the linker structure have better selectivity [3]. The compounds were prepared using the recently worked-out methodology for regioselective alkylation of N^6-acetyl-2′,3′,5′-tri-O-acetyladenosine with alcohols under Mitsunobu reaction conditions or with alkyl halides promoted by a base. The removal of acetyl groups with 4M $PrNH_2$ in MeOH affords the desired nucleosides in good overall yields after chromatographic purification [2,3].

Modification of the phenyl ring in BAPR structure is another perspective approach for the optimization. Therefore, a series of BAPR analogs with different substituents at the phenyl ring has been obtained [4]. The traditional approach for the preparation of N^6-alkylated or N^6-arylated adenosines is the substitution of the chlorine atom in commercially available 6-chloropurine riboside with alkyl- or arylamines. To simplify the separation procedure, we have used 2′,3′,5′-tri-*O*-isobutyroyl-6-chloropurine riboside directly in the substitution reactions as an initial synthon [4]. Our SAR study clearly shows that the presence of small substituents at phenyl ring of BAPR significantly increases antiviral effect. Monofluorination of phenyl ring leads to the high cell toxicity. Interestingly, the incorporation of a second fluorine atom resulted in a significant improvement of selectivity. Moreover, N^6-trifluoromethylbenzyladenosines exhibited also high antiviral activity with low cytotoxicity. As a result, the lead compound containing trifluoromethyl group at position 3 of the phenyl ring exhibited selectivity index 230 times higher than that in BAPR [4].

Thus, we have modified the structure of the natural cytokinin nucleoside BAPR to obtain a number of compounds with high antiviral activity on the human enterovirus EV71 and low cell toxicity.

This work was supported by Russian Foundation for Basic Research (grant No 16-04-01594).

References

1) M.S. Drenichev, V.E. Oslovsky, S.N. Mikhailov. Curr. Top. Med. Chem. 2016, 16, 2562–2576.
2) V.I. Tararov, A. Tijsma, S.V. Kolyachkina, V.E. Oslovsky, J. Neyts, M.S. Drenichev, P. Leyssen, S.N. Mikhailov. Eur. J. Med. Chem. 2015, 90, 406–413
3) M.S. Drenichev, V.E. Oslovsky, L. Sun, A. Tijsma, N.N. Kurochkin, V.I. Tararov, A.O. Chizhov, J. Neyts, C. Pannecouque, P. Leyssen, S.N. Mikhailov. Eur. J. Med. Chem. 2016, 111, 84–94
4) V.E. Oslovsky, M.S. Drenichev, L. Sun, N.N. Kurochkin, V.E. Kunetsky, C. Mirabelli, J. Neyts, P. Leyssen, S.N. Mikhailov. Molecules. 2017, 22, 1219

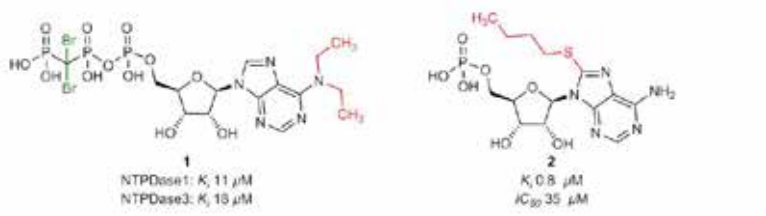
SYNTHESIS OF NUCLEOTIDE DERIVATIVES AS POTENT AND SELECTIVE NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE 1 (CD39) INHIBITORS

Constanze Schmies, Xihuan Luo, Sang-Yong Lee, Ali El-Tayeb, Christa E. Müller

Pharmaceutical Chemistry I, Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

Nucleotides such as adenosine triphosphate (ATP), and nucleosides, in particular adenosine, are not only utilized as intracellular building blocks or as a source of energy (ATP), but also have important functions as extracellular signalling molecules. ATP may be envisaged as a danger signal, and upon its release, various signalling pathways can be induced leading to platelet aggregation and proinflammatory effects.[1,2] In order to terminate nucleotide signalling, the released ATP is hydrolyzed yielding the anti-inflammatory and immunosuppressive nucleoside adenosine.[1] This is achieved by *ecto*-nucleotidases, which are located in the cell membrane with an extracellular catalytic site.[1-3] Nucleoside triphosphate diphosphohydrolase1 (NTPDase1, CD39) and nucleotide pyrophosphatase/phosphodiesterase1 (NPP1) are the main enzymes that convert ATP to AMP, which is subsequently dephosphorylated by *ecto*-5'-nucleotidase (e5NT, CD73) yielding adenosine.[3] NTPDase1 is mainly expressed on endothelial and immune cells, and its expression is upregulated, together with that of CD73, in inflamed tissues and on cancer cells. The production of immunosuppressive, tumour growth-stimulatory and angiogenic adenosine contributes to the immune escape of cancer cells.[2,3] Due to their pathophysiological roles, NTPDases represent potential drug targets that require further validation. For this purpose, potent, selective and metabolically stable antagonists need to be identified, which is the main goal of this project.

In the literature, several NTPDase1 inhibitors have already been described. These are, however, only weakly potent or non-selective. Therefore, based on the structures of two known nucleotide-based inhibitors, ARL-67156 (**1**)[4] and 8-butylthio-AMP (8-BuS-AMP, **2**)[5], new compounds were designed and synthesized in order to study their structure-activity relationships as NTPDase1 inhibitors, and to improve their potency and selectivity.



References

1) Burnstock, G. Physiology and pathophysiology of purinergic neurotransmission. Physiol. Rev. 2007, 87, 659-797.
2) Vitiello, L.; Gorini, S.; Rosano, G.; la Sala, A. Immunoregulation through extracellular nucleotides. Blood 2012, 120, 511-518.
3) Zimmermann, H.; Zebisch, M.; Sträter, N. Structure of Ecto-nucleotidases. Purineric. Signal. 2012, 8, 437-502.
4) Lévesque, S.A.; Lavoie, E.G.; Lecka, J.; Bigonnesse, F.; Sévigny, J. Specificity of the ecto-ATPase inhibitor ARL 67156 on human and mouse ectonucleotidases. Brit. J. Pharmacol. 2007, 152, 141-150.
5) Lecka, J.; Gillerman, I.; Fausther, M.; Salem, M.; Munkonda, M.N.; Brosseau, J.P.; Cadot, C.; Martín-Satué, M.; D'Orléans-Juste, P.; Rousseau, E.; Poirier, D.; Künzli, B.; Fischer, B.; Sévigny, J. 8-BuS-ATP derivatives as specific NTPDase1 inhibitors. Brit. J. Pharmacol. 2013, 169, 179-196.

THIENO-FUSED 7-DEAZAPURINE RIBONUCLEOSIDES: SYNTHESIS AND BIOLOGICAL ACTIVITIES

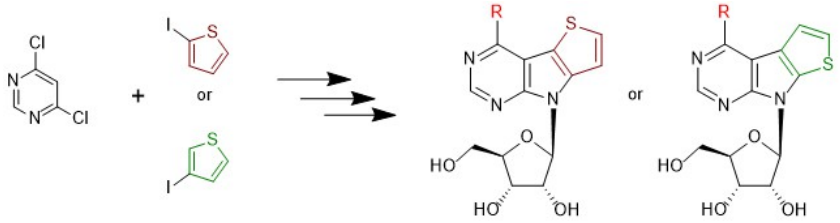
Michal Tichý (1), Michal Hocek (1,2)

1) Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Gilead Sciences & IOCB Research Center, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic
2) Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12843 Prague 2, Czech Republic

Recent discoveries of new classes of highly potent and selective deazapurine nucleoside cytostatics (6-hetaryl-7-deazapurine ribonucleosides[1], 7-hetaryl-7-deazaadenosines[2] or 7-hetaryl-7-deazapurine nucleosides bearing methoxy, methyl or methylsulfanyl groups in position 6[3]) indicate that there is a space for modification in the „major groove" part of the molecule. These results inspired us to design and synthesis of (het)aryl-fused 7-deazapurine ribonucleosides. First class of such tricyclic nucleosides, pyrimidoindole nucleosides, was not cytotoxic, however, few derivatives showed interesting antiviral (especially anti-dengue) activities.[4,5] In order to expand the space of such fused deazapurines and to investigate the effect of the size of the fused ring on biological activities, we designed and synthesized two series of isomeric thieno-fused 7-deazapurine ribonucleosides bearing various groups in position 4 of the pyrimidine ring.

The target nucleosides were synthesized in 5–6 steps starting from simple 4,6-dichloropyrimidine and 2- or 3-iodothiophene by a sequence involving Negishi coupling, nucleophilic azidation, cyclization of tetrazoles, glycosylation and Suzuki or Stille coupling or nucleophilic substitution.

Two series of the final nucleosides were synthesized and tested for cytostatic and antiviral activities.[6] Several compounds from both series (especially methyl, methoxy and methylsulfanyl derivatives) exerted sub-micromolar cytostatic activities against broad panel of leukemia and cancer cell lines with lower toxicity to normal fibroblasts. Detailed synthesis, biological activities as well as results from investigation of mechanism of action will be discussed on the poster.



Acknowledgment: This work was supported by the Academy of Sciences of the Czech Republic (RVO 61388963 and the Praemium Academiae award to M. Hocek), by the Czech Science Foundation (16-001785) and by Gilead Sciences, Inc.

References

1) Nauš, P.; Pohl, R.; Votruba, I.; Džubák, P.; Hajdúch, M.; Ameral, R.; Birkus, G.; Wang, T.; Ray, A.S.; Mackman, R.; Cihlar, T.; Hocek, M.: J. Med. Chem. 2010, 53, 460–470.
2) Bourderioux, A.; Nauš, P.; Perlíková, P.; Pohl, R.; Pichová, I.; Votruba, I.; Džubák, P.; Konečný, P.; Hajdúch, M.; Stray, K. M.; Wang, T.; Ray, A. S.; Feng, J. Y.; Birkus, G.; Cihlar, T.; Hocek, M. J. Med. Chem. 2011, 54, 5498–5507.
3) Nauš, P.; Caletková, O.; Konečný, P.; Džubák, P.; Bogdanová, K.; Kolář, M.; Vrbková, J.; Slavětínská, L.; Tloušťová, E.; Perlíková, P.; Hajdúch, M.; Hocek, M. J. Med. Chem. 2014, 57, 1097–1110.
4) Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y. L.; Yokokawa, F.; Shi, P. Y.; Hocek, M. Bioorg. Med. Chem. 2012, 20, 6123–6133.
5) Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Lee, Y. J.; Hocek, M. Bioorg. Med. Chem. 2013, 21, 5362–5372.
6) Tichý, M.; Smoleń, S.; Tloušťová, E.; Pohl, R.; Oždian, T.; Hejtmánková, K.; Lišková, B.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. J. Med. Chem. 2017, 60, 2411–2424.

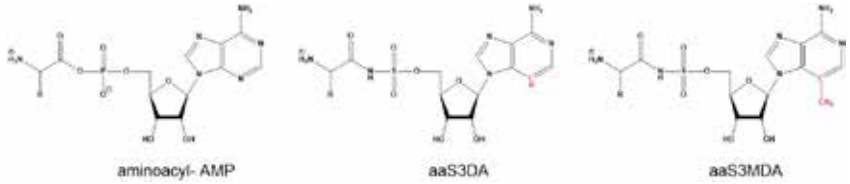
AMINOACYLATED SULFAMOYL-3-DEAZAADENOSINE ANALOGUES: DETAILED ANALYSIS OF THE aaSA SCAFFOLD FOR AMINOACYL tRNA SYNTHETASE INHIBITION

Baole Zhang, Steff De Graef, Manesh Nautiyal, Luping Pang, Bharat Gadakh, Sergei Strelkov, Stephen Weeks, Arthur Van Aerschot

Medicinal Chemistry, Rega Institute for Medical Research, Herestraat 49 box 1041, B-3000 Leuven, Belgium

Aminoacyl-tRNA synthetases (aaRSs) catalyze an important step in protein translation, attaching an amino acid to its cognate tRNA. These essential enzymes are therefore considered as viable targets for the development of novel antimicrobial agents.[1] A typical organism has 20 different aaRSs, which can be split into two distinct structural classes. Taking the well-known aminoacyl-sulfamoyl adenosines (aaSA) as lead compounds, we have evaluated the importance of the N^3-position of the adenine by synthesizing a number of 3-deaza congeners (aaS3DA) and assessing their inhibitory activity. We observed a clear class bias, with a dramatic loss of activity for aaS3DA analogues targeting class II enzymes when compared to the equivalent aaSA. Crystallographic studies indicated a conserved water molecule to be important for base recognition within class II enzymes.[2]

We have therefore now synthesized six aaSA analogues in which the adenine is substituted by 3-methyl-3-deazaadenine, thus providing the methylated congeners (aaS3MDA) of the 3-deaza derivatives. Again, both aaRS classes have been targeted to obtain a comparative inhibitory profile. Where we expect only a small influence of the additional methyl moiety for class I enzymes, increased hydrophobic contacts and entropic gain by the release of water should govern enzyme-ligand interactions, and could potentially restore inhibitory activity against class II enzymes. The newly synthesized compounds are presently under evaluation and we will report on their interaction studies.



References

1) Gaston H.M. Vondenhoff and Arthur Van Aerschot (2011), Aminoacyl-tRNA synthetase inhibitors as potential antibiotics – Invited review; Eur. J. Med. Chem., 46, 5227-5236.
2) Zhang B., De Graef S., Nautiyal M., Pang L., Gadakh B., Froeyen M., Van Mellaert L., Strelkov S., Weeks S., Van Aerschot A. (2018). Family-wide analysis of aminoacyl-sulfamoyl-3-deazaadenosine analogues as inhibitors of aminoacyl-tRNA synthetases. Eur. J. Med. Chem., 148, 384-396.

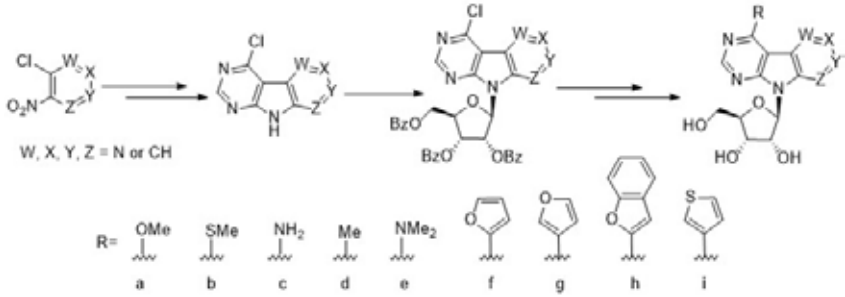
SYNTHESIS OF NOVEL HETERO-FUSED 7-DEAZAPURINE RIBONUCLEOSIDES

Lucia Veselovská (1), Michal Hocek (1,2)

1) Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead & IOCB Research Center, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic
2) Dept. of Organic Chemistry, Faculty of Science, Charles University, Hlavova 8,CZ-12843 Prague 2, Czech Republic

Although dozens of antiproliferative drugs already exist, the treatment of many types of leukemia and tumors still has a low success rate. Many substituted 7-deazapurine nucleosides showed various biological activities, but, despite extensive study of this type of compounds, there is still a potential for the development of new antiviral and anticancer drugs. Recently, our group discovered, patented and published several classes of potent cytostatic compounds – substituted (het)arylo-fused 7-deazapurine ribonucleosides with a fused benzene, thiophene, furan or 5-methylpyrrole ring. Some of these derivatives showed micromolar, even nanomolar cytostatic and cytotoxic activities against a broad panel of cancer cells and promising antiviral activities against HCV and Dengue viruses.[1]

Based on these results, we decided to prepare and explore biological activities of similar pyrido-fused 7-deazapurine ribonucleosides possessing nitrogen atom in different positions in the fused pyridine ring. Desired nucleobases were synthesized starting from corresponding chloronitropyridines. The synthesis employs key nucleophilic substitution of chlorine atom with ethyl cyanoacetate, reduction with zinc dust followed by cyclisation using formamide and chlorination. Obtained nucleobases were then subjected to Vorbrüggen glycosylation to provide the benzoylated β-nucleosides. Free ribonucleosides were subsequently prepared using different palladium-catalyzed coupling reactions and nucleophilic substitutions with subsequent or simultaneous deprotection. All final nucleosides are being tested for their biological activities.



This work was supported by the Academy of Sciences of the Czech Republic (RVO 61388963 and the Praemium Academiae award to M. Hocek), by the Czech Science Foundation (16-001785 to M. Hocek) and by Gilead Sciences, Inc.

References

1) a) Tichý, M.; Pohl, R.; Xu, H. Y.; Chen, Y.-L.; Yokokawa, F.; Shi, P.-Y.; Hocek, M. Bioorg. Med. Chem. 2012, 20, 6123–6133; b) Tichý, M.; Pohl, R.; Tloušťová, E.; Weber, J.; Bahador, G.; Lee, Y.-J.; Hocek, M. Bioorg. Med. Chem. 2013, 21, 5362–5372; c) Tichý, M.; Smoleń, S.; Tloušťová, E.; Pohl, R.; Oždian, T.; Hejtmánková, K.; Lišková, B.; Gurská, S.; Džubák, P.; Hajdúch, M.; Hocek, M. J. Med. Chem. 2017, 60, 2411–2424; d) Hocek, M.; Smoleń, S. B.; Tokarenko, A.; Džubák, P.; Hajdúch, M.: WO201802465A1..

MECHANISM OF MOLECULAR RECOGNITION OF RNA APTAMER TO HUMAN IMMUNOGLOBULIN G

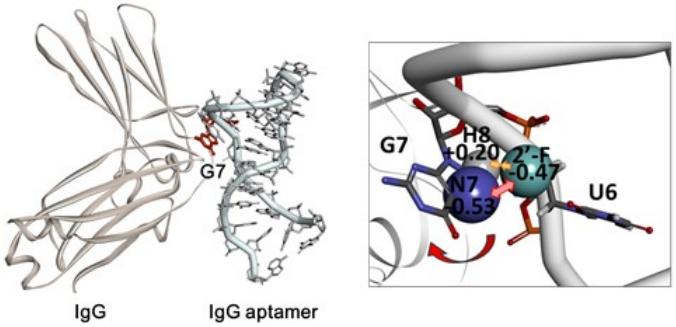
Hisae Yoshida (1), Takeshi Ishikawa (2), Taiichi Sakamoto (3), Kenji Yamagishi (1)

1) Graduate School of Engineering, Nihon University 1 Nakagawara, Tokusada, Tamuramachi, Koriyama, Fukushima, Japan
2) Graduate School of Biomedical Sciences, Nagasaki University 1-12-4 Sakamoto, Nagasaki, Nagasaki, Japan
3) Faculty of Advanced Faculty of Advanced Engineering, Chiba Institute of Technology 2-17-1 Tsudanuma, Narashino, Chiba, Japan

RNA aptamers are short single-stranded nucleic acids with high affinity and specificity for their target molecules, which can be nucleic acids, proteins, or small organic compounds. Aptamers, therefore, have many potential applications in medicine and technology. RNA aptamers can take a variety of three dimensional structures and the single stranded regions are commonly used as recognition sites and building blocks.

Recently, a RNA aptamer that binds to the Fc portion of human IgG1 (hFc1) has been identified. The crystal structure complexed with the hFc1 has been solved. This aptamer contains six Watson-Crick type base pairs, two non-canonical base pairs, and one base triple. Base are continuously stacked in G1-U6, G7-G12 and A13-C23, whereas the flipping bases of U6 and G7 are not stacked. The flipped base of G7 stabilized following interactions: the stacking interaction between the G7 base and the side chain of Tyr373, the hydrogen bonds between N2 of G7 base and the carbonyl oxygen of Gly402; and van der Waals contacts between the G7 base and 2'-fluoro of U6 ribose. These results indicate that base flipping motif of RNA aptamer plays important role to recognize the target molecule.

In this study, to elucidate the mechanism of molecular recognition of RNA aptamer to human IgG1, we carried out ab initio fragment molecular orbital (FMO) calculations for the aptamer/hFc1 complex. We analyzed the interaction energy of all base-residue pairs by using the inter–fragment interaction energy (IFIE) analysis based on FMO calculations.



References

1) S. Miyakawa, et al., RNA, 14:1154-1163, 2008.
2) Y. Nomura, et al., Nucleic Acids Research, 38:7822-7829, 2010.

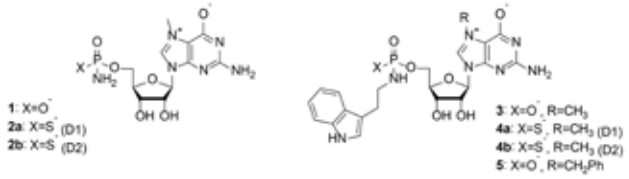
SYNTHESIS OF NOVEL PHOSPHORAMIDATE AND THIOPHOSPHORAMIDATE CAP ANALOGS - POTENTIAL PRONUCLEOTIDE TRANSLATION INHIBITORS

Sebastian Golojuch (1,2), Michal Kopcial (1,3,4), Dominika Strzelecka (4), Joanna Kowalska (4), Jacek Jemielity (1)

1) Centre of New Technologies, University of Warsaw, Banacha 2C, 02-097, Warsaw, Poland
2) Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
3) College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Banacha 2C 02-097 Warsaw, Poland
4) Division of Biophysics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland

The cap structure, which is modification present at the 5'-end of eukaryotic mRNA has at least two major functions. It protects RNA chain from hydrolysis caused by exonucleases and it interacts with eukaryotic Translation Initiation Factor 4E (eIF4E) creating the eIF4F translation initiation complex, which initiates protein biosynthesis process.[1] It has been shown, that aberrant cap dependent translation in cancer cells is related to overexpression of eIF4E.[2] Furthermore it has been also shown, that inhibition of eIF4E expression reduces tumor growth and malignancy without eliciting toxicity.[3] The cap structure is thereby an attractive starting point for the drug design. However, although several analogs of cap have demonstrated utility as therapeutics, their usability is limited by the low membrane permeability.[4] As one of the solutions to overcome this obstacle, development of prodrug methodology involving pronucleotides has been proposed.[4]

We report chemical synthesis of novel 7-methylguanosine monophosphate (m^7GMP) pronucleotide analogs bearing phosphoramidate or thiophosphoramidate moiety. Phosphoramidate moiety was introduced through Yoshikawa phosphorylation followed by addition of ammonia to obtain analog **1** or via Mukaiyama-Hashimoto activation of m^7GMP and coupling with a tryptamine to obtain compound **3**. Analogs **2a, 2b, 4a, 4b,** as two pairs of diastereomers bearing thiophosphoramidate moiety were synthesized through modified Yoshikawa phosphorylation method followed by addition of an ammonia or a tryptamine. Each pair of diastereomers were separated using the RP-HPLC. Introduction of the phosphoramidate moiety, which concerns prodrug methodology was expected to increase cell permeability owing to the phosphate charge masking effect.[5] The thiophosphate modification was introduced to increase the affinity for eIF4E. We also synthesized previously reported translation inhibitor pronucleotide **5** as a reference for further biophysical and biological studies. Enzymatic activation of the pronucleotides were investigated in HEK extracts. It has demonstrated that obtained compunds are transformed enzymatically from their pronucleotide form to corresponding nucleotide in cell extracts.



References

1) Ziemniak, M. et al. Potential therapeutic applications of RNA cap analogs. Future Med. Chem. 5, 1141–1172 (2013),
2) Sun, S. et al. Activation of Akt and eIF4E Survival Pathways by Rapamycin- Mediated Mammalian Target of Rapamycin Inhibition. Cancer Res. 65, 7052–7059 (2005),
3) Graff, J. R. et al. Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. 117, 2638–2648 (2007),
4) Wagner, C. R. et al. Pronucleotides: Toward the In Vivo Delivery of Antiviral and Anticancer Nucleotides. Med. Res. Rev. 20, 417–451 (2000),
5) Li, S. et al. Treatment of breast and lung cancer cells with a N-7 benzyl guanosine monophosphate tryptamine phosphoramidate pronucleotide (4Ei-1) results in chemosensitization to gemcitabine and induced eIF4E proteasomal degradation. Mol. Pharm. 10, 523–531 (2013).

ANALYSIS OF DINUCLEOTIDE ANALOGUES MODIFIED IN THE OLIGOPHOSPHATE BRIDGE USING TANDEM MASS SPECTROMETRY

Agnieszka Brzezinska (1), Dominika Strzelecka (1), Sebastian Chmielinski (1,2), Sylwia Bednarek (3), Jacek Jemielity (2), Joanna Kowalska (1)

1) Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, Warsaw, Poland
2) Centre of New Technologies, University of Warsaw, Banacha 2C, Warsaw, Poland
3) Laboratory of Neuroinformatics, Depratment of Neurophysiology Nencki Institute of Experimental Biology of Polish Academy of Sciences, Pasteura 3, Warsaw, Poland

Dinucleotides are a subclass of nucleotides which play many biological functions in leaving organisms. The nucleotides fulfil various regulatory and signaling functions, for example purine dinucleotide polyphosphates act as agonists of receptors involvedin various cellular processes[1]. Compounds that perform such different and important functions and their synthetic analogs are interesting from the therapeutic point of view and the elucidation of cellular mechanisms [2].

The aim of our work was to analyze qualitatively, understand fragmentation pathways, and propose general rules for the fragmentation of dinucleotides, as previously reported for mononucleotides [3]. In this work, we analyzed parent ions of mono- and doubly charged dinucleotides in negative and positive ion mode using tandem mass spectrometry (MS/MS) with electrospray ionization and triple quadrupole analyser. We especially focused on the fragmentation of biologically and therapeutically important dinucleotides with different modifications in the oligophosphate bridge such as phosphorothioate, boranophosphate, fluorophosphate, and methylenebisphosphonate.Based on the results we were able to find characteristic signals for introduced modification and determine probable fragmentation pathways for dinucleotides. The results of our research can be useful in analysis of natural dinucleotide modifications in biological samples, investigation and identification of nucleotide-based drugs and prodrug metabolites and studying the pathways of their metabolism, as well as identification of dinucleotide analogs produced by chemical synthesis.

This project was supported bythe National Science Centre(NCN, UMO-2015/18/E/ST5/00555).

References

1) A.Khvorova, J.K.Watts, Nature biotechnology, Vol 35, 2017
2) S.Shaver , J.Rideout, W.Pendergast, J.Douglass, E.Brown, J.Boyer, R.Patel, C.Redick, A.Jones, M.Picher&B.Yerxa, PurinergicSignalling, Vol 1, 2005
3) D.Strzelecka, S.Chmielinski, S.Bednarek, J.Jemielity&J.Kowalska, Scientific Reports, Vol 7, 2017

NOVEL N7-BENZYLATED DINUCLEOTIDE 5'CAP ANALOGUES - SYNTHESIS, PROPERTIES AND CAP-PROTEIN INTERACTIONS

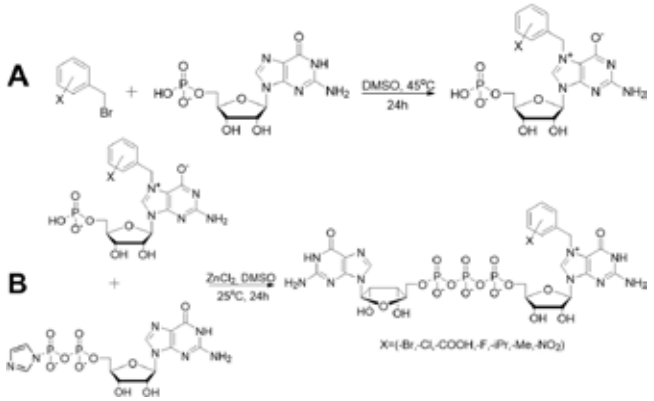
Radosław Wójcik (1,2), Marek R. Baranowski (3), Anna Nowicka (3), Paweł J. Sikorski (1), Joanna Kowalska (3), Jacek Jemielity (1)

1) Centre of New Technologies, University of Warsaw, Warsaw, Poland
2) College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland
3) Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

All eukaryotic mRNAs have a unique structure located at their 5'end, called 5'cap. It consists of a modified nucleoside – N^7-methylguanosine – linked to the first transcribed nucleotide of mRNA through 5'-5' triphosphate bridge. Cap influences mRNA stability and functions owing to its specific interactions with numerous proteins and enzymes associated with mRNA maturation, translation and degradation. The significant biological role of the cap makes it a good object of chemical modifications.

Synthetic cap analogues may find applications in medicine as specific binders to eIF4E (eukaryotic translational initiation factor), a protein overexpressed in cancer cells. Previous studies showed that replacement of the N^7-methyl group of the N^7-methylguanosine monophosphate with a benzyl group increased binding affinity to eIF4E.[1]

We synthesised and studied the properties of a new series of dinucleotide cap analogues containing differently substituted benzyl groups in N^7-position of guanosine. To obtain the analogues, N^7-benzylated guanosine monophosphates were synthesised by N-alkylation of guanosine with respective benzyl bromide (Fig.A). Then guanosine diphosphate was converted into P-imidazolide through Mukaiyama-Hashimoto reaction. Finally, the N^7-benzylated guanosine was coupled with P-imidazolide of guanosine diphosphate in the presence of $ZnCl_2$ (Fig.B) giving the respective cap analogue.[2]



Our approach resulted in obtaining fifteen new N^7-benzylated cap analogues, which differ in the type and position of the substituents in benzyl moiety. The synthesised compounds were characterised by high resolution mass spectrometry (HRMS) and ^{1}H NMR spectroscopy. Thereafter all analogues were preliminary tested in biochemical context, including measurement of binding affinity to eIF4E, incorporation into mRNA by SP6 RNA Polymerase during transcription in vitro, and ability to promote the expression of luciferase protein in HeLa cells.

References

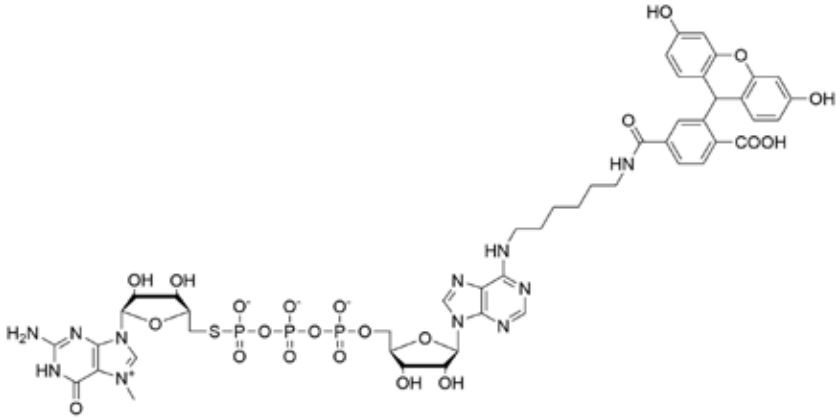
1) C.J. Brown, et al. J Mol Biol. Vol. 372, pp. 7-15, 2007
2) E. Grudzien, et al. RNA Vol.10 pp. 1479-1487, 2004

SYNTHESIS OF CLEAVAGE-RESISTANT, FLUORESCENTLY LABELED CAP ANALOGUE AS A MOLECULAR PROBE FOR HDCPS

Teodor Olejko (1,2), Sylwia Walczak (1,2), Anna Nowicka (2,3), Joanna Kowalska (3), Jacek Jemielity (2)

1) College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland
2) Centre of New Technologies, University of Warsaw, Warsaw, Poland
3) Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

The 5' mRNA cap protects mRNA from 5' to 3'-exonuclease degradation and has an important role in gene expression processes including translation initiation. The cap interacts with various proteins involved in mRNA metabolism which have often been linked to disease development. Therefore, synthetic cap analogues are useful in wide range of applications, including modulating activity of several key cap-dependent enzymes. One of them is Decapping Scavenger enzyme (DcpS) – a pyrophosphatase degrading cap structures released from mRNA 3' to 5' decay, cleaving the triphosphate chain within the cap to release m^7GMP and a 5′-diphosphate from the rest of the molecule. DcpS is also a molecular target in Spinal Muscular Atrophy (SMA) treatment, hence its inhibitors are potential therapeutic agents. Fluorescent molecular probes that bind to DcpS with high affinity can be used in search of tightly binding DcpS inhibitors as potential therapeutics for SMA. Here, we designed and synthesized a fluorescently labelled mRNA cap analogue with resistance and high binding affinity to DcpS (Figure). The key modification to achieve both features was phosphorothiolate group neighbouring to 7-methylguanosine moiety. Carboxyfluoresceine dye was attached to the base of second nucleoside via diamine linker using NHS chemistry. The preliminary spectroscopic and biochemical properties of the probe and its applications for discovery of DcpS inhibitors by fluorescence polarization method also will be presented.



References

1) Wojtczak, B. A. et al. 5′-Phosphorothiolate Dinucleotide Cap Analogues: Reagents for Messenger RNA Modification and Potent Small-Molecular Inhibitors of Decapping Enzymes. J. Am. Chem. Soc. 140, 5987–5999 (2018).

POSTERS - TECHNOLOGIES

PET Imaging as a Tool in for in Vivo Drug Evaluation and Development

IDENTIFICATION OF IN VIVO ACTIVE HITS FROM THE GSK TRES-CANTOS ANTI-KINETOPLASTID SET (TCAKS) AGAINST CHAGAS DISEASE

Gigante A. (1,2), Orr D. (1), Padilla A. M. (1), Kessler A. (2), Gonzalez S. (2), Fiandor J. M. (2), Tarleton R. L. (1)

1) Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia (USA)
2) Diseases of the Developing Word (DDW), Tres Cantos Medicines Development Campus, GlaxoSmithKline, Tres Cantos (Spain)

Chagas disease, a major cause of cardiac disease in many countries of Latin America, is caused by *Trypanosoma cruzi*, a kinetoplastid protozoan parasite.[1] At present there is no vaccine against this illness and the current available drugs (benznidazole and nifurtimox) present severe side effects and show variable efficacy.[2] Therefore, new drugs to prevent this disease are needed.

In 2015, GSK Tres Cantos identified and published the TCAKS, which contains 222 compounds as potential hits against *T. cruzi*.[4] These compounds shown high potencies, low host cell cytotoxicities and good physico-chemical properties *in vitro*. Previously, Tarleton *et at.* reported development of a rapid and efficient *in vivo* assay in which up to 30 compounds can be evaluated in less than 1 week to screen for compounds with *in vivo* activity.[3] This protocol uses a single oral dose administration using a small quantity of compound (approx. 10 mg) and whole animal imaging pre-and post-treatment to determine *in vivo* efficacy in mice. Hits which show activity in this model have a high probability of success in the Chagas *in vivo* chronic model (3 months).

In this work we present four hits which have been identified from the TCAKS. They have shown similar *in vivo* efficiency indexes as the current chemotherapies. These promising results have allowed us to start a SAR of one of these families for lead identification.

References

1) Trend. Parasitol. 2016, 22, 583-588
2) N. Engl. Med. 2014, 370:20, 1899-1908
3) PLoS Negl. Trop. Dis. 2010, e74
4) Sci. Rep. 2015, 5: 8771

STEREOSELECTIVITY OF THE BINDING OF (R)-[11C]ME-NB1 OVER (S)-[11C]ME-NB1 FOR THE GLUN2B RECEPTOR SUBUNIT DEMONSTRATED BY AUTORADIOGRAPHY AND PET IMAGING

Linjing Mu (1,2), Ahmed Haider (1), Adrienne Müller Herde (1), Stefanie D. Kraemer (1), Yves P. Auberson (3), Roger Schibli (1,2), Bernhard Wünsch (4), Simon M. Ametamey (1)

1) Institute of Pharmaceutical Sciences, ETH Zürich, Vladimir-Prelog-Weg 4, CH-8093 Zürich, Switzerland
2) Department of Nuclear Medicine, University Hospital Zürich, CH-8091 Zürich, Switzerland
3) Novartis Institutes for BioMedical Research, CH-4057 Basel, Switzerland
4) Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, D-48149 Münster, Germany

Background. In the course of our search for a suitable radioligand for the positron emission tomography (PET) imaging of the GluN2B-subunit of the NMDA receptor, we have identified a racemic benzazepine compound denoted *(rac)*-[^{11}C]Me-NB1 as a suitable GluN2B imaging agent[1]. We have now established in a recent study that the two enantiomers *(R)*-[^{11}C]Me-NB1 and *(S)*-[^{11}C]Me-NB1 exhibit distinct behaviors in in vitro autoradiographic studies on murine brain tissues as well as in vivo PET imaging using Wistar rats. These results were unexpected although it is well known that enantiomers can have distinct pharmacodynamic profile when compared to their respective racemic mixtures.

Methods. Separation of the phenol precursor (rac)-NB1 was achieved by a chiral Reprosil column. Both enantiomeric pure precursors (R)-NB1 and (S)-NB1 were radiolabeled with [^{11}C]CH_3I. The absolute configurations of (R)/(S)-Me-NB1 were determined by X-ray crystallography. In vitro autoradiographic studies were performed on rat and mouse brain slices, and blocking experiments were performed in order to determine the selectivity and specificity of the radioligands. In vivo PET experiments were performed with male Wistar rats and PET data was evaluated with PMOD (PMOD Ltd., Switzerland). Preclinical receptor occupancy studies with CP101,606, a GluN2B-antagonist, was included in the radiotracer evaluation.

Results. Carbon-11 labeling of the R- and S-enantiomers of Me-NB1 was accomplished using [^{11}C] iodomethane in 42 ± 9 % radiochemical yield (decay corrected). The molar activity was 204 ± 80 GBq/µmol (n = 66) at the end of synthesis and radiochemical purity was >99%. Autoradiographic experiments revealed significant differences between the binding patterns of *(R)*-[^{11}C]Me-NB1 and *(S)*-[^{11}C]Me-NB1 on murine brain tissue sections. *(R)*-[^{11}C]Me-NB1 showed a heterogeneous distribution pattern with high binding to GluN2B-rich regions such as the cortex, striatum, thalamus and hippocampus. The specificity and selectivity of (R)-[^{11}C] Me-NB1 towards GluN2B were confirmed by using both GluN2B and σ1 receptor selective ligands. *(S)*-[^{11}C] Me-NB1 displayed a homogenous distribution across the whole rat and mouse brain. We established that the S-enantiomer binds predominantly to the σ1 receptor.

In PET experiments with rats, the brain uptake of (R)-[^{11}C]Me-NB1 was generally higher than (S)-[^{11}C] Me-NB1. Efficient blockade was observed for *(R)*-[^{11}C]Me-NB1 following the injection of the GluN2B-antagonist eliprodil (2 mg/kg), but not in the case of *(S)*-[^{11}C]Me-NB1. Receptor occupancy study with *(R)*-[^{11}C] Me-NB1 and CP101,606, a GluN2B-selective antagonist, revealed a value of 158 nM CP101,606 plasma concentration for 50 % receptor occupancy.

Conclusion. A remarkable stereoselectivity of the GluN2B receptor for the R- over the S-enantiomer of benzazepine compound [^{11}C]Me-NB1 was demonstrated in autoradiographic studies on murine brain tissues as well as in vivo dose-response experiments. *(S)*-[^{11}C]Me-NB1 binds predominantly to the σ1 receptor. *(R)*-[^{11}C] Me-NB1 is a promising PET radioligand for imaging the GluN2B subtype of ionotropic NMDA receptor.

References

1) S. D. Krämer, et al. J. Nucl. Med. 2018, in print

RADIOCHEMICAL SYNTHESIS OF 18F-PROTIDES FOR POSITRON EMISSION TOMOGRAPHY (PET) IMAGING

Alessandra Cavaliere (1,2), Katrin Probst (2), Abdul Karim Haji Dheere (3), Franklin I. Aigbirhio (3), Chris McGuigan (1), Andrew D. Westwell (1)

1) School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3NB, Wales, U.K.
2) Wales Research and Diagnostic Positron Emission Tomography Imaging Centre (PETIC), University Hospital of Wales, Heath Park, Cardiff, CF14 4XN, Wales, UK.
3) Wolfson Brain Imaging Centre, School of Clinical Medicine, University of Cambridge, Cambridge Biomedical Campus, Cambridge, CB2 0QQ

Positron Emission Tomography (PET) is a highly sensitive imaging technique used in cancer diagnosis, treatment planning and monitoring of therapy response. ^{18}F is an optimal PET label considering its half-life (110 min.) and imaging resolution. One of the major challenges in ^{18}F PET research is the installation of the weakly nucleophilic $^{18}F^-$ into a precursor molecule at a late stage in the synthetic route.[1]

Fluorinated nucleoside analogues such as gemcitabine represent an important class of therapeutic agents for different types of solid tumours. Unfortunately issues such as poor cellular uptake (*via* nucleoside transporters), the requirement for kinase-mediated intracellular (tri)phosphorylation, and drug resistance (e.g. *via* catabolism) represent major problems limiting their therapeutic efficacy. The phosphoamidate ProTide approach is a strategy to circumvent the limitations of nucleoside analogues to deliver the monophosphate nucleotide to the cellular target and overcome resistance mechanisms. The gemcitabine ProTide NUC-1031 (Acelarin) provides an outstanding example of a fluorinated anticancer ProTide currently in Phase III clinical trials.[2]

In this study we present the first radiochemical syntheses of ^{18}F-ProTides *via* the fluorination of advanced precursors bearing sulfonyl-based (nosyl, tosyl) leaving groups. Both 2'-fluoro (FIAU) and 3'-fluoro (FLT) ^{18}F model compounds have been synthesised. An automated synthetic Modular Lab placed into a shielded hot cell has been used to perform the hot fluorination. The final isolated products have been analysed by radio-HPLC, LC-MS and a radio TLC, comparing to cold synthetic standards.

^{18}F-ProTides represent new PET imaging agents for the direct visualization of uptake and biodistribution of ProTides *in vivo* for the first time, providing further confidence in the mechanism of action and uptake kinetics of this powerful and ciinically validated pro-nucleotide delivery strategy.

References

1) Meyer J, Probst K, Westwell A. J. Label Compd. Radiopharm. 2014, 57, 333–337.
2) Slusarczyk M, Lopez MH, Balzarini J, Mason M, Jiang WG, Blagden S, Thompson E, Ghazaly E, McGuigan C. J. Med. Chem. 2014, 57, 1531-1542.

NOTES

POSTERS - THERAPEUTIC AREAS

Immuno-Oncology: Novel Therapeutic Opportunities

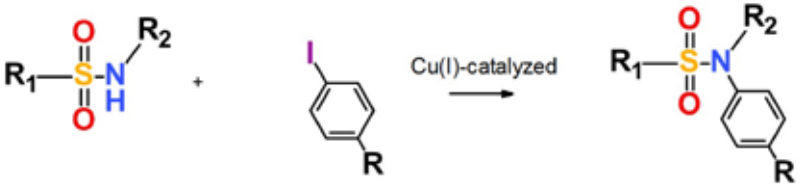
ULLMAN-DERIVED INHIBITORS OF ER-AMINOPEPTIDASES (ERAPS)

Bao Vy Lam (1), Ronan Gealageas (1), Sandrine Warenghem (1), Valentin Guillaume (1), Virgyl Camberleyn (1), Julie Dumont (1), Damien Bosc (1), Leroux Florence (1), Benoit Deprez (1), Rebecca Deprez-Poulain (1,2)

1) INSERM U1177 Drugs and Molecules for Living Systems; Drug Discovery unit; Pasteur Institute Lille, University of Lille, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE
2) Institut Universitaire de France, IUF

Endoplasmic reticulum aminopeptidases (ERAP1 and 2) are M1 family zinc metalloproteases playing a key role in the antigen presentation pathway. These intracellular aminopeptidases trim peptide precursors resulting from proteins degradation by the proteasome and generate mature antigenic epitope of appropriate length for presentation on the cell surface by major histocompatibility complex class I (MHCI) molecules. The cytotoxic T-cells recognition of the extracellular peptide triggers immune response against infected or diseased cells through biological cascades that lead to cell apoptosis. Thereby ERAPs are major regulators of adaptive immune responses in humans. GWAS studies have associated polymorphism of ERAPs with predisposition to immune diseases (i.e. ankylosing spondylitis, Behcet, Birdshot uveitis and type1 diabetes). ERAP1 inhibitor was shown to delete Th17 response in a model of spondylarthritis (Chen, L., et al. (2016) Annals of the Rheumatic Diseases 75(5): 916-923).Conversely, cancer cells can evade the immune system by stopping the generation of antigenic peptides and inhibitors of ERAP1 have been shown to affect antigen processing in cultured cells and elicit cytotoxic T-cell responses in a dose-dependent and affinity-dependent manner (Zervoudi E. *et al. PNAS.* 2013, *110*, 19890-5). Thus ERAPs have emerged in the past years as potential target for cancer immunotherapy and treatments for autoimmune diseases upstream inflammatory chemokines production.

So far, the ERAP2 inhibitors bear either a phosphinic group or a 1,4-diaminobenzoic acid motif, to bind the catalytic zinc atom. They display good to excellent activities against ERAP2 (IC_{50} of 240 to 11nM). However, these inhibitors need to be optimized to achieve better selectivity and druggable properties. In parallel to these efforts, we chose to develop a fast enzyme-efficient 384-well plate HTS assay, and screen a focused in-house library to discover new chemical templates able to inhibit ERAP2. One of the hits identified in during the screening campaign displayed an N-aryl-sulfonamide group. We thus designed and synthesized analogues of this hit and explored their potency to inhibit ERAP1, 2 and their selectivity towards related IRAP and LAP enzymes.



References

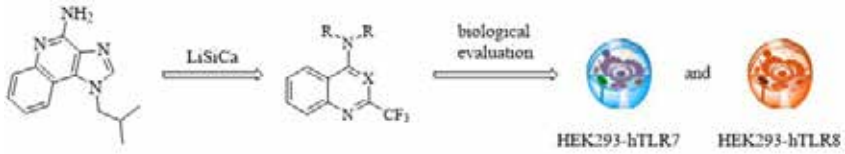
1) Mpakali, A., Giastas, P., Deprez-Poulain, R., Papakyriakou, A., Koumantou, D., Gealageas, R., Tsoukalidou, S., Vourloumis, D., Mavridis, I. M., Stratikos, E., & Saridakis, E. Crystal Structures of ERAP2 Complexed with Inhibitors Reveal Pharmacophore Requirements for Optimizing Inhibitor Potency. ACS Medicinal Chemistry Letters,2017, 8(3): 333-337
2) Deprez-Poulain, R., Cousaert, N., Toto, P., Willand, N., & Deprez, B. Application of Ullmann and Ullmann-Finkelstein reactions for the synthesis of N-aryl-N-(1H-pyrazol-3-yl) acetamide or N-(1-aryl-1H-pyrazol-3-yl) acetamide derivatives and pharmacological evaluation. Eur J Med Chem,2011, 46: 3867-3876.
3) Toto, P., Gesquiere, J.-C., Cousaert, N., Deprez, B., & Willand, N. UFU (Ullmann-Finkelstein-Ullmann): a new multicomponent reaction. Tetrahedron Letters,2006, 47(28): 4973-4978.

DESIGN, SYNTHESIS AND EVALUATION OF TOLL-LIKE RECEPTOR 7 AGONISTS WITH 2-(TRIFLUOROMETHYL)QUINOLINE-4-AMINE AND 2-(TRIFLUOROMETHYL)QUINAZOLINE-4-AMINE SCAFFOLDS

Ana Dolšak (1), Urban Švajger (1,2), Samo Lešnik (3), Janez Konc (3), Stanislav Gobec (1), Matej Sova (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
2) Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, 1000 Ljubljana, Slovenia
3) National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

Toll-like receptors (TLRs) are pattern-recognition receptors that play an important role in the innate immune responses against a number of pathogens.[1] TLR7, one of the 12 functional TLRs discovered up to date, is recognized as a promising target for the treatment of viral infections, autoimmune diseases and cancer.[2] For identification of potential novel ligands of TLR7 our ligand-based virtual screening protocol named LiSiCA was used, with imiquimod as a query compound.[3] 22 compounds, topologically most similar to the reference compound, were obtained from different vendors. After biological evaluation of their agonist activity two hit compounds with similar scaffolds, namely 2-(trifluoromethyl)quinoline-4-amine and 2-(trifluoromethyl)quinazolin-4-amine, were discovered. Concurrently, a simple three-step synthetic procedure was developed to resynthesize initial hits and prepare a focused library of their analogs. 22 novel compounds were synthesized and evaluated for TLR7 agonist activity on the HEK293 cell line, co-transfected with hTLR7 gene and an inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Activation of hTLR7 receptors triggers higher secretion of SEAP, which could be measured colorimetrically in the supernatant using Quanti-Blue™ reagent. EC_{50} values of the most potent agonists were determined in the micromolar range, with the most potent one of 53.1 μM. All active compounds were further tested on HEK293-hTLR8 cells using the same assay protocol. None of our TLR7 agonists showed any activity on TLR8. Even though our compounds are less potent TLR7 agonists compared to imiquimod, they show selectivity toward TLR7, thus representing an important starting point for further studies of small-molecule agonists with novel 2-(trifluoromethyl)quinoline-4-amine and 2-(trifluoromethyl)quinazolin-4-amine scaffolds.



References

1) Kawai T, Akira S: Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. Immunity 2011; 34 (5): 637-650.
2) Hennessy EJ, Parker AE, O'Neill LAJ: Targeting Toll-like receptors: emerging therapeutics? Nature Reviews Drug Discovery 2010; 9 (4): 293-307.
3) Lešnik S et al: LiSiCa: A Sotware for Ligand-Based Virtual Screening and Its Application for the Discovery of Butyrylcholinesterase Inhibitors. Journal of Chemical Information and Modeling 2015; 55 (8): 1521-1528.

MPL-7097, AN ESM(TM) P38 MAPK INHIBITOR

Nelly Piton (1), Samuel Brookes (1), Jonas Calleja (1), Katie Chapman (1), Duncan Hannah (1), Mark Jackman (1), Alistair McNair (1), Gary Newton (1), Philip Skone (1), Claire Tebbutt (1), Natalie Winfield (1), David Moffat (2), Martin Perry (2)

1) Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, CB10 1XL, UK, nelly.piton@domainex.co.uk
2) Macrophage Pharma Limited, Gainsborough House, 59-60 Thames Street, Windsor, SL4 1TX, UK

Tumour-associated macrophages (TAMs) contribute significantly to enhanced malignancy in multiple cancers by generating an immunosuppressive tumour microenvironment through production of cytokines such as IL-10. Polarization of these immunosuppressive M2 macrophages toward a pro-inflammatory M1 phenotype is capable of activating an effective anti-tumour immune response. p38 MAPK has been shown to play a role in polarising macrophages toward an immunosuppressive M2 phenotype, however, it also has a pro-inflammatory effect in other immune cells such as T-cells. Macrophage Pharma's Esterase Motif Technology™ (ESM™) targets myelomonocytic cells whilst sparing other immune cells. The application of this technology to p38 MAPK will be described to generate a series of potent ESM™ p38 inhibitors that selectively target myelomonocytic cells.

HIGH-THROUGHPUT-SCREENING TO IDENTIFY ECTO-5'-NUCLEOTIDASE (CD73) INHIBITORS WITH POTENTIAL FOR THE IMMUNOTHERAPY OF CANCER

Christian Renn (1), Herbert Zimmermann (2), Christa E. Müller (1)

1) PharmaCenter Bonn, Pharmaceutical Sciences Bonn (PSB), Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, D-53121 Bonn, Germany
2) Institute of Cell Biology and Neuroscience, Molecular and Cellular Neurobiology, Goethe University, 60438 Frankfurt am Main, Germany

Extracellular ATP acts as a proinflammatory signaling molecule via G protein-coupled P2Y receptors and ATP-gated ion channels (P2X receptors).[1,2] It is hydrolyzed by several types of ecto-nucleotidases: in a first step ecto-nucleoside triphosphate diphosphohydrolases (NTPDases) and nucleotide pyrophosphatases/phosphodiesterases (NPPs) convert ATP to AMP, which is then further hydrolyzed by ecto-5'-nucleotidase (CD73) to adenosine. Adenosine activates G protein-coupled adenosine (P1) receptors; it exerts powerful immunosuppressive properties via A_{2A} and A_{2B} adenosine receptor activation.[3,4] The well-balanced system of pro-inflammatory ATP and immunosuppressive adenosine is disturbed under several pathological conditions. Many tumors overexpress ecto-nucleotidases which leads to high levels of adenosine in the tumor microenvironment resulting in tumor immune escape.[5] Thus, inhibition of ecto-nucleotidases has been proposed as a novel strategy in cancer immunotherapy.[6] The present study was aimed at identifying novel scaffolds for CD73 inhibitors. Therefore, we established a CD73 assay suitable for high throughput screening based on the detection of phosphate by malachite green. We adapted the assay to a robotic screening platform and initially screened a purine target-focused library of 6.000 small molecules. A primary screen resulted in 85 hit compounds (hit rate: 1.4%). For hit validation, a previously developed sensitive radioassay was employed,[7] which led to the confirmation of 27% of the hit compounds. Several new scaffolds were identified, which inhibited the enzymatic activity by more than 50% at a concentration of 10 µM. Among these dual CD73 inhibitors / adenosine A_{2A} receptor antagonists were identified that showed similar potency at both targets.

References

1) Save, S.; Persson, K. Infect. Immun. 2010, 78, 3609–3615.
2) Burnstock, G. Purinergic Signal. 2016, 12, 59–67.
3) Zimmermann, H.; Zebisch, M.; Sträter, N. Purinergic Signal. 2012, 8, 437–502.
4) Thiel, M.; Caldwell, C. C.; Sitkovsky, M. V. Microbes Infect. 2003, 5, 515–526.
5) Hoskin, D.; Mader, J.; Furlong, S.; Conrad, D.; Blay, J. Int. J. Oncol. 2008.
6) Stagg, J.; Divisekera, U.; McLaughlin, N.; Sharkey, J.; Pommey, S.; Denoyer, D.; Dwyer, K. M.; Smyth, M. J.. PNAS 2010, 107, 1547–1552.
7) Freundlieb, M.; Zimmermann, H.; Müller, C. E. Anal. Biochem. 2014, 446, 53–58.

IN SILICO DESIGN, SYNTHESIS AND BIOCHEMICAL EVALUATION OF NOVEL SMALL-MOLECULE INDOLEAMINE 2,3-DIOXYGENASE 1 INHIBITORS WITH A PYRIMIDIN-4(3H)-ONE SCAFFOLD

Matej Sova (1), Ana Dolšak (1), Matic Proj (1), Damijan Knez (1), Samo Lešnik (2), Janez Konc (2), Stanislav Gobec (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
2) National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia

The discovery of indoleamine 2,3-dioxygenase as an important immunotherapy target in cancer treatment has led to the intensive search for inhibitors in academia and pharmaceutical industry. Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme catalyzing the oxidation of L-tryptophan to *N*-formylkynurenine. High IDO1 expression found in tumor cells triggers the escape from immune system and has been associated with poor prognosis in several types of cancer [1]. Therefore, our aim was to design and develop novel small-molecule inhibitors of IDO1 as potential anticancer agents.

The compounds of interest were designed by advanced *in silico* drug design approaches. Firstly, ligand-based virtual screening using IDO1 inhibitor epacadostat as a query was performed with our software LiSiCA [2]. Secondly, structure-based screening protocol on the human form of IDO1 enzyme using complementary docking methodologies (Glide, Fred and our novel ProBiSdock algorithm) was carried out to obtain new structurally diverse IDO1 inhibitors. 65 commercially available *in silico* hit compounds were purchased and biochemically evaluated for IDO1 inhibitory activity in an optimized highly sensitive fluorescence-based end-point assay. Compounds **1** and **2** (Figure 1) showed promising inhibitory potency against IDO1 with IC_{50} values of 30.8 μM and 41.5 μM, respectively. According to biochemical evaluation and predicted binding mode from docking studies, pyrimidin-4(3H)-one scaffold seems to be important for IDO1 inhibitory activity. Furthermore, the appropriate synthetic procedures were developed, optimized and used for the preparation of a focused library of analogs of **1** and **2** to systematically explore structure-activity relationships of novel IDO1 inhibitors. Altogether, pyrimidin-4(3H)-one based IDO1 inhibitors represent an important starting point for further optimization and development of novel small-molecule cancer immunotherapeutics.

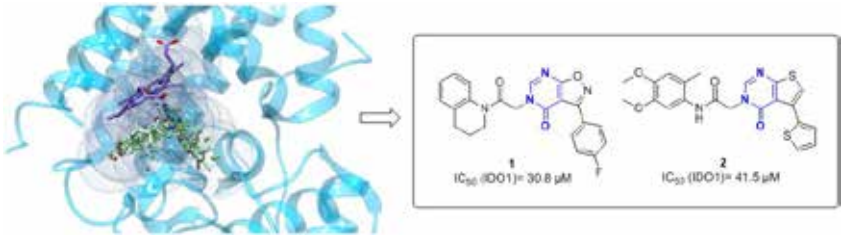


Figure 1. In silico design of IDO1 inhibitors and two hit compounds with pyrimidin-4(3H)-one scaffold.

References

1) Röhrig UF, Majjigapu SR, Vogel P, et al. Challenges in the Discovery of Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitors. J Med Chem. 2015;58(24):9421-37.
2) Lešnik S, Štular T, Brus B, et al. LiSiCA: A Software for Ligand-Based Virtual Screening and Its Application for the Discovery of Butyrylcholinesterase Inhibitors. J Chem Inf Model. 2015;55(8):1521-8.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - THERAPEUTIC AREAS

Life Science at the Interface of Agro and Pharma

P158

DEVELOPMENT OF BIOTRANSFORMATION PROTOCOL FOR VALORIZATION OF FOOD INDUSTRIES' WASTE TO PRODUCE COMPOUNDS WITH ENHANCED PHARMACOLOGICAL PROPERTIES

Nikitia Mexia (1,2), Meryem Benohoud (3), Christopher M. Rayner (2,3), Richard S. Blackburn (1,3)

1) Sustainable Materials Research Group, School of Design, University of Leeds, UK
2) School of Chemistry, University of Leeds, UK
3) Keracol Limited, Leeds, UK

Anthocyanins are a large group of phenolic compounds with distinct structural characteristics, well known for their coloring properties, and are the one of the main pigment categories found in nature. They also have interesting pharmacological properties, including antioxidant, anti-inflammatory and anticancer activity [1,2,3]. In general, anthocyanins display a lack of stability [4], but acylated anthocyanins are more stable than their non-esterified counterparts [5]. On the other hand, enzymes have been used extensively over the past decades to catalyze numerous reactions and they offer several advantages such as regio- and enantio-selectivity in reactions, along with the possibility of recovering and recycling the catalyst; they are also potentially eco-friendly and sustainable. Amongst them, lipases can be used for ester formation and hydrolysis on fatty acids, as they are capable of catalyzing both reactions depending on substrate's nature and water availability. In this project we focused on the development of an acylation protocol for natural compounds with enzyme-catalyzed biotransformation reactions and the investigation of the optimal conditions for their evolution by using lipases of microbial origin, aiming ultimately to the valorization of anthocyanin-rich food industries' waste.
The flavonoid disaccharide rutin was chosen as the model system to allow us to establish an efficient, environmentally friendly procedure while offering, simultaneously, the possibility to develop quick, clear and robust methodologies, using all the state-of-the-art techniques for the analysis and purification of the synthesized compounds, which could subsequently be transferred to anthocyanins. After many trials under differentiated conditions, where various factors were modified, we managed to clarify the role of each component individually and come up with the parameters that lead to the higher conversion of our substrate with Lipase B immobilized on acrylic resin to catalyze this reaction. We were then able to translate this method to anthocyanins acylation and evaluate all the produced compounds for their physicochemical as well as their pharmacological properties before attempting to incorporate them in novel industrial products.
As scale-up is the key barrier in biotransformations, the procedure developed in this work can ensure a viable process leading to compounds with ameliorated characteristics that can be formulated in novel cosmeceuticals and nutraceuticals.

References

1) H. Wang et al., J. Nat. Prod. 1999, 62, 294-296
2) A. Rossi et al., Free. Radic. Res. 2003, 37, 891–900
3) N.S. Yarla et al., Semin. Canc. Biol. 2016, 40–41, 48–81
4) L. Mäkilä et al., J. Agric. Food Chem. 2016, 64, 4584–4598
5) C.-L. Zhao et al., Food Chem. 2017, 214, 119–128

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - THERAPEUTIC AREAS

Targeting Aggregated Proteins in Neurodegenerative Diseases with Small Molecules

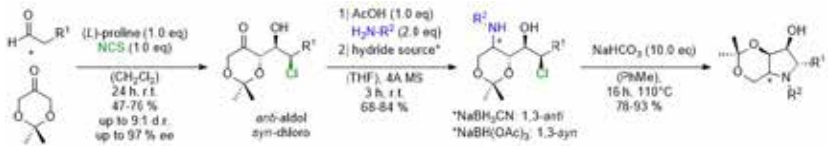
DEVELOPMENT OF NOVEL IMINOCYCLITOL INHIBITORS FOR HUMAN O-GLCNACASE

Johannes Lehmann (1,2), Bharanishashank Adluri (1), Alexandra Males (3), Gideon Davies (3), David Vocadlo (2), Robert Britton (1)

1) Department of Chemistry, Simon Fraser University, Burnaby, Canada
2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, Canada
3) Department of Chemistry, University of York, York, United Kingdom

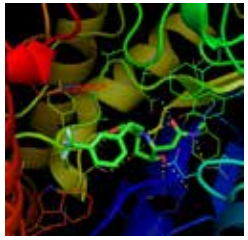
O-glycoside hydrolase (OGA) is an essential physiological enzyme. It catalyzes the hydrolysis of glycosylated proteomic serine- and threonine-residues and plays a key role in neurodegeneration[1] acting on APP and tau-protein of Alzheimer's diseased tissues. Its inhibition has been shown to affect glycosylation levels by preventing aggregation and lowering the toxicity of tau-protein.[2]

Application of an established proline-catalysed tandem α-chlorination aldol-reaction, that is controlled by a dynamic-kinetic resolution, enabled access to a variety of highly enantioenriched chlorohydrin building blocks. Subsequent reductive amination and cyclization readily gave rise to a diverse set of iminocyclitols with full control over three of the four stereocenters (Scheme 1).[3]



In an effort to rapidly expand the library of compounds, we utilize robust reactions such as cross metathesis, alkyne-azide cyclization or reductive amination. Furthermore, late-stage photochemical modifications of C-H bonds developed in our laboratories (i.e. fluorination)[4] are applied to improve overall pharmacokinetic properties of the final molecules.

All compounds are evaluated for in-vitro affinity to the human OGA-enzyme. Structures with promising inhibitory activity are forwarded to our collaborators for co-crystallization. Analysis of the structure revealed binding of the cyclitol backbone to the active site pocket, which competes with the natural substrate and consequently blocks the catalytically active residues (Figure 1).[5]



Our current studies aim to develop a highly potent inhibitor with improved brain permeability and metabolic stability in ongoing mice studies.

References

1) L. S. Griffith, M. Mathes, B. Schmitz, J. Neurosci. Res., 41, 270-8 (1995)
2) F. Liu, K. Iqbal, I. Grundke-Iqbal, G. W. Hart, C. X. Gong, PNAS. 101, 10804-9 (2004)
3) M. Bergeron-Brlek, M. Meanwell, R. Britton, Nat. Commun., 6, 6903-6 (2015)
4) M. B. Nodwell, A. Bagai, S. D. Halperin, R. E. Martin. H. Knust, R. Britton, Chem Commun. 51, 11783-6, (2015)
5) C. Roth, S. Chan, W. A. Offen, G. R. Hemsworth, L. I. Willems, D. T. King, V. Varghese, R. Britton, D. J. Vocadlo, G. J. Davies, Nat. Chem. Bio. 6, 610-2 (2017)

INHIBITION OF PI5P4 KINASES TO UPREGULATE AUTOPHAGY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

Timothy Rooney

Alzheimers Research UK Cambridge Drug Discovery Institute, University of Cambridge, CB2 0AH

Autophagy is a major intracellular process that facilitates the lysosomal degradation of damaged organelles, invasive bacteria and aggregate prone proteins. Upregulation of autophagy has been proposed as a strategy to clear misfolded and aggregated proteins for the treatment of a wide range of neurodegenerative diseases.[1]

Phosphoinositides are a class of membrane phospholipids involved in intracellular signalling mechanisms which are interconverted through phosphorylation and dephosphorylation of the hydroxyl groups on the inositol ring. Phosphatidylinositol 5-phosphate (PI5P) has been shown to increase numbers of autophagosomes and autolysosomes in a dose dependent manner.[2] The activity of the kinases PI5P4K, which convert PI5P to $PI(4,5)P_2$, are closely linked to the cellular levels of PI5P and, as a result, to the autophagic activity of the cell. In support of this, knockdown of PI5P4K has been shown to lead to the clearance of mutant huntingtin (mHTT) aggregates.[2]

Here we describe our work towards the development of inhibitors of the PI5P4K lipid kinases (α, β and γ isoforms). By screening diversity, kinase-focused and fragment libraries using multiple techniques a number of hit compounds were identified for these targets. The resulting inhibitors have been further developed in order to improve potency and selectivity for each isoform of PI5P4K. Compounds with favourable ADME properties, including high oral bioavailability and brain penetration *in vivo*, have been identified. These inhibitors have been shown to increase autophagy and mHTT clearance in cell based assays, supporting this approach as a tractable strategy for the treatment of neurodegenerative diseases.

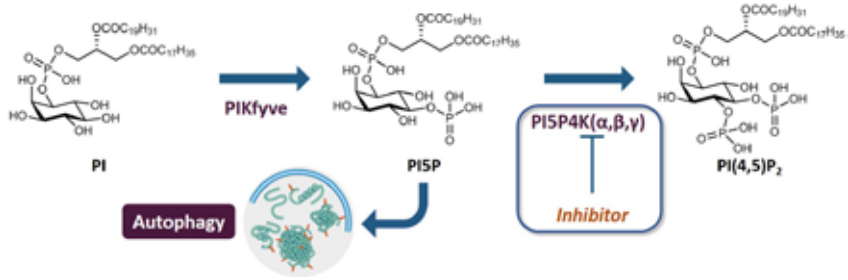


Figure: Inhibition of the lipid kinase PI5P4K leads to accumulation of PI5P, causing an increase in autophagy and the clearance of aggregated proteins.

References

1) Menzies, F. et al., Neuron 2017, 93, 1015-1034
2) Vicinanza, M. et al., Mol. Cell 2015, 57, 219-234

MULTI-TARGET-DIRECTED LIGANDS WITH POTENTIAL DISEASE MODIFYING AND SYMPTOMATIC EFFECTS IN ALZHEIMER'S DISEASE

Anna Wieckowska (1), Tomasz Wichur (1), Justyna Godyn (1), Dawid Panek (1), Jakub Jonczyk (1), Krzysztof Wieckowski (2), Damijan Knez (3), Stanislav Gobec (3), Marcin Kolaczkowski (1), Adam Bucki (1), Monika Marcinkowska (1), Barbara Malawska (1)

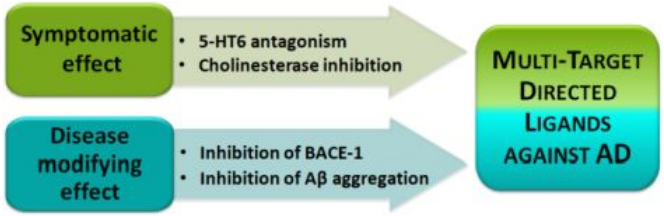
1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jagiellonian University Collegium Medicum, 9 Medyczna Str. 30-688 Krakow, Poland
2) Department of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Collegium Medicum, 9 Medyczna Str. 30-688 Krakow, Poland
3) Faculty of Pharmacy, University of Ljubljana, Askerceva 7, 1000 Ljubljana, Slovenia

The number of people suffering from Alzheimer's disease (AD) is growing and it is estimated to reach 70 million worldwide in 2030. Unless we find an effective therapy [1]. According to the current state of knowledge, it is unlikely to indicate a single therapeutic mechanism that would allow to cure AD. Therefore, in our studies we applied multi-target-directed ligands (MTDLs) strategy [2]. Selection of the adequate biological targets for MTDLs gives the opportunity to develop drugs that would treat both the causes and symptoms of the disease.

According to amyloid hypothesis, aggregation of amyloid β (Aβ) is a crucial pathognomonic process in AD. Aβ is a product of proteolytic cleavage of the amyloid precursor protein by β secretase (BACE-1) and γ-secretase. In AD, Aβ peptides aggregate to soluble oligomers and insoluble plaques causing multidirectional neurotoxicity. Inhibition of the synthesis and the aggregation of Aβ are the most attractive approaches in the development of amyloid-lowering therapies - a promising disease modifying treatment [3].

Substantial cholinergic deficits observed in the brains of AD patients led to cholinergic hypothesis of AD and the development of cholinesterase inhibitors (donepezil, rivastigmine, galantamine) as the mainstay of AD pharmacotherapy. Inhibition of cholinesterases increases the cholinergic neurotransmission and masks the cholinergic deficits. This effect, however, is only temporary, limited to 6–12 month delay in the progress of the disease. An improvement of this therapy was shown in clinical trials with 5-HT_6 receptor antagonist (idalopirdine) as the add-on therapy [4]. 5-HT_6 antagonism is a complementary mechanism that potentiate acetylcholine release but may also alleviate behavioral and psychological symptoms, thereby significantly improving the quality of life in AD patients.

Herein, we present the design, synthesis and biological evaluation of a series of MTDLs, that on one hand can have disease modifying effect by inhibition of BACE-1 and Aβ aggregation, and on the other hand by cholinesterase inhibition and 5-HT_6 receptor antagonism can release symptoms of Alzheimer's disease.



Acknowledgments: this work was supported by the National Science Centre, Poland, grant number 2016/23/D/NZ7/01328 and 2016/21/B/NZ7/01744.

References

1) M. Prince et. al Alzheimer's Dis. Int. (2015) 84.
2) R. Morphy, Z. Rankovic J. Med. Chem. 48 (2005) 6523–6543.
3) D.J. Selkoe, J. Hardy EMBO Mol. Med. 8 (2016) 595–608.
4) D. Wilkinson et. al Lancet. Neurol. 13 (2014) 1092–9.

NOTES

POSTERS - THERAPEUTIC AREAS

Breakthroughs in Polypharmacology Towards Neurological Disorders

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF THE FIRST DUAL MODULATORS OF DOPAMINE D3 RECEPTOR AND GSK-3β AS PROMISING AND INNOVATIVE TOOLS FOR BIPOLAR DISORDER TREATMENT

Rita M. C. Di Martino (1), Debora Russo (2), Ilaria Penna (2), Alessio De Simone (3), Tiziano Bandiera (2), Giovanni Bottegoni (4), Andrea Cavalli (1,5)

1) Computational and Chemical Biology, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova (GE), ITALY
2) PharmaChemistry Line, Istituto Italiano di Tecnologia, via Morego 30, 16163 Genova (GE), ITALY
3) EaStCHEM School of Chemistry, Joseph Black Building, David Brewster Road, EH9 3FJ Edinburgh, United Kingdom (current)
4) Heptares Therapeutics Ltd, BioPark, Broadwater Road, AL7 3AX, Welwyn Garden City, United Kingdom (current)
5) FaBit, University of Bologna, via Belmeloro 6, 40126 Bologna (BO), ITALY

Bipolar affective disorder (BD) is a neuropsychiatric disorder characterized by spontaneously alternating episodes of mania and depression.[1]

With an estimated worldwide prevalence up to 4 %, BD is one of the leading causes of disability. The effectiveness of the standard care is limited (currently used medications only treat symptoms) and it is oftentimes associated with significant side effects. Hence, there is an urgent need for the discovery of truly disease-modifying drugs for BD.[2,3]

Dopamine D3 receptor (D3R) and glycogen synthase kinase-3β (GSK-3β) are structurally unrelated targets that, through independent physiological pathways, are believed to play a crucial role in cognition and mood. In this work, inspired by the idea that the concurrent modulation of these targets could represent a viable strategy for achieving an effective BD treatment, we applied our recently reported multi-target directed ligands rational design approach.[4,5] In particular, combining computer-aided drug design protocols, synthetic efforts, and *in vitro* pharmacological evaluation, we developed the first set of analogues endowed with both partial agonist efficacy at D3R and potent inhibitory activity against GSK-3β (**Figure 1**).

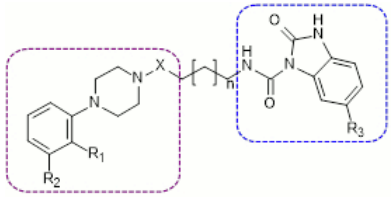


Figure 1. General structure of the newly synthesized dual D3R and GSK-3β modulators.

References

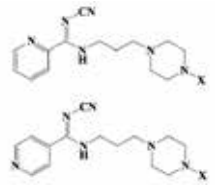
1) Ashok A. H. et al. The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. Molecular Psychiatry 2017, 22 (5), 666-679.
2) Harrison P. G. et al. Innovative approaches to bipolar disorder and its treatment. Ann. N.Y. Acad. Sci. 2016, 1366 (1), 76-89.
3) Beyer D. K. E. and Freund N. Animal models for bipolar disorder: from bedside to the cage. Int. J. Bipolar Disord. 2017, 5 (1):35, 1-26.
4) De Simone A. et al. Applying a multitarget rational drug design strategy: the first set of modulators with potent and balanced activity toward dopamine D3 receptor and fatty acid amide hydrolase. Chem. Commun. (Camb). 2014, 50 (38), 4904-7.
5) De Simone A. et al. Design, synthesis, structure-activity relationship studies, and three-dimensional quantitative structure-activity relationship (3D-QSAR) modeling of a series of O-biphenyl carbamates as dual modulators of dopamine D3 receptor and fatty acid amide hydrolase. J. Med. Chem. 2017, 60 (6), 2287-2304.

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SEROTONINERGIC LIGANDS CONTAINING N'-CYANOPICOLINAMIDINE AND N'-CYANOISONICOTINAMIDINE FRAGMENTS

Elisa Magli (1), Ferdinando Fiorino (1), Angela Corvino (1), Beatrice Severino (1), Elisa Perissutti (1), Francesco Frecentese (1), Irene Saccone (1), Vincenzo Santagada (1), Giuseppe Caliendo (1), Ewa Kedzierska (2), Jolanta Orzelska-Gorka (2), Jolanta Helena Kotlinska (2), Paola Massarelli (3)

1) Department of Pharmacy - University of Naples Federico II - Via D. Montesano, 49, 80131 Naples, Italy
2) Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, 20-093 Lublin, Poland
3) Departiment of Scienze Mediche, Chirurgiche e Neuroscienze University of Siena - Strada delle Scotte, 6 - 53100 Siena, Italy

Serotonin (5-hydroxytryptamine, 5-HT), one of the most important neurotransmitter in the central and peripheral nervous systems (CNS and PNS, respectively), has been implicated in numerous physiological and physiopathological processes. Serotonin receptors may be involved in the regulation of impulsivity and alcoholism, in the different phases of sleep, sexual behavior, appetite control, thermoregulation, cardiovascular function and recently it has been found to show growth-promoting activity and to be functionally related to oncogenes. In particular 5-HT_{2C} receptor subtype is considered to be an attractive target for the design of novel drugs for treatment of CNS-related diseases such as obesity, obsessive compulsive disorders and sexual dysfunction. Furthermore, the 5-HT_{2C} receptor displays multiple actions on various neurotransmitters and receptors; abnormalities of 5-HT_{2C} receptors are associated with psychiatric diseases such as depression, schizophrenia, drug abuse, anxiety and eating disorder. Finally it's already known that 5-HT_{2C} blockade can prevent the extrapyramidal side effects induced by atypical antipsychotics. Several chemical classes of agents are already known for their high affinity toward 5-HT receptors (aminotetralines, ergolines, arylpiperazine, indolylalkylamines, indoles, etc.) and one of the most studied group is the long-chain arylpiperazine (LCAPs) one, that have provided interesting drugs acting on CNS (Buspirone) and compounds with a potential therapeutic profile (Flesinoxan). In continuation of our research program, we designed and synthesized new set of derivatives where the piperazine-N-alkyl moiety has been linked, via three methylene spacing units, to a N'-cyanopicolinamidine or N'-cyanoisonicotinamidine fragments as terminal part of LCAPs (Figure 1).



The multireceptor profiles of promising new N'-cyanopicolinamidine or N'-cyanoisonicotinamidine derivatives towards 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors were also evaluated in terms of binding affinities for D1, D2 and α1, α2 receptors. The binding data presented in this study have shed additional light on the influence of the LCAPs on the 5-HT receptors affinity and selectivity. Finally, compounds with a better affinity/selectivity profile towards 5-HT_{2C} have been evaluated by *in vivo* assay (e.i. behavioural tests), to determine their functional activity.

References

1) Fiorino, F.; Severino, B.; Magli, E.; Ciano, A.; Caliendo, G.; Santagada, V.; Frecentese, F.; Perissutti, J. Med. Chem. 2014, 57(11), 4407-4426.
2) Fiorino, F.; Severino, B.; Magli, E.; Perissutti, E.; Frecentese, F.; Esposito, A.; Incisivo, G. M.; Ciano, A.; Massarelli, P.; Nencini, C.; Santagada, V.; Caliendo, G. Eur. J. Med. Chem. 2012, 47, 520-529.
3) Kimura, Y.; Hatanaka, K.; Naitou, Y.; Maeno, K.; Shimada, I.; Koakutsu, A.; Wanibuchi, F.; Yamaguchi, T. Eur. J. Pharmacol. 2004, 483(1), 37-43.
4) Fiorino, F.; Severino, B.; De Angelis, F.; Perissutti, E.; Magli, E.; Frecentese, F.; Esposito, A.; Massarelli, P.; Nencini, C.; Santagada, V.; Caliendo, G. Bioorganic Med. Chem. Lett. 2010, 20, 2978-2982.

DESIGN OF COMPOUNDS THROUGH COUPLING ANTIOXIDANT ACIDS WITH CNS-ACTING MOIETIES FOR NOOTROPIC ACTIVITY

Georgios Papagiouvannis, Panagiotis Theodosis-Nobelos, Christina Damianidou, Anastasia Gerasimidou, Ioanna Gouliou, Eleni Rekka

Department of Medicinal Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Alzheimer's Disease (AD) is the most common neurodegenerative disorder and a major health problem to society, with a rising number of patients worldwide. AD has a multifactorial character and develops as a complex network of interconnected events leading to the evolution of the disease. Thus, the concept of the multitarget approach is particularly applicable to AD.

Oxidative stress is one of the main causes of neuronal death in AD and oxidative damage is a key process in AD pathogenesis. Increased hydrogen peroxide formation and elevated free iron concentrations, due to decreased amount of ferritin, observed in AD patients, generate more reactive oxygen species (ROS). ROS oxidise lipids and damage membranes in the AD brain. Protein, DNA and RNA oxidation products are increased in several brain regions in AD patients.

Inflammation and glia activation are observed in AD patients, thus, inflammation is a key target in AD drug development. 5-Lipoxygenase is overexpressed in AD and contributes to neuronal vulnerability.

Proline amides have been found to improve cognition deficits induced by neurodegenerative diseases. GABA is the main inhibitory neurotransmitter in brain and decreased GABA levels have been detected in brain regions of patients with AD, suggesting that abnormalities of the GABAergic system may also contribute to the pathogenesis of AD.

In this research, we have designed and synthesised novel compounds that contain phenolic acids with antioxidant activity, such as trolox or ferulic acid, and moieties, such as proline and GABA, aiming to multitarget ligand design for AD. The compounds were synthesized by amidation of acids using N,N-dicyclohexylcarbodiimide or carbonyldiimidazole as coupling agents. They were purified by flash column chromatography and identified (^{1}H-NMR, ^{13}C-NMR, MS).

The synthesised compounds were found to have in vitro antioxidant activity as lipid peroxidation inhibitors (IC_{50} values as low as 1.5μM) and DPPH radical scavengers, to inhibit lipoxygenase activity and to exert in vivo anti-inflammatory activity, assessed as paw oedema reduction (40-55% inhibitory activity). Furthermore, some introductory calculations concerning the blood-brain-barrier penetration were performed, in order to obtain an indication of their ability to enter the brain.

With the design of the described derivatives we aimed to compounds that would acquire a series of biological properties able to prevent or restore a number of pathological changes implicated in AD and appearing in the demented brain. This study has demonstrated that, in general, the synthesised compounds possess a combination of the desired properties integrated in their molecules.

G. Papagiouvannis and P. Theodosis-Nobelos acknowledge the General Secretariat for Research and Technology (GSRT) of Greece and the Hellenic Foundation for Research and Innovation (HFRI) for a grant supporting their PhD research.

IDENTIFICATION OF NOVEL DJ-1 TARGETING SMALL MOLECULES WITH PROTECTIVE ACTIVITY IN CELLULAR AND IN VIVO MODELS OF PARKINSON'S DISEASE

Gergely Tóth (1,5), Balázs Herberth (1), Balázs Fórizs (1), Eva Moravcsik (1), Fanni Tolnai (1), Jean-Christophe Rochet (2), Gennady Smagin (3), Thomas Neumann (4)

1) Cantabio Pharmaceuticals, Sunnyvale, California, USA
2) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana, United States of America
3) Melior Discovery, Exton, Pennsylvania, United States of America
4) Novalix SAS, Illkirch Cedex, France
5) MTA-TTK-NAP B - Drug Discovery Research Group – Neurodegenerative Diseases, Institute of Organic Chemistry, Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Familial mutations in the DJ-1 gene have been linked to the early-onset of Parkinson's disease (PD). Moreover, results from studies of neurotoxicant- and alpha synuclein-based *in vivo* PD models suggest a role for DJ-1 in sporadic PD. Herein, we describe a drug discovery approach to identify small molecule therapeutic candidates for the treatment of PD by targeting DJ-1. Our approach is based on the concept that specific binding of small molecules to wild-type native dimeric DJ-1 can result in enhancing DJ-1 function under oxidative stress conditions in PD. Our drug discovery approach involved the use of a high-throughput chemical microarray surface plasmon resonance imaging method to screen over 110,000 immobilized drug-like fragments and lead-like compounds to detect the binding between small molecules and the DJ-1 protein. This screen identified a novel set of drug-like fragment and lead-like compounds that bound to DJ-1 protein. We report herein on one selected hit compound, and its analogues, which had substantial biological activity in cellular and *in vivo* models of oxidative stress. A selected analogue compound alleviated neuroblastoma cell toxicity and dopaminergic neuronal loss mediated by paraquat, MPP+, 6-OHDA and MG132 treatment. In addition, this compound protected from dopamine loss in a MPTP mice model of Parkinson's disease when administered orally. Moreover, differential scanning fluorimetry studies showed that this compound increased the melting temperature of native DJ-1 suggesting that the compound can stabilize the protein. In conclusion, our studies show that the DJ-1 protein can be targeted by a variety of drug-like small molecules, and that the presented selected compound is a novel biologically active DJ-1 modulator that serves as a promising drug candidate for further optimization and development for the treatment of PD.

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - THERAPEUTIC AREAS

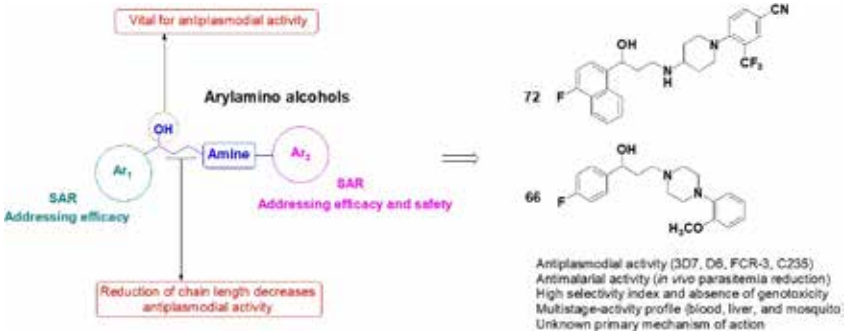
Addressing Infectious Diseases in the Developing Countries

STRUCTURE-ACTIVITY RELATIONSHIP OF NEW ANTIMALARIAL 1-ARYL-3-SUSBTITUTED PROPANOL DERIVATIVES: SYNTHESIS, PRELIMINARY TOXICITY PROFILING AND PARASITE LIFE CYCLE STAGE STUDIES

Miguel Quiliano (1), Adriana Pabón (2), Leonardo Bonilla-Ramirez (2), Diego A. Nieto-Acoa (1), Ariane Vettorazzi (5), Adela López de Cerain (5), Eric Deharo (3), Giovanny Garavito (4), Ignacio Aldana (1), Silvia Galiano (1)

1) Universidad de Navarra, Facultad de Farmacia y Nutrición, Departamento de Química Orgánica y Farmacéutica, Campus Universitario, 31008 Pamplona, Spain
2) Grupo Malaria, Universidad de Antioquía, Medellín, Colombia
3) UMR 152 PHARMA-DEV, Université Toulouse, IRD, UPS, 31062, Toulouse, France
4) Universidad Nacional de Colombia, Sede Bogotá, Facultad de Ciencias, Departamento de Farmacia (DFUNC), Grupo de investigación FaMeTra (Farmacología de la Medicina tradicional y popular), Carrera 30 45-03, Bogotá D.C., Colombia
5) Universidad de Navarra, Facultad de Farmacia y Nutrición, Department of Pharmacology and Toxicology, Campus Universitario, 31008 Pamplona, Spain

The design, synthesis, structure-activity relationship, cytotoxicity studies, *in silico* drug-likeness, genotoxicity, and *in vivo* studies of new 1-aryl-3-substituted propanol derivatives led to the identification of nine compounds with a promising *in vitro* and *in vivo* antimalarial profile against *Plasmodium falciparum*. In general compounds exhibited potent antiplasmodial activity against chloroquine resistant strain FCR-3 (IC_{50}s < 0.28 µM). Meanwhile, the most active compounds showed potent antimalarial activity in chloroquine sensitive and multidrug resistant strains (IC_{50}s < 0.7 µM for 3D7, D6, FCR-3 and C235). All of them share appropriate drug-likeness profile, adequate selectivity index (77 < SI < 184), and absence of genotoxicity. *In vivo* efficacy in mouse model showed two compounds as promising candidates exhibiting a significant parasitemia reduction (80.4 - 96.4 %). Additional studies such as liver stage and sporogony inhibition, target exploration of Hsp90 P. falciparum, targeted delivery by immunoliposomes, and enantiomer characterization were performed and strongly reinforce the hypothesis of APD as promising antimalarial compounds.



THE DEVELOPMENT OF NEW TREATMENTS FOR MULTI-DRUG RESISTANT TUBERCULOSIS

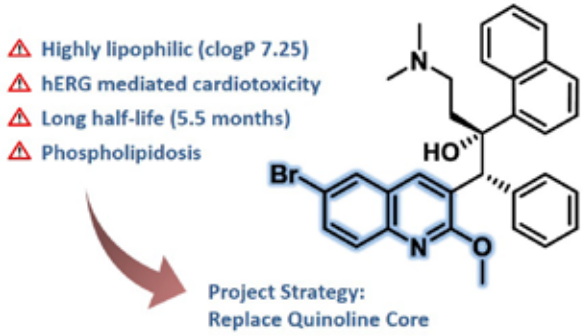
Lisa Barbaro (1), Daniel Preibbenow (1), Gayathri Nagalingam (2), Jamie Triccas (2), Jonathan Baell (1)

1) Monash Institute of Pharmaceutical Sciences, Melbourne, Australia
2) University of Sydney, Sydney, Australia

Tuberculosis (TB) is the curable disease that continues to kill, fuelled by the recent increase in multi-drug resistant infections.[1] In response to the urgent need to combat the rise of resistant infections, the novel diarylquinoline drug Bedaquiline (BDQ) received accelerated approval from the FDA in 2012. Despite being highly effective against drug-resistant TB as a result of its unique mode of action (inhibition of mycobacterial ATP-synthase),[2,3] BDQ has been associated with significant toxicities and issues (hERG mediated cardiotoxicity, phospholipidosis, long half-life) and as such, safety concerns are limiting its clinical use.[1]

The key objective of this project was to synthesise novel and distinct analogues of BDQ with modified structural features, designed to retain high potency whilst improving the safety profile and limiting current side effects. To date, a series of analogues have been synthesised and examined for their activity.

This presentation will outline the development of the synthetic pathways utilised to access these analogues, with our initial focus being on the replacement of the quinoline core. The latest results on the activity of these modified BDQ analogues will also be presented.



References

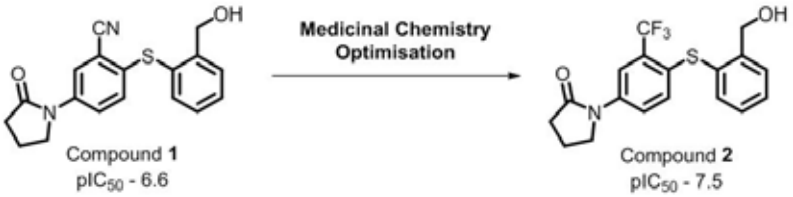
1) World Health Organisation, Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis, Geneva, Swizerland, 2014.
2) K. Andries, P. Verhasselt, J. Guillemont, H. W. H. Göhlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, et al., Science 2005, 307, 223–7.
3) L. Preiss, J. D. Langer, O. Yildiz, L. Eckhardt-Strelau, J. Guillemont, A. Koul, T. Meier, Sci. Adv. 2015, 1, e1500106–e1500106.

HIT-TO-LEAD OPTIMISATION: SINGLE AGENTS FOR THE TREATMENT OF CHAGAS DISEASE

Julia Beveridge (1), Jonathan Baell (1), Jitendra Harjani (1), Albane Kessler (2)

1) Monash Institute of Pharmaceutical Sciences, Parkville, Vic, Australia
2) GSK, Tres Cantos, Madrid, Spain

In 2014, GSK performed a high-throughput-screen of 1.8 million compounds against three kinetoplastid parasites. This data was published as an open source in an effort to encourage research and drug development for these neglected diseases.[1] A simple arylthioether compound (compound **1**) was found to have desirable activity against *Trypanosoma cruzi* (the parasite responsible for Chagas disease) and was selected as the hit compound for this project. The initial investigation led to the discovery of an even more potent compound, with a superior pIC_{50} of 7.5 (compound **2**).



This class of compounds showed promising results in acute *in vivo* efficacy studies and even more potent compounds have been developed since. However, several issues have been identified for this chemical series, such as toxicity and low oral exposure. A full toxicity study was undertaken and several alerts were identified that relate to CNS and cardiovascular toxicity. In order to address these concerns, future analogues have been focused to decrease toxicity and increase exposure. This will be achieved by exploring lipophilicity, solubility and increasing microsomal stability.

References

1) Pena I, Pilar Manzano M, Cantizani J, Kessler A, Alonso-Padilla J, Bardera AI, et al. New Compound Sets Identified from High Throughput Phenotypic ScreeningAgainst Three Kinetoplastid Parasites: An Open Resource. Sci Rep. 2015; 5

FOCUS LIBRARY OF QUINOXALINE 1,4-DI-N-OXIDE DERIVATIVES AS FASCIOLA HEPATICA CATHEPSIN L INHIBITORS

Florencia Ferraro (1,2), Alicia Merlino (2), Ileana Corvo (1), Mauricio Cabrera (1)

1) Laboratorio de I + D de Moléculas Bioactivas, CENUR Litoral Norte, Universidad de la República. Paysandú, Uruguay.
2) Laboratorio de Química Teórica y Computacional, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.

Increased reports of human infections have led fasciolosis, a widespread disease of cattle and sheep caused by the liver flukes Fasciola hepatica and F. gigantica, to be considered an emerging zoonotic disease. Chemotherapy is the main control measure available, and triclabendazole is the preferred drug since is effective against both juvenile and mature parasites. However, resistance to triclabendazole has been reported in several countries urging the search of new chemical entities and target molecules to control fluke infections.

Quinoxaline 1,4-di-*N*-oxides derivatives has been described such as antitubercular, antimalarial, antileishmania, antichagas among others neglected diseases but to the best of our knowledge, no one has evaluated them as fasciolicidal agents. Taking all of this into account and as a continuation of our search of new anthelmintic drugs from our in-house chemical library we selected a serie of twenty-eight quinoxaline 1,4-di-*N*-oxides derivatives in order to study their ability to inhibit essential cathepsin L of *Fasciola hepatica*.

We have identified four quinoxaline 1,4-di-*N*-oxides derivatives as novel inhibitors of the two main cathepsins secreted by juvenile and adult liver flukes that interestingly were active towards the newly excysted juveniles juvenile.

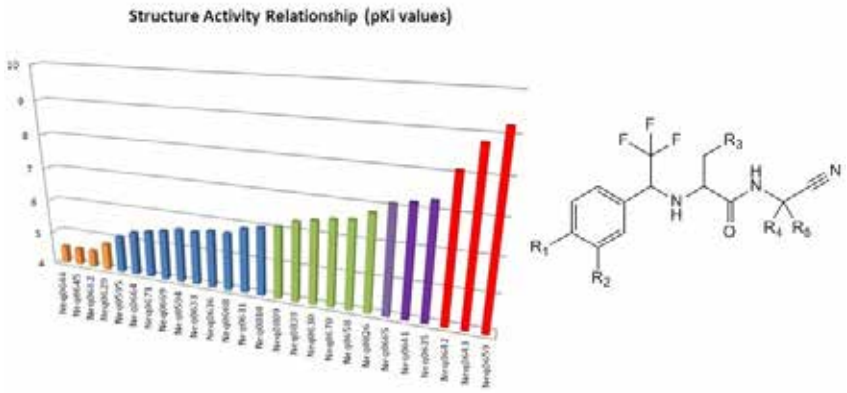
These findings open new avenues for the development of novel agents to control fluke infection and possibly other helminthic diseases.

ANTI-TRYPANOSOMAL ACTIVITY OF NON-PEPTIDIC NITRILE-BASED CYSTEINE PROTEASE CRUZAIN INHIBITORS

Lorenzo Cianni (1), Daniela De Vita (1), Juliana C. Gomes (1), Jean Ribeiro (1), Samelyn Da Costa (1), Martins Silva (2), Pedro H. J. Batista (1), Carla Duque (2), Carolina Moraes (2), Caio H. Franco (2), Lucio H. G. Freitas (2), Sergio de Alburqueque (2), Andrei Leitao (1), Peter Kenny (1), Antonio C. B. Burtoloso (1), Carlos A. Montanari (1)

1) Institute of Chemistry of Sao Carlos, University of Sao Paulo, Av. Trabalhador Sancarlense, 400, 13566-590 - Sao Carlos/S, Brazil
2) Faculty of Pharmaceutical Science of Riberao Preto, University of Sao Paulo, Avenida do Cafe s/n Monte Alegre, 14040-903 – Ribeirao Preto, SP, Brazil

Chagas disease, caused by the protozoan parasite Trypanosoma cruzi, remains a serious health problem due to inadequate therapy and lack of an effective vaccine. New drugs that are safe and efficacious are critically needed.Cruzipain (Cz) the major cysteine protease of the T.cruzi, is the most studied biological target for Chagas disease; three dimensional structures of the enzyme with a variety of ligands have been resolved.Our recent study1 showed key interactions of P1, P2 and P3 portion of dipeptidyl nitrile ligands with the respective subsites S1, S2 and S3 of the enzyme. Specifically, it is already known the importance of hydrogen bonds between the ligand and residues Asp161, Gly66 and His162 located in S2 and S1 subsites, respectively, for the inhibition of Cz 2. However dipeptidyl nitrile compounds lack of metabolic stability and selectivity. Therefore we replaced the amide group in P3/P2 with trifluoromethyl amine group. So we designed, synthesized and characterized over 25 different non-peptidic nitrile based compounds, in order to perform an accurate and extensive SAR based on kinetic assays values (pKi); in particular we evaluated the importance of stereochemistry in the recognition and inhibition process. Furthermore all compounds were tested in vitro against T.cruzi and their logP were measured using RLPC. Compounds Neq0683, Neq0684 and Neq0659 showed the ability to inhibit the Cz in a nanomolar scale, while compounds Neq0662 and Ne0663presented antitripanosomal activity in the low micromolar range.



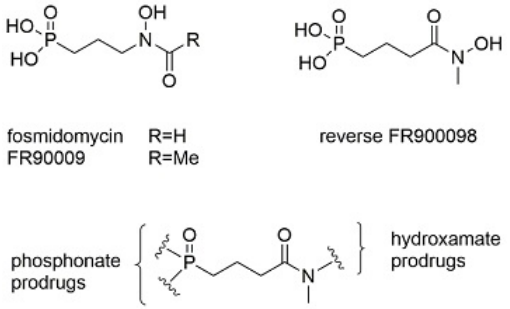
References

1) Wiggers, Helton J., et al. "Non-peptidic cruzain inhibitors with trypanocidal activity discovered by virtual screening and in vitro assay." PLoS neglected tropical diseases 7.8 (2013): e2370. Aug2013. PLoS NTD
2) Avelar, Leandro AA, et al. "Molecular design, synthesis and trypanocidal activity of dipeptidyl nitriles as cruzain inhibitors." PLoS neglected tropical diseases 9.7 (2015): e0003916

FOSMIDOMYCIN ANALOGS AS ANTIMALARIAL AND ANTITUBERCULAR AGENTS - A PRODRUG APPROACH

Charlotte Courtens, Martijn Risseeuw, Serge Van Calenbergh

Laboratory for Medicinal Chemistry, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium



Antimalarial and antitubercular agents with new mechanisms of action are necessary to tackle infections by *Plasmodium* parasites and *Mycobacteria* that are resistant to current therapies. Fosmidomycin has been shown to be a well-tolerated, safe and efficacious antimalarial drug in combination treatment. However, its pharmacokinetic (PK) properties are less than ideal, with only moderate bioavailability and a short plasma half-life. Moreover, because of the unique highly lipophilic cell wall of *Mycobacteria*, fosmidomycin cannot cross the cell wall and thus, is not active against *Mycobacteria*.

A lot of research has been dedicated to improve the potency of fosmidomycin analogs. However, the problem of low bioavailability remains. The development of hydrophobic phosphonate and/or hydroxamate prodrugs of fosmidomycin could improve both oral bioavailability and cell penetration by passive diffusion. To date, only acyloxymethyl- and alkoxy-carbonyloxymethyl phosphonate prodrugs have been reported, both with only moderate in vivo activity. The aim of this research is to design and synthesize a broad range of potential prodrugs with different bioactivation mechanisms in order to enhance in vivo antimalarial and antitubercular activity as a result of optimized PK properties.

This research demonstrates that a prodrug approach may allow to convert fosmidomycin into agents with improved permeability characteristics, opening avenues for its use as antimalarial and/or antitubercular drug. Further optimization of the prodrug pro-moiety is however still needed to obtain more potent analogs, especially with regard to whole cell antitubercular activity.

References

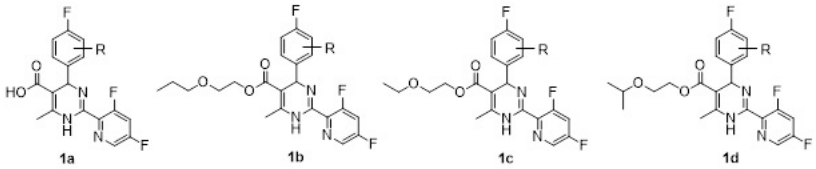
1) Mombo-Ngoma, G.; Remppis, J.; Sievers, M.; Zoleko Ma-nego, R.; Endamne, L.; Kabwende, L.; Veletzky, L.; Ngu-yen, T. T.; Groger, M.; Lötsch, F.; et al. Efficacy and Safety of Fosmidomycin–Piperaquine as Nonartemisinin-Based Combination Therapy for Uncomplicated Falciparum Malaria: A Single-Arm, Age De-Escalation Proof-of-Concept Study in Gabon. Clin. Infect. Dis.
2) Murakawa, T.; Sakamoto, H.; Fukada, S.; Konishi, T.; Nishida, M. Pharmacokinetics of Fosmidomycin, a New Phosphonic Acid Antibiotic. Antimicrob. Agents Chemother. 1982, 21 (2), 224–230.
3) Brown, A. C.; Parish, T. Dxr Is Essential in Mycobacte-rium Tuberculosis and Fosmidomycin Resistance Is Due to a Lack of Uptake. BMC Microbiol. 2008, 8, 78.
4) Masini, T.; Hirsch, A. K. H. Development of Inhibitors of the 2C-Methyl-d-Erythritol 4-Phosphate (MEP) Pathway Enzymes as Potential Anti-Infective Agents. J. Med. Chem. 2014, 57 (23), 9740–9763.
5) Ponaire, S.; Zinglé, C.; Tritsch, D.; Grosdemange-Billiard, C.; Rohmer, M. Growth Inhibition of Mycobac-terium Smegmatis by Prodrugs of Deoxyxylulose Phos-phate Reducto-Isomerase Inhibitors, Promising Anti-Mycobacterial Agents. Eur. J. Med. Chem. 2012, 51, 277–285.
6) Brücher, K.; Gräwert, T.; Konzuch, S.; Held, J.; Lienau, C.; Behrendt, C.; Illarionov, B.; Maes, L.; Bacher, A.; Wittlin, S.; et al. Prodrugs of Reverse Fosmidomycin Analogues. J. Med. Chem. 2015, 58 (4), 2025–2035.

SYNTHESIS AND EVALUATION OF 1,4-DIHYDROPYRIMIDINONE DERIVATIVES - HEPATITIS B VIRUS CAPSID SELF-ASSEMBLY INHIBITORS

Anda Sīpola (1), Unda Nelda Dubova (2), Brigita Vīgante (1), Karīna Spunde (2), Tatjana Kozlovska (2), Gunārs Duburs (1)

1) Latvian Institute of Organic Synthesis, Riga, Latvia
2) Latvian Biomedical Research and Study Centre, Riga, Latvia

At least 2 billion people have been exposed to date to the Hepatitis B virus (HBV), according to World Health Organization. HBV exposure leads to chronic infection in 85-95% of infected neonates/children and 5-15% of infected adults [1]. HBV infection is especially heavy burden for the developing countries [2]. Life-long nucleos(t)ide therapy is necessary, but still the complete cure has not been achieved. Effective approaches to chronic hepatitis B virus require complete suppression of viral replication. The HBV capsid protein (Cp) is an essential component and regulator of the HBV life cycle and it has been recognized as an attractive antiviral target. Now heteroaryldihydropyrimidines (HAP) are emerged as a promising class of Cp targeted antivirals. The first HAP compound Bay 41-4109 affects Cp assembly and leads to irregular particles and causes protein degradation. Structural optimizations have been done by structure-activity relationship studies [3;4].



So far, we have found structures that partially (**1a** and **1b**) or even dramatically (**1c** and **1d**) suppress HBV replication *in vitro*, presumably, through different mechanisms of action. Direct analysis of intracellular Cp assembly products using native agarose gel electrophoresis showed, that compounds **1a** and **1d** diminish production of correct HBV capsids, and along with increase of HAP concentration accumulation of misassembled Cp particles have not been detected. On the contrary, **1c** and **1b** derivatives induce production of Cp aggregates.

Acknowledgements: Support by Latvian State Programme „Biomedicine 2014-2018” is gratefully acknowledged.

References

1) Diab, A. et.al. (2018) Antivir. Res. 149, 211
2) Zampino, R. et. al. (2015) World J. Gastroent. 21, 11941
3) Zhou, Z. et. al. (2017) Sci. Rep. 7, 42374
4) Qiu, Z. et. al. (2016) J. Med. Chem. 59, 7651

METAL-CHELATING ACETOHYDROXAMIC ACIDS AGAINST HEPATITIS C VIRUS AND FLAVIVIRUSES

Erofili Giannakopoulou (1), Vasiliki Pardali (1), Efseveia Frakolaki (2), Vassilios Myrianthopoulos (1,3), Emmanuel Mikros (1,3), Ralf Bartenschlager (4), Niki Vassilaki (2), Grigoris Zoidis (1)

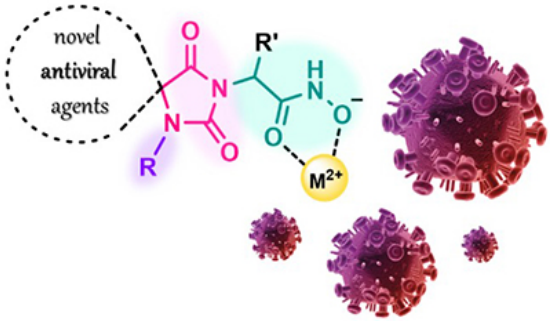
1) School of Health Sciences, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Greece
2) Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece
3) Athena Research Center, Athens, Greece
4) Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Germany

Hepatitis C Virus (HCV) infections pose a major public health threat globally, with infected individuals being at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. There is no vaccine available and despite advances in current chemotherapy, the global burden of HCV infections remains high, due to their partial effectiveness or resistance. The flaviviruses Dengue (DENV), Yellow fever (YFV), and Zika (ZIKV) cause diseases ranging from mild febrile illness to severe encephalitis or hemorrhagic syndromes. Despite the extensive research on flaviviral diseases, there is no clinically approved therapy, thus, they constitute high priority targets for drug discovery. Because of all the above and based on literature reports on metal-chelating agents inhibiting HCV NS5B-polymerase,[1] the development of novel scaffolds of metal-chelators with antiviral properties was undertaken.

By utilizing docking-scoring calculations, structural insight regarding HCV inhibition was obtained, prompting the rational design and synthesis of novel carbocyclic-(spiro)substituted hydantoin-derivatives, bearing the acetohydroxamic acid metal-chelating group upon the imidic nitrogen, and a variety of lipophilic substitutions at the amidic nitrogen.

The compounds were evaluated for their effect on HCV RNA replication and cell viability (ATP and luciferase assays), exhibiting EC_{50} values ranging from 0.08 to 4.50 μM, in Huh7 reporter subgenomic replicon cell lines of genotype 1b, and remarkable Selectivity Indexes rising up to 781. As flaviviruses are members of the Flaviviridae family, along with HCV, and share several similarities among their homologous metalloenzymes (NS5B/NS5 RNA-dependent RNA polymerase and NS3 protease/helicase)[2],[3] prompted us to evaluate the most potent anti-HCV compounds against DENV, YFV and ZIKV.

The preliminary anti-flaviviral results, of low μM EC_{50} values, observed for many compounds (EC_{50} 0.07 μM, 2.76 μM, and 0.44 μM for DENV, YFV and ZIKV respectively) are highly encouraging and, along with theoretical simulations, suggest that the novel framework of metal-chelators we developed, offers a highly promising starting point for the design of potent and broadly effective antiviral agents with dual-target potential. Analysis of resistance mutations and modeling studies are currently underway to further characterize their inhibition mechanism.



References

1) Sofia M.J., Chang W., Furman P.A., Mosley R.T., Ross B.S. J. Med. Chem. 2012, 55, 2481-531
2) Caillet-Saguy C., Lim S.P., Shi P.Y., Lescar J., Bressanelli S. Antiviral Res. 2014, 10, 8-16
3) Palanisamy N., Akaberi D., Lennerstrand J. Mol. Phylogenet. Evol. 2018, 118, 58-63

OPTIMIZING THE TORIN SCAFFOLD AS A DUAL-STAGE ANTIMALARIAL: TOWARDS PARASITE SELECTIVITY

Jorge Grilo (1,2), Lara Fidalgo (1), André Dias (1), Pedro Gonçalves (1), Diana Fontinha (2), Miguel Prudêncio (2), Ana S. Ressurreição (1)

1) iMed.ULisboa – Research Institute for Medicines, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
2) iMM – Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal

Malaria, a mosquito-borne infectious disease, caused by protozoan parasites of the Plasmodium genus, is an endemic disease in most tropical regions of the globe that still represents a major public health problem with nearly half a million deaths reported just in 2016.[1] Plasmodium infection progresses initially through a liver stage of parasite development, followed by a blood stage cycle, responsible for disease symptoms. Moreover, two species of Plasmodium, P. vivax and P. ovale, can remain latent in infected hepatic cells and are responsible for relapses and therapeutic failure.[2]

Despite the existing therapeutic arsenal, parasite resistance is an established concern for most drug classes, with growing reports of increased tolerance to artemisinin in some parasite strains. These hurdles clearly demonstrate the necessity for the development of drugs displaying novel mechanisms of action that can overcome both the resistance cases and fill the existing void of liver stage active compounds. [3]

Torin2, an ATP-competitive mTOR kinase inhibitor, has been recently disclosed as a potent antimalarial with in vivo activity against both liver and blood stages. [4] Although no Plasmodium orthologs of mTOR exist, some proteins show a relatively high sequence similarity to the human mTOR at the kinase catalytic domain, corroborating the hypothesis that Torin2 acts by a different mechanism of action compared to the drugs already in clinic. Still, due to its strong interaction with the human mTOR, Torin2 cannot be regarded as an ideal lead compound.

In order to unveil the structural features responsible for the antimalarial activity as well as those that relate to parasite-host selectivity, we built up a library of new Torin2 analogues, which were screened against both liver and blood stage parasites cultures, and we report the synthetic methodology as well as the structure-activity relationships (SAR) obtained in order to identify suitable lead compounds for further development (Figure 1). Equipped with that knowledge, and through minimally disruptive insertions of a photoreactive moiety and a handle for “click chemistry” we prepared a library of photoaffinity-based probes aimed at identifying the molecular target for this class of compounds.

Figure 1: Development of a chemically diverse library of Torin2 analogues, for the establishment of the SAR on *Plasmodium* spp. and the rationale for the development of the corresponding photo-affinity based probes.

Acknowledgements: This work is supported by Fundação para a Ciência e Tecnologia (FCT), Portugal through grants: PTDC/QEQ-MED/7097/2014, Pest-OE/SAU/UI4013/2014, ROTEIRO/0028/2013 and PhD fellowship awarded to JG (PD/BD/128260/2016) through the MedChemTrain PhD Programme. ASR is a FCT Investigator (IF/01034/2014).

References

1) WHO. World Malaria Report. 2016.
2) Prudêncio M.; Rodriguez, A.; Mota, M. M. Nature Rev. Microbiol. 2006, 4, 849.
3) Lu, F.; Culleton, R.; Zhang, M.; Ramaprasad, A.; von Seidlein, L.; Zhou, H. et al. N. Engl. J. Med. 2017, 376, 991.
4) a) Liu, Q.; Wang, J.; Kang, S. A. et al. J. Med. Chem. 2011, 54, 1473 b) Hanson, K. K.; Ressurreição, A. S.; Buchholz, K.; Prudêncio, M. et al. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, E2838.

STRUCTURE OF MEMBRANE BOUND PYROPHOSPHATASE FROM THERMOTOGA MARITIMA IN COMPLEX WITH IMIDODIPHOSPHATE AND N-[(2-AMINOBENZO[d]THIAZOL -6-YL)METHYL]-1H-INDOLE-2-CARBOXAMIDE

Keni Vidilaseris (1), Alexandros Kiriazis (2), Ainoleena Turku (2), Ayman Katthab (3), Niklas G. Johansson (2), Teppo O. Leino (2), Paula S. Kiuru (2), Gustav Boije af Gennäs (2), Seppo Meri (3), Jari Yli-Kauhaluoma (2), Henri Xhaard (2), Adrian Goldman (1,4)

1) Department of Biosciences, Division of Biochemistry, University of Helsinki, Helsinki, Finland
2) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
3) Malaria Research Laboratory, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland
4) School of Biomedical Sciences and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Membrane-bound pyrophosphatases (mPPases) can be found in many human pathogens including *Plasmodium* species, the protozoan parasite causing malaria.[1] These large homodimeric integral membrane proteins generate an ion gradient across the acidocalcisomal membrane by hydrolysis of pyrophosphate (PP_i). The mPPases are essential for the parasites as PP_i is a by-product from many biosynthetic pathways and too high concentrations of PP_i may disturb physiological reactions. Although mPPases can be found in many pathogenic protozoan parasites they do not exist in humans, thereby making them promising drug targets. The first structure of a mPPase was solved in the Goldman laboratory.[2]

To date, only phosphorus-containing inhibitors of mPPases have been reported, limiting their therapeutic utility. Our aim is to develop novel protozoan mPPase inhibitors capable of disrupting the essential ion gradient of the pathogenic parasites in order to decrease their viability. Herein, we present a novel organic inhibitor of the *Thermotoga maritima* PPase through screening efforts. The compound inhibited the enzyme activity uncompetitively with an IC_{50} of 1.7 mM. In addition the binding mode was solved by X-ray crystallography at 3.7 Å resolution together with the substrate analogue, imidodiphosphate. The hit compound binds to the protein monomer near the exit channel, forming a hydrophobic clamp that lock the enzyme conformation in the closed state thus preventing hydrolysis and sodium pumping activity.

References

1) Shah, R.N.; Vidilaseris, K.; Xhaard, H.; Goldman, A. AIMS Biophysics 2016, 3 (1), 171–194.
2) Kellosalo J.; Kajander T.; Kogan K.; Pokharel K.; Goldman A. Science 2012, 337 (6093), 473–476.

SAR OF A NOVEL SCAFFOLD THAT INHIBITS MOTILITY AND DEVELOPMENT OF PARASITIC STAGES OF HAEMONCHUS CONTORTUS

Thuy Le (1), Banfeng Ruan (1), Nghi Nguyen (1), Yaqing Jiao (2), Sarah Preston (2), Jose Garcia-Bustos (2), Robin Gasser (2), Jonathan Baell (2)

1) Medicinal Chemistry, Monash Institute of Pharmaceutical Science, Monash University, Parkville, Australia.
2) Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, Australia

Recently from a whole-organism motility screen of the “open scaffolds” library against *Haemonchus contortus* (*H. contortus*), we identified an oxadiazole-carboxamide hit, namely SN00797439 with an initial IC_{50} of 5.9 µM and 11 µM against the exsheathed L3 (xL3) and L4 larval stages respectively. The hit represented a promising space for medicinal chemistry optimization. To probe the first-generation anthelmintic structure-activity relationships (SAR), a set of analogs had been synthesised and assessed for their activities. One analogue from the first-generation SAR with improved activity was chosen for second-generation SAR elaboration that we will herein discuss in details.

References

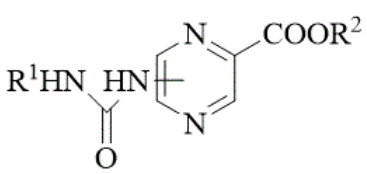
1) Sarah Preston, Yaqing Jiao, Jonathan B. Baell, Jennifer Keiser, Simon Crawford, Anson V. Koehler, Tao Wang, Moana M. Simpson, Ray M. Kaplan, Karla J. Cowley, Kaylene J. Simpson, Andreas Hofmann, Abdul Jabbar, Robin B. Gasser, Screening of the ‘Open Scaffolds’ collection from Compounds Australia identifies a new chemical entity with anthelmintic activities against different developmental stages of the barber's pole worm and other parasitic nematodes, International Journal for Parasitology: Drugs and Drug Resistance, Volume 7, Issue 3, 2017, Pages 286-294, ISSN 2211-3207, https://doi.org/10.1016/j.ijpddr.2017.05.004. (http://www.sciencedirect.com/science/article/pii/S2211320717300398)
Keywords: ‘Open Scaffolds’ compound collection; Whole organism screening; Haemonchus; Nematodes; Anthelmintic

TOWARD A BETTER PYRAZINAMIDE; NEW STRUCTURAL MODIFICATIONS, NEW BIOLOGICAL ACTIVITIES

Martin Dolezal, Ghada Bouz, Jan Zitko

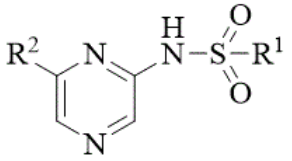
Department of Pharmaceutical Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy in Hradec Kralove, Charles University, Heyrovského 1203, Hradec Kralove 500005, Czech Republic

Despite being an old disease, tuberculosis remains the leading cause of death from infectious diseases at present time[1]. Among anti-tuberculars, pyrazinamide particularly has captured research attention. Several new specific mechanisms have been recently identified by which pyrazinamide exerts its antimycobacterial effect. This achievement opened a window for possible structural modifications in order to improve its biological activity and overcome emerging resistance. We will discuss two derivatization approaches of pyrazinamide. In the first series **(1)**, urea moiety was introduced to the pyrazine core. Among all prepared compounds, Propyl 5-(3-phenylureido)pyrazine-2-carboxylate (MIC_{Mtb} = 1.56 g/mL, 5.19 M) and propyl 5-(3-(4-methoxyphenyl)ureido)pyrazine-2-carboxylate (MIC_{Mtb} = 6.25 g/mL, 18.91 M) had high antimycobacterial activity against *Mtb* H37Rv with no *in vitro* cytotoxicity on HepG2 cell line [2]. In the second series **(2)**, different pyrazine sulfonamides were prepared. Synthesized compounds are being evaluated for their biological activities, including anti-infective and any possible anti-cancer properties. Obtained results will be discussed in the poster presentation.



R^1: Alkyl/Aryl Substituents
R^2: H/C_3H_5

(1)



R^1: Aryl Substituents
R^2: H/Cl

(2)

The study was supported by the Grant Agency of Charles University (projectC-C3/1572317) and Czech Science Foundation (project No. 17-27514Y).

References

1) World Health Organization, Global Tuberculosis, Report 2016. WHO/HTM/TB/2016.10.
2) BOUZ, G., JUHAS, M., NIKLOVA, P., JANDOUREK, O., PATEROVA, P., JANOUSEK, J., TUMOVA, L., KOVALIKOVA, Z., KASTNER, P., DOLEZAL, M., ZITKO, J.: Molecules, 22, 2017, 1797.

DISCOVERY OF A NEW CLASS OF POTENT AND NON CYTOTOXIC TRYPANOSOMA BRUCEI GROWTH INHIBITORS

Jesus Maria Ontoria (1), Andreina Basta (1), Ilaria Biancofiore (1), Alina Ciammaichella (1), Federica Ferrigno (1), Savina Malancona (1), Valentina Nardi (1), Simona Ponzi (1), Giacomo Paonessa (1), Rita Graziani (1), Marcel Kaiser (2), Alberto Bresciani (1), Edith Monteagudo (1), Annalise Di Marco (1), Ralph Laufer (1), Sergio Altamura (1), Vincenzo Summa (1), Steven Harper (1)

1) IRBM Science Park, Departments of Chemistry and Biology, Via Pontina km 30,600, 00071 Pomezia, Rome, Italy
2) Swiss Tropical and Public Health Institute, Socinstrasse 57, 4051 Basel, Switzerland, University of Basel, Petersplatz 1, 4003 Basel, Switzerland

Human African Trypanosomiasis (HAT) is a fatal parasitic disease caused by infection with either Trypanosoma brucei gambiense, responsible for the chronic infection, or *Trypanosoma brucei rhodesiense*, responsible for the acute infection. HAT is endemic in sub-Saharan Africa where it is transmitted to humans by tsetse flies. Over 65 million people living in endemic areas are at risk of contracting HAT and about 5000 new HAT cases are reported annually. The disease has two stages: a first-stage hemolymphatic infection where the parasites replicate in blood and lymphatic system, and a second-stage infection after parasite migration to the CNS. There is no effective vaccine and current treatment options are limited and often inadequate as they require hospitalization and show toxicity and reduced efficacy due to parasite resistance. T brucei infections are always fatal if untreated highlighting that the development of new drugs against HAT remains a strong humanitarian need.

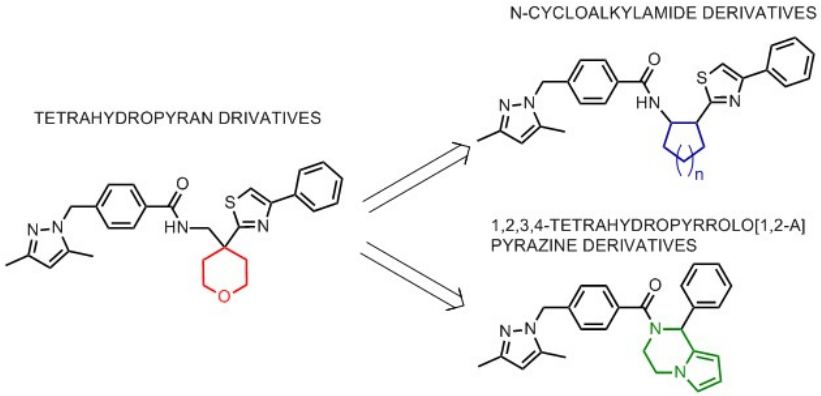
The identification of a new series of potent and non-cytotoxic *T. brucei* growth inhibitors will be reported. Phenotypic screening of a subset of compounds from IRBM collection against the parasite led to the identification of several compounds that inhibited growth of *T. brucei* at submicromolar concentrations and were nontoxic to mammalian cells. SAR around one of our hit compounds led to analogs with low nanomolar growth inhibitory activity and no cytotoxicity in different cell lines that show promising permeability of the blood-brain barrier (BBB). Data from *in vitro* biological profiling and *in vitro* and *in vivo* stability and metabolism studies will be disclosed together with studies on the mechanism of action and work toward obtaining proof-of-concept in an *in vivo* efficacy model will be presented.

DESIGN AND SYNTHESIS OF NEW DIRECT INHIBITORS OF INHA BASED ON N-CYCLOALKYLAMIDE AND 1,2,3,4-TETRAHYDROPYRROLO[1,2-A]PYRAZINE SCAFFOLD

Stane Pajk, Izidor Sosič, Kaja Rožman, Martina Hrast, Marko Jukič, Andrej Šterman, Sebastjan Nemec, Stanislav Gobec

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenija

Tuberculosis (TB) remains a major global health problem, with an estimated 9 million new cases and 1.5 million deaths per year. Although progress has been made to reduce the global incidence of TB, the emergence and spread of drug resistance threatens to undermine these efforts.1
One of the enzymes involved in the mycobacterial fatty-acid biosynthesis pathway II (FAS-II) in Mycobacterium tuberculosis (Mtb) is InhA, a NADH-dependent, enoyl-acyl carrier protein reductase. This is the target of isoniazid, a first-line drug for treatment of TB. Isoniazid is a pro-drug that is enzymatically activated by KatG, a mixed function catalase/ peroxidase. Resistance to isoniazid is mainly the result of mutations in KatG that reduce its activation of isoniazid, and to a lesser extent, to mutations in the InhA active site. Therefore, compounds that directly target InhA are promising candidates for treatment of infections caused by isonoazid-resistant strains. Tetrahydropyran derivatives was one of several compound classes of direct InhA inhibitors discovered by GlaxoSmithKline trough a high-throughput screening campaign.2,3 During the EU-funded 7th Framework Project ORCHID, limited structure-activity relationship (SAR) study of tetrahydropyran derivatives was undertaken, without significant improvement of activity.4
In the next round of SAR studies we focused on substitution of tetrahydropiran moiety for more rigid structures. N-cycloalkylamide derivatives and 1,2,3,4-tetrahydropyrrolo[1,2-a] pyrazine derivatives (Figure 1.) retained InhA inhibitory activity and are currently subject of further optimization.



ACKNOWLEDGMENTS: This study received funding from the Global Alliance for TB Drug Development, the European Union's 7th Framework Programme (FP7-2007-2013; under the Orchid grant agreement No. 261378), and the Slovenian Research Agency. Authors would also like to thank Roman Šink, Matej Živec, Raquel Fernandez-Menendez, Lourdes Encinas, Daniel Alvarez-Gomez, Eva Maria Lopez-Roman, Alfonso Mendoza-Losana, Julia Castro-Pichel, Joaquin Rullas-Trincado, Inigo Angulo-Barturen, David Barros, Lluis Ballell-Pages, and Robert J. Young for their contribution to this work.

[image]

References

1) WHO Global tuberculosis report 2017, http://www.who.int/tb/publications/global_report/en/, accessed March 26th 2018.
2) Ballell Pages, L.; Castro Pichel, J.; Fernandez Menendez, R.; Fernandez Velando, E. P.; et al. WO/2010/118852.
3) Encinas, L.; O'Keefe, H.; Neu, M.; et al. J. Med. Chem. 2014, 57(4), 1276-1288.
4) Pajk, S.; Živec, M.; Šink, R.; Sosič, I. et al. Eur. J. Med. Chem. 2016, 112, 252-257.

REPURPOSING HUMAN MTOR INHIBITORS FOR NEGLECTED TROPICAL DISEASES: TOWARD OPTIMIZATION OF A SINGLE CHEMOTYPE WITH ACTIVITY AGAINST MULTIPLE PROTOZOAN PARASITES

Lara Fidalgo (1), Jorge Grilo (1), Rosario Diaz (2), Tânia Cruz (3), Dolores G. Pacanowska (2), Ana M. Tomás (3), Miguel Navarro (1), Rui Moreira (1), Ana S. Ressurreição (1)

1) iMed.ULisboa – Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
2) Instituto de Parasitologia y Biomedicina "Lopez-Neyra", Consejo Superior de Investigaciones Cientificas (CSIC), Granada 18100, Spain
3) i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

Neglected tropical diseases (NTDs) affect a large proportion of the world's population and impose a huge economic and health burden on developing countries. From the 17 core NTDs defined by the World Health Organization, 3 are caused by protozoan pathogens: Leishmaniasis (*Leishmania spp.*), Human African Trypanosomiasis (*Trypanosoma brucei*), and Chagas Disease (*Trypanosoma cruzi*). One powerful approach to fight the dearth of drugs for NTDs has been directed at repurposing established knowledge about classes of molecular targets that the pathogen holds in common with humans, being protein kinases one of the main focuses of target repurposing strategies in parasitic diseases.[1]

We have recently disclose Torin2, an ATP-competitive mTOR kinase inhibitor,[2] as a potent antimalarial with *in vivo* activity against both liver and blood stages, capable of curing liver stage infection with a single, well-tolerated oral dose and presenting a distinct mode of action compared with currently used antimalarials.[3] These findings inspired us to further explore this kinase inhibitor in other protozoan parasites and our results showed that the compound is consistently efficient against *T. brucei*, *T. cruzi* and *L. amazonensis* (IC_{50} in the nM region).

In this report, we will described the optimization of a single chemotype capable of targeting several NTDs (**Figure 1**), disclosing a library of novel Torin2 analogues which was screened for in vitro activity against multiple protozoan parasites, as well as, against human cell lines. The structure-activity relationships (SAR) obtained unveiling the structural features which control selectivity and potency towards the parasites, allowing to identify suitable lead compounds for further development. We will also report our preliminary findings regarding

in vivo efficacy and our efforts concerning the development of biocompatible click probes for live-cell imaging and target-drug profiling of these inhibitors.

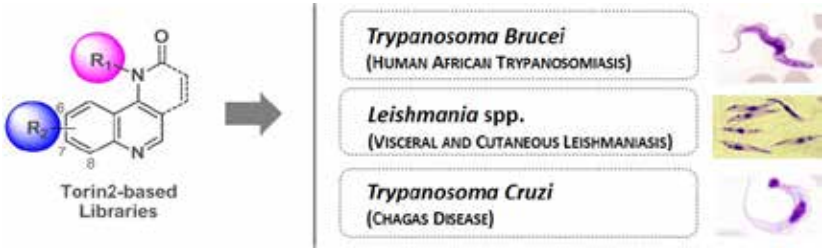


Figure 1: Optimization of Torin's chemotype with activity against multiple protozoan parasites.

Acknowledgements: This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal, through grants: PTDC/QEQ-MED/7097/2014, PhD fellowship awarded to JG (PD/BD/128260/2016) through the MedChemTrain PhD Programme. ASR is a FCT Investigator (IF/01034/2014).

References

1) Merritt, C.; Silva, L. E.; Tanner, A. L.; Stuart, K.; Pollastri, M. P. Chem. Rev. 2014, 114, 11280.
2) Liu, Q.; Wang, J.; Kang, S. A.; Thoreen, C. C.; Hur, W.; Sabatini, D. M.; Gray, N. S. J. Med. Chem. 2011, 54, 1473.
3) Hanson, K. K.; Ressurreição, A. S.; Buchholz, K.; et al. Proc. Natl. Acad. Sci. U. S. A. 2013, 110, E2838.

DESIGN AND SYNTHESIS DE NEW PHTHALOYL DERIVATIVES AS TRANS-SIALIDASE INHIBITORS

Muhammad Kashif (1), Karla F. Chacon-Vargas (2), Benjamin Nogueda-Torres (2), Alma D. Paz-Gonzalez (1), Rosalia Agusti (3), Maria Laura Uhrig (3), Alicia Reyes-Arellano (4), Gildardo Rivera (1)

1) Laboratorio de Biotecnologia Farmaceutica, Centro de Biotecnologia Genomica, Instituto Politecnico Nacional, Reynosa, Mexico
2) Departamento de Parasitología, Escuela Nacional de Ciencias Biológicas, Instituto Politecnico Nacional, Ciudad de México, México
3) Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
4) Departamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas, Instituto Politecnico Nacional, Ciudad de México, México

Chagas disease affects 8 to 10 million people worldwide, mainly in South and Central America. Currently, only two drugs are used for the pharmacological treatment, however, them efficacy is limited, therefore, is urgent obtain new therapeutic options. In the last years, trans-sialidase of *Trypanosoma cruzi* (TcTS), has been considered as a good drug target, because this enzyme play an important role in catalyses, transfering sialic acids from host surface glycoconjugates to *Trypanosoma cruzi (T. cruzi)* mucin-like surface glycoproteins. In the search of new non-sugar based inhibitors of TcTS, our research group proposed novel phthaloyl derivatives as potential trans-sialidase inhibitors.

Forty five novel phthaloyl derivatives (serie A, B and C) were systematically designed and synthetized with excellente yields (80-90%). The molecular docking analysis on TcTS active site revealed that the compounds B-11 and C-11 showed the highest predicted binding affinity (-11.1 Kcal/mol) and hydrogen bond, π-π stacking and Van der Waals interactions with key amino acid residues Tyr342, Trp312, Arg53 and Glu230. An analysis by high performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD) was used to determine the inhibition of selected compounds toward TcTS enzyme and found the following trend of inhibition, series C > B >A. Compounds C-11 and C-4 also exhibited the highest predicted binding affinities (86.9% and 82.6% respectively). This trend suggests that the high C log P and lipophilic substituents (CH_3) at the phthaloyl group are important in order to increase the TcTS inhibition. The *in vitro* trypanocidal activity of all compounds showed not correlation among NINOA and INC-5 strains due to a different expression of proteins, but series C compounds showed the better trypanocidal and LC_{50} values for both strains.

References

1) Buschiazzo A, Amaya MF, Cremona ML, Frasch AC, Alzari PM. The crystal structure and mode of action of trans-sialidase, a key enzyme in Trypanosoma cruzi pathogenesis. Molecular Cell. 2002; 10(4): 757-68.
2) Amaya MF, Watts AG, Damager I, Wehenkel A, Nguyen T, Buschiazzo A, Paris G, Frasch AC, Withers SG, Alzari PM. Structural insights into the catalytic mechanism of Trypanosoma cruzi trans-sialidase. Structure. 2004; 12(5): 775-84.
3) Kashif M, Herrera AM, Lara-Ramirez EE, Ramírez-Moreno E, García VB, Ashfaq M, Rivera G. Recent developments in trans-sialidase inhibitors of Trypanosoma cruzi. Journal of Drug Target. 2017; 25; 485-498.
4) Lima AH, Lameira J, Alves CN. Protein–ligand interaction of T. cruzi trans-sialidase inhibitors: a docking and QM/MM MD study. Structural Chemistry. 2012; 23(1): 147-52.
5) Neres J, Bonnet P, Edwards PN, Kotian PL, Buschiazzo A, Alzari PM, Douglas KT. Benzoic acid and pyridine derivatives as inhibitors of Trypanosoma cruzi trans-sialidase. Bioorganic & Medicinal Chemistry, 2007; 15(5): 2106-2119.
6) Neres J, Brewer ML, Ratier L, Botti H, Buschiazzo A, Edwards PN, Mortenson PN, Charlton MH, Alzari PM, Frasch AC, Bryce RA. Discovery of novel inhibitors of Trypanosoma cruzi trans-sialidase from in silico screening. Bioorganic & Medicinal Chemistry Letters. 2009; 19(3): 589-96.

FOCUSING ON VIRAL SURFACE GLYCOPROTEINS AS TARGET AGAINST HIV-2 INFECTIONS - STRUCTURAL ELUCIDATION AND MOLECULAR DYNAMICS STUDY

Patrícia Serra (1), Andreia Martins (1), Nuno Taveira (1,2), Rita C. Guedes (1)

1) iMed.ULisboa, Research Institute for Medicines and Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
2) Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM), Quinta da Granja, 2829-511 Monte da Caparica, Portugal

The ubiquitin proteasome system is a nonlysosomal pathway by which cells regulate the controlled degradation of several proteins, not just in cell cycle and apoptosis but also in inflammatory and immune processes, carcinogenesis, among other clinical situations. Usually in protein homeostasis the defective proteins are ubiquitinated and are proteolysed into short peptides by the proteasome. Proteasome substrates include, for example, signalling molecules, tumour suppressors, cell cycle regulators and transcription factors. Proteasome inhibition results in an interruption of the degradation of these substrates, leading to activation of apoptotic pathways and, eventually, cell death. Rapidly growing cells, such as cancer cells, are particularly susceptible to proteasome inhibition mechanisms.[1][2]

This work relies on a computational-based drug discovery approach to find alternative new, selective (and more effective) small molecules as reversible proteasome inhibitors that can overcome the severe adverse drug reactions demonstrated by in use drugs. The efforts to discover new anticancer drugs described here combine different computer-aided drug design techniques (i.e. molecular docking, pharmacophore modeling, structure-based virtual screening and molecular descriptors calculation) in order to identify potential hit compounds (picture below). The selected compounds were tested in cell growth inhibition assays, being also performed inhibition assays for the chymotrypsin-like, trypsin-like and caspase-like activities of the proteasome using fluorogenic substrates.

[image]

*Acknowledgements:*Fundação para a Ciência e a Tecnologia (SFRH/BD/104441/2014, PTDC/QEQ-MED/7042/2014, UID/DTP/04138/2013, SAICTPAC/0019/2015). Salvador J.A.R thanks PT2020 (Programa Operacional do Centro 2020), and the financial support by FEDER (COMPETE 2020 Programme), project CENTRO-01-0247-FEDER-003269, drugs2CAD.

References

1) Kwong, P. D. et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393, 648–659 (1998)
2) Uchtenhagen, H. et al. Crystal structure of the HIV-2 neutralizing Fab fragment 7C8 with high specificity to the V3 region of gp125. PLoS One 6, e18767 (2011)
3) Davenport, Y. W., West, A. P. & Bjorkman, P. J. Structure of an HIV-2 gp120 in Complex with CD4. J. Virol. 90, 2112–2118 (2016)

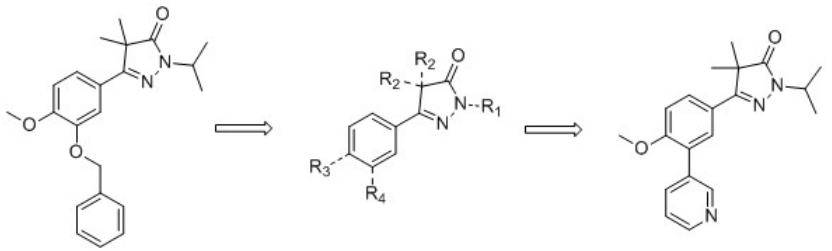
PHENOTYPIC OPTMIZATION OF TRYPANOSOMA CRUZI INHIBITORS

Maarten Sijm (1), Ewald Edink (1), Kristina M. Orrling (1), Julianna Siciliano de Araujo (2), Susanne Schroeder (3), An Matheeussen (4), Tiffany van de Meer (1), Payman Sadek (1), Marco Siderius (1), Louis Maes (4), Dave Brown (3), Maria de Nazare Correia Soeiro (2), Geert Jan Sterk (1), Iwan de Esch (1), Rob Leurs (1)

1) Division of Medicinal Chemistry, Faculty of Sciences, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
2) Laboratório de Biologia Celular, Oswaldo Cruz Institute (Fiocruz), Rio de Janeiro, Brazil
3) School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK
4) Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Belgium

Trypanosoma cruzi (Tcr) is a parasite transferred by the kissing bug and is the causative agent of Chagas' disease. Currently, Chagas' disease is treated with nifurtimox or benznidazole, however dosage regimes of three times daily for 60-90 days and only a moderate anti-trypanosomal effect in Chagas' heart disease make these drugs far from perfect.

From a phenotypic screening NPD-0583 and some analogues were identified as a hit; from these the phenyl-pyrazolone scaffold was identified and optimized on 4 positions. While a whole range of derivatives were synthesized, current optimized hit (NPD-0227, pIC_{50} *T.cruzi* = 6.4) is remarkably close to the original hit (NPD-0583).



Several potentital targets of these compound series were investigated, TcrPDEB, TcrPDEC and TcrCYP51. However, inhibition of these enzymes did not correlate with the observed phenotypic activity. The optimised hit (NPD-0227) showed significant differences in activity on different strains and forms of the parasite. While inactive against the bloodstream trypamastigote (pIC_{50} < 4.3), the potency against the intracellular form of the Y-strain is surpassing (pIC_{50} 7.0 vs 5.4) Benznidazole, the current drug of choice against Chagas disease.

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF DIRECT INHA INHIBITORS

Izidor Sosič, Stane Pajk, Kaja Rožman, Andrej Šterman, Sebastjan Nemec, Stanislav Gobec

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenija

According to World Health Organization data, tuberculosis (TB) is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, 6.3 million new cases of TB were reported, which is an increase from 6.1 million in 2015. Drug-resistant TB is also a continuing threat and such infections can significantly complicate the treatment. Despite noted progress in the pipeline for new diagnostics, drugs, and treatment regimens, a need for novel agents is obvious and urgent.[1]

Mtb enoyl acyl carrier protein reductase (InhA) is an NADH-dependent enzyme that facilitates the reduction of long-chain *trans*-2-enoyl-acyl carrier protein fatty acids. It is a key component of the *Mtb* FAS II pathway and widely recognized as a validated drug target. The initial discovery of two compound classes as direct InhA inhibitors, which represent the foundation for this work, was made by GlaxoSmithKline through a high-throughput screening campaign. These classes are the thiadiazoles[2] (represented by the general structure **1**, Figure 1) and the tetrahydropyran derivatives (such as compound **2**[3]). During the EU-funded 7th Framework Project ORCHID, the structure-activity relationship (SAR) studies of both series of compounds were undertaken. These efforts culminated in the compound **GSK693**[4] and a series of pyridinyl-2-thiadiazole based inhibitors of InhA (such as compound **3**[5]). In the latter case, we reduced the aromatic ring count of compounds with the aim to improve their physicochemical properties, while retaining their InhA inhibitory potency and antimycobacterial activity. Several other strategies to explore the chemical space of these direct inhibitors were attempted, such as substitution of the thiadiazole central core with other heterocycles, yielding extensive SAR data.

Because of the realization of the need for new antimycobacterial compounds, the work in this field is continuing after the formal completion of the EU-funded project. Our current focus is devoted into further SAR definition of InhA inhibitors by combining the pharmacophores (i.e. scaffold merging approach) of both compound classes into a single molecule.

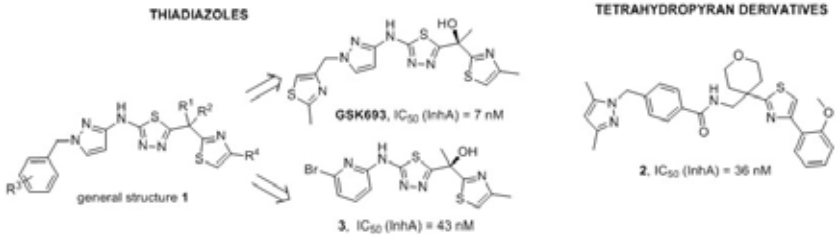


Figure 1. Schematic representation of the studies of direct InhA inhibitors.

ACKNOWLEDGMENTS: This study received funding from the Global Alliance for TB Drug Development, the European Union's 7th Framework Programme (FP7-2007-2013; under the Orchid grant agreement No. 261378), and the Slovenian Research Agency. Authors would also like to thank Roman Šink, Matej Živec, Raquel Fernandez-Menendez, Lourdes Encinas, Daniel Alvarez-Gomez, Eva Maria Lopez-Roman, Alfonso Mendoza-Losana, Julia Castro-Pichel, Joaquin Rullas-Trincado, Inigo Angulo-Barturen, David Barros, Lluis Ballell-Pages, and Robert J. Young for their contribution to this work.

References

1) WHO Global tuberculosis report 2017, http://www.who.int/tb/publications/global_report/en/, accessed March 26th 2018.
2) Ballell Pages, L.; Castro Pichel, J.; Fernandez Menendez, R.; Fernandez Velando, E. P.; et al. WO/2010/118852.
3) 3. Pajk, S.; Živec, M.; Šink, R.; Sosič, I. et al. Eur. J. Med. Chem. 2016, 112, 252-257.
4) 4. Martínez-Hoyos, E.; Perrez-Herran, E.; Gulten, G.; Encinas, L. et al. EBioMedicine 2016, 8, 291-301.
5) 5. Šink, R.; Sosič, I.; Živec, M.; Fernandez-Menendez, R. et al. J Med Chem 2015, 58, 613-324.

Structure based design of glycomimetic ligands of bacterial N-acetylglucosaminidase AtlE

Tjaša Tibaut (1,2), Marko Anderluh (2), Nives Hribernik (2), Vesna Hodnik (3), Sara Pintar (4), Marjana Novič (1), Dušan Turk (4)

1) National Institute of Chemistry, Theory department, Laboratory of Cheminformatics, Hajdrihova ulica 19, SI-1000 Ljubljana, Slovenia
2) University of Ljubljana, Faculty of Pharmacy, Chair of Pharmaceutical Chemistry, Aškerčeva cesta 7, SI-1000 Ljubljana, Slovenia
3) University of Ljubljana, Biotechnical Faculty, Department of Biology, Jamnikarjeva 101, SI-1000 Ljubljana
4) Jozef Stefan Institute, Department of Biochemistry and Molecular and Structural Biology, Jamova cesta 39, SI-1000 Ljubljana, Slovenia

In our study, *de novo* structure based approach is applied for the development of inhibitors of autolysin E (AtlE), a bacterial *N*-acetylglucosaminidase. Autolysins are peptidoglycan hydrolases responsible for the degradation of the bacterial cell wall [1, 2]. Several studies indicate that autolysins are implicated in cell division, cell growth [2] and biofilm formation [3], and hence their inhibition could be a promising approach for the development of a novel group of therapeutics against human pathogens [1, 4, 5].

According to crystal structures of the enzyme-ligand complexes (PDB ID: 4PI7, 4PI9) [6], *de novo* design of glycomimetics was one of the approaches for the initial studies. Designed compounds contain monosaccharide unit (*N*-acetylglucosamine) and aglycone unit, composed of the linker and cation and/or lipophilic part. We have performed *in silico* studies, where designed molecules were docked into the binding site of the *Staphylococcus aureus* AtlE (PDB ID: 4PIA [6]). Hits from docking results were synthetized and subjected to quantitative binding analysis, using surface plasmon resonance (SPR). Several compounds show interactions with AtlE according to SPR response curves.

Ligands of *N*-acetylglucosaminidase designed in our study offer valuable information for further development of autolysin inhibitors, an emerging class of future antibacterial agents.

References

1) S. Zoll, B. Patzold, M. Schlag, F. Gotz, H. Kalbacher, and T. Stehle, Structural basis of cell wall cleavage by a staphylococcal autolysin, PLoS Pathog. 6 (2010), e1000807.
2) W. Vollmer, B. Joris, P. Charlier and S. Foster, Bacterial peptidoglycan(murein) hydrolases, FEMS Microbiol. Rev. 32 (2008), 259-286.
3) R. Biswas, L. Voggu, U.K. Simon, P. Hentschel, G. Thumm, and F. Gotz, Activity of the major staphylococcal autolysin Atl, FEMS Microbiol. Lett. 259 (2006), 260–268.
4) F.M. Büttner, S. Zoll, M. Nega, F. Götz, and T. Stehle, Structure-function analysis of Staphylococcus aureus amidase reveals the determinants of peptidoglycan recognition and cleavage, J. Biol. Chem. 280 (2014), 11083–11094.
5) S. Nayyab, M. O'Connor, J. Brewster, J. Gravier, M. Jamieson, E. Magno, R. D. Miller, D. Phelan, K. Roohani, P Williard, Amit Basu, and C. W. Reid, Diamide Inhibitors of the Bacillus subtilis N-Acetylglucosaminidase LytG That Exhibit Antibacterial Activity, ACS Infect. Dis. 3 (2017), 421–427.
6) M. Mihelič, K. Vlahoviček-Kahlina, M. Renko, S. Mesnage, A. Doberšek, A. Taler-Verčič, A. Jakas and D. Turk, The mechanism behind the selection of two different cleavage sites in NAG-NAM polymers. IUCrJ.4 (2017), 185-198.
7) Kelley, B.P.; Brown, S.P.; Warren, G.L.; Muchmore, S.W. POSIT: Flexible Shape-Guided Docking For Pose Prediction. J. Chem. Inf. Model. 55 (2015) , 1771-1780.
8) McGann, M. FRED Pose Prediction and Virtual Screening Accuracy. J. Chem. Inf. Model., 51 (2011) , 578-596.

3D-PHARMACOPHORE MODELS FOR IDENTIFICATION AND OPTIMIZATION OF ATP-COMPETITIVE BACTERIAL DNA GYRASE AND TOPOISOMERASE IV INHIBITORS

Tihomir Tomašič (1), Riccardo Martini (2), Danijel Kikelj (1), Sharon D. Bryant (2)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, Ljubljana, Slovenia
2) Inte:Ligand Softwareentwicklungs- und Consulting GmbH, Mariahilferstrasse 74B, Vienna, Austria

The increasing emergence of pathogenic bacteria resistant to antibacterial drugs is a serious threat to global health and represents the continuous need for development of novel antibacterial drugs. Bacterial DNA gyrase and topoisomerase IV are heterotetrameric proteins consisting of two GyrA or ParC subunits, which are involved in DNA transit, and two GyrB or ParE subunits containing the ATPase domains, respectively. Structural similarity of GyrB and ParE ATP binding sites enables the discovery of dual targeting inhibitors, which makes them attractive targets for antibacterial drug discovery. Although ATP-competitive inhibitors of GyrB and ParE are among the most studied classes of antibacterial agents, there is currently no representative in the antibacterial drug pipeline. Moreover, selectivity of ATP-competitive GyrB and ParE inhibitors against closely related human ATP-binding enzymes should be evaluated early in the development to avoid off-target binding of advanced compounds in later stages.

Recently, we prepared several series of novel GyrB and ParE inhibitors with inhibitory activities in the low nanomolar range and antibacterial activity against Gram positive and Gram negative bacterial strains.[1-3] To assess their selectivity profiles and support hit-to-lead optimization,we developed 3D-chemical feature pharmacophore models for on-target (GyrB and ParE) and off-target (e.g. topoisomerase II, Hsp90, pyruvate dehydrogenase kinase) predictions using LigandScout (ref). Structure-based pharmacophore models were created based on x-ray derived enzyme-inhibitor complexes, while ligand-based models were created based on the known potent ligands. The models were validated and trained using sets of known active, inactive and decoy molecules and are important for hit finding, hit optimization support and activity profiling of previously prepared and novel GyrB and ParE inhibitors. Results from activity profiling of our GyrB and ParE inhibitors using the on- and off-target pharmacophore models were experimentally confirmed by testing a representative set of our library of DNA gyrase and topoisomerase IV inhibitors against human topoisomerase IIα and Hsp90. These results confirmed that the 3D-pharmacophore models are useful tools for prediction and discrimination of compounds with activities at DNA gyrase, topoisomerase IV and topoisomerase II and useful for hit finding in virtual screening protocols and lead optimization studies.

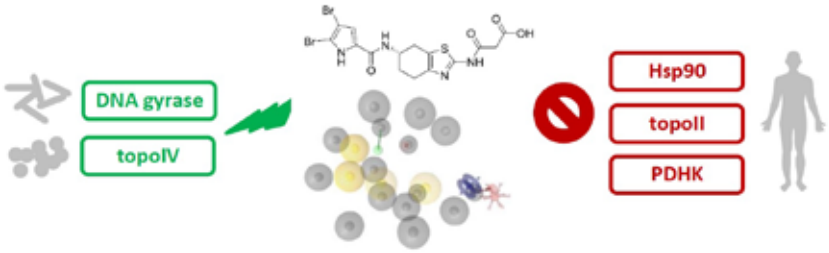


Figure 1. Activity profiling of DNA gyrase and topoisomerase IV inhibitors.

References

1) Tomašič, T.; Katsamakas, S.; Hodnik, Ž.; Ilaš, J.; Brvar, M.; Šolmajer, T.; Montalvao, S.; Tammela, P.; Banjanac, M.; Ergović, G.; Anderluh, M.; Peterlin Mašič, L.; Kikelj, D. Discovery of 4,5,6,7-tetrahydrobenzo[1,2-d]thiazoles as novel DNA gyrase inhibitors targeting the ATP-binding site. J. Med. Chem., 2015, 58, 5501-5521.
2) Gjorgjieva, M.; Tomašič, T.; Barančoková, M.; Katsamakas, S.; Ilaš, J.; Tammela, P.; Peterlin Mašič, L.; Kikelj, D. Discovery of benzothiazole scaffold-based DNA gyrase B inhibitors. J. Med. Chem., 2016, 59, 8941-8954.
3) Durcik, M.;Tammela, P.; Barančoková, M.; Tomašič, T.; Ilaš, J.; Kikelj, D.; Zidar, N. Synthesis and evaluation of N-phenylpyrrolamides as DNA gyrase B inhibitors. ChemMedChem., 2018, 13, 186-198.

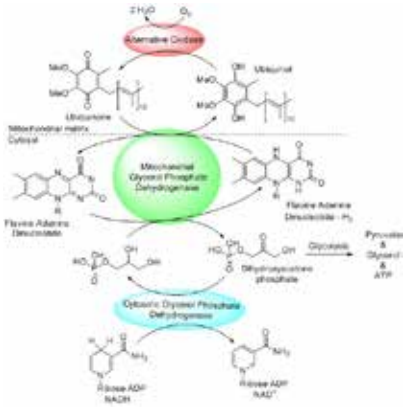
TRYPANOSOME ALTERNATIVE OXIDASE INHIBITORS FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

Ryan West (1), Simon Ward (1), Thomas Cunningham (1), Lewis Pennicott (1), Srinivasa Rao (2)

1) University of Sussex, Brighton, BN1 9RH, UK
2) Novartis Institutes for Tropical Diseases, 5300 Chiron Way, California, 94608-2916, USA

Human African Trypanosomiasis (HAT) is a parasitic disease that is transmitted by the bite of a Genus Glossina (Tsetse fly). Two strains of the parasite, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense,* are responsible for HAT. In 2012 there were 20,000 estimated cases with an at risk population of 60 million.[1] Without treatment HAT is fatal,[2] treatments for HAT are available but either require complicated and prolonged administration of drugs that have poor pharmacokinetic and central nervous system penetration properties, or are drugs that are highly toxic resulting in unacceptable side effects.[3]

Trypanosome Alternative Oxidase (TAO) has been investigated by a variety of academic groups as a potential target for treating HAT.[4–7] The oxidase is present in the mitochondria of long slender bloodstream forms of trypanosomes.[8,9] TAO oxidises ubiquinol to ubiquinone in tandem carrying out the four electron reduction of oxygen to water. Ubiquinone is then reduced by mitochondrial glycerol phosphate dehydrogenase (GPDH) as part of the glycerol phosphate oxidase system. This system is responsible for the generation of dihydroxyacetone phosphate, which is vital for glycolysis and the re-oxidation of NADH that are both essential for cellular respiration and survival of the trypanosome.[8,10]



Our work has identified novel inhibitors of TAO based upon the natural product ascofuranone. They retain good inhibitory potency of TAO and show potent growth inhibition of the parasite, whilst removing two undesirable chemical functionalities present in the natural product. These novel analogues provide an avenue for further exploration of structure activity relationships and the opportunity to rationally design new molecules with improved pharmacokinetic properties to provide more drug like leads for inhibitors of TAO.

References

1) WHO. Control and Surveillance of Human African Trypanosomiasis; Geneva, 2013.
2) Checchi, F. et al. PLoS Negl. Trop. Dis. 2008, 2, e303.
3) Fairlamb, A. H. Trends in Parasitology, 2003, 19, 488–494.
4) Grady, R. W. et al. Mol. Biochem. Parasitol. 1986, 21, 55–63.
5) Grady, R. W. et al. Antimicrob. Agents Chemother. 1993, 37, 1082–1085.
6) Nihei, C. et al. Biochim. Biophys. Acta 2002, 1587, 234–239.
7) Ott, R. et al. Acta Trop. 2006, 100, 172–184.
8) Clarkson, A. B. et al. J. Biol. Chem. 1989, 264, 17770–17776.
9) Opperdoes, F. R. et al. Eur. J. Biochem. 1977, 76, 29–39.
10) Minagawa, N. et al. Mol. Biochem. Parasitol. 1996, 81, 127–136.

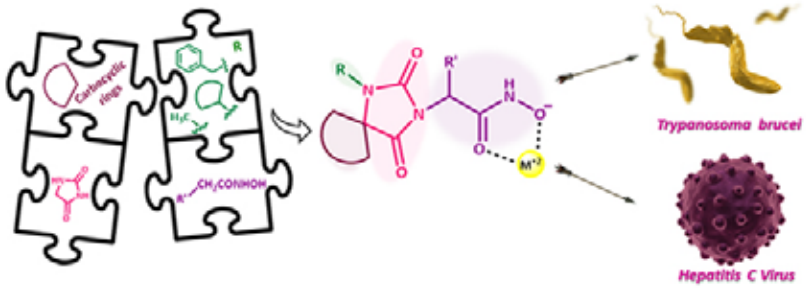
NOVEL HYDANTOIN-BASED ACETOHYDROXAMIC ACID DERIVATIVES, AS METAL CHELATING AGENTS WITH DUAL-TARGETING FUNCTION AGAINST T. BRUCEI AND HEPATITIS C VIRUS

Vasiliki Pardali (1), Erofili Giannakopoulou (1), Efseveia Frakolaki (2), Martin C. Taylor (3), Vassilios Myrianthopoulos (1,4), Emmanuel Mikros (1,4), Ralf Bartenschlager (5), Niki Vassilaki (2), John M. Kelly (3), Grigoris Zoidis (1)

1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Athens, Greece
2) Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens, Greece
3) Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, U.K
4) Athena Research Center, Athens, Greece
5) Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Germany

Human African Trypanosomiasis (HAT) is a neglected tropical disease, exerting a considerable health burden on 36 countries in sub-Saharan Africa. Moreover, Hepatitis C Virus (HCV) infection is the most common liver disease and the leading cause of liver transplantation for the 71 million infected individuals globally. Current treatment for both diseases is characterized by poor efficacy, high toxicity and increasing levels of resistance. Thus, there is a great need to develop new agents with an acceptable efficacy and safety profile.
Several Fe^{2+}/Zn^{2+} metalloenzymes have been identified in Trypanosoma brucei,[1] the causative agent of HAT. Concerning HCV, its NS5B polymerase contains a "two-metal-ion" catalytic center.[2] The importance of these metalloenzymes, and the fundamental role of the divalent cations in their activity, along with the fact that they have no counterparts in the host cell,[1,2] prompted the development of novel scaffolds, bearing a metal-chelating motif, as potent inhibitors of these enzymes.

Based on previously reported derivatives of the 2,6-diketopiperazine structure which showed high potency against T. brucei,[3] we incorporated the acetohydroxamic acid motif, a metal-chelating group, at the imidic nitrogen atom of the 2,4-diketoimidazolidine scaffold (hydantoin). Taking into consideration that the activity profile covering more than one microorganisms is favorable, and several anti-HCV metal chelators have been reported in the literature,[2]the newly synthesized analogues were tested for both their trypanocidal activity and their effect on HCV RNA replication and cell viability as well. The novel acetohydroxamic acid derivatives were potently active against T. brucei, with IC_{50} values ranging from 0.008 to 0.88 μM and the cytotoxicity of the compounds against mammalian cells was low to negligible (Selectivity Indices up to 1100). The effects of the synthesized analogues on HCV RNA replication were highly encouraging, with EC_{50} values rising up to 9.28 μM; moreover promising safety profiles were detected. Additional theoretical studies and docking calculations will contribute to acquire more structure-activity relationship (SAR) data, offering to the design of more agents with broad-spectrum activity.



References

1) A.L. Santos, C.L. Sodre, R.S. Valle, B.A. Silva, E.A Abi-Chacra, L.V Silva, A.L. Souza-Goncalves, L.S. Sangenito, D.S. Goncalves, L.O. Souza, V.F. Palmeira, C.M. d'Avila-Levy, L.F. Kneipp, A. Kellett, M. McCann, M.H. Branquinha. Curr. Med. Chem. 2012, 19, 2715-2737.
2) M.J. Sofia, W. Chang, P.A. Furman, R.T. Mosley, B.S. Ross. J. Med. Chem. 2012, 55, 2481-2531.
3) C. Fytas, G. Zoidis, N. Tzoutzas, M.C. Taylor, G. Fytas, J.M. Kelly. J. Med. Chem. 2011, 54, 5250-5254.

ADJUVANT APPROACHES SUPPORTING THE ERADICATION OF RESISTANT AND PERSISTENT M. TUBERCULOSIS STRAINS

Marco Pieroni (1), Elisa Azzali (1), Miriam Girardini (1), Diana Machado (2), Miguel Viveiros (2), Gabriele Costantino (1)

1) Food and Drug Department, Università di Parma, Parco Area delle Scienze 27, 43124 Parma, Italy
2) Grupo de Micobactérias, Unidade de Microbiologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa (IHMT/UNL), Rua da Junqueira 100, 1349-008 Lisboa, Portugal

Tuberculosis (TB), brought by *M. tuberculosis* (*Mtb*) is one of deadliest diseases ever occurred on Earth and the toll of deaths is still remarkable.[1] TB is thought to represent a concern only in developing countries, however global warming, business travels and the increasing migration flows from regions where TB is endemic, pose the threat to contract TB also in the developed ones; the whole scenario is further worsened by the presence of resistant strains. After decades of drug discovery oblivion, bedaquiline and delamanid have eventually upgraded the antituberculosis arsenal. Although they are new chemical entities (NCE), and hit unexplored targets, the whole approach to their discovery was rather conventional and the degree of innovation limited. It is therefore not surprising that mutations causing resistance to these two agents have already been reported.[2] Mycobacterial efflux pumps have recently triggered the interest of many scientists, as their inhibition might lead to shorten the duration of the treatment and prevent the transmission of resistance genes.[3] Verapamil, the most potent *Mtb* efflux pumps inhibitor (EPI) known so far, has shown to increase the efficacy of existing regimens and to inhibit *Mtb* drug-tolerance. Also thioridazine (TDZ), a neuroleptic drug known to inhibit *Mtb* efflux pumps, has been used under compassionate bases in combination with first- and second-line antituberculars to treat resistant infections.[4] In spite of these experimental evidences, the use of both verapamil and TDZ is strongly limited by the potential raise of severe side effects.[5] Considering these findings, the rational design and synthesis of EPIs with improved cytotoxic profile could have a significant impact in the treatment of mycobacterial infections, maintaining the concentration of a given drug at the therapeutic dose, and minimizing the possibility to select mutants. This would strongly affect both the cure duration and the emergence of resistances. In addition, since the lack of intrinsic killing activity for many EPIs, the onset of resistance toward these agents is highly unlikely to occur. Finally, the mechanism of macrophage killing is aspecific, therefore the antibiotic-resistant status of the bacillus is irrelevant. Using TDZ and verapamil as the chemical template, we have prepared a preliminary set of inhibitors of the Mtb efflux pumps, that were tested for their cytotoxicity and their ability to inhibit ethidium bromide efflux, to enhance the potency of first- and second-line antitubercular agents, and to eradicate intramacrophageal infection.[6] Further chemical manipulation led to the development of analogues that resulted to work better than TDZ and even verapamil, holding promise as effective tools for an innovative antituberculosis regimen.

References

1) World Health Organization. (2017) Global tuberculosis report 2017. WHO/HTM/TB/2017.23. pp. 1-262. World Health Organization, Geneva, Switzerland.
2) Bloemberg, G. V. et al. ; Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. N. Engl. J. Med. 2015, 373 (20), 1986–1988.
3) Bhardwaj, A. K. et al. Bacterial Efflux Pumps Involved in Multidrug Resistance and Their Inhibitors: Rejuvinating the Antimicrobial Chemotherapy. Recent Patents Anti-Infect. Drug Disc. 2012, 7 (1), 73–89
4) Abbate, E. et al. Successful Alternative Treatment of Extensively Drug-Resistant Tuberculosis in Argentina with a Combination of Linezolid, Moxifloxacin and Thioridazine. J. Antimicrob. Chemother. 2012, 67 (2), 473–477.
5) Martins, M. et al. The Curative Activity of Thioridazine on Mice Infected with Mycobacterium Tuberculosis. Vivo Athens Greece 2007, 21 (5), 771–775.
6) Pieroni, M. et al. Rational Design and Synthesis of Thioridazine Analogues as Enhancers of the Antituberculosis Therapy. J. Med. Chem. 2015, 58 (15), 5842–5853.

HOW TO FIGHT ANTIMICROBIAL RESISTANCE: DESIGN AND SYNTHESIS OF FTSZ INHIBITORS AS NOVEL POTENT GRAM-POSITIVE ANTIBIOTICS

STRANIERO VALENTINA, CASIRAGHI ANDREA, VALOTI ERMANNO

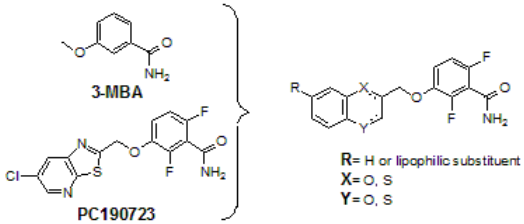
Department of Pharmaceutical Sciences, University of Milan, via Luigi Mangiagalli 25, 20133 Milano, Italy

Nowadays, antimicrobial resistance is a global threat to public health. This well know plague only recently burst out, prompting to the urgent need of developing efficient antibiotics with innovative mechanisms of action.

In this context, the bacterial divisome turned out to be an interesting and promising target (1). Cell division proteins are indeed crucial for bacteria viability, are widely conserved among several species and are completely absent in eukaryotic cells, thus strengthening the selectivity of the novel antimicrobics. FtsZ (Filamentous temperature sensitive Z) is one of the essential cell division proteins; FtsZ is a tubulin homologue (2) and is the first protein that localizes to the mid-point of the cell and undergoes polymerization in a GTP-dependent manner, bringing to the formation of the Z-ring. It recruits at least ten other cell division proteins, which enable cell constriction, the formation of mesosome and two daughter cells (3).

Recently, we studied and developed FtsZ inhibitors, starting from the most significant results of other research groups and confirming that FtsZ inhibition results in a bactericidal effect.

We prepared 3-Methoxybenzamide (3-MBA) derivatives, structurally similar to the FtsZ inhibitors lead compound: PC190723 (4-6).



Our derivatives (which general structure is depicted above) were designed replacing the thiazolopyridine of PC190723 with differently substituted 1,4-benzodioxane or 1,4-benzoxathiane. We further assessed the Structure Activity Relationship (SAR) of this class, through a series of isosteric, positional or substituent modifications (7-9).

These molecules proved to strongly inhibit *S. aureus, E. faecalis and M. tuberculosis* viability and to target FtsZ. We specifically performed two different biochemical assays, aimed at studying GTPase and polymerization activities of S. aureus FtsZ, when incubated with our compounds.

References

1) Nature Reviews Drug Discovery 2008, 7, 324-338.
2) Journal of Molecular Biology 2004, 342, 953–970.
3) Nature Reviews Molecular Cell Biology 2005, 6, 862-872.
4) Science 2008, 321, 1673-1675.
5) B.M.C.L. 2009, 19, 524-527.
6) B.M.C.L. 2014, 24, 353-359.
7) E.J.M.C. 2015, 89, 252-265.
8) E.J.M.C. 2016, 120, 227-243.
9) ChemMedChem 2017, 12, 1303-1318.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - THERAPEUTIC AREAS

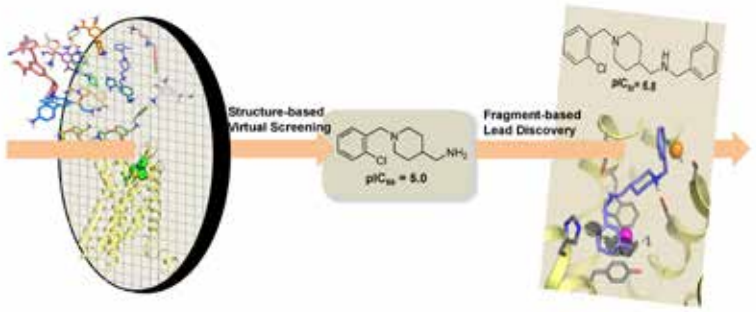
Inflammatory and Autoimmune Diseases

STRUCTURE-BASED DISCOVERY OF CXCR4 CHEMOKINE RECEPTOR ANTAGONISTS

Ilze Adlere (1), Shanliang Sun (2), Aurélien Zarca (2), Marta Arimont (2), Henry Vischer (2), Martine Smit (2), Maikel Wijtmans (2), Chris De Graaf (2), Iwan de Esch (1,2), Rob Leurs (1,2)

1) Griffin Discoveries BV, Department of Medicinal Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands
2) Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands

Chemokines and chemokine G protein-coupled receptors (GPCRs) play an important role in cell migration and are important targets for drug discovery for various immune-related diseases, including chronic obstructive pulmonary disease, multiple sclerosis, rheumatoid arthritis, HIV-1 infection and cancer. Despite recent breakthroughs in obtaining crystal structures of chemokine receptors, structure-based small-ligand discovery for these peptide-binding GPCRs is still challenging. We present a fragment-based lead discovery (FBLD) approach to identify and optimize CXCR4 antagonists. For this, CXCR4 crystal structure-based virtual fragment screening is followed by hit optimization that is guided by structure-activity relationships. This is illustrated by one of the hit molecules that was identified by *in silico* screening and that was used as a starting point for the design and synthesis of 31 molecules to explore and optimise structural interactions with the CXCR4 binding site. We demonstrate that through structure-based design applications, new CXCR4 ligands can be identified and optimised from micromolar affinity hits to potent antagonists.



This work was partially supported by European Union's Horizon2020 MSCA Programme under grant agreement 641833 (ONCORNET)

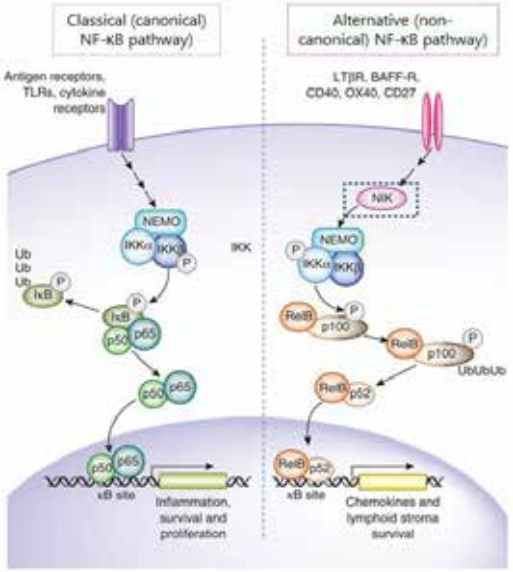
ADDRESSING METABOLISM THROUGH STRUCTURE-BASED DESIGN: IDENTIFICATION OF POTENT AND SELECTIVE INHIBITORS OF NF-KB INDUCING KINASE (NIK)

James Crawford

Senior Scientist, Discovery Chemistry, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA

NF-kB-inducing kinase (NIK) is a protein kinase that is central to the non-canonical NF-kB pathway and mediates NF-kB signaling through IKKa activation and p100 processing to nuclear transcription factors p52 and RelB. This non-classical pathway is downstream from multiple TNF receptor family members ,including BR3/BAFF-R, CD40, LT-bR, OX40, RANK, CD27, and Fn14 (TWEAK-R), which have been associated with B cell survival and maturation, dendritic cell activation, secondary lymphoid organ development, and bone metabolism. Increased serum BAFF levels are associated with autoimmunity and disorders such as lupus erythematosus, and inhibition of BAFF signaling has been shown to be efficacious in murine models of lupus. It is thought that inhibition of NIK could provide additional benefit over BAFF inhibition in the blockade of the non-canonical NF-kB pathway through modulation of signaling of multiple receptors at once.

A lead chemical series was identified through the optimization of a high throughput screening hit. Structure-based design led to the identification of several potent and selective NIK inhibitors which reach past the methionine-471 gatekeeper residue. These compounds exhibited selective inhibition of LTβR-dependent p52 translocation and transcription of NF-kB2 related genes. Guided by scaffold-dependent toxicity observations and results from genetic models, we focused our efforts towards the identification of a low clearance compound from within a specific sub-series, aided by metabolite identification and further structure-guided design. The identification and optimisation of these compounds will be described.



References

1) Castanedo et al., Structure-Based Design of Tricyclic NF-κB Inducing Kinase (NIK) Inhibitors That Have High Selectivity over Phosphoinositide-3-kinase (PI3K). J. Med. Chem., 2017, 60 (2), pp 627–640

DESIGN, SELECTION AND IN VITRO EVALUATION OF POTENTIAL, SMALL-MOLECULE COMPLEMENT C1S INHIBITORS

Katalin Szilágyi (1), István Hajdú (1,2), Beáta Flachner (1), Zsolt Lőrincz (1,2), Péter Gál (2), Péter Závodszky (2), Sándor Cseh (1), György Dormán (1,3)

1) Targetex Biosciences, H-2120 Dunakeszi, Madách Imre utca 31/2. Hungary
2) Research Centre for Natural Sciences, Institute of Enzymology, Hungarian Academy of Sciences, H-1117 Budapest, Magyar tudósok körútja 2., Hungary
3) Institute of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Szeged, H-6720 Szeged Zrínyi u. 9., Hungary

The complement system is a key component of innate immunity, which is involved in several physiologic and pathologic processes. Dysregulated or impaired complement is involved in an increasing list of human diseases (many autoimmune, inflammatory, and neurodegenerative diseases, as well as ischemia-reperfusion injury and cancer). The complement system consists of over forty protein components that are present in the blood or on cell surfaces. The complement system is activated by infection or by injury. Complement activation may be prolonged or misdirected to healthy cells and can lead to inflammatory or auto-immune diseases. Complement-targeted drugs could provide novel therapeutic intervention against the above mentioned diseases and conditions.

Nine serine proteases are integral elements of the complement cascade (C1r, C1s, C2, MASP-1, MASP-2, MASP-3, factor D, factor B, factor I). C1s is present as a proenzyme within the C1 molecule in complex with C1q and C1r, thus forming the C1 complex of the classical activation pathway of the complement system. Activation of the classical complement pathway is initiated by the interaction of C1q with immunoglobulin (Ig) antigen complexes. The activation signal is mechanically transmitted by C1q to C1r dimers; activated C1r proteases then cleave and activate the C1s proenzymes. Activated C1s protease forwards the activation signal by cleaving C4 and C4b-associated C2 to form the classical pathway C3 convertase C4b2a, so an inhibitor that targets the C1s protease domain could be effective in blocking the activation of the classical pathway of the complement system.

The C-terminal catalytic region of C1s consists of two complement control protein (CCP) modules and a trypsin-like serine protease domain. Only few small molecule inhibitors of these proteases are described up to present, including Nafamostat, which is currently approved for use in human pancreatitis and disseminated intravascular coagulation.

One of the main challenges is the selectivity over other serine proteases (including the proteases of the blood coagulation and fibrinolysis etc.). The typical architecture of a C1s inhibitor contains a heterocyclic amidine (or guanidine) which interacts with active site asparagines in the S1 substrate binding subdomain. In other proteases replacement of the amidine (guanidine) warheads with bioisosteric groups has been proven as a successful strategy to identify novel inhibitors, however, to the best of our knowledge this strategy has not been investigated with C1s inhibitors.

In an ongoing research program, we generated first the bioisosters of the key recognition motifs of known C1s inhibitors and based on the resulting novel structural motifs a potential focused library was selected from commercial vendor libraries (6 M compounds). A diverse selection of the focused library is screened in vitro for C1s inhibitory activity.

In the present poster the initial results of the above research is discussed and presented.

References

1) Iyer, A., Xu, W., Reid, R. C., & Fairlie, D. P. (2017). Chemical Approaches to Modulating Complement-Mediated Diseases. J. Med. Chem. Article ASAP
2) Qu, H., Ricklin, D., & Lambris, J. D. (2009). Recent developments in low molecular weight complement inhibitors. Molecular Immunology, 47(2-3), 185-195.

IN VITRO ACTIVITY OF MICONAZOLE AGAINST CANDIDA BIOFILM

Barbara Dorocka-Bobkowska (1), Senait Gebremedhin (2), Krystyna Konopka (2), Nejat Duzgunes (2)

1) University of Medical Sciences, Department of Gerodontology and Oral Pathology, Bukowska 70, 60-812 Poznan, Poland
2) University of the Pacific, Department of Biomedical Sciences, 155 Fifth Street, San Francisco, CA, USA

Oral candidiasis in the form of *Candida*-associated denture stomatitis (*Ca*DS) is associated with *Candida* adhesion and biofilm formation on the fitting surface of poly (methyl methacrylate) (PMMA) dentures. *Candida* biofilms show considerable resistance to most conventional antifungal agents, a phenomenon that is considered a developmental-phase-specific event that may help explain the high recurrence rates associated with *Ca*DS. *C. albicans* is still considered to be the major etiologic agent of oral candidiasis. *C. glabrata* is the most prevalent non-*albicans Candida* species isolated in oral candidiasis in patients with diabetes, advanced cancer, HIV infection and patients suffering from *Ca*DS.

The aim of this study was to examine the activity of miconazole towards *in vitro*- grown mature *Candida* biofilms formed on heat-cured PMMA discs as a standardized model.

The effect of miconazole nitrate obtained from Sigma-Aldrich (Switzerland), on *Candida* biofilms developed on acrylic discs was determined for *C. albicans* MYA-2732 (ATCC), *C. glabrata* MYA-275 (ATCC), and clinical isolates, *C. albicans* 6122/06, *C. glabrata* 7531/06, *C. tropicalis* 8122/06, and *C. parapsilosis* 11375/07. *Candida* biofilms were developed on heat-cured poly(methyl methacrylate) discs (5 mm diameter x 1.5 mm thick), obtained from Hing Lung Engineering, Inc. (Hong Kong, China). *Candida* biofilms were treated with miconazole (0.5 – 96 µg/ml). The metabolic activity of the biofilms was measured by the XTT reduction assay. The minimum inhibitory concentrations (MICs) of miconazole against *Candida* species were determined by the microdilution method. The MICs for miconazole for the investigated strains ranged from 0.016 – 32 µg/ml. Treatment with miconazole resulted in a significant reduction of biofilm metabolic activity for all strains. The highest inhibition was observed at 96 µg/ml miconazole. In the case of *C. glabrata* MYA-275 and *C. tropicalis* 8122/06 this corresponded to 83.7% and 75.4% inhibition, respectively. The lowest reduction was observed for *C. parapsilosis* 11375/07 – 46.1%.

For all *Candida* strains there was a strong correlation between MIC values and miconazole concentrations corresponding to a reduction of metabolic activity of the biofilm by 50%.

Miconazole exhibits high antifungal activity against *Candida* biofilms developed on the surface of PMMA discs. The study provides support for the use of miconazole as an effective agent for the treatment of oral candidiasis.

DEVELOPMENT OF THE NOVEL BARBITURIC ACID-BASED TOTAL INHIBITORS OF LEUKOCYTE TRANSMIGRATION

Tamar Getter (1), Raanan Margalit (2), Genia Alpert (3), Sophia Zilber (4), Paul Bradfield (5,7), Archana Vijaya Kumar (6), Shirin Kahremany (1), Hanoch Senderowitz (1), Ron Lahav (3), Thomas Matthes (6), Beat Imhof (7), Arie Gruzman (1)

1) Division of Medicinal Chemistry, Department of Chemistry, Faculty of Exact Sciences, Bar-Ilan University, Ramat-Gan, Israel
2) Science in Action, Ness-Ziona, Israel
3) AltA-ZuZ Therapeutics, Ness-Ziona, Israel
4) Department of Pathology, Shaare Zedek Medical Center, Jerusalem, Israel
5) MESENFLOW Technologies, Geneva, Switzerland
6) Hematology Service, University Hospital Geneva, Geneva, Switzerland
7) Department of Pathology and Immunology, University Medical Center, Geneva University, Geneva, Switzerland

Leukocyte transmigration is one of the most important events in the physiological tissue immune response. However, over-activation of the immune system leads to damage of healthy tissues. Thus, effective leukocyte migration inhibitors are considered as very promising potential therapeutic agents against inflammatory and autoimmune diseases. In addition, inhibition of the homing of B-lymphocytes to lymphoid organs may be envisioned as a new therapeutic strategy to reduce B-cell lymphoma proliferation and their capacity to reach supportive lymphoid microenvironments. Junctional adhesion molecules (JAM) belonging to the immunoglobulin superfamily, localize to inter-endothelial surfaces and regulate monocyte transmigration by binding to integrins. Based on a pharmacophore model derived from the JAM-C and integrin's interaction sites, fifteen new molecules with modified barbituric acid scaffold were designed in-silico, synthetized and tested in vitro. Human endothelial cells and human monocytes were used for the evaluation of the effect of synthetized compounds on the leucocyte transmigration. Three out of 15 compounds were active in a pharmacological concentration range. Importantly, one of the compounds (GT-73) completely blocked leukocyte transmigration, without damaging monocytes or endothelial cells (IC_{50}=2.4 µM). So far, even pan-antibody blockers of the beta-1 and 2 integrins were not able to block completely monocyte transmigration. GT-73 (10 mg/kg) was also active in-vivo using Crohn's disease and Multiple Sclerosis models. Finally, a possible effect on the rolling of lymphocytes was tested using a B-Cell lymphoma homing assay. GT-73 was injected together with human B-lymphoma cells IV to NOD mice. GT-73 significantly reduced the amount of cancer cells in the spleen and liver. Detailed acute toxicity profile of the compound was also studied and demonstrated not to have any toxic effects in the administrated doses. Such type of molecules might therefore provide a unique starting point for designing a novel class of leukocyte transmigration blocking agents with broad therapeutic applications.

EVOLUTION OF SELECTIVE FLAP INHIBITOR BRP-7 INTO MULTI-TARGET INHIBITOR OF FLAP, 5-LO AND mPGES-1 IN THE ARACHIDONIC ACID PATHWAY

Zehra Tuğçe Gür (1), Burcu Çalışkan (1), Abdurrahman Olgaç (1), Jana Gerstmeier (2), Ulrike Garscha (2), Oliver Werz (2), Erden Banoglu (1)

1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, 06330 Ankara, Turkey
2) Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany

Arachidonic acid (AA) pathway plays important role mainly for mediating a wide spectrum of inflammatory conditions. AA is released from membrane phospholipids by the action of phospholipase A_2 (PLA_2), and further processed by cyclooxygenases (COXs) to produce prostaglandins (PGs) and by 5-lipoxygenase (5-LO) to produce leukotrienes (LTs), which eventually elicit a wide array of physiological and pathological effects. Microsomal PGE_2 synthase-1 (mPGES-1), 5-LO and 5-LO-activating protein (FLAP) inhibitors seem to be promising therapeutic agents intervening with AA pathway at different nodes to generate potent anti-inflammatory effect. By this way, dual and balanced inhibition of AA cascade to prevent both PG and LT formation are anticipated to cause lower side effects while generating potent anti-inflammatory response. During our longstanding efforts to develop efficient inhibitors in the AA cascade, we have recently identified BRP-7, a benzimidazol derivative, that selectively inhibits FLAP to intervene with the LT biosynthesis with an IC_{50} of 0.31 µM [2].

To introduce pharmacophore groups on BRP-7 skeleton that might transform this selective FLAP inhibitor into a dual inhibitor of both PG and LT biosynthesis, structure activity relationship (SAR) studies involving (C)5 and (C)2 positions of benzimidazole ring were performed. Introducing polar substituents including carboxylic acid moieties and its bioisosters at C(5) resulted in compounds with multi-ligand properties. To improve drug-like properties of BRP-7 derivatives, heteroarylamine groups were also introduced at C(2) position. As a result, our SAR studies concluded that the BRP-7 core bearing oxadiazol-2-thione ring at C(5)-BI were able to inhibit FLAP, mPGES-1 and 5-LO activities with IC_{50} values of 0.05, 0.4 and 0.6 mM, respectively (This study was supported by TUBITAK Research Grant 112S596).

References

1) Ricciotti, E.; FitzGerald G. Arterioscler Thromb Vasc Biol. 2011, 31(5), 986–1000.
2) Banoglu, E., Caliskan, B.; Luderer, S.; Eren, G.; Ozkan, Y.; Altenhofen, W.; Weinigel, C.; Barz, D.; Gerstmeier, J.; Pergola, C.; Werz, O. Bioorg. Med. Chem. 20 (2012), 3728-3741.

4''-O-ALKYLATED α-GALACTOSYLCERAMIDE ANALOGUES AS iNKT CELL ANTIGENS: SYNTHETIC, BIOLOGICAL AND STRUCTURAL STUDIES

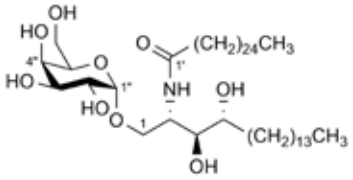
Jonas Janssens (1,2), Jing Wang (3), Aruna Bitra (3), Tine Decruy (4), Koen Venken (4), Johan Van der Eycken (2), Dirk Elewaut (4), Dirk M. Zajonc (3), Serge Van Calenbergh (1)

1) Laboratory for Medicinal Chemistry, Department of Pharmaceutics (FFW), Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium
2) Laboratory for Organic and Bioorganic Synthesis, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281 (S4), B-9000 Ghent, Belgium
3) Division of Cell Biology, La Jolla Institute for Allergy and Immunology (LJI), 9420 Athena Circle, La Jolla, CA 92037, USA
4) Department of Internal Medicine, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium

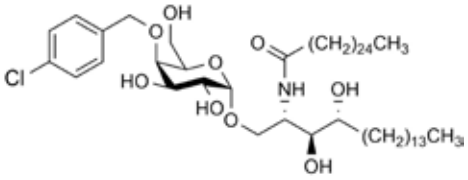
Invariant natural killer T-cells (*i*NKT) represent a unique subset of T-lymphocytes that play an important regulatory role in the protection against tumour cells, auto-immune diseases and certain infections. *i*NKT cells recognize the prototypical ligand α-galactosylceramide (α-GalCer, **1**), a synthetic glycolipid, presented by the MHC class I-like non-polymorphic glycoprotein CD1d.[1] After recognition by the T-cell receptor (TCR) and formation of a ternary CD1d-glycolipid-TCR complex, the *i*NKT cells secrete vast amounts of Th1- and Th2-cytokines, which serve as small-protein modulators in the immune system.

This presentation will focus on our attempts to design α-GalCer analogues that polarize the cytokine response towards Th1, which is desirable for defense against tumours and various intracellular pathogens. Towards this end we carefully investigated modifications of the galactose ring, in particular the 4"-position, which has remained underexplored up until now.[2]

We will demonstrate the ability of analogues modified at the 4”-position of the galactose ring to induce a polarized Th1 response in an *in vivo* mouse model. Crystallographic studies indicate that benzyl-type ethers, such as *p*-ClBn-α-GalCer (**2**), undergo additional Vanderwaals interactions with CD1d. In all, we have shown that judiciously chosen modifications of the carbohydrate moiety of α-GalCer may lead to an enhanced release of Th1-cytokines in mice.



1



2

References

1) A. Banchet-Cadeddu et al., Org. Biomol. Chem. 2011, 9, 3080–3104
2) J. Janssens et al., ACS Med. Chem. Lett. 2017, 8, 642–647

TARGETED METABOLOMICS PROFILING AS A BASIS FOR PREDICTIVE MODEL BUILDING IN MULTIPLE SCLEROSIS RESEARCH

Marat Kasakin (1,2), Artem Rogachev (2,3), Vladimir Koval (1), Elena Predtechenskaya (2), Andrey Pokrovsky (2)

1) Institute of Chemical Biology and Fundamental Medicine of SB RAS, 8 Lavrentiev Avenue, Novosibirsk, 630090, Russia
2) Novosibirsk State University, 1 Pirogova street, Novosibirsk, 630090, Russia
3) N. N. Vorozhtsov Novosibirsk institute of organic chemistry, 9 Lavrentiev Avenue, Novosibirsk, 630090, Russia

Multiple sclerosis (MS) is the most common immune-mediated disorder impacting on central nervous system. Metabolomics is a powerful approach for discovering biomarkers and investigation of human disease mechanism. It is known that amino and fatty acids metabolism is disturbed in this disease. We performed targeted metabolomics approach based on quantitative LC-MS/MS analysis of amino acids and acylcarnitines in dried plasma spots samples followed by multivariate statistical analysis using R integrated suite for discovering differences between MS (n=16) and control (n=12) groups.

It was found that asparagine level to be increased in MS group (p=0.0022), L-octenoyl-carnitine (C8:1) level to be decreased (p=0.0406). Partition least square discriminant analysis (PLS-DA) method widely used in metabolomics studies gives better separation between the groups compared to principal component linear discriminant analysis (PCA-LDA) algorithm (Figure 1), although it could be overestimated during leave-one-out cross-validation and needs to evaluation on the test group. Predictive models yield to AUC = 0.79, Sensitivity = 0.67, Specificity = 0.75 for PCA-LDA; 0.98, 0.81, 1 for PLS-DA and 0.80, 0.64, 0.80 for random forest algorithm (RF), respectively. PLS-DA model performs preliminarily excellent results as a potential screening test for MS, PCA-LDA and RF models produce results close to each other. All three models detect noticeable changes in amino acids and acylcarnitines profile in MS group in comparison with control group.

The data obtained and the methods of analysis developed are a reliable basis for the diagnosis of multiple sclerosis.

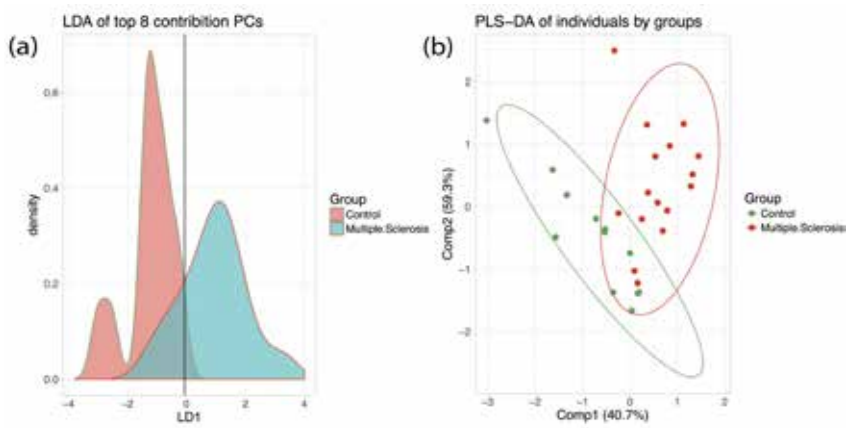


Figure 1. Supervised multivariate analysis for Multiple Sclerosis and Control groups
(a) Linear discriminant analysis preliminary pre-processed PCA (b) Partial least square discriminant analysis

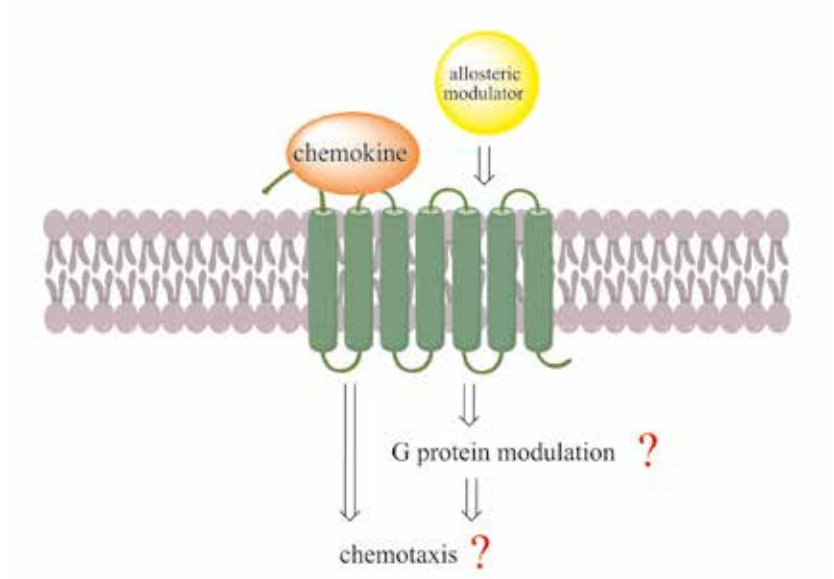
P204

DEVELOPMENT OF SELECTIVE AND/OR DUAL CXCR3 AND CXCR4 ALLOSTERIC MODULATORS

Anja Kolarič (1,2), Urban Švajger (3), Tihomir Tomašič (2), Nikola Minovski (1), Nuška Tschammer (4), Marko Anderluh (2)

1) Theory Department, Laboratory for Cheminformatics, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
2) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
3) Blood Transfusion Center of Slovenia, Šlajmerjeva 6, 1000 Ljubljana, Slovenia
4) Department of Chemistry and Pharmacy, Medicinal Chemistry, Emil Fischer Center, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Numerous diseases and disorders were associated with the malfunction of chemokine receptors signaling, which makes them interesting and promising drug targets.[1] Based on the previously published dual negative allosteric modulator of CXCR3 and CXCR4 chemokine receptors[2], we designed, synthesized and biologically characterized a set of novel not only negative, but also positive allosteric modulators with preserved pyrazolopyridine core. We successfully identified a dual negative modulator, inhibiting G protein activity of both receptors. For CXCR4 receptor we postulate that *para*-substituted aromatic group of compounds distinguishes between negative and positive modulation. *Para*-methoxy substitution leads to functional antagonism, while *para*-chloro stimulates agonism. Additionally, we discovered that chemotaxis is not necessarily in a strong correlation with G protein signaling pathways. In this work we have successfully demonstrated the discovery of selective as well as dual-acting CXCR3/CXCR4 modulators, which provide valuable information for future discovery of chemokine receptor modulators.



References

1) P.A. Insel, C.M. Tang, I. Hahntow, M.C. Michel, BBA Biomemb., 2007, 1768, 994-1005.
2) D. Schmidt, V. Bernat, R. Brox, N. Tschammer and P. Kolb, ACS Chem. Biol., 2014, 10, 715-724.

P206

AMPK ACTIVATORS AS NOVEL DRUG CANDIDATES FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES

Luca Quattrini (1), Luca Antonioli (2), Francesco Angelucci (1), Vito Coviello (1), Matteo Fornai (2), Carolina Pellegrini (2), Corrado Blandizzi (2), Won-Keun Oh (3), Concettina La Motta (1)

1) Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126, Pisa, Italy
2) Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa, Via Savi 10, 56126, Pisa, Italy
3) College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea

Inflammatory bowel diseases (IBDs), mainly represented by ulcerative colitis and Crohn's disease, are chronic and idiopathic diseases of the digestive tract. Their incidence and prevalence is raising significantly in both developed and developing countries, thus representing a major challenge for the worldwide healthcare systems. The pharmacological armamentarium for the treatment of IBDs is far from being satisfactory, as the therapeutic success of the available drugs is still limited. Accordingly, the development of novel and effective compounds is highly requested. In this context, the serine/threonine heterotrimeric kinase AMPK (adenosine monophosphate-activated protein kinase) seems a sound target to strike.

Known as the central hub of energy homeostasis in eukaryotic cells, AMPK contributes also to the modulation of immune/inflammatory cell functions. Actually, alterations in AMPK expression and/or activity play a key role in the pathophysiology of immune-mediated inflammatory diseases characterized by abnormal immune cell functions, like IBDs. Moreover, AMPK is able to improve intestinal health by enhancing para-cellular junctions, nutrient transporters, autophagy and apoptosis. Accordingly, AMPK activation represents a promising therapeutic strategy for the treatment of intestinal inflammatory disorders.[1]

Here we describe a novel heterocyclic derivative, developed as AMPK activator.[2]

Tested in C2C12 myoblast cell lines, our compound significantly increased AMPK activity, in a concentration-dependent manner, turning out to be more effective than the well-known activator acadesine (ACA). Moreover, assayed in a mouse model of acute DNBS-induced colitis, the novel heterocycle displayed a relevant anti-inflammatory efficacy, proving to ameliorate both systemic- and tissue-related inflammatory parameters like body and spleen weight, colon length, macroscopic damage, TNF and MDA levels. Also in this case, our compound turned out to be significantly more active that the known reference ACA, thus imposing itself as a novel and valuable drug candidate for the treatment of IBDs.

References

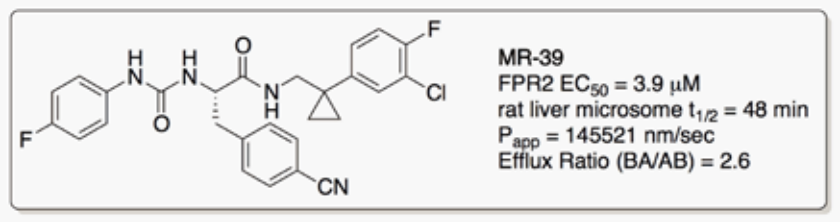
1) Séverine Oliviera, S.; Foretza, M.; Violleta, B. Promise and challenges for direct small molecule AMPK activators. Biochem. Pharmacol. 2018.
2) F. Angelucci, L. Quattrini, V. Coviello, L. Antonioli, M. Fornai, C. Blandizzi, W.K. Oh, C. La Motta. Italian Patent Application, 102017000039329.

OPTIMIZATION OF FORMYL PEPTIDE RECEPTOR 2 (FPR2) AGONISTS WITH NEUROPROTECTIVE PROPERTIES BASED ON UREIDOPROPANAMIDE SCAFFOLD

Enza Lacivita (1), Margherita Mastromarino (1), Igor A. Schepetkin (2), Liliya N. Kirpotina (2), Ewa Trojan (3), Mark T. Quinn (2), Agnieszka Basta-Kaim (3), Marcello Leopoldo (1)

1) Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari, Via Orabona, 4, 70125 Bari, Italy
2) Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717, USA
3) Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Kraków, Poland

Chronic or unresolved inflammation is a central pathological process in various diseases, including neurodegenerative disorders. Successful resolution of inflammation requires the activation of endogenous pathways, which can switch from production of pro-inflammatory to specialized pro-resolving mediators (SPMs). New insights into such pathways are offering novel opportunities to pharmacologically manipulate the resolution of inflammation and, eventually, to open new therapeutic approaches for chronic inflammation [1]. Formyl peptide receptor 2 (FPR2), a receptor modulated by several SPMs, such as lipoxin A4 and resolvins, is one of the key players in the resolution of inflammation [2]. Recently, we identified a class of non-peptidic FPR2 agonists with a ureidopropanamide scaffold, exemplified by compound MR-39, which shows neuroprotective properties in an *in vitro* model of neuroinflammation. In fact, MR-39 is able to reduce nitric oxide (NO) release and attenuate tumor necrosis factor (TNF) and IL-1b release in rat primary microglial cells stimulated with bacterial lipopolysaccharide (LPS). In addition, MR-39 has good *in vitro* pharmacokinetic properties, such as resistance to oxidative metabolism in rat microsomes and passive diffusion and permeation rate in a hCMEC/D3 cell monolayer, which is a model of blood brain barrier [3].



Here we report further optimization of the MR-39 structure that has led to the identification of a set of new FPR2 agonists with improved potency and *in vitro* pharmacokinetic properties. We will discuss the structure-activity and structure-property relationships of new FPR2 agonists. The influence of the most potent compounds on viability/metabolic activity, necrotic death, and production of pro-inflammatory mediators in microglial cells under normal conditions and after stimulation with LPS will be illustrated by highlighting the potential of these agonists in the treatment of CNS diseases characterized by neuroinflammation.

References

1) Fullerton, J. N.; Gilroy, D. W. Resolution of inflammation: a new therapeutic frontier. Nat. Rev. Drug Discov. 2016, 15(8):551-67.
2) Corminboeuf, O.; Leroy, X. FPR2/ALXR agonists and the resolution of inflammation. J. Med.Chem. 2015, 58, 537.
3) Stama, M. L.; Ślusarczyk, J.; Lacivita, E.; Kirpotina, L. N.; Schepetkin, I. A.; Chamera, K.; Riganti, C.; Perrone, R.; Quinn, M. T.; Basta-Kaim, A.; Leopoldo, M.Novel ureidopropanamide based N-formyl peptide receptor 2 (FPR2) agonists with potential application for central nervous system disorders characterized by neuroinflammation. Eur. J. Med. Chem. 2017, 141:703-720.

DEVELOPMENT OF PEPTIDES AS THERAPEUTICS FOR PHARMACOLOGICAL INTERVENTION IN VITAL PROTEIN CASCADES

Christina Lamers (1), Christian Heinis (2), Daniel Ricklin (1)

1) Molecular Pharmacy Group, University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland
2) Laboratory of Therapeutic Peptides and Proteins, École polytechnique fédérale de Lausanne, Av. F.-A. Forel 2, 1015 Lausanne, Switzerland

As a therapeutic molecule class, peptides combine advantages of protein-based biomolecules, such as high affinity, specificity and ability to target “undruggable” targets, with the easy synthetic accessibility and chemical modification of small molecules. Cyclic peptides in particular can utilize a high surface area for binding, which makes them suitable for targeting protein-protein interactions. With their comparatively constrained structures, they are reducing the entropic penalty upon binding and their rigidity renders cyclic peptides more resistant to proteolytic degradation than their linear counterparts. Several cyclization methods are used, such as on-resin side specific lactamization, disulfide bridging, thioether cyclization or usage of thiol-reactive linker.

In this study, we describe the development of phage display-derived cyclic peptides for the therapeutic modulation of vital protein cascades within the blood circulation. Both the complement system and the coagulation cascade serve as “first line of defense” against injurious stimuli and microbial invaders: upon activation, a series of cascading enzymatic reactions lead to an amplification of the initial signal, resulting in fibrin deposition (coagulation cascade), pathogen clearance and opsonic cell killing (complement cascade). In several thrombo-inflammatory conditions[1], including transplant rejection, stroke and reperfusion injury, both host defense systems may be inadvertently triggered and contribute to clinical complications. Therapeutic control of complement and coagulation activation has therefore gained attention.

For example, blocking coagulation factor XII (FXII) has been shown to reduce thrombosis in various animal models without increasing the risk for bleeding[2], a major problem of current anti-coagulants. Moreover, plasma kallikrein (PK) amplifies FXII activity and is also considered an important target due to the generation of proinflammatory kinins. By employing a bicyclic peptide phage display approach[3] with on-phage chemical cyclization, we obtained bispecific FXII/PK inhibitors, which were improved to inhibit both targets in the nanomolar range using additional structure-activity relationship studies.

A similar approach can also be applied to the development of complement inhibitors. Cyclic peptides have shown great promise as protein-protein interaction inhibitors in the complement cascade[4]. Through incorporation of unnatural amino acids and other modifications, we aim to improve affinity, selectivity and pharmacokinetic properties of such leads for a use in a broad range of disease models.

References

1) Ricklin D, et al. Nature Reviews Nephrology 2016; 12: 383-401.
2) Kenne E, et al. Drug discovery today. 2014; 19:1459-64.
3) Heinis C, et al. Nature chemical biology. 2009; 5: 502-7.
4) Ricklin D, et al. Nature Reviews Nephrology 2018; 14: 26-47.

P209

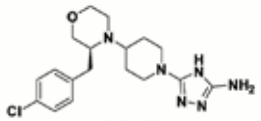
DEVELOPMENT OF DUAL AMCase AND CHIT1 INHIBITOR OAT-870 AS A POTENTIAL THERAPEUTIC FOR INTERSTITIAL LUNG DISEASES

Marzena Mazur (1), Sylwia Olejniczak (1), Robert Koralewski (1), Barbara Dymek (1), Magdalena Salamon (1), Wojciech Czestkowski (1), Bartłomiej Borek (1), Agnieszka Bartoszewicz (1), Gleb Andryianau (1), Michał Kowalski (1), Krzysztof Matyszewski (1), Elżbieta Pluta (1), Agnieszka Zagożdżon (1), Piotr Sklepkiewicz (1), Jakub Golab (2), Michał Piotrowicz (1), Piotr Niedziejko (1), Mariusz Gruza (1), Jacek Olczak (1), Karolina Dzwonek (1), Adam Gołębiowski (1)

1) OncoArendi Therapeutics SA, Żwirki i Wigury 101, 02-089 Warsaw, Poland
2) Department of Immunology, Medical University of Warsaw, 1A Banacha Str., 02-097 Warsaw, Poland

Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinases, which have been shown to be involved in various lung pathologies such as idiopathic pulmonary fibrosis, sarcoidosis, chronic obstructive pulmonary disease and asthma. Elevated CHIT1 levels and activity were found in the plasma and bronchoalveolar lavage (BAL) fluids from patients with interstitial lung diseases (IPF and sarcoidosis). AMCase is activated during type 2 inflammatory responses in both murine models of airway inflammation and in asthma patients.

Herein we present design and synthesis of a series of potent dual AMCase and CHIT1 inhibitors. Among this series, OAT-870 was identified as a lead compound with good in vitro and in vivo efficacy. OAT-870 is a highly potent dual AMCase and CHIT1 small molecule inhibitor with a nanomolar activity for both human and murine enzymes.



OAT-870

hAMCase	IC_{50}	19nM
hCHIT1	IC_{50}	47nM
mAMCase	IC_{50}	20nM
mCHIT1	IC_{50}	77nM

In vitro structure-activity relationship data, ADME, pharmacokinetic properties as well as in vivo data showing strong anti-inflammatory effects of compound OAT-870 in house dust mite (HDM) induced airway inflammation model is reported.

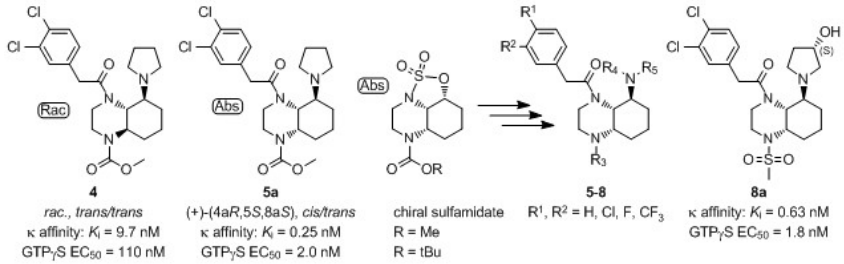
P210

DESIGN AND SYNTHESIS OF ENANTIOMERICALLY PURE DECAHYDROQUINOXALINES AS POTENT AND SELECTIVE K-OPIOID RECEPTOR AGONISTS WITH ANTI-INFLAMMATORY ACTIVITY IN VIVO

Menno Monnee (1), Anita Wegert (1), Peter Molenveld (1), Roy Storcken (1), Renaud Bouzanne des Mazery (1), Geert Jan Sterk (1), Reshma Autar (1), Sonja Ständer (2), Bernhard Wünsch (3), Michael Soeberdt (4)

1) Mercachem, Kerkenbos 1013, NL-6546 BB Nijmegen, The Netherlands
2) Klinik für Hautkrankheiten, Universitätsklinikum Münster, Von-Eschmarch-Straße 58, D-48149 Münster, Germany
3) Westfälische Wilhelms-Universität Münster, Corrensstraße 48, D-48149 Münster, Germany
4) Dr. August Wolff GmbH & Co. KG Arzneimittel, Sudbrackstraße 56, D-33611 Bielefeld, Germany

Opioid receptor agonists activating especially μ receptors are clinically used for their analgesic efficacy. Compared with μ agonists, κ agonists show a different side effect profile with minimal respiratory depression, negligible inhibition of gastrointestinal motility and reduced physical dependence.[1] Recently, racemic *trans/trans* decahydroquinoxaline **4**, a conformationally restricted analogue of the well-known κ-opioid agonists U-50,488 and GR-89,696, was reported as a potent and selective κ-opioid receptor agonist. We synthesized all diastereoisomers of decahydroquinoxaline **4**, and separated them into enantiomers. Enantiomer **5a** was identified as a high affinity and selective κ ligand (K_i = 0.25 nM) and full agonist (EC_{50} 2.0 nM). We have developed an enantioselective synthesis via a chiral cyclic sulfamidate scaffold, and fine-tuned the physicochemical and pharmacological properties by structural modifications on three positions.[2] The methanesulfonamide **8a** was identified as potent (K_i = 0.63 nM) and peripherally restricted κ agonist (EC_{50} = 1.8 nM) with dose-dependent anti-inflammatory activity in acute and chronic skin inflammation.



4	**5a**		**5-8**	**8a**
rac., trans/trans	(+)-(4a*R*,5*S*,8a*S*), *cis/trans*	chiral sulfamidate	R^1, R^2 = H, Cl, F, CF_3	κ affinity: K_i = 0.63 nM
κ affinity: K_i = 9.7 nM	κ affinity: K_i = 0.25 nM	R = Me		$GTP_\gamma S$ EC_{50} = 1.8 nM
$GTP_\gamma S$ EC_{50} = 110 nM	$GTP_\gamma S$ EC_{50} = 2.0 nM	R = tBu		

References

1) (i) Stein, C.; Machelska, H. Pharm. Rev. 2011, 63, 860; (ii) Trescot, A. M.; Datta, S.; Lee, M.; Hansen, H. Pain Physician 2008, Opioid Special Issue 11, S133.
2) (i) Soeberdt, M.; Knie, U.; Abels, C., patent WO2014/184355 A1 and WO2014/184356 A1. (ii) Soeberdt, M.; Molenveld, P. et al., J. Med. Chem. 2017, 60 (6), 2526-2551.

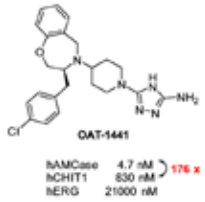
DISCOVERY OF SELECTIVE, ORALLY BIOAVAILABLE INHIBITOR OF HUMAN ACIDIC MAMMALIAN CHITINASE (hAMCase)

Piotr Niedziejko, Gleb Andryianau, Michał Kowalski, Michał Piotrowicz, Barbara Dymek, Magdalena Salamon, Agnieszka Zagożdżon, Marcin Mazurkiewicz, Marzena Mazur, Sylwia Olejniczak, Robert Koralewski, Krzysztof Matyszewski, Wojciech Czestkowski, Agnieszka Bartoszewicz, Elżbieta Pluta, Mariusz Gruza, Filip Stefaniak, Karolina Dzwonek, Jacek Olczak, Adam Gołębiowski

OncoArendi Therapeutics, Żwirki i Wigury 101, 02-089 Warsaw, Poland

Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinases, which have been shown to be involved in various lung pathologies such as idiopathic pulmonary fibrosis, sarcoidosis, chronic obstructive pulmonary disease and asthma. Elevated CHIT1 levels and activity were found in the plasma and bronchoalveolar lavage (BAL) fluids from patients with interstitial lung diseases (IPF and sarcoidosis). AMCase is activated during type 2 inflammatory responses in both murine models of airway inflammation and in asthma patients.

Herein we present design and synthesis of a series of potent dual AMCase and CHIT1 inhibitors. Among this series, **OAT-870** was identified as a lead compound with good *in vitro* and *in vivo* efficacy. **OAT-870** is a highly potent dual AMCase and CHIT1 small molecule inhibitor with a nanomolar activity for both human and murine enzymes.



In vitro structure-activity relationship data, ADME, pharmacokinetic properties as well as *in vivo* data showing strong anti-inflammatory effects of compound **OAT-870** in house dust mite (HDM) induced airway inflammation model is reported.

References

1) Zhu, Z.; Zheng, T.; Homer, R. J.; Kim, Y. K.; Chen, N. Y.; Cohn, L. Hamid, Q.; Elia;s, J. Science 2004, 304, 1678-1682.
2) Shen, C. R.; Juang, H. H.; Chen, H. S.; Yang, C. J.; Wu, C. J.; Lee, M. H.; Hwang, Y. S.; Kuo, M. L.; Chen, Y. S.; Chen, J. K.; Liu, C. L. Int. J. Mol. Sci. 2015, 16, 27371-27377.
3) Matsumoto, T.; Inoue, H.; Sato, Y.; Kita, Y.; Nakano, T.; Noda, N.; Eguchi-Tsuda, M.; Moriwaki, A.; Kan-O, K.; Matsumoto, K.; Shimizu, T.; Nagasawa, H.; Sakuda, S.; Nakanishi, Y. Biochem. Biophys. Res. Commun. 2009, 390, 103-108.

CONJUGATION OF NSAIDS WITH ACTIVE ALCOHOLS AND THEIR EFFECT ON INFLAMMATION

Paraskevi Tziona , Panagiotis Theodosis-Nobelos, Georgios Papagiouvannis , Panos N. Kourounakis , Eleni Rekka

Department of Medicinal Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Inflammation is a defensive mechanism of the organism to cope with chemical or cellular challenges. However, it is well documented that inflammation is implicated in several pathological conditions, such as metabolic syndrome, cardiovascular and neurodegenerative diseases.

Non steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed classes of drugs for pain and inflammation. They are responsible for approximately 5-10% of all medications prescribed each year, although their use is connected with serious undesired effects, mainly from the gastrointestinal tract and the kidneys.

Atherosclerosis, a condition affecting arterial blood vessels, is the main risk factor for cardiovascular disease, one of the most widespread diseases in the modern western world. Hyperlipidemia can lead to the formation of multiple plaques within the artery. Oxidation of LDL promotes inflammatory responses.

In this investigation, we synthesised a series of esters of well known non specific COX-1 and -2 inhibitors, such as ibuprofen or ketoprofen, with a number of alcohols. (3,4,5-Trimethoxyphenyl)methanol was selected since gallic acid and related compounds have been reported to possess antioxidant, anti-mutagenic, anti-allergic and anti-inflammatory activities. In addition, trimethazidine, a trimethoxybenzyl-derivative, is used for cardiovascular events. 2-Methoxy-4-methyl-phenol was also used, based on the reported potential anti-inflammatory and cytoprotective action of a number of derivatives of this compound. 2,6-Di-*tert* -butyl-4-(hydroxymethyl)phenol was used, since butylated hydroxytoluene (BHT, 2,6-di-*tert* -butyl-4-methylphenol) is a well-known antioxidant with low toxicity. For a number of structures, natural amino acids were used as linkers, due to the potentially low toxicity of the derivatives. All compounds were isolated and their structures identified.

The anti-inflammatory activity of compounds was assessed from their ability to inhibit the paw oedema induced by carrageenan in rats. The compounds were administered ip at a dose of 150 μmol/kg and demonstrated significant inhibition of oedema, ranging for 25 to 90%. Their in vivo effect on inflammation enzymes was also tested.

In addition, the hypolipidemic properties were examined for selected compounds. Their effect on plasma cholesterol and triglyceride levels was estimated in rats with Triton-WR1339 induced hyperlipidemia. The synthesised compounds could reduce plasma lipidemic indices from 50 to 88%.

The obtained results indicate that the design of the described derivatives of non steroidal anti-inflammatory drugs with potentially active alcohols gave novel compounds that acquire a series of biological properties able to prevent or restore pathological changes in conditions related to inflammation, with potentially wider safety margin.

P. Theodosis-Nobelos and G. Papagiouvannis acknowledge the General Secretariat for Research and Technology (GSRT) of Greece and the Hellenic Foundation for Research and Innovation (HFRI) for a grant supporting their PhD research.

NOVEL ANTIINFLAMMATORY STEROIDAL COMPOUNDS.

Jesus Sandoval-Ramírez (1), Reyna Zeferino-Díaz (1), María A. Fernández-Herrera (2), Nuvia Kantún-Moreno (3), Leticia Olivera-Castillo (3)

1) Laboratorio de Síntesis y Modificación de Productos Naturales, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. Puebla, Puebla, México
2) Departamento de Física Aplicada, CINVESTAV-Mérida, Laboratorio de Biomoléculas. Mérida, Yucatán, México
3) Departamento de Recursos del Mar, CINVESTAV-Mérida, Laboratorio de Nutrición Acuícola, Mérida, Yucatán, México

Introduction. Inflammation is a natural response for the protection of organisms, against any damage or infection and involves cellular events and chemical signals. Despite of many undesirable consequences, the most prescribed anti-inflammatory compounds are corticosteroids[1] and their analogs. In this sense, the search of new drugs has been undertaken. A new family of hydroxyimino steroids[2] has been synthesized and evaluated by means of an acute mouse ear edema assay (induced by 12-O-tetradecanoylphorbol 13-acetate **TPA**). The oximes were synthetized from diosgenin (Dg) and their anti-inflammatory activity was evaluated. According to these results, the best active compounds were selected to be analyzed vis a vis the gene markers, expressed in inflammation processes: Tumor Necrosis Factor alpha (TNF-*a*), Interleukin 6 (IL-6), Cyclooxygenase 2 (COX-2) and Macrophage Inhibition Factor (MIF)[3,4]

Development. The steroidal oximes were synthesized from Dg. First, the regioselective opening of E and F rings was performed, obtaining the (25*R*)-26-hydroxy-22-oxocholest-5-en-3β,16β-diyl diacetate, which was selectively oxidized on the hydroxyl at C-26 to obtain the corresponding aldehyde (25*R*)-22,26-dioxocholest-5-en-3β,16β-diyl diacetate. From the latter, two methodologies were used to obtain the target oximes; the first one, under classic oximation conditions hydroxylamine was employed to obtain the steroidal 26*E* and 26*Z* oximes (25*R*)-26-hydroximino-22-oxocholest-5-en-3β,16β-diyl diacetate, **1** and **2**. The second methodology involved the use of 3,4,5-trimethoxyaniline. Under this basic condition, the loss of C-26 was promoted. In this way, the 27-nor-22,25-dioxocholest-5-en-3b,16b-diyl diacetate was obtained. From the latter compound, the *E* oxime **3,** at C-25 was chemoselectively prepared.[5]

The biological evaluation was carried out under a murine model (*Mus musculus*), which consisted in the topical application of the proto-inflammatory TPA, at the mouse right ear. This procedure increases the relative expression of the genes COX -2, TNF-α, IL-6 and MIF, involved in the inflammatory process. The new steroidal oximes **1, 2**, and **3** were tested to reverse the inflammation, comparing their activity with dexamethasone (**DXA**).

Interesting results were obtained in all studied parameters. TNF-a: The expression of this gene decreased considerably at the topically treated ears with **DXA** and with oximes **1** and **3**, even when using lower doses than the positive control **DXA**. IL-6: The expression of this gene decreased in the ears treated with **DXA**, **1** and **3**; the latter showing the best effect at the transcriptional level on interleukin 6. COX-2: On the other hand, the expression of this gene decreased drastically in the topically treated ears with **DXA** (74%) as well as oximes **1** (63%) and **3** (77%). The reduction in COX-2 expression was recorded for oximes when using half dose of **DXA**. MIF: The expression of this gene decreased with the **DXA** (67%), oximes **1** (84%) and **3** (84%) application.

Conclusions. Oximes **1** and **3** showed higher anti-inflammatory activity than **DXA** in an acute mouse ear model. Oximes **1** and **3** inhibit the expression of the TNF-α, COX-2, Il-6 and MIF genes. MIF is associated with several cutaneous pathologies. Steroidal oximes inhibit the expression of MIF, so these substances have a high dermatological potential.

References

1) Rodríguez, J.; Nuñez, L.; Peixinho, S.; Jiménez, C. Tetrahedron Lett. 1997, 38, 1833.
2) Coutinho AE, Chapman KE. Mol. Cell Endocrinol. 2011, 335, 2.
3) Murakawa, M.; Yamaoka, K.; Tanaka, Y.; Fukuda, Y. Biochem. Pharmacol. 2006, 71, 1331.
4) Gabor, M. (2003). Models of acute in the ears. In: Winyard P.G., Willoughby D.A. (eds) Inflammation Protocols. Methods in Molecular Biology, 225. Humana Press
5) Sanchez-Sanchez, L.; Hernandez-Linares, M. G.; Escobar, M. L.; Lopez-Munoz, H.; Zenteno, E.; Fernandez-Herrera, M. A.; Guerrero-Luna, G.; Carrasco-Carballo, A.; Sandoval-Ramirez, J. Molecules 2016, 21, 17.

DESIGN OF NOVEL β-HYDROXY-β-ARYLALKANOIC ACIDS WITH IMPROVED GASTROINTESTINAL ABSORPTION BASED ON QSRR STUDIES

Jelena Savić, Vladimir Dobričić, Katarina Nikolic, Jasmina Brborić

Department of Pharmaceutical Chemistry, University of Belgrade-Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

Gastrointestinal absorption of thirteen synthetized β-hydroxy-β-arylalkanoic acids which exhibited anti-inflammatory activity [1,2] was predicted and compared to ibuprofen using biopartitioning micellar chromatography [3]. BMC experimental conditions were chosen in a way to simulate gastrointestinal tract. The mobile phase consisted of aqueous phase (40 mM solution of Brij35 in 7 mM disodium hydrogen phosphate) and acetonitrile (80:20, v/v). pH of the mobile phase was adjusted to 5.5 to mimic the upper portion of duodenum, and the column temperature was set to 36.5 ºC. Working solutions of analyzed compounds were injected in triplicates and retention factors (*k*) were calculated (solution of KI in the mobile phase was used for the determination of column dead time).

All tested acids had lower *k,* so expected gastrointestinal absorption is lower than for ibuprofen (18.92±0.13).

Quantitative Structure Retention Analysis (QSRR) of obtained results was performed in order to identify molecular descriptors with the highest influence on *k*. ANN(*k*) and MLR(*k*) models were created. Based on statistical analysis MLR(*k*) model was selected as an optimal. Regression equation of this model is:

$$y = (18.19\pm1.47)-(2.44 \pm 0.06)\cdot nBM+(0.04\pm0.01)\cdot P\_VSA\_LogP\_8+(4.02\pm0.28)\cdot Eta\_L.$$

Interpretation of descriptors (nBM, P_VSA_LogP_8 and EtaL) included into the equation, indicated that introduction of saturated or partially unsaturated rings instead of phenyl rings, as well as introduction of nitro group or halogens into another ring could positively affect *k* value. Based on these conclusions, six novel β-hydroxy-β-arylalkanoic acids were designed. *K* values for these compounds calculated using the selected MLR(*k*) model were higher than for synthetized compounds indicating that designed compounds should have better gastrointestinal absorption than synthetized ones.

References

1) J. S. Savić, S. P. Dilber, B. D. Marković, M. T. Milenković, S. M. Vladimirov, I. O. Juranić, Molecules 2011, 16, 6645-6655.
2) J.Savić, S. Dilber, M. Milenković, J. Kotur-Stevuljević, S.Vladimirov, J. Brborić J. Med. Chem. 2017, 13, 186-195.
3) Dobričić V., Savić J., Nikolic K., Vladimirov S., Vujić Z., Brborić J. Eur. J. Pharm. Sci. 2017, 100, 280-284.

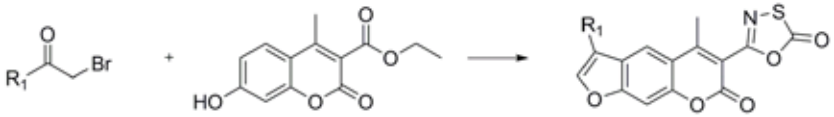
SYNTHESIS AND CHARACTERISATION OF PSORALEN DERIVATES AS INHIBITORS OF THE β5i SUBUNIT OF THE IMMUNOPROTEASOME

Eva Shannon Schiffrer, Izidor Sosič, Martina Gobec, Irena Mlinaric-Rascan, Stanislav Gobec

Faculty of Pharmacy, University of Ljubljana, Askerceva 7, 1000 Ljubljana, Slovenia

The eukaryotic 26S proteasome represents the heart of the ubiquitin-proteasome system. The system is responsible for maintaining protein homeostasis and regulation of many cellular processes, such as antigen processing, signal transduction, cell differentiation and apoptosis. Its 20S core particle has three enzymatically active subunits which have distinct substrate specificities. The β5i (chymotrypsin-like) subunit prefers neutral, hydrophobic residues at the cleavage site and β5i-selective compounds are investigated for possible application in autoimmune and inflammatory diseases related to the immunoproteasome. The majority of currently available inhibitors have a peptidic backbone which makes them prone to poor metabolic stability and low bioavailability.

Previous studies established psoralen derivates with an oxathiazolone 'warhead' as nonpeptidic covalent inhibitors of the β5i subunit.1 With the intent to deepen structure-activity relationship knowledge for psoralens, we synthesised a series of compounds with variations at the R1 position on the parent psoralen. Interestingly, despite seemingly straightforward reactions, several synthetic difficulties arose during preparation of some derivatives with substitutions at the R1 position. Our focus was also devoted to the replacement of the oxathiazolone 'warhead'. Besides previously published1 succinimidyl esters, acrylamides, and nitrile-based electrophiles we introduced several other 'warheads', such as α',β'-epoxyketones, 3-bromo-4,5-dihydrooxazole, vinyl, and ketoaldehyde; in all cases, a phenyl group was maintained as a substituent at R1. All successfully prepared psoralens were characterised in in vitro and cell-based assays to assess their selectivity and potency.



References

1) I. Sosič, M. Gobec, B. Brus, D. Knez, M. Živec, J. Konc, S. Lešnik, M. Ogrizek, A. Obreza, D. Žigon, D. Janežič, I. Mlinarič-Raščan, S. Gobec, Angew. Chem. Int. Ed. 2016, 55, 5745-5748.

FIDELTAMACRO™: MACROLIDE INSPIRED MACROCYCLIC LIBRARY

Linda Tomašković on behalf of Fidelta

Fidelta Ltd, Prilaz baruna Filipovića 29, 10000 Zagreb, Croatia

Novel macrolide inspired macrocyclic library is prepared using FideltaMacro™ technology. It comprises macrocycles designed to diversify and enrich chemical space with different ring sizes, a variety of 3D shapes and potential pharmacophoric features.[1]

Macrolides are an exceptional starting point for constructing macrocycles. It has been reported that certain macrolide antibiotics also possess anti-inflammatory properties. These properties are considered fundamental for their efficacy in the treatment of chronic inflammatory diseases. However, the long-term low-dose treatment with macrolide antibiotics presents a considerable risk for promotion of bacterial resistance. Although they do not follow conventional Lipinski Ro5, they are druggable and possess demonstrated clinical relevance.[2]

Fidelta has developed chemistry exploiting macrolides to afford new diverse macrocycles. The aim was to maintain the attractive pharmacokinetic and permeability properties of macrolides. A diversed macrocyclic library was designed and prepared in order to modulate anti-inflammatory and anti-infective properties. The library was screened to select compounds with ability to inhibit IL-6 production in vitro. Moreover, this novel macrolide derivatives showed the anti-inflammatory activity as demonstrated by inhibition of TNF-alpha overproduction induced by bacterial lipopolysaccharide (LPS) *in vivo*.[3]

Therefore, such macrocycles are a good candidates for development of novel anti-inflammatory agents, which will fill the gap in existing anti-inflammatory therapeutics and significantly broaden treatment possibilities.

References

1) Alihodžić S. et al. Current Trends in Macrocyclic Drug Discovery, Prog Med Chem, in press.
2) Stepanić V. et al. Physico-chemical profile of macrolides and their comparison with small molecules, Eur J Med Chem 2012, 47:462-72.
3) Fajdetić A. et al. Seco Macrolide Compounds, WO2017194452A1.

DRUG DESIGN AND BIOLOGICAL EVALUATION OF NOVEL ARYL HYDROCARBON RECEPTOR (AhR) ANTAGONISTS

Carlos Henrique Tomich de Paula da Silva, Larissa Pernomian, Joao Gabriel Curtolo Poiani

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil

Aryl hydrocarbon receptor (AhR) is a transcription factor activated by ligand, which regulates genes of the cytochrome P4501. However, AhR also modulates many physiological and pathological processes that affect inflammatory and immunological responses. There is a growing interest in discovery of selective competitive antagonists for AhR, where the most potent ones exhibit acceptable antagonist properties but they also show partial agonist activity. Other AhR pure competitive antagonists also exhibit agonist activity on estrogen receptors (ER), such as the resveratrol2 and kaempferol3. Also, limited availability of selective and pure competitive AhR antagonists and scarce structural information regarding AhR binding domain (located in PAS-B structure within the PAS domain) are reported.

In this work, a preliminary search in the Protein Data Bank (PDB) for AhR structures revealed only one – a PAS-A domain, but not PAS-B. A homology model of the PAS-B domain of the AhR receptor was then carried out, using PAS-B structures of ARNT as templates, which belongs to the same family of AhR proteins and whose PAS-B domain share a sequence identity with PAS-B of AhR around 30% (50% for the binding residues). A flexible docking approach was then used with the most potent AhR antagonists reported, allowing us to derive (and to validate) a pharmacophoric pattern common to the compounds thus aligned.

Two subsequent virtual screening experiments were then performed in databases of commercially available compounds, using the pharmacophore model built for the most potent AhR antagonists reported. In sequence, the compounds were filtered regarding to both atoxicity and good pharmacotherapeutic profile, thus predicted in silico, and tested. Finally, 7 novel atoxic AhR antagonists have been thus discovered, which experimentally showed atheroprotective efficacy correlated to the AhR antagonism, since they inhibited, almost completely, AhR-mediated oxLDL uptake by murine macrophages, induced by TCDD.

References

1) TV Beischlag, et al., Crit. Rev. Eukaryot. Gene Expr. 18 (2008) 207-250.
2) RF Casper, et al., Mol. Pharmacol. 56 (1999) 784-790.
3) J Wang, et al., FEBS Letters 583 (2009) 643-647.

NOVEL TRIAZOLE BASED MANNICH BASES AS ANALGESIC AND ANTIINFLAMMATORY AGENTS

Hayrünnisa Taşçı (1), Ahmet Avcı (1), Özgür Devrim Can (2), Nesrin Gökhan Kelekçi (1), Birsen Tozkoparan (1)

1) Hacettepe University Faculty of Pharmacy Dept of Pharmaceutical Chemistry, 06100 Ankara-Turkey
2) Anadolu University, Faculty of Pharmacy, Department of Pharmacology, 26470, Eskisehir-Turkey

Molecular hybridization is a powerful tool in the rational drug design approaches where new chemical entities are obtained by combining two or more pharmacophores of bioactive scaffolds into a single molecule with the aim of both improved biological potential and reduced undesirable side effects. 1,2,4-Triazole scaffold has been subjected to the many researches for their variety biological activities. Over the last twenty years, our interest has focused on the synthesis of novel heterocyclic systems derived from 3-substituted-1,2,4-triazole-5-thiones having analgesic/antiinflammatory activity. Analgesic and antiinflammatory properties of some Mannich bases derived from 3-substituted-1,2,4-triazole-5-thiones were also examined previously[1,2]. Some of these compounds were shown to exert higher analgesic/antiinflammatory activity and lower ulcerogenic risk in the stomach. Prompted by these promising results, and in continuation of the efforts toward the development of new molecules having analgesic/antiinflammatory activity, here, we synthesized new analogue Mannich bases starting from a hybride molecule involving both 1,2,4-triazol and naproxen which is a member of the 2-arylpropionic acid family of NSAIDs.

The synthesis of target compounds was performed in two steps: Initially, 3-[1-(6-methoxy-2-naphthyl) ethyl]-1,2,4-triazole-5-thione was synthesized by dicyclohexylcarbodiimide (DCC)-promoted amide formation reaction, starting from naproxen according to pathway that was reported in our previous articles[2,3]. In the second step, target compounds were acquired via the classical Mannich reaction, a one-pot three component condensation reaction, by reacting triazole molecule, formaldehyde and diverse secondary amines in ethanole[1]. The synthesized compounds were characterized and elucidated by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopies and elemental analysis. Preliminary activity results of the compounds will be discussed in the poster.

Acknowledgments

Funding for this project was provided by the Hacettepe University, Scientific Research Projects Coordination Unit [Project number: THD-2018-16721].

References

1) B. Tozkoparan, E. Küpeli, E. Yeşilada, Ş. Işık, M. Özalp, M. Ertan, Synthesis and Evaluation of Analgesic/Antiinflammatory and Antimicrobial Activities of 3-Substituted-1,2,4-triazole-5-thiones, Arzneim. Forsch./Drug Res. 55(9), 533 (2005).
2) S. Sert-Ozgur, B. C. Tel, E. I. Somuncuoglu, I Kazkayasi, M. Ertan, B. Tozkoparan, Design and Synthesis of 1,2,4-Triazolo[3,2-b]-1,3,5-Thiadiazine Derivatives as a Novel Template for Analgesic/Anti-Inflammatory Activity, Arch Pharm (Weinheim), 350 (7) (2017).
3) B. Tozkoparan, N. Gokhan, E. Kupeli, E. Yesilada, M. Ertan, Synthesis, Characterization and Antiinflammatory-Analgesic Properties of 6-(Alpha-amino-4-chlorobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ols. Arzneimittel-forsch. 54, 35 (2004).

NEW IMIDAZOPYRIDINE DERIVATIVES AS PHOSPHODIESTERASE 4 AND/OR 7 INHIBITORS WITH ANTI-INFLAMMATORY ACTIVITY

Veronika Ručilová (1), Artur Świerczek (2), Krzysztof Pociecha (2), Miroslav Soural (1), Maciej Pawłowski (3), Elżbieta Wyska (2), Paweł Zajdel (3), Grażyna Chłon-Rzepa (3)

1) Institute of Molecular and Translational Medicine, Palacký University, 5 Hněvotínska´Street, 779-00 Olomouc, Czech Republic
2) Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
3) Department of Medicinal Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

Heterocyclic compounds based on imidazopyridine scaffold have been extensively studied in medicinal chemistry and chemical biology due to their diverse effects. This widespread biological activity is attributed particularly to their bioisostery to purine and indole moieties. The letter points to possible modulation of phoshodiesterases (PDEs), enzymes which control intracellular level of cAMP and cGMP. In recent years PDE4 and PDE7 have emerged as a promising molecular targets for the treatment of neurological, inflammatory and immune disorders [1]. Regarding the mentioned-above, we designed novel trisubstituted imidazo[4,5-*b*]pyridines and imidazo[4,5-*c*]pyridines as potential PDE4 and/or PDE4/7 inhibitors. The synthetic strategy was inspired by our previous experience in solid-phase synthesis of imidazopyridines, however solid-phase approach had to be applied due to the specific properties of the final compounds [2]. Synthetized compounds were biologically evaluated *in vitro* using PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE4B and PDE7A expressed in Sf9 cells, and displayed potent inhibitory activity at concentrations close to those of the reference compounds – rolipram and BRL 50481, respectively. The inhibition of both cAMP-specific isoenzymes resulted in a strong anti-TNF-a effect in vitro. In a rat whole blood assay, several studied compounds decreased concentrations of this cytokine by 82.3–92.7%. Finally, anti-inflammatory activity of the most promising compound was tested in LPS-induced endotoxemia model and collagen-induced arthritis in rats. This compound at a dose of 20 mg/kg was able to reduce TNF-a levels in rat plasma by approximately 50% and significantly decreased the paw size of arthritic rats in all time points measured. In addition, the studied PDE4/7 inhibitor revealed a favorable pharmacokinetic profile following intraperitoneal administration to rats. Further studies are warranted to gather more data regarding pharmacokinetic and pharmacodynamic properties of these derivatives in order to indicate their potential therapeutic applications.

This project was supported by the National Science Centre, Poland, grant No 2014/15/B/NZ7/00885, and the statutory activity of Jagiellonian University Medical College (K/ZDS/007214).

References

1) Jankowska, A. et al. Curr. Med. Chem. 2017, 24, 673-700
2) Lemrova, B. et al ACS Comb. Sci. 2014, 16, 558-565

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - THERAPEUTIC AREAS

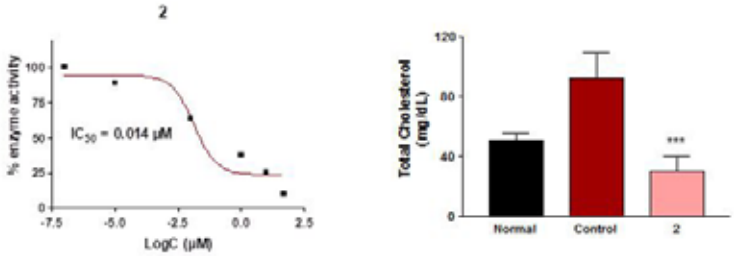
Cardiometabolic Diseases

BIFUNCTIONAL ANTIHYPERLIPIDEMIC-ANTIOXIDANT MORPHOLINE DERIVATIVES: OPTIMIZING THEIR PHARMACOLOGICAL PROFILE BY FOCUSING ON SQUALENE SYNTHASE INHIBITION

Angeliki Kourounakis, Alexios Matralis

Department of Medicinal Chemistry, School of Pharmacy, University of Athens, 15771 Athens, Greece (angeliki@pharm.uoa.gr)

Increased plasma levels of ROS and LDL are considered causal risk factors directly promoting the onset and progression of coronary and peripheral atherosclerosis. Using previously developed morpholine derivatives as a starting point[1-3], we performed extensive structural changes by either substituting or by modifying the morpholine ring, with the purpose of refining the structural elements required for an improved SQS-antioxidant pharmacological profile. The most active compounds emerged from this effort display IC_{50} values for SQS inhibition between 0.014 and 0.51 μM, comparable to TAK-475 (IC_{50}=0.078 μM), the first SQS inhibitor entered in advanced clinical trials. Moreover, they exhibit good inhibitory activity against the lipid peroxidation of hepatic microsomal membranes induced by Fe^{2+}/ascorbate with IC_{50}values much lower than known antioxidants such as probucol.



In vivo proof-of-principle studies corroborated our initial design since the most promising derivative of this series produced an outstanding antihyperlipidemic and antioxidant effect, affording at the same time a significant anti-inflammatory activity estimated as protection offered against the edema induced by carrageenan. In conclusion, rational design accompanied by SAR studies produced compounds combining improved antioxidant and SQS inhibitory activity that may serve as multifunctional agents against atherosclerosis.

References

1) A.N. Matralis, A.P. Kourounakis. Design of novel antihyperlipidemic agents with antioxidant/anti-inflammatory properties: exploiting phenothiazine's strong antioxidant activity, J. Med. Chem. 57 (2014) 2568-2581.
2) E.M. Ladopoulou, A.N. Matralis, A. Nikitakis, A.P. Kourounakis. Antihyperlipidemic morpholine derivatives with antioxidant activity: an investigation of the aromatic substitution, Bioorg. Med. Chem. 23 (2015) 7015-7023
3) M.G. Katselou, A.N. Matralis, A.P. Kourounakis. Developing potential agents against atherosclerosis: Design, synthesis and pharmacological evaluation of novel dual inhibitors of oxidative stress and Squalene Synthase activity. Eur J Med Chem.138 (2017) 748-760.

NITRIC OXIDE RELEASING NSAID DERIVATIVES AS ANTIATHEROGENIC COMPOUNDS

Panagiotis Theodosis-Nobelos , Paraskevi Tziona , Antonios Gavalas , Eleni Rekka

Department of Medicinal Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Atherosclerosis is an inflammatory disease accompanied by endothelial dysfunction, oxidative stress and a decline in nitric oxide (NO) production. In diabetic macroangiopathies there is an increase in superoxide anion radical production and a decrease in NO released by endothelial cells. Aging is an important risk factor for cardiovascular diseases and in aged animals, increased levels of proinflammatory molecules are expressed in senescent cells.

Inflammation plays an essential part in the development of atherosclerosis, through upregulating endothelial adhesion receptors, promoting platelet aggregation, altering vascular permeability and inducing oxidative stress. Furthermore, genetic deletion of lipoxygenase decreases LDL oxidation and atherosclerotic lesions in animal models.

Endothelial NO promotes vascular smooth muscle relaxation and inhibits platelet adhesion.In addition to these properties, NO also exerts multiple antiatherosclerotic effects, such as inhibition of LDL oxidation, prevention of leukocyte adhesion to vascular endothelium and leukocyte migration into the vascular wall.

All known risk factors for atherosclerosis enhance oxidative stress and reduce endothelial NO.

Therefore, prevention of vascular inflammation and improvement of endothelial NO activity represent rational therapeutic approaches for atherosclerosis.

In this investigation, we have designed and synthesised novel derivatives of known NSAIDs, such as ibuprofen and naproxen, with 2-hydroxyethyl nitrate. In a number of compounds, an aminoacid linkage between the two molecular entities was introduced. The synthesis was performed using the intermediate acyl-chlorides or dicyclohexylcarbodiimidazole, with yields up to 85%. The structures were identified spectroscopically.

The synthesised compounds were tested in vitro for lipoxygenase inhibition and found to be more potent than the parent drugs. Their ability to liberate NO was also determined and found to demonstrate considerable NO donating activity. Their effect on acute inflammation, applying the carrageenan rat paw oedema model, was examined and verified that they can inhibit inflammation up to 75%. Their effect on plasma cholesterol and triglyceride levels was also estimated in rats with Triton-WR1339 induced hyperlipidemia. The synthesised compounds could reduce plasma lipidemic indices more than 50%.

This study has demonstrated that integration of a nitric oxide releasing moiety with anti-inflammatory drug molecules results in compounds which retain or augment the anti-inflammatory activity of the parent drugs, while they acquire significant hypolipidemic effect. This combination of activities is considered useful towards the development of agents for the prevention and treatment of atherosclerosis.

P. Theodosis-Nobelos acknowledges the General Secretariat for Research and Technology (GSRT) of Greece and the Hellenic Foundation for Research and Innovation (HFRI) for a grant supporting his PhD research.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - CHEMICAL BIOLOGY

Glycans in Medicinal Chemistry : Carbohydrate-Based Vaccines

STRUCTURAL INSIGHTS GUIDING THE DESIGN OF A VACCINE CANDIDATE AGAINST OTITIS MEDIA PATHOGENS

Sanjesh Singh (1,3), Martin Frank (2), Patrick Collins (1), Ian Peak (1,3), Jennifer Wilson (3,4), Darren Grice (1,3)

1) Institute for Glycomics, Gold Coast, 4222 Queensland, Australia
2) Biognos AB, Generatorsgatan 1, 41705 Gothenburg, Sweden
3) School of Medical Science, Gold Coast, 4222 Queensland, Australia
4) Menzies Health Institute, Gold Coast, 4222 Queensland, Australia

Otitis media is a very common childhood infection of the middle ear caused predominantly by a cocktail of bacteria including *Moraxella catarrhalis, Streptococcus pneumoniae and Haemophilis influenzae.* Repeated episodes of otitis media can lead to hearing loss and developmental issues in young infants. Moreover, recently an oligosaccharide (OS) antigen from *Moraxella catarrhalis* and a protein antigen from *Haemophilus influenzae* have been combined into a single vaccine candidate and tested for their ability to invoke an immune response in a mouse model. The results of these investigations will be presented.

In addition, we have used NMR and molecular modelling to study the conformation of potential carbohydrate antigens derived from the OS component of the lipooligosccharide from *M. catarrahlis* [1]. These studies show that the highly-branched glucose-rich inner core of the OS has an altered conformation compared to the most truncated tetra-glucose-Kdo lgt1/4Δ OS structure. Addition of one residue to each of the (1-4) and (1-6) chains to give the lgt2Δ OS is the minimum requirement for this conformational change to occur. The occurrence of a significant conformational change between a truncated and extended OS structure may have important implications on the strategy utilized to design carbohydrate vaccines composed of truncated OS that, although may contain the core residues common to many strains, may not necessary possess the same 3D structure of a wild type OS. The results of extensive molecular modeling and NMR investigations showing significantly altered conformational preferences between the two structures will be presented.

References

1) Frank, Collins, Peak, Grice & Wilson. Molecules (2015) 20, 14234-14253.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - CHEMICAL BIOLOGY

Recent Advances in Targeted Protein Degradation

P233

VALIDATING NOVEL TARGETS FOR PROTEIN DEGRADATION

Grant McGonagle

Protein Degradation DPU, GlaxoSmithKline, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK

The heterobifunctional molecules referred to as proteolysis targeting chimeras (PROTACs), were identified as promoters of cellular protein degradation over fifteen years ago. PROTACs contain one moiety that binds an E3 ligase and another that binds a desired cellular target protein of interest. This induced proximity results in ubiquitination of the target followed by its degradation at the proteosome.[1-5] With interest in this drug discovery paradigm rapidly increasing throughout the industry, this poster will describe efforts within the Protein Degradation DPU to validate new targets.

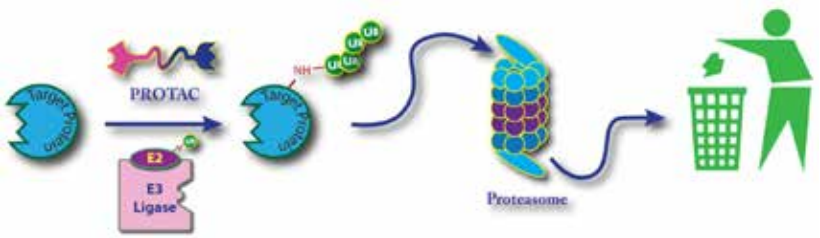


Figure 1: General mechanism of action of PROTACs

The Interleukin-1 receptor-associated kinases (IRAKs) are key mediators of the toll-like receptor (TLR) and interleukin-1 receptor (IL1R) signalling processes.[6] TLR/IL1R-mediated signalling controls diverse cellular processes including inflammation, apoptosis and cellular differentiation. TLR/IL1R signalling is achieved through differential recruitment of adaptor molecules such as MyD88. In addition to performing a scaffolding role, these adaptors function in the subsequent recruitment and activation of IRAK family kinases. Four IRAK genes exist in the human genome (IRAK1, IRAK2, IRAK3 and IRAK4), and studies have revealed biological roles in inflammation and oncology.

Using an IRAK4 ligand described in the literature,[7] the synthesis and biological data of a number of PROTACs will be discussed in order to determine their ability to bind to and degrade IRAK4.

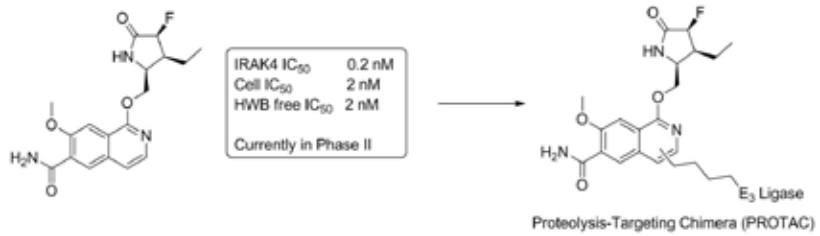


Figure 2: IRAK4 PROTACs based on literature inhibitor

References

1) Nat. Chem. Biol., 2017, 13, 514–521.
2) Nat. Rev. Drug. Discov., 2014, 13, 889-903
3) Med. Chem. Comm., 2016, 7, 2206-2216.
4) Nat. Chem. Biol., 2015, 11, 611-617.
5) Science 2015, 348, 1376-1381.
6) Nat. Rev. Immunol., 2004, 4, 499-511.
7) J. Med. Chem., 2017, 60(13), 5521-5542.

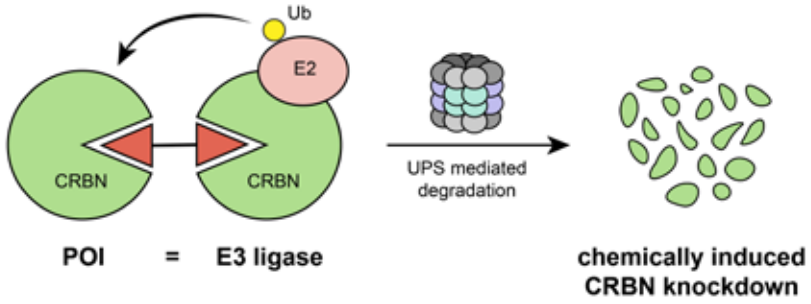
P234

GEMINI-TYPE PROTACS FOR THE CHEMICAL KNOCKDOWN OF CEREBLON

Christian Steinebach (1), Stefanie Lindner (2), Jan Krönke (2), Michael Gütschow (1)

1) Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany
2) Department of Internal Medicine III, University Hospital Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany

The immunomodulatory drugs (IMiDs) thalidomide, lenalidomide, and pomalidomide, all approved for the treatment of multiple myeloma, induce targeted ubiquitination and degradation of Ikaros (IKZF1) and Aiolos (IKZF3) via the cereblon (CRBN) E3 ubiquitin ligase.[1,2] IMiD-based proteolysis targeting chimeras (PROTACs) can efficiently recruit CRBN to a protein of interest leading to its ubiquitination and proteasomal degradation.[3]



By linking two pomalidomide molecules, we designed and synthesized a series of homobifunctional, so called gemini-type PROTACs and investigated their ability to induce self-directed ubiquitination and degradation of cereblon. One of our gemini-type compounds was characterized as a highly potent and efficient CRBN degrader with only minimal effects on IKZF1 and IKZF3. By performing a global proteomic analysis, we found several proteins whose levels were increased after PROTAC or pomalidomide treatment that may represent potential endogenous CRBN substrates. Inactivation by our degrader did not affect proliferation of different cell lines, prevented pomalidomide-induced depletion of IKZF1 and IKZF3 and antagonized the effects of pomalidomide on multiple myeloma cells. Homobifunctional CRBN degraders will be useful tools for future biomedical investigations on CRBN-related signaling and may help to further elucidate the molecular mechanism of thalidomide analogs.

References

1) Krönke et al., Science 2014, 343, 301
2) Krönke et al., Nature 2015, 523, 183
3) Tinworth et al., Med. Chem. Commun. 2016, 7, 2206

GENERATING A CHEMICAL TOOLBOX TO SUPPORT PROTAC R&D

Hannah Maple, Graham Marsh, Simon Pridmore, Nat Clayden, Didier Bruyere, Mark Norley, Robert Felix

Bio-Techne (Tocris), The Watkins Building, Atlantic Road, Avonmouth, Bristol, BS11 9QD

PROTACs (PROteolysis TArgeting Chimeras) are bifunctional small molecules that harness the Ubiquitin Proteasome System (UPS) to selectively degrade target proteins within cells. They represent an exciting new modality, repurposing small molecule chemical tools to achieve selective degradation (knock-down) of target proteins. Moreover, they have the potential to expand the 'druggable proteome', since they can be used to degrade proteins that although bound, are not effectively inhibited, by small molecules. (1) (2)

PROTACs are modular in design and consist of three, covalently linked components:

1. E3 ubiquitin ligase ligand
2. Linker
3. Ligand for a target protein of interest

The development of small molecule ligands for E3 ligases has been pivotal to enabling successful PROTAC development. Availability of these compounds, together with knowledge of their binding mode, has facilitated the burgeoning interest from research groups to enter this field and develop PROTACs as chemical tools and potential therapeutics. To date, however, only a handful of E3 ligases have been successfully harnessed for this application, using a set of well characterized small molecule E3 ligase ligands. Published data has shown that the choice of E3 ligand can impact the activity and selectivity of the final PROTAC, and as such, it can be beneficial to explore different E3 ligands early on in PROTAC discovery projects. (3)

Controlled, PROTAC-mediated, ubiquitination of proteins requires the formation of a ternary complex between the E3 ligase, PROTAC and target protein. The choice of linker is critical for enabling ternary complex formation, and in addition can confer beneficial physicochemical properties, such as improved solubility/cell permeability.

We present the design of an initial collection of functionalized E3 ligase ligands plus linkers for a modular 'PROTAC toolbox'. The components are building blocks, designed to support and enable early stage PROTAC discovery projects. In addition we compare the current landscape of clinically and chemically 'druggable' proteomic space covered with commercially available, biologically active small molecule tools to highlight opportunities for novel PROTAC development.

References

1) Targeted protein degradation by PROTACs. Neklesa, T. K., Winkler, J. D., Crews, C. M. 2017, Pharmacol. Ther., Vol. 174, pp. 138-144.
2) Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? Churcher, I. 2018, J. Med. Chem., Vol. 61, pp. 444-452.
3) Modular PROTAC design for the degradation of oncogenic BCR-ABL. Lai, A. C., Hellerschmied, D., Salami, J., Jaime-Figueroa, S., Ko, E., Hines, J., Crews, C. M. 2016, Angew. Chem. Int. Ed., Vol. 55, pp. 807-810.

NOTES

POSTERS - CHEMICAL BIOLOGY

Chemical Biology Approaches to Target Identification

RHOMBOID PROTEASES: DOES THE ENVIROMENT MATTER?

Marta Barniol-Xicota (1), Steven Verhelst (1,2)

1) Laboratory of Chemical Biology, Department of Cellular and Molecular Medicine, KU Leuven - University of Leuven, Herestraat 49, box 802, 3000 Leuven, Belgium
2) AG Chemical Proteomics, Leibniz Institute for Analytical Sciences ISAS, Otto-Hahn-Str. 6b, 44227 Dortmund, Germany

Intramembrane proteases (IMP) are proteolytic enzymes that are embedded in the lipid bilayer. The serine subclass of IMPs are also called **rhomboid proteases** (ROMs). ROMs are the most ubiquitous IMPs in nature and occur in all three kingdoms of life [1]. Their functional roles include important cell signaling events, such as quorum sensing in some prokaryotes. Recently, ROMs have been linked to several human diseases, such as Parkinson's disease and cancer [2]. Despite this, their specific role and their druggability are unclear.

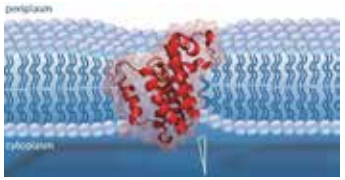


Figure 1. Model ROM in a membrane.

IMPs cleave their substrates, which are also membrane proteins, in a TM or in a juxtamembrane region. The weak transmembrane packing interactions of ROMs, are responsible for their low intrinsic thermodynamic stability, which translates in a high dependence on the environment. Hence, rhomboid protease activity is substantially influenced by membrane composition [3].

Unfortunately the study of these ROMs in their native environment has rendered impractical to date. The bottleneck is the current purification techniques use detergents that ravage the physiological membrane, yielding low enzyme stability [4] and, in some cases, activity. In its turn, this rules out the use of activity assays and chemical probes to study their function. Encapsulating these proteins in their lipid environment will address these shortcomings.

Figure 2. SMALP schematic mechanism. SMA copolymer extracts the membrane protein in a SMALP nanoparticle.

We have developed a detergent free purification method, based on maleic acid copolymers: SMA and DIBMA. Those function as a "molecular cookie cutter", creating polymer-lipid-protein nanodiscs, which retain their biological properties upon purification. Here we present the results of the comparative study (detergent vs nanodiscs) of two rhomboids: GlpG (*E.Coli*) and VcROM (*V.Cholerae*).

References

1) Rawson RB, et al. 1997. Mol Cell, (1):47-57.
2) Strisovsky K. 2016. The FEBS Journal, 283(10):1837-45.
3) Baker, R. P. & Urban, S. 2012. Nat. Chem Bio. 8, 759-768.
4) Wolf, E.; Zeissler, A. & Verhelst, S. H. L. 2015. ACS Chem. Biol. 10, 10, 2325-2333.

EU-OPENSCREEN: THE EUROPEAN INFRASTRUCTURE FOR CHEMICAL BIOLOGY

Bahne Stechmann

EU-OPENSCREEN ERIC, Robert-Rössle-Str. 10, 13125 Berlin

The academic Chemical Biology initiative EU-OPENSCREEN (www.eu-openscreen.eu) integrates high-capacity screening platforms throughout Europe, which jointly use a rationally selected compound collection, comprising up to 140.000 commercial and proprietary compounds collected from European chemists. EU-OPENSCREEN offers to researchers from academic institutions, SMEs and industrial organisations open access to its shared resources. EU-OPENSCREEN will collaboratively develop novel molecular tool compounds with external users from various disciplines of the life sciences.

External chemists are invited to include their proprietary compounds into the jointly used EU-OPENSCREEN compound collection, which is screened against a wide range of biological assays, thereby delivering extensive information about the biological activities of their compounds. This also opens the perspective that some of these donated compounds will be identified as hits. In that case, the respective chemist will be asked to be involved in the following research projects and novel collaborations with the assay providers from all over Europe and beyond. Furthermore, EU-OPENSCREEN bioprofiles these donated compounds in a set of standard assays to annotate them for basic physico-chemical (e.g. identity, solubility, light absorbance and fluorescence) and essential biological properties (e.g. cytotoxicity, antibiotic activity, antifungal activity). Thus, chemists will rapidly receive data on the biological activities of their donated compounds.

PROMS: A CONSTRUCTION KIT FOR POTENTIAL METASTATIC INHIBITORS INVOLVING PROLINE-RICH SEGMENT RECOGNITION

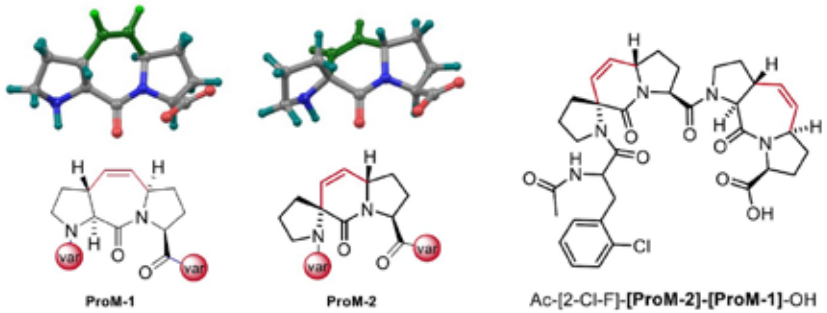
Slim Chiha (1), Ronald Kühne (2), Hans-Günther Schmalz (1)

1) University of Cologne, Department of Chemistry, Greinstrasse 4, 50939 Köln, Germany
2) Leibniz-Institut für Molekulare Pharmakologie (FMP), Robert-Rössle-Str. 10, 13125 Berlin, Germany

Protein domains specialized in the recognition of proline-rich segments (PRS) adopting a left-handed polyproline type II helix (PPII) conformation are particularly abundant, yet so far undruggable.[1] Considering that these domains (e.g. Ena/VASP EVH1) often play a significant role in the expansion of invasive cancer, they represent a target of choice for the development of a potential metastatic inhibitor.

We established efficient syntheses of proline-derived modules (**ProMs**), i.e. polycyclic dipeptide units structurally rigidified in a PPII conformation.[2] These were then used as building blocks in the synthesis of tailored small molecule ligands, which selectively bind to the target domain with remarkable affinity.

As a proof-of-principle, we developed a highly selective, non-peptidic inhibitor of protein-protein interactions involving Ena/VASP EVH1 domains. Highly invasive breast cancer cells treated with this ligand showed displacement of VASP from focal adhesions at the front of lamellipodia and caused a strong suppression of cell motility and chemotaxis, as reflected by an inhibition of cancer cell invasion by 66%.[3]



References

1) L. Ball, R. Kühne, J. Schneider-Mergener, H. Oschkinat, Angew. Chem. Int. Ed. 2005, 44, 2852.
2) (a) J. Zaminer, C. Brockmann, P. Huy, R. Opitz, C. Reuter, M. Beyermann, C. Freund, M. Müller, H. Oschkinat, R. Kühne, H.-G. Schmalz, Angew. Chem. Int. Ed. 2010, 40, 7111; (b) C. Reuter, P. Huy, J.-M. Neudörfl, R. Kühne, H.-G. Schmalz, Chem. Eur. J. 2011, 17, 12037; (c) C. Reuter, R. Opitz, et. al. R. Kühne, H.-G. Schmalz, Chem. Eur. J. 2015, 21, 8464; (d) S.Chiha, A. Soicke, R. Kühne, H.-G. Schmalz, Eur. J. Org. Chem. 2018, 455-460.
3) R. Opitz, M. Müller, C. Reuter, H.-G. Schmalz, R. Kühne, PNAS 2015, 112, 5011.

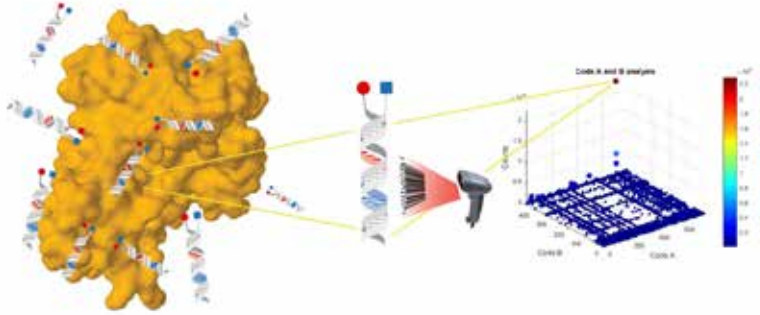
ENCODED SELF-ASSEMBLING CHEMICAL (ESAC) LIBRARIES: A POWERFUL TECHNOLOGY FOR LIGANDS DISCOVERY AND AFFINITY MATURATION

Etienne Donckele (1), Florent Samain (1), Martina Bigatti (1), Arnel Hodzic (1), Dario Neri (1,2), Jörg Scheuermann (2)

1) Philochem AG, Libernstrasse 3, CH-8112 Otelfingen, Switzerland
2) ETH Zürich, Institut für Pharmazeutische Wissenschaften, Vladimir-Prelog-Weg 1-5/10, 8093 Zürich, Switzerland

DNA-encoded chemical library (DECL) technology has become a useful tool for ligand discovery in chemical biology and in pharmaceutical research. The encoding of individual organic molecules with DNA fragments, serving as amplifiable identification barcodes, allows the construction and screening of compound libraries of unprecedented size.

DNA-encoded chemical libraries can be classified in terms of their synthesis strategy (e.g., “DNA-recorded” and “DNA-templated” synthesis) or on terms of the number of molecules displayed on DNA (e.g., “single-pharmacophore” and “dual-pharmacophore” libraries). They make use of DNA hybridization in order to generate DNA-encoded assemblies of small-molecule fragments in a sequence-programmed fashion. Encoded Self-Assembling Chemical (ESAC) technology allows the identification of synergistic binding pairs of fragments, capable of interacting with adjacent epitopes of the target protein of choice in a chelate fashion. Here, we describe novel advances in ESAC technology, including an experimental demonstration for the isolation of high-affinity ligands directed against acid-1 glycoprotein (AGP). The strategy relied on the discovery of synergistic fragments, binding to adjacent sites on the AGP surface, followed by the identification of optimal linkers to connect the two fragments. The best ligand had a dissociation constant of 9.9 nM to the target, which was confirmed both by fluorescence polarization and by BIAcore methods.



References

1) Franzini, R.; Neri, D.; Scheuermann, J. Acc. Chem. Res. 2014, 47 1247-1255.
2) Wichert, W.; Krall, N.; Decurtins, W.; Franzini, R. M.; Pretto, F.; Schneider, P.; Neri, D.; Scheuermann, J. Nat. Chem. 2015, 7, 241-249.
3) Bigatti, M.; Dal Corso, A. Vanetti, S. Cazzamalli, S.; Rieder, U.; Scheurmann, J.; Neri, D.; Sladojevich, F. ChemMedChem. 2017, 12, 1748-1752.
4) Neri, D.; Lerner, R. A. Annu. Rev. Biochem. 2018, 87, 5.1-5.24.

SENSING G-QUADRUPLEXES BY USING INFRARED PROBES

Gonzalez-Garcia Jorge (1), Delcamp Jared (2), Gabelica Valerie (1)

1) European Institute of Chemistry and Biology (IECB), University of Bordeaux, 2 Rue de Escarpit, Pessac 33600, France.
2) Department of Chemistry and Biochemistry, 405 Coulter Hall, University of Mississippi, USA.

In the past few years, non-canonical nucleic acid structures have emerged as molecular controlling gates of biological processes acting as epigenetic markers. Unusual nucleic acid structures include triplexes, i-motifs, three-way junctions, holiday junctions or G-quadruplexes (G4). The later one is formed from stacks of two or more planar guanine tetrads that arise from hydrogen bonding network of four guanines whereas these structures are assembled and stabilized by alkali metal cations. A large number of putative G-quadruplex forming sequences have been identified in the human genome and evidences suggest their pivotal role in key biological processes.[1] Therefore, these G4 structures have been proposed as potential targets by small molecules for therapeutic intervention.[2]

Even G-quadruplexes have been fully proved to exist *in vitro*; their existence *in vivo* still remains an active debate. Some of the most direct evidence has been obtained by using antibodies to visualize G4 structures in fixed cells.[3] Because of the limitation of the antibody technology, a large number of optical probes has been reported to date to visualize these structures in live cells (rather than fixed cells). Mostly, small-molecule optical probes are based on changes in the emission intensity in the visible range.[4] However, this approach in microscopy has important drawbacks such as photon scattering, high absorption and autofluorescence of cells. To overcome this issue, we recently developed a series of small near infrared fluorescent probes which emission intensity is tightly regulated by the interaction with G-quadruplexes.

In this communication, we will present our most recent studies in this area including: (i) development of new NIR optical probes for targeting G4s; (ii) interaction of these probes towards a panel of G-quadruplexes of different topology in addition to duplexes and other non-canonical DNA structures; (iii) demonstration that these probes can be used in live cells to visualize G4 formation processes.

Acknowledgment: This work was supported by an *IDex fellowship* (University of Bordeaux).

References

1) D. Rhodes, H. J. Lipps, Nucleic Acids Res., 2015, 43(18), 8627.
2) S. Neidle, S. Balasubramanian (Eds.), Quadruplex Nucleic Acids, RSC (Cambridge, UK), 2006.
3) G. Biffi, D. Tannahill, J. McCafferty, S. Balasubramanian, Nat. Chem., 2013, 5, 182.
4) Largy, E. et al., Top. Curr. Chem., 2013, 330, 111.

MONOAMINE OXIDASE A/B INHIBITING EFFECT AND MOLECULAR MODELING OF SOME SYNTHESIZED HYDRAZONE DERIVATIVES

Hayrunnisa Tasci (1), Begum Nurpelin Saglik (2), Yusuf Ozkay (2), Birsen Tozkoparan (1), Nesrin Gokhan Kelekci (1)

1) Hacettepe University Faculty of Pharmacy Dept of Pharmaceutical Chemistry, 06100 Ankara-Turkey
2) Anadolu University Faculty of Pharmacy Dept of Pharmaceutical Chemistry, 44280 Eskişehir-Turkey

The monoamine oxidases (MAO-A/B) from family of oxidoreductase enzyme is responsible for the deamination of monoamine neurotransmitters such as noradrenalin, serotonin, dopamin. Increased activity of these enzymes lead to imbalance in the concentration of these neurotransmitters in the brain. This circumstance is linked with the biochemical pathology of various neurologic disorders and neurodegenerative diseases (1). There have been many reports that show increased level of hMAO-B in the brain of AD patients (2, 3) and a number of research reports have suggested that the depletion of monoamine neurotransmitters concentration in severe depression cases (4,5). In this regard, MAO inhibition is a important target for in therapies and prophylaxis of these neurogenic defects.

Considering the pharmacological importance of MAO inhibitors, different chemical structures have been designed and evaluated in terms of MAO inhibition. Hydrazones which are one of the these structures are open chain pyrazolines in whch the two aromatic rings are linked by C=O-NH-N=CH system. There have been many reports on the antidepresant / MAO-inhibition activity of hydrazones derived from substituted hydrazides and their reduction products(6).

As a major goal was to obtain a novel lead compound, in this paper, we designed a series of hydrazones containing substituted-2-benzoxazolinone derivatives. Molecular modelig studies were carried out on recent and high resolution hMAO-A and hMAO-B crystallographic structures to better justify the enzyme-inhibitor interaction toward hMAO isoforms and to explain the structure-activity relationship of this kind of inhibitors. Synthesized compounds were assayed for their in vitro hMAO inhibitory activity and selectivity and all compounds showed IC50 values in the micromolar range against hMAO-B.

Acknowledgments

Funding for this project was provided by the Hacettepe University, Scientific Research Projects Coordination Unit [Project number: THD-2018-16821].

References

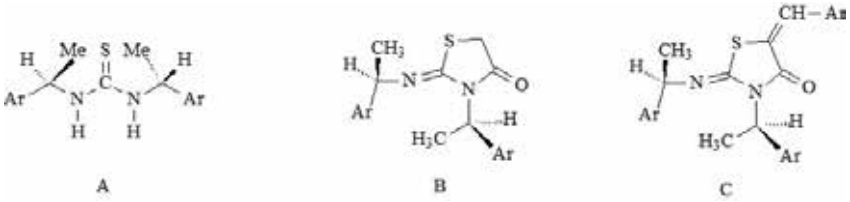
1) Wimbiscus M., et al. MAO inhibitors: risks, benefits, and lore. Clev Clin J Med 2010; 77(12): 859-82.
2) Kennedy B., et al. Early and persistent alterations in prefrontal cortex MAO A and B in Alzheimer's disease. J Neural Transm 2003; 110(7): 789-801.
3) Anand R. and Wesnes K. Cognition-enhancing effects of moclobemide, a reversible MAO inhibitor, in humans. Adv Neurol 1990; 51: 261-8.
4) Benedetti MS. and Dostert P. Monoamine oxidase, brain ageing and degenerative diseases. Biochem Pharmacol 1989; 38(4): 555-61.
5) Emilsson L., et al. Increased monoamine oxidase messenger RNA expression levels in frontal cortex of Alzheimer's disease patients. Neurosci Lett 2002; 326(1): 56-60.
6) Salgin-Gökşen U., et al. Synthesis, molecular modeling and in vitro screening of monoamine oxidase inhibitory activities of some novel hydrazone derivatives. J. Neural Transm., 2013. 120(6): p. 883-891.

ANTIMICROBIAL ACTIVITIES OF CHIRAL THIOUREAS AND THEIR CYCLIZED DERIVATIVES THIAZOLIDINONES

Melike Ekizoglu (1), Senel Teke Tuncel (2), Sule Erol Gunay (2), İlknur Dogan (2), Nesrin Gokhan Kelekci (1)

1) Hacettepe University, Faculty of Pharmacy, 06100 Sıhhiye, Ankara, Turkey
2) Bogazici University, Department of Chemistry, Bebek, 34342 Istanbul, Turkey

The emergence of resistance to the major classes of antibacterial agents is recognized as a serious health problem. Particularly, in recent years much attention has been focused on the multi-drug resistant bacteria and fungi resulting from the widespread use and misuse of classical antimicrobial drugs. Thioureas, both with symmetrical and unsymmetrical structure, have attracted much attention as antimicrobial drug candidates[1]. Many organic connections of thiourea were found to be cytotoxic against different cell lines derived from human tumors[2-5]. Thiourea derived compounds have also been reported as inhibitors of herpes virus family as well as they are effectively used in the antiretroviral therapy[6-7]. Cyclized thiourea forms named imino-thiazolidin-4-one derivatives have also been attracting considerable attention due to their biological importance and the biological investigation of thiazolidinones has revealed that substitution at 2,3 and 5 positions imparts different activities[8]. It was reported that napthylthiourea, phenylthiourea and 1,3-diphenylthiourea were to be highly cytotoxic in rat hepatocytes and the methylene unit insertion between phenyl and thiourea remarkably reduced the cytotoxic activity[9,10]. Keeping this in mind and as a part of research program to investigate the role of substitutions by functional groups attached to the thiourea bridge and thiazolidinone ring, synthesized compounds were evaluated for antibacterial and antifungal activities. The compounds A-2-RR, C-2-SS and C-2-RR are the ones with the highest antifungal and antibacterial activity in all compounds. The chiral thiourea structure A-2-RR, and the benzylidenthiazolidin-4-one structure C-2-SS and C-2-RR compounds have the highest antifungal and antibacterial activity in all compounds. These compounds have antifungal activity of 8-2 μg / ml and antibacterial activities of 64-2 μg / ml. The compounds were found to be effective all bacterial strains. These three compounds are considered to be promising compounds that can be used therapeutically due to their high antifungal and antibacterial activity.



Acknowledgments

This study was supported by Scientific and Technological Research Council of Turkey (Project Number: 114Z172).

References

1) Mishra A. and Batra, S. Curr.Top.Med.Chem. (2013), 13, 2011-2025.
2) Vega-Perez J.M., et al. Eur. J. Med. Chem. (2012) 58, 591-612.
3) Kumbhare R.M., et al. Bioorg. Med. Chem. Lett. (2012), 22 453-455
4) Li, W.Q., et al. Bioorg. Med. Chem. (2013), 21 2363-2369
5) Koca I., et al. Bioorg. Med. Chem. (2013), 21 3859-3865
6) Di Grandi, M.J., et al., Bioorg. Med. Chem. Lett. (2004), 14, 4157-4160.
7) Bloom J.D., et al. Bioorg. Med. Chem. Lett. (2003), 13.
8) Manjal SK., et al. Bioorganic Chemistry (2017), 75, 406–423
9) Bielenica A., et al. European Journal of Medicinal Chemistry. (2015), 101, 111-125.
10) Onderwater R.C., et al., Toxicology (1998), 125, 117-129.

BIOTRANPORT AND BIODISTRYBUTION OF [60]FULLERENE DERIVATIVE IN MURINE ORTHOTROPIC MODEL OF BREAST ADENOCARCINOMA MODULATED BY NON-INVASIVE HYPERTHERMIA

Krzykawska-Serda Martyna (1,2), Lapin Norman A. (2), Dilliard Sean (2,3), Mackeyev Yuri (4), Serda Maciej (4,5), Wilson Lon J. (4), Curley Steven A. (2,6), Corr Stuart J. (2,4,7)

1) Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow 30-387, Poland
2) Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX 77030, USA
3) Department of Chemical and Biomolecular Engineering, Rice University, Houston 77005, TX, USA
4) Department of Chemistry, Rice University, Houston 77005, TX, USA
5) Institute of Chemistry, University of Silesia in Katowice, 40-006, Katowice, Poland
6) Department of Mechanical Engineering and Materials Science, Rice University, Houston, TX 77005, USA
7) Department of Biomedical Engineering, University of Houston, Houston 77204, TX, USA

The water-soluble and fluorescent [60]fullerene derivative (C_{60}-serPF) was designed to be an amphiphilic nanostructure, which is able to cross biological membranes and accumulate in tumor tissues by passing through abnormally leaky tumor blood vessels. The goal of the design of [60]fullerene nanoparticle was to enhance drug delivery systems in targeting the micro-vasculature and micro-environments of breast cancer tumors. Additionally, the ability to real-time fluorescence imaging of C_{60}-serPF allow us to use Intravital microscopy (IVM) and perform quantitative analysis of particle extravasation. With this tool, we understand the combined and differential biokinetic effects of radiofrequency (RF) electric-field hyperthermia as an adjunctive therapy to [60]fullerene nanoparticle-based drug delivery systems. To adequately elucidate the coupled effects of the highly permeable, but heterogeneous tumor vasculature, with the permeabilizing effects of mild (40-42 ^oC) hyperthermia produced by a local RF field, we controlled variables across tumor and non-tumor mammary gland microvasculature with and without application of RF hyperthermia in each condition. The analysis of a permeability parameter (P_{app}), C_{60}-serPF velocity, and the time of compound influx into the intra- and extra-vascular space suggest that mild RF hyperthermia can suggestively improve nanoparticle delivery into tumor tissue. We proofed that tumor tissue is characterized by more intense drug extravasation than in contralateral mammary fad pad tissue.

References

1) Lapin NA, Krzykawska-Serda M, Dilliard S, Mackeyev Y, Serda M, Wilson LJ, et al. The effects of non-invasive radiofrequency electric field hyperthermia on biotransport and biodistribution of fluorescent [60]fullerene derivative in a murine orthotopic model of breast adenocarcinoma. J Control Release. 2017 Aug;260:92–9.

P244

A NOVEL VIRUS-INSPIRED APPROACH TO DISCOVER FIRST-IN-CLASS PRECLINICAL ASSETS FOR A RANGE OF THERAPEUTIC AREAS

Benoit de Chassey, Laurene Meyniel-Schiklin, Melanie Denizot, Laetitia Lines, Pierre Mallinjoud, Jacky Vonderscher, Eric Meldrum

ENYO Pharma SA, Bâtiment Domilyon, 321 avenue Jean Jaurès, 69007 LYON, FRANCE

ENYO Pharma has developed an innovative systems biology approach to identify patentable chemistries directed at new human disease targets. Viruses are obligate intracellular pathogens that must modulate host cell pathways involved in countless cellular processes to complete their replication cycle. ENYO's approach identifies the host targets of a virus, and develops therapeutics mimicking the viral molecular mechanisms. As the target is a human pathway, ENYO's molecules have a therapeutic application beyond infectious diseases.

ENYO Pharma has built a library of virus-derived peptides known to be necessary for virus-host protein-protein interactions. From this library, one sequence has been selected for study due its ability to induce autophagy and inhibit influenza replication. A 3D structure based approach was used to design small molecules that mimic the pharmacophores on the bioactive peptide and screening of a small collection of mimics identified a structurally related, active cluster. Optimisation of this starting point has generated a novel class of molecules that potently inhibit Influenza replication (IC50 in whole cellsin vivomodels including inhibition of tumor growth in mouse xenograft models. Data communicating the properties of this chemical series will be presented.

ENYO Pharma has received funding from the European Union's Horizon 2020 program to scale-up our approach to discovery research. The aim is to discover original preclinical assets in both infectious and non-infectious disease contexts. ENYO Pharma has designed a proprietary library of 10,000 developable small molecules that mimic the pharmacophores on bioactive peptides targeting multiples cellular pathways. The library has been screened in phenotypic assays against four viruses (Influenza, RSV, Zika and HRV), one mycobacterium (Tuberculosis) and also screened for inducers of Immunogenic Cell Death in a triple negative breast cancer cell line. The characteristics of the library and output of screening will be presented. Our drug discovery engine can be leveraged through partnership to further exploit the capacity of the library to deliver first-in-class drug candidates.

P245

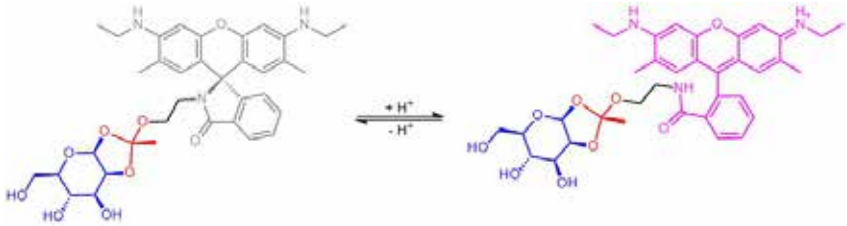
PROBING DENDRITIC CELLS WITH pH-SENSITIVE PROBE

Janez Mravljak (1), Zoran Arsov (2), Urban Švajger (3), Stane Pajk (1,2), Anita Kotar (4), Iztok Urbančič (2), Janez Štrancar (2), Marko Anderluh (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
2) Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
3) Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, 1000 Ljubljana, Slovenia
4) Slovenian NMR Centre, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

DC-SIGN is a type II C-type lectin expressed exclusively on dendritic cells (DCs) that has a clear role in the immune response as an antigen-uptake receptor but, conversely, can also facilitate infection by providing entry of pathogens into DCs.[1] By binding to DC-SIGN pathogens are internalized in DCs, but escape lytic processing in DCs' endosomes until delivered to the cells to be infected.[2] The key action in both processes is internalization into acidic endosomes and lysosomes. Prevention of pathogen adhesion to DC-SIGN by its inhibitors is thus a plausible mechanism towards novel antiviral or antibacterial agents. DC-SIGN inhibitors offer one of the possible alternatives towards topical microbicides for blocking HIV-1 transmission. Molecular probes that bind to DC-SIGN could thus provide a useful molecular tool to study internalization and constitute potential antagonists against pathogens. So far, only large molecules have been used to directly observe DC-SIGN-mediated internalization into DCs by fluorescence visualization.

We designed and synthesized a small, rhodamine-based glycomimetic probe that is pH-activatable and exhibits aggregation-induced spectral shift.[3] As the shift is very small, a recently developed, highly spectrally sensitive fluorescence microspectrometer was used to evaluate local shifts in live-cell samples. The time dependence of fluorescence emission intensity and spatial dependence of the spectral shape confirmed that this probe targets and accumulates in DC compartments with low pH. A competitive gp120 displacement assay established a relatively high DC-SIGN affinity for the probe. Internalization was significantly lower in monocytes that do not express the C-type lectin receptors (CLRs) to induce internalization, thus supporting the notion of CLR-mediated internalization of the probe. This indicates that small molecules can be internalized by the same mechanism as pathogens, and that inhibition of binding is not restricted to the extracellular space, but might also involve cytosolic compartments. The newly synthesized compound, therefore, might serve as a model compound for other receptor-specific and environment-sensitive smart probes, as well as for further rational design of DC-SIGN antagonists. Our results indicate that small glycomimetic molecules could compete with antigen/pathogen for binding not only outside but also inside the DC, thus preventing the harmful action of pathogens that are able to intrude into DCs, for example, HIV-1.



References

1) Banchereau J et al., Ann. Rev. Immunol., 2000, 18, 767-811.
2) Švajger U et al., Cell. Signal., 2010, 22 (10), 1397-1405.
3) Arsov Z et al., ChemBioChem., 2015, 16(18), 2660-2667.

INVESTIGATION OF THE INTERACTION BETWEEN GLUCOCORTICOID RECEPTOR AND 14-3-3

Claire Munier (1), Anders Gunnarsson (2), Lisa Wissler (2), Karl Edman (2), Matthew Perry (1), Christian Ottmann (3)

1) Respiratory, Inflammation and Autoimmunity, IMED Biotech Unit, AstraZeneca Gothenburg, Pepparedsleden 1, SE-431 83 Mölndal, Sweden
2) Discovery Sciences, IMED Biotech Unit, AstraZeneca Gothenburg, Pepparedsleden 1, SE-431 83 Mölndal, Sweden
3) Department of Biomedical Engineering Technische Universiteit Eindhoven 5600 MB Eindhoven, The Nederlands

It has been estimated there may be as many as 650 000 protein-protein interactions (PPI) in human cells. Modulation of these interactions would potentially significantly enlarge the "drugable genome". 14-3-3 is a family of seven highly conserved regulatory proteins and has been reported to interact with the glucocorticoid receptor (GR), a nuclear receptor which functions as a ligand dependent transcription factor, and modulate its activity[1]. Different reports however have described both positive and negative regulatory roles to GR/14-3-3 interactions.[2,3,4,5] Given the importance of GR agonists in medicine it is of great interest to better understand the role(s) of these interactions and to study their modulation.

In this work, the interaction between GR and 14-3-3 has been studied. Phosphopeptides, centered on putative 14-3-3 binding sites of GR, were synthesized and their affinity was measured with 14-3-3. Two peptides centred around T524 and S617 were the most active. A dimeric peptide based on these two joined by a pentaglycine linker was synthesized and determined to bind to 14-3-3 in the low nM range. The SAR picture for the importance of different residues to the binding was built up by an alanine scan. Finally these peptides have been crystallized with 14-3-3 (**Figure 1**).

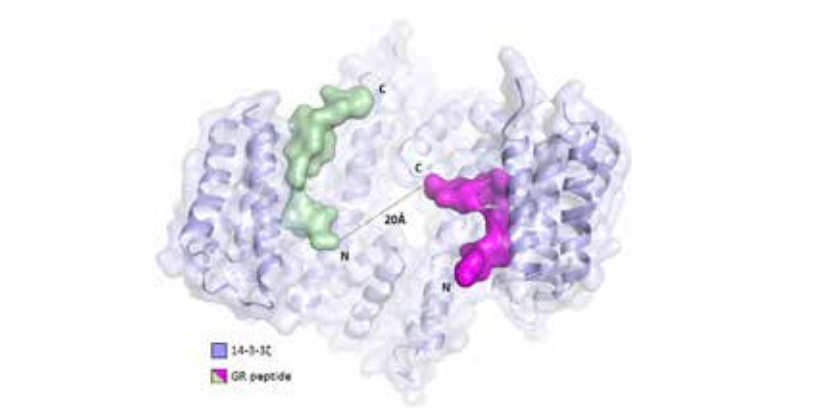


Figure 1. Crystal structure of GR_T524-S617 and 14-3-3ζ

References

1) Widén, C., Zilliacus, J., Gustafsson, J. & Wikström, A. Glucocorticoid Receptor Interaction with 14-3-3 and Raf-1, a Proposed Mechanism for Cross-talk of Two Signal Transduction Pathways. J. Biol. Chem. 275, 39296–39301 (2000).
2) Wakui, H., Wright, A. P. H., Gustafsson, J. & Zilliacus, J. Interaction of the Ligand-activated Glucocorticoid Receptor with the 14-3-3η Protein. J. Biol. Chem. 272, 8153–8156 (1997).
3) Galliher-Beckley, A. J., Williams, J. G. & Cidlowski, J. A. Ligand-Independent Phosphorylation of the Glucocorticoid Receptor Integrates Cellular Stress Pathways with Nuclear Receptor Signaling. Mol. Cell. Biol. 31, 4663–4675 (2011).
4) Kino, T., Souvatzoglou, E., De Martino, M. U., Tsopanomihalu, M., Wan, Y. & Chrousos, G. P. Protein 14-3-3σ Interacts with and Favors Cytoplasmic Subcellular Localization of the Glucocorticoid Receptor, Acting as a Negative Regulator of the Glucocorticoid Signaling Pathway. J. Biol. Chem. 278, 25651–25656 (2003).
5) Kim, Y. S. et al. Role of 14-3-3η as a Positive Regulator of the Glucocorticoid Receptor Transcriptional Activation. Endocrinology. 146, 3133–3140 (2005).

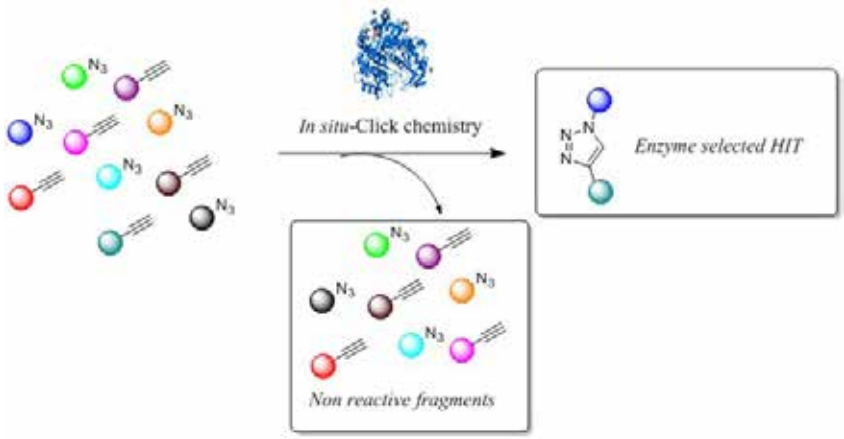
IN SITU-CLICK CHEMISTRY: AN IDEAL TARGET BASED APPROACH FOR THE GENERATION OF MULTITARGET DRUGS

Vallle Palomo (1), Alfonso Garcia-Rubia (1), Vanesa Nozal-Garcia (1), Carlos Roca (1), Concepción Pérez (2), Ana Martínez (1)

1) Centro de Investigaciones Biológicas-CSIC, Ramiro de Maeztu 9, 28040 Madrid
2) Instituto de Química Médica-CSIC, Juan de la Cierva 3, 28006 Madrid

In situ-click chemistry brings together an advantageous procedure for generating new molecules with an ideal macromolecular template to generate high specific binders to a selected target.[1] 1,3-dipolar Huisgen reaction usually requires a copper catalyst to be performed at room temperature with short reaction times. However, if reactants (alkyne and azide derivatives) are brought together in an appropriate fashion, the reaction undergoes successfully without the need of a metal catalysis. Using an enzyme as a template allows for the formation of those unique triazoles that constitute good enough binders to react, presuming the newly formed products will be good binders of the enzyme.

We therefore envisioned that *in situ*-click chemistry would be an ideal methodology not only to discover new enzyme-targeted drugs, but a method in which combining fragments with an complementary activity would lead to the formation of bi or tri functional molecules. Specifically, our work is centered in discovering new BACE-1 inhibitors formed by fragments active in other key enzymes involved in neurodegenerative diseases. These molecules emerge as a potential approach to treat these disorders, were multiple factors are involved in onset and disease progression and an efficient therapy requires tackling several pathological pathways simultaneously.[2]



References

1) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radić, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. Angew. Chem. 2002, 41, 1053-7.
2) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. J. Med. Chem. 2008, 51, 347-72.

DESIGN AND PHARMACOCHEMICAL EVALUATION OF NOVEL SUBSTITUTED-CINNAMATE AND COUMARIN DERIVATIVES AS PLEIOTROPIC AGENTS

Katerina Peperidou (1,2), Hadjipavlou- Litina Dimitra (1), Silvia Bua (2), Murat Bozdag (3), Claudiu Supuran (2)

1) Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Science, Aristotle University of Thessaloniki, University Campus, 54124 Greece
2) Department Neurofarba , Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Via Ugo Schiff 6, 50019 Sesto Fiorentino (Florence), Italy
3) Department of Chemistry, Bioinorganic Chemistry Laboratory, University of Florence, Via della La struccia 3, 50019 Sesto Fiorentino (Florence), Italy

Recently, intensive research has been conducted on cinnamic acid scaffold, seeking to create new polyfunctional drugs acting as inhibitors on multiple biological targets. [1] Substituted cinnamic acid hybrids as well as natural coumarinyl derivatives exhibit a wide range of biological activities whereas hybrids combining both scaffold are used as drugs with anticoagulant , anti-inflammatory , antimicrobial, antioxidant and anticancer properties [2,3]. In our laboratory the last decade several derivatives of cinnamic acids have been designed and synthesized as potent pleiotropic agents e.g. lipoxygenase inhibitors, antioxidants and anti-inflammatories.

In continuation of our research, we made an attempt to design and synthesize two series of new multitarget agents cinnamic acid-based drug candidates : a) hybrids of substituted cinnamic acids with known drugs and drug-like molecules, such as paracetamol, hymechromone, propranolol, atenolol, 7- or 4- or 6-OH-coumarin, and b) acetic acid derivatives of 6- and 7-hydroxycoumarins with several amines. [4]

For the synthesis of the novel hybrid compounds we applied known synthetic procedures and simple techniques. The compounds have been identified using spectroscopic methods and they were tested in vitro: a) as antioxidant and scavenging agents, b) as inhibitors of multiple biological targets implicated in inflammation e.g. lipoxygenase, trypsin.

References

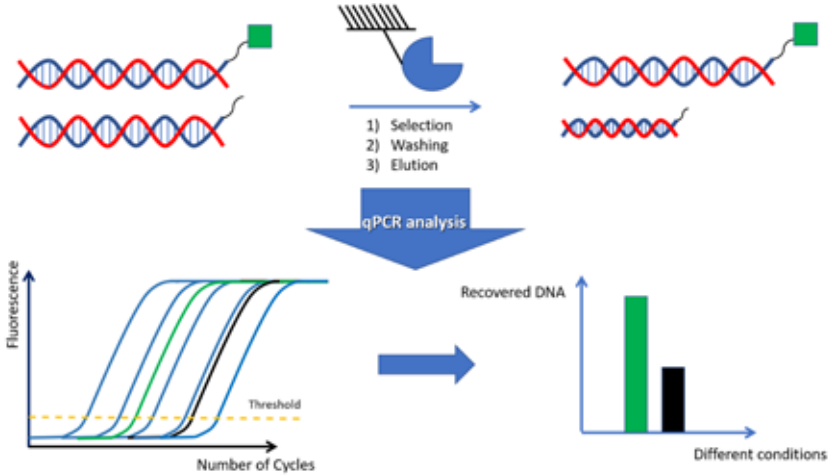
1) Teixeira J.; Silva T.; Benfeito S.; Gaspar A.; Garrido E.M.; Garrido J.; Borges F. Exploring nature profits: Development of novel and potent lipophilic antioxidants based on galloyl-cinnamic hybrids. European Journal of Medicinal Chemistry 2013, 62, 289-296;
2) A. Peperidou, D. Kapoukranidou, C. Kontogiorgis, D. Hadjipavlou-Litina, “Multitarget Molecular Hybrids of Cinnamic Acids” Molecules 2014, 19, 20197-20226.
3) Aikaterini Peperidou, Eleni Pontiki, Dimitra J Hadjipavlou-Litina, Efstathia Voulgari, Konstantinos Avgoustakis. Multifunctional Cinnamic Acid Derivatives. Molecules 2017, 22(8), 1247 DOI 10.3390/molecules22081247.

THE RECOVERY OF DNA-TAGGED LIGANDS: IMPACT OF DIFFERENT EXPERIMENTAL PARAMETERS THROUGH AFFINITY SELECTIONS

Alessandro Sannino, Florent Samain, Dario Neri

Philochem AG, Libernstrasse 3, CH-8112 Otelfingen

The encoding of organic molecules with distinctive DNA tags allows the construction and screening of DNA-encoded chemical libraries (DECL) of unprecedented size, thus facilitating ligand discovery. Library selections are typically performed by affinity capture against purified proteins of interest, followed by PCR amplification and DNA sequencing. Although these methodologies may enable the discovery of useful binders, their successful implementation requires an adequate application of various experimental parameters. Stringent washing steps, presence of detergent, and elution conditions may significantly impact the selection outcome.[1-3] The assessment and optimization of DECL selections using qPCR methodologies represents an important step in order to facilitate ligand discovery.[4] Here, we describe the quantification of DNA input/recovery in model selection experiments, performed on immobilized tagged-modified carbonic anhydrase-IX (CAIX). The recovery of different DNA-tagged ligands of known biochemical properties and compounds of irrelevant specificity used as negative controls was assessed using qPCR methodologies, providing invaluable information regarding the contribution of experimental parameters towards recovery yields and selectivity in screening procedures. The model selection experiments allowed the implementation of optimized selection methodologies both to single- and to dual-pharmacophore libraries. Results of these selection experiments will be presented.



References

1) Neri, D.; Lerner, R. A. Annu. Rev. Biochem. 2018, 87, 5.1–5.24.
2) Decurtins, W.; Wichert, M.; Franzini, R.M.; Buller, F.; Stravs, M.A. Zhang. Y.; Neri. D.; Scheuerman, J. Nat. Protoc. 2016, 11, 764–780.
3) Leimbacher, M.; Zhang, Y.; Mannocci, L.; Stravs, M.; Geppert, T.; Scheuermann, J.; Schneider, G.; Neri, D. Chem. Eur. J. 2012, 18, 7729–7737.
4) Li, Y.; Zimmermann, G.; Scheuermann, J.; Neri, D. ChemBioChem. 2017, 18, 848–852.

DRUGGING THE FBW7 E3 LIGASE WITH A FRAGMENT-BASED APPROACH

Salvatore Scaffidi (1), Miriam Martinez (1), Xavier Barril (1,2), Carles Galdeano (1)

1) Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Biomedicina (IBUB), Universitat de Barcelona. Av. Joan XXIII 27-31, 08028, Barcelona, Spain
2) Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys, 23, 08010 Barcelona, Spain

Fbw7 is an important E3 ligase and one of the most commonly deregulated proteins in human cancers. 6% of cancers have mutations in the *fbw7* gene. In one hand, the loss of activity of the mutated Fbw7 results in a loss of its tumour suppressor function and an upregulation of the natural and oncogenic substrate proteins, such as c-Myc, cyclin-E, and Notch.[1] On the other hand, the inhibition of Fbw7 has been proposed as an approach to sensitize cancer stem cells to chemotherapies.[2] Given the key role of Fbw7 in tumorgenesis, a small molecule directly targeting Fbw7 would have a large impact on the clinic. However, so far, no potent small-molecules that directly bind to Fbw7have been reported, in part because modulating their activity and regulation requires targeting protein-protein interactions.[3]

Our goal is to identify and characterize fragments that bind to the Fbw7 E3 ligase and can be further developed as chemical probes. These fragments may turn *on* or *off* the activity of the protein. Fbw7 binders could serve as anchors to develop disease-specific PROTAC molecules, leading to proximity-induced ubiquitylation and subsequent degradation of proteins of interest.[4] Our group has built a library of around 700 fragments. Surface Plasmon Resonance (SPR) has been carry out. Potential fragment-hits have been identified and they are being validated using orthogonal biophysical techniques. Furthermore, in order to elucidate the binding mode of the fragments, it is crucial to perform x-ray crystallography. Crystal structure of fragments binding to the protein will not only show the key points for the interaction but also it can provide the starting point for a rational design to grow the molecules in order to improve their affinity and specificity.

References

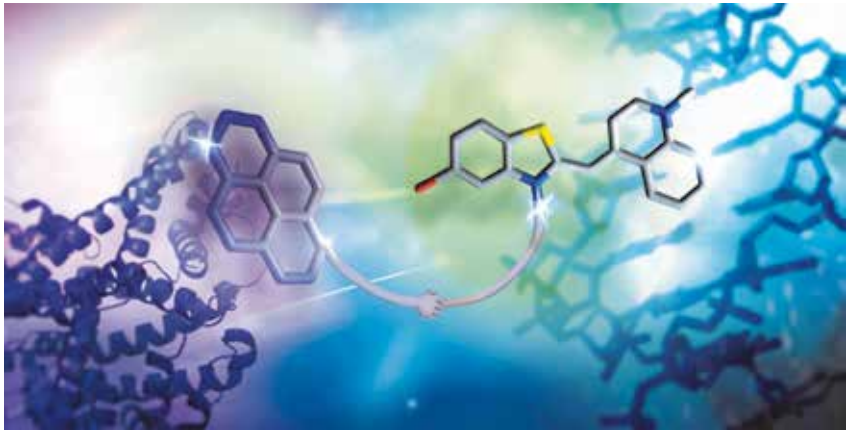
1) Hao, B., Oehlmann, S., Sowa, M. E., Harper, J. W. & Pavletich, N. P. Structure of a Fbw7-Skp1-Cyclin E Complex: Multisite-Phosphorylated Substrate Recognition by SCF Ubiquitin Ligases. Mol. Cell 26, 131–143 (2007).
2) King, B. et al. XThe ubiquitin ligase FBXW7 modulates leukemia-initiating cell activity by regulating MYC stability. Cell 153, 1552–1566 (2013).
3) Galdeano, C. Drugging the undruggable: targeting challenging E3 ligases for personalized medicine. Future Med. Chem. 9, 347–350 (2017).
4) Lai, A. C. & Crews, C. M. Induced protein degradation: An emerging drug discovery paradigm. Nat. Rev. Drug Discov. 16, 101–114 (2017).

PYRENE-CYANINE DIPEPTIDES: ONE MOLECULE - DUAL FLUORESCENCE RESPONSE

Tamara Šmidlehner, Marko Badovinac, Ivo Piantanida

Ruđer Bošković Institute, Bijenička cest 54, 10000 Zagreb, Croatia

Two novel conjugates of pyrene and cyanine were constructed by linking them with a rigid triazole–peptide linker. These new probes bind very strongly (with 0.1 mM affinity) to both ds-DNA(RNA) and proteins (BSA), giving significantly different fluorimetric responses: a strong pyrene emission change is highly selective for proteins and the "switch-on" of cyanine fluorescence is highly selective for DNA(RNA). Moreover, the new probes yield induced CD bands only with DNA/RNA, but not with BSA, which allowed an independent check of DNA presence in DNA/protein mixtures. Furthermore, these probes contain a FRET pair of chromophores, whereby FRET is silent in a free molecule solution and is activated by binding of the small molecule to the biomacromolecular target. The efficiency of FRET is to some extent related to the secondary structure of DNA/RNA and only for one of the probes is FRET activated in proteins. The two probes show distinctively different induced CD patterns in the 400–600 nm range (attributed to a different position of linker attachment on the cyanine core), allowing differentiation between various secondary structures of DNA or RNA, which are shown to be additionally enhanced by combining pyrene and cyanine into one molecule. Due to their low cytotoxicity and efficient cellular uptake, these probes are good candidates for further biological studies.



References

1) T. Šmidlehner, M. Badovinac, I. Piantanida, New J Chem (2018) 42, 6655-6663

GENERAL AND MODULAR STRATEGY FOR DESIGNING POTENT, SELECTIVE, AND PHARMACOLOGICALLY COMPLIANT INHIBITORS OF RHOMBOID PROTEASES

Anežka Tichá (1), Stancho Stanchev (1), Kutti R. Vinothkumar (2), David C. Mikles (1), Petr Pachl (1), Jakub Began (1), Jan Škerle (1), Kateřina Švehlová (1), Minh T.N. Nguyen (3), Steven H.L. Verhelst (3,4), Darren C. Johnson (5), Daniel A. Bachovchin (5), Martin Lepšík (1), Pavel Majer (1), Kvido Stříšovský (1)

1) Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo n. 2, Prague, 166 10, Czech Republic
2) Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, United Kingdom
3) Leibniz Institute for Analytical Sciences ISAS, Otto-Hahn-Str. 6b, 44227 Dortmund, Germany
4) KU Leuven - University of Leuven, Herestraat 49, box 802, 3000 Leuven, Belgium
5) Chemical Biology Program, Memorial Sloan Kettering Cancer Center, 1275 York Ave., Box 428, New York, NY 10065, USA

Intramembrane proteases control important biological processes by cleaving membrane proteins in their transmembrane helices. Rhomboid-family intramembrane serine proteases have been associated with malaria, cancer, and Parkinson's disease. They have emerging medical potential, but their specific inhibitors have been lacking. Here we bridge this gap, building on structural understanding of rhomboid protease specificity and mechanism [1-2], and discover that peptidyl alpha-ketoamides substituted at the ketoamide nitrogen by hydrophobic groups are potent rhomboid inhibitors [3]. They are active in the nanomolar range, surpassing the currently used rhomboid inhibitors by up to three orders of magnitude. Such peptidyl ketoamides show selectivity for rhomboids, leaving most human serine hydrolases unaffected. Crystal structures show that these compounds bind the active site of rhomboid covalently in a substrate-like manner, and kinetic analysis reveals their reversible, slow-binding, non-competitive mechanism. Since ketoamides are clinically used pharmacophores, our findings uncover a straightforward modular way for the design of specific inhibitors of rhomboid proteases, which are widely applicable in cell biology and drug discovery.

References

1) Zoll, S.; Stanchev, S.; Began, J.; Skerle, J.; Lepsik, M.; Peclinovska, L.; Majer, P.; Strisovsky, K., EMBO J. 2014, 33 (20), 2408-21
2) Ticha, A.; Stanchev, S.; Skerle, J.; Began, J.; Ingr, M.; Svehlova, K.; Polovinkin, L.; Ruzicka, M.; Bednarova, L.; Hadravova, R.; Polachova, E.; Rampirova, P.; Brezinova, J.; Kasicka, V.; Majer, P.; Strisovsky, K., J. Biol. Chem. 2017, 292 (7), 2703-2713.
3) Ticha, A.; Stanchev, S.; Vinothkumar, K. R.; Mikles, D. C.; Pachl, P.; Began, J.; Skerle, J.; Svehlova, K.; Nguyen, M. T. N.; Verhelst, S. H. L.; Johnson, D. C.; Bachovchin, D. A.; Lepsik, M.; Majer, P.; Strisovsky, K., Cell Chem Biol 2017, 24 (12), 1523-1536 e4

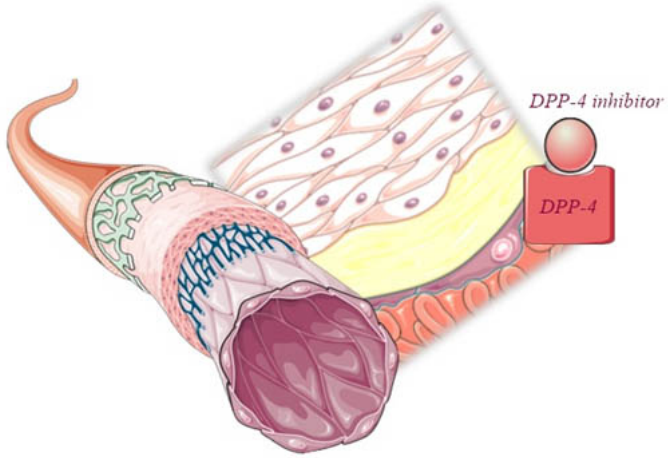
BENEFICIAL EFFECTS OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS ON VASCULAR DYSFUNCTION

Katarina Tomović (1), Gordana Kocić (2), Marko Anderluh (3), Andrija Šmelcerović (4)

1) Department of Pharmacy, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia
2) Institute of Biochemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia
3) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000, Slovenia
4) Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Niš, Serbia

Endothelial dysfunction that may result from multiple factors including loss of balance between vasoconstrictors and vasodilators, oxidative stress, inflammation, dysfunctional immunity, dyslipidemia and hyperglycemia, alters vascular homoeostasis and contributes to progression of vasculopathies and complications to a wide spectrum of disorders and organ damage. Endothelial cells show significant expression of dipeptidyl peptidase-4, besides its soluble circulating form. Inhibition of dipeptidyl peptidase-4 might participate in preservation of endothelial function, its integrity and vasculoprotection. Mechanisms underlying beneficial effects of dipeptidyl peptidase-4 inhibitors on vascular dysfunction are ascribed to its catalytic and receptor-like activity, improvement of glyco- and lipometabolic profiles, impacts on mediators of oxidative stress, apoptotic markers, inflammatory signaling, number and mobilization of endothelial progenitor cells, vascular smooth muscle cells proliferation and vascular tone.

We pointed to beneficial effects of dipeptidyl peptidase-4 inhibitors in the repair after myocardial infarction by the prevention of the cleavage of chemoattractant cytokine stromal cell-derived factor-1,[1] and this work represents the continuation with the aim to gain more detailed insight into multiple favorable effects of dipeptidyl peptidase-4 inhibition in the improvement of vascular dysfunction.



References

1) Anderluh M, Kocic G, Tomovic K, Kocic R, Deljanin Ilic M, Smelcerovic A. Cross-talk between the dipeptidyl peptidase-4 and stromal cell-derived factor-1 in stem cell homing and myocardial repair: potential impact of dipeptidyl peptidase-4 inhibitors. Pharmacology and Therapeutics 2016; 167: 100-107.

IN SITU LABELING OF POLYHISTIDINE-TAGGED PROTEINS FOR QUANTITATIVE PROTEIN INTERACTION ANALYSIS BY MICROSCALE THERMOPHORESIS

Nuska Tschammer

NanoTemper Technologies GmbH, Floessergasse 4, 81369 Munich

MicroScale Thermophoresis (MST) is a versatile method for the quantitative characterization of intermolecular interactions. In this technique a variation in the fluorescence signal is detected, which is a result of a temperature gradient induced by an infrared laser [1]. Because the extent of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target, this signal can be translated into the equilibrium dissociation constant (K_d). Although MST measurements can be performed using intrinsic fluorescence of proteins, labeling of the target proteins with a suitable fluorophore is often required. To enable near-native, site-specific *in situ* labeling strategy of proteins, we employed the combination of oligohistidine tag and its high-affinity ligand tris-NTA. Tris-NTA is comprised of three NTA moieties coupled to a cyclic scaffold and can thus simultaneously bind six histidine residues of a His6-tag, yielding subnanomolar binding affinity and a well-defined 1:1 stoichiometry. During our first iteration, three different three DYE-tris-NTA conjugates (NT647, NT547 and Oregon Green®488) were synthesized and their performance evaluated in the MST binding assays [2]. RED-tris-NTA conjugate (NT647-tris-NTA) arose as the optimal dye conjugate yielding the best signal-to-noise ratio. Owing to its red emission spectrum, it enabled also reliable measurements in complex biological matrices such as cell lysates, which display substantial autofluorescence in the blue and green part of the spectrum. To further optimize the signal-to-noise ratio and the assay window, we fine-tuned the properties of the red fluorophore. This resulted in the RED-tris-NTA 2nd generation conjugate with superior signal-to-noise ratio and amplitude compared to RED-tris-NTA (Figure 1). Overall, we generated a set of DYE-tris-NTA conjugates that enable site-specific labeling of proteins and thus permit the study of biomolecular interactions in near-native environment, and the studies of sensitive proteins, which do not tolerate covalent labeling (like SIRT5). Importantly, the use of this RED-tris-NTA conjugates may eliminate the need for protein purification for many MST assay setups, enabling shorter workflows and easier investigation of difficult-to-purify proteins.

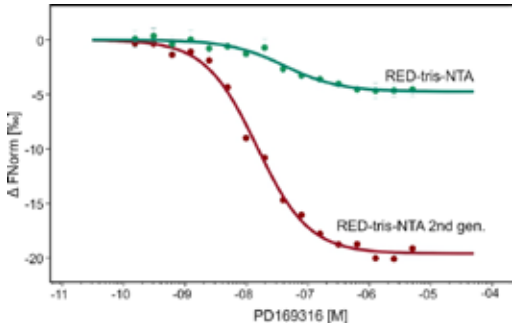


Figure 1: Comparison RED-tris-NTA and RED-tris-NTA 2nd generation. The p38alpha inactive kinase was labeled with the indicated dyes and the MST measurement performed at LED 60% and high MST power. The data were analyzed after 5 s MST-on time. The signal-to-noise ratio increased from 14.5 to 35.4 with the use of RED-tris-NTA 2nd generation.

References

1) Baaske, P. et al. Optical thermophoresis for quantifying the buffer dependence of aptamer binding. Angew. Chemie - Int. Ed. 49, 2238–2241 (2010).
2) Bartoschik, T., et al. "Near-native, site-specific and purification-free protein labeling for quantitative protein interaction analysis by MicroScale Thermophoresis." Scientific Reports 8.1 (2018): 4977.

KNOCKING ON THE BACKDOOR: SEARCHING FOR ALLOSTERIC POCKETS IN NUCLEAR RECEPTORS

Iris van de Gevel, Luc Brunsveld

Laboratory of Chemical Biology, Department of Biomedical Engineering
Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands

Nuclear Receptors (NRs) are a family of multi-domain transcription factors that reside within the cell. In here, NRs are able to bind directly to DNA and regulate gene expression. NRs play a role in processes ranging from embryonic development till homeostasis, and diseases from cancer to diabetes.[1] Because of this they are a popular drug target. Targeting their highly conserved orthosteric pocket comes with several disadvantages. These include specificity problems, where it is hard to target one out of 48 NRs, competition with endogenous ligands and mutation induced antagonist/agonist switching.

In 2015 Scheepstra et al. discovered an allosteric binding pocket in the ligand binding domain of the constitutively active retinoic-acid-receptor-related orphan receptor γt (RORγt).[2] This pocket only forms upon compound binding and induces a reorientation of helix 12 which blocks coactivator binding and therefore leads to inhibition. Up till now, this is the only NR known for which this allosteric inverse agonist pocket exists. It would be of great pharmacological interest to find such pockets in other NRs as well, since this could overcome a lot of the existing problems inherent to orthosteric modulators.

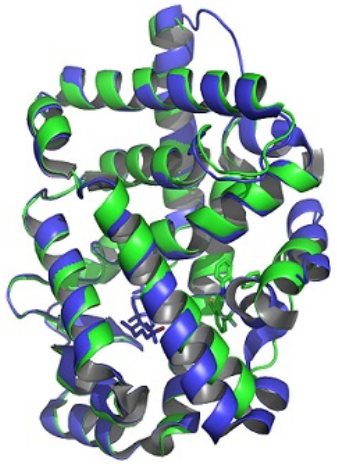


Figure 1: RORγt (blue) in complex with natural ligand 25-hydroxycholesterol and cofactor peptide SCR2 (PDB: 3L0L) superimposed with RORγt (green) in complex with allosteric compound MRL-871 (PDB: 4YPQ)

The project described here aims to explore if other NRs would be able to form a similar pocket. This is done via several routes presented here. Using mutagenesis the allosteric pocket and its boundaries in RORγt are evaluated and pushed to their limit. Besides that, RORα and RORβ are screened for allosteric activity and RORγt mimicking pockets are engineered into these isoforms. Finally, observed cross-reactivity of RORγt inverse agonists on the NR Peroxisome proliferator-activated receptor γ (PPARγ) is investigated using covalent orthosteric blockers.

References

1) H. Gronemeyer, J.-Å. Gustafsson, and V. Laudet, “Principles for modulation of the nuclear receptor superfamily,” Nat. Rev. Drug Discov., vol. 3, no. 11, pp. 950–964, Nov. 2004
2) M. Scheepstra et al., “Identification of an allosteric binding site for RORγt inhibition,” Nat. Commun., vol. 6, p. 8833, Dec. 2015

TOWARDS THE ELUCIDATION OF THE MECHANISM OF ACTION OF SMALL MOLECULE UPREGULATORS OF UTROPHIN USING CHEMICAL PROTEOMICS

Aini Vuorinen (1), Noelia Araujo (1), Maria Chatzopoulou (1), Stephen G. Davies (1), Enrico Emer (1), Josh Milner (1), Jessica Rowley (1), Graham M. Wynne (1), Kay E. Davies (2), Hannah Dugdale (2), Kelly Perkins (2), Sarah E. Squire (2), Nandini Shah (2), Kilian V. M. Huber (3), Kathryn Pugh (3), Francis X. Wilson (4), Angela J. Russell (1,5)

1) Department of Chemistry, Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, United Kingdom
2) Department of Physiology, Anatomy and Genetics, MRC Functional Genomics Unit, University of Oxford, Parks Road, Oxford OX1 3PT, United Kingdom
3) Target Discovery Institute, University of Oxford, NDM Research Building, Old Road Campus, Oxford OX3 7FZ, United Kingdom
4) Summit Therapeutics plc., 136a Eastern Avenue, Milton Park, Abingdon OX14 4SB, United Kingdom
5) Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, United Kingdom

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive and progressive muscle-wasting disease caused by lack of the cytoskeletal protein dystrophin. There is currently no cure for DMD, although various promising approaches (e.g. exon skipping, read through of stop codons, gene therapy) are being developed. We aim to develop an orally delivered small molecule modulator that replaces and compensates for the missing dystrophin with its autosomal paralogue utrophin. This therapy will be applicable to all patients regardless of their dystrophin mutation and will target skeletal muscle, heart and diaphragm. In partnership with Summit Therapeutics, ezutromid (SMT C1100), a first-in-class small molecule utrophin modulator that reduces dystrophic symptoms in the mdx mouse,[1,2] is in a Phase 2 clinical trial.[3,4] Preliminary data from a 24 week evaluation of patient muscle biopsies has recently been released, and encouraging signs of on-target activity have been demonstrated.[5]

Ezutromid demonstrates proof of principle for the strategy, but we still need to rapidly progress follow-on compounds which have better efficacy, pharmaceutical properties and/or complementary mechanisms to maximise the success of the utrophin modulation approach. We have discovered novel utrophin modulator chemotypes using an improved *in vitro* screening assay based on immortalised myoblasts from the dystrophin-null, utrophin luciferase knock-in mouse (LU*mdx*). Multiple structural classes, which significantly modulate utrophin expression in both murine and human DMD myoblasts, have been identified and are now being optimised. However, the precise mechanism by which these small molecules increase levels of utrophin is not understood. Importantly, initial evidence suggests that some of these small molecules modulate utrophin transcription through an alternative regulatory mechanism to ezutromid.

To discover the molecular mechanism of action of these novel utrophin modulators we have conducted structure-activity relationship studies using one of the compound classes, and a variety of chemical probes have been designed and synthesised in order to undertake chemoproteomic analyses. Initial pull-down studies were carried out using cell lysates treated with biotin-tagged probe molecules. As a result, a number of potential target proteins were identified, and the validation of these is ongoing. Additionally, to overcome the issue of limited cell-permeability of biotin-tagged probes and possible weak binding affinity, we have synthesised a range of improved dual-tagged probes, which bear a photoaffinity label (diazirine) and an alkyne handle for click chemistry. *In situ* pull-down experiments using these probes are ongoing. In addition to pull-down experiments, RNAseq and ATACseq experiments have been conducted, and analysis focusing on the utrophin A promoter pathway is ongoing. Recent results and next steps for this work will be presented.

References

1) J. Med. Chem., 2011, 54, 3241-3250
2) PLoS One, 2011, 6(5), e19189
3) J. Clin. Pharmacol., 2015. 55, 698-707
4) PLoS One, 2016, 11(4), e0152840
5) Press release, 25th January 2018, Summit Therapeutics plc.

DEVELOPMENT OF COVALENT INHIBITORS OF KDM5B

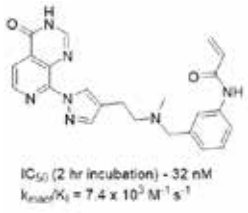
Miranda Wright

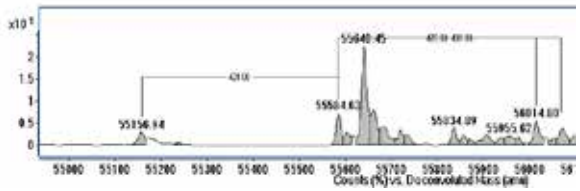
Nuffield Department of Medicine Research Building, University of Oxford, Old Road Campus, Roosevelt Drive, Oxford, United Kingdom, OX3 7FZ.

Lysine demethylases (KDMs) catalyse the removal of methyl modifications on histone tails which regulates gene expression. Over twenty KDMs have been discovered and linked to tumour growth and stem cell differentiation. JmjC-domain containing KDMs require 2-oxoglutarate (2-OG) and molecular oxygen as cosubstrates and Fe(II) as a cofactor to function. Current inhibitors of JmjC-KDMs are generally limited to metal-chelating scaffolds which inhibit enzymatic activity through chelation to the active site Fe(II) and compete with 2-OG. The main challenges have been achieving cellular activity and selectivity between KDMs due to similarity in their active sites.

The aim of this project was to design and synthesise irreversible inhibitors of KDM5B to reduce competition with cellular 2-OG. Cysteine 480 in KDM5B is not conserved across KDM5 subfamily and across other KDM families so targetting this cysteine could result in a selective covalent inhibitor. The designed compounds incorporated a core scaffold, 8-pyridopyrimidinone and different cysteine-selective electrophiles such as acrylamide and chloroacetamide in order to fine-tune the covalent reactivity.

The synthesised inhibitors were confirmed to bind covalently to KDM5B and were very potent against KDM5B in biochemical assays. Clickable analogues of the most potent inhibitor were also synthesised for use in pull-down assays to determine target engagement of the compounds with KDM5B in the cell.





EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - CHEMICAL BIOLOGY

Recent Developments in Kinase Inhibitors

TARGETING SPECIFIC INTERACTIONS TO IMPROVE EGFR-LIGAND BINDING

Markus Kossner

Chemical Computing Group, Kaiser-Wilhelm-Ring 11, 50672 Koeln, Germany

The epidermal growth factor receptor (EGFR) is implicated in many cancers, and its kinase activity is the target of commercial anti-cancer agents such as Tarceva and Iressa. However, despite their effectiveness, EGFR kinase inhibitors often show only moderate antiproliferative activity against certain tumour types in the clinic. Resistance to EGFR inhibitors is mediated by mutation in the ATP site and often through activation of the MAPK pathways by other receptor tyrosine kinases. This inspired the investigation of agents directed not only at EGFR kinase but also at divergent targets such as Src kinase or DNA, with the purpose of producing single compounds termed “combi-molecules”, with greater potency than the single EGFR inhibitor. A structure-based drug design modeling program, combined with PDB data-mining, protein structural fingerprints and pharmacophore searches was used to help identify and characterize linkers for connecting EGFR-binding moieties to DNA and Src targeting functionalities. The resulting compounds showed EGFR inhibitory potency in the low micromolar to nM range and retained significant activity against their divergent targets.

THE DEVELOPMENT OF MICROTUBULE AFFINITY REGULATING KINASES (MARK) INHIBITORS AND THEIR IN-VIVO PROFILE

Martin Ambler (1), Edward McIver (1), Stephen Lewis (1), Joanne Osborne (1), Kevin Gillen (1), Denise Tsagris (1), Nisha Mistry (1), Chido Mpamhanga (1), Debra Taylor (1), Janet Brownlees (1), Alison Levy (1), Michelle Newman (1), Emilie Bureau (1), Fenghua Fu (2), Zhenhua Wang (2), Zongliang Liu (2)

1) LifeArc, Stevenage Bioscience Catalyst,Gunnels Wood Road, Stevenage, SG1 2FX, UK
2) BIOasis, Shandong International Biotechnology Park Development Co.,Ltd., 39, Keji Avenue, Yantai High-Tech Zone, Shandong Province, 264670 China

Inhibition of microtubule affinity regulating kinases (MARK) may represent the potential to stop the formation of neurofibrillary tangles (NFTs), a pathology associated with Alzheimer’s disease (AD). Accumulation of NFTs in the neurons of AD patients correlates well with the degree of dementia. The normal function of tau protein is to stabilize microtubules, however, phosphorylation at S262 and S356 by MARK kinases results in dissociation from tau, which in turn becomes hyper phosphorylated by a range of kinases including GSK3 and CDK5. Hyper phosphorylated tau protein results in the formation of insoluble aggregates and NFTs.

Development of selective MARK inhibitors may provide a valuable therapy for the treatment of AD. On the other hand it is known that MARK kinases have important functions such as regulation of cell polarity which may result in undesirable effects *in vivo*.

We will describe the development of potent and selective series of MARK inhibitors with good ADME properties. These inhibitors were developed through a template hopping strategy and subsequent optimisation adjacent to the hinge binding motif. Through a successful research collaboration between MRCT (UK) and Bioasis (China) we were able to profile these inhibitors in a range of cellular and *in-vivo* assays probing the effects of MARK inhibition.

SYNTHESIS OF BENZAZULENES AS POTENT PIM-1 AND PIM-3 INHIBITORS

Ralica Arnaudova (1), Alexandros Kiriazis (1), Ingo Aumüller (1), Jari Yli-Kauhaluoma (1), Päivi Koskinen (2)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, Viikinkaari 5 E, FI-00014 Helsinki, Finland
2) Department of Biology, Section of Genetics and Physiology, University of Turku, Vesilinnantie 5, FI-20014 Turku, Finland

Cancer remains one of the most treated human diseases in the last decade and despite the numerous investments, there is limited overall improvement in treatment outcome. The discovery of anti-cancer drugs is undergoing and represents a highly challenging endeavor. Dysregulation of kinase function is one of the major mechanisms through which cancer cells escape from normal constraint of growth and proliferation. Overexpression of one of the kinase families, i.e. Pim kinase family (Pim-1, Pim-2 and Pim-3), is implicated with tumorigenesis, inflammatory states and chemo- and radio-resistance.[1,2] This, only three-member family exhibits high homology between its members and possesses a unique consensus hinge region sequence, ERPXPX.[3] Moreover, the hinge region keeps Pim kinases in a constitutively active form and therefore they do not require phosphorylation for activation once transcribed.[4] The main role of Pim kinases in cellular transformation, metabolic programming, engineering immune cells to blunt their anti-cancer action, protecting cancer cells from apoptosis and rescuing apoptotic cells by defending mitochondria makes these kinases as a prime target for cancer therapy. Furthermore, gene knockout studies have demonstrated that Pim deficient mice are viable and fertile.[5] Despite the unique architecture of the ATP-binding site of Pim kinases, there is no currently available anti-Pim kinase drug on the market.

In the last years many pharmaceutical companies have highlighted the significance of natural products to the drug discovery process. Most nature-derived medicines today lead their outset from plants, fungi and bacteria. Guaiazulene is an azulene derivative belonging to the bicyclic sesquiterpene class of natural products. There are literature reports of anti-fungal, antibacterial, immunomodulatory and anti-cancer activity possessed by azulene derivatives.[6] Benzazulenes refer to a class of fused 5-6-7-member rings and they contain an azulene moiety embedded in their tricyclic framework. Our previously designed and synthesized benzazulene derivatives exhibit selective inhibitory activity against Pim family members. In vitro auto-phosphorylation of Pim-1 kinase is diminished up to 90% at 10 μM concentration by our best inhibitor. These compounds efficiently impair intracellular anti-apoptotic effect of Pim-1 and Pim-3 and moreover, they significantly slow down the migration of cancer cells. Therefore, benzazulenes and their derivatives provide a novel group of small molecules that exhibit a potent and selective inhibitory activity towards Pim family members.[7-10]

References

1) a) Nawijn et al., Nat.Rev. Cancer, 2011, 11, 23. b) Merkel et al., Expert Opin. Invest. Drugs, 2012, 21. c) Drygin et al., J. Med. Chem., 2012, 55, 8199.
2) a) Drygin et al., J. Med. Chem., 2012, 55, 8199. b) Isaac et al., Drug Resist. Updates, 2011, 14, 203.
3) a) Qian et al., J. Biol. Chem. 2005, 280, 6130-6137. b) Brault et.al., Haematologia, 2010, 95, 1004-1015.
4) Qian et al., J. Biol. Chem., 2005, 280 (7), 6130-6137.
5) a) Tursynbay et al.; Biomedical Reports 2016, 4, 140-146. b) Jinesh et al.; Apoptosis, 2016, 21:1203–1213. c) Mikkers et al.; Mol. Cell. Biol., 2004, 24, 6104-6115.
6) Balunas et al.; Life Sci., 2005, 78, 431-441.
7) Aumüller et al., Org. Lett., 2009, 11, p.5363-5365.
8) Aumüller et al., Org. Lett., 2011, 13, p. 1670-1673.
9) Kiriazis et al., PLoS One, 2013, 8, p. e55409.
10) Kiriazis et al., Org. Lett., 2017, 19, p. 2030-2033.

DISCOVERY OF THE CLINICAL CANDIDATE AZD1390: A HIGH QUALITY, POTENT AND SELECTIVE INHIBITOR OF ATM KINASE WITH THE ABILITY TO CROSS THE BLOOD BRAIN BARRIER

Bernard Barlaam (1), Kurt Pike (1), Nicola Colclough (1), Stephen Durant (1), Elaine Cadogan (1), Martin Pass (1), Thomas Hunt (1), Andrew Eatherton (1), Kristin Goldberg (1), Ollie Steward (1), Willem Nissink (1), Kan Chan (2), Yumei Yan (2), Li Zheng (2), Peter Johnström (3)

1) Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, UK
2) Asia and Emerging Markets, IMED Biotech Unit, AstraZeneca, Shanghai, P.R. China
3) PHB, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

Glioblastoma multiforme (GBM) is the most common and lethal form of primary brain tumor and current treatment (surgery followed by fractionated radiotherapy and temozolomide) provides a median survival of just 12-15 months.[1,2] The poor prognosis associated with GBM is attributed to an extensive infiltration into surrounding brain tissue (thereby limiting the effectiveness of surgical excision), an intrinsic chemo/radioresistance of the tumor and the presence of the blood-brain barrier (BBB) which limits the ability of certain chemotherapies to reach the tumor. Ataxia telangiectasia mutant (ATM) is a serine/threonine protein kinase from the phosphatidylinositol 3-kinase-related kinase (PIKK) family of protein kinases and plays a crucial role in the cellular DNA damage response signalling activated by DNA double strand breaks (DSB). Activated ATM promotes DNA repair and S/G1-cell cycle checkpoints to prevent premature mitosis, maintain genomic integrity and promote appropriate cell survival or death pathways. DSBs arise intrinsically through the collapse of stalled replication forks, which are induced by a wide range of chemotherapies, or extrinsically through exposure to ionising radiation. Therefore, ATM inhibition represents an exciting clinical opportunity as a target to hyper-sensitize tumors to chemo/radiotherapy.

The optimization of compound properties suitable to allow efficient BBB penetration remains a significant challenge within Medicinal Chemistry and failure to consider these can severely restrict the utility of an agent for CNS disease. Herein, we describe the identification of AZD1390, a first in class orally available and CNS penetrant ATM inhibitor suitable for the treatment of intracranial malignancies. This presentation will focus on the Medicinal Chemistry strategies employed to optimize BBB-penetration, alongside the SAR for ATM potency, selectivity and pharmacokinetic properties. AZD1390 is an exceptionally potent inhibitor of ATM in cells (IC_{50} = 0.78 nM) with >10,000 fold selectivity over closely related members of the PIKK family of enzymes and excellent selectivity across a broad panel of kinases. AZD1390 displays excellent oral bioavailability in preclinical species (66% in rat and 74% in dog), is not a substrate for human efflux transporters and has been shown to efficiently cross the BBB in Non-Human Primate PET studies. Profound tumor regressions and increased animal survival (>50 days) have been observed in orthotopic xenograft models of brain cancer following just 2 or 4 days combination treatment of AZD1390 with radiotherapy, compared to radiotherapy treatment alone. These data support the potential of CNS penetrant ATM inhibitors to provide an important new therapeutic agent for the treatment of intracranial malignancies. AZD1390 is currently undergoing early clinical assessment.

References

1) Stupp, R., Hegi, M.E., Gilbert, M.R., Chakravarti, A., Chemoradiotherapy in malignant glioma: standard of care and future directions, J. Clin. Oncol. (2007) (25) 4127-4136
2) Ajaz, M.; Jefferies, S.; Brazil, L.; Watts, C.; Chalmers, A., Current and investigational drug strategies for glioblastoma, Clinical Oncology (2014) (26) 419 – 430

DISCOVERY OF NOVEL CLASS OF ALPHA SELECTIVE PI3K INHIBITORS

Marie-Gabrielle Braun

Genentech, 1 DNA Way, South San Francisco, CA 94080, USA

The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays a critical role in regulating tumor cell growth, proliferation and survival. Hotspot mutations in *PIK3CA*, the gene that encodes for the p110-alpha catalytic subunit of phosphatidylinositol-3-kinase, are highly prevalent in cancer and thus PI3K-alpha is a promising target for the treatment of cancer. Herein we report the discovery of a novel and highly potent series of PI3K-alpha inhibitors that are highly selective over the other Class I isoforms as well as the broader kinome. A scaffold hopping approach, followed by a structure based design approach using PI3K alpha structures allowed the identification of highly potent compounds, which nevertheless suffer from poor permeability and poor oral bioavailability. These challenges were addressed through the use of modulation of physicochemical properties and in-silico ADME property modelling. Increasing permeability while maintaining solubility culminated in the identification of a compound which was suitable for in vivo efficacy studies. This compound shows a dose dependent tumor growth inhibition and effectively suppressed growth of tumors in a mouse xenograft model. Overall, the high potency and selectivity make this series of great interest, as selective inhibitors have the potential to allow for treatment of P13K-alpha-driven cancers with a greater therapeutic index.

SMALL MOLECULE INHIBITORS OF IRAK4

Marian C. Bryan, Hans Brightbill, Edna Choo, Steven Do, Joy Drobnick, Claire Emson, Ross Francis, Alberto Gobbi, Zhiyu Huang, Patrick Lupardus, Swathi Sujatha-Bhaskar, James Kiefer

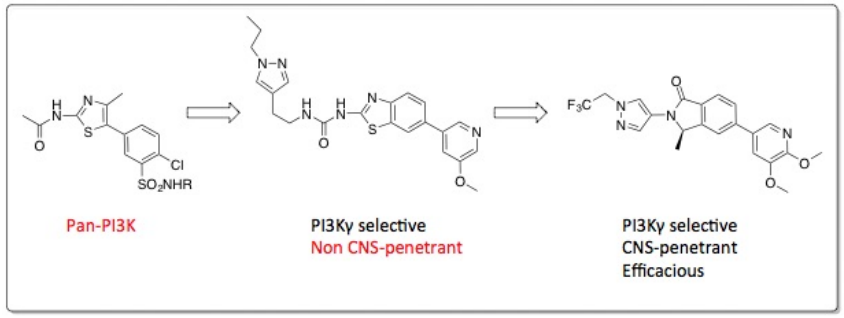
Genentech, One DNA Way, South San Francisco CA 94080, USA

The innate immune response enables cells to quickly respond to inflammatory cytokines by mounting the initial protective response through the activation and downstream function of the interleukin-1 receptor activated kinase (IRAK) family. Small molecule kinase inhibitors of IRAK4 have long been sought to block this response in autoinflammatory diseases such as lupus. In an effort to block disease progression, several scaffolds of IRAK4 small molecule inhibitors were explored using structure-based drug design. These efforts lead to our lead IRAK4 small molecule scaffold of potent and stable molecules. The discovery of this scaffold along with how it was influenced by other scaffolds will be presented including structural understanding of the binding site and in vivo PK and PD.

A ROADMAP FOR PI3Kγ SELECTIVITY DESIGN: DISCOVERY OF CNS-PENETRANT PI3Kγ INHIBITORS FOR THE POTENTIAL TREATMENT OF MULTIPLE SCLEROSIS

Philip Collier, Jon Come, Alex Aronov

Vertex Pharmaceuticals Inc., 50 Northern Avenue, Boston, MA 02210, USA



The lipid kinase PI3Kγ has attracted attention as a potential target to treat a variety of autoimmune disorders including Multiple Sclerosis (MS). Herein, we describe the evolution of a pan-PI3K inhibitor into a family of potent and selective benzothiazole inhibitors and detail the structural determinants of PI3K intra-family selectivity. We also outline the design strategy that provided CNS-penetrant inhibitors, without the efflux liabilities associated with our earlier scaffolds. Further optimization led to the discovery of a compound which demonstrated efficacy in a mouse model of MS, providing support for the further evaluation of the PI3Kγ pathway for this indication.

References

1) P.N. Collier et al. J. Med. Chem. 2015, 58, 517
2) P.N. Collier et al. J. Med. Chem. 2015, 58, 5684
3) P.N. Collier et al. J. Med. Chem. 2018, submitted

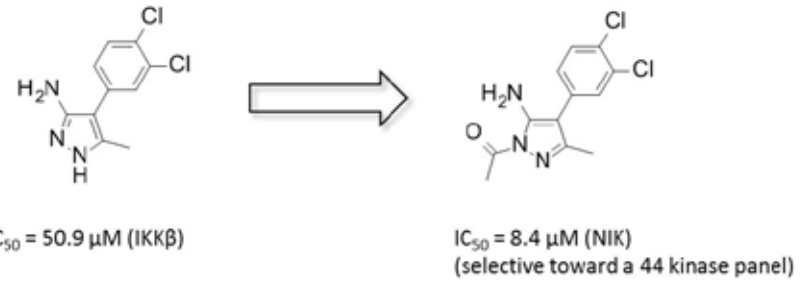
IDENTIFICATION OF SELECTIVE NF-KB KINASE (NIK) INHIBITORS

Jean-Marie Contreras (1), Christophe Morice (1), Agnese C. Pippione (2), Stefano Sainas (2), Antonella Federico (2), Elisa Lupino (3), Marco Piccinini (3), Michael Kubbutat (4), Alessandro Barge (2), Alex Ducime (2), Donatella Boschi (2), Salam Al-Karadaghi (5), Marco L. Lolli (2)

1) Prestwick Chemical, 220 Blvd Gonthier d'Andernach, 67400 Illkirch, France
2) Department of Science and Drug Technology, University of Torino, via Pietro Giuria 9, 10125 Torino, Italy
3) Department of Oncology, University of Torino, via Michelangelo 27/B, 10126 Torino, Italy
4) ProQinase GmbH, Breisacher Str. 117, 79106 Freiburg, Germany
5) SARomics Biostructures, Medicon Village, Scheelevägen 2, 223 63 Lund, Sweden

The NF-κB pathway is a validated target that influence multiple human diseases, including many cancers. NF-κB is a ubiquitously expressed family of transcription factors known to be constitutively activated in a variety of malignancies, resulting in uncontrolled apoptosis, cell cycle deregulation and metastatic growth. NF-κB-inducing kinase (NIK) is a central signalling component of the non-canonical pathway that integrates signals from a subset of TNF receptor family members. NIK may provide a means to directly inhibit the non-classical NF-κB pathway and thus potentially influence cancer proliferation.

The present poster describes the identification of new chemotypes by a blind screening conducted on the four kinases, IKKβ, IKKα, IKKε and NIK. An aminopyrazole showed weakly but selective inhibition on IKKβ and was the starting point of a hit-to-lead optimization study. More than 40 aminopyrazole derivatives were synthesized and evaluated and led to the identification of selective and micromolar NIK inhibitors.



References

1) Med. Chem. Commun. 2018, 9, 963-968

DISCOVERY OF AZD0364, A POTENT AND SELECTIVE ORAL INHIBITOR OF ERK1/2 THAT IS EFFICACIOUS IN BOTH MONOTHERAPY AND COMBINATION THERAPY IN MODELS OF NSCLC

Iain Simpson (1), Mark J. Anderton (2), David M. Andrews (1), Jason Breed (3), Emma Davies (1), Judit E. Debreczeni (3), Vikki Flemington (1), Francis D. Gibbons (4), Mark A. Graham (1), Philip Hopcroft (3), Tina Howard (3), Julian Hudson (1), Christopher Jones (1), Clifford D. Jones (1), Nicola Lindsay (1), J. Elizabeth Pease (1), Philip Rawlins (3), Karen Roberts (3), Steve Swallow (1), Steve St-Gallay (1), Michael Tonge (3), Richard A. Ward (1)

1) Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom
2) Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom
3) Discovery Sciences, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom
4) Oncology, IMED Biotech Unit, Gatehouse Park, Waltham, MA, USA

The RAS/MAPK pathway is a major driver in oncogenesis and is dysregulated in approximately 30% of human cancers, primarily by mutations in BRAF or RAS genes. The extracellular-signal-regulated kinases (ERK1 and ERK2) serve as key central nodes within this pathway. The feasibility of targeting the RAS/MAPK pathway has been demonstrated by the initial clinical responses observed to BRAF and MEK inhibitors in BRAF V600E/K metastatic melanoma, however resistance frequently develops by reactivation of the pathway. Direct targeting of ERK1/2, may provide another therapeutic option in tumours with mutations in BRAF or RAS genes. Importantly, ERK1/2 inhibition may have clinical utility in overcoming acquired resistance to RAF and MEK inhibitors where RAS/MAPK pathway reactivation has occurred, such as relapsed BRAF V600E/K melanoma.

Building on our published work,[1] we will describe a scaffold hopping approach leading to the identification of AZD0364, a pre-clinical ERK1/2 inhibitor candidate drug. Driven by conformational modelling and structure-based design, and by utilising novel sulfamidate ring opening chemistry, a high lipophilicity efficiency core was identified. Structure based, multi-parameter based optimisation of this improved core ultimately led to AZD0364. AZD0364 exhibits high cellular potency against a direct downstream substrate on the MAPK pathway (*e.g.* inhibition of phospho-p90RSK1 in BRAFV600E mutant A375 cells, $IC_{50} = 6$ nM). The molecule is a highly selective kinase inhibitor (10/329 kinases tested are inhibited at >50% at a 1 μM) and has long residence time on the protein (as determined by SPR on human unphosphorylated-ERK2: $pK_d = 10$; $t_{1/2} = 277$ mins). The good *in vitro* potency and selectivity is complemented by excellent physico-chemical properties and good oral pharmacokinetics across species, leading to a low predicted dose to man.

In xenograft models, AZD0364 inhibits phospho-p90RSK1 in tumours in a dose-dependent manner. AZD0364 induces regressions in the KRAS mutant NSCLC Calu 6 xenograft model. AZD0364 can also be combined safely and effectively with the MEK1/2 inhibitor selumetinib in KRAS mutant NSCLC xenograft models.

References

1) Richard A. Ward et. al. Structure-Guided Discovery of Potent and Selective Inhibitors of ERK1/2 from a Modestly Active and Promiscuous Chemical Start Point, J. Med. Chem. 2017, 60, 3438−3450.

DISCOVERY OF POTENT AND SELECTIVE COVALENT ITK INHIBITORS

Saskia Verkaik, Diana Mittag, Maaike Emmelot - van Hoek, Bas van de Kar, Anouk de Jong, Niels Hoogenboom, Dennis Demont, Edwin de Zwart, Gerjan de Bruin, Allard Kaptein, Todd Covey, Tjeerd Barf

Acerta Pharma BV, Kloosterstraat 9, 5349 AB, Oss, the Netherlands

Interleukin-2 inducible T-cell kinase (Itk) is thought to play a key role in T-cell and NK cell signaling. Itk inhibitors may have utility in disorders such as inflammation and cancer. Itk features a cysteine in the ATP binding pocket that allows for a targeted covalent approach. Our aim is to develop potent and selective Itk inhibitors, utilizing the covalent binding technology.

We identified potent lead compounds with moderate to good selectivity over all kinases with a cysteine in the same position as Itk. These leads significantly reduced anti-CD3-induced IL2-secretion in an in vivo PD mouse model following 10 mg/kg intravenous administration.

Further optimization cycles resulted in a covalent Itk inhibitor with low nanomolar potency and an excellent kinase selectivity profile.

PLASMODIUM PI4K. THE DISCOVERY AND DEVELOPMENT OF KINASE INHIBITORS AS ANTIMALARIAL DRUGS

James Duffy

Medicines for Malaria Venture (MMV), Route de Pré-Bois 20, 1215 Geneva, Switzerland

Despite substantial scientific progress since the start of the 21st century, new, affordable and safe malaria medicines are urgently required to overcome increasing resistance against artemisinin based combination treatments, treat vulnerable populations, interrupt the parasite life cycle by blocking transmission to the vectors, prevent infection and target malaria species that transiently remain dormant in the liver.(1)

The Plasmodium lipid kinase, phosphatidylinositol 4-kinase type III beta (PI4K), has been validated with in vitro and in vivo models as an antimalarial drug target (2),(3). Plasmodium PI4K is a ubiquitous eukaryotic enzyme that phosphorylates lipids to regulate intracellular signaling and trafficking. Inhibitors of Plasmodium PI4K have activity against all stages of the Plasmodium life-cycle, except liver-stage hypnozoites and can achieve high selectivity over their mammalian orthologues.(4)

The most advanced PfPI4K inhibitor, MMV048, is currently in clinical development. Furthermore, in partnership with Medicines for Malaria Venture (MMV) research groups from University of Cape Town (South Africa) and the University of Campinas (Brazil) have identified potential back up series. This presentation will summarize the current state of the field and highlight the best opportunities for the development of the next generation of PfPI4K inhibitors with potential for clinical development.

References

1) Wells, T. N. C., van Huijsduijnen, R. H. & Van Voorhis, W. C. Malaria medicines: a glass half full? Nat. Rev. Drug Discov. 14, 424–442 (2015).
2) McNamara CW, Lee MC, Lim CS, Lim SH, Roland J, Nagle A, et al. Targeting Plasmodium PI(4)K to eliminate malaria. Nature. 504, 248–253 (2013).
3) Paquet T, Le Manach C, Cabrera DG, Younis Y, Henrich PP, Abraham TS, et al. Antimalarial efficacy of MMV390048, an inhibitor of Plasmodium phosphatidylinositol 4-kinase. Sci Transl Med. 9:eaad9735 (2017).
4) Doerig C. Protein kinases as targets for anti-parasitic chemotherapy. BBA-Proteins Proteomics. 1697, 155–68 (2004).

DEVELOPMENT OF HIGHLY POTENT AND SELECTIVE PREVENTION OF ACTIVATION (POA) MK2 INHIBITORS

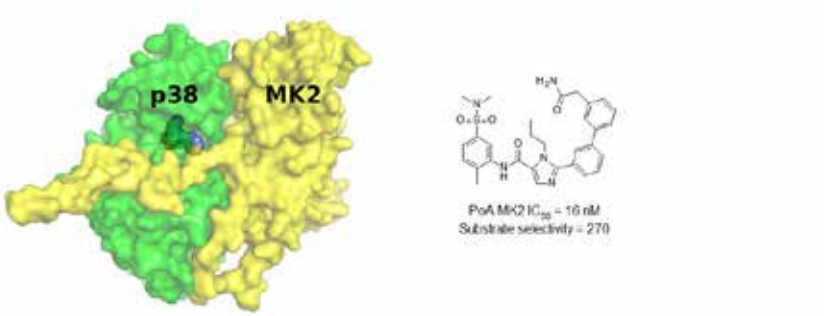
Emma Evertsson, Mickael Mogemark, Helena Käck, Monica Norberg, Magnus Munck af Rosenschöld, Sara Lever, Peter Bold, Katerina Pardali, Ulf Hedström, Andy Davis

Respiratory, Inflammation & Autoimmunity, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden, Emma.Evertsson@astrazeneca.com

Mitogen activated protein kinases such as p38 MAPK (p38) and MAPK-activated kinase-2(MK2) are attractive targets for inflammatory diseases such as rheumatoid arthritis (RA), Crohn's disease, inflammatory bowel syndrome (IBS) and chronic obstructive pulmonary disease (COPD).

P38α plays a dual role in the inflammation cascade, due to activation of MK2 and MSK1. Inhibition of p38α should not only decrease the pro-inflammatory mediator TNFa which signals through MK2, but also the anti-inflammatory mediator IL-10, through inhibition of the MSK1 pathway. Inhibiting MK2 on the other hand, should block only the production of TNFa, whilst sparing the anti-inflammatory mediator IL-10.

In our MK2 program we conducted a high throughput screening, where we identified two series of compounds, which inhibited the phosphorylation of non-activated MK2 by p38α via a prevention of activation (PoA) mechanism. It was evident from X-ray structures, that the compounds bound to the ATP binding pocket of p38α, but they had a higher affinity to the heterodimeric complex of p38α-MK2 compared to p38α alone. The two novel lead series were developed using structural chemistry and rational design to achieve compounds with excellent potency, good physicochemical properties and kinase selectivity.



TARGETING GLUCOSE METABOLISM FOR CANCER TREATMENT: A STRUCTURE-BASED VIRTUAL SCREENING TOWARD HEXOKINASE 2 INHIBITORS

Sara N. Garcia (1,2), M. Matilde Marques (1), Rita C. Guedes (2)

1) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
2) iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Glucose is regarded as the main fuel of cancer cells and the glycolytic pathway has been demonstrated as a potential target to be explored for cancer treatment. Several enzymes involved in glycolysis are overexpressed in different types of cancer cells, namely hexokinase 2 (HK2)[1]. This enzyme is involved in the first and most determinant step of the process, catalysing the phosphorylation of glucose to glucose-6-phosphate, also involved in the pentose phosphate pathway[2,3]. Therefore, the inhibition of the HK2 catalytic centre (**Figure 1**) is proposed as a strategy to reduce the main source of energy to cancer cells, thus substantially decreasing cancer cell proliferation. As an effort to find hit compounds able to interfere with the HK2 catalytic centre and thereby block its activity, a structure-based drug design strategy was implemented, leading to the virtual screening of several general databases such as DrugBank (~2000 molecules), NCI (~265 000 molecules), Chemoteca (~800 molecules) and some specific natural products databases such as Inter Bio Screen Natural Products (~84 000 molecules), Human Metabolome Database Food (~40 000 molecules) and Enzyme Function Initiative - Phosphate sugars (~100 molecules). The virtual screening was carried out using molecular docking calculations through Gold 5.20 software. Molecules were prepared using Molecular Operating Environment (MOE2016 0802) and then docked into the HK2 catalytic site. Prior validation of the above-mentioned protocol was conducted, by testing different three-dimensional (crystallographic) HK2 structures, the amino acids at the catalytic pocket centre, scoring functions and catalytic pocket radius. Our results have suggested several hit compounds with the potential to act as new HK2 inhibitors that may progress to biological evaluation.

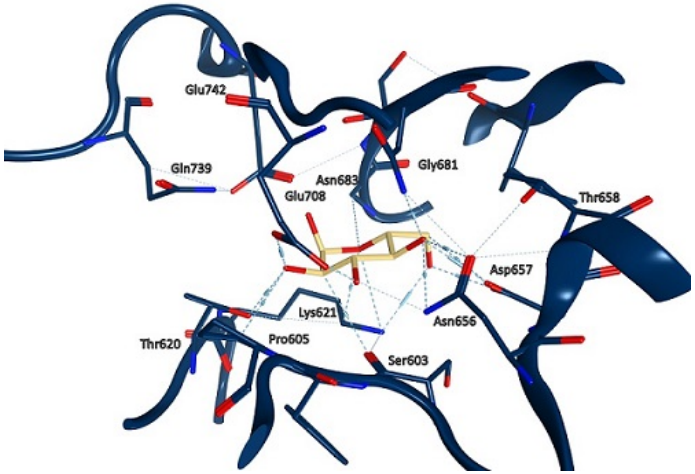


Figure 1 -Representation of the HK2 catalytic centre (C-terminus – dark blue) in interaction with a glucose molecule (yellow) (PDB code: 2NZT).

References

1) Hay, N. Nat. Rev. Cancer 2016, 16 (10), 635–649.
2) Martinez-Outschoorn, U. E.; Peiris-Pagès, M.; Pestell, R. G.; Sotgia, F.; Lisanti, M. P. Nat. Rev. Clin. Oncol. 2017, 14 (1), 11–31.
3) Hamanaka, R. B.; Chandel, N. S. J. Exp. Med. 2012, 209 (2), 211-215.

POTENT FLT3 KINASE INHIBITORS FOR ACUTE MYELOID LEUKEMIA WITH FLT3 MUTATIONS

Tomas Gucky (1), Eva Reznickova (2), Radek Jorda (2), Martin Lepšík (3), Vladimir Divoky (4), Vladimír Kryštof (2)

1) Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic
2) Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University and Institute of Experimental Botany AS CR, Šlechtitelů 27, 783 71 Olomouc, Czech Republic
3) Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
4) Department of Biology, Faculty of Medicine and Dentistry, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech

FLT3 tyrosine kinase is a potential drug target in acute myeloid leukemia (AML) because patients with FLT3-ITD mutations respond poorly to standard cytotoxic agents and there is a clear link between the disease and the oncogenic properties of FLT3. We prepared novel purine derivatives with potent FLT3 inhibitory activity. Molecular docking to FLT3 suggests a type I binding mode and explains the structural determinants of its potency. The lead compound displays nanomolar activity in biochemical assays and selectively blocks proliferation of AML cell lines harboring FLT3-ITD mutations, whereas other transformed and normal human cells are several orders of magnitude less sensitive. The treated MV4-11 cells suppressed the phosphorylation of FLT3 and its downstream signaling pathways, with subsequent G1 cell cycle arrest and apoptosis. Additionally, a single dose of the lead compound in mice with subcutaneous MV4-11 xenografts caused sustained inhibition of FLT3 and STAT5 phosphorylation over 48 hours, in contrast to the shorter effect observed after administration of the reference FLT3 inhibitor quizartinib. Experiments with subcutaneously implanted MV4-11 xenografts confirmed that a single dose of the tested compound induced sustained inhibition of FLT3 *in vivo*. In conclusion, we suggest this series to be followed for development of potent and specific FLT3 inhibitors for use as drug candidates for treating AML.

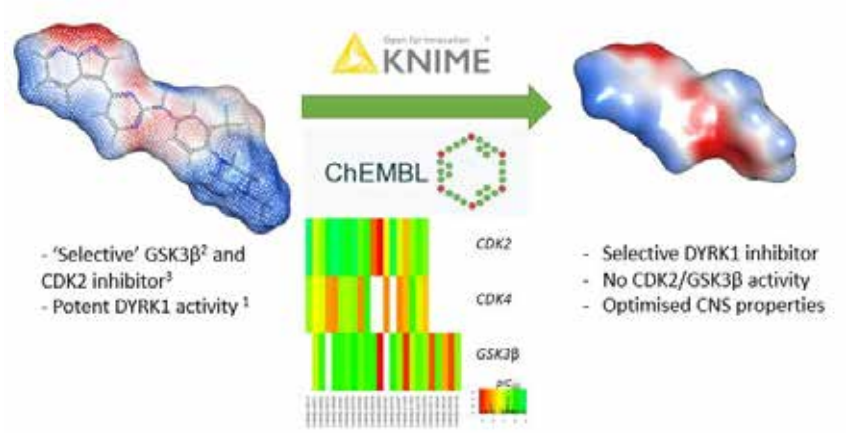
The work has been supported by the Ministry of Health of the Czech Republic (15-28951A).

MAKING THE MOST OF PUBLIC DOMAIN DATA WITH KNIME: LIGAND-BASED DESIGN OF SELECTIVE DYRK1 INHIBITORS

Scott Henderson (1), Fiona Sorrell (2), James Bennett (3), Paulo Godoi (4), Jonathon Elkins (2,4), Simon Ward (5)

1) SDDC, University of Sussex, Brighton, BN1 9RH,UK
2) SGC, University of Oxford, Oxford, OX3 7DQ, UK
3) TDI, University of Oxford, Oxford, OX3 7FZ
4) SGC-UNICAMP, Universidade Estadual de Campinas, Campinas, CEP: 13083-886
5) Medicines Discovery Institute, Cardiff University, Cardiff, CF10 3AT

A recent publication describes the screening of a set of diverse drug-like kinase inhibitors.[1] Several novel DYRK1 inhibitor templates have been identified from this set. Hit-validation and small-scale expansion of a number of these templates has led to the synthesis of nanomolar inhibitors of DYRK1. The open source Knime® Analytics Platform is being used to mine ChEMBL in order to predict off-target activity and guide inhibitor design. Whilst some of the inhibitors are reported to exhibit off-target activity against other CMGC (including cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAP kinases), glycogen synthase kinases (GSK) and CDK-like kinases) kinases, the aforementioned chemoinformatics and ligand-based approach has successfully removed these liabilities.[2] In combination with iterative high resolution X-ray crystallography we are successfully furnishing more selective inhibitors of DYRK1.



DYRK1 is over-expressed in the central nervous system (CNS) of individuals with Trisomy 21 (Down's Syndrome) and has been linked with early-onset Alzheimer's disease observed in the Down's Syndrome population. The drug-like properties of classical kinase inhibitors are inconsistent with those of marketed CNS drugs and until recently there were no reports of CNS kinase inhibitors [3]. Knime® is being utilised to increase the chances of target engagement *in vivo* through the use of workflows that predict properties such as central nervous system multiparameter optimization (CNS MPO) score and overall drug likeness amongst others. Using this method we hope to furnish a selective, CNS penetrant tool from which further inhibitors can be derived.

References

1) Elkins, J. M., Fedele, V., Szklarz, M., Abdul Azeez, K. R., Salah, E., Mikolajczyk, J., Romanov, S., Sepetov, N., Huang, X.-P., Roth, B. L., et al. (2016). Nat. Biotechnol. 34, 95–103.
2) Francis X. Tavares, Joyce A. Boucheron, Scott H. Dickerson, Robert J. Griffin,Frank Preugschat, Stephen A. Thomson, Tony Y. Wang, and Hui-Qiang Zhou . J. Med. Chem., 2004, 47 (19), pp 4716–4730
3) Heffron, T. J. Med. Chem., 2016, 59 (22), pp 10030–10066

DESIGN AND OPTIMIZATION OF NOVEL INHIBITORS OF NOTCH ACTIVATION COMPLEX KINASE (NACK)

Tanya T. Kelley (1), Xiaoxia Zhu (2), Anthony J. Capobianco (2), Stephan C. Schürer (1,3)

1) Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine, Miami Fl 33136
2) Department of Surgery, University of Miami Miller School of Medicine, Miami Fl 33136
3) The Center for Computational Science, University of Miami, Coral Gables Fl 33146

The pseudokinase Notch Activation Complex Kinase (NACK) was recently identified as a key player in Notch-mediated tumorigenesis by Capobianco *et al*[1], fashioning NACK an attractive novel target for the treatment of esophageal adenocarcinoma. However, there is no co-crystal protein ligand structure in the Protein Data Bank, and no reported biological data or known endogenous ligand, positioning NACK drug discovery difficult from traditional approaches. To identify a hit compound for NACK inhibition, machine learning classifiers for nineteen similar kinases were established, and over 6 million commercially available compounds were screened against these classifiers. Nearly 8000 compounds were prioritized based on the predicted probability of being active. A structure model of the NACK kinase domain was generated through homology modeling and further optimized with molecular dynamics (MD) simulations, followed by virtual screening of pre-prioritized compounds. Top-scoring compounds were purchased and screened in biochemical and cell-based assays. Commercially available compound Z271-0326 (iNACK) displayed the best inhibitory activity and was further validated in several xenograft mouse models. Recently, a robust novel chemical synthesis for iNACK was accomplished in six steps with an overall yield of 26%. A preliminary library of 20 analogues was synthesized and assayed, where analogue UM_004 displayed enhanced bioactivity and binding affinity over iNACK. Currently, we continue to optimize iNACK into the first NACK molecular probe, with the ultimate goal of creating an advanced pre-clinical lead compound. This is accomplished by improving the NACK kinase domain structure model *via* extended molecular dynamics studies with known active and inactive compounds to better understand binding interactions and increase model stability to inform our rational chemical library design. Additionally, we are employing computationally-driven structure-activity relationship (SAR) studies to improve inhibitory activity, as well as using interactive optimization platforms to increase affinity and favorable ADMET.

References

1) Weaver, K. L., Capobianco, A. J., et al. Cancer Res. 2014, 74, 4741

NEW DERIVATIVES OF IMATINIB WITH ANTI-MYELOPROLIFERATIVE ACTIVITY

Andressa Paula de Oliveira (1,2), Stefany de Castro Bazan (1), Monica Macedo Bastos (1,2), Núbia Boechat (1,2), Floriano P. S. Junior (3), Rafael F. Dantas (3)

1) Fundacao Oswaldo Cruz, Instituto de Tecnologia em Farmacos, Farmanguinhos - Fiocruz, Departamento de Sintese de Farmacos, Manguinhos, CEP 21041-250, Rio de Janeiro, RJ, Brazil
2) Programa de Pos-Graduação em Farmacologia e Quimica Medicinal do Instituto de Ciencias Biomedicas – ICB- UFRJ, Centro de Ciências da Saúde - CCS, Bloco J, Ilha do Fundão, CEP 21941-902 Rio de Janeiro-RJ, Brasil
3) Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Bioquímica Experimental e Computacional de Fármacos, 21040-360 Rio de Janeiro, RJ, Brazil

We have synthesized a new series of quinoline-sulfadoxine hybrids, planned by molecular hybridization between the quinoline ring and the benzenesulfonamide moiety present in chloroquine and sulfadoxine. Compound **I** exhibited selectivity index (SI) values (1102.2) and IC_{50} (0.09 mM) higher than chloroquine (834.74; 0.46 mM). When evaluated against *P. berghei* malaria, it was inhibited the parasitemia by 49% on day 5 after inoculation, contributing to the discovery of new prototype.[1]

In order to obtain new compounds with anti-*P. falciparum* activity, we used the compound **I** to design the new 1*H*-pyrazolo[3,4-*d*]pyrimidine-sulfonamide derivatives (**1-9**). The quinoline ring was replaced by the system 1*H*-pyrazolo[3,4-*d*]pyrimidine by ring isosterism. An *N*-(4-aminobutyl)benzenesulfonamide moiety was attached at the 4-position of the 1*H*-pyrazolo[3,4-*d*]pyrimidine ring (Figure 1).

The compounds **11a-c** could be prepared from the reaction of suitable 5-amino-pyrazoles **(10a-c)** and formic acid.[2] The derivatives **11a-c** were refluxed with $POCl_3$ to produce **12a-c**. The compounds **13a-c** were synthesized by the nucleophilic substitution reaction between **12a-c** and butane-1,4-diamine. The reaction[1] between **13a-c** and the appropriate sulfonyl chloride produced the target compounds **1-9** (Figure 2).

[image]

Among the 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **1-9** synthesized none of these were toxic to BGM cells. The compound **3** (R_1 = F / R_2 = CH_3) presented SI value 62.90 and IC_{50} = 5.13 μM lower than the sulfadoxine drug control (SI = 20.70; IC_{50} = 15.00 μM), in the anti-HRPII assay. The chloroquine and the prototype **I** is still more potent than **1-9** derivatives. The pyrazolo[3,4-*d*]pyrimidine is promising for further studies of antimalarial.

The authors thank the CNPq, CAPES and FAPERJ for financial support.

References

1) Instituto Nacional do Câncer (INCA). Available in: http://www.inca.gov.br/wcm/dncc/2015/estimativa-2016.asp. Access in: April 2018.
2) O' Dwyer, M. E.; Druker, B. J. Lancet. Oncol., 2000, 1, 207.

TRISUBSTITUTED PURINE INHIBITORS OF PDGFRA WITH HIGH SELECTIVITY TOWARD HUMAN EOSINOPHILIC CELL LINE EOL-1

Veronika Malínková (1), Eva Řezníčková (1), Radek Jorda (1), Tomáš Gucký (2), Vladimír Kryštof (1)

1) Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Šlechtitelů 27, 783 71 Olomouc, Czech Republic
2) Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic

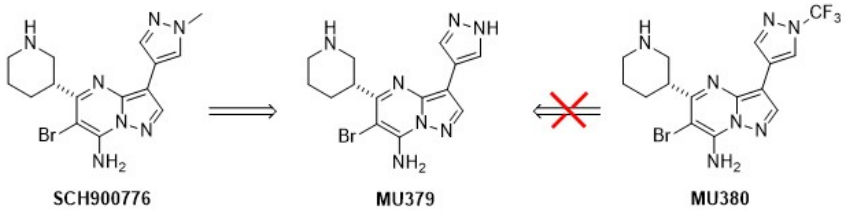
Inhibition of protein kinases is a validated concept of pharmacological intervention in cancers. We have synthesized a collection of 23 novel 2,6,9-trisubstituted purine derivatives with nanomolar inhibitory activities against receptor tyrosine kinase PDGFRA. The compounds demonstrated strong and selective cytotoxicity in human eosinophilic leukemia cell line EOL-1, whereas several other cancer cell lines were noticeably less sensitive. EOL-1 cell line expresses FIP1L1-PDGFRA fusion oncogenic kinase and we found that the cytotoxicity of compounds in this cell line correlates significantly with PDGFRA inhibition. We further studied cellular effects in EOL-1 by immunoblotting and flow cytometry. Dose-dependent inhibition of PDGFRA autophosphorylation and suppression of its downstream signaling pathways in treated EOL-1 cells confirmed the cellular mechanism of action. The results suggest that trisubstituted purines can serve as a source of tyrosine kinase inhibitors with specific activity towards eosinophilic leukemia and other cancers expressing constitutively activated PDGFRA mutants.

ENANTIOSELECTIVE SYNTHESIS AND PROFILING OF A NOVEL POTENT SELECTIVE INHIBITOR OF CHK1 KINASE

Prashant Khirsariya (1,2), Ondřej Hylse (1), Tereza Suchánková (4), Pounami Samadder (2,3), Lumír Krejčí (2,3), Karel Souček (2,4), Kamil Paruch* (1,2)

1) Masaryk University, Faculty of Science, Department of Chemistry, Kamenice 5/A8, 623 00 Brno, Czech Republic
2) International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, Czech Republic
3) Department of Biology, Faculty of Medicine, Masaryk University, 625 00 Brno, Czech Republic
4) Department of Cytokinetics, Institute of Biophysics CAS, v.v.i., Královopolská 135, 612 65 Brno, Czech Republic
**paruch@chemi.muni.cz*

The pivotal role of CHK1 (Checkpoint Kinase 1) in maintaining genomic stability offers attractive opportunity for increasing the selectivity, effectivity and reduced toxicity of chemotherapy. The attractiveness of targeting CHK1 is illustrated by the number of pre clinically and clinically profiled inhibitors, typically progressed in combination with standardly used antimetabolites.[1] Herein, we report enantioselective synthesis and profiling of **MU380**, a non-trivial analog of clinically profiled compound **SCH900776** possessing the highly unusual *N* -trifluoromethylpyrazole motif, which was envisioned not to undergo metabolic oxidative dealkylation and thereby provide greater robustness to the compound.[2]



MU380 is a selective and potent inhibitor of CHK1 which significantly sensitizes a variety of tumor cell lines to hydroxyurea or gemcitabine, shows extended inhibitory effects in the cell, and, unlike **SCH900776**, does not undergo *in vivo* N-dealkylation to the significantly less selective metabolite **MU379**. Compared to **SCH900776**, **MU380** in combination with gemcitabine is more efficacious in the A2780 xenograft mouse model.[2]

Funding: *CZOPENSCREEN: National Infrastructure for Chemical Biology (LM2015063) and European Union - project ICRC-ERA-HumanBridge (316345).*

References

1) Samadder, P.; Aithal, R.; Belan, O.; Krejci, L. Pharmacol. Ther. 2016, 161, 111.
2) Samadder, P.; Suchánková, T.; Hylse, O.; Khirsariya, P.; Nikulenkov, F.; Drápela, S.; Straková, N.; Vaňhara, P.; Vašíčková, K.; Kolářová, H.; Binó, L.; Bittová, M.; Ovesná, P.; Kollár, P.; Fedr, R.; Ešner, M.; Jaroš, J.; Hampl, A.; Krejčí, L.; Paruch, K.; Souček, K. Mol. Cancer Ther. 2017, 16, 1831.

DISCOVERY OF NOVEL INDAZOLE-3-CARBOXAMIDE GSK-3B INHIBITORS FOR THE TREATMENT OF MOOD DISORDERS \r\n

Federica Prati (1), Rosella Ombrato (1), Guido Furlotti (1), Francesca Mancini (1), Lucia Durando (1), Angelo Reggiani (2), Serena Tongiani (1)

1) Angelini RR&D (Research, Regulatory & Development) - Angelini S.p.A. Piazzale della Stazione snc, 00071, S. Palomba-Pomezia (Rome), Italy
2) Istituto Italiano di Tecnologia, D3 Research Line, Via Morego 30, 16163, Genova, Italy

Glycogen synthase kinase 3β (GSK-3β) is a constitutively active serine/threonine protein kinase mediating phosphorylation of several specific substrates.[1]

GSK-3β is highly expressed in the central nervous system and is involved in the regulation of many signalling pathways and cellular processes, such as cell cycle, inflammation, and cellular proliferation.

Importantly, aberrant GSK-3β activity has been linked to several disease conditions. Specifically, there is growing evidence on the role of GSK-3β in the pathophysiology of mood disorders with special regard to bipolar disease and depression.[2]

On this basis, Angelini proprietary compound libraries were virtually screened in a 3D model of GSK-3β enzyme to identify novel inhibitors as potentialmood stabilizers. In silico hits were then confirmed by human GSK-3β biochemical assay, leading to a new class of 1H-indazole-3-carboxamide inhibitors active in low micromolar range.[3] Subsequent structure-based optimization efforts afforded a promising lead compound, which showed a remarkably enhanced GSK-3β inhibitory potency (IC_{50} = 18 nM), interesting selective activity against a focused kinase panel and a clean *in vitro* cytotoxic profile. It also presented encouraging plasma and brain exposure levels in mouse PK studies, as well as *in vivo* efficacy in mouse models of depression.[4]

Therefore, the identified lead was selected for further *in vitro/in vivo* pharmacological evaluation, to elucidate the potential of GSK-3β inhibitors in the development of new treatments for mood disorders.

References

1) Doble, B. W.; Woodgett, J. R., GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 2003, 116, 1175-86.
2) Mao, Y.; Ge, X.; Frank, C. L.; Madison, J. M.; Koehler, A. N.; Doud, M. K.; Tassa, C.; Berry, E. M.; Soda, T.; Singh, K. K.; Biechele, T.; Petryshen, T. L.; Moon, R. T.; Haggarty, S. J.; Tsai, L. H., Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. Cell 2009, 136, 1017-31.
3) Ombrato, R.; Cazzolla, N.; Mancini, F.; Mangano, G., Structure-Based Discovery of 1H-Indazole-3-carboxamides as a Novel Structural Class of Human GSK-3 Inhibitors. J Chem Inf Model 2015, 55, 2540-51.
4) Furlotti, G.; Alisi, M. A.; Cazzolla, N.; Dragone, P.; Durando, L.; Magaro, G.; Mancini, F.; Mangano, G.; Ombrato, R.; Vitiello, M.; Armirotti, A.; Capurro, V.; Lanfranco, M.; Ottonello, G.; Summa, M.; Reggiani, A., Hit Optimization of 5-Substituted-N-(piperidin-4-ylmethyl)-1H-indazole-3-carboxamides: Potent Glycogen Synthase Kinase-3 (GSK-3) Inhibitors with in Vivo Activity in Model of Mood Disorders. J Med Chem 2015, 58, 8920-37.

DISCOVERY OF A NEXT GENEARTION B/C RAF KINASE INHIBITOR TO TREAT RAS/RAF MUTANT SOLID TUMORS: THE PATH TO THE CLINIC

Savithri Ramurthy (1), Benjamin A. Taft (1), Robert J. Aversa (2), Paul A Barsanti (1), Matthew T. Burger (2), Michael P. Dillon (8), Shenlin Huang (3), Gisele Nishiguchi (2), Alice Rico (8), Lina Setti (1), Aaron Smith (1), Sharadha Subramanian (1), Victoriano Tamez (2), Huw Tanner (1), John E Tellew (3), Lifeng Wan (1), Valery Polyakov (1), Mulugeta Mamo (1), Hanne Merritt (5), Johanna M. Jansen (1), Emma Lees (4,10), Mohammad Hekmat-Nijjad (5), Vijay Sethuraman (5), Kalyani Gampa (4), Fei Feng (4), Jing Yuan (11), Yingyun Wang (11), Jacob R. Haling (3), Sepideh Vaziri (3), Anne Van-Abbema (11), Richard Zang (7), Lesley Mathew Griner (4), Payman Amiri (11), Mallika Singh (9), Amy Lambert (4), Laura LaBonte (4), Vesselina G. Cooke (4), Wenlin Shao (6), Darrin D. Stuart (4), William R. Sellers (12)

1) Global Discovery Chemistry, Novartis Institutes for BioMedical Research, 5300 Chiron Way, Emeryville, California 94608, United States
2) Global Discovery Chemistry, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States
3) Genomics Institute of the Novartis Research Foundation, 10675 John Hopkins Dr., San Diego, C California 92121
4) Oncology, Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States
5) Infectious Diseases, Novartis Institutes for BioMedical Research, Emeryville, CA, USA
6) iMED Oncology, AstraZeneca, Waltham, MA, USA
7) Genentech Inc., South, San Francisco, CA, USA
8) IDEAYA Biosciences, South San Francisco, CA,USA
9) Revolution Medicines, Redwood City, CA, USA
10) Jounce Therapeutics, Cambridge, MA, USA
11) Oncology, Novartis Institutes for BioMedical Research, Emeryville, CA, USA
12) Broad Institute, Cambridge, MA, USA

RAS oncogenes have been implicated in >30% of human cancers, all representing high unmet medical need. The exquisite dependency of CRAF kinase in RAS mutant tumors has been established in genetically engineered mouse models and human tumor cells. To date, many small molecule approaches are under investigation to target CRAF, yet kinase-selective and cellular potent inhibitors remain challenging to identify. Herein, we present the discovery of LXH254, a selective B/C RAF inhibitor, which was developed through a hypothesis-driven approach focusing on drug-like properties. We heavily utilized structure-based design approach to improve potency. Applying number of medicinal chemistry principles, we further optimized compounds towards cellular potency, selectivity, efficacy and therapeutic index that led to the discovery of LXH254. It proved to be efficacious in multiple animal xenograft models, including N/KRAS, RAF mutant models with excellent tolerability and favorable projected PK parameters, suitable for QD/BID administration in humans.

THE 3-OXABICYCLO[4.1.0]HEPTANE ISOSTERE FOR MORPHOLINE AS A KINASE HINGE BINDING MOIETY

Declan Summers (1,3), Simon Peace (1), Heather Hobbs (1), Sandeep Pal (2), Craig Jamieson (3)

1) Fibrosis and Lung Injury DPU, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK
2) Molecular Design UK Team, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK
3) Department of Pure and Applied Chemistry, WestCHEM, Thomas Graham Building, University of Strathclyde, 295 Cathedral Street, Glasgow, G1 1XL, UK

Kinases are attractive drug targets due to their key role in various cellular activities including proliferation, survival, apoptosis, metabolism and differentiation.[1] Commonly small molecule inhibitors compete directly with ATP and form vital H-bond interactions with the kinase hinge region. In Phosphoinositide 3-kinase (PI3K) inhibitors this interaction is often made *via* an aryl- morpholine hinge binding moiety (**1**). Co-planarity between these two rings is a requirement for activity and thus ring systems which adopt orthogonal conformations such as aryl- tetrahydropyran (**2**) are ineffective as morpholine isosteres,[2] whereas unsaturated systems (**3**) are often considered undesirable.

We have identified 3-oxabicyclo[4.1.0]heptane (**4**) as the first example of a saturated carbon linked hinge binding moiety for the PI3K family of kinases. Cyclopropyl carbon-carbon bonds are known to form stabilising interactions with adjacent π-systems.[3] DFT conformational studies suggested low energy co-planar conformations for aryl- 3-oxabicyclo[4.1.0]heptane systems and we have subsequently synthesised a series of tool compounds to aid investigation into the application and limitation of this potential morpholine isostere.

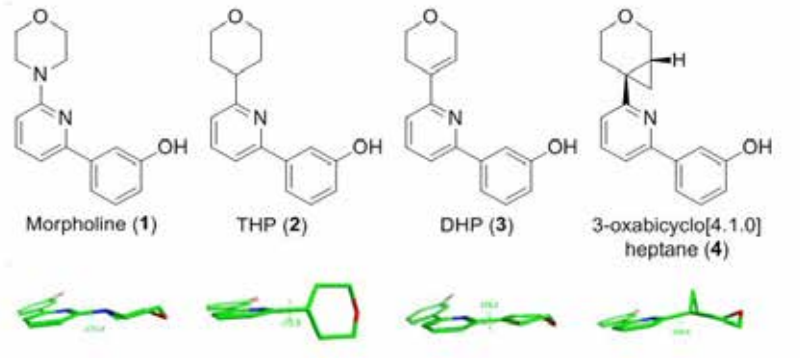


***Figure 1**: 2-pyridyl tool compound Small molecule XRC structures to investigate the conformational preference of various hinge binding fragments.*

We have utilised; predictive DFT calculations, X-ray crystallography (**figure 1**), NMR studies and biological evaluation of these tool compounds to comprehensively investigate the conformational preference of 3-oxabicyclo[4.1.0]heptane when attached to a range of 6-membered heterocyclic rings. We will report our findings which suggest a subtle stereoelectronic balance to conformation with a significant impact for applicability of 3-oxabicyclo[4.1.0]heptane as a general morpholine isostere.

References

1) Science 2002, 298, 1912-1934
2) Bioorg. Med. Chem. Lett. 2010, 20, 640-643
3) Angew. Chem. Int. Ed. 1979, 18, 809-826.

DISCOVERY OF NOVEL CYCLIN G ASSOCIATED KINASE (GAK) INHIBITORS WITH BROAD-SPECTRUM ANTIVIRAL ACTIVITY

Randy Wouters (1), Szu-Yuan Pu (2), Piet Herdewijn (1), Shirit Einav (2), Steven De Jonghe (1)

1) Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Herestraat 49 – bus 1041, 3000 Leuven, Belgium
2) Department of Medicine, Division of Infectious Diseases and Geographic Medicine, and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California, USA

The [60]fullerene molecule (C_{60}) and its derivatives are attractive constructs for biomedical applications. Glycoconjugated C_{60} derivatives are of particular interest as potential cancer targeting agents due to an upregulated metabolic glucose demand, especially in the case of pancreatic adenocarcinoma and its dense stroma, which is known to be driven by a subset of pancreatic stellate cells (PSCs). Herein we describe the synthesis and characterization of two D-glucosamine derivatives of [60]fullerene and their biological properties. The [60]fullerenes are inherently non-toxic up to concentrations of 10 mg/ml and are photoactive when illuminated with blue and green LED light, allowing its use as a photodynamic therapy agent.

It was observed that all fullerenes form two aggregate fraction 20-30 nm and 400-500 nm. Initial dark cytotoxicity studies on pancreatic cell lines PSCs and PANC-1 have been carried out using flow cytometry and propidium iodide (PI) apoptosis staining. It has been shown that all two glycofullerenes are non-toxic even in high concentrations (up to 10 mg/ml, incubation 3 and 24 hours). Moreover, synthesized [60]fullerene derivatives localizes preferentially in the nucleus of PSC cells, with some localization in the cell cytoplasm. Additionally, designed nanotherapeutics were tested for SRC kinase inhibition. Conducted experiments have shown that synthesized [60]fullerene derivatives selectively inhibited two kinases FYN A and LCK.

Figure 1 The structures of glycofullerene **1** and **2**.

[image]

Maciej Serda thanks National Science Center (Poland) for the support (grant UMO- 2016/23/D/NZ7/00912)

References

1) Bhatt, S.; Gething, P. W.; Brady, O. J.; Messina, J. P.; Farlow, A. W.; Moyes, C. L.; Drake, J. M.; Brownstein, J. S.; Hoen, A. G.; Sankoh, O.; et al. The Global Distribution and Burden of Dengue. Nature 2013, 496, 504–507.
2) Bekerman, E.; Neveu, G.; Shulla, A.; Brannan, J.; Pu, S. Y.; Wang, S.; Xiao, F.; Barouch-Bentov, R.; Bakken, R. R.; Mateo, R.; et al. Anticancer Kinase Inhibitors Impair Intracellular Viral Trafficking and Exert Broad-Spectrum Antiviral Effects. J. Clin. Invest. 2017, 127 (4), 1338–1352.
3) Kovackova, S.; Chang, L.; Bekerman, E.; Neveu, G.; Barouch-Bentov, R.; Chaikuad, A.; Heroven, C.; Sála, M.; De Jonghe, S.; Knapp, S.; et al. Selective Inhibitors of Cyclin G Associated Kinase (GAK) as Anti- Hepatitis C Agents. J. Med. Chem. 2015, 58, 3393–3410.
4) Li, J.; Kovackova, S.; Pu, S.; Rozenski, J.; De Jonghe, S.; Einav, S.; Herdewijn, P. Isothiazolo[4,3-B]pyridines as Inhibitors of Cyclin G Associated Kinase: Synthesis, Structure–activity Relationship Studies and Antiviral Activity. MedChemComm 2015, 6, 1666–1672

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL AMINOPYRIMIDINYLISOINDOLINES AS AXL KINASE INHIBITORS

Kyung Ho Yoo (1), Min Jung Choi (1,2), Juseung Kim (1,2), Juri Suh (1,2), Jong Seung Kim (2)

1) Chemical Kinomics Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
2) Department of Chemistry, Korea University, Seoul 136-701, Republic of Korea

In recent years, AXL kinase has emerged as a key facilitator of immune escape and drug-resistance by eliminating intercellular antibodies and regulating the secretion, and release of cytokines. The AXL protein was latter classified as a RTK belonging to the TAM (TYRO3, AXL, and MER) subfamily. AXL has been implicated as a cancer driver and correlated with poor survival in numerous aggressive tumors including TNBC, AML, NSCLC, pancreatic cancer, and ovarian cancer. Therefore, AXL has recently been proposed as an attractive target for cancer therapeutics and a number of small molecule inhibitors have been developed.

The purpose of this study is to develop the potent inhibitors against AXL kinase. In this work, a novel series of aminopyrimidinylisoindoline derivatives having an aminopyrimidine scaffold as a hinge region binding motif were designed and synthesized. Among them, six compounds showed potent inhibitory activities against AXL with IC_{50} values of sub-micromolar range. Especially, KIST 215121 possessing acetylpiperazinylphenylamino moiety exhibited extremely excellent efficacy (IC_{50} = < 0.000508 μM). Their *in vitro* antiproliferative activities were tested over five cancer cell lines. Most compounds showed good antiproliferative activities against HaLa cell. The kinase panel profiling of 50 different kinases for the representative compound KIST 215121 and the determination of IC_{50} values against selected protein kinases were carried out. KIST 215121 exhibited excellent inhibitory activity with IC_{50} values of 0.0502 μM (TYRO3), < 0.000508 μM (AXL), and 0.0257 μM (MER), respectively, against TAM subfamily. It can be used as a promising lead for the development of potent AXL kinase inhibitors.

References

1) Lemke, G.; Rothlin, C. V. Nat. Rev. Immunol. 2008, 8, 327.
2) Baladi, T.; Abet, V.; Piguel, S. Eur. J. Med. Chem. 2015, 105, 220.
3) Myers, S. M.; Brunton, V. G.; Unciti-Broceta, A. J. Med. Chem. 2016, 59, 3593.

MODELLING AND EXPERIMENTAL DETERMINATION OF KINASE UNBINDING PARAMETERS

Anna Maria Capelli (1), Andrea Rizzi (1), Daniele Pala (1), Serena Bertolini (1), Matteo Biagetti (1), Silvia Capacchi (1), Laura Carzaniga (1), Valentina Mileo (1), Donatella Callegari (2), Alessio Lodola (2), Silvia Rivara (2), Marco Mor (2), Beatrice Oliosi (3), Cristiana Griffante (3), Filippo Visentini (3), Mauro Corsi (3), Claudio Fiorelli (1)

1) Chiesi Farmaceutici S.p.A., Largo F. Belloli 11/A, 43122 Parma, Italy
2) Dipartimento di Farmacia, Universita' degli Studi di Parma, Viale delle scienze 27/A, 43124 Parma, Italy
3) Drug Discovery & Design, Aptuit, Verona, Italy

Nowadays several techniques are routinely used to model and predict in silico drug target affinity and selectivity indexes during lead optimization. On the contrary, the prediction of the duration of drug efficacy in vivo is still elusive. This complex property depends on the rate of receptor-ligand association (on-rate, k_{on}) and, most critically, on the dissociation rate constant (off-rate, k_{off}), which in turn can be translated into a dissociative half-life ($t_{1/2}$) for receptor-ligand complex as a direct measure of residence time [1]. Considering that the duration of drug efficacy in vivo is a key parameter to be optimized during lead optimization, the availability of robust computational approaches able to qualitatively predict or rank derivatives according to their residence time at a target would accelerate candidate selection. At the same time the availability of robust experimental methods would allow quantitation of these properties. Herein we present a computational protocol, based on metadynamics, suitable to predict unbinding kinetics of kinase ligands [2]. Besides, we will provide highlights regarding a non-radioactive method to detect the binding kinetics of kinase reference inhibitors [3].

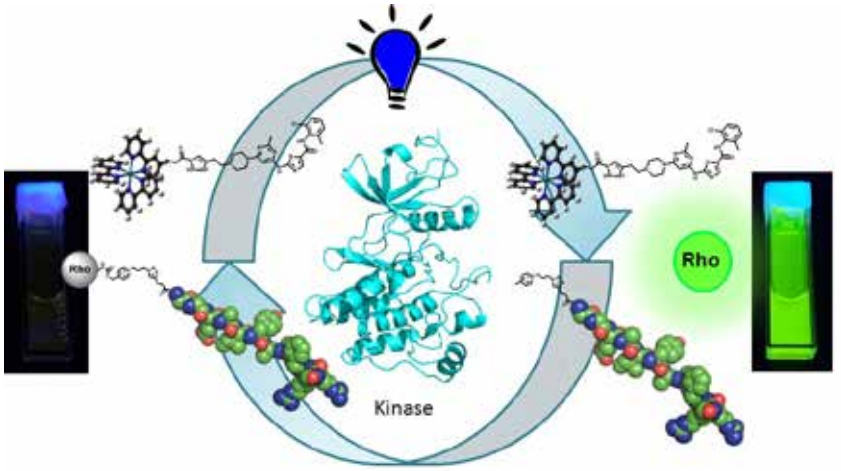
References

1) Copeland, R.A.; Pompliano, D.L.; Meek, T.D. Drug Target Residence Time and its Implications in Lead Optimization. Nature Rev. Drug Discovery 2006, 5, 730-9.
2) Callegari, D.; Lodola, A.; Pala, D.; Rivara, S.; Mor, M.; Rizzi, A.; Capelli, A.M. Metadynamics Simulations Distinguish Short- and Long-Residence-Time Inhibitors of Cyclin-Dependent Kinase 8 J.Chem.Inf.Model. 2017, 57, 159-69.
3) Oliosi B.; Griffante, C.; Visentini, F.; Bertolini, S.; Biagetti, M.; Capacchi, S.; Capelli, A.M.; Carzaniga, L.; Mileo, V.; Corsi, M. Determination of Association (kon) and Dissociation (koff) Rates of inhibitors of the PI3Kδ isoform using the LanthaScreen® technology. British Pharmacology Society, Pharmacology 2017 meeting 11-13 December 2017, poster PB049.

KINASE TEMPLATED ABIOTIC REACTION

Jacques Saarbach (1), Eric Lindberg (1), Sarah Folliet (1), Sandrine Georgeon (2), Oliver Hantschel (2), Nicolas Winssinger (1)

1) Faculty of Science, Department of Organic Chemistry, NCCR Chemical Biology, University of Geneva, 30 quai Ernest Ansermet, Geneva, Switzerland
2) Swiss Institute for Experimental Cancer Research (ISREC), NCCR Chemical Biology, School of Life Sciences, École Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland



Protein kinases are essential regulators of cellular signalling and have been at the centre stage of drug discovery for the past decade. The successful development of kinase inhibitors demonstrated that kinases were drugable and triggered tremendous research effort in this area. However, inhibitors developed so far often target the conserved ATP binding site of the protein and thus are lacking selectivity[1], and the more selective ones are targeting an inactive form of the protein. These features limit their use as chemical probes to sense kinase activity. Herein we report a strategy based on two reacting probes[2] targeting both nucleotide and substrate binding sites[3]. The reaction used[4] allows to use fluorescence readout to selectively sense Abl of Src kinase activity both in biochemical and fixed whole cell experiments.

References

1) O. Hantschel, U. Rix, T. Buerckstuemmer, U. Schmidt, M. Kneidinger, K. L. Bennett, I. Kaupe, W. Ellmeier, P. Valent and G. Superti-Furga, Blood, 2007, 110, 207b-207b
2) K. Gorska, I. Keklikoglou, U. Tschulena and N. Winssinger, Chem Sci, 2011, 2, 1969-1975
3) K. K. Sadhu, T. Eierhoff, W. Römer and N. Winssinger, J Am Chem Soc, 2012, 134, 20013-20016
4) D. Chang, E. Lindberg and N. Winssinger, J Am Chem Soc, 2017, 139, 1444-1447

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - CHEMICAL BIOLOGY

Enzyme Activators and Positive Allosteric Modulators

TUNING HUMAN 20S PROTEASSOME ACTIVITY BY PROLINE- AND ARGININE-RICH PEPTIDES

Malgorzata Gizynska (1), Julia Witkowska (1), Przemyslaw Karpowicz (1), Andrew M. Pickering (2), Pawel Osmulski (3), Maria Gaczynska (3), Elzbieta Jankowska (1)

1) Department of Biomedical Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland
2) Department of Molecular Medicine, The Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center, 15355 Lambda Dr, San Antonio, Texas 78245, USA
3) Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center, 15355 Lambda Dr, San Antonio, Texas 78245, USA

The proteasome is a main protease in the ubiquitin-proteasome pathway responsible for degradation of majority of intracellular proteins, such as damaged, mutated, oxidized and short-lived regulatory proteins. Dysfunction of the proteasome is involved in development of diverse diseases including cancer, autoimmune, neurodegenerative and rheumatoid disorders. These pathological states can be a result of uncontrolled degradation of functional proteins or inhibition of degradation of the damaged proteins [1]. Tens of the proteasome inhibitors have been developed so far. Three of them are already approved for clinical use in the treatment of hematologic malignancies. In the contrast to proteasome inhibitors only few activators have been reported to date [2-3].

Proline and arginine rich peptides (PR) are established inhibitors of 20S proteasome that require a set of positively charged N-terminal residues for their activity [4]. However, the role of other parts of PR peptides is not clear. We tested the significance of a proline rich internal segment and the C-terminal sequence of PRs. We found that extending PR sequence at the C-terminus with bulky tryptophan or phenylalanine residues substantially improved inhibitory capacity of PRs with activated 20S proteasome, while peptides with a HbYX motif (HbYX = hydrophobic, tyrosine, any residue) stimulated activity of the proteasome in a dose dependent manner. HbYX peptide was also capable to activate proteasome in cultured fibroblasts. Besides, we observed by means of atomic force microscopy that PR peptides influenced conformational dynamics of the proteasome and affected the opening of a gate leading to the catalytic chamber. These observations indicate that compounds designed based on PR peptide can be used as potential inhibitors or activators.

Acknowledgments:

This study was financially supported by NCN-funded grants 2016/23/N/ST5/02812 and 2014/15/B/NZ7/01014

References

1) Jung T., Catalgol B., Grune T., The proteasomal system, Mol Aspects Med, 2009, 30, 191–296
2) Witkowska J., Giżyńska M., Grudnik P., Golik P., Karpowicz P., Giełdoń A., Dubin G., Jankowska E., Crystal structure of a low molecular weight activator Blm-pep with yeast 20S proteasome - insights into the enzyme activation mechanism, Sci Rep, 2017, 7, 6177
3) Kisselev A. F., van der Linden W. A., Overkleeft H. S., Proteasome inhibitors: an expanding army attacking a unique target, Chem Biol, 2012, 19, 99-115
4) M. Gaczynska, P.A. Osmulski, Y. Gao, M.J. Post, M. Simons, Proline- and arginine-rich peptides constitute a novel class of allosteric inhibitors of proteasome activity, Biochemistry. 42 (2003) 8663–8670

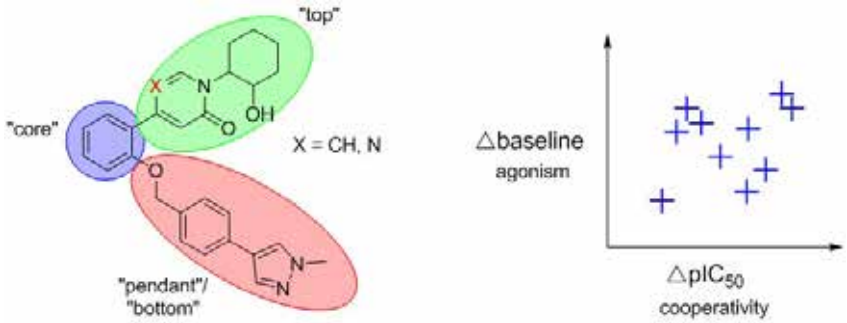
STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF 4-PHENYLPYRIDINONE AND 4-PHENYLPYRIMIDINONE-BASED POSITIVE ALLOSTERIC MODULATORS OF THE M1 MUSCARINIC ACETYLCHOLINE RECEPTOR

Manuela Jörg (1), Emma van der Westhuizen (2), Elham Khajehali (2), Kwok Choy (2), Celine Valant (2), Ben Capuano (1), Arthur Christopoulos (2), Peter Scammells (1)

1) Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria, 3052, Australia
2) Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria, 3052, Australia

The muscarinic M_1 acetylcholine receptor (M_1 AChR) has been recognized as a promising target for the treatment of the cognitive symptoms observed in patients with Alzheimer's disease and schizophrenia. However, the design of selective orthosteric ligands of the M_1 AChR has proven to be extremely challenging due to the highly conserved orthosteric site of all the muscarinic receptor subtypes (M_1-M_5). Consequently, the concept of targeting the less conserved allosteric region of the muscarinic receptors has gained more attention. Previously our group has disclosed three novel families of M_1 mAChR PAMs that are based on an arylpyrimidinone,[1] 4-phenylpyridin-2-one and 6-phenylpyrimidin-4-one scaffold,[2,3] respectively.

Herein, we report an extensive structure-activity relationship study of our previously established lead compounds looking at modification to the top, core and pendant part of the scaffold (Figure 1, left). We developed a unique pharmacological method allowing a higher throughput characterisation by comparing cooperativity with ACh and intrinsic agonism plotting the delta pEC_{50} versus the delta baseline of the novel synthesized analogues (Figure 1, right). [4] Furthermore, we have tested selected compounds for biased agonism,[3] in pharmacokinetic and toxicology studies as well as in mouse primary culture neurons, a more physiologically relevant systems.



References

1) S. N. Mistry, H. Lim, M. Jörg, B. Capuano, A. Christopoulos, J. R. Lane, P. J. Scammells, ACS Chem. Neurosci. 2016, 7, 647-661.
2) S. N. Mistry, M. Jörg, H. Lim, N. B. Vinh, P. M. Sexton, B. Capuano, A. Christopoulos, J. R. Lane, P. J. Scammells, J. Med. Chem. 2016, 59, 388−409.
3) E.T. van der Westhuizen, A. Spathis, E. Khajehali, M. Jörg, S.N. Mistry, B. Capuano, P.M. Sexton, P.J. Scammells, C. Valant, A. Christopoulos, Mol. Phar. 2018, in press.
4) J.C.C. Dallagnol, E. Khajehali, E.T. van der Westhuizen, M. Jörg, Manuela; C. Valant; A.G. Goncalves, B. Capuano, A. Christopoulos, P.J. Scammells, J. Med. Chem. 2018, DOI: 10.1021/acs.jmedchem.7b01812.

DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF PFKFB3 PHOSPHATASE ACTIVITY ALLOSTERIC MODULATORS

Helena Macut (1), Xiao Hu (1), Delia Tarantino (2), Luca Giovanni Regazzoni (3), Ettore Gilardoni (3), Sara Pellegrino (1), Maria Luisa Gelmi (1)

1) DISFARM - Section of General and Organic Chemistry "A. Marchesini", University of Milan, Via Venezian 21, 20133 Milan, Italy
2) Department of Biosciences, University of Milan, Via Celoria 26, 20133, Milan, Italy
3) DISFARM- Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133, Milan, Italy

Cardiovascular disease is a severe health problem, especially in the Western world, and its primary cause is atherosclerosis, which is characterized by the arterial wall thickening. Modern therapeutic strategies have restricted efficacy and mortality still remains high[1]. Current research has supported the idea of targeting disregulated endothelial cell (EC) metabolism as a novel therapeutic strategy[2]. In the scope of MSCA Moglynet EJD, we aim to further explore the possibilities for an improved treatment of this life threatening disease.

EC glycolytic flux is up-regulated during angiogenesis and it is controlled by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3)[3], which is hence an innovative target for atherosclerosis therapy. PFKFB3 is a homodimeric bifunctional enzyme that has a very high kinase to phosphatase activity ratio. Its activity is controlled by the N-terminus autoregulatory domain in the kinase region [4].

Virtual screening was performed on the targeted allosteric binding site and here we present the synthesis and biological evaluation of the selected libraries of PFKFB3 phosphatase modulators deriving from two design strategies. *In vitro* activity and binding assays were performed on the isolated recombinant enzyme. Phosphatase activity method was developed in-house using LC-MS instrument and binding assay was performed using Microscale thermophoresis.

References

1) J. Goveia, P. Stapor, P. Carmeliet, EMBO Mol Med 2016, 6, 1105–1120.
2) D. J. Rader, A. Daugherty, Nature 2008, 541, 904-913.
3) J. Chesney, R. Mitchell, F. Benigni, M. Bacher, L. Spiegel, Y. Al-Abed, J.H. Han, C. Metz, R. Bucala, Proc Natl Acad Sci U S A 1999, 96, 3047–3052.
4) S.G. Kim, N.P. Manes, M.R. El-Maghrabi, Y.H. Lee, J.Biol.Chem 2006, 281, 2939-2944.

ALLOSTERIC MODULATION OF THE NUCLEAR RECEPTOR RORGT USING SMALL MOLECULES

Femke Meijer, Lech-Gustav Milroy, Luc Brunsveld

Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands

Nuclear Receptors (NRs) are a large family of transcription factors in the human body, controlling several essential functions of the cell such as metabolism, development and reproduction. The retinoic acid receptor-related orphan receptor (ROR) is a subclass of these NRs which demonstrates high therapeutic potential, in particular the RORγt isoform. RORγt is expressed in lymphoid organs such as the thymus, where active RORγt is required for the differentiation and proliferation of T helper 17 (Th17) cells.[1,2] The inappropriate activation of Th17 cells has been linked to the pathology of numerous autoimmune disorders. The inhibition of RORγt by use of small molecules could therefore represent a promising strategy for the treatment of autoimmune diseases.[3]

Most of the reported modulators target the orthosteric binding site in the ligand binding domain (LBD) of RORγt. However, recently a novel type of ligand (MRL-871) was explored, acting as an inverse agonist. As observed in the co-crystal structure, this compound occupies an alternative, previously undisclosed binding site in the LBD of RORγt, called allosteric site.[4] Binding to this site results in repositioning of Helix 12 towards a less stable state, preventing the binding of co-activators and therefore leading to inhibition, similar to orthosteric inhibition. This allosteric modulation could be advantageous over orthosteric modulation in terms of selectivity issues and mutation-induced resistance.

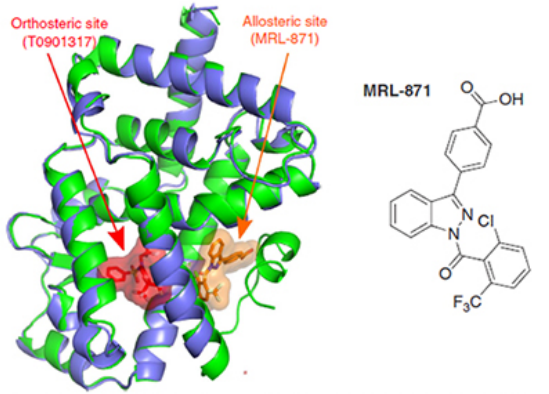


Figure 1: Superposition of RORgt (blue) in complex with orthosteric ligand T0901317 shown in red and RORgt (green) in complex with allosteric compound MRL-871, shown in orange.

Following this, we have further explored the allosteric binding pocket of RORγt. We present the design and synthesis of novel allosteric ligands with pharmaceutical potential, the possibility of an interplay between the orthosteric and allosteric site, the physiological effect of allosteric inverse agonists in cells, and the design of covalent orthosteric ligands that could be used to permanently block the orthosteric site.

References

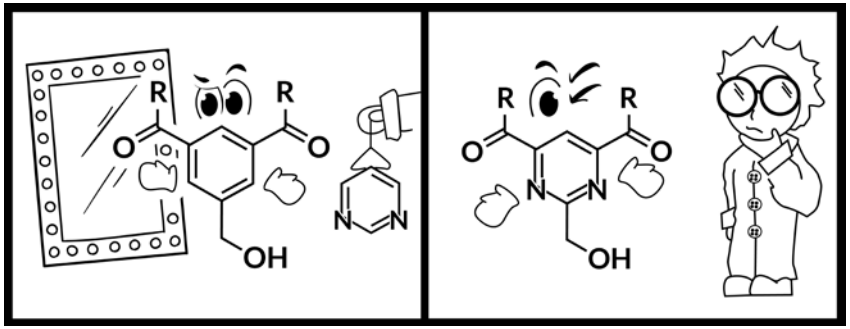
1) Fauber B, Magnuson S et al. Modulators of the Nuclear Receptor Retinoic Acid Receptor-Related Orphan Receptor-γ (RORγ or RORc). J Med Chem. 57(14), 5871-5892 (2014).
2) Solt L, Burris T. Action of RORs and their Ligands in (Patho)physiology. Trends Endocrinol Metab. 23(12), 619-627 (2012).
3) Cyr P, Bronner S et al. Recent Progress on Nuclear Receptor RORγ modulators. Bioorg Med Chem Lett. 26(18), 4387-4893 (2016).
4) Scheepstra M, Leysen S et al. Identification of an Allosteric Binding Site for RORγt Inhibition. Nat Comm. 6:8833, DOI: 10.1038/ncomms9833 (2015).

SCAFFOLD HOPPING FROM ISOPHTHALATE TO MULTISUBSTITUTED PYRIMIDINE DIMINISHES BINDING AFFINITY OF LIGANDS TARGETED TO THE C1 DOMAIN OF PKC

Riccardo Provenzani (1), Ilari Tarvainen (2), Giulia Brandoli (1), Antti Lempinen (1), Sanna Artes (1), Ainoleena Turku (1), Maria Jäntti (2), Virpi Talman (2), Jari Yli-Kauhaluoma (1), Raimo K. Tuominen (2), Gustav Boije af Gennäs (1)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
2) Drug Research Program, Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

Protein kinase C (PKC) isoforms regulate numerous cellular functions, making them highly attractive drug targets.[1] Utilizing the crystal structure of the PKCδ C1B domain,[2] we have developed hydrophobic isophthalic acid derivatives which allosterically modulate PKC activity by targeting the C1 domain of the enzyme.[3, 4] In the present study,[5] we aimed to improve the drug-like properties of the isophthalic acid derivatives by increasing their solubility and enhancing the binding affinity. We synthesized a series of multisubstituted pyrimidines as analogs of C1 domain–targeted isophthalates and characterized their binding affinities to the PKC α isoform. In contrast to our computational predictions, the scaffold hopping from phenyl to pyrimidine core diminished the binding affinity. However, the present results provide useful structure-activity relationship data for further development of ligands targeted to the C1 domain of PKC.



References

1) Mochly-Rosen D, Das K, Grimes KV. Protein kinase C, an elusive therapeutic target? Nat Rev Drug Discov. 2012;11(12):937-57.
2) Zhang G, Kazanietz MG, Blumberg PM, Hurley JH. Crystal structure of the cys2 activator-binding domain of protein kinase C delta in complex with phorbol ester. Cell. 1995;81(6):917-24.
3) Boije af Gennäs G, Talman V, Aitio O, Ekokoski E, Finel M, Tuominen RK, et al. Design, synthesis, and biological activity of isophthalic acid derivatives targeted to the C1 domain of protein kinase C. Journal of Medicinal Chemistry. 2009;52(13):3969-81.
4) Talman V, Provenzani R, Boije af Gennas G, Tuominen RK, Yli-Kauhaluoma J. C1 domain-targeted isophthalates as protein kinase C modulators: structure-based design, structure-activity relationships and biological activities. Biochem Soc Trans. 2014;42(6):1543-9.
5) Provenzani R, Tarvainen I, Brandoli G, Lempinen A, Artes S, Turku A, et al. Scaffold hopping from (5-hydroxymethyl) isophthalates to multisubstituted pyrimidines diminishes binding affinity to the C1 domain of protein kinase C. PLoS One. 2018;13(4):e0195668.

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - DIVERSIFIED TOPICS

First Time Disclosures

ACTIVATION OF LSD1 INHIBITOR PRODRUGS BY NITROREDUCTASE EXPRESSED IN CANCER CELLS

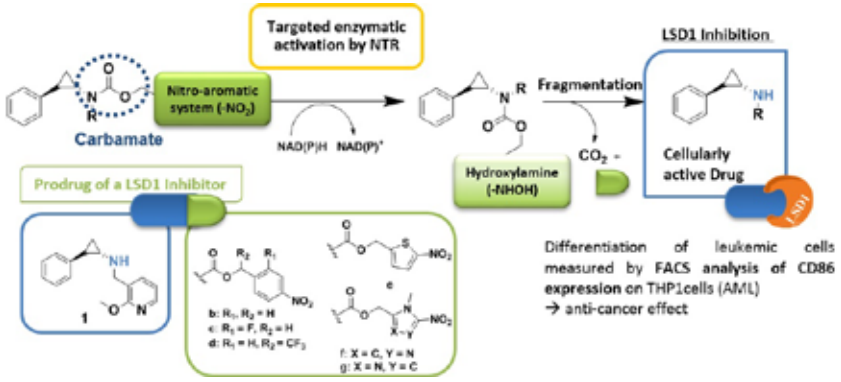
Eva-Maria Herrlinger (1), Mirjam Hau (1), Desiree Redhaber (2), Cornelius Miething (2), Gabriele Greve (2), Michael Luebbert (2), Roland Schuele (3), Manfred Jung (1)

1) Institute of Pharmaceutical Sciences, University of Freiburg, Albertstraße 25, 79104 Freiburg im Breisgau, Germany
2) Division of Hematology, Oncology and Stem Cell Transplantation, Department of Internal Medicine, University of Freiburg Medical Center, Hugstetter Str. 55, 79106 Freiburg, Germany
3) Department of Urology and Center for Clinical Research, University of Freiburg Medical Center, Breisacherstrasse 66, 79106 Freiburg, Germany

So far, known small molecule inhibitors of epigenetic proteins lack selectivity in affecting only tumour cells, often resulting in acute damage to healthy and rapidly cycling cells. The specific targeting of tumor cells should increase therapeutic effectiveness and decrease toxic side effects during treatment. A showcase model for the use of bacterial Nitroreductase (NTR) in enzyme-prodrug systems for epigenetic targets, the lysine-specific demethylase 1 (LSD1 or KDM1A) was selected.

To achieve target-specificity, pharmacologically inactive and nontoxic forms of known LSD1 inhibitors with a nitro-aromatic system, so-called *bioreductive prodrugs*, are designed, synthesized and tested against LSD1 activity *in-vitro* and on cultured AML THP1 cells. The LSD1 inhibitors are protected by a carbamate linked to the nitro-aryl bioreductive system which is reduced by the NTR, leading to subsequent release of the active drug.

We identified promising prodrug/drug pairs by measuring the expression of CD86 surface marker and by performing colony-forming unit assays with THP1 cells.[1] Several prodrugs are converted into the active parent drug by the NTR, which is solely expressed in transduced tumour cells. Depending on the nitro-aryl system, different activation patterns can be observed both *in vitro* and *in vivo*. By applying different targeting techniques such as antibody-directed enzyme-prodrug therapy (ADEPT) and gene-directed enzyme-prodrug therapy (GDEPT),[2] these prodrugs provide a direction for more selective anti-cancer drugs.



Funding by DFG CRC992 Medical Epigenetics, Project A04

www.jungm.de

References

1) Schulz-Fincke, J. et al. Structure-activity studies on N -Substituted tranylcypromine derivatives lead to selective inhibitors of lysine specific demethylase 1 (LSD1) and potent inducers of leukemic cell differentiation. Eur. J. Med. Chem. 144, 52–67 (2018).
2) Rautio, J. et al. Prodrugs: design and clinical applications. Nat. Rev. Drug Discov. 7, 255–270 (2008).

BENZAMIDES: A NEW SERIES OF POTENT, BBB PERMEABLE AND REVERSIBLE MAO-B INHIBITORS WITH NEUROPROTECTIVE EFFECT ON CORTICAL NEURONS

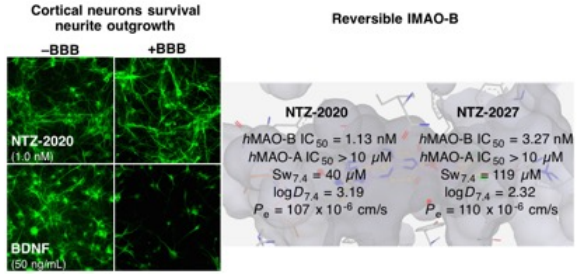
Nikolay T. Tzvetkov (1), Hans-Georg Stammler (2), Liudmil Antonov (3), Marcus Gastreich (4)

1) NTZ Lab Ltd., Krasno Selo 198, 1618 Sofia, Bulgaria, ntzvetkov@ntzlab.com
2) Bielefeld University, Department of Chemistry, Universitaetsstr. 25, 33615 Bielefeld, Germany
3) Bulgarian Academy of Sciences, Institute of Organic Chemistry, Acad. G. Bonchev Str. 9, Sofia 1113, Bulgaria
4) BioSolveIT GmbH, An der Ziegelei 79, 53757 St. Augustin, Germany

We report on a new series of structurally optimized (pyrrolo-pyridin-5-yl)benzamide derivatives, developed as potential drug and radioligand candidates for the treatment and diagnosis of Parkinson's Disease (PD) and other neurodegenerative diseases such as Alzheimer's Disease (AD). Recently, we reported on remarkably potent, *selective* MAO-B and *dually active* MAO-A/B inhibitors, acting through a different mechanism of action than the standard treatment with levodopa [1,2]. As a consequence, we performed further exploration within compounds with a privileged indazole template [3]. The most potent derivatives were compounds that belong to the best-balanced and best-in-class MAO-B inhibitors reported to date.

The new series of benzamide derivatives are not only highly potent and reversible MAO-B inhibitors, but also brain penetrant neuroprotectants. Compounds NTZ-2020 and NTZ-2027 can be highlighted because of their remarkable *in vitro* MAO-B inhibitory activity and selectivity - combined with a well-balanced physicochemical profile and BBB penetration ability. The reversible MAO-B inhibitor NTZ-2020 exhibits a neuroprotective effect on cortical neuron survival and induces neurite network outgrowth. These effects are associated with a good BBB penetration of NTZ-2020 that was confirmed in a triple cell neurovascular unit model co-culturing with cortical neurons, primary human brain microvascular endothelial cells (HBMEC), and astrocytes. Thus, due to their excellent pharmacological profile combined with acceptable physicochemical and drug-like properties, compounds NTZ-2020 and NTZ-2027 are considered for advanced optimization and study in relevant AD and PD models.

To rationalize the SAR detected and investigate further exploration steps, we analysed the binding mode of selected benzamide derivatives within the binding pocket of the human MAO-B enzyme using the novel SeeSAR [4]; basic concepts behind the estimations and visualizations will be reported in this contribution.



References

1) Tzvetkov et al. J. Med. Chem., 2014, 57(15), 6679-6703.
2) Tzevtkov et al. Eur. J. Med. Chem., 2017, 127, 470-492.
3) Tzvetkov PCT Int. Appl. 2015/013777.
4) www.biosolveit.de/SeeSAR

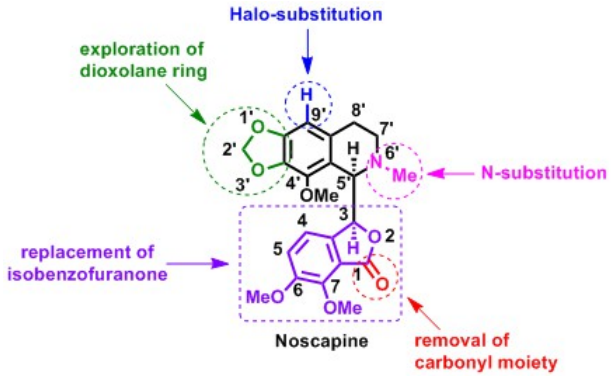
NOSCAPINE AND ITS DERIVATIVES AS CHEMOTHERAPEUTICS

Cassandra Yong (1), Shane Devine (1), Colin Pouton (2), Ben Capuano (1), Peter Scammells (1)

1) Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia
2) Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

Noscapine is a naturally-occuring phthalideisoquinoline alkaloid derived from the opium poppy, *Papaver somniferum*. Since the discovery of its antitussive properties in the 1950s, noscapine has been widely used as a cough suppressant. Four decades later, the anti-mitotic activity of noscapine was identified, unlocking the potential of noscapine and its analogues as chemotherapeutic agents. Noscapine is orally bioavailable and is effective against cancer, with no visible toxicities in vital organs of tumour-implanted mice.[1] In addition, with the gradual resistance development against current clinically available anti-mitotic agents, the low susceptibility of noscapinoids against overexpression of drug-efflux pumps is of significant importance.[2] Despite its weak cytotoxic activity, several semi-synthetic noscapine derivatives have shown a vast improvement against various cancer cell lines, indicating the potential of noscapine to be developed for clinical use.[2,3]

The predominant focus of our research is on the dioxolane moiety, an unexplored region of noscapine. We probed the region through ring expansion and introduction of hydrophobic aryl groups at the 1′-position. It has previously been elucidated that the “southern” isobenzofuranone ring is not crucial for activity.[4] Our synthetic efforts led to the a series of noscapine-inspired 5-substituted tetrahydroisoquinoline (THIQ) with methoxy-substituted phenyl and benzyl groups in place of isobenzofuranone. In continuation of previous work conducted by Debono *et al.*, we have also further explored substitution at the *N*6′-position.[3] Through subsequent pharmacology evaluation in cytotoxicity assays, we have discovered noscapinoids with significant improvement in potency against human breast and pancreatic cell lines, in comparison to noscapine.



References

1) Ye, K., Ke, Y., Keshava, N., Shanks, J., Kapp, J., Tekmal, R., Petros, J., Joshi, H. C., Proc. Natl. Acad. Sci. USA, 1998, 95, 1601-6
2) Debono, A., Mistry, S., Xie, J, Muthiah, D., Phillips, J., Ventura, S., Callaghan, R., Pouton, C. W., Capuano, B., Scammells, P. J., ChemMedChem, 2014, 9, 399-410
3) Debono, A., Xie, J., Ventura, S., Pouton, C. W., Capuano, B., Scammells, P. J., ChemMedChem, 2012, 7, 2122-33
4) Zimmermann, T, Roy, S., Martinez, N., Ziegler, S., Hedberg, C., Waldmann, H., ChemBioChem, 2013, 14, 295-300

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

POSTERS - DIVERSIFIED TOPICS

Emerging Topics

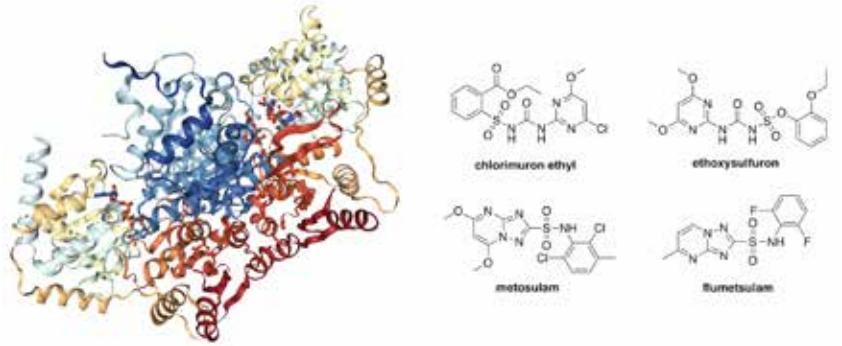
REPURPOSING COMMERCIAL AHAS-INHIBITING HERBICIDES AS ANTIFUNGALS TO TARGET THE EMERGING PATHOGEN, CANDIDA AURIS.

Kylie A. Agnew-Francis, Craig M. Williams, Luke W. Guddat, James A. Fraser

School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD 4072, Australia

Candida auris is a diploid yeast and human fungal pathogen first documented in 2009, in Japan.[1] This emergent species, though new, poses a great risk to human health owing to its extensive drug-resistance profile, and high mortality rates (~30-60%).[2-3]

Previous work by our group has identified acetohydroxyacid synthase (AHAS), an enzyme responsible for *de novo*synthesis of branched-chain amino acids (BCAA's) and currently used as a target for many commercial herbicides, as a viable target for anti-fungal drug development.[4-5] Here we have shown that commercially available herbicides in the sulfonylurea and triazolopyrimidine family can act as potent inhibitors of a drug-susceptible and drug-resistant strain of *C. auris*, with MIC_{50}'s as low as 97 nM.



References

1) Satoh, K.; Makimura, K.; Hasumi, Y.; Nishiyama, Y.; Uchida, K.; Yamaguchi, H., Microbiology and Immunology 2009, 53 (1), 41-44
2) Lockhart, S. R.; Etienne, K. A.; Vallabhaneni, S.; Farooqi, J.; Chowdhary, A.; Govender, N. P.; Colombo, A. L.; Calvo, B.; Cuomo, C. A.; Desjardins, C. A.; Berkow, E. L.; Castanheira, M.; Magobo, R. E.; Jabeen, K.; Asghar, R. J.; Meis, J. F.; Jackson, B.; Chiller, T.; Litvintseva, A. P., Clin. Infect. Dis. 2017, 64 (2), 134-140
3) Lockhart, S. R.; Berkow, E. L.; Chow, N.; Welsh, R. M., Clin. Microbiol. Newsletter 2017, 39 (13), 99-103.
4) Lee, Y.-T.; Cui, C.-J.; Chow, E. W. L.; Pue, N.; Lonhienne, T.; Wang, J.-G.; Fraser, J. A.; Guddat, L. W., Journal of Medicinal Chemistry 2013, 56 (1), 210-219.
5) Nason, P.; Luke, W. G., Curr. Pharm. Des. 2014, 20 (5), 740-753.

SHAPING THE PROSPECTS OF LABORATORY WORK: THE LAB OF THE FUTURE INITIATIVE

Nuria Aiguabella Font (1), Marcus Rhenius (2), Tobias Thaler (3), Thomas Neubauer (4), Nicolas Bernsmeier (2), Andreas Taske (6), Michael McLeod (5), Florian Erver (5)

1) Bayer AG, Müllerstraße 178, 13353 Berlin (Germany)
2) Bayer Business Services GmbH, Aprather Weg, 42113 Wuppertal (Germany)
3) Bayer AG, Kaiser-Wilhelm-Allee, 51368 Leverkusen (Germany)
4) Bayer AG, Aprather Weg, 42113 Wuppertal (Germany)
5) Bayer AG, Industriepark Höchst, 65926 Frankfurt (Germany)
6) Fraunhofer-Institut für Angewandte Informationstechnik FIT, Abteilung Kooperationssysteme, MARS Gruppe, Schloss Birlinghoven, 53754 Sankt Augustin (Germany)

The world is moving into a new era at a very fast pace, with disruptive technologies rapidly changing everything we do, from how we communicate with each other to how we shop, travel or drive. The pharmaceutical industry is not an exception to these changes, and we already anticipate how the way we do research and develop drugs can benefit from newly developed technologies.

Digitalization is one of the driving forces to these new processes, and in spite of its strong presence in our private everyday lives, laboratory digitalization still finds itself at a very early experimentation phase. At Bayer, we see this is as an opportunity for us to shape today how we envision laboratory work in the not so distant future.

The *Lab of the Future* initiative is an in-house project that pursues - through rapid prototyping - the integration of state of the art technologies in a scientist's and laboratory technician's daily laboratory life in order to **optimize cost efficiency and throughput, safety, and quality** of documentation. In this poster, we will showcase our efforts towards making our laboratories "smarter" through the use of smart devices and the creation of a *Lab App* that can integrate our current tools and databases in an easy to use and customizable platform.

CONFORMATIONAL SAMPLING AND BINDING AFFINITY PREDICTION OF MACROCYCLES

Daniel Cappel

Schrödinger GmbH, Q7 23, 68161 Mannheim, Germany

When optimizing ligand binding to a target protein during the drug design process a macrocyclic structure of the ligand can provide advantages. Macrocyclisation is an effective way to restrict a compound's conformational space compared to acyclic inhibitors with the potential to improve potency, selectivity and metabolic stability.

In the context of computationally-driven drug design this diverse class of chemical structures provides some challenges when it comes to conformational flexibility. Here we will discuss a method for exploring macrocyclic conformational space and the results of a benchmarking study[1] for this algorithm. A dataset of 208 structures was curated from the Cambridge Structural Database, the Protein Data Bank and the Biologically Interesting Molecule Reference Dictionary. A conformational search algorithm using the program Prime reproduces the crystal structure conformations in a highly accurate way and is fast compared to other published approaches. The sampling algorithm is also used in the context of a membrane permeability prediction protocol for macrocyles.

Furthermore, results for binding affinity prediction using the FEP+ framework for macrocycles are presented.[2] We have applied the method to 7 pharmaceutically interesting data sets taken from recent drug discovery projects including 33 macrocyclic ligands covering a diverse chemical space. The predicted binding free energies are in excellent agreement with experimental data, with an overall root mean square error (RMSE) of the predictions below 1 kcal/mol.

References

1) Sindhikara, D.; Spronk, S. A.; Day, T.; Borelli, K.; Cheney, D. L.; Posy, S. L. Improving Accuracy, Diversity, and Speed with Prime Macrocycle Conformational Sampling. J. Chem. Inf. Model. 2017, 57, 1881-1894.
2) Yu, H. S.; Deng, Y.; Wu, Y.; Sindhikara, D.; Rask, A. R.; Kimura, T; Abel, R.; Wang, L. Accurate and Reliable Prediction of the Binding Affinities of Macrocycles to Their Protein Targets. J. Chem. Theory Comput. 2017, 13, 6290-6300

ENHANCING STRUCTURE-BASED DRUG DESIGN WITH MEASURED FREE LIGAND CONFORMATIONS FOR CLASSICAL AND EMERGING DRUG CLASSES

Nichola L. Davies (1), Rodrigo J. Carbajo Martinez (1), Amber Balazs (2), Martin Packer (1), Elisabetta Chiarparin (1)

1) Oncology, IMED Biotech Unit, AstraZeneca, Cambridge, UK
2) Oncology, IMED Biotech Unit, AstraZeneca, Boston, MA, US

The determination of free ligand conformations of small molecules in solution is becoming a vital contributor to both ligand- and structure-based drug design. Measured solution conformations by NMR spectroscopy enhances drug design programs by focusing hypotheses of compound affinity and physical chemistry, reducing design make test cycles, and ultimately reducing synthesis efforts.

Design advantages gained from knowledge of free ligand conformations also extends to emerging drug classes. Synthetic macrocycles are increasingly considered as a source of potential drug molecules. However, their often long and challenging synthesis can limit their broader exploitation in drug discovery programs. Conformationally guided macrocycle design can improve this process; knowing when such a strategy will be most beneficial, and ensuring accurate design for potency and physico-chemical property enhancements. Another exciting drug class is the PROTACs approach - a proteolysis targeting chimera (PROTAC) is a two-headed molecule capable of removing unwanted proteins by inducing selective intracellular proteolysis. With the molecular weight falling in the 700-1000Da range, the delivery and bioavailability of PROTACs remain the largest hurdles to their progress. In the absence of x-ray crystallography to guide design and rationalise SAR, the utility of NMR free ligand conformations to optimise the linker design will be discussed.

In this presentation we will highlight, with examples of classical and emerging drug classes from the AstraZeneca Oncology portfolio, how measured free ligand conformations can enhance the drug design process.

HOW CAN WE INHIBIT A PROTEIN THAT IS INTRINSICALLY DISORDERED? ANDROGEN RECEPTOR – EPI-001 A CASE STUDY

Marta Frigolé-Vivas (1), Alessandro Ruffoni (1), Paula Martínez-Cristóbal (1), Jesús García (1), Elzbieta Szulc (1), Isabelle Burn-Heath (1), Antoni Riera (1,2), Xavier Salvatella (1,3)

1) Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Baldiri Reixac 10, 08028 Barcelona, Spain.
2) Departament de Química Orgànica, Universitat de Barcelona, Parc Científic de Barcelona Baldiri Reixac 10, 08028 Barcelona, Spain.
3) ICREA, Passeig Lluís Companys 23, 08010 Barcelona, Spain.

Intrinsically disordered proteins (IDPs) are highly attractive drug targets[1]. However, targeting them is a major challenge as their lack of defined secondary and tertiary structures hinders conventional structure-based drug discovery.

Androgen Receptor (AR) is a hormone-activated transcription factor. AR N-terminal domain (AR-NTD) is intrinsically disordered. Its function is to recruit the basal transcription machinery to express genes related to the development of the male phenotype. AR over-activation leads to prostate cancer (PC) and, eventually, castration-resistant prostate cancer (CRPC) for which there is currently no treatment[2].

EPI-001 is the only small molecule inhibitor of the AR-NTD and was identified by phenotypic screening[3]. A derivative of EPI-001 entered clinical trials for CRPC treatment. However, not much is known about its mechanism of action. In this project we want to understand how EPI-001 can specifically interact with the disordered AR-NTD. Also we are rationally building improved analogues and designing a screening assay to find new small-molecule scaffolds with the same mechanism.

So far, by NMR spectroscopy we have showed that EPI-001 interacts with a region of the NTD called Transactivation Unit-5, although with very low affinity[4]. In the symposium I will provide evidence that EPI-001 interacts with a specific conformational state of this domain that can be stabilized *in vitro* and closely resembles the state that this domain adopts in its biological milieu. Our results help understand the mode of action of this experimental drug and suggest general avenues for targeting proteins rich in intrinsic disorder such as transcription factors.

References

1) Metallo, S. J. Curr. Opin. Chem. Biol. 2010, 14, 481–488.
2) Gelmann, E.P. J. Clin. Oncol. 2002, 20, 3001–3015.
3) Andersen, R.J., et al. Cancer Cell. 2010, 17(6), 535–546.
4) De Mol, E. et al. ACS Chem. Biol., 2016, 11 (9), 2499–2505.

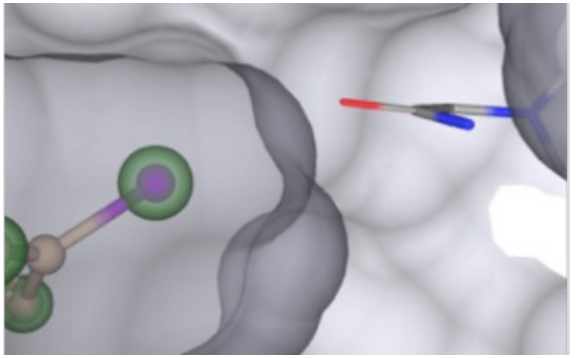
SIGMA HOLES — REALLY THAT INFLUENTIAL?

Marcus Gastreich

BioSolveIT GmbH, An der Ziegelei 79, 53757 St.Augustin, Germany
gastreich@biosolveit.de

Over the past years, sigma-holes[1] (the localized electron deficiency of polarizable halogen atoms leading to favorable electronic interactions with Lewis bases) have experienced vivid discussions and broad published awareness. Some drug researchers have recently started to incorporate the halogen binding concept into their rationalizing of lead optimization.[2]

In this presentation we will shine light on the fine difference between correlation versus causality, and — using a multitude of examples — we will analyze the impact of these clearly physical, electronic effects on binding affinity.



We will balance the effect of water versus sigma-holes onto substrate and drug binding using affinity measurements that shall be compared to both an empirical, logP-based model [3] and advanced quantum chemical computations. A broad geometric analysis of complexes in the PDB using a recently developed academic tool [4] supports the assumption that the overall energetic contributions are almost negligible in an aqueous environment, and that the expected geometries are only very rarely found in protein-ligand crystal structures. Conclusions and consequences for rational design shall be discussed.

Whereas most electron structure calculations quantify sigma-hole interactions in an in vacuo context, it is important to note that water plays an additional, very important role in the definition and thus calculation of binding affinities in a drug design context.

References

1) Wilcken et al, J. Med. Chem. 2013, 56, 1363−1388
2) a) Hardegger et al, Angew. Chem. Int. Ed. 2011, 50, 314 –318 and references therein; b) Derek Lowe in http://blogs.sciencemag.org/pipeline/archives/2013/01/17/halogen_bonds; c) Lam et al., ACS National Meeting, Org Div abs. 58, Aug 16th, 2009
3) a) Reulecke I, Lange G, Albrecht J, Klein R, Rarey M, ChemMedChem 2008, 3, 885 – 897, b) Schneider et al., J. Comput.-Aided Mol. Des. 27 (2013) 15e29. c) HYDE in SeeSAR v7, BioSolveIT GmbH, St. Augustin, Germany, 2017
4) Inhester and Rarey, Pelikan, J Chem Inf Model. 2017 57(2), 148-158; retrieved from zbh.uni-hamburg.de

TARGETED PHOTODYNAMIC THERAPY OF LUNG CANCER

Zoë R. Goddard, Maria J. Marin, Isabelle Chambrier, David Russell, Mark Searcey

University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK

Lung cancer is the third most common cancer in the UK and the prognosis for sufferers is extremely poor. It is treated with surgery, chemotherapy and radiotherapy, with both the latter treatments having dose limitations and severe side effects.[1] Photodynamic therapy (PDT) provides an attractive alternative for lung cancer treatment. It involves the use of a photosensitive drug which, when activated by visible light, causes the formation of singlet oxygen within cells. Singlet oxygen is highly toxic and causes cell death with minimal side effects.[2]

Zinc phthalocyanines have been shown to be efficient photosensitisers. Attachment to PEGylated gold nanoparticles has been shown to improve their potency by increasing their water solubility and therefore increasing their bioavailability.[3]

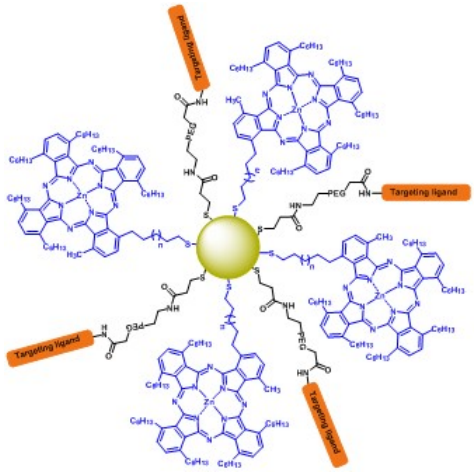


Figure 1: Structure of gold nanoparticle conjugates

We have designed and synthesised a series of gold nanoparticle-based photosensitisers, and actively targeted these towards non-small cell lung cancer through antibodies and small molecules. The synthesis of these conjugates will be discussed and preliminary biological studies will be disclosed.

References

1) Moghissi, K & Dixon, K, Eur. Resp. J., 2003, 22, 535-541
2) Ethirajan, M, et al., Chem. Soc. Rev., 2011, 40, 340-362
3) Hone, D.C, et al., Langmuir, 2002, 18, 2985-2987

IDENTIFICATION OF PYRAZOLIDINE-3,5-DIONES AND PYRROLIDINE-2,4-DIONES AS NOVEL POTENT LDHA INHIBITORS

Andrzej Gondela (1), Michał Gałęzowski (1), Joanna Fogt (1), Oleksandr Levenets (1), Łukasz Dudek (1), Alicja Obara (1), Jakub Wojciechowski (1), Marta Sowińska (1), Joanna Szeremeta-Spisak (1), Andrzej Dzienia (1,2), Marcin Król (1), Artur Biela (1,3), Aleksandra Poczkaj (1), Małgorzata Żurawska (1), Kamil Sitarz (1), Hans-Michael Eggenweiler (4), Krzysztof Brzózka (1), Tomasz Rzymski (1)

1) Selvita S.A.
ul. Bobrzyńskiego 14
30-348 Krakow, Poland
2) Institute of Chemistry, University of Silesia
ul. Szkolna 9
40-003 Katowice, Poland
3) Department of Cell Biology and Imaging
Institute of Zoology and Biomedical Research
Jagiellonian University
ul. Gronostajowa 9
30-387 Krakow, Poland
4) Medicinal Chemistry, Merck Biopharma
Merck KgaA
Frankfurter Straße 250
64293 Darmstadt, Germany

It is well described in literature that many solid tumors exhibit altered metabolic pathway utilizing glucose by glycolysis to produce energy,[1] relative to normal tissues wherein glucose catabolism is mainly occurring by oxidative phosphorylation.[2] These differences represent attractive therapeutic opportunities to selectively target tumor cells.[3]

Human lactate dehydrogenase A (LDHA) among other enzymes and transporters plays a crucial role in the promotion of glycolysis. LDHA uses NADH as a cofactor to transfer a hydride to the pyruvate ketone moiety, converting pyruvate into lactate.[4] Cancer cells experiencing glycolytic shift show elevated expression of LDHA and higher lactate production. The resulting extracellular acidosis, facilitates tumor invasion, metastasis and immune evasion.[5] It has been shown that silencing of the LDHA expression results in proliferation inhibition of tumor cell lines in vitro and TGI in in vivo xenograft models.[6]

Herein we report the synthesis, biochemical evaluation and SAR of novel pyrazolidine-3,5-diones and pyrolidin-2,4-diones developed as selective LDHA inhibitors. Docking studies were applied for rational structure optimization that resulted in the identification of LDH inhibitors in the sub-micromolar range (IC_{50} 0.65 µM). Repression of biochemical LDHA activity in cancer cells resulted in effective inhibition of cellular lactate production and reduced viability. Observed on-target efficacy in cancer cells warrants further development and testing in vivo.

References

1) Warburg, O. Science 1956, 123, 309
2) Vander Heiden, M. G.; Cantley, L. C.; Thompson, C. B. Science 2009, 324, 1029
3) R. Rani and V. Kumar, J. Med. Chem., 2016, 59, 487–496
4) D. Neri and C. T. Supuran, Nat. Rev. Drug Discovery,2011, 10, 767–777
5) Sheng, S. L.; Liu, J. J.; Dai, Y. H.; Sun, X. G.; Xiong, X.P.; Huang, G.FEBS J. 2012, 279, 3898–3910; Wang, Z. Y.; Loo, T. Y.; Shen, J.G.; Wang, N.; Wang, D. M.; Yang, D. P.; Mo, S. L.; Guan, X. Y.; Chen,J. P. Breast Cancer Res. Treat. 2012, 131, 791–800
6) K. Augoff, A. Hryniewicz-Jankowska and R. Tabol, Cancer Lett., 2015, 358, 1–7

SYNTHESIS, ANTIBACTERIAL ACTIVITY AND DOCKING STUDIES OF NEW THIOSEMICARBAZONE CONJUGATES OF MACROLIDE ANTIBIOTICS

Ivan Grgičević (1), Mirjana Bukvić (1), Vedrana Radovanović (1), Mihailo Banjanac (1), Predrag Novak (2), Branimir Bertoša (2), Iva Habinovec (2), Nina Čorak (3)

1) Fidelta Ltd., Prilaz baruna Filipovića 29, 10000 Zagreb
2) University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102A, 10000 Zagreb
3) University of Zagreb, Faculty of Science, Department of Biology, Roosveltov trg 6, 10000 Zagreb

One of the 20th century's significant achievements is discovery of azithromycin and its development to a commercial product for effective treatment of various infective diseases. Considering azithromycin's beneficial pharmacokinetic properties, our group have led the widespread modification of the azalide scaffold in a search for new azalides active against resistant bacterial strains [1-5]. In spite of a number of existing macrolide antibiotics, the emerging multi-drug resistant microbial pathogens present serious and challenging problems in medical treatment which demand novel and more effective antimicrobial agents to be discovered. Here we present a short overview of the work that has led to discovery of novel thiosemicarbazones of 15-membered azalides as a new class of compounds. The new compounds were evaluated *in vitro* against a panel of sensitive and resistant Gram-positive and Gram-negative bacterial strains. Synthesized compounds have shown good activity against macrolide sensitive Gram positive strains, comparable to azithromycin. Activity was also observed against two Gram negative strains (*Escherichia coli* and *Haemofilus influenzae*). Among macrolide resistant strains, compounds were modestly active only against efflux-mediated resistant *Streptococcus pneumoniae*. In order to achieve better insight into the molecular interactions responsible for compounds binding into the ribosome's active site, docking study of chosen compounds was performed. This approach can afford fast and effective preparation of a library of novel compounds with the goal of identifying new class bacterial inhibitors. Although the limited number of compounds studied here cannot allow for a comprehensive SAR analysis, they can serve as a good platform to explore the nature of bacterial resistance, especially against resistant *S. pneumoniae*.

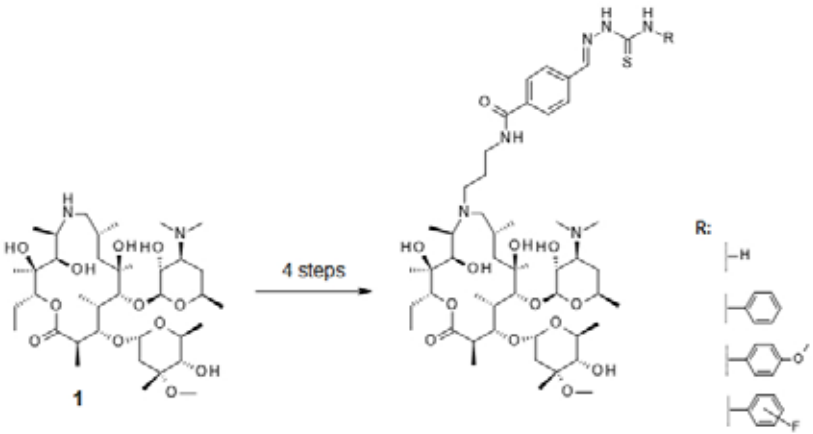


Figure 1. Route of synthesis for new macrolide thiosemicarbazone conjugates

References

1) Marušić Ištuk et. al., Bioorg Med Chem Lett 2011, 19, 556-566
2) Bukvić Krajačić et. al., J Med Chem 2011, 54, 3595-3605
3) Fajdetić et. al., Eur J Med Chem 2011, 46, 3388-3397
4) Matanović Škugor et. al., Bioorg Med Chem 2010, 18, 6547-6558
5) Hutinec et. al., Bioorg Med Chem Lett 2010, 20, 3244-3249

A BIDIRECTIONAL PHOTO-ANTAGONIST TOOLBOX FOR HISTAMINE H3 RECEPTOR PHOTOPHARMACOLOGY

Niels Hauwert, Tamara Mocking, Daniel Da Costa Pereira, Albert Kooistra, Bert De Boer, Chris De Graaf, Henry Vischer, Martine Smit, Iwan de Esch, Maikel Wijtmans, Rob Leurs

Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands

Histamine receptors are G protein-coupled receptors which are key regulators of a plethora of pathophysiological processes ranging from inflammation and itching to obesity. The histamine H_3 receptor (H_3R) is highly expressed in the central nervous system (CNS). It modulates the release of histamine and of other neurotransmitters[1] and has been identified as a potential target in diseases such as obesity, narcolepsy, Alzheimer's and ADHD.[1] Despite advances in H_3R medicinal chemistry and pharmacology, the complex H_3R signaling is still poorly understood and studies addressing this would benefit from molecules enabling temporal and spatial control of signaling. Therefore we developed molecules which have the ability to reversibly switch affinity and potency upon illumination for H_3R. A variety of azobenzene-containing ligands has been prepared by 3 to 5-step syntheses and characterized for both their photochemistry (NMR, LC-MS, UV-Vis) and pharmacology. In this presentation, we will show our key compounds VUF14862 and VUF14738 which represent a highly complementary bidirectional photo-antagonist toolbox.[2] These key compounds show more than 10-fold decrease or increase, respectively, in H_3R binding affinity upon illumination, and ultimately allow dynamic regulation of H_3R in *Xenopus* oocytes. These photopharmacological tools can be of aid in spatio-temporal studies to dissect the complex signaling cascade of H_3R.

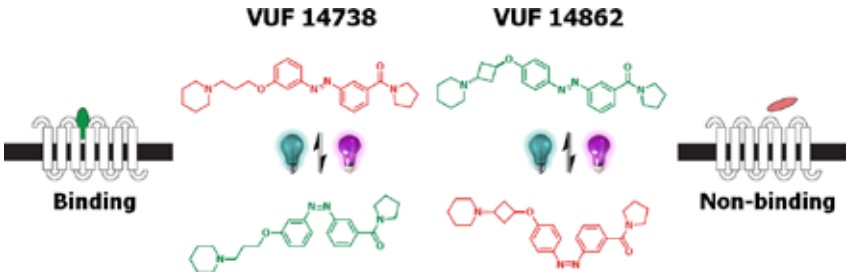


Figure 1: Characteristics of photoswitchable H_3R antagonists

References

1) Leurs et al., Nat. Rev. Drug. Discov., 2005, 4, 107-120
2) Hauwert et al., J. Amer. Chem. Soc., 2018, 140, 4232-4243

COVALENT FRAGMENT-BASED DISCOVERY OF NEW MURA INHIBITORS

Martina Hrast (1), Péter Ábrányi-Balogh (2), Hélène Barreteau (3), György M. Keserű (2), Stanislav Gobec (1)

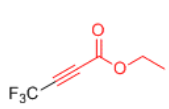
1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia
2) Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt 2, H-1117 Budapest, Hungary
3) Equipe Enveloppes Bactériennes et Antibiotiques, Institut de Biologie Intégrative de la Cellule (I2BC) UMR 9198 CEA-CNRS-UPSud, Bâtiment 430, Université Paris-Sud, F-91405 ORSAY cedex France

The covalent drugs are compounds containing electrophile moiety that can form a covalent bond with nucleophilic amino acid residues of proteins. Despite major concerns about toxicity including lack of specificity and potential immunogenecity, covalent inhibitors offer a number of advantages over non-covalent compounds[1]. High potency and prolonged duration of action may result in lower and less frequent dosing and reduced off-target activity. Additionally, covalent compounds can even decrease risk for development of resistance, which is extremely important in antibacterial drug discovery[2]. In the last years, a number of compounds with covalent mode of action has increased significantly, resulting in several blockbuster drugs[3].

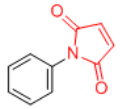
We focused on UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA) that takes part in the early steps of peptidoglycan biosynthesis and is essential for bacteria. MurA catalyses the transfer of enolpyruvate from phosphoenolpyruvate to UDP-*N*-acetylglucosamine[4]. It is well-validated target for antibacterial drug discovery, as it is inhibited by clinically used antibiotic fosfomycin, which forms a covalent adduct with the cysteine residue within the active site of MurA[5].

A covalent fragment library containing a large set of different warheads was assayed against MurA from *E. coli* and *S. aureus*. First, the assays were performed in the presence and absence of dithiothreitol (DTT) to decouple covalent binding from non-covalent interactions. The analysis of the results showed that the majority of fragments bound covalently to the target. Additionally, for the active fragments IC_{50} values were determined, followed by detailed enzyme kinetic evaluation that revealed exact mechanism of inhibition. We discovered fragments that inhibit both MurAs in low micromolar and sub-micromolar concentrations. The best compounds had the similar potency as clinically used MurA inhibitor fosfomycin.

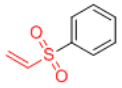
The data presented in this study revealed the reactivity and specificity of various covalent warheads that bind to MurA enzymes. This will allow us to select the appropriate warhead and optimize it to yield covalent inhibitor with sufficient potency and selectivity.



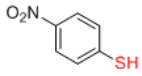
IC_{50} ($MurA_{EC}$) = 24 μM
IC_{50} ($MurA_{SA}$) = 47μM



IC_{50} ($MurA_{EC}$) = 0.851 μM
IC_{50} ($MurA_{SA}$) = 0.167 μM



IC_{50} ($MurA_{EC}$) = 15 μM
IC_{50} ($MurA_{SA}$) = 34 μM



IC_{50} ($MurA_{EC}$) = 0.125 μM
IC_{50} ($MurA_{SA}$) = 0.346 μM

References

1) Johnson, D. S., et al., Future Medicinal Chemistry 2010, 2 (6), 949-964.
2) (a) Bauer, R. A., Drug Discovery Today 2015, 20 (9), 1061-1073; (b) Singh, J., et al., Nature Reviews Drug Discovery 2011, 10, 307.
3) (a) De Cesco, S., et al., European Journal of Medicinal Chemistry 2017, 138, 96-114; (b) Baillie, T. A., Angewandte Chemie International Edition 2016, 55 (43), 13408-13421.
4) Barreteau, H., et al., FEMS Microbiology Reviews 2008, 32 (2), 168-207.
5) Hrast, M., et al., Bioorganic Chemistry 2014, 55, 2-15.

DESIGNED MULTIPLE LIGANDS - NOVEL ANTITHROMBOTIC COMPOUNDS WITH DUAL ACTIVITY TARGETING GPIIb/IIIa RECEPTOR AND THROMBIN

Janez Ilaš

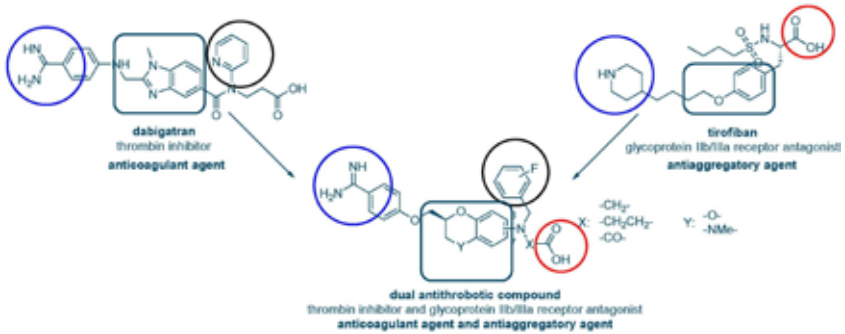
University of Ljubljana, Department of Medicinal Chemistry, Askerceva cesta 7 1000 Ljubljana, Slovenia

The development of effective and patient friendly antithrombotic agents, new anticoagulants as well antiplatelet drugs remains a permanent challenge to medicinal chemists. The rational design of compounds with designed multiple mode of action towards multiple targets is a widely used approach in drug design. In the field of antithrombotic drugs several multiple ligands were published, however, they were mainly acting on the targets (eg. fXa and thrombin), which had strong structural resemblance.

We developed for the first time compounds possessing thrombin inhibitory activity and fibrinogen receptor antagonism as novel antithrombotic drugs, combining enzyme and receptor as molecular targets. Thrombin inhibitors (e.g. *D*-Phe-Pro-Arg or *dabigatran*) possess basic centre, central scaffold and aromatic moiety, while GPIIb/IIIa receptor antagonists (e.g. Arg-Gly-Asp tripeptide (RGD) or *tirofiban*) possess basic centre, linker and acidic moiety.

Designed multiple ligands, novel antithrombotic compounds with dual activity targeting GPIIb/IIIa receptor and thrombin and possessing anticoagulant and antiaggregatory activity in the same molecule combine basic centre, central scaffold, aromatic moiety and acidic moiety. Benzamidine moiety was used for the P1 part of the molecule; various heterocycles were used as central scaffold/linkers, aromatic P3 moiety was optimized using various fluorine substituents on aromatic ring, and P4 carboxyl group moiety was optimized using optimal substitution on heterocyclic ring and the length of the alkyl chain.

Animal studies were performed to demonstrate *in vivo* activity. Thus we are presenting compounds having nanomolar thrombin inhibitory activity as well nanomolar fibrinogen receptor antagonistic activity as novel antithrombotic compounds and potential drug candidates.



References

1) Ilaš, J.; Jakopin, Ž.; Borštnar, T.; Stegnar, M.; Kikelj, D. 3,4-Dihydro-2H-1,4-benzoxazine derivatives combining thrombin inhibitory and glycoprotein IIb/IIIa receptor antagonistic activity as a novel class of antithrombotic compounds with dual function. J. med. chem., 2008, 18, 5617-5629.
2) Ilić, M.; Kikelj, D.; Ilaš, J. Fluorinated dual antithrombotic compounds based on 1,4-benzoxazine scaffold. Eur. j. med. chem. 2012, 50, 255-263.
3) Trstenjak U, Ilaš J, Kikelj D. Low molecular weight dual inhibitors of factor Xa and fibrinogen binding to GPIIb/IIIa with highly overlapped pharmacophores. Eur J Med Chem, 2013, 64, 302-13.
4) Trstenjak U, Ilaš J, Kikelj D. Transformation of selective factor Xa inhibitor rivaroxaban to a dual factor Xa/thrombin inhibitor by modification of the morpholin-3-one moiety. MedChemComm, 2014, 5, 197-202.

IDENTIFICATION OF SMALL MOLECULES THAT INDUCE SELECTIVE DIFFERENTIATION OF CANCER STEM-LIKE CELLS

Laia Josa Cullere (1), Thomas Cogswell (1), Laura Mola Sola (1), Thomas Jackson (2), Thomas Milne (2), Paresh Vyas (2), Graham Wynne (1), Angela Russell (1), Steve Davies (1)

1) Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK
2) Weatherall Institute of Molecular Medicine, Molecular Haematology Unit, University of Oxford, Oxford OX3 9DS, UK

The ability to harness adult stem cells for the treatment of human disease could revolutionise the field of medical therapeutics. They are remarkable cells characterised by their capacity to divide and to differentiate into cell types constituting adult tissue in the body. Moreover, many examples are now described where these cells contribute to tissue repair in the event of injury. Such cells thus hold enormous promise both for use as in vitro screening tools for drug efficacy and toxicity testing, but especially for their application in regenerative therapies treating a wide range of disorders with high unmet medical need such as neurodegenerative diseases, diabetes, heart disease, and vision loss.[1,2]

Currently, most regenerative medicine therapies are based on manipulation of stem cells in vitro followed by transplantation into the patient. Our approach is to stimulate the adult stem and precursor cells with small molecules in situ, taking advantage of the endogenous repair mechanisms that already exist within the body. This would have several advantages, such as avoiding manufacturing of cells in vitro. We are using phenotypic high throughput screens based on cultures of tissue-specific cells to identify and optimise new classes of compounds with novel mechanisms of action.

In the first instance, we are applying this unique approach to a number of debilitating conditions with significant unmet medicinal need across several therapeutic areas. OxStem Neuro are identifying new classes of drug that stimulate de novo neuron production from neural stem cells that can compensate for disease pathology in neurodegenerative diseases and restore cognitive function; OxStem Cardio aims to stimulate resident cardiac precursor cells using small molecules to increase cardiac muscle regeneration and improve functional recovery following myocardial infarction (MI); OxStem Ocular is working on the stimulation of appropriate precursor cells within the retina of patients with a range of retinopathies to activate retinal repair to restore vision. This poster will highlight the cutting-edge approach of our work in this field, displaying an overview of each of the four areas, with specific focus on the oncology project.

In Oncology, we are targeting the manipulation of 'cancer stem-like cells' (CSLCs) for the development of novel cancer therapeutics. CSLCs are tumorigenic cells that have the ability to self-renew and differentiate to grow and replenish the bulk tumour.[3] The resistance of CSLCs to cytotoxic chemotherapy regimens, characterised in a range of cancer types, is a key reason for the high rates of relapse and remission seen in numerous cancers. This is very evident in Acute Myeloid Leukemia (AML), a cancer of the haematopoietic system, resulting in a long-term survival rate of only 20-30%. Our aim is to use a small molecule approach to induce differentiation of CSLCs to more benign states to improve clinical outcomes and prevent resistance/relapse. With this goal in mind, we have developed a robust in vitro screening assay which has been used to identify a number of validated hit compounds that show differentiation of AML cells in several subtypes. A lead generation campaign is currently underway as well as in-depth RNA sequencing experiments to shed light on the target pathways in this process.[3]

References

1) Regenerative Medicinal Chemistry: The in Situ Control of Stem Cells, Angela. J. Russell, ACS Med. Chem. Lett. 2013, 4, 365−368.
2) Stemistry: The Control of Stem Cells in Situ Using Chemistry, Stephen G. Davies, Peter D. Kennewell, Angela J. Russell, Peter T. Seden, Robert Westwood, and Graham M. Wynne, J. Med. Chem. 2015, 58 (7), 2863-2894.
3) Stem cells, cancer, and cancer stem cells, Tannishtha Reya, Sean J. Morrison, Michael F. Clarke and Irving L. Weissman, Nature 2001, 414, 105-111.

LEAD OPTIMIZATION OF ISOXAZOLE DERIVATIVES TARGETING GATA4-NKX2-5 PROTEIN-PROTEIN INTERACTION RELEVANT FOR CARDIAC REMODELLING

Mikael Jumppanen (1), Sini Kinnunen (2), Mika Välimäki (2), Samuli Auno (1), Gustav Boije af Gennäs (1), Henri Xhaard (1), Virpi Talman (2), Heikki Ruskoaho (2), Jari Yli-Kauhaluoma (1)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland
2) Drug Research Program, Division of Pharmacology and Pharmacotheraphy, Faculty of Pharmacy, University of Helsinki, Finland

Ischemic heart disease leads to irreversible cell loss and is characterized by unmet medical need. Cardiac transcription factors, such as GATA4 and NKX2-5, regulate both physiological and pathophysiological processes in the heart. For example, a physical interaction of these two TFs leads to stretch-induced cardiomyocyte hypertrophy.[1] In our previous studies we have demonstrated that inhibition of this protein-protein interaction (PPI) with a small molecule compound inhibits cardiomyocyte hypertrophy in vitro and improves cardiac function in vivo in experimental models of myocardial infarction and hypertension.[2,3]

In this study, we continued optimization of the original isoxazole hit compound by modifying its northern, central and southern parts. The new compounds were tested in the luciferase assay to examine the inhibition of the transcriptional synergy of the GATA4 and NKX2-5. Additionally, the most potent compounds were tested in luciferase assays for NKX2-5 and GATA4 separately. The generated three-dimensional activity data was analyzed by using hierarchical clustering to identify compounds capable of inhibiting PPI but not interfering with GATA4 or NKX2-5 DNA binding. Furthermore, toxicity of the compounds was studied with MTT and LDH assays in the COS-1 cell line.

In summary, we have synthesized and identified a group of non-toxic compounds, which inhibit transcriptional synergy of GATA4 and NKX2-5 without interfering with GATA4 transcriptional activity.

References

1) Pikkarainen, S.; Tokola, H.; Majalahti-Palviainen, T.; Kerkelä, R.; Hautala, N.; Bhalla, S. S.; Charron, F.; Nemer, M.; Vuolteenaho, O.; Ruskoaho, H. GATA-4 Is a Nuclear Mediator of Mechanical Stretch-Activated Hypertrophic Program. J. Biol. Chem. 2003, 278 (26), 23807–23816.
2) Kinnunen, S. M.; Tölli, M.; Välimäki, M. J.; Gao, E.; Szabo, Z.; Rysä, J.; Ferreira, M. P. A.; Ohukainen, P.; Serpi, R.; Correia, A.; Mäkilä, E.; Salonen, J.; Hirvonen, J.; Santos, H. A.; Ruskoaho, H. Cardiac Actions of a Small Molecule Inhibitor Targeting GATA4–NKX2-5 Interaction. Sci. Rep. 2018, 8 (1), 4611.
3) Välimäki, M. J.; Tölli, M. A.; Kinnunen, S. M.; Aro, J.; Serpi, R.; Pohjolainen, L.; Talman, V.; Poso, A.; Ruskoaho, H. J. Discovery of Small Molecules Targeting the Synergy of Cardiac Transcription Factors GATA4 and NKX2-5. J. Med. Chem. 2017, 60 (18), 7781–7798.

ARYLIDENE-SUBSTITUTED IMIDAZOTHIAZINONES: POTENT AND SELECTIVE ANTAGONISTS OF THE ORPHAN G PROTEIN-COUPLED RECEPTOR GPR18

Maria Kaleta (1), Clara Schoeder (2), Victor Rempel (2), Andhika Mahardhika (2), Gniewomir Latacz (1), Agnieszka Olejarz-Maciej (1), Małgorzata Frankowska (3), Magdalena Zaniewska (3), Karolina Wydra (3), Beata Bystrowska (4), Ewelina Honkisz-Orzechowska (1), Marek Grosicki (1), Kamil Kuder (1), Tadeusz Karcz (1), Małgorzata Filip (3), Christa Müller (2), Katarzyna Kieć-Kononowicz (1)

1) Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Faculty of Pharmacy, 9 Medyczna Str., 30-688 Kraków, Poland
2) Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Faculty of Pharmacy, 9 Medyczna Str., 30-688 Kraków, Poland
3) Department of Drug Addiction Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12 Str., 31-343 Krakow, Poland
4) Department of Toxicology, Jagiellonian University Medical College, Faculty of Pharmacy, 9 Medyczna Str., 30-688 Kraków, Poland

G-protein coupled receptors (GPCRs) are the most common targets for currently available therapeutics. GPCRs without an identified endogenous ligand are considered “orphan receptors” and may represent novel therapeutic targets. GPR18 is an example of such an orphan receptor. On the basis of mRNA transcripts, there is evidence of GPR18 expression in immune cells and tissues, gastrointestinal and testicular tissues, human sperm, cancer cells, in particular cells associated with the immune system as well in various brain tissues (mainly hypothalamus, cerebellum, brain stem) and microglial cells [1,2]. Since the cannabinoid agonist Δ^9-THC is an agonist at GPR18, it was suggested that GPR18 could be considered a third cannabinoid (apart from CB_1 and CB_2) receptor subtype. Cannabinoid receptors, a part of the endocannabinoid system, are involved in a variety of physiological and pathophysiological processes such as appetite regulation, and energy homeostasis, cognitive and mental functions, drugs addiction, effects on pain transmission and inflammation. GPR18 with yet unknown biological function may be considered as a potential novel drug target. In our studies bicyclic imidazole-4-one derivatives were discovered as the first synthetic scaffolds active that block GPR18 receptor function as detected in b-arrestin assays. [3]. Structure-activity relationships were analyzed leading to the development of PSB-CB-5 ((*Z*)-2-(3-(4-chlorobenzyloxy)benzylidene)-6,7-dihydro-2*H*-imidazo[2,1-*b*][1,3]thiazin-3(5*H*)-one)and PSB-CB-27, ((*Z*)-2-(3-(6-(4-chlorophenoxy)hexyloxy)benzy-lidene)-6,7-dihydro-2*H*-imidazo[2,1-*b*][1,3]thiazin-3(5*H*)-one) showing the best potency and selectivity profile (IC_{50}=0.279 μM and 0.650 μM, respectively). Their selectivity against GPR55 (another orphan receptor that interacts with cannabinoids) and cannabinoid CB_1 and CB_2 receptors was confirmed. In *in vitro* assays these compounds displayed antiproliferative activity in several cancer cells (Hec-1B; HuT102, M10, BLM, SH-SY5Y and HT-1080) at 10 μM concentration. The compounds display drug-like properties (e.g. interaction with CYP3A4, no mutagenicity Ames test, metabolic stability). Pharmacokinetic studies of PSB-CB-5 indicatedits penetration into brain tissue. *In vivo* tests confirmed their effect on food intake (PSB-CB-5) and antinociceptive properties (hot plate test) (PSB-CB-27).

Acknowledgements: Financial support by Polish National Science Center DEC. 2013/11/8/NZ7/04865, Jagiellonian University statutory funds K/ZDS/007121 and the statutory funds of the Institute of Pharmacology is greatly acknowledged. C.T.S. was supported by a Bayer fellowship, A.O-M was supported by STSM of MuTaLig COST Action CA15135. C.E.M. and C.T.S. are grateful for support by the DFG (GRK1873) and the BMBF (BIGS DrugS).

References

1) D. McHugh et al., Br. J. Pharmacol., 2012, 167, 1575-1582
2) C. Flegel, Sci. Rep., 2016, 6, 32255
3) V. Rempel et al., MedChemComm., 2014, 5, 632-649

PROBISDOCK: PROTEOME-SCALE DOCKING USING EXISTING KNOWLEDGE FROM THE PROTEIN DATA BANK

Janez Konc (1,2), Samo Lesnik (1), Matej Sova (3), Matic Proj (3), Stanislav Gobec (3), Dusanka Janezic (2)

1) National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
2) Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška 8, 6000, Koper, Slovenia
3) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

The co-crystallized ligands in Protein Data Bank (PDB) represent a great quantity of information about protein binding sites. There are more than 100,000 ligands all-together and more than 20,000 unique ligands in the PDB in 2017; however, this information is not used explicitly in the existing docking algorithms. Docking has had persistent problems in predicting binding free energies and related to that scoring and ranking of docked poses is still an open problem.

We developed ProBiSdock, a docking algorithm that scores the docked poses using unique new scoring function in which the pose’s score depends on its overlap with the existing ligands' atom field. This force field is generated for each query protein specifically from existing co-crystallized ligands in other protein structures in the PDB transposed to the query protein using ProBiS binding sites alignment algorithm. To account for conformational changes in protein upon ligand binding, both compounds and proteins are treated as flexible. ProBiSdock enables fast docking of large databases containing millions of compounds and has been successfully validated on the DUD-E benchmark and on cross-docking examples where the treatment of protein flexibility is required. It was already used to perform proteome-scale docking as well as to discover new experimentally confirmed inhibitors of IDO-1 enzyme, an attractive target in cancer therapy. ProBiSdock enables researchers to quickly search for new active compounds or, inversely, for new target proteins of existing drugs taking into account knowledge in the PDB and has been successfully validated *in silico* and *in vitro*.

P322

DESIGN AND SYNTHESIS OF A LIBRARY OF CHROMOGENIC CHEMICAL ENTITIES FOR THE DISCOVERY OF NEW THERMOSTABLE ENZYMES RELEVANT FOR THE CHEMICAL INDUSTRY

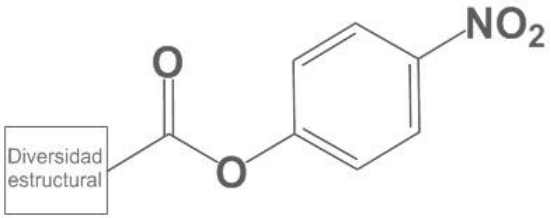
Begoña Pampín Casal (1), Jacobo Cruces Colado (1), Julie Masse (1), María Luisa Rúa Rodríguez (2), María Isabel González Siso (3)

1) Galchimia, S.A. R&D Department, Cebreiro s/n 15823 O Pino, A Coruña, Spain
2) Grupo de Bioquímica, Departamento de Química Analítica y Alimentaria, Universidad de Vigo, As Lagoas, 32004 Ourense, Spain
3) Grupo EXPRELA, Universidade da Coruña, Rúa da Fraga 10, Campus da Zapateira, 15071, A Coruña, Spain

Thermostable enzymes are in widespread use for a broad range of applications. The global market for industrial enzymes has surpassed the USD 7.1 billion. Most than 75% of the enzymes used commercially are coming from the hydrolase family. Apart from its use in food, detergents, starch and others, hydrolases are also intensely used in organic synthesis, such as:

- Acylases for the production of 6-aminopenicillinanic acid, use for semisynthetic penicillins
- Lipases for the resolution of enantiopure carboxylic acids,
- Nitrilases for the synthesis of chiral amionoacids

Extremophilic colonies are rich sources of new biocatalysts for the industry. One of the main objective of the Marie Curie HOTDROPS project was to look for new thermostable esterases and lipases. Once they were isolated, a specific library of compounds was designed and synthesized in order to screen and characterize their activity. Therefore a serie of compounds were prepared based on the following structure:



P323

MECHANISMS OF BIASED SIGNALLING IN HUMAN MU OPIOID RECEPTOR.

Damian Bartuzi, Magdalena Litka, Karina Liszka, Agnieszka A. Kaczor, Dariusz Matosiuk

Department of Synthesis and Chemical Technology of Medicinal Substances with Computer Modelling Lab, Medical University of Lublin, Chodzki 4A Str., PL20093 Lublin, Poland

In human, G protein-coupled receptors (GPCRs) constitute the largest family of receptors and one of the largest families of proteins in general. They are responsible for a vast part of signal transmission within organism, as well as for sensing external stimuli such as light or odour. Their function was once thought to be plain and straightforward, being considered as simple one-function relays, recognizing particular stimulus and responding to it with one particular intracellular signal. Today it is known, however, that nature designed GPCR structures for complex signal processing, with one single receptor molecule capable of inducing different signalling cascades in response to different ligands – particular ligands can 'bias' signalling toward particular effector. Understanding this complexity is crucial for design of modern drugs selective toward selected signalling pathway rather than receptor only, which is believed to bring less side effects. Engineering such drugs is problematic for many reasons, including probe dependence (modulators affect different transmitters in a very different way) and species selectivity (drug candidate performing well in rodent models may behave differently in humans).

Opioid receptors are among the most intensively investigated GPCRs in allosteric and/or biased drug design. Such drugs could greatly improve current therapies, cursed with dangerous side effects. In recent years, some prototypical biased compounds were reported, e.g. TRV-130, PZM21 or SHR9352. To understand their mechanisms and allow for rational design of further analogues, we created a native-like in silico environment, including μ opioid receptor in complex with G protein, immersed in a raft-like asymmetric membrane. The system was then set in motion with molecular dynamics simulations. Number of simulations with different G protein-biased or β-arrestin-biased derivatives bound to the receptor were performed. Subsequently, Principal Component Analysis was then used to sift relevant information on the underlying principles of functional selectivity. Importantly, our simulations were performed on the human receptor model in a native-like environment, so the results may eventually help to overcome difficulties related to differences in properties of drug candidates in mouse models and humans.

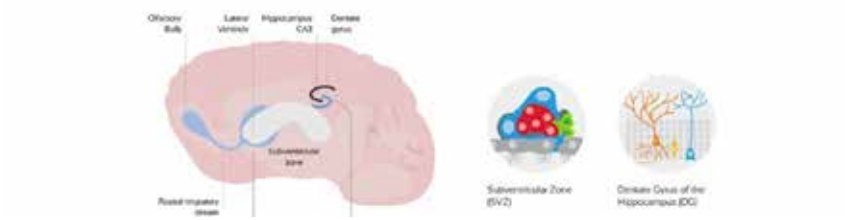
DISCOVERY OF PRONEUROGENIC DRUG CANDIDATES: A NEW THERAPEUTIC STRATEGY FOR NEURODEGENERATIVE DISORDERS

Laura Mola Sola (1), Maria Blanca Torroba (2), Liam Bromhead (1), Tom Carter (1), Julie Davies (2), Penelope Fouka (2), Sebastien Galan (1), Laia Josa Cullere (1), Kate Madden (1), Ksenia Musaelyan (2), Erin Shepherd (1), Graham Wynne (1), Steve Davies (1), Francis Szele (2), Angela Russell (1,3)

1) Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK
2) Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, OX1 3PT, UK
3) Department of Pharmacology, University of Oxford, Mansfield Road, Oxford, OX1 3QT, UK

Neurodegenerative diseases exert a vast physical, emotional and economic cost on patients and society, and with an aging population their prevalence is rapidly increasing. There are currently estimated to be 47m people living with dementia globally, costing over $800b each year, but this is predicted to rise nearly three-fold by 2050.[1]The only treatments currently available for these conditions are symptomatic, with none targeting underlying causes; thus there is an enormous unmet medical need.

We aim to activate neuroregeneration by targeting the endogenous neural stem cells (NSCs) already present within the adult brain and stimulating natural repair mechanisms. This could be utilised as a novel treatment for a range of conditions such as Alzheimer's disease, Parkinson's disease, and traumatic brain injury. NSCs are found within two main neurogenic niches; the subgranular zone (SGZ) of the hippocampal dentate gyrus, and the subventricular zone (SVZ) of the lateral ventricles. [2–4] These cells are known to become activated upon injury, and their progeny to then migrate toward the damaged area, but only to a very limited extent. Enhancement of this process has been observed during treatment with a range of drugs, molecules and genetic manipulations this provides precedent that our approach is feasible.[5]



We have developed a semi-automated in vitro phenotypic assay, using a monolayer of primary murine NSCs (isolated from SGZ or SVZ) and measuring the appearance of mature neurons. We have used this assay to perform a pilot screen of 1500 compounds, from which we identified 30 compounds which induced a significant increases in neurogenesis. The use of a phenotypic assay gives us the opportunity to utilise a hypothesis-free and target agnostic approach, whilst also allowing a more direct translation of results into in vivo studies. Following preliminary pharmacokinetic evaluation, early in vivo efficacy work was conducted, wherein one lead compound was found to give a significant enhancement in SGZ neurogenesis after oral administration to wild-type mice. As a result, this compound has now been progressed to Alzheimer's disease models. Work is ongoing to optimise the ADME / PK and efficacy properties of this and other series, and in parallel to identify and study their mechanism(s) of action.

References

1) Prince, M et al (2015). World Alzheimer's Report 2015, The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends. Alzheimer's Disease International
2) Sanai, N.; Nguyen, T.; Ihrie, R. A.; Mirzadeh, Z.; Tsai, H.-H.; Wong, M.; Gupta, N.; Berger, M. S.; Huang, E.; Garcia-Verdugo, J.-M. M.; Rowitch, D. H.; Alvarez-Buylla, A. Nature 2011, 478 (7369), 382.
3) Ernst, A.; Alkass, K.; Bernard, S.; Salehpour, M.; Perl, S.; Tisdale, J.; Possnert, G.; Druid, H.; Frisen, J. Cell 2014, 156 (5), 1072
4) Bonaguidi, M. A.; Wheeler, M. A.; Shapiro, J. S.; Stadel, R. P.; Sun, G. J.; Ming, G. L.; Song, H. Cell 2011, 145 (7), 1142.
5) Malberg J. E.; Blendy J. A., Trends Pharmacol. Sci. 2005, 26, 631

SALICYLIC ACID DERIVATIVES: A NOVEL AND PROMISING PHARMACOLOGICAL APPROACH FOR THE TREATMENT OF PRIMARY HYPEROXALURIA TYPE 1

María Dolores Moya-Garzón (1), Cristina Martín-Higueras (2), Miguel X. Fernandes (2), Francisco Franco-Montalbán (1), José A. Gómez-Vidal (1), Eduardo Salido-Ruiz (2), Mónica Díaz-Gavilán (1)

1) Medicinal and Organic Chemistry Department, School of Pharmacy, University of Granada, Granada, Spain.
2) Pathology Department, School of Medicine, University of La Laguna, Tenerife, Spain.

Calcium oxalate accumulation provokes generalized life-threatening tissue damage in primary hyperoxaluria type 1 (PH1) patients. This genetic rare disease is caused by the diminished function of the enzyme alanine-glyoxylate aminotransferase (AGT), which is in charge of glyoxylate detoxification.[1] These conditions promote glyoxylate accumulation and its oxidation to oxalate by other enzymes, like glycolate oxidase (GO).[2]

Substrate reduction therapy (SRT) in PH1 is currently being studied as a strategy for preventing glyoxylate accumulation by inhibiting those enzymes involved in its formation.[3,4] In this sense, recent studies point at glycolate oxidase (GO) as one of those promising targets for SRT in PH1.[2]

We have recently found that furylsalicylates are moderate GO-inhibitors and efficient agents reducing oxalate output on hyperoxaluric mouse hepatocytes culture, which represents a novel aspect to be added to the biological profile of salicylic acids.[5] Our hit compound, with an EC_{50} at the low micromolar range for oxalate decrease, presents a polar salicylate head which is directly attached to a moiety consisting on a furan ring. So as to identify the ideal distance between the polar functionalities and the furan ring, structural analogs have been designed, synthesized and tested on *m*GO and hyperoxaluric mouse hepatocytes. Both flexible and rigid nitrogen-based linkers have been introduced in order to space the two different moieties of our molecules and to increase water solubility, what constitutes an important feature of useful drug candidates. In addition, docking studies have been carried out in order to gain advantageous information about the suitable orientation of the binding groups.

References

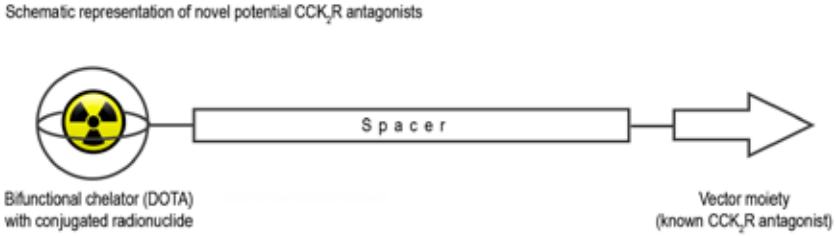
1) Zhang, X.; Roe, S.M.; Hou, Y.; Bartlam, M.; Rao, Z.; Pearl, L.H.; Danpure, C.J. Crystal Structure of Alanine:Glyoxylate Aminotransferase and the Relationship Between Genotype and Enzymatic Phenotype in Primary Hyperoxaluria type 1. J. Mol. Biol. 2003, 331 (3), 643-652.
2) Martin-Higueras, C.; Luis-Lima, S.; Salido, E. Glycolate Oxidase Is a Safe and Efficient Target for Substrate Reduction Therapy in a Mouse Model of Primary Hyperoxaluria Type I. Mol. Ther. 2016, 24 (4), 719-725.
3) Martin-Higueras, C.; Torres, A.; Salido, E. Molecular Therapy of Primary Hyperoxaluria. J. Inherit. Metab. Dis. 2017, 40 (4), 481-489.
4) Cellini, B. Treatment Options in Primary Hyperoxaluria Type I. Expert Opin. Orphan D. 2017, 5 (4), 309–319.
5) Díaz-Gavilán, M.; Gómez-Vidal, J.A.; Moya-Garzón, M.D.; Salido-Ruiz, E.; Martín-Higueras, Cristina; Fernandes, M. X. Compuestos para el tratamiento de enfermedades causadas por la acumulación de oxalato. Patent number: P201730326. Date of application: May 2017.

RATIONAL DESIGN AND SYNTHESIS OF NOVEL POTENTIAL CCK2R ANTAGONISTS

Doroteja Novak (1), Marko Anderluh (2), Tihomir Tomašič (2), Marko Krošelj (1), Petra Kolenc Peitl (1)

1) Department of Nuclear Medicine, University Medical Centre Ljubljana, Zaloška 7, SI-1000 Ljubljana, Slovenia
2) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

Aim. The CCK_2/gastrin receptor (CCK_2R) is expressed in high densities in several tumour types. These tumours can thus be visualized with nuclear medicine imaging techniques, such as PET and SPECT, by using radiolabelled CCK_2/gastrin receptor binding ligands. Currently, CCK_2R binding radioligands are either minigastrin or cholecystokinin (CCK) analogues with agonistic properties, thus exhibiting pentagastrine test-like adverse effects even in quantities below those required for targeted peptide receptor radionuclide therapy (PRRT). On the other side, it was recently shown in the SSTR/somatostatin system that internalization is not prerequisite for high tumour uptake and retention. Switching from agonists to antagonists led to higher tumour uptake due to higher number of binding sites[1]. The aim of the present study was to design and synthesize novel CCK_2R antagonists suitable for further radiolabelling with different radiometals for SPECT, PET and PRRT.



Methods. First step of the development of the novel CCK_2R antagonists was *in silico* structure-based drug design. The homology modelling approach was used and the crystal structure of β_2 adrenergic receptor (PDB code: 2RH1) served as the template. The best model with the lowest DOPE score and PDF energy score was selected for further optimization, and already known CCK_2R antagonists were docked into the modelled CCK_2R on the basis of previous mutagenesis data enabling refinement of the model. Docking of energy minimized structures of the molecules, comprised of known antagonist Z-360 and different linkers, was performed using GOLD 5.5 (The Cambridge Crystallographic Data Centre). Best scored potential DOTA-conjugated antagonists were synthesized using standard Fmoc based solid phase peptide synthesis. The products were purified using semi preparative high-performance liquid chromatography (HPLC) and evaluated with HRMS.

Results. Based on the values of GoldScore scoring function and visual inspection of docked structures, we have determined the minimal number of amino acids that are necessary for the unhindered binding of DOTA-conjugated molecules and linker to exit the binding gap, and selected the potential DOTA-conjugated antagonists that showed additional interactions in the spacer region for synthesis. All synthesized conjugates showed purities over 95% as confirmed by reversed-phase HPLC. The characterization was performed by electrospray ionization mass spectrometry (ESI-MS), high resolution mass spectrometry (HRMS), and RP-HPLC.

Conclusion. Several novel potential CCK2R antagonists were synthesized and will be radiolabelled and further evaluated for their binding and agonistic/antagonistic properties.

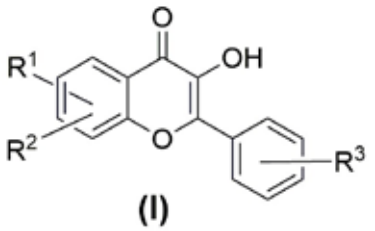
References

1) Ginj, M. et al. Radiolabeled somatostatin receptor antagonists are preferable to agonists for in vivo peptide receptor targeting of tumors. Proc. Natl. Acad. Sci. U. S. A. 103, 16436–41 (2006).

3-FLAVONOLS AS NOVEL QUORUM SENSING INHIBITORS

Jayendra Patel (1), Tina Seifert (2), Malena Skogman (3), Kirsi Savijoki (3), Fallarero Fallarero (3), Kristina Luthman (2), Jari Yli-Kauhaluoma (1)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, PL 56, Viikinkaari 5, FI-00014 University of Helsinki, Finland
2) Department of Chemistry and Molecular Biology, Medicinal Chemistry, University of Gothenburg, SE-412 96, Gothenburg, Sweden
3) Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, PL 56, Viikinkaari 5, FI-00014 University of Helsinki, Finland



(I)

R^1 & R^2 = H, Cl, Br, OBn, OH, OMe, etc.
R^3 = H, Cl, Br, OBn, OH, OMe, etc.

Bacterial biofilms are a major obstacle in the treatment of severe infections, especially cystic fibrosis and urinary tract infections.[1] The low membrane permeability of antibiotics through bacterial biofilm results in enhanced resistance and ineffective treatment.[2] Since bacterial cell-to-cell communication network, termed as quorum sensing (QS), plays a major role in the biofilm formation as well as pathogenicity and virulence, targeting QS will be more beneficial to tackle these bacteria.[3,4] The goal of this Jane and Aatos Erkkos foundation-supported project is to develop novel QS inhibitors (QSIs). Earlier, our colleagues have reported identification of 3-flavonols as low micromolar quorum-sensing inhibitors (QSIs).[5] Here in, we present our recent findings related to key structural features of 3-flavonols (Fig. 1, general structure **I**) required to maintain anti-quorum sensing activity. Our work show potential of 3-flavonols as potential QSIs.

References

1) Finnan S., Morrissey J. P., Gara F. O., Boyd F., Boyd E. F., O'Gara F., Genome diversity of Pseudomonas aeruginosa isolates from cystic fibrosis patients and the hospital environment. J. Clin. Microbiol. 2004, 42, 5783-5792.
2) Olson M. E., Ceri H., Morck D. W., Buret A. G., Read R. R., Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can. J. Vet. Res., 2002, 66, 86–92.
3) Miller M. B, Bassler B. L. Quorum sensing in bacteria. Annu. Rev. Microbiol. 2001, 55, 165–99.
4) Suga, H.; Smith, K. M. Molecular mechanisms of bacterial quorum sensing as a new drug target. Curr. Opin. Chem. Biol. 2003, 7, 586–591.
5) Skogman, M. E., Kanerva S., Manner S., Vuorela, P. M., Fallarero, A., Flavones as Quorum Sensing Inhibitors Identified by a Newly Optimized Screening Platform Using Chromobacterium violaceum as Reporter Bacteria. Molecules 2016, 21, 1211.

PHARMACOPHORE-BASED VIRTUAL SCREENING TOWARD THE DISCOVERY OF EZH2 INHIBITORS

Filipa Ramilo-Gomes (1,2), Riccardo Martini (3), Sharon D. Bryant (3), Luís Sobral (4), Matilde Marques M. (1), Rita C. Guedes (2)

1) Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
2) iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
3) Inte:Ligand Software Entwicklungs und Consulting, Mariahilferstrasse 74B, 1070 Vienna, Austria
4) Hovione, 2674- 506 Loures, Portugal

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder worldwide, affecting approximately 1.5% of the population above 60 years old and 4% of the population at the age of 80 [1].

Although PD is primarily a sporadic disorder of unclear aetiology, it is now clear that genetic factors contribute to the pathogenesis of the disease. For example, mutations in the *parkin* gene, which encodes Parkin protein, are a relatively frequent cause of autosomal recessive early-onset forms of PD [1].

Parkin is a ring-in-between-ring (RBR) E3 ubiquitin ligase, composed by six distinct domains. The catalytic module of PARKIN has a multidomain architecture consisting of RING1, IBR and RING2 domains (the latter harbouring the catalytic cysteine), and is responsible for the ubiquitination and consecutive proteasome degradation of a number of protein substrates [2,3].

The ubiquitination-proteasome system is fundamental to several cellular events and its malfunction induces impairment in mitophagy and accumulation of dysfunctional mitochondria, indicating that loss-of-function of Parkin protein may be a key to the neurodegeneration process and to the pathogenesis of PD. Therefore, restoring Parkin function using rationally designed peptides and small molecules has been emerging as a potential therapy for Parkin-linked PD.

However, medicinal chemistry approaches to regulate this pathway have always been hindered by the lack of suitable robust methodologies for screening endeavours [2,3].

To address this challenge, a series of activity-based probes for profiling Parkin activity is being developed. Concurrently, a yeast-based phenotypic assay [4] is being implemented and the biological activity of selected probes evaluated.

These novel chemical tools hold promise as innovative biomarkers for Parkin activation, providing the bases for Parkin high-throughput screening campaigns.

Acknowledgments

We thank the Fundação para a Ciência e Tecnologia for financial support of the iMed.ULisboa UID/DTP/04138/2013, UCIBIO/REQUIMTE UID/MULTI/04378/2013 and Sofia Domingos PhD grant PD/BD/114281/2016 from FCT PhD Programme in Medicines and Pharmaceutical Innovation (i3DU).

References

1) Laugesen, A., Hojfeldt, J. W., Helin, K. Cold Spring Harb. Perspect. Med. 2016, 6.
2) Brooun, A., Gajiwala, K. S., Deng, Y.-L., et al. Nature Communications. 2016, 7, 11384.
3) LigandScout Molecular Design Software from InteLigand GmbH (http://www.inteligand.com).

A DNA-ENCODED LIBRARY TECHNOLOGY BASED PLATFORM FOR HIT DISCOVERY, OPTIMIZATION AND ANALYSIS ACROSS DIVERSE TARGET FAMILIES

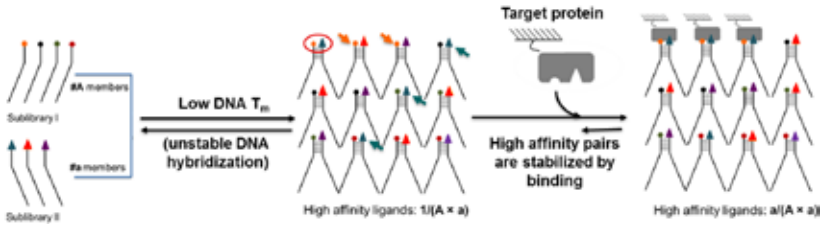
F.V. Reddavide (1), S. Heiden (1), L. Krämer (2), M. Cui (2), W. Lin (2), Z. Zhang (2), M. Thompson (1)

1) DyNAbind GmbH, Dresden, Germany
2) Technische Universität Dresden, Dresden, Germany

DNA-Encoded Library (DEL) technology is an elegant method for rapidly probing a large chemical space for binding moieties to a target protein in a single experiment. The DEL consists of a collection of small organic molecules, each conjugated to a unique oligonucleotide strand which functions as an identifying barcode. A simple workflow consisting of incubating the immobilized target with the library, washing off the non-binding members and then eluting and PCR amplifying the DNA tags for sequencing and deconvolution allows the simultaneous interrogation of massive (>10^9) amounts of substances. Compared to a traditional high-throughput screening approach, a DEL campaign can operate at about ten times the speed, with one-tenth the running costs.

Though this technology has been promising in both academic and industrial use over the last decade, a number of inherent flaws have held back the data quality, reliability and maximum speed of the method. For example, the common split-and-pool methods applied for library synthesis prevent any sort of meaningful quality control, often resulting in mixtures containing truncates and side products, where a single DNA tag can encode multiple compounds. Likewise, differing synthetic yields can generate misleading artifacts which appear to suggest structure-activity relationship data where there is none. The process is slowed down by the hit validation phase, where hundreds of hit compounds are typically resynthesized off-DNA for individual kinetic analysis, a process which can take many months and incur significant expenses.

Here we present a new DEL platform of technologies designed to significantly increase the data quality, reliability and speed of the method while also preserving access to a large chemical space. The first component, the Dynamic Library architecture, consists of two DNA sublibraries, which unstably interact and reshuffle, to randomly present two chemical structures to the target, until stabilized by binding.[1] In this way, diversity is preserved while permitting meaningful quality control, with additional benefits to the signal-to-noise ratio from the dynamic generation of stronger binding pairs. The schematic of the technology principle is shown in the figure. This technology is supported by a novel algorithmic encoding/decoding method that offers built-in error proofing of the DNA sequencing and a hit validation technology that precludes the necessity for the vast majority of resynthesis, allowing hit full kinetic profiles to come back in days instead of months.[2]



Here we show how the combination of these technologies presents a new, powerful platform for drug discovery and hit optimization. The performance of our system is first benchmarked against more traditional DEL approaches, before applications in drug discovery, including *de novo* hit discovery and affinity maturation are shown against multiple targets. Carbonic anhydrase II (CAII) is used to demonstrate how an affinity maturation approach with our system can improve the performance of known binding moieties. Tumor necrosis factor alpha (TNFa) is used to demonstrate our capacities for finding hits against difficult targets, while Sirtuins 1 and 3 are used to demonstrate how counterscreens can select for specificity within a protein family.

References

1) Reddavide, F. V.et al., Angew Chem Int Ed Engl 2015, 54 (27), 7924-8.
2) Lin, W. et al., Analytical chemistry 2014.

TARGETING BASIC DEFECT IN CYSTIC FIBROSIS: DISCOVERY AND DEVELOPMENT OF NOVEL NANOMOLAR F508-del CFTR CORRECTORS

Alejandra Rodríguez-Gimeno (1), Federico Sorana (1), Francesco Berti (1), Nicoletta Brindani (1), Sine Mandrup Bertozzi (1), Giuliana Ottonello (1), Andrea Armirotti (1), Raffaele Spanò (1), Maria Summa (1), Rosalia Bertorelli (1), Ilaria Penna (1), Natasha Margaroli (1), Debora Russo (1), Emanuela Caci (2), Loretta Ferrera (2), Valeria Tomati (2), Elvira Sondo (2), Emanuela Pesce (2), Paolo Scudieri (3), Nicoletta Pedemonte (2), Luis J. V. Galietta (3), Fabio Bertozzi (1), Tiziano Bandiera (1)

1) D3-PharmaChemistry, Istituto Italiano di Tecnologia (IIT), Genova, Italy
2) U.O.C. Genetica Medica, Istituto Giannina Gaslini (IGG), Genova, Italy
3) Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (Napoli), Italy

Cystic Fibrosis (CF) is a lethal genetic disease caused by mutations in the CF Transmembrane conductance R egulator (CFTR) chloride channel, resulting in reduced anion conductance on epithelial cells of multiple organs. Nearly 2000 mutations of the CFTR gene have been identified [1]; the most frequent is the deletion of phenylalanine at position 508 (F508-del). This mutation causes a severe defect in protein folding and stability, and affects the gating behavior. An effective treatment for F508-del CF patients requires at least two CFTR modulators: a *corrector* to increase CFTR levels at the cell surface, and a *potentiator* to increase the opening frequency of the mutant CFTR channel [2,3]. At the moment only two correctors for the treatment of CF patients bearing the F508del-CFTR mutation have been approved, i.e. *lumacaftor* (*VX-809*) and *tezacaftor* (VX-661), in combination with a potentiator, *ivacaftor* (*VX-770*). The therapeutic benefit of these combinations is however still unsatisfactory. There is, therefore, the need of new, more effective correctors [4].

Following a HTS approach, we screened a collection of about 15,000 maximally diverse commercial small-molecule compounds, in two different cell types (FRT and CFBE41o-) stably expressing F508del-CFTR, using high-throughput functional phenotypic assays based on the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP) [5]. This activity yielded some primary hits, belonging to different chemical classes. One of these chemo-types was investigated extensively. Rounds of chemical modifications of the hit and functional evaluation in different secondary assays provided the information to build the Structure-Activity Relationships (SARs) within the class. Hit-to-Lead and Lead-Optimization campaigns led to compounds with high potency and efficacy in rescuing the activity of F508del-CFTR in bronchial epithelial cells from CF patients homozygous for the F508del mutation, as measured by electrophysiological assays. The best correctors showed potency in the low nanomolar range, retaining very good efficacy in the single-digit nanomolar range. Several compounds showed drug-like properties suitable for further development upon evaluation in *in vitro* DMPK assays. The data generated on the most promising correctors will be presented and discussed.

This work was supported by the Italian Foundation for Cystic Fibrosis (FFC) as part of the "Task Force for Cystic Fibrosis" project.

References

1) Mutations database: http://www.genet.sickkids.on.ca/app
2) Li et al, J. Cyst. Fibros., 2018,17, S14-S21
3) Zegarra-Moran and Galietta, Cell. Mol. Life Sci., 2017, 74, 117-128 ; Rowe and Verkman, Cold Spring Harb. Perspect. Med. 2013; 3:a009761
4) Cholon et al., Expert Rev. Precis. Med. Drug Dev., 2016, 1, 235-243
5) Galietta et al., Am. J. Physiol. Cell Physiol., 2001, 281, C1734–C1742

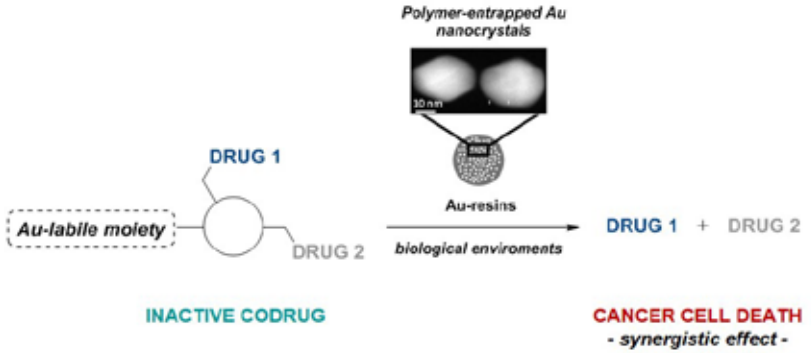
DEVELOPMENT OF PRECURSORS OF COMBINATION THERAPY THAT ARE SPECIFICALLY ACTIVATED BY GOLD-MEDIATED BIOORTHOGONAL CHEMISTRY

Belén Rubio-Ruiz (1), Ana M. Pérez-López (1), Víctor Sebastian (2), Jesús Santamaria (2), Asier Unciti-Broceta (1)

1) Cancer Research UK Edinburgh Centre, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XR, UK
2) Department of Chemical Engineering and Environmental Technology and Institute of Nanoscience of Aragon (INA), University of Zaragoza, 50018 Zaragoza, Spain

In recent years several research groups have tried to exploit the catalytic properties of non-biological transition metals to mediate bioorthogonal organometallic (BOOM) reactions.[1] Despite the challenge that implies to achieve such kind of chemistry in living systems, abiotic metals have been successfully used for different applications in, on and outside cells, e.g. for the synthesis of small molecules (e.g. fluorophores), the functionalization and uncaging of enzymes and *in situ* prodrug activation.[2] Regarding the latter, our group has explored new chemistries and deactivation strategies to develop novel caged chemotherapeutic agents that are specifically released *via* heterogeneous metal catalysis in order to minimize adverse effects associated to chemotherapy.[3,4]

One of the most promising approaches to address cancer heterogeneity is the use of combination therapy, which is based on the simultaneous use of drugs with different mode of actions and synergistic effect. In this communication, we will present the design and development of an unprecedented class of bioorthogonal prodrug able to release two drugs in biological settings *via* biocompatible Au-functionalized resins. This bioorthogonal activation method would offer a safer way to treat locally-advanced cancers through drug combinations and potentially, overcome chemoresistance.



References

1) Unciti-Broceta, A et al. Nat Protocols 2012, 7, 1207-1218.
2) Li J and Chen PR. Nature Chemical Biology 2016, 12, 129-137.
3) (a) Weiss, JT et al. Nat.Commun. 2014, 5, 3277; (b) Weiss, JT et al. J Med Chem. 2014, 57, 5395-5404 (c) Weiss, JT et al. Sci Rep. 2015, 5:9329 (d) Rubio-Ruiz, B et al. J Med Chem. 2016, 59, 9974-9980.
4) Perez-Lopez, A et al. Angew Chem Int Ed Engl. 2017, 56, 12548-12552.

DEVELOPMENT OF INHIBITORS OF THE NUDIX HYDROLASE NUDT22

Tobias Koolmeister (1), Karl S.A. Vallin (1), Evert J Homan (1), Megan Carter (2), Ann-Sofie Jemth (1), Jordi Carreras-Puig (1), Patrick Herr (1), Markel Martınez Carranza (2), Adam Throup (1), Pål Stenmark (2), Thomas Helleday (1)

1) Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, 171 21 Stockholm, Sweden
2) Department of Biochemistry and Biophysics, Stockholm University, 106 91 Stockholm, Sweden

Human NUDT22 belongs to the diverse NUDIX family of proteins, but has, until now, remained uncharacterized. NUDIX family proteins play an important role in the regulation of diverse nucleotide-linked moieties involved in cellular signaling and homeostasis.

Herein, we disclose new roles of NUDT22, the crystal structure of NUDT22 in complex with the substrate UDP-glucose and development of new sub micro-molar inhibitors.

References

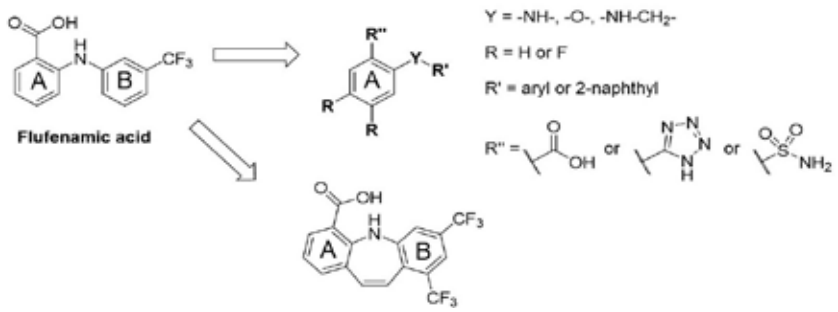
1) Structure 26, 295–303, February 6, 2018

SYNTHESIS OF NEW LIGANDS FOR BITTER TASTE RECEPTOR TAS2R14

Lukas A. W. Waterloo (1), Antonella Di Pizio (2), Maik Behrens (3), Masha Y. Niv (2), Peter Gmeiner (1)

1) Department of Chemistry and Pharmacy, Friedrich-Alexander-University Erlangen-Nürnberg, Germany
2) Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel
3) Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

The perception of bitterness is of particular importance in order to prevent the unintentional uptake of toxic food components that often have bitter taste.[1] Responsible for this are a group of 25 G-protein-coupled receptors (GPCRs), called bitter taste receptors (TAS2Rs).[2] Intriguingly, TAS2Rs are also expressed in extra-oral tissues, such as human airway smooth muscle and the heart, making them a potential novel drug target.[3,4] TAS2R14 is one of the most broadly tuned receptors, as it is activated by natural and synthetic compounds which vary greatly in their structure.[5]



In this work, flufenamic acid, one of the most potent and selective agonists for TAS2R14[1], served as lead structure for the synthesis of new derivatives, using methods of computational docking, bioisosteric exchange and rigidization, to gain further information on the receptors binding pocket. *In vitro* testing of the synthesized molecules revealed a few compounds with similar or higher potency compared to the parent compound. Interestingly, 5-substituted tetrazoles were identified as bioisosteres serving as a novel lead compounds for the development of high affinity TAS2R14 ligands.

References

1) Levit, A. et al. The bitter pill: clinical drugs that activate the human bitter taste receptor TAS2R14. Faseb j 28, 1181-1197, doi:10.1096/fj.13-242594 (2014).
2) Chandrashekar, J. et al. T2Rs function as bitter taste receptors. Cell 100, 703-711 (2000).
3) Deshpande, D. A. et al. Bitter taste receptors on airway smooth muscle bronchodilate by a localized calcium flux and reverse obstruction. Nat Med 16, 1299-1304, doi:10.1038/nm.2237 (2010).
4) Foster, S. R. et al. Expression, Regulation and Putative Nutrient-Sensing Function of Taste GPCRs in the Heart. PLOS ONE 8, e64579, doi:10.1371/journal.pone.0064579 (2013).
5) Behrens, M. et al. The human taste receptor hTAS2R14 responds to a variety of different bitter compounds. Biochem Biophys Res Commun 319, 479-485, doi:10.1016/j.bbrc.2004.05.019 (2004).

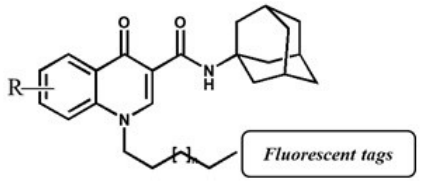
DEVELOPMENT OF FLUORESCENT LIGANDS TO DETECT CB2 RECEPTORS IN CANCER AND NEURODEGENERATIVE DISEASES

Francesco Spinelli (1), Angela Stefanachi (1), Nicola Antonio Colabufo (1), Francesco Leonetti (1), Francesco Berardi (1), Carmen Abate (1), Roberta Giampietro (1), Chiara Riganti (2), Peter J. Mccormick (3), Marialessandra Contino (1)

1) Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari ALDO MORO
2) Dipartimento di Oncologia, Università di Torino
3) Centre for Endocrinology, William Harvey Research Institute, Bart's and The London School of Medicine and Dentistry, Queen Mary University of London

The endogenous cannabinoid system (ECS) is a complex system including: 1) the two "canonical" cannabinoid receptor subtypes 1 (CB1R) and 2 (CB2R), belonging to the GPCRs family; 2) the endocannabinoids anandamide and 2-arachidonoylglycerol; 3) the enzymes involved in the biosynthesis and degradation of the endocannabinoids (e.g., FAAH and MAGL); 4) the "ionotropic cannabinoid receptors" (TRP channels); 5) other GPCRs, such as GPR55; 6) some receptors belonging to the PPAR family (e.g., PPARα); and 7) protein transporters, such as FABP family.[1,2]

The CB1R is the most abundant GPCR expressed in the CNS, in neurons and glial cells, where it modulates several functions such as memory and cognition, emotion and pain control.[3] On the other side, the CB2R is mainly localized in the peripheral immune system (e.g., spleen and macrophages), and recent studies demonstrated its selective upregulation in response to inflammatory insults, as seen during neurodegeneration and in several types of cancers.[2,3] In order to better define the CB2R-mediated pathways in the different types of diseases characterized by inflammation, we designed several fluorescent ligands linking two different fluorescent tags (the nitrobenzoxadiazole and the 4-dimethylamminophthalimide) to a well-known CB2R active scaffold (*N*-adamantil-4-oxo-1-alkyl-1,4-dihydroquinoline-3-carboxamide) (Figure 1).[3] Studies are currently ongoing in term of both binding affinity and fluorescent properties aiming to find the best ligand, that will be consequently tested through cytofluorimetric and BRET (bioluminescent resonance energy transfer) analyses, potentially giving more insights into the CB2R signaling pathways in physiological and pathological conditions. Moreover, these new tools may also serve as fluoligands in a fluorescence binding assay replacing the less safe radioligand binding study.



References

1) Howlett, A. C. Cannabinoid receptor signaling. Handb. Exp. Pharmacol. 2005, 168, 53-79.
2) Schwarz, et al. Targeting the Endocannabinoid System as a Potential Anticancer Approach. Drug Metab. Rev. 2018, 50, 26-53.
3) Spinelli et al. Perspectives of Cannabinoid type 2 receptor (CB2R) ligands in neurodegenerative disorders: Structure Affinity Relationship (SAfiR) and Structure Activity Relationship (SAR) Studies. J Med Chem. 2017, 60, 9913-9931

SOLUBLE EPOXIDE HYDROLASE INHIBITION AS A NEW THERAPEUTIC STRATEGY FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Carles Galdeano (1), Christian Griñan-Ferré (2), Sandra Codony (3), Eugènia Pujol (3), Jun Yang (4), Rosana Leiva (3), Carmen Escolano (3), Dolors Puigoriol-Illamola (2), Júlia Companys-Alemany (2), Rubén Corpas (5,6), Coral Sanfeliu (5,6), Christophe Morisseau (4), Bruce D. Hammock (4), Santiago Vázquez (3), Merce Pallàs (2)

1) Facultat de Farmàcia i Ciències de l'Alimentació, Institute of Biomedicine (IBUB), Universitat de Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain.
2) Secció de Farmacologia i Toxicologia and Institut de Neurociències, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain.

3) Laboratori de Química Farmacèutica (Unitat Associada al CSIC) and Institute of Biomedicine (IBUB), Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII, 27-31, 08028 Barcelona, Spain.

4) Department of Entomology and Nematoplogy and Comprehensive Cancer Center, University of California, Davis, California, USA.

5) Institut d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC and IDIBAPS, Barcelona, Spain.

6) CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

Alzheimer's Disease (AD) is the most common form of dementia, accounting for about 60% of cases. All drugs approved for the treatment of AD modulate neurotransmitters, trying to counterbalance the neurotransmitter disturbances of the disease, but they do not tackle the neuroinflammation process associated with AD. Today, 19 % of the focus of R&D investment in AD is devoted to neuroinflammation.**[1]** One relevant enzyme in the inflammation response is the soluble epoxide hydrolase (sEH). The sEH, widely expressed in relatively high abundance in the human brain, converts epoxyeicosatrienoic acids (EETs) to their corresponding dihydroxyeicosatrienoic acids, whereby diminishing, eliminating, or altering the beneficial anti-inflammatory, angiogenic and antiatherosclerotic effects of the natural EETs.[2] Taking into account that several lines of evidences underline a broad involvement of EETs signaling in central nervous system (CNS) function and disease, we hypothesized that brain penetrant sEH inhibitors would stabilize EETs in the brain, resulting in reduction of reactive oxygen species, diminished inflammation and neurodegeneration, leading to a positive outcome in AD.

We have evaluated the cognitive impairment and the pathological hallmarks in two models of neurodegeneration and AD (SAMP8 and 5xFAD) using three structurally different sEH inhibitors, including UB-EV52, a novel inhibitor. Our results confirmed our expectations on the beneficial effects of central sEH inhibition, improving cognitive decline, reducing neuroinflammation and leading to a reduction in Aβ plaque and neurofibrillary tangles accumulation. Moreover, we have demonstrated, using CETSA,[3] compound-induced target stabilization *in vivo*.

References

1) Kodamullil AT, Zekri F, Sood M, et al. Trial watch: Tracing investment in drug development for Alzheimer disease. Nat Rev Drug Discov. 16(12), 819–819 (2017).
2) Morisseau C, Hammock BD. Impact of Soluble Epoxide Hydrolase and Epoxyeicosanoids on Human Health. Annu. Rev. Pharmacol. Toxicol. 53(1), 37–58 (2013).
3) Molina DM, Jafari R, Ignatushchenko M, et al. Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay. Science. 341(6141), 84–87 (2013).

POSTERS - DIVERSIFIED TOPICS

Late Breaking News

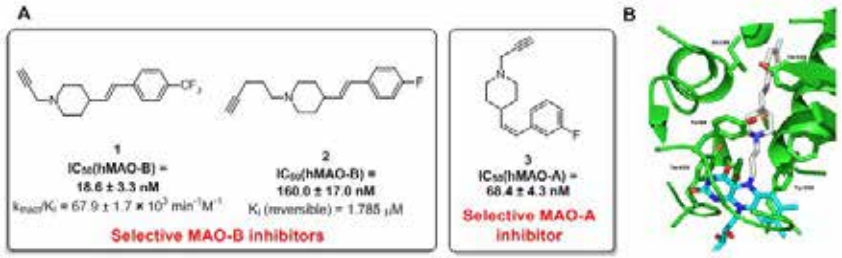
GEOMETRIC ISOMERS OF STYRLPIPERIDINES SELECTIVELY INHIBIT MONOAMINE OXIDASE ISOFORMS A AND B

Damijan Knez (1), Matej Sova (1), Anja Pišlar (1), Simon Žakelj (1), Jurij Trontelj (1), Natalia Colettis (2), Nora Mariel Marder (2), Janko Kos (1), Claudia Binda (3), Stanislav Gobec (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
2) Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956 (C1113AAD), Buenos Aires, Argentina
3) Department of Biology and Biochemistry, University of Pavia, Via Ferrata 1, 27100 Pavia, Italy

Flavin-dependent oxidoreductases monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) are validated targets in the therapy of several neurological disorders, e.g. depression, Parkinson's disease, Alzheimer's disease.[1,2] As a part of our screening program devoted to discovery of new compounds targeting neurodegenerative diseases, styrilpiperidines were found to inhibit MAO-A and MAO-B. A comprehensive series of over 90 *novel* styrilpiperidines was therefore synthesized by applying systematic structural modifications on the benzene ring and by replacing piperidine with other saturated rings. Interestingly, 1,4-disubstituted *N*-propargylstyrilpiperidines with *trans*-vinyl linker connecting piperidine and benzene ring irreversibly inhibit human (h)MAO-B with low nanomolar IC_{50} values. On the other hand, *cis* isomers irreversibly inhibit human (h)MAO-A with high selectivity over hMAO-B (Figure 1A). In contrast, derivatives with prolonged substituents (butinyl/pentinyl) on piperidine nitrogen displayed reversible inhibition of hMAO-B, as demonstrated by 100-fold dilution assay. To characterize the mechanism of MAO inactivation, UV/visible spectroscopy and co-crystallization experiments were performed. Crystal structures of several *N*-propargylstyrilpiperidines in complex with human MAO-B were resolved, further confirming irreversible covalent modification of FAD cofactor (Figure 1B).

Compounds are not cytotoxic to neuroblastoma SH-SY5Y cell line (EC_{50} > 100 μM) and display neuroprotective properties in cell based 6-hydroxydopamine model of Parkinson's disease. They also display favorable *in vitro* pharmacokinetic parameters in terms of oral bioavailability and BBB permeability. *Ex vivo* experiments further on demonstrate MAO-A and MAO-B inhibition after i.p. administration in mice brain homogenates. Importantly, selective hMAO-A inhibitor **3** (Figure 1A) shows antidepressant activity in mice after i.p. administration (0.3 mg/kg) in chronic 10-day treatment regime.



References

1) Youdim, M.B.H et al., Nat Rev Neurosci 2006, 7, 295
2) Robakis, D. et al., CNS Drugs 2015, 29, 433

POSTERS - DIVERSIFIED TOPICS

Other

SYNTHESIS AND EVALUATION OF 7β-HYDROXY-8-KETONE OPIOID DERIVATIVES

Tiina J. Ahonen (1), Maiju Rinne (1), Peter Grutschreiber (1), Kert Mätlik (2), Mikko Airavaara (2), Dieter Schaarschmidt (3,4), Heinrich Lang (3), David Reiss (5), Henri Xhaard (1), Claire Gaveriaux-Ruff (5), Jari Yli-Kauhaluoma (1), Vânia Moreira (1,6)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland. Email: tiina.j.ahonen@helsinki.fi
2) Institute of Biotechnology, HiLife Unit, University of Helsinki, Finland
3) Technische Universität Chemnitz, Faculty of Natural Sciences, Institute of Chemistry, Inorganic Chemistry, 09107 Chemnitz, Germany
4) University of Hamburg, Institute of Inorganic and Applied Chemistry, Germany
5) Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; Université de Strasbourg, Illkirch, France; Centre National de la Recherche Scientifique, UMR7104, Illkirch, France; Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France
6) Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, UK

A large number of semisynthetic derivatives of opioid compounds have been developed over the years with many of them resulting from derivatization at positions 3, 6 and 17 of the opioid core. Modifications on the 7,8-double bond have been scarcely reported in the literature, and have mostly consisted of double bond reduction.[1] Oxidations of opioids are generally challenging and they have focused on the production of 14-hydroxy derivatives from thebaine and oripavine, in route to the preparation of drugs such as naloxone and naltrexone.[2-3]

To study the effect of modifying the 7,8-double bond of opioids, we have developed a convenient, one-step, heterogeneous oxidation method for conversion of $\Delta^{7,8}$-opioids into the corresponding 7β-hydroxy-8-ketones with potassium permanganate supported on iron(II) sulfate heptahydrate.[4] We have demonstrated that the oxidation reaction can be performed in the presence of various protecting groups, and studied the effect of the C6-substituent on the reaction outcome. 7β-Hydroxy-8-ketone opioids can be regarded as versatile intermediates for the synthesis of other opioids of interest.

The binding to and activation of opioid mu, delta and kappa receptors by the synthesized opioid hydroxy ketones was evaluated. The compounds acted as antagonists at the mu- and delta-receptors. Docking simulations and structure-activity analysis suggest that the newly introduced 7β-hydroxy-8-ketone functionality results in gain of activity towards the delta opioid receptor.

References

1) I. Hutchinson, S. Archer, K.P. Hill, J.M. Bidlack, Bioorg. Med. Chem. Lett. 1996, 6, 1563–1566. doi:https://doi.org/10.1016/S0960-894X(96)00274-0
2) G.B. Kok, P.J. Scammells, RSC Adv. 2012, 2, 11318-11325. doi:10.1039/c2ra21693k
3) A. MacHara, D.P. Cox, T. Hudlicky, Adv. Synth. Catal. 2012, 354, 2713–2718. doi:10.1002/adsc.201200677
4) T.J. Ahonen, M. Rinne, P. Grutschreiber, K. Mätlik, M. Airavaara, D. Schaarschmidt, H. Lang, D. Reiss, H. Xhaard, C. Gaveriaux-Ruff, J. Yli-Kauhaluoma, V.M. Moreira, Eur. J. Med. Chem. 2018, doi.org/10.1016/j.ejmech.2018.02.074

ROLE OF THE FIRST TRANSMEMBRANE HELIX OF THE OPIOID RECEPTOR IN RESPONSE TO FULL AND PARTIAL AGONISTS

Damian Bartuzi (1), Justyna Żuk (1), Agnieszka A. Kaczor (1,2), Dariusz Matosiuk (1)

1) Department of Synthesis and Chemical Technology of Medicinal Substances with Computer Modelling Lab, Medical University of Lublin, Chodzki 4A Str., PL20093 Lublin, Poland
2) University of Eastern Finland, School of Pharmacy, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland

G protein-coupled receptors (GPCRs) are a broad and diverse family of receptor proteins. Despite the variety of perceived stimuli, they all share the same scaffold of seven transmembrane helices. Such conservation of the fold, together with a number of very conserved sequence motifs would suggest important role of the complete scaffold in signalling. However, there are known examples of six-transmembrane variants of GPCRs, deprived of the first helix (1TM). Therefore, the role of 1TM becomes ambiguous.

In this study, we employed molecular dynamics simulations to investigate behaviour of the 1TM of the human mu opioid receptor in presence of different ligands – full agonist, partial agonists and an antagonist. Structure of the receptor was co-modelled with G protein and immersed in raft-like membrane to ensure native conditions. Subsequently, Gromacs tools were used to analyse relative motions of transmembrane helices. Our results suggest, that behaviour of 1TM is connected to efficacy of the ligand. Interestingly, antagonist seems to induce similar effects as full agonist, but these behaviors differ from those observed in signalling induced by partial agonists.

PROBING THE STRUCTURE OF FRENTIZOLE-LIKE 17ß-HSD10 INHIBITORS TO INCREASE THEIR POTENCY

Ondrej Benek (1,2,3), Lucie Vinklarova (1,2), Monika Schmidt (1,2), Lukas Hroch (2), Laura Aitken (4), Terry K. Smith (4), Frank Gunn-Moore (4), Kamil Musilek (1,2)

1) University Hospital in Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic
2) University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic
3) National Institute of Mental Health, Topolova 748, 250 67 Klecany, Czech Republic
4) University of St. Andrews, School of Biology, Medical and Biological Sciences Building, North Haugh, St. Andrews KY16 9TF, United Kingdom

Amyloid-beta peptide (Aß), considered the main causative factor for the development of Alzheimer's disease, has been shown to interact with the mitochondrial 17beta-hydroxysteroid dehydrogenase type 10 (17ß-HSD10), also known as amyloid-binding alcohol dehydrogenase (ABAD) [1]. *In vitro* experiments have suggested that this interaction is cytotoxic and that enzyme activity is necessary for the cytotoxicity to be observed [2]. Thus, the inhibition of the 17ß-HSD10 may be of therapeutic merit for treatment of Alzheimer's disease.

Based on the structure of recently identified benzothiazolyl urea inhibitors [3] we have designed, synthesized and evaluated a plenty of novel compounds (Fig. 1). The SAR study indicated the key structural motifs responsible for inhibitory ability. Several compounds were found more potent inhibitors of 17ß-HSD10 compared to the parent molecules.

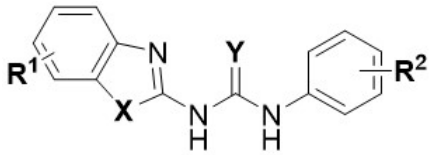


Figure 1: General structure of prepared 17ß-HSD10 inhibitors.

This work was supported by the Ministry of Health of the Czech Republic (no. NV15-28967A), Biotechnology and Biological Sciences Research Council (BBSRC; no. BB/J01446X/1), the Alzheimer's Society UK and the Barcopel Foundation.

References

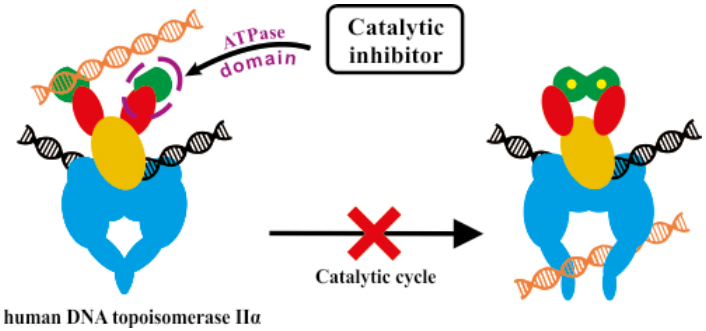
1) Yan SD, Fu J, Soto C, et al. An intracellular protein that binds amyloid-beta peptide and mediates neurotoxicity in Alzheimer's disease. Nature. 389(6652), 689–695 (1997).
2) Yan SD, Shi Y, Zhu A, et al. Role of ERAB/L-3-hydroxyacyl-coenzyme A dehydrogenase type II activity in Abeta-induced cytotoxicity. J. Biol. Chem. 274(4), 2145–2156 (1999).
3) Hroch L, Benek O, et al. Design, synthesis and in vitro evaluation of benzothiazole-based ureas as potential ABAD/17β-HSD10 modulators for Alzheimer's disease treatment, Bioorg. Med. Chem. Lett. 26, 3675–3678 (2016).

SUBSTITUTED 4,5'-BITHIAZOLES AS CATALYTIC INHIBITORS OF THE HUMAN DNA TOPOISOMERASE IIα

Kaja Bergant (1), Matej Janežič (2), Andrej Perdih (1)

1) National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
2) Structural Bioinformatics Team, Division of Structural and Synthetic Biology, Center for Life Science Technologies, RIKEN, 1-7-22 Suehiro, Tsurumi, Yokohama, Kanagawa, 230-0045, Japan

The human DNA topoisomerase IIα (human topo IIα) is one of the major anticancer targets due to its role in the cell proliferative process. It catalyses topological changes of the DNA molecule, and plays an important role in biological processes, such as DNA replication, transcription and chromosome segregation, and its concentration is higher in rapidly dividing cells [1]. Because of its complex catalytic mechanism, several possibilities exist how to tackle this established anticancer target. Active agents targeting the human topo IIα are divided into two large groups; the established topoisomerase poisons [2] and an emerging group of catalytic inhibitors [3]. In our research we are using available structural information of the human topo IIα ATPase domain to rationally design new catalytic human topo IIα inhibitors that target the ATP-binding site on its ATPase domain. Such inhibitors prevent the native ATP ligand from binding, consequently stopping its catalytic cycle [2-4].



The starting point of this study comprised our discovered 4,5'-bithiazole compounds that were discovered to bind to the ATP binding site of the DNA Gyrase from *E. Coli,* the bacterial analogue of the human topo IIα [5]. By aligning the ATPase domains of the human topo IIα and that of the DNA Gyrase we determined the structural differences between their corresponding ATP binding sites. Based on these results a small focused library of 4,5'-bithiazoles was selected and screened against the human topo IIα ATP binding site. Analysis using obtained binding modes coupled with LigandScout-generated pharmacophores resulted in a selection of small series of compounds that were evaluated for its *in vitro* inhibitory activity. The best compounds showed activity in the lower micromolar range. In subsequent investigation we confirmed that these compounds do not act as topoisomerase poisons and further functional and biophysical assays suggested that they bind to the topo IIα ATPase domain. Compounds also displayed promising cytotoxicity and are a promising class for further development.

References

1) Nitiss, J.L. DNA topoisomerase II and its growing repertoire of biological functions. Nature Reviews Cancer, 2009, 9(5), 327-337.
2) Pogorelčnik, B.; Perdih, A.; Solmajer, T. Recent Developments of DNA Poisons - Human DNA Topoisomerase II alpha Inhibitors - as Anticancer Agents. Curr. Pharm. Des., 2013, 19(13), 2474-2488
3) Pogorelčnik, B.; Perdih, A.; Solmajer, T. Recent Advances in the Development of Catalytic Inhibitors of Human DNA Topoisomerase II alpha As Novel Anticancer Agents. Curr. Med. Chem., 2013, 20(5), 694-709.
4) Pogorelcnik, B.; Brvar, M.; Zegura, B.; Filipic, M.; Solmajer, T.; Perdih, A. Discovery of Mono- and Disubstituted 1H-Pyrazolo[3,4]pyrimidines and 9H-Purines as Catalytic Inhibitors of Human DNA Topoisomerase II alpha. ChemMedChem, 2015, 10(2), 345-359.
5) Brvar, M.; Perdih, A.; Renko, M.; Anderluh, G.; Turk, D.; Solmajer, T. Structure-Based Discovery of Substituted 4,5'-Bithiazoles as Novel DNA Gyrase Inhibitors. J. Med. Chem., 2012, 55(14), 6413-6426.

SYNTHESIS AND BIOLOGICAL PROFILING OF MEPHEDRONE METABOLITES AND DERIVATIVES

Daniela Cintulova (1), Felix Mayer (2), Marco Niello (2), Laurin Wimmer (1), Eva Hellsberg (3), Gerhard F. Ecker (3), Harald H. Sitte (2), Marko D. Mihovilovic (1)

1) TU Wien, Institute of Applied Synthetic Chemistry, Getreidemarkt 9/163-OC, 1060, Vienna, Austria
2) Medical University of Vienna, Center for Physiology and Pharmacology, Institute of Pharmacology, Währingerstrasse 13A, 1090 Vienna, Austria
3) University of Vienna, Department for Pharmaceutical Chemistry, Althanstraße 14 (UZA II), 1090 Vienna, Austria

$GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the central nervous system. These GABA-gated chloride channels are composed of five subunits that can belong to different subunit classes. Several pyrazoloquinolinone ligands have already been described as high affinity ligands of the benzodiazepine (Bz) binding site but also, they exert a positive modulatory effect at the alpha+beta- interfaces.[1,2] Previously, it was shown that some pyrazoloquinolinone derivatives showed preference towards beta1 containing receptors in terms of potency. Further studies in homology models and mutant receptors confirm that the amino acid located in position 41 of segment G in the beta1 and beta3 subunits strongly influences the potency and efficacy of the tested ligands.[3] In the present study, further pyrazoloquinolinone derivatives were studied and results showed that they possess improved functional selectivity. The results of this study are herein presented and the properties of these compounds will be further investigated.

References

1) Meltzer, P. C., Butler, D., Deschamps, J. R., Madras, B. K.: J. Med. Chem. 2006, 49, 1420-1432
2) Sitte, H. H., Freissmuth, M.: Trends in Pharmacological Sciences, 2015, 36, 41-50
3) Pedersen, A. J., Reotzel, L. A., Johansen, S. S., Linnet K.: Drug Test. Analysis 2012, 5, 313-319

ENAMINONES AND THEIR MOLYBDENUM(VI) COMPLEXES AS NON-CYTOTOXIC COMPOUNDS WITH ANTIBACTERIAL ACTIVITY

Danijela Cvijanović (1), Vladimir Damjanović (1), Jana Pisk (2), Mirta Rubčić (2), Luka Bilić (2), Marijana Đaković (2), Tomica Hrenar (2), Jasna Lovrić (1), Višnja Vrdoljak (2), Marina Cindrić (2)

1) Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Šalata 3, Zagreb, Croatia
2) Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, Zagreb, Croatia

Enaminones as a class of compounds containing conjugated N–C=C–C=O system represent a promising group of potential therapeutics showing anticonvulsant, antimicrobial, antioxidant, antitumor and cytogenetic biological activity. Due to their lipophilicity, sensitivity to minor structural changes in the N–C=C–C=O backbone and stability in solution under physiological conditions they are used as suitable starting material for the synthesis of bioactive molecules in the field of medicinal chemistry. These tridentate ONO donors also represent a significant group of ligands in the coordination chemistry of transition metals.

Trying to give more insight into the correlation between chemical structure and biological activity, six non-symmetric acyclic enaminones, 4-[(2-hydroxy-5-methylphenyl)amino]pent-3-en-2-one (**H_2L^1**), 4-[(2-hydroxy-4-methylphenyl)amino]pent-3-en-2-one (**H_2L^2**), 4-[(4-hydroxy-2-methylphenyl)amino)]pent-3-en-2-one (**H_2L^3**), 3-[(2-hydroxy-5-methylphenyl)amino]-1-phenylbut-2-en-1-one (**H_2L^4**), 3-[(2-hydroxy-4-methylphenyl)amino]-1-phenylbut-2-en-1-one (**H_2L^5**) and 3-[(4-hydroxy-2-methylphenyl)amino]-1-phenylbut-2-en-1-one (**H_2L^6**), have been synthesized and characterized (Fig. 1). The cytotoxicity of enaminones was investigated against THP-1 and HepG2 cells *in vitro* and corresponding IC_{50} values were determined by MTS assay. The antibacterial activity was tested by microdilution method against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Moraxella catarrhalis* bacterial strains to assess their MIC values. To investigate the coordination ability and influence of metal ion complexation on cytotoxicity and antibacterial activity of prepared enaminones, twelve molybdenum(VI) complexes of different nuclearity containing enaminone ligands **H_2L^4** or **H_2L^5** were prepared. The obtained complexes, $[MoO_2(L^{5\text{ or }4})(MeOH)]\times MeOH$ (**1**×MeOH and **2**×MeOH), $[MoO_2(L^{5\text{ or }4})(D)]$ [D = pyridine, (**1a** and **2a**), 3-methylpyridine, (**1b** and **2b**) and 4-methylpyridine, (**1c** and **2c**)], $[\{MoO_2(L^{5\text{ or }4})\}_2(D)]$ [D = 4,4'-bipyridine (**1d** and **2d**)], and complexes $[MoO_2(L^{5\text{ or }4})]_n$ (**3** and **4**) were characterized and tested for cytotoxic and antibacterial activities. Enaminones and their Mo(VI) complexes were characterized by thermal analysis, IR spectroscopy and X-ray diffraction.

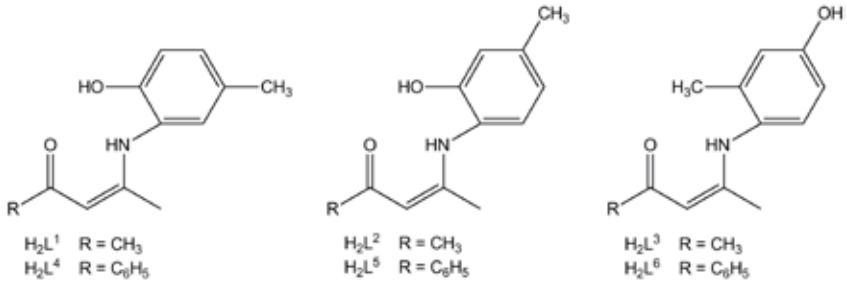


Figure 1. Structural formulas of enaminones H_2L^1 – H_2L^6

RATIONAL DRUG DESIGN OF HISTONE DEACETYLASE 6 INHIBITORS

Dusan Ruzic (1), Katarina Nikolic (1), Milos Petkovic (2), A Ganesan (3), Danica Agbaba (1)

1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
2) Department of Organic Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
3) School of Pharmacy, University of East Anglia, Norwich Research Park, NR4 7TJ Norwich, United Kingdom

Activity of the histone deacetylases (HDACs) has an essential influence on histone posttranslational modifications. Therefore, alterations in the structure and expression of HDACs isoforms are strongly related to the pathogenesis of inflammation, cancer, and neurodegeneration. The HDACs became extensively examined targets in novel drug discovery. The HDAC6 isoform is a non-histone cytoplasmic deacetylase, manly involved in deacetylation of α-tubulin, cortactin, and heat shock protein 90 (Hsp90) [1]. Our rational drug design study was focused on identification of selective histone deacetylase 6 (HDAC6) inhibitors by use of combined ligand and structure based methodologies. Based on the 3D-QSAR (Quantitative Structure Activity Relationship) modeling of HDAC6 inhibitors were defined specific molecular determinants for selective HDAC6 inhibition and further applied for fragment based design of selective HDAC6 inhibitors. Recently resolved crystal structure of second human catalytic domain of HDAC-6 enzyme (5EDU) [2] was used in virtual docking study of the examined inhibitors.

References

1) Lee, J. H., Mahendran, A., Yao, Y., Ngo, L., Venta-Perez, G., Choy, M. L., Kim N., Ham WS, Breslow R. and Marks, P. A. (2013). Development of a histone deacetylase 6 inhibitor and its biological effects. Proceedings of the National Academy of Sciences, 110(39), 15704-15709.
2) Hai, Y., Christianson, D. W. (2016). Histone deacetylase 6 structure and molecular basis of catalysis and inhibition. Nature chemical biology, 12(9), 741.

COMBINED MOLECULAR DYNAMICS AND VIRTUAL SCREENING STUDIES TO IDENTIFY NOVEL SIRTUIN 2 INHIBITORS

Nemanja Djokovic (1), Katarina Nikolic (1), Maija Lahtela-Kakkonen (2), Danica Agbaba (1)

1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
2) School of Pharmacy (Pharmaceutical Chemistry) University of Eastern Finland, Kuopio, Finland

Sirtuins are highly conserved class of NAD+-dependent lysine deacetylases. Altered function of sirtuin 2 (Sirt2) is related to pathogenesis of cancer, inflammation and neurodegeneration, which makes Sirt2 very attractive drug target in novel epigenetic research [1]. A number of Sirt2 inhibitors have been recently developed, but for most of them are missing structural information of their interaction with the enzyme [2, 3]. Our molecular dynamic (MD) study was performed on recently resolved crystal structures of selective ligand-Sirt2 complexes [1]. In the MD study were defined significant interactions with novel inhibitors, one of key residues responsible for conformational stability of cofactor-binding pocket, and residue acting as gate-keeper for cofactor-binding loop. Some residues completely changed orientation after the MD simulation, compared to the starting crystal structures. This result indicates on the errors in the X-ray structures that may have influence on structure-based design of novel inhibitors. After clustering of MD trajectory, 20 conformations (centroids) from 20 clusters of Sirt2 have been selected as representative conformations for retrospective structure based virtual screening. The virtual screening performances were significantly improved by use of the ensemble of conformations, selected with this MD methodology, compared to screening against available X-ray structures.

References

1) Rumpf, T.; Schiedel, M.; Karaman, B.; Roessler, C.; North, B.J.; Lehotzky, A.; Oláh, J.; Ladwein, K.I.; Schmidtkunz, K.; Gajer, M.; Pannek, M.; Steegborn, C.; Sinclair, D.A.; Gerhardt, S.; Ovádi, J.; Schutkowski, M.; Sippl, W.; Einsle, O.; Jung, M. Selective Sirt2 inhibition by ligand-induced rearrangement of the active site. Nat Commun. 2015, 6, 6263.
2) Seifert, T.; Malo, M.; Kokkola, T.; Engen, K.; Fridén-Saxin, M.; Wallén, E.A.; Lahtela-Kakkonen, M.; Jarho, E.M.; Luthman, K. Chroman-4-one- and chromone-based sirtuin 2 inhibitors with antiproliferative properties in cancer cells. J Med Chem. 2014, 57, 9870-88.
3) Schiedel, M.; Rumpf, T.; Karaman, B.; Lehotzky, A.; Oláh, J.; Gerhardt, S.; Ovádi, J.; Sippl, W.; Einsle, O.; Jung, M. Aminothiazoles as Potent and Selective Sirt2 Inhibitors: A Structure-Activity Relationship Study. J Med Chem. 2016, 59, 1599-612.

P348

MEDCHEM STRUCTURE GENIUS : FREE MOBILE APPS TO LEARN DRUG STRUCTURES

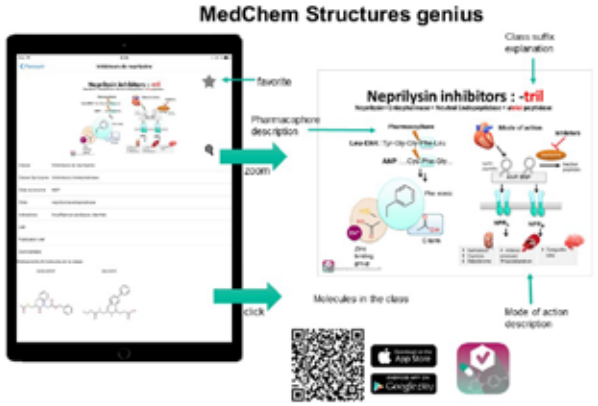
Julie Charton (1), Damien Boscc (1), Rebecca Deprez-Poulain (1,2)

1) INSERM U1177 Drugs and Molecules for Living Systems; Drug Discovery unit; Pasteur Institute Lille, University of Lille, 3 rue du Professeur Laguesse, F-59000 LILLE, FRANCE
2) Institut Universitaire de France, IUF

During the past years, we have identified a decrease in the ability of the students of Pharmacy and of Medicinal chemistry Masters to remember the chemical structure of drugs. As a consequence, the students try to learn by heart the structures as meaningless drawings and show difficulties to recognize (not only learn) the structures and their functions, to sort the drugs into the right pharmacologic class, to identify sets of properties that are professionally relevant and to associate the International Nonproprietary Name (INN) with structure.

This results in an opportunity loss to aggregate the properties of drugs that are embedded in their chemical structure, like binding and potency, ADME properties, side-effects… Indeed, the structures are very important vertices that help to consolidate a multidisciplinary learning and knowledge, as exemplified in drug-discovery.

We designed a free e-learning tool to support the medicinal chemistry and pharmacology face-to-face courses in a blended learning approach. The innovative pedagogic project entitled “MedChem Structures Genius” available on mobile stores and also on web. So far, more than 400 drugs and 100 pharmacological classes have been implemented in the database and available for students and medicinal chemistry professionals to review and test themselves.



P349

DISCOVERY OF BENZOTHIAZOLE-BASED DNA GYRASE AND TOPOISOMERASE IV INHIBITORS WITH BROAD SPECTRUM ANTIBACTERIAL ACTIVITY

Martina Durcik (1), Tihomir Tomašič (1), Ákos Nyerges (2), Michaela Barančoková (1), Žiga Skok (1), Janez Ilaš (1), Päivi Tammela (3), Tamás Révész (2), Csaba Pál (2), Nace Zidar (1), Anamarija Zega (1), Danijel Kikelj (1), Lucija Peterlin Mašič (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
2) Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged H-6726, Hungary
3) Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, 00014 Helsinki, Finland

DNA gyrase and topoisomerase IV are validated targets for discovery of antibacterial drugs. They are heterotetrameric enzymes composed of two catalytic GyrA/ParC subunits and two GyrB/ParE subunits with ATPase activity. The latter have become attractive targets in many drug discovery projects in pharmaceutical industry, especially after successful introduction of the novobiocin into the therapy. However, novobiocin was later withdrawn from the clinic due to its toxicity and development of bacterial resistance. In recent decades, several new GyrB and ParE inhibitors with antibacterial activity, mainly against Gram-positive bacteria, have been discovered. Although some of these new inhibitors have advanced to phase I trials, none have so far reached clinical practice.

Recently, we have discovered and optimized several structural classes of potent DNA gyrase and topoisomerase IV inhibitors with activity mainly against Gram-positive pathogens.[1-4] Our latest optimization efforts resulted in the benzothiazole class of potent dual DNA gyrase and topoisomerase IV inhibitors with activities in the low nanomolar range (5-20 nM) against GyrB, which is the primary target of compounds in bacteria. The most potent compounds possess antibacterial activity with MIC values lower than 1 μg/mL against many Gram-positive strains (e.g. *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Enterococcus faecalis*) and low μg/mL values against Gram-negative strains (e.g. *Escherichia coli, Klebsiella pneumoniae, Shigella sonnei, Pseudomonas aeruginosa*). The best compounds display activity also against plasmid-mediated quinolone resistant *E. coli* strains, therefore, showing no cross-resistance with the fluoroquinolones. In addition, resistance potential in *E. coli* was determined and mutations were mapped to the residues in the ATPase domain of GyrB.

References

1) Tomašič, T.; Katsamakas, S.; Hodnik, Ž.; Ilaš, J.; Brvar, M.; Šolmajer, T.; Montalvao, S.; Tammela, P.; Banjanac, M.; Ergović, G.; Anderluh, M.; Mašič, L.P.; Kikelj, D. Discovery of 4,5,6,7-tetrahydrobenzo[1,2-d]thiazoles as novel DNA gyrase inhibitors targeting the ATP-binding site. J. Med. Chem., 2015, 58, 5501-5521.
2) Zidar, N.; Macut, H.; Tomašič, T.; Brvar, M.; Montalvão, S.; Tammela, P.; Solmajer, T.; Peterlin Mašič, L.; Ilaš, J.; Kikelj, D. N-Phenyl-4,5-dibromopyrrolamides and N-phenylindolamides as ATP competitive DNA gyrase B inhibitors: Design, synthesis, and evaluation, J. Med. Chem., 2015, 58, 6179-6194.
3) Gjorgjieva, M.; Tomašič, T.; Barančoková, M.; Katsamakas, S.; Ilaš, J.; Tammela, P.; Peterlin Mašič, L.; Kikelj, D. Discovery of benzothiazole scaffold-based DNA gyrase B inhibitors. J. Med. Chem., 2016, 59, 8941-8954.
4) Durcik, M.; Tammela, P.; Barančoková, M.; Tomašič, T.; Ilaš, J.; Kikelj, D.; Zidar, N. Synthesis and Evaluation of N-Phenylpyrrolamides as DNA gyrase B inhibitors. ChemMedChem, 2018, 13, 1-15.

TANKYRASE INHIBITOR DEVELOPMENT: EVIDENCE FOR POTENTIAL IN CANCER IMMUNE THERAPY

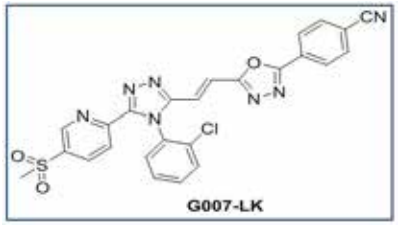
Rutger Folmer (1), Anita Wegert (1), Jo Waaler (5), Ruben Leenders (1), Sven Sowa (2), Upendra Rao Anumala (3), Piotr Nieczypor (1), Sjoerd Aertssen (1), Eddy Damen (1), Marc Nazaré (3,4), Lari Lehtiö (2), Stefan Krauss (5)

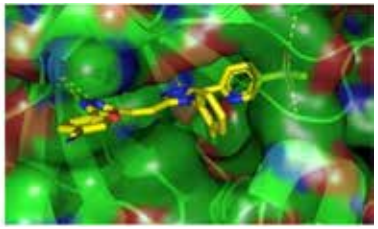
1) Mercachem, Kerkenbos 1013, 6546 BB Nijmegen, Netherlands
2) Faculty of Biochemistry and Molecular Medicine, Biocenter Oulu, University of Oulu, Finland
3) Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Campus Berlin-Buch, 13125 Berlin, Germany
4) Berlin Institute of Health (BIH), Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany
5) Hybrid Technology Hub - Centre of Excellence, University of Oslo and Oslo University Hospital, Norway

WNT/β-catenin signaling regulates key cellular functions including proliferation, differentiation, migration, apoptosis, stem cell renewal and immune system modulation. Abberrant WNT/ β-catenin signaling is found in multiple cancers. In particular, the recently described role of the WNT/β-catenin pathway in regulating immune cell infiltration in the tumor micro-environment suggests an impact of the pathway on immunotherapy [1]. Hence, WNT-directed therapeutic intervention represents an area of significant developmental focus.

The Poly-ADP-ribosylases tankyrase 1 and 2 are cental biotargets in the WNT/β-catenin signaling pathway, regulating the turnover of the protein complex that controls β-catenin stability and in adition impacting the hippo signaling pathway. Several small molecules have been identified that inhibit tankyrases 1 and 2 [2], and we have earlier shown efficacy of tankyrase inhibitors in WNT dependent adenoma and tumor models [3, 4].

Here we describe the successful discovery of a selective tankyrase inhibitor from a hit stage to a late lead stage with potential as a preclinical candidate [5, 6]. In addition, we show proof of concept for our tankyrase inhibitor as an immune modulatory agent.





Crystal structure of G007-LK in complex with tankyrase 2 (PDB-code 4HYF)

References

1) Pai et al. J Hematol Oncol. 2017 May 5;10(1):101;
2) Ferri et al. Eur J Med Chem. 2017 Dec 15;142:506-522;
3) Waaler J, et al. Cancer Res. 2012 Jun 1;72(11):2822-32;
4) Lau T, et al. Cancer Res. 2013 May 15;73(10):3132-44
5) Voronkov et al. J Med Chem. 2013 Apr 11;56(7):3012-23;
6) Anumala et al. J Med Chem. 2017 Dec 28;60(24):10013-10025

DEVELOPMENT OF NEW ZINC CHELATING POLYAMINES WITH ANTIPROLIFERATIVE ACTIVITY

Cristina Galiana-Roselló (1), Clara Aceves-Luquero (2), Ruth Villalonga-Planells (2), Guillem Ramis (2), Silvia Fernández de Mattos (2), Concepción Soriano (3), Enrique García-España (1), Jose Miguel Llinares (3), Mª Eugenia González-Rosende (4), Priam Villalonga (2)

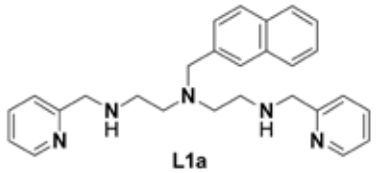
1) Departamento de Química Inorgánica, ICMOL, Universitat de València, C/Catedrático José Beltrán, 2, 46980-Paterna, València, Spain
2) Cancer Cell Biology Group, Universitat de les Illes Balears, Ctra. Valldemossa Km 7.5, 07122 Palma de Mallorca, Spain
3) Departamento de Química Orgánica, ICMol, Universitat de València, C/Catedrático José Beltrán, 2, 46980-Paterna, València, Spain
4) Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad CEU Cardenal Herrera, Edificio Seminario s/n, 46113-Moncada, València, Spain

Polyamines are essential biological compounds in eukaryotes, participating in a variety of cellular processes such as cell growth, proliferation, and differentiation. These effects are caused by various specific molecular interactions with polynucleotides, proteins, metals ions, ionic channels, membranes and cytoskeletal structures among others.[1] Consequently, they represent a universal template in drug design, particularly to afford new anticancer agents.[2]

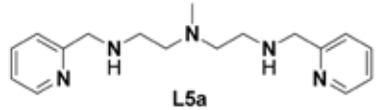
According to recent efforts in the polyamine-based drug design focused on anticancer compounds, we designed and synthesized a series of 4-N-substitued 1,4,7-triazaheptane compounds symmetrically substituted with picolyl groups. Among the compounds, **L1a** and **L5a** showed noteworthy low micromolar potency against a panel cancer cell lines. These lead compounds promoted G0/G1 arrest of cell cycle, which was followed by cellular senescence as indicated by the detection of senescence-associated β-galactosidase (SA-β-gal) in **L1a** and **L5a** -treated cells. For large treatments, they also activated strong apoptotic response, as indicated by externalization of phosphatidylserine, caspases 3/7 induction and a decrease in mitochondrial membrane potential.

Structure–Activity Relationship of polyamines **L1a** and **L5a** agrees with the well-known apoptosis-inducing ability and metal affinity of zinc chelating agents.[3] Thus, we investigated, as a potential target of compounds **L1a** and **L5a**, the modulation of intracellular zinc homeostasis by fluorescence *in vitro* studies with the zinc-specific probe *Zinquin*. **L1a** and **L5a** reduced the intracellular labile zinc ions in LN229 glioma cancer cells in contrast with the inactive compounds. Additionally, the intracellular zinc depletion was in a dose-dependent manner.

These results support the development of new zinc-chelating agents as a potential strategy for the treatment of several cancer types and represent a promising new class of antitumor agents.



L1a



L5a

References

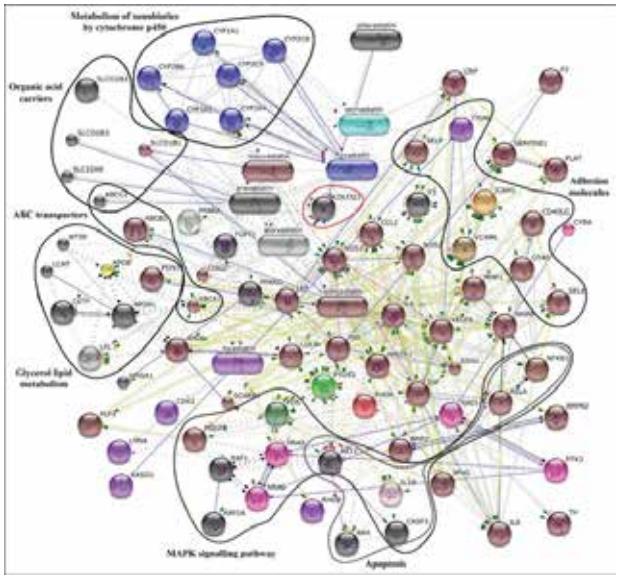
1) Miller-Fleming, L.; Olin-Sandoval, V.; Campbell, K.; Ralser, M. J Mol Biol 2015, 427 (21), 3389-406.
2) Melchiorre, C.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V. J Med Chem 2010, 53 (16), 5906-14.
3) Åstrand, O. A. H.; Aziz, G.; Ali, S. F.; Paulsen, R. E.; Hansen, T. V.; Rongved, P. Bioorg Med Chem 2013, 21 (17), 5175-5181

EFFECT OF STATINS ON THE GENES´ EXPRESSION

Helena Gbelcová (1,4), Petra Priščáková (1), Soňa Režnáková (1), David Végh (2), Michal Kolář (3), Jan Kubovčiak (3), Silvie Rimpelová (4), Vanda Repiská (1)

1) Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Bratislava, Slovakia
2) Department of Genetics, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia
3) Institute of Molecular Genetics, Czech Academy of Science, Prague, Czech Republic
4) Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Czech Republic

Statins (inhibitors of the hydroxy-methylglutaryl-coenzyme A reductase) have various anticancer effects and potentially they could be used in the treatment of tumor diseases. However, it is important to ascertain their possible side effects on non-cancerous cells, e.g. on stem cells, since cancer- and stem cells have similar properties that include the ability of the unlimited dividing. In the past we screened the changes in expression of 48 000 genes induced by all commercially available statins on pancreatic cancer cells MiaPaCa-2. Recently we have applicated the microarray experiment on adipose-derived mesenchymal stem cells. We analyzed, compared and interpreted the significance of the effects of statins on expression of individual genes as well as the effect on complete metabolic and signaling cascades. Predicted functional association networks are shown in figure. Individual nodes represent drug and genes products. Individual node colors indicate the type of the interaction: binding - blue ball, activation - green arrow, inhibition - red bar, catalysis - magenta ball, same activity - cyan, reaction - black ball.



This work was supported by the projects APVV-15-0217, VEGA1/0168/18 and RVO: 68378050.

INSIGHT INTO THE SELECTIVE BINDING OF NOVEL INHIBITORS OF FUNGAL CYP51

Urška Zelenko (1), Darko Kocjan (1), Milan Hodošček (2), Damjana Rozman (3), Simona Golič Grdadolnik (1)

1) Laboratory of Biomolecular Structure, National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
2) Laboratory for Molecular Modeling, National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
3) Centre for Functional Genomics and Bio-Chips, Institute of Biochemistry, Faculty of Medicine,University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia

The most widely used antifungal agents in clinical medicine and agriculture are azoles that act as reversible inhibitors of sterol 14α-demethylase (CYP51), the key enzyme in the sterol biosynthetic pathway. The basis for azole efficacy is their selective inhibition of fungal CYP51 over the human ortholog. However, worldwide increase in the incidence of opportunistic fungal infections and emergence of resistance to available antifungal drugs, raise the need to develop new, more selective, and efficient inhibitors of fungal CYP51.

The aim of our studies is to provide new directions for the design of compounds that would selectively inhibit CYP51 from a yeast *C. albicans*, a major human fungal pathogen. We investigated the binding of a group of synthesised pyridylethanol(phenylethyl)amine derivatives to CYP51 orthologs. We expressed wild-type human and *C. albicans* CYP51 proteins in *E. coli* and purified both recombinant proteins. We also optimized eukaryotic CYP51s expression in *E. coli*. Spectrophotometric titrations of both othologs with a group of these derivatives showed that the length of amine nitrogen alkyl chain is important for the binding strenght to CYP51 and that the phenyl ring substitutions are important for the selective binding to *C. albicans* CYP51. We confirmed the selectivity of compounds by half maximal inhibitory concentration (IC50) determinations to human and *C. albicans* CYP51. Using a combination of the solution-state NMR spectroscopy and molecular modeling methods the binding mode of selected derivatives was determined at the atomic level. This is the first determination of the location and interactions of any of these derivatives in the CYP51 active site at the atomic level. Previous attempts to determine the crystal structure of these compounds in complex with CYP51 were unsuccessful, probably because of their dynamic nature. Our results reveal the unique binding properties of the investigated derivatives in comparison to the azoles. Most importantly the halogenated phenyl ring is located in the substrate access channel forming a unique set of interactions with the hydrophobic side chains. Especially informative are the interactions of chlorine atoms with the unconserved residue Met381 in human CYP51, which corresponds to Phe380 in *C. albicans* CYP51. These can explain the structural requirements for selectivity of the examined pyridylethanol(phenylethyl)amine derivatives and provide novel directions for the design of selective fungal inhibitors.

This work was supported by the Slovenian Research Agency (Grant numbers P1-0010 and J1-8145) and EN-FIST Centre of Excellence.

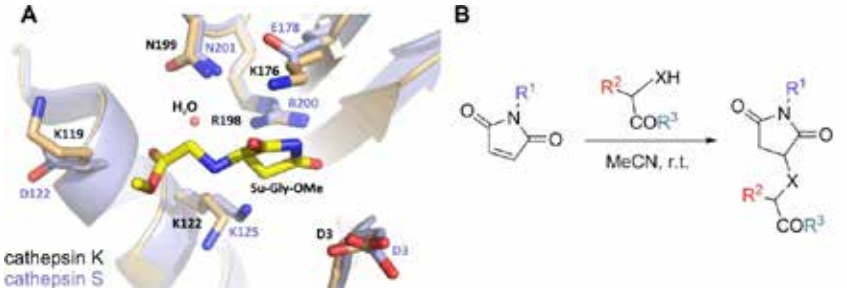
OPTIMIZATION OF ALLOSTERIC EFFECTORS OF CATHEPSINS K AND S BASED ON A SUCCINIMIDE-GLYCINATE SCAFFOLD

Tjaša Goričan, Nejc Petek, Jurij Svete, Marko Novinec

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

Cysteine cathepsins are a family of proteolytic enzymes which have fundamental roles in the degradation of proteins in (endo)lysosomes. Some of them, including cathepsins K and S, are also promising drug targets. In bone tissue, cathepsin K participates in the process of bone resorption and its elevated enzyme activity is associated with bone diseases. On the other hand, cathepsin S plays an important role in the immune response and represents a target for the treatment of rheumatoid arthritis, psoriasis and cancer [1]. Multiple orthosteric inhibitors targeting the active sites of cathepsins K and S are being developed as drugs for the treatment of several diseases and a few of them have already reached clinical trials, however none of them have yet been approved. Allosteric regulation is an alternative way to regulate enzyme activity. It is emerging as an important strategy for drug discovery and development since allosteric drugs bind to evolutionary less conserved sites, making them more specific compared to orthosteric drugs [2]. Furthermore several allosteric drugs in development have already been approved for the treatment.

Cathepsin K is a model enzyme for allosteric regulation in cysteine cathepsins. Apart from its natural effectors glycosaminoglycans two synthetic effectors NSC13345 and NSC94914 are known to bind to an allosteric site of cathepsin K [3, 4]. We recently synthesized and characterized a novel allosteric effector of cathepsin K, Su-Gly-OMe (methyl (*R*)-(2,5-dioxopyrrolidine-3-il)glycinate), which has a mode of action consistent with the aforementioned effectors NSC13345 and NSC94914. We confirmed that it binds to the same allosteric site and showed that it partially inhibits not only cathepsin K but also cathepsin S. We hypothesize that it binds to the same site on cathepsin S and that on the basis of structural differences between both sites we can develop compounds specific for each enzyme. For this purpose, we prepared compound libraries with three sites of diversification on the Su-Gly-OMe scaffold. We tested the effects of the synthesized compounds on the activity of cathepsins K and S and determined the affinities of those compounds which acted as inhibitors. Thus far we have shown that one site of diversification can be used to increase the affinity of the effector for cathepsin K and another site to optimize its specificity. Both of them can also be used to optimize the specificity of the effector for cathepsin S, whereas all three sites of diversification can be used to increase affinity for cathepsin S.



References

1) Novinec, M. in Lenarčič, B. Papain-like peptidases: structure, function, and evolution. Biomol Concepts, 2013, 4(3), str. 287-308.
2) Lindsley, C. W. 2013 Philip S. Portoghese Medicinal Chemistry Lectureship: drug discovery targeting allosteric sites. J Med Chem, 2014, 57(18), str. 7485-7498.
3) Novinec, M., Korenč, M., Caflisch, A., Ranganathan, R., Lenarčič, B. in Baici, A. A novel allosteric mechanism in the cysteine peptidase cathepsin K discovered by computational methods. Nat Commun, 2014, 5, str. 1-10.
4) Novinec, M., Rebernik, M. in Lenarčič, B. An allosteric site enables fine-tuning of cathepsin K by diverse effectors. FEBS Lett, 2016, 590(24), str. 4507-4518.

RATIONALISATION OF ALPHAV-BETA6 SELECTIVITY FOR CLINICAL CANDIDATE GSK3008348 IN THE TREATMENT OF IDIOPATHIC PULMONARY FIBROSIS

Heather Gray (1), Timothy Ritchie (2)

1) Fibrosis Discovery Performance Unit, GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, UK
2) Zerlavanz Consulting Ltd, St Albans, UK

GSK3008348 is currently in clinical trials for the treatment of idiopathic pulmonary fibrosis (IPF), a disease which leads to aberrant scarring in the lung, resulting in shortness of breath, persistent coughing and eventually death.[1] A key target for this treatment is the αvβ6 integrin, which activates profibrotic cytokine transforming growth factor (TGF-β). Selectivity towards αvβ6 over other integrins is believed to be important to regulate TGF-β inhibition. GSK3008348 (Figure 1) delivers excellent potency at αvβ6 in cell adhesion and radioligand binding assays, with >100-fold selectivity over the other RGD integrins in the latter (Table 1).[2]

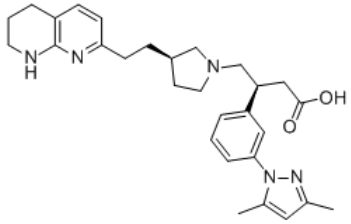


Figure 1. Structure of GSK3008348.

Table 1. Potency of GSK3008348 against RGD integrins.[2]

	Cell pIC_{50}	Binding pK_d
αvβ6	8.7	10.8
αvβ1	7.4	8.6
αvβ3	6.0	7.7
αvβ5	7.0	7.7
αvβ8	8.0	8.6
α5β1	6.1	7.0

This work aims to investigate the excellent selectivity of GSK3008348 for αvβ6 over the other RGD integrins. The key intermolecular interactions when GSK3008348 is docked into a crystal structure of αvβ6 will be explored.[3] The difference in potencies at αvβ6 and αvβ3 will be rationalised by comparison of the amino acid residues in the receptor binding pockets. The binding of GSK3008348 in protein homology models of other integrin receptors will also be investigated, and the key interactions described to explain the excellent selectivity of GSK3008348, which is hoped will ultimately deliver a more efficacious medicine to patients.

References

1) P. W. Noble, C. Albera, W. Z. Bradford, U. Costabel, M. K. Glassberg, D. Kardatzke and T. E. King, Lancet, 2011, 377, 1760-1769.
2) E. R. Hall, L. I. Bibby and R. J. Slack, Biochem. Pharmacol., 2016, 117, 88-96.
3) X. Dong, N. E. Hudson, C. Lu, T. A. Springer, Nat. Struct. Mol. Biol., 2014, 21, 1091–1096.

DOCKTHOR-VS: A FREE DOCKING SERVER FOR PROTEIN-LIGAND VIRTUAL SCREENING

Isabella A. Guedes (1), André M. S. Barreto (1), Eduardo Krempser (2), Camila S. de Magalhães (3), Laurent E. Dardenne (1)

1) Laboratório Nacional de Computação Científica, LNCC, Petrópolis 25651-075, Brazil
2) Fundação Oswaldo Cruz, Fiocruz, Rio de Janeiro 21040-361, Brazil
3) Universidade Federal do Rio de Janeiro, UFRJ, Xerém, 25245-390, Brazil

INTRODUCTION. Receptor-ligand molecular docking is a structure-based drug design approach widely used by the scientific community in Medicinal Chemistry [1]. The main objective is to assist the process of drug discovery, searching for new lead compounds against relevant therapeutic targets with known three-dimensional structures. The program DockThor [2], developed by our group GMMSB/LNCC, has obtained promising results in comparative studies with other well established docking programs for predicting experimental binding modes, considering diverse molecular targets and chemical classes of compounds. The DockThor Portal was developed to enable the use of the docking program by the scientific community using the computational facilities provided by the SINAPAD Brazilian high-performance platform and the supercomputer Santos Dumont. Furthermore, we recently developed several scoring functions with protein-ligand interaction-driven features trained with machine learning techniques for predicting binding affinities of protein-ligand complexes. Such new scoring functions will be available on the free portal for large-scale virtual screening experiments, the DockThor-VS.

METHODS. The DockThor program has implemented a grid-based method that employs a steady-state genetic algorithm for multiple solutions as the search engine and the MMFF94S force field as the scoring function for pose prediction. The DockThor-VS portal provides the major steps for ligand and protein preparation, being possible to change the residues protonation states and to define the degree of flexibility of the ligand. The user can also customize the main parameters of the grid box and the genetic algorithm. Recently, we developed general and specific scoring functions for target-classes, the last to account for binding characteristics associated with a target class of interest, focusing on proteases, kinases and protein-protein interactions complexes (PPIs). The scoring functions were derived using linear regression (MLR) and more sophisticated machine learning techniques for nonlinear problems using the PDBbind refined set 2013 (N = 2959) for training and testing. Currently, the affinity prediction implemented in the DockThor-VS portal is given by the linear general scoring function. Guest users are able to submit virtual screening experiments within a limit of 100 compounds, while registered users with approved projects are able to submit up to 1000 compounds per job.

DISCUSSION AND RESULTS. In the DockThor-VS portal, the docking results are automatically analyzed and clustered by an internal analysis tool. The parameters of the analysis step may be also customized by the user, such as the number of binding poses shown and comparing them with a reference conformation of the ligand through the RMSD calculation. The DockThor program obtained very satisfactory results in redocking experiments using benchmarking datasets, achieving performances of 78%, 83.33% and 78% in the Astex diverse (N = 85), Iridium-HT (N = 120) and PDBbind 2013 core set (N = 195) for the top-ranked energy pose, respectively. Furthermore, our scoring functions obtained promising performances when evaluated in both experimental and docked structures, with the best one achieving a high correlation with measured binding data (R = 0.705), and the linear-general model obtaining a competitive performance (R = 0.602) when compared with the state-of-the-art linear scoring functions.

CONCLUSION. The competitive performance of the DockThor program for binding mode prediction and the accuracy of the affinity functions recently developed encouraged us to develop the portal DockThor-VS as a free and reliable tool for virtual screening. The portal utilizes the computational facilities provided by the SINAPAD Brazilian high-performance platform and the petaflop supercomputer Santos Dumont. The DockThor-VS portal is freely available for the scientific community at the address www.dockthor.lncc.br.

References

1) Guedes, I. A., de Magalhães, C. S., and Dardenne, L. E. (2014). Receptor–ligand molecular docking. Biophys. Rev. 6, 75–87. doi:10.1007/s12551-013-0130-2
2) de Magalhães, C. S., Almeida, D. M., Barbosa, H. J. C., and Dardenne, L. E. (2014). A dynamic niching genetic algorithm strategy for docking highly flexible ligands. Inf. Sci. 289, 206–224. doi:10.1016/j.ins.2014.08.002

INHIBITORS OF THE RAS CONVERTING ENZYME RCE1 DISRUPTS RAS LOCALISATION IN HUMAN CELLS

Shahienaz Hampton (1), Idrees Mohammed (1), Louise Ashall (1), Emily Hilderbrandt (2), Robert Kutlik (3), Surya Manandhar (2), Brandon Floyd (3), Haley Smith (1), Jonathan Dozier (4), Mark Distefano (4), Walter Schmidt (2), Timothy Dore (1,3)

1) New York University Abu Dhabi, Abu Dhabi, UAE
2) Department of Biochemistry and Molecular Biology, University of Georgia, Athens, USA
3) Department of Chemistry, University of Georgia, Athens, USA
4) Department of Chemistry, University of Minnesota, Minneapolis, USA

Mutations in the Ras family of proto-oncogenes resulting in constitutively active Ras are implicated in 20-30% of human cancers.Inhibition of Ras maturation is therefore considered to be a potential anticancer strategy. The Ras converting enzyme (Rce1), an integral membrane protein, mediates CaaX proteolysis, a key modifying step required for Ras maturation and localization. There has been limited development of Rce1 inhibitors, which would greatly aid in the investigation of the physiological role of Rce1 in Ras regulation. NSC1011, a previously reported inhibitor of Rce1, identified in a medium throughput assay, was used as a starting point to develop a small library of new compounds. These exhibit moderate potency and improved selectivity against the human Rce1 (*Hs*Rce1) and were shown to induce EGFP-Ras isoform mislocalisation from the plasma membrane in a human colon carcinoma cell line. Importantly, several of these analogues were also shown to mislocalise EGFP-K-Ras more effectively than a known farnesyl transferase inhibitor (FTI). To identify new scaffolds, we are using computational methods to virtually screen chemical libraries to identify alternate starting points. These may be used to further develop new analogues and further increase the potency of inhibitors towards *Hs*Rce1.

DEHYDROABIETIC ACID DERIVATIVES TARGET BACTERIAL BIOFILMS

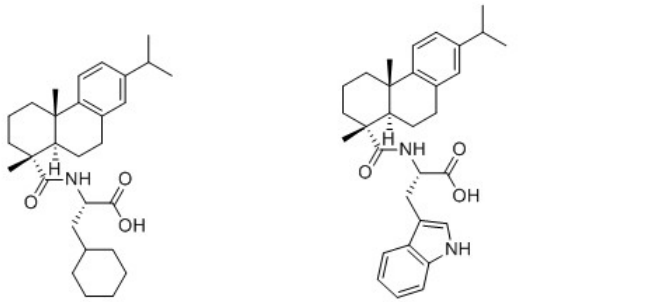
Ghada Hassan (1), Leena Keurulainen (1), Mikko Vahermo (1), Suvi Manner (2), Malena Skogman (3), Pia Vuorela (3), Adyary Fallarero (3), Jari Yli-Kauhaluoma (1), Vânia Moreira (1,4)

1) Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Viikinkaari 9, 00790 Helsinki, Finland
2) Pharmaceutical Sciences Laboratory, Faculty of Science and Engineering, Abo Akademi University, BioCity, Artillerigatan 6 A, FI-20520 Turku, Finland
3) Pharmaceutical Biology, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5 E, FI-00014 Helsinki, Finland
4) Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom

Bacterial biofilms represent a major threat due to their remarkable resistance to conventional antibiotics and involvement in hospital-acquired infections (HAIs).[1] For example, hospital-acquired pneumonia has been related to a mortality rate of 70% and above, either directly or by contributing to other factors. In addition, other HAIs lead to 7-9 additional days of hospitalization which causes a worldwide financial burden.

Given the facts, it is necessary to synthesize new effective anti-biofilm agents that can inhibit biofilm formation and/or kill established biofilms. Recently, our group discovered a new class of hybrid compounds using dehydroabietic acid, a diterpenoid from coniferous trees, as a starting material. Two of the designed compounds are the most potent abietane-type anti-biofilm agents reported so far in literature (Figure. 1), targeting staphylococci including *Staphylococcus aureus*.[2]

The results discovered showed that diterpenoids from coniferous trees represent an excellent starting material for anti-biofilm agents. The ongoing research in our lab focuses on exploring and optimizing more diterpenoid derivatives to target bacterial biofilms. Standard structural elucidation techniques are used to confirm the structure of the synthesized compounds.



N-(Abiet-8,11,13-trien-18-oyl) cyclohexyl-l-alanine *N*-(Abiet-8,11,13-trien-18-oyl) d-tryptophan

Figure 1.The two most potent anti-biofilm and antimicrobial abietane-type derivatives reported so far in literature.[2]

References

1) MASTERTON, R.G., GALLOWAY, A., FRENCH, G., STREET, M., ARMSTRONG, J., BROWN, E., CLEVERLEY, J., DILWORTH, P., FRY, C., GASCOIGNE, A.D., KNOX, A., NATHWANI, D., SPENCER, R. and WILCOX, M., 2008. Guidelines for the management of hospital-acquired pneumonia in the UK: report of the working party on hospital-acquired pneumonia of the British Society for Antimicrobial Chemotherapy. J. Antimicrob. Chemother., 62(1), pp. 5-34.
2) MANNER, S., VAHERMO, M., SKOGMAN, M.E., KROGERUS, S., VUORELA, P.M., YLI-KAUHALUOMA, J., FALLARERO, A. and MOREIRA, V.M., 2015. New derivatives of dehydroabietic acid target planktonic and biofilm bacteria in Staphylococcus aureus and effectively disrupt bacterial membrane integrity. Eur. J. Med. Chem., 102, pp. 68-79.

IDENTIFICATION OF SUB-MICROMOLAR LIGANDS OF MCL-1

F. Berst (1), J. Blank (1), W. Breitenstein (1), P. Floersheim (1), A. Floersheimer (1), C. Freslon (1), P. Furet (1), P. Grosche (1), K. Herlihy (2), J. Hinrichs (1), B. Hurley (2), R. Kulathila (2), A. Marzinzik (1), J. Ottl (1), G. Paris (2), D. Porter (2), M. Schaefer (1), X. Xie (2)

1) Novartis Institutes for BioMedical Research, Novartis Campus, CH-4056 Basel, Switzerland, juergen.hinrichs@novartis.com
2) Novartis Institutes for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139, United States

Mcl-1 is a Bcl-2 family member and critical negative regulator of apoptosis. Mcl-1 is overexpressed in many cancers and contributes to tumor progression and chemo-resistance by binding to and sequestering pro-apoptotic BH3 domain-containing proteins. Disrupting Mcl-1/BH3 protein interactions with a small molecule is predicted to initiate apoptosis or sensitize cancer cells to cytotoxic inducers of apoptosis.

While a number of Mcl-1 inhibitors have been described,[1] new chemical matter is still needed. Here we describe the discovery of a new series of Mcl-1 ligands and their structure-based optimization to sub-micromolar potency.

References

1) J. P. Taygerly, D. W. Robbins, Med. Chem. Reviews 2017, 52, 263-277.

SYNTHESIS AND IN VITRO EVALUATION OF HYDROXYFATTY ACIDS AND SYNTHETIC ANALOGUES ON GPR84

Mads Holmgaard Kaspersen (1,3), Laura Jenkins (2), Julia Dunlop (2), Graeme Milligan (2), Trond Ulven (1,3)

1) Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark
2) Centre for Translational Pharmacology, Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
3) Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

The orphan G protein-coupled receptor 84 (GPR84) was discovered over a decade ago but has only recently been linked to a variety of inflammatory diseases and Alzheimer-type dementia.[1] GPR84 is a receptor for medium-length fatty acids (MCFAs) and to a greater extent their 2- and 3-hydroxylated counterparts but its pathophysiological roles have not yet been fully clarified. [2,3] Despite reported agonists, modulators and antagonists for GPR84, the current research in the field is at an early stage.[4] Here we present the synthesis of hydroxyfatty acids and synthetic analogues of these and their activity on GPR84.

References

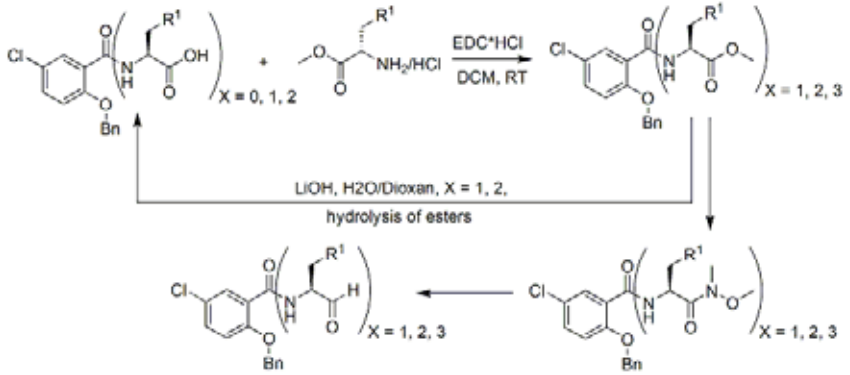
1) Wittenberger, T.; Schaller, H. C.; Hellebrand, S. Journal of Molecular Biology 2001, 307, 799.
2) Suzuki, M.; Takaishi, S.; Nagasaki, M.; Onozawa, Y.; Iino, I.; Maeda, H.; Komai, T.; Oda, T. Journal of Biological Chemistry 2013, 288, 10684.
3) Kaspersen, M. H.; Jenkins, L.; Dunlop, J.; Milligan, G.; Ulven, T. MedChemComm 2017, 8, 1360.
4) Milligan, G.; Shimpukade, B.; Ulven, T.; Hudson, B. D. Chem Rev 2016.

NOVEL DIPEPTIDE SALICYLAMIDES, THEIR BIOLOGICAL ACTIVITIES, CYTOTOXICITY AND PROTEASOMAL INHIBITION ACTIVITY

Aleš Imramovský (1), Pratibha Magar (1), Karel Pauk (1), Eva Řezníčková (2), Radek Jorda (2), Vladimír Kryštof (2)

1) Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 53210 Pardubice, Czech Republic
2) Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University & Institute of Experimental Botany ASCR, Šlechtitelů 27, 78371 Olomouc, Czech Republic

Combination of salicylic moiety, amino acids and basic anilines form specific type of compounds are able to induce apoptosis in cancer cell lines *in vitro*[1] as well as sensitizes cancer cells to metabolic stress by disrupting actin cytoskeleton and inhibiting autophagic flux.[2] Under base of this results a new salicylamide dipeptides were designed, synthesized and fully characterized. The biological screening of antiproliferative and cytotoxic activities in cancer cell lines *in vitro* for targeted molecules as well as for chosen intermediates was provided.



Biological evaluation shown interesting results for antiproliferative properties. Several intermediates were modified to obtain new compounds with various functional groups. These compounds were tested for their inhibition of protesomal activity. Chosen members shown significant inhibition of proteasome.[3] This group was further extended with novel functional groups, which were tested. Results will be discussed in presented poster.

Acknowledgements: the authors wish to acknowledge for the financial support to the project GA 18-03847S, institutional financial support by the Ministry of Education youth and sports of the Czech Republic. Also to SG-project of the Faculty of Chemical Technology, *University of Pardubice.*

References

1) Imramovský A., Jorda R., Pauk K., Řezníčková E., Dušek J., Hanusek J., Kryštof V. Substituted 2-hydroxy-N-(arylalkyl)benzamides induce apoptosis in cancer cell lines. Eur. J. Med. Chem. 2013, 68, 253-259.
2) Páchniková, G.; Uldrijan, S.; Imramovský, A.; Kryštof, V.; Slaninová, I. Substituted 2-hydroxy-N-(arylalkyl)benzamide sensitizes cancer cells to metabolic stress by disrupting actin cytoskeleton and inhibiting autophagic flux. Toxicology in Vitro 2016, 37, 70-78.
3) Jorda, R.; Dušek, J.; Řezníčková, E.; Pauk, K.; Magar, P. P.; Imramovský, A.; Kryštof, V. Synthesis and proteasomal activity of novel O-benzyl salicylamide-based inhibitors built from leucine and phenylalanine. European Journal of Medicinal Chemistry 2017, 135, 142–158

EFFECT OF THE TRIAZOLOPYRIMIDINE COMPOUND ON ENDOGENOUS H2S LEVELS IN LUNG TISSUE HOMOGENATES: A SCAFFOLD HOPPING APPLICATION ON RESVERATROL DERIVATIVES

Huseyin Istanbullu (1), Elif Alancay (2), Umran Kizrak (2), Gunes Coban (3), Gulnur Sevin (2), Gunay Yetik-anacak (2)

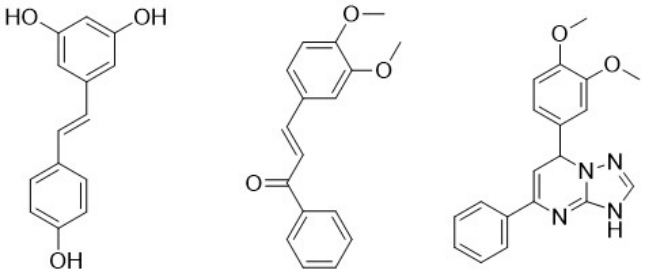
1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Izmir Katip Celebi University, Izmir, Turkey
2) Department of Pharmacology, Faculty of Pharmacy, Ege University, Izmir, Turkey
3) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ege University, Izmir, Turkey

Hydrogen sulfide (H_2S), an important gasotransmitter, vasodilator and neuromodulator agent, is generated by cystathionine-gamma-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulphurtransferase (MPST) enzymes in mammals [1, 2]. The mitochondrial enzyme MPST is an endogenous source of H_2S in various cells and tissues. Previously, we showed resveratrol's (3,5,4'-trihydroxy-*trans*-stilbene) effect on H_2S formation under oxidative stress[3]. The biological activity of resveratrol may be limited by poor absorption and first-pass metabolism: only low plasma concentrations of resveratrol are seen following oral administration, and metabolism to glucuronide and sulfate conjugates is rapid.

In the view of this data, synthetic analogs and isosteres are the subjects of research for increased bioavailability of resveratrol derivatives. For this purpose, we tested a compound (H2) from our previous studies on chalcone family; with a linker bond that connects the two benzenes (as a results of scaffold hopping approaches) and their triazolopyrimidine derivative (H1; restricted turnover of the benzene rings with cyclization and increased heteroatoms for the possible interactions)[4].

We confirmed that the addition of substrate L-cysteine (10 mM) together with cofactor pyridoxal phosphate (10 mM) causes an increase in endogenous H_2S formation in mice lung homogenates. Further, we showed that aminooxyacetic acid (AOAA)(10 mM) the inhibitor of H_2S synthesis enzymes CSE and CBS inhibits endogenous H_2S formation in lung homogenates significantly by Unisense H_2S microsensor time dependently. Finally, we found that incubation of resveratrol derivative H1 (100 μM, 30 minutes) stimulates L-cysteine-induced endogenous H_2S formation (p2S inhibitor AOAA confirmed that the increase in H_2S produced by H1 with L-cysteine was endogenous(p2S synthesis in lung.

These results reveal the activity potential of the thiazolopyrimidine scaffold and it is necessary to focus on this scaffold; more derivatives will be synthesized and detailed SAR will be generated.



Resveratrol H2 H1

References

1) Modis, K.; Asimakopoulou, A.; Coletta, C.; Papapetropoulos, A.; Szabo, C. Biochemical and Biophysical Research Communications, 2013, 433, 401–07
2) Yetik-Anacak, G.; Dereli, M. V.; Sevin, G.; Ozzayim, O.; Erac, Y.; Ahmed, A. J Sex Med, 2015, 12, 2004–12
3) Yetik-Anacak, G.; Sevin, G.; Ozzayim, O.; Dereli, M. V.; Ahmed, A. Vascular Pharmacology, 2016, 87, 76–82.
4) Srinivasan, B.; Rodrigues, J. V.; Tonddast-Navaei, S.; Shakhnovich, E.; Skolnick, J. ACS Chem. Biol., 2017, 12(7), 1848-57.

INHIBITORY ACTIVITIES OF CONSTITUENTS FROM GLECHOMA HEDERACEA VAR. LONGITUBA ON 3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE

Dong Hyeon Kang (1,2), Hyoung Ja Kim (1), Hyunbeom Lee (1), Dae Sik Jang (2), Yong Sup Lee (2), Changbae Jin (1)

1) Molecular Recognition Research Center, Future Convergence Research Division, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 02792, Korea
2) College of Pharmacy, Kyung Hee University, Kyungheedae-ro 26, Dongdaemun-gu, Seoul 02447, Korea

Glechoma hederacea var. *longituba* (Labiatae), a perennial vine plant, has been used for centuries in traditional oriental medicine for the treatment of cholelithiasis, urolithiasis, dropsy, asthma, bronchitis, cold and inflammation (1). Moreover, it has been reported that its extract produces weight loss and reduction in blood sugar and lipid levels (2). Since the inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is known to be one of the most effective approaches for treating hypercholesterolemia and eventually cardiovascular diseases (3), the present study was aimed to search for HMGCR inhibitory constituents from *G. hederacea* var. *longituba*. The methanolic extract from the stems and leaves of the plant was fractionated with CH_2Cl_2, ethyl acetate, *n*-butanol and H_2O. Only the ethyl acetate soluble fraction showed potent inhibitory effect on HMGCR (IC_{50} = 37.7 μg/mL). To identify HMGCR inhibitory components, various chromatographic separations of the ethyl acetate soluble fraction led to the isolation of four known rosmarinic acid derivatives, two flavonoids, nine triterpenes and a sterol. All isolated substances were evaluated for their inhibitory activities on HMGCR. Among them, rosmarinic acid methyl ester and ursolic acid, a major component, showed the most potent inhibitory activities with IC_{50} values less than 100 μM. In addition, pygenic acid A, pygenic acid B and maslinic acid showed relatively weak inhibitory activities. The results suggest that *G. hederacea* var. *longituba* has potential to be a new source of agents for controlling cholesterol biosynthesis.

References

1) Kim JP, Song SB, Lee IS, Kim YH, Yoo ID, Ryoo IJ, Bae KH. Anti-inflammatory activity of constituents from Glechoma hederacea var. longituba. Bioorg Med Chem Lett 2011;21:3483-3487.
2) Zhu W, Wang H, Zhang Y, Sun P, Li B, Xu Z, Jiang H, Chen K. Glechoma longituba extract, preparation method for same, and use thereof in sugar reduction, weight loss, and lipid reduction. WO Patent 2015139603 A1 20150924.
3) Pallottini V. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase modulator: toward age- and sex-personalized medicine. Expert Opin Ther Pat 2015;25:1079-1083.

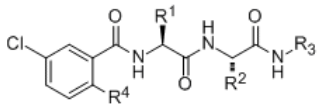
P365

SYNTHESIS AND BIOLOGICAL CHARACTERIZATION OF NOVEL SALICYLAMIDES WITH POTENTIAL ANTICANCER ACTIVITY

Radek Jorda (1), Pratibha Magar (2), Aleš Imramovský (2), Denisa Hendrychová (1), Vladimír Kryštof (1)

1) Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacky University & Institute of Experimental Botany ASCR, Šlechtitelů 27, 78371 Olomouc, Czech Republic
2) Institute of Organic Chemistry and Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic

Our previous studies revealed that 2-hydroxy-N-(arylalkyl)benzamides induce potently apoptosis in cancer cell lines [1,2,3,4]. Chemically, these compounds consist of a short dipeptide or tripeptide chain bonded to *O*-benzyl salicylic acid on the N-terminus and carrying various functional groups on the C-terminus; therefore we designate them as pseudopeptides. Because their mechanism of proapoptotic activity was unknown, we applied several independent approaches (including chemoinformatics and small scale phenotypic high content screening) with the aim to identify molecular targets in cancer cells. We have shown that some compounds disrupt the dynamics of actin cytoskeleton, affecting processes essential for the maintenance and expansion of tumours such as cell adhesion, motility, proliferation, vesicular transport, and autophagic flux [3]. Newly, we prepared 32 salicylamides and screened them for antiproliferative activity in 4 cancer cell lines *in vitro*. Eight compounds showed single-digit micromolar GI_{50} and we chose the most potent candidate that was evaluated in more detail. Our candidate reduced proliferation and induced apoptosis in the melanoma cell line G361 in a dose-dependent manner, as shown by decrease in 5-bromo-2'-deoxyuridine incorporation and increase in several apoptotic markers, including subdiploid population increase, activation of caspases and site-specific poly-(ADP-ribose)polymerase (PARP) cleavage. Here, we describe an innovative synthesis of novel salicylamides and their anticancer activities in vitro.



R^1, R^2 = Amino acids
R^3 = further substitution
R^4 = O-Benzyl, OH

Fig. 1 General structure of triamides

This research was supported by the Czech Science Foundation (GA 18-03847S) and further by grants No. LO1204 (Sustainable development of research in the Centre of the Region Haná) from the National Program of Sustainability I, MEYS, IGA_PrF_2017_013 and IGA_PrF_2018_006.

References

1) Eur J Med Chem. 2013 Oct;68:253-9.
2) Eur J Med Chem. 2017 Jul 28;135:142-158.
3) Toxicol In Vitro. 2016 Dec;37:70-78.
4) Letters in Drug Design & Discovery 14 (6), 662-671

P367

HOW TO MAKE NEW OUT OF OLD?

Laurence Jung (1), Jean-Marie Contreras (1), Christophe Morice (1), Marie-Louise Jung (1), Bruno Didier (2)

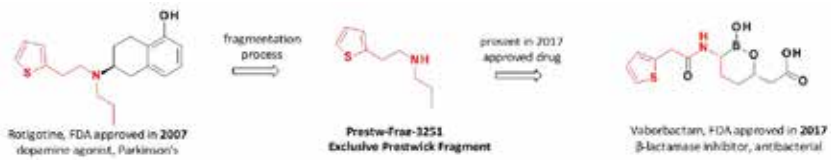
1) Prestwick Chemical, 220 bd Gonthier d'Andernach, 67400 ILLKIRCH, FRANCE
2) University of Strasbourg, therapeutic innovation laboratory, UMR7200 CNRS, Faculty of Pharmacy, 74 route du Rhin, 67400 Illkirch, FRANCE

Developing a brand-new drug takes an enormous amount of time, money and efforts. However, there is a wide consensus that new drugs in many therapeutic areas are urgently needed meaning that it is crucial to advance strategies to reduce time frame, decrease costs and improve success rates.

Sir James Black, winner of the 1988 Nobel Prize in Physiology and Medicine, famously stated that: “the most fruitful basis for the discovery of a new drug is to start with an old drug”. Disillusioned with HTS and struggling to bring new chemical entities to market, many companies are turning back to Sir James’ wisdom.[1] In this perspective, a range of valuable tools based on marketed drugs have been developed at Prestwick to support strategies such as:

- **Drug repurposing**

- **Fragment-Based Drug Discovery (FBDD)**



- **Selective Optimization of a Side Activity (SOSA approach)**

The design, properties and advantages of Prestwick tools are presented and discussed in the present poster

References

1) Innovations in Pharmaceutical Technology, issue 42, 10-12

THE COMPUTER-AIDED SEARCH FOR NOVEL CHEMOSENSITIZERS OF STAPHYLOCOCCUS AUREUS MDR STRAINS AMONG 3-AMINEALKYL DERIVATIVES 5-ARYLIDENEIMIDAZOL-4-ONE

Aneta Kaczor (1), Karolina Witek (1,2,3), Ewa Żesławska (4), Sabina Podlewska (5), Wojciech Nitek (6), Katarzyna Kieć-Kononowicz (1), Markus Bischoff (3), Claus Jacob (2), Jadwiga Handzlik (1)

1) Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University, Medical College, ul. Medyczna 9, 30-688 Cracow, Poland
2) Bioorganic Chemistry, School of Pharmacy, University of Saarland, Campus B2.1, D-66123 Saarbrüecken, Germany
3) Institute of Medical Microbiology and Hygiene, Saarland University, D-66421, Homburg, Germany
4) Pedagogical University, Department of Chemistry, ul. Podchorążych 2, 30-084 Kraków, Poland
5) Department of Medicinal Chemistry Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Krakow, Poland
6) Faculty of Chemistry Jagiellonian University, Ingardena 3, PL 30-060 Krakow, Poland

The progressive evolution of bacterial multi-drug resistance (MDR) to the most available antibiotics is the significant worldspread health problem. One of the approaches is to find new chemosensitizers able to inactivate PBP2 and PBP2a proteins which display essential role in resistance of *S.aureus* strains. These compounds, also called adjuvants, should not have antibacterial activity itself. In previous studies several 5-arylidenoimidazolones were obtained that displayed significant action in other potential antibiotic adjuvant target [1]. Following the aforementioned concept, the purpose of these studies included: (i) computer-aided design of new potential adjuvants, (ii) chemical synthesis, (iii) crystallographic studies, (iv) biological assays, (v) docking and molecular dynamic simulations. New arylidenoimidazolones with amine at position 3 were synthesized in the 4-step synthesis pathway, i.e. Knoevenagel condensation, S-methylation, condensation with methylpiperazinepropylamine and Dimroth rearrangement, which was confirmed in crystallographic studies. Final products were investigated in the microbiological studies, in two methicillin susceptible *S.aureus* (MSSA) and seven methicillin resistance *S.aureus* strains. Their adjuvant activity was investigated in 1/4 of their intrinsic minimal inhibitory concentration (MIC) to avoid their antibacterial activity. Compound **1** displayed significant (up to 64-fold) reduction of oxacillin MIC in MRSA strains. The highest (192-fold) reduction of oxacillin MIC showed compound **2** in MM-O021 (MRSA) strain. Reduction of MIC for ampicillin was lower than for oxacillin and up to 24-fold. Compound **3** did not display potent antibiotic adjuvant action. None of new compounds displayed significant action with erythromycin, ciprofloxacin and vancomycin in *S.aureus* strains. All the compounds were docked to the crystal structure of PBP2a protein in two modes: (I) both to the active and allosteric site, (II) to allosteric site and to the active site, where there was oxacillin fitted. Docking studies confirmed that **3** did not form pi-pi interaction, which is present with remaining products. Molecular dynamic simulations demonstrate that active compounds (**1** and **2**) were more stable in the active site. These results give hope to find an arylidenoimidazolone adjuvant for antibiotics therapy. Partly supported by grant of Polish Ministry of Science no 0169/DIA/2017/46.

References

1) J. Bohnert et al. Antimicrob. Agents Chemother. 60 (2016) 1974-83.

NOVEL BETULINIC CARBOXAMIDES AS POTENTIAL CYTOTOXIC AGENTS

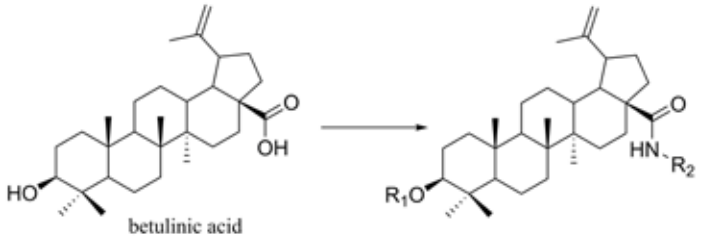
Michael Kahnt, Lucie Heller, René Csuk

Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

Up to now, great progress has been made in cancer therapy and several breakthroughs have been recorded in recent years.[1] Nevertheless, some types of cancer can still not be cured successfully. Therefore, the development of new chemotherapeutics is still of great importance.

Since many natural products show a wide range of pharmacological properties, including antiviral, antimalarial, anti-inflammatory and antitumor activity, they are considered as ideal lead structures for the development of new bioactive substances. One class of pharmacologically interesting natural products are triterpenes, which also exhibit cytotoxic properties among several other biological activities.[2]

In the following we used the easily accessible, natural occurring triterpenoid, betulinic acid as starting material for the synthesis of novel cytotoxic agents. More than 10 different betulinic carboxamides were prepared and biologically screened to evaluate their cytotoxic activity against several human tumor cell lines using SRB-assays. Some derivatives showed remarkable cytotoxic properties, as indicated by EC_{50} values lower than 1 µM.



References

1) J. Heymach et al., J. Clin. Oncol. 2018, DOI: 10.1200/JCO.2017.77.0446
2) M. N. Laszczyk, Planta med. 2009, 75 (15), 1549-1560.

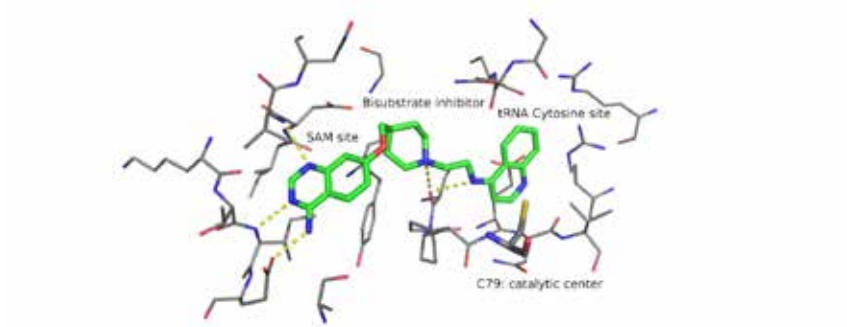
RNA METHYLATION IN EPIGENETIC GENE REGULATION: STRUCTURE-BASED DESIGN OF DNMT2 INHIBITORS

Christian Kersten, Tim Fischer, Edmond Fleischer, Tanja Schirmeister, Mark Helm

Institute of Pharmacy and Biochemistry, Staudinger Weg 5, Johannes Gutenberg University, 55128 Mainz, Germany

Differently from the closely related enzymes Dnmt1 and Dnmt3, Dnmt2 is not a DNA but an **RN**A **m**ethyl**t** ransferase that catalyzes the transfer of a methyl group from the cofactor *S*-adenosylmethionine (SAM) to its substrates. In human beings it has a high specificity for cytosine 38 of $tRNA^{Asp}$ (m^5C), but further RNA (and DNA) substrates were found in other organisms. Recent publications identified altered expression levels of tRNA methyltransferase activity and an upregulation of Dnmt2 (mutants) in various tumor cells. Therefore, Dnmt2 is not only relevant for the understanding of the epigenetic role of RNA methylation, but also a potential target for cancer treatment.[1]

In the development of Dnmt2 inhibitors, we use different approaches of structure based design. Bisubstrate inhibitors,[2] that were designed for closely related Dnmt1 and Dnmt3A, adress both binding pockets of RNA and the cofactor and were selected as a starting point for Dnmt2 inhibitor design. Another approach focuses on SAM-site inhibitors of the Catechol-*O*-´Methyltransferase (COMT).[3] Additionally to these drug repurposing strategies, a virtual screening of the commercial chemical space was conducted to identify completely novel scaffolds. The subsequent optimization aims on the combination of these different inhibitor classes and the introduction of an electrophilic warhead to increase affinity and residence time by covalent(-reversible) binding to the catalytic cysteine 79 of the target.[4]



References

1) Jeltsch, A.; Ehrenhofer-Murray, A.; Jurkowski, T. P.; Lyko, F.; Reuter, G.; Ankri, S.; Nellen, W.; Schaefer, M.; Helm, M. Mechanism and Biological Role of Dnmt2 in Nucleic Acid Methylation. RNA Biology. 2017, pp 1108–1123.
2) Halby, L.; Menon, Y.; Rilova, E.; Pechalrieu, D.; Masson, V.; Faux, C.; Bouhlel, M. A.; David-Cordonnier, M. H.; Novosad, N.; Aussagues, Y.; et al. Rational Design of Bisubstrate-Type Analogues as Inhibitors of DNA Methyltransferases in Cancer Cells. J. Med. Chem. 2017, 60, 4665–4679.
3) Lerner, C.; Jakob-Roetne, R.; Buettelmann, B.; Ehler, A.; Rudolph, M.; Sarmiento, R. M. R. Design of Potent and Druglike Nonphenolic Inhibitors for Catechol O-Methyltransferase Derived from a Fragment Screening Approach Targeting the S-Adenosyl- L -Methionine Pocket. J. Med. Chem. 2016, 59, 10163–10175.
4) Schirmeister, T.; Kesselring, J.; Jung, S.; Schneider, T. H.; Weickert, A.; Becker, J.; Lee, W.; Bamberger, D.; Wich, P. R.; Distler, U.; et al. Quantum Chemical-Based Protocol for the Rational Design of Covalent Inhibitors. J. Am. Chem. Soc. 2016, 138, 8332–8335.

NON-SYMMETRIC HETEROCYCLIC NS5A INHIBITORS FOR THE TREATMENT OF HEPATITIS C VIRUS

Hwa Li (1), Youjin Jeong (2), Jeong Tae Lee (2), Hee Sun Kim (3), Kyung Il Choi (1), Sung Key Jang (3), Gyochang Keum (1)

1) Korea Institute of Science and Technology (KIST), Seoul, Republic Korea
2) Hallym University, Chuncheon, Republic Korea
3) Pahang University of Science and Technology (POSTECH), Pohang, Republic of Korea

GungHepatitis C virus (HCV) infection is a leading cause of acute and chronic liver disease. About 180 million people worldwide are infected with HCV, which can lead to liver cirrhosis, hepatocellular carcinoma and consequently death. In addition to NS5B and NS3/4A protease inhibitor, NS5A which is a multifunctional protein essential for HCV replication was proved to be and a promising target for the treatment of HCV.[1] Several NS5A inhibitors such as BMS-790052 were launched recently and some were under clinical trials. We prepared a series of symmetric and non-symmetric heterocyclic NS5A inhibitors possessing new scaffold and evaluated their activities against HCV cell lines. Among them, several compounds showed potent anti-proliferative activity against HCV cell lines, less than 5 pM EC_{50}.Non-symmetric analogues showed broader genotype activity, and higher activity against resistant strains. Herein, we will present anti-HCV activity of the non-symmetric hit compounds against various HCV genotypes and resistant strains mutated at L31V, Y93H of NS5A.

References

1) Meanwell, N. A. J. Med. Chem., 2016, 59, 16, 7311–7351

A NOVEL ORALLY ACTIVE INVERSE AGONIST OF ESTROGEN-RELATED RECEPTOR GAMMA (ERRγ), DN200434, ENHANCES SODIUM IODIDE SYMPOTER FUNCTION

Kim Jina (1), Chin Jungwook (1), Jeon Yong Hyun (2), Cho Sung Jin (1)

1) New Drug Development Center, DGMIF, 80, Cheombok-ro, Dong-gu, Daegu 41061, Korea
2) Laboratory Animal Center, DGMIF, 80, Cheombok-ro, Dong-gu, Daegu 41061, Korea

New strategies to restore sodium iodide symporter (NIS) expression and function in anaplastic thyroid cancers (ATCs) that are refractory to radioiodine therapy are urgently required. Based on the structural motifs of GSK5182, we have successfully demonstrated the synthesis of compound libraries that are more selective against ERRγ inverse agonists with improved absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles. Moreover, we have broadened the therapeutic scope of these compounds based on our findings that GSK5182 facilitates the responsiveness to radioiodine therapy by modulating NIS function in ATC cells via ERRγ and MAP kinase signaling pathway. Herein, we have validated the most promising ERRγ inverse agonist, DN200434, from our previous studies for its ability to enhance NIS protein function, which is a key protein for radioiodine therapy, and improve susceptibility to the therapy in *in vitro/vivo* ATC models.

DISCOVERY OF BENZOPYRAN DERIVATIVES AS A NOVEL CLASS OF 11BETA-HYDROXYSTEROID DEHYDROGENASE TYPE1(11BETA-HSD1) FOR THE TREATMENT OF DIABETES

Shinae Kim (1,2), Sujeong Kim (1), YeEun Noh (1), Taeho Lee (1)

1) College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, 80 Daehak-ro, Buk-gu, Daegu 41566, Korea
2) New Drug Development Center 80, Cheombok-ro, Dong-gu, Daegu 41061, Korea

11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) has attracted considerable attention as a promising therapeutic target for the treatment of Type 2 diabetes. We discovered a novel class of 11beta-HSD1 inhibitors bearing benzopyran scaffold by high-throughput screening. The initial hit exhibited a good in vitro and selective inhibitory activity against 11beta-HSD1. Further optimization was performed in an effort to identify various potent compounds. Compound 1 is discovered as a very potent with an IC_{50} value of 42.6 nM.

P375

NOVEL TOOLS IN DRUG DISCOVERY: LISICA AND BOBER

Samo Lešnik, Blaž Škrlj, Nika Eržen, Janez Konc

National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

We developed two novel tools useful in the process of drug discovery. LiSiCA is a ligand-based virtual screening software implemented as a freely available user friendly PyMOL plugin. BoBER is a method for identifying and implementing bioisosteric and scaffold hopping replacements, also freely available as a web tool. LiSiCA was successful in obtaining novel compounds with diverse scaffolds which are active upon the butyrylcholinesterase enzyme and tool-like receptor 7. With BoBER we successfully optimized a covalent inhibitor of the monoamine oxidase B enzyme. Both tools are available at http://insilab.org.

P376

CARBON MONOXIDE-RELEASING MOLECULES AS AN ALTERNATIVE APPROACH TOWARDS THE THERAPY OF TRIPLE-NEGATIVE BREAST CANCER

Malamati Kourti (1,2), Andrew D. Westwell (2), Andrea Brancale (2), Jun Cai (1)

1) Cardiff-China Medical Research Collaborative, School of Medicine, Cardiff University, Heath Park, CF14 4XN, Cardiff, Wales, UK
2) School of Pharmacy & Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Ave, CF10 3NB, Cardiff, Wales, UK

Triple-negative breast cancer (TNBC) is defined by the lack of expression of the oestrogen and progesterone receptors and the overexpression of HER-2[1]. Recently, carbon monoxide (CO) was found to behave as an important endogenous signalling molecule and interestingly, to suppress VEGF receptor-2 (VEGFR-2) and protein kinase B (Akt) phosphorylation[2]. Given that anti-angiogenic drugs exist as one of the few available targeted therapies against TNBC, we want to enhance their activity by combining them with new CO-releasing molecules (CORMs), in order to reduce the cancer-driven angiogenesis. Therefore, the aim of this project is to study the effects of CORMs on TNBC cell lines and reveal any potential anti-angiogenic properties of these molecules. New analogues will then be synthesized and evaluated in similar assays.

Four commercially available CORMs were screened for their effects against TNBC by means of cytotoxicity, cell metabolism, migration, VEGF expression, tube formation and VEGFR-2 activation assays. The results were crucial for the selection of one leading compound, which was subjected to structural modifications in order to produce 15 new analogues. A panel of two breast cancer cell lines served as a model of TNBC, namely MDA-MB-231 and MDA-MB-436, alongside the non-cancerous human epithelial breast cells MCF-10A and the human endothelial cells (ECs) HECV.

The results so far indicate that the four commercial CORMs are slightly cytotoxic against the TNBC cell lines in the μM concentration range. More specifically, 100μM of CORM-1 seem to be the most active, reducing cell viability by 60% and 40% for MDA-MB-231 and MDA-MB-436, respectively. Glycolysis levels of cancer cells are reduced, for example 100μM of CORM-3 reduce the ECAR/pg protein level of MDA-MB-231 cells up to 40% compared to control DMSO-treated cells, suggesting an interesting impairment of the cellular metabolism due to the presence of CORMs. Another interesting observation indicates the reduction of VEGF levels expressed from CORM-treated TNBC cells. This proposes a potential decrease in the angiogenic signal sent towards ECs, in order to stimulate angiogenesis. This reduction in excreted VEGF reached 61% after treatment of MDA-MB-231 with CORM-2 for 24h. Inhibition of the migratory activity of CORM-treated ECs was also observed, especially after CORM-2 and CORM-1 treatments. Finally, activation of VEGFR-2 was also shown to be affected by treatment with CORMs, especially with 100μM CORM-2 and CORM-3.

Ongoing studies need to evaluate the ability of CORMs to alter the potential of ECs to form tubes and the in vitro testing of the new analogues will be also finalized soon. From these studies, one final leading compound will be suggested and can be subsequently used for further research.

References

1) Saha, P. and Nanda, R. 2016. Concepts and targets in triple-negative breast cancer: Recent results and clinical implications. Therapeutic Advances in Medical Oncology 8(5), pp. 351-359. doi: 10.1177/1758834016657071
2) Ahmad, S. et al. 2015. Carbon monoxide inhibits sprouting angiogenesis and vascular endothelial growth factor receptor-2 phosphorylation. Thrombosis and Haemostasis 113(2), pp. 329-337. doi: 10.1160/TH14-01-0002

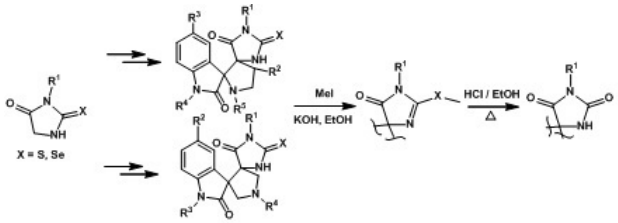
DESIGN, SYNTHESIS AND BIOLOGICAL TESTING OF NOVEL POTENT MDM2/P53 SMALL MOLECULE INHIBITORS

Kukushkin Maxim (1), Filatov Vadim (1), Novotortsev Vladimir (1), Vorobyeva Natalia (2), Skvortsov Dmitriy (1), Beloglazkina Elena (1), Zyk Nikolay (1), Majouga Alexander (1)

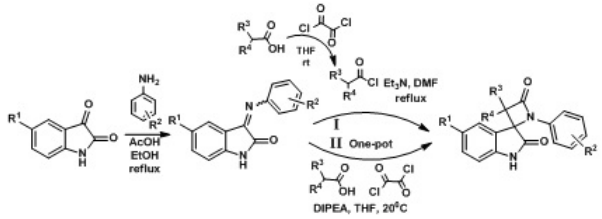
1) Moscow State University, Chemistry Dept., 119991 Moscow, Leninskie Gory, Building 1/3, GSP-1, Russian Federation
2) National University of Science and Technology MISiS, Moscow 119049, Leninskiy prosp. 4, Russian Federation

Every year the number of cases detected and the death rate from cancer are increasing, which leads to a wave of research in the medical chemistry. The wrestle with cancer, there are many approaches, however more and more popular is becoming targeted therapy that can fight oncology with much less side effects. One of the targets for this kind of therapy is the protein-protein interaction of p53-MDM2 presented in about 50% of tumors, the violation of which leads to the release of p53, which triggers to apoptosis of the tumor cells. In the course of studying this protein-protein interaction, it was found that compounds having in their structure a spiroindolinone fragment are able to show good affinity to the binding site of these proteins, which leads to activation of the target p53 [1].

In our study, we propose approaches to the synthesis of three different classes of spiroindolinones from commercially available reagents according to the reactions of 1,3-dipolar cycloaddition [2]:



as well as for different variations of Staudinger reactions:



The structure of all target molecules is proved by X-Ray data. In addition, the obtained compounds were tested on the HCT116 $p53^{(+/+)}$, HCT116 $p53^{(-/-)}$, LNCap and PC3 cell lines, and spiro-β-lactams also on BW25113 and dtolC, which led to conclusions about the structure-activity relationship.

The work was supported by the RFBR, grant number 16-33-60166

References

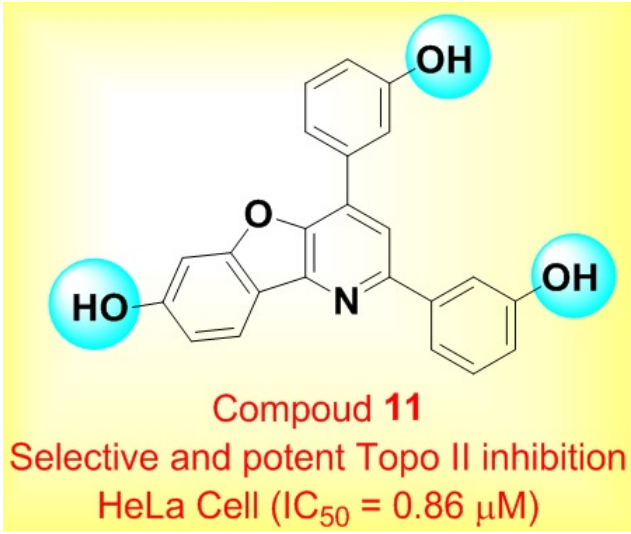
1) K. Ding, Y., et al. Structure-Based Design of Potent Non-Peptide MDM2 Inhibitors. JACS, 2005, V.127, 29, 10130-10131
2) Y. Ivanenkov, et al. Design, synthesis and biological evaluation of novel potent MDM2/p53 small molecule inhibitors. Bioorg.Med.Chem.Lett, 2015,V.25, 2, 404.

STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL BENZOFURO[3,2-b]PYRIDIN-7-OLS AS DNA TOPOISOMERASE II INHIBITORS AND ANTIPROLIFERATIVE AGENTS

Eung-Seok LEE , Aarajana SHRESTHA, Pramila KATILA , Ritina SHRESTHA , Youngjoo KWON

College of Pharmacy, Yeungnam University, Gyeongsan 38541, Korea

DNA topoisomerases are important biological target that solve the topological problems occurred during cellular processes. Topoisomerase inhibitors still remain frontline interventions for the treatment of cancer. For the development of new anticancer agents with improved activity, we have been working on pyridine derivatives which showed topo I and II inhibitory activity, and antiproliferative activity. Herein we systematically designed and synthesized a new series of sixteen, benzofuro[3,2- *b*]pyridin-7-ol derivatives containing hydroxyl moiety in 2- and/or 4-phenyl position of central pyridine and evaluated for their topo I and II inhibitory activity, and antiproliferative activity. Structure-activity relationships revealed the position of *ortho*- and *para*-hydroxyl group at 2-phenyl ring, and *meta*-hydroxyl group at 4-phenyl ring of benzofuro[3,2-*b*]pyridin-7-ol are important for potent and selective topo II inhibitory activity. Compound **11** which contains hydroxyl group at meta- position of 2- and 4- phenyl ring of benzofuropyridin-7-ol showed the most potent and selective topo II inhibition (100% inhibition at 100 μM) and antiproliferative activity (IC_{50} = 0.86 μM) in HeLa cell as compared to all the tested positive controls. Further mechanistic study on compound **11** is underway, and the results will be presented.

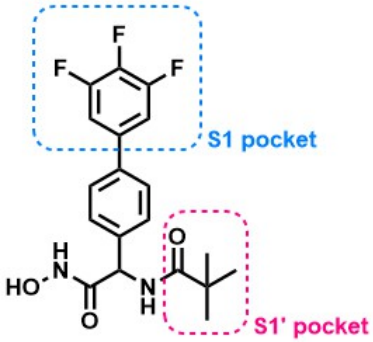


REPURPOSING AN ANTI-MALARIAL AGENT AS A NOVEL AMINOPEPTIDASE N INHIBITOR FOR THE TREATMENT OF CANCER

Jisook Lee (1), Natalie B. Vinh (1), Nyssa Drinkwater (2), Komagal K. Sivaraman (2), Luke S. Schembri (3), Sheena McGowan (2), Peter Scammells (1)

1) Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, VIC 3052, Australia.
2) Infection and Immunity Program, Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton Campus, Melbourne, VIC 3800, Australia.
3) Preparative Medicinal Chemistry, Uppsala Biomedicinska Centrum, Uppsala University, Uppsala, Sweden

Irreversible ligands have been proven to be useful pharmacological tools in the study of structural and functional features in drug receptor pharmacology of G protein-coupled receptors (GPCRs).[1] Recent advances in the field, which made it possible to obtain ligand-bound X-ray structures by co-crystallizing GPCRs with covalently bound probes, have been one of the major drivers behind the increased interest in the development of novel irreversible probes targeting GPCRs. Here, we will present our quest to solve the first X-ray structure of the adenosine A_1 receptor. This includes our efforts to obtain the first X-ray structure of the adenosine A_1 receptor, which was stabilized using DU-172, an irreversible antagonist (Figure 1).[2] Furthermore, we have successfully designed, synthesized and evaluated novel irreversible agonists of the adenosine A_1 receptor (Figure 2).[3] Four of these compounds, were shown to possess similar potency and efficacy to the reference high efficacy agonist, NECA, in an assay of ERK1/2 phosphorylation assay and two irreversible agonists demonstrated an ability to stabilize purified, detergent-solubilised adenosine A_1 receptors in a ThermoFluor assay to a significantly higher degree than NECA. Thus, these results offer an attractive starting point for a range of experiments including our quest to solve the first active-state X-ray structure of the adenosine A_1 receptor.



MIPS1700

References

1) L. Torre, F. Bray, R. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, CA Cancer J. Clin. 2015, 65, 87-108.
2) S. Valastyan, R. Weinberg, Cell, 2011, 147, 275-92.
3) A. Wong, D. Zhou,J. Rini, J. Biol. Chem. 2012, 287, 36804-13.
4) L. Su, J. Cao, Y. Jia, X. Zhang, H. Fang, W. Xu, ACS Med. Chem. Lett. 2012, 3, 959-64.
5) K. Fukasawa, H. Fujii, Y. Saitoh, K. Koizumi, Y. Aozuka, K. Sekine, M. Yamada, I. Saiki, K. Nishikawa, Cancer Lett. 2006, 243, 135-43.
6) N. Drinkwater, N. Vinh, S. Mistry, R. Bamert, C. Ruggeri, J. Holleran, S. Loganathan, A. Paiardini, S. Charman, A. Powell, V. Avery, S. McGowan, P. Scammells, Eur. J. Med. Chem. 2016, 110, 43-64.

CRA13 ANALOGUES: DESIGN, SYNTHESIS AND IN VITRO EVALUATION

Yong Sup Lee (1,2,3), Ahmed H.E. Hassan (1), Min Chang Cho (2), Hye In Kim (2), Ji Seul Yang (2), Yoon Mi Yoon (2), Kun Won Lee (2), Seung Yeun Yoo (2), Ki Duk Park (3)

1) Medicinal Chemistry Laboratory, Department of Pharmacy, College of Pharmacy, Kyung Hee University, Seoul 02447, Korea
2) Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 02447, Korea
3) KHU-KIST Department of Converging Science and Technology, Kyung Hee University, Seoul 02447, Korea

CRA13, originally developed by Novartis Pharma, elicits an effective agonistic activity on both of peripheral CB_1 and CB_2 receptors. It was advanced to clinical trials as a pain killer that does not stimulate the central cannabinoid receptors responsible for the undesirable psychotropic and addictive effects (1). Nevertheless, when administered in high dose, it could cross the blood brain barrier resulting in potentially unwanted effects. A more polar compound might be a safer candidate. We describe in this work our efforts to develop more polar analogues of CRA13 as potential cannabinoid receptor ligands with less probability to produce unwanted central effects. The developed molecules incorporate polar functional groups to increase the total polar surface area without impairing binding to cannabinoid receptor.

References

1) Gardin A, Kucher K, Kiese B, Appel-Dingemanse S. Cannabinoid receptor agonist 13, a novel cannabinoid agonist: First in human pharmacokinetics and safety. Drug Metab Dispos 2009;37:827-833.

AZULENE-BASED COMPOUNDS TARGETING OREXIN RECEPTORS

Teppo O. Leino (1), Ainoleena Turku (1,2), Jari Yli-Kauhaluoma (1), Jyrki P. Kukkonen (2,3), Henri Xhaard (1), Erik A. A. Wallén (1)

1) Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, P.O. Box 56, FIN-00014 University of Helsinki, Finland
2) Department of Veterinary Biosciences, Faculty of Veterinary Medicine, P.O. Box 66, FIN-00014 University of Helsinki, Finland
3) Department of Physiology, Faculty of Medicine, University of Helsinki, P.O. Box 63, FI-00014 University of Helsinki, Finland

Orexin-A and orexin-B are the activating peptide ligands of G protein-coupled orexin receptors, OX_1R and OX_2R.[1] The orexin signaling system has a central role in sleep-wake regulation. Therefore the orexin receptors could provide a clinical target for antagonism and agonism, to treat insomnia and narcolepsy, respectively.[2] In recent years, the orexin receptor antagonists have been successfully developed, but the agonists have gained minor attention. Still most of the existing agonists are peptides, which are well known to be unsuitable therapeutic molecules and only one series of effective non-peptide orexin receptor agonists has been published to date.[3]

In order to discover novel ligands for orexin receptors, we designed a virtual library consisting of 70 000 azulene-based compounds with substituents in the 1-, 3- and 6-position, which can be synthesized by our efficient synthetic methods for 1,3,6-trisubstituted azulenes.[5,6] After docking the database to OX_2R[4] and visual examination of the top-scoring compounds, we selected a series of compounds for synthesis. With this approach, we identified novel orexin receptor ligands: both antagonists with K_i values in the low micromolar range and weak agonists.[7] In addition, we discovered compounds that potentiated the orexin-A response to OX_1 receptors two-fold at 10 µM.

References

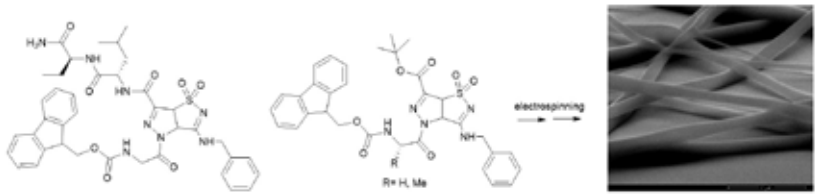
1) Sakurai, T.; Amemiya, A; Ishii, M.; Matsuzaki, I.; Chemelli, R.; Tanaka, H.; Williams, S.; Richardson, J.; Kozlowski, G.; Wilson, S.; Arch, J.; Buckingham, R.; Haynes, A.; Carr, S.; Annan, R.; McNulty, D.; Liu, W.-S.; Terret, J.; Elshourbagy, N.; Bergsma, D.; Yanagisawa, M. Orexins and Orexin Receptors: A Family of Hypo-Thalamic Neuropeptides and G Protein-Coupled Receptors That Regulate Feeding Behavior. Cell 1998, 92 (92), 573–585.
2) Gotter, A. L.; Roecker, A. J.; Hargreaves, R.; Coleman, P. J.; Winrow, C. J.; Renger, J. J. Orexin Receptors as Therapeutic Drug Targets. Prog. Brain Res. 2012, 198, 163–196.
3) Nagahara, T.; Saitoh, T.; Kutsumura, N.; Irukayama-Tomobe, Y.; Ogawa, Y.; Kuroda, D.; Gouda, H.; Kumagai, H.; Fujii, H.; Yanagisawa, M.; Nagase, H. Design and Synthesis of Non-Peptide, Selective Orexin Receptor 2 Agonists. J. Med. Chem. 2015, 58 (20), 7931–7937.
4) Yin, J.; Mobarec, J. C.; Kolb, P.; Rosenbaum, D. M. Crystal Structure of the Human OX2 Orexin Receptor Bound to the Insomnia Drug Suvorexant. Nature 2015, 519 (7542), 247–250.
5) Leino, T. O.; Baumann, M.; Yli-Kauhaluoma, J.; Baxendale, I. R.; Wallén, E. A. A. Synthesis of 1,3,6-Trisubstituted Azulenes. J. Org. Chem. 2015, 80 (22), 11513–11520.
6) Leino, T. O.; Johansson, N. G.; Devisscher, L.; Sipari, N.; Yli-Kauhaluoma, J.; Wallén, E. A. A. Synthesis of 1,3,6-Trisubstituted Azulenes Based on the 1-Acyloxyazulene Scaffold. Eur. J. Org. Chem. 2016, 2016 (33), 5539–5544.
7) Leino, T.O.; Turku, A.; Yli-Kauhaluoma, J.; Kukkonen, J.P.; Xhaard, H.; Wallén, E.A.A. Azulene-Based Compounds for Targeting Orexin Receptors. Submitted.

NANOFIBERS FROM SMALL MOLECULES

Silvia Locarno (1), Davide Garanzini (1), Alexander Bittner (2), Francesca Clerici (1)

1) University of Milan, Department of Pharmaceutical Sciences, Milan Italy
2) CIC Nanogune, Donostia, Spain

Electrospinning is a simple and versatile technique used for the fabrication of continuous micro and nanofibers. This approach is inexpensive, scalable, reliable and mainly used from polymer solutions and polymer melts.[1] Nonpolymeric molecules can usually not be electrospun, as only polymer solutions or melts are sufficiently viscous to provide the required degree of molecular entanglement.[2] However, recent studies have demonstrated that high molar mass polymers are not essential for production of uniform electrospun fibers but that sufficient intermolecular interactions acting as chain entanglements is the primary criterion.[3] Recently it was demonstrated that the dipeptide phenylalanine-phenylalanine (FF), and two Fmoc derivatives, *i.e.* Fmoc glycine (Fmoc-Gly) and Fmoc-phenylalanyl-glycine (Fmoc-Phe-Gly), in spite of their small size, can assemble by electrospinning to nanofibers basing solely on noncovalent interactions.[2,4]Starting from this observation we focused on the exploitation of sulfur/nitrogen containing heterocycles having particular features that can improve the self-assembling propensity of the system. Several compounds containing natural amino acids (Gly, Ala, Leu, Val) together with a heterocyclic scaffold properly functionalized, were synthesized and a study on their electrospinnability was developed. The compounds were dissolved in high concentration in HFIP and the experiments executed on a



References

1) D. B. Khadka, D. T. Haynie; Nanomedice: nanotechnology, biology and medice (2102), 8, 1242-1262
2) W. Nuasing, A. Rebollo, J. M. Mercero, J. Zuniga, A. Bittner; J. Raman Spectrosc. (2012), 43, 1397-1406
3) M. G. McKee, J. M. Layman, M. P. Cashion, T. E. Long, Science (2006), 311, 353-355
4) G. Singh, A. Bittner, S. Loscher, N. Malinowski, K. Kern; Adv. Mater. (2008), 20, 2332-2336

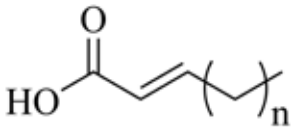
IN VITRO AChE INHIBITORY ACTIVITY OF (E)-α,β-UNSATURATED FATTY ACIDS

Anne Loesche, Jana Wiemann, Julia Karasch, René Csuk

Martin-Luther-University Halle-Wittenberg, Organic Chemistry, Kurt-Mothes-Str. 2, D-06120 Halle (Saale), Germany

An improved standard of living and falling mortality rates lead to an increase in the number of people suffering from aging-associated diseases, such as atherosclerosis, hypertension and age-related dementia. Today, the number of persons with clinically diagnosted Alzheimer's disease (AD) is greater than ever. Worldwide, 47 million people live with dementia, and this number is expected to increase to more than 131 million by 2050.[1] AD is a progressive neurodegenerative disease, characterized by the irreversible decline in cognitive functions. According to the "cholinergic hypothesis", impairment in the cholinergic function is of crucial importance in AD. The level of neurotransmitter acetylcholine, which is responsible for the conduction of electrical impulses between nerve cells, is decreased due to its rapid enzymatic hydrolysis.[2] Alzheimer's disease can't be cured, but treatment with cholinesterase inhibitors can slow the course of the malady and improve the patient's quality of life.

In this study, the inhibitory potency and selectivity of (*E*)-2-mono-unsaturated fatty acids (MUFAs) are tested. Therefore, a series of (*E*)-2-MUFAs differing in chain length was synthesized and screened for their inhibitory action against acetylcholinesterase (AChE, from electric eel) und butyrylcholinesterase (BChE, from equine serum). *Trans*-2-eicosenoic acid was shown to be a selective and efficient mixed-type inhibitor for acetylcholinesterase (K_i = 1.51 ± 0.09 µM, K_i' = 7.15 ± 0.55 µM).



Structure of tested *trans*-mono-unsaturated fatty acids

References

1) Prince, M., Comas-Herrera, A., Knapp, M., Guerchet, M., Karagiannidou, M. World Alzheimer Report 2016. Alzheimer's Disease International, London, 2016.
2) Anand, P., Singh, B. Arch. Pharm. Res. 2013, 36, 375.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SMALL-MOLECULE PSMA-TARGETED TAXANE CONJUGATES

Aleksei Machulkin (1), Anton Ber (1), Dmitriy Skvortsov (1), Ekaterina Plotnikova (2), Raisa Yakubovskaya (2), Elena Beloglazkina (1), Nikolay Zyk (1), Viktor Kotelyansky (1), Alexander Majouga (1,3)

1) Lomonosov Moscow State University, Chemistry Dept., Leninskie gory, Building 1/3, GSP-1, Moscow, 119991, Russian Federation
2) Branch, National Medical Radiology Research Center, P. A. Herzen Moscow Oncology Research Institute , 3 2nd Botkinskiy Proezd, Moscow 125284, Russia.
3) National University of Science and Technology MISiS, 9 Leninskiy pr, Moscow, 119049, Russian Federation

The last year, statistical output was greatly unfavorable and sad since prostate carcinoma (PCa) was the most spread malignant tumor commonly diagnosed around the world and as a leading cause of cancer-related lethal outcomes registered among men in the US with an estimated 233K diagnoses and 30K deaths.[1]

Glutamate carboxypeptidase II (GCPII), also known as prostate specific membrane antigen (PSMA) has recently emerged as a prominent biomarker of this pathological state and as an attractive protein trap for drug targeting. [2, 3]

In present work we synthesized the series paclitaxel/docetaxel conjugates, modified with residues of 5-hexynoic acid with different PSMA ligands. As PSMA ligand different ε-Lys substituted or non-substituted residues with long hydrophobic linker of Glu-urea-Lys (DCL) ligands were synthesized. Synthesis of final conjugates was performed via [3+2] azide alkyne cycloaddition reaction (click reaction). All ligands and conjugates in this work were isolated individually and described with ^{1}H and ^{13}C NMR methods, high resolution mass-spectrometry, purity was confirmed by LC/MS.

Cytotoxic effect of these conjugates was estimated on prostate cancer cells (LNCaP, 22Rv1 and PC-3 cell lines) and non-prostate cell lines (HEK-293, Va-3, MCF-7, A549) structure-activity relationships were studied. On the basis of *in vitro* studies 3 PSMA targeted conjugates were selected for subsequent studies.

PSMA targeted conjugates demonstrated the same tumor growth inhibition as for paclitaxel, in this experiment.

Synthetic approaches and biological evaluation of synthesized structures would be minutely discussed in the report.

This work was kindly supported by Ministry of Education and Science of the Russian Federation № IP-MSU/10-14 (NKR 185/17) and Russian scientific fund № 17-14-01316

References

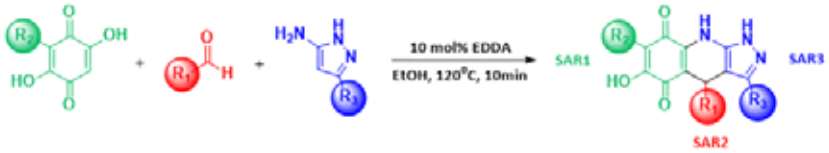
1) R. Siegel, J. Ma, Z. Zou, A. Jemal, CA Cancer J. Clin. 2014, 64, 9–29.
2) C.A. Foss et al., Curr. Med. Chem. 2012, 19, 1346–1359.
3) A.E. Machulkin, et al. J. Drug Target. 2016, V. 24, P. 679-93

MULTICOMPONENT SYNTHESIS OF NEW CYTOTOXIC DIHYDRO-1H-PYRAZOLO[1,3-b]PYRIDIN EMBELIN DERIVATIVES

Pedro Martín-Acosta (1), Ángel Amesty (1), Patricia Martín-Rodríguez (2), Miguel Guerra-Rodríguez (2), Haideé Aranda-Tavío (2), Borja Guerra (2), Leandro Fernández-Pérez (2), Ana Estévez-Braun (1)

1) Instituto Universitario de Bio-Orgánica Antonio González (CIBICAN). Departamento de Química Orgánica, Universidad de La Laguna. Avda. Astrofísico Fco. Sánchez Nº 2, 38206, La Laguna, Spain
2) Instituto Universitario de Investigaciones Biomédicas y Sanitarias (IUIBS), Departamento de Ciencias Clínicas, BIOPHARM, Universidad de Las Palmas de Gran Canaria, Spain

Cancer in one of the main health problems faced for the medical community. The search of new compounds with promising activities in oncology represents one of the main goals of the pharmaceutical industry in our days. Embelin (**1**) is a natural benzoquinone isolated as the active principle of the medicinal plant *Oxalis erythrorhiza* (Oxalidaceae) which displays many biological activities remarking its antitumor effect. This molecule is able to interact with multiple biological targets such as XIAP, STAT3, CK2 or Akt, etc. All of this make embelin an interesting scaffold for synthesizing new therapeutic agents with increased molecular complexity that could led to more selective compounds against specific biological targets. Herein we present the synthesis of new dihydro-1*H*-pyrazolo[1,3-b]pyridine embelin derivatives through MCRs under microwave irradiation with potent cytotoxic activity against several hematological and non-hematological cancer cell lines.



Acknowledgments: We thank MINECO (SAF2015-65113-C2-1-R and SAF2015-65113-C2-2-R), ERDF and Gobierno Autónomo Canario (ProID 2017010071) for financial support. P.M.A thanks ACIISI-EU for a predoctoral grant.

References

1) Martín-Acosta, P., Haider, S., Amesty, A., Aichele, D., Jose, J., Estévez-Braun, A. Eur. J. Med. Chem. 2018, 144, 410-423.
2) Martín-Acosta, P., Feresin, G. E., Tapia, A., Estévez-Braun, A. J. Org. Chem. 2016, 81, 9738-9756.
3) Guerra, B., Martín-Rodríguez, P., Díaz-Chico, J. C., McNaughton-Smith, G., Jiménez-Alonso, S., Hueso-Falcón, I., Montero, J. C., Blanco, R., León, J., Rodríguez-González, G., Estévez-Braun, A., Pandiella, A., Díaz-Chico, B. N., Fernández-Pérez, L. Oncotarget. 2017, 8, 29679-29698

MOLECULAR MODELING AND SYNTHESIS OF BACLOFEN ANALOGUES AS POSSIBLE GABAB RECEPTOR AGONISTS.

Zuleyma Martínez Campos (1), Rodrigo Said Razo Hernández (2), Mario Fernández Zertuche (1)

1) Instituto de Investigación en Ciencias Básicas y Aplicadas, Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Cuernavaca 62209, Morelos, México
2) Instituto de Investigación en Ciencias Básicas y Aplicadas, Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos. Cuernavaca 62209, Morelos, Mexico

γ –Aminobutyric acid **1** (GABA) is the major inhibitory neurotransmitter in the brain, where it exerts its physiological action through the interaction with specific receptors called: $GABA_A$, $GABA_B$ and $GABA_C$.[1]

$GABA_B$ receptors have an important function in the neuronal physiology of many central nervous system diseases and disorders including anxiety, depression, epilepsy, autism spectrum disorder, stroke, drug addiction, and the neurodegenerative disorders as Huntington's, Parkinson's, and Alzheimer's diseases.[2-4] Actually, the only FDA-approved drug to target the $GABA_B$ receptor is Baclofen **2**, a drug used as a muscle relaxant.[5]

The aim of this work is the rational designed and synthesis of Baclofen analogues by molecular modelling. Analyzing the crystal structure of the $GABA_B$ receptor with Baclofen (PDB:4MS4), we observed that the aromatic ring, the amino and the carboxylic groups are part of the pharmacophore of the Baclofen molecule. In this context, we make some structural modifications that do not alter the pharmacophore. The proposed modifications are shown in figure 1. The nitrogen in γ position was included within heterocyclic systems such as Isoxazole and Oxadiazole. Additionally, bioisosteric replacements of the aromatic ring were made.

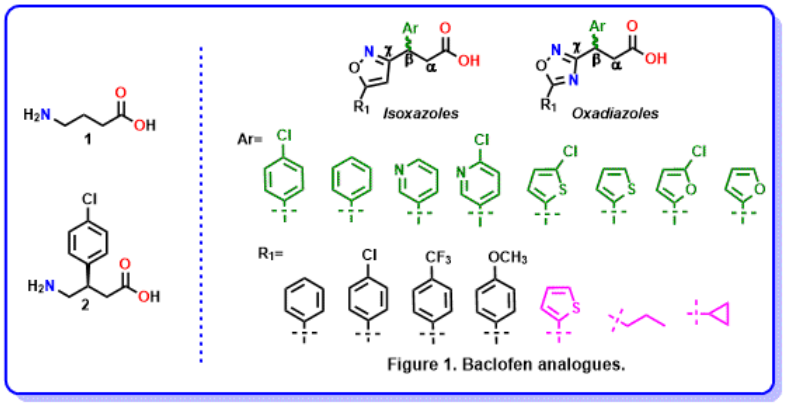


Figure 1. Baclofen analogues.

These Baclofen analogues were submitted to a QSAR model- previously constructed- concluding that analogues whose R_1 substituent should be cyclopropyl, propyl and thiophene, to be active as $GABA_B$ agonists.

References

1) Brown, K. M; Roy, K. K; Hockerman, G. H; Doerksen, R. J; Colby, D. A. J. Med. Chem. 2015, 58, 6336–6347.
2) Kumar, K; Sharma, S; Kumar, P; Deshmukh, R. Pharmacol., Biochem. Behav. 2013, 110, 174–184.
3) Tyacke, R. J; Lingford-Hughs, A; Reed, L. J; Nutt, D. J. Adv. Pharmacol. 2010, 58, 373–396.
4) Bowery, N. G; Froestl, B; Bettler, B; Gallagher, J. P; Marshall, F; Raiteri, M; Bonner, T. I; Enna, S. J. Pharmacol. Rev. 2002, 54, 247–264.
5) Froestl, W. Adv. Pharmacol. 2010, 58, 19–62.

IDENTIFICATION OF NOVEL BACE1 INHIBITORS: A COMBINED PROTOCOL OF PHARMACOPHORE MODELING, VIRTUAL SCREENING AND STRUCTURE-BASED DRUG DESIGN

Judite R. M. Coimbra (1,2), Salete J. Baptista (1,2,3), Armanda E. Santos (2,4), Maria M. C. Silva (1,2), Teresa C. P. Dinis (2,4), Jorge A. R. Salvador (1,2)

1) Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
2) Center for Neuroscience and Cell Biology, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal
3) Chem4Pharma, Edifício IPN Incubadora, Rua Pedro Nunes, 3030-199 Coimbra, Portugal
4) Laboratory of Biochemistry, Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

Alzheimer's Disease (AD) is a severe neurodegenerative disorder that remains without effective therapies available to prevent the advance of the disease. The main neuropathological features of AD consist in extracellular Amyloid-β (Aβ) plaques deposition and aggregates of intracellular Neurofibrillary Tangles (NFTs). Understanding the pathophysiological mechanisms that underlie neurodegeneration in AD is essential for rational design of therapies to slow or halt disease progression. A critical molecular event in the pathogenesis of AD is the accumulation of Aβ peptide, which is produced by sequential Amyloid Precursor Protein (APP) proteolytic cleavage by β- and γ-secretase. The β-secretase responsible for the proteolytic processing of APP in the brain is the β-site APP-cleaving enzyme 1 (BACE1).[1] Since processing of APP by BACE1 is the rate-limiting step in the production of Aβ, BACE1 is considered a major therapeutic target to tackle AD.[2,3] Therefore, the last two decades witnessed intensive efforts to discover inhibitors that can reach the brain and effectively block BACE1.[4,5] Furthermore, different classes of inhibitors have been described and some of them are currently being tested in clinical trials.[6]

The main goal of this research project is the discovery of new small molecules that effectively inhibit BACE1. The project has been conducted by using different Computer-Aided Drug Design (CADD) methodologies, such as pharmacophore modeling, virtual screening and molecular docking. First, both structure-based and ligand-based pharmacophores models were designed to identify novel and potent BACE1 inhibitors. The former structure-based approach was based on receptor-ligand key interactions, while ligand-based pharmacophore allows mapping the essential features of a set of known active compounds against BACE1. The pharmacophore models were further applied for virtual screening of large druglike compound databases, in order to identify the most promising hit compounds. Afterwards, molecular docking studies enabled the selection of the best candidates for *in vitro* and *in vivo* evaluation.

Acknowledgements: We gratefully acknowledge PT2020 (Programa Operacional do Centro 2020), and the financial support by FEDER (European Regional Development Fund) through the COMPETE 2020 Programme (Operational Programme for Competitiveness and Internationalization), project CENTRO-01-0247-FEDER-003269, drugs2CAD. This work was also financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme: project CENTRO-01-0145-FEDER-000012-HealthyAging2020, the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation, and the Portuguese national funds via FCT – Fundação para a Ciência e a Tecnologia, I.P.: project POCI-01-0145-FEDER-007440.

References

1) Masters, C. L. et al. Alzheimer's disease. Nat. Rev. Dis. Primers, 2015, 1, 1-18.
2) De Strooper, B., Vassar, R. & Golde, T. The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat. Rev. Neurol., 2010, 6, 99-107.
3) Karran, E., Mercken, M. & De Strooper, B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat. Rev. Drug Discov., 2011, 10, 698-712.
4) Ghosh, A. K. & Osswald, H. L. BACE1 (beta-secretase) inhibitors for the treatment of Alzheimer's disease. Chem. Soc. Rev., 2014, 43, 6765-6813.
5) Yan, R. & Vassar, R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol., 2014, 13, 319-329
6) Yan, R. Stepping closer to treating Alzheimer's disease patients with BACE1 inhibitor drugs. Transl. Neurodegener., 2016, 5, 1-11.

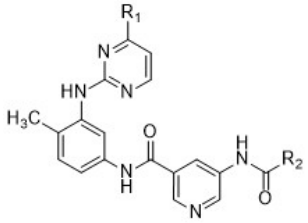
IDENTIFICATION OF POLY(ADP-RIBOSE) ACCUMULATOR MO2455 AS POTENTIAL ANTICANCER AGENT

Kenji Matsuno (1), Tomoko Hashimoto (1), Rafiqul Islam (2), Tadashi Okawara (2), Takeji Takamura (3), Yuka Sasaki (4,5), Takae Onodera (4,5), Hiroaki Fujimori (4,5), Fumiaki Koizumi (6), Tatsu Shimoyama (6), Takeshi Sawada (6), Mari Akimoto (6), Yoshinobu Ishikawa (7), Tetsumi Irie (8), Kengo Inoue (9), Mitsuko Masutani (4,5)

1) Laboratory of Medicinal Chemistry, Department of Chemistry and Life Science, School of Advanced Engineering, Kogakuin University (Institute of Technology), 2665-1 Nakano-machi, Hachi-oji, Tokyo, 192-0015, Japan
2) Department of Medical Technology, Kumamoto Health Science University, 325 Izumi-machi, Kumamoto 861-5598, Japan
3) Faculty of Engineering, Kanagawa Institute of Technology, 1030 Shimo-ogino, Atsugi, Kanagawa, 243-0292, Japan
4) Division of Cellular Signaling, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
5) Department of Frontier Life Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan
6) Division of Clinical Research Support, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, 3-18-22 Hon-komagome, Bunkyo, Tokyo 113-8677, Japan
7) Graduate School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
8) Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe, Chuo-ku, Kumamoto, 862-0973, Japan
9) Pharma Valley Center, 1002-1 Shimo-nagakubo, Nagaizumi, Sunto, Shizuoka, 411-0934, Japan

Poly(ADP-ribosyl)ation is a post-translational modification in which an ADP-ribose unit from nicotinamide adenine dinucleotide (NAD^+) is transferred to specific amino acids of its target proteins. This modification influences protein–protein interactions and regulates various cellular processes including DNA repair and cell death. The synthesis and degradation of poly(ADP-ribose) (PAR) are catalyzed by two types of enzymes: PAR polymerase (PARP) family of proteins and PAR glycohydrolase (PARG), respectively. It is established that PARP inhibitor could be anticancer agent through the success of olaparib. Also, dysfunction of PARG in particular cancer cells leads to enhanced cell death with inducing PAR accumulation after treatments with DNA alkylating agents or irradiation, suggesting that PARG or PAR accumulation would be a potential target for cancer therapy.

We identified MO2455 with significant PAR accumulation and cytotoxicity to various cancer cell lines through our screening campaign for in-house chemical libraries and further structural optimizations. In this symposium, identification of MO2455 as novel PAR accumulator and the biological effects will be presented.



TARGETING OREXIN RECEPTOR TYPE 2 IN THE TREATMENT OF NARCOLEPSY

Eva Mezeiova (1), Jan Korabecny (1), Eugenie Nepovimova (1,2), Rafael Dolezal (1), Jana Janockova (1), Katarina Spilovska (1,2), Ondrej Soukup (1)

1) Biomedical Research Centre, University Hospital of Hradec Kralove, Hradec Kralove, Czech Republic
2) University of Hradec Kralove, Faculty of Sciences, Department of Chemistry, Hradec Kralove, Czech Republic

Narcolepsy is a chronic neurologic disorder characterized by excessive daytime sleepiness and other symptoms such as cataplexy, vivid hallucinations and paralysis.[1] Narcolepsy is considered as the rare disease affecting approximately 1 in 3000 people.[2] It is believed that narcolepsy is based on autoimmune response mediated by loss of a specific hypothalamic neuropeptide, orexin (also called hypocretin).[3,4] Two orexins have been described – orexin A and orexin B. Accordingly, there are two specific receptors for the orexin peptides, orexin receptor tape 1 (OX1R) and orexin receptor type 2 (OX2R). However, patients with narcolepsy are currently treated only symptomatically. Compounds such as modafinil (non-amphetamine wake promoting compound for excessive daytime sleepiness) and sodium oxybate (short-acting sedative for fragmented nighttime sleep and cataplexy) are preferentially used.[1] An alternative to the symptomatic treatment of narcolepsy with cataplexy would be a direct orexigenic system-targeted therapy in the form of non-peptide small-molecule orexin agonists able to cross the blood brain barrier.

The aim of this work was to design, synthetize and biologically evaluate a novel class of the orexin receptor 2 type agonists. From the group of proposed novel structures, we selected those that fulfill several criteria including CNS multiparameter optimization desirability with predicted proper interaction with OX2R as shown by *in silico* methods.[5,6] Solubility profile was also one considered as one of the key parameters with logS values higher than -4. Within our contribution, all the achieved results in syntheses and biological evaluations of prepared derivatives will be presented.

This study was supported by grant GA CR No. 17-08596S given by Grant Agency CR. Authors declare no conflict of interest.

References

1) Wurtmann RJ. Narcolepsy and the hypocretins. Metabolism. 55, S36-S39 (2006).
2) Leschziner G. Narcolepsy: a clinical review. Pract. Neurol. 14(5), 323-331 (2014).
3) Dauvilliers Y, Billiard M; Montplaisir J. Clinical aspects and pathophysiology of narcolepsy. Clin. Neurophysiol. 114(11), 2000-2017 (2003).
4) Kornum BR, Faraco J, Mignot E. Narcolepsy with hypocretin/orexin deficiency, infections and autoimmunity of the brain. Curr. Opin. Neurobiol. 21(6), 897-903 (2011).
5) Wager TT, Hou X, Verhoest PR, Villalobos A. Moving beyond Rules: The Development of a Central Nervous System Multiparameter Optimalization (CNS MPO) Approach To Enable Aligment of Druglike Properties. ACS Chem. Neurosci. 1(6), 435-449 (2010).
6) Wager TT, Hou X, Verhoest PR, Villalobos A. Central Nervous System Multiparameter Optimalization Desirability: Application in Drug Discovery. ACS Chem. Neurosci. 7(6), 767-775 (2016).

KINETIC AND THERMODYNAMIC CHARACTERIZATION OF PI-CATION INTERACTIONS FOR GALECTIN-3 BY VARIOUS BIOPHYSICAL TOOLS

Cédric Atmanene, Céline Ronin, Emmanuel Klein, Dominique Roecklin, Christina Muller, Nathalie Weidner, Thomas Neumann, Fabrice Ciesielski, Valérie Vivat, Cyrille Grandjean

NovAliX, Bld Sébastien Brant, Bioparc, 67405 Illkirch Cedex, France

Combination of biophysical and structural techniques allowed characterizing and uncovering the mechanisms underlying increased binding affinity of lactosamine derivatives for galectin 3. In particular, complementing information gathered from X-ray crystallography, native mass spectrometry, isothermal microcalorimetry, Biacore SPR, MST, NanoDSF and others is compared to each other.

Our studies showed favorable enthalpic contribution of cation-pi interaction between lactosamine aryl substitutions and arginine residues from the carbohydrate recognition domain, which resulted in two log increase in compound binding affinity. This incrementing strategy allowed individual contribution of galectin inhibitor moieties to be dissected. Altogether, our results suggest that core and substituents of these saccharide-based inhibitors can be optimized separately, providing valuable tools to study the role of galectins in diseases.

BIVALENT LIGANDS TARGETING THE CANNABINOID RECEPTOR TYPE 2

Paula Morales (1), Gemma Brugal (2), Laura Redondo-Gallego (1), Maria Gomez-Cañas (3), Ruth Pazos (3), Pilar Goya (1), Javier Fernandez-Ruiz (3), Rafael Franco (2), Nadine Jagerovic (1)

1) Instituto de Química Médica, CSIC, Juan de la Cierva 3, Madrid, Spain
2) Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
3) Departamento Bioquímica y Biología Molecular, CIBERNED, IRICYS, Facultad de Medicina, Universidad Complutense de Madrid, Spain

G-protein-coupled cannabinoid receptors, CB_1 and CB_2, have emerged as promising therapeutic targets with a high potential for the treatment of cancer, pain or neurological disorders among others. As other GPCRs, CB_1 and CB_2 present a rather complex molecular pharmacology. The existence of distinct binding sites, different effector-coupling proteins, biased modulation, or oligomerization processes govern their intricate functionality. In this context, bivalent ligands may allow the study of multifunctional receptor activy and can provide receptor type-selectivity.

Few bivalent ligands have been described for the cannabinoid receptors; most of them target the CB_1 receptor. Heterobivalent ligands targeting CB_1 and opioid receptors have previously been developed by us.[1] Herein we report the identification of CB_2 selective bivalent ligands based on the chromenopyrazole scaffold previously described by us as cannabinoid ligand.[2]

A series of homobivalent chromenopyrazoles containing alkyl chains as spacers and their respective univalent 9-alkoxychromenopyrazole analogs have been synthesized. Their ability to bind to cannabinoid receptors was measured through radioligand assays observing full selectivity towards the CB_2 type eliminating the psychotropic effects related to the CB_1 type. Functional cAMP assays performed in HEK293 cells overexpressing recombinant human CB_2 receptors showed their CB_2 agonist profile. Interestingly, their univalent analogs were not able to orthosterically displace[^{3}H]-CP55940 in radioligand binding assays. However, functional studies are currently ongoing to assess their potential allosterism. To further investigate if the bivalent ligands act as dualsteric/bitopic CB_2 agonists modeling and mutational studies are being undertaken.

ACKNOWLEDGMENTS: Grant SAF2015-68580-C2-2-R (MINECO/FEDER).

References

1) Fernández-Fernández, C. et al. Drug Des. Devel. Ther. (2014) 8, 263–277
2) Cumella, J. et al. ChemMedChem (2012) 7, 452–463.

PHYTOCHEMICAL ANALYSIS OF AN AUSTRALIAN NATIVE PLANT AGAINST COMMON WOUND-COLONISING BACTERIA

Marzieh Negahban, Trudi Collet, Christopher Collet

Indigenous Medicines Group, Institute of Health & Biomedical Innovation, School of Clinical Sciences, Queensland University of Technology, Brisbane, Australia.

Background

Many diseases and disease agents that were once controlled by antibiotics have evolved into new resistant forms which are no longer susceptible to the original antimicrobial therapy [1]. The global emergence of multidrug-resistant forms of bacteria continues to limit the efficacy of current therapeutics, resulting in treatment failure and infection recurrence [2]. Bacterial resistance to current therapeutics continue to increase, hence, alternative antimicrobial agents derived from natural sources are being targeted. Plants produce different types of biologically active compounds, many of which have been shown to have antimicrobial effects [3]. These bioactive compounds may form new therapeutic agents which can be used as a source of antibacterial, anti-inflammatory and wound-healing treatments [4] .

Methods

Ground dried leaves of an Australian native plant (denoted species 8484) were extracted with different solvents. The extracts were screened against nineteen wound-colonising bacteria using the well diffusion assay. Sterile Milli-Q water was used as the negative control and standard antibiotic discs acted as the positive control. Each assay was performed in triplicate and final values were expressed as mean values ± SEM. The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the effects of plant extracts on the formation of monomicrobial and polymicrobial biofilms were also determined. Further, extracted compounds were separated by reverse phase high performance liquid chromatography (HPLC) which were then identified and evaluated by nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Discussion

The extracts obtained from the selected plant (100 mg/mL) showed an antimicrobial activity against wound-colonising bacteria. The ethyl acetate extract (100 mg/mL) was superior with regards to producing antimicrobial activity compared with other solvents at the same concentration. Of the 19 bacteria screened, MRSA clinical isolates and vancomycin-resistant *E. faecalis*, *E. gallinarum* and *E. casseliflavus*, were shown to be especially susceptible to the plant extracts as all exhibited greater zones of inhibition subsequent to treatment when compared to their cognate control. The methanolic extract of plant species 8484 had a MIC of 2µg/mL against *E. faecalis*. In contrast, a MBC of 20 mg/mL from the methanolic extract was shown to exert the greatest bactericidal effect against *S. pyogenes*. Results of both the monomicrobial and polymicrobial biofilms showed that with increasing concentrations (0, 20, 30 and 40 mg/mL) of the methanolic plant extract, the formation of monomicrobial (*E.coli*) and polymicrobial (MRSA and *P. aeruginosa*) biofilms significantly decreased. The isolation and identification of bioactive compounds is currently in the preliminary phase.

References

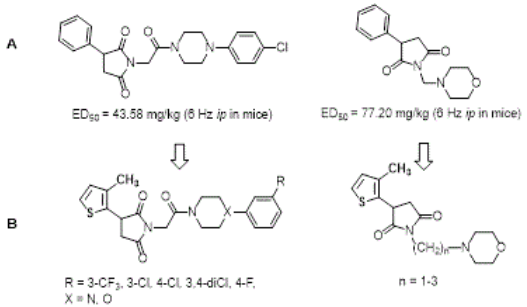
1) Levy, S.B., The Challenge of Antibiotic Resistance. Scientific American, 1998. 278(3): p. 46-53.
2) Tanwar, J., et al., Multidrug Resistance: An Emerging Crisis. Interdisciplinary Perspectives on Infectious Diseases, 2014. 2014: p. 7.
3) Palombo, E.A. and Semple, S.J., Antibacterial activity of traditional Australian medicinal plants. Journal of ethnopharmacology, 2001. 77(2): p. 151-157.
4) Cock, I.E., The phytochemistry and chemotherapeutic potential of tasmannia lanceolata (tasmanian pepper): A review. Pharmacognosy Communications, 2013.

SYNTHESIS AND PHYSICOCHEMICAL PROPERTIES OF NEW DERIVETIVES OF 3-(3-METHYL-THIOPHEN-2-YL)-PYRROLIDINE-2,5-DIONE WITH POTENTIAL ANTICONVULSANT ACTIVITY

Jolanta Obniska (1), Małgorzata Góra (1), Anna Czopek (1), Krzysztof Kamiński (1), Anna Rapacz (2)

1) Department Medicinal Chemistry Jagiellonian University, Medical College, 9 Medyczna Street, 30-688 Kraków, Poland
2) Department of Pharmacodynamics, Jagiellonian University, Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

The previous research from our laboratory have demonstrated the various anticonvulsant activity among the derivatives of pyrrolidine-2,5-dione with different substituents at position-3. In this series the most active were compounds with phenyl group at position-3 of the imide ring and with 4-phenylpiperazines or morpholine at position-1 (Fig. 1A).[2] Taking into consideration these results, in the present studies we have obtained a new series of compounds in which phenyl ring was replaced by the 3-methyl-tiophene substituent (Fig. 1B). Notably, this heterocyclic ring is one of the essential structural fragments of known AED – tiagabine.



The starting material 2-(3-methylthiophen-2-yl)-succinic acid (**1**) was prepared according to the method described previously.[3] In the next step, the condensation reaction of **1** with 2-amineacetic acid yielded the 3-(3-methyl-thiophen-2-yl)-2,5-dioxo-pyrrolidin-1-yl-acetic acid. This intermediate was converted to final compounds in the coupling reaction with appropriate 4-phenylpiperazines or morpholine in the presence of carbonyldiimidazole. In the second step the 3-(3-methyl-thiophen-2-yl)-pyrrolidine-2,5-dione (**2**) was obtained in the cyclization reaction of **1** with 25% ammonia. Intermediate **2** was used in the aminoalkylation reaction with formaldehyde, and morpholine to obtain target Mannich-type compounds. Ethylene or propylene derivatives were synthesized using the cyclization reaction of the starting acid (**1**) with appropriate amino-alkyl-morpholines.

All synthesized compounds were evaluated for their anticonvulsant activity in the maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ) and 6 Hz seizure tests in mice after ip. administration. With the aim of explaining the possible mechanism of action, for selected molecules, their influence on sodium and calcium channels will be evaluate in the *in vitro* assays.

This study was supported by the grant of National Science Centre, Poland (Grant No **2017/25/B/NZ7/01048**).

References

1) A. Rapacz, J. Obniska, B. Wiklik-Poudel et al., Eur. J. Pharmacol., 2016, 781, 239-249.
2) K. Kamiński, J. Obniska, I.Chlebek et al., Bioorg.Med. Chem., 2013, 21, 6821-6830
3) P. Abeijón, J. M. Blanco, F. Fernández et al. Eur. J. Org. Chem., 2006, 3, 759-764.

DISCOVERY AND DEVELOPMENT OF RAC1-GEF INTERACTION INHIBITORS USING IN SILICO FRAGMENT MAPPING METHOD

Shin-ichiro Ozawa, Yuma Kitamura, Noriyuki Yamaotsu, Shuichi Hirono

School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 1088641 Japan

Rac1, also known as Ras-related C3 botulinus toxin substrate 1, is a member of the Rho family of GTPases. Rac1 is a pleiotropic regulator of many cellular processes, including the cell cycle, cell-cell adhesion, motility and epithelial differentiation. Aberrant activation of Rac1 is associated with tumorigenesis, cancer progression, invasion and metastasis. Importantly, a part of this aberrant signaling is driven by alterations in its regulatory proteins, guanine nucleotide exchange factors (GEFs). Thus, the interaction between Rac1 and GEFs appears to be a promising and relevant target for the development of novel anticancer drugs. In this study, we aimed to identify novel inhibitors targeted to protein-protein interaction (PPI) between Rac1 and GEFs, by virtual screening based on our *in silico* fragment mapping method.
First, small fragments derived from the ligands in the protein-ligand complexes of the PDBbind database were mapped onto the GEF binding site of apo Rac1 (PDB : 2P2L) based on the similarity of the subsites. Compounds conforming to the three-dimensional (3D) pharmacophore model constructed from the mapped fragments were then retrieved from several commercial databases (containing approximately 15 million compounds). Next, we performed docking calculation of the compounds, and then molecular dynamics (MD) simulations were conducted for the top-ranked compounds to examine the stability of the binding poses of them. As a result, four compounds were selected and subjected to the nucleotide exchange assay of GDP for mant-GTP in the presence of Rac1 and its GEF, Tiam1. Among them, two compounds showed 30-40% inhibition of Rac1-Tiam1 interaction at the concentration of 100 μM. Currently we are carrying out a structural optimization of the compounds in order to design more potent PPI inhibitors of Rac1 and GEFs.

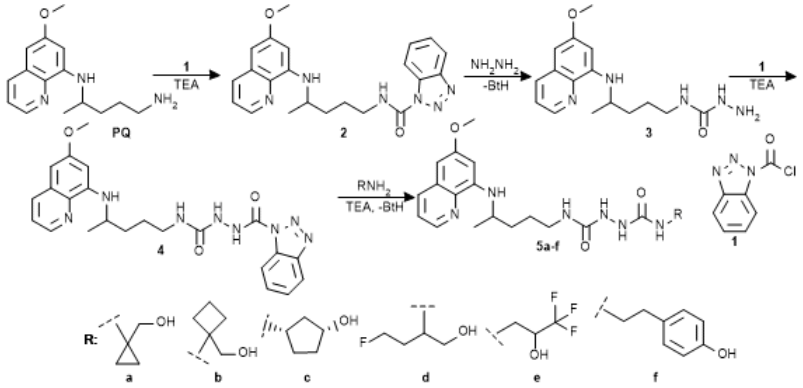
SYNTHESIS OF THE SECOND GENERATION OF PRIMAQUINE BIS-UREAS BEARING HYDROXYALKYL/HYDROXYARYL MOIETIES

Kristina Pavić, Zrinka Rajić Džolić, Ivana Perković, Branka Zorc

Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, Zagreb, Croatia

In several papers published by our research group, we have described the preparation of various primaquine (PQ) derivatives and reported their antiproliferative, antioxidative, antimalarial, antimicrobial and/or antiviral activities [1]. A great number of compounds, members of different classes of PQ derivatives, namely amides, ureas, *bis* -ureas, acylsemicarbazides, showed prominent antiproliferative activities, with acylsemicarbazides and *bis*-ureas being more active than corresponding amides and ureas. Among synthesized PQ derivatives, urea with 5-hydroxypentyl substituent exerted remarkable antiproliferative activity against human colon adenocarcinoma SW620 cell line (IC_{50} = 0.2 μM). Having in mind all the above mentioned facts, we have designed and synthesized a second generation of *bis*-urea PQ derivatives where PQ core and a spacer type are preserved, but aminoalcohol part of the molecule is replaced by (a) more rigid aminoalcohols bearing small cycloalkane moieties, (b) fluoro substituted aminoalcohols or (c) aminophenol. In particular, organofluorine compounds are very interesting candidates in drug discovery since they tend to have improved metabolic stability, physicochemical properties, bioavailability and/or biological activity[2].

In the first reaction step, PQ-benzotriazolide **2** was synthesized in the reaction of PQ and 1-benzotriazole carboxylic acid chloride (BtcCl, **1**). Following our previously described procedure, PQ semicarbazide **3** was prepared from compound **2** and hydrazine. Compound **3** reacted with BtcCl giving benzotriazolide **4** which was used without further purification in the next reaction step with aminoalcohols/aminophenol yielding *bis*-ureas **5a-f**. This reaction was performed in dioxane in the presence of an equimolar amount of triethylamine (TEA) if the reaction included aminoalcohol or aminophenol, or two equivalents of TEA if aminoalcohol was in the form of a salt. Structures of all new compounds were confirmed by standard methods (IR, ^{1}H, ^{13}C NMR, MS). Evaluation of their antiproliferative and antimalarial activity is in progress.



This work has been fully supported by the Croatian Science Foundation under the project number IP-09-2014-1501.

References

1) G. Džimbeg et al., Eur. J. Med. Chem. 2008, 43, 1180; M. Šimunović et al., Bioorg. Med. Chem. 2009, 17, 5605; I. Perković et al., J. Enzyme Inhib. Med. Chem. 2013, 28, 601; K. Pavić et al., Eur. J. Med. Chem. 2014, 86, 502; I. Perković et al., Eur. J. Med. Chem. 2016, 124, 622; K. Pavić et al., Molecules 2016, 21, 1629; K. Pavić et al., Eur. J. Med. Chem. 2018, 143, 769; J. Vlainić et al., J. Enzyme Inhib. Med. Chem. 2018, 33, 376; J. Levatić et al., Eur. J. Med. Chem. 2018, 146, 651.
2) V. Prakash Reddy, Oganofluorine Compounds in Biology and Medicine, Elsevier, Amsterdam, 2015.

CATHEPSIN X SELECTIVE AND REVERSIBLE INHIBITOR IMPAIRS TUMOR CELL MIGRATION AND NEURITE OUTGROWTH

Ursa Pecar Fonovic (1), Ana Mitrovic (1), Damijan Knez (1), Tanja Jakos (1), Anja Pišlar (1), Boris Brus (1), Jure Stojan (2), Bojan Doljak (1), Simon Žakelj (1), Jurij Trontelj (1), Stanislav Gobec (1), Janko Kos (1,3)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, Ljubljana, Slovenia
2) Institute of Biochemistry, Medical Faculty, University of Ljubljana, Vrazov trg 2, Ljubljana, Slovenia
3) Department of Biotechnology, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia

A cysteine peptidase cathepsin X is a carboxymonopeptidase found mainly in immune cells, where it regulates migration, adhesion, proliferation, maturation, phagocytosis and signal transduction. In the last 10 years several of its molecular targets were identified and characterized including beta-chain of integrin receptors, gamma-enolase, chemokine CXCL-12, bradykinin, kallidin, huntingtin and profilin 1. Besides, cathepsin X is highly elevated in certain types of cancer, neurodegenerative disorders, inflammatory diseases and other and it became an object of interest as a possible therapeutic target. Till now, an epoxysuccinyl-based inhibitor AMS36 was the only one that showed selectivity toward cathepsin X. It is an irreversible inhibitor that also inhibits cathepsin B in prolonged incubation times. For treatment of various pathologic conditions associated with excessive proteolytic cleavage, reversible small molecular inhibitors are usually the preferred option.

In our study 579 compounds from the in-house library were tested for the relative inhibition of cathepsin X. Ki value was determined for a group of several compounds exhibiting the highest relative inhibition. Binding type, determined by the washout experiment, showed reversible inhibition of cathepsin X for all new inhibitors. Inhibitors with the lowest Ki values were further tested for the cathepsin specificity (cathepsins L, H, S and B exo- and endo-peptidase activity). A reversible and cathepsin X specific inhibitor Z9 with Ki 2.45 ± 0.05 uM was validated on PC-3 prostate cancer cells and PC-12 pheochromocytoma cells. It showed significant inhibition of PC-3 migration and PC-12 neurite outgrowth, two processes that are under the control of cathepsin X carboxypeptidase activity.

SYNTHESIS OF NOVEL 7-(1-AMINOALKYL)PYRAZOLO[1,5-a]PYRIMIDINES AS POTENTIAL INHIBITORS OF CATHEPSIN K

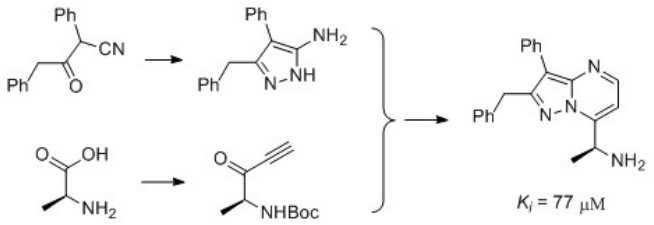
Nejc Petek, Jurij Svete, Marko Novinec, Bogdan Štefane, Franc Požgan, Uroš Grošelj

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

Cathepsin K is a collagenase secreted by osteoclasts, which plays an important role in bone resorption and therefore in degenerative bone diseases such as osteoporosis. Selective reduction of bone resorption rather than both resorption and formation is possible by inhibition of cathepsin K. A number of inhibitors have already been successfully tested as potential drugs for treatment of osteoporosis, with some specificity problems as other cathepsins can also be inhibited[1].

Novel pyrazolo[1,5-*a*]pyrimidines were synthesized and tested for inhibition of cathepsin K. As starting materials (*S*)-amino acids were used, namely glycine, alanine and phenylalanine. Their amine group was protected with *tert*-butyloxycarbonyl (Boc) protecting group. They were converted to Weinreb amides and 1,3-dielectrophilic ynones[2]. 5-Aminopyrazoles were prepared by cyclisation of ketonitriles with hydrazine hydrate[3]. Subsequent cyclization of 5-aminopyrazoles and ynones in methanol at room temperature afforded pyrazolo[1,5-*a*]pyrimidines. The products were purified by column chromatography or simple filtration. The diversity of starting compounds led to pyrazolo[1,5-*a*]pyrimidines with different side chains. Pyrazolo[1,5-*a*]pyrimidine with an ester group on position 3 was of particular interest. It was hydrolyzed under basic conditions to carboxylic acid and then transformed into different carboxamides. In some cyclization products Boc protecting group was removed by acidolysis with HCl in ethyl acetate.

Molecular docking was carried out for all synthesized compounds. Potential binding to the active site of cathepsin K was found and some of the compounds were tested for inhibition of cathepsin K by spectrofotometrically measuring the activity of cathepsin K in the presence of substrate and synthesized pyrazolo[1,5-*a*]pyrimidines. The resulting data were analyzed and inhibition constants for some compounds were calculated. The mechanism of action was found to be competitive for all tested pyrazolo[1,5-*a*]pyrimidines. The best inhibition was measured for a compound with a free amine group with $K_i = 77 \pm 5$ μM. It represents a promising lead compound for further development of cathepsin K inhibitors with such structure.



References

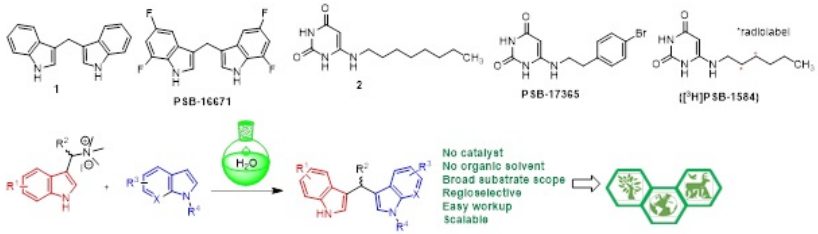
1) Boonen, S., Rosenberg, E., Claessens, F., Vanderschueren, D., Papapoulos, S., Inhibition of Cathepsin K for Treatment of Osteoporosis. Curr Osteoporos Rep 2012, 10, 73-79.
2) Šenica, L., Petek, N., Grošelj, U., Svete J., A four-step synthesis of novel (S)-1-(heteroaryl)-1-aminoethanes from (S)-Boc-alanine. Acta Chim. Slov. 2015, 62, 60-71.
3) Ji, N., Meredith, E., Liu, D. L., Adams, C. M., Artman, G. D., Jendza, K. C., Ma, F. P., Mainolfi, N., Powers, J. J., Zhang, C., Syntheses of 1-substituted-3-aminopyrazoles. Tetrahedron Lett. 2010, 51, 6799-6801.

DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF AGONISTS FOR THE IMMUNOSTIMULATORY ORPHAN G PROTEIN-COUPLED RECEPTOR GPR84

Thanigaimalai Pillaiyar, Meryem Köse , Christa E. Müller

PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, E-mails: thanigai@uni-bonn.de; christa.mueller@uni-bonn.de

G protein-coupled receptor GPR84, a G_i protein-coupled class A, δ-branch GPCRs that is activated by medium chain (hydroxy)fatty acids, has gained much attraction due to its significant role in immunological functions.[1] GPCR84 is expressed on leukocytes and its expression in markedly enhanced under various inflammatory conditions. Thus, it appears to be a promising drug target for inflammatory diseases, and diseases associated with a dysregulation of immunological processes, such as sepsis, neurodegenerative diseases and cancer. 3,3'-Diindolylmethane (**1**, DIM)[2] and 6-octylaminouracil (6-OAU, **2**)[3] were previously identified as small molecule GPR84 agonists.[2,3] We have extensively studied the structure-activity relationships (SARs) of both chemical classes, which interact with different binding sites on the receptor protein.[4,5]



To explore the SARs of DIM derivatives at GPR84, we developed an efficient green synthesis for (un)symmetrically substituted 3,3'-diindolylmethanes derivatives, which allowed a broad variation of the scaffold.[4,6,7] Starting from 6-octylaminouracil (**2**) as a lead molecule, broad structural modification was performed to improve potency, selectivity and metabolic stability, and develop G_i protein-biased agonists.[5] The products were evaluated at the human GPR84 in cAMP (a) and *β*-arrestin (b) assays. For DIMs, the SARs were steep. DIM bearing small lipophilic residues at the 5- and/or 7-position of the indole rings displayed the highest activity in cAMP assays, the most potent agonist being di-(5,7-difluoro-1*H*-indole-3-yl)methane (PSB-16671, EC_{50} 41.3 nM). In β-arrestin assays, SARs were different, indicating biased agonism.

For uracil derivatives, which represent a lipid-like structure, the length of the lipophilic tail attached at the 6-position of the uracil core determined their potency.[5] Further introduction of an aromatic residue into the lipophilic tail improved potency. 6-Hexylamino-2,4(1*H*,3*H*)-pyrimidinedione (PSB-1584, EC_{50} 5.0 nM (a), 3.2 nM (b)) and 6-((*p*-bromo-phenylethyl)amino)-2,4(1*H*,3*H*)-pyrimidinedione (PSB-17365, EC_{50} 2.5 nM (a), 100 nM (b)) was found to be the most potent GPR84 agonist showing high efficacy. The new compounds from both chemical classes (DIMs and uracils) were selective versus related fatty acid receptors and showed high metabolic stability in comparison to the lead structures.

Based on the uracil core, we developed the first radioligand ([^{3}H]PSB-1584) for GPR84, which has allowed evaluation of the binding affinity of ligands.[8] Radioligand binding studies clearly showed that DIM derivatives behave as ago-allosteric ligands, increasing the affinity of the lipid-like uracil derivative and also activating the receptor by themselves in the absence of a lipid-like agonist.

References

1) J. Wang, et al. J. Biol. Chem., 2006, 281, 34457
2) S. Takeda, et al. Life Sci., 2003, 74, 367.
3) M. Suzuki, et al. J. Biol. Chem., 2013, 288, 10684.
4) T. Pillaiyar, et al. J. Med. Chem., 2017, 60, 3636.
5) T. Pillaiyar, et al. ACS Omega, 2018, 3, 3365.
6) T. Pillaiyar, et al. Arkivoc, 2018, iii, 1.
7) T. Pillaiyar, et al. 2018 (submitted).
8) M. Köse, et al. 2018 (manuscript in preparation).

PHARMACOLOGICAL EVALUATION OF ALPRENOLOL ANALOGUES AT THE SECONDARY CONFORMATION OF THE BETA1-ADRENOCEPTOR

Emanuel P. Sousa (1,2), Peter Scammells (3), Jillian G. Baker (2), Shailesh N. Mistry (1)

1) School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom
2) Cell Signalling, School of Life Science, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, United Kingdom
3) Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville 3052, Victoria, Australia

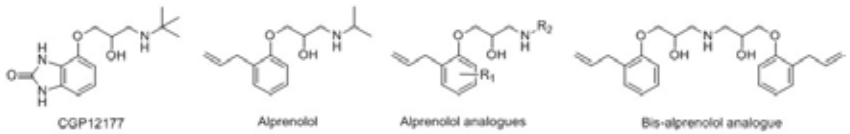
The β_1-adrenergic receptor (β_1-AR) exists in at least two different agonist conformations:

1) A primary conformation where endogenous catecholamine agonists and β-blockers bind. Agonist responses from this conformation are blocked by low concentrations of antagonists.

2) A secondary conformation, for which the precise nature is unknown. Agonist responses from this conformation are more resistant to blockade by conventional primary conformation antagonists. (1)

Conventional agonists (e.g. isoprenaline and cimaterol) stimulate a response mainly through the primary conformation. However, some ligands, such as alprenolol can stimulate agonist responses through both conformations of the β_1-AR, though the response mediated through the secondary conformation requires a higher concentration of ligand. (2) CGP12177 (a prototypical secondary conformation agonist) acts as a high affinity antagonist at the primary conformation, but mediates an agonist response through the secondary conformation at higher concentrations. (3)

In order to identify the molecular features that influence the interaction/functional response at both conformations of the β_1-AR, a set of alprenolol analogues were synthesised and pharmacologically evaluated. In this communication, we report the affinity of these analogues for each conformation of the β_1-AR, determined via inhibition of cimaterol and CGP12177 responses in CRE-SPAP reporter gene assays using CHO cells stably expressing the human β_1-AR.



References

1) Kaumann and Molenaar (2008). Pharmacol. Ther. 118: 303-336.
2) Baker et al. (2003). Mol. Pharmacol. 63: 1312-1321.
3) Pak and Fishman (1996) J Recept Signal Transduct Res. 16: 1-23

NOVEL SULFATED COMPOUNDS AS INHIBITORS OF HUMAN ENTEROVIRUS A71

Gergely Pipa, Benjamin Bailly, Chih-Wei Chang, Robin J. Thomson, Mark von Itzstein

Institute for Glycomics, Griffith University, Gold Coast Campus, Gold Coast, Queensland 4222, Australia

Human enterovirus A71 (EV71) belongs to the genus Enterovirus within the family *Picornaviridae*. EV71 is one of the major causative agents of hand-foot-and-mouth disease, a generally mild childhood disease, however particularly for children under the age of six severe cases of the infection can result in fatal neurological complications. No approved, specific antiviral is available against EV71 to date, although several small molecules have shown inhibitory activity.[1]

Heparan sulfate (HS) is a glycosaminoglycan polymer characterized by highly anionic regions that can be found on the cell surface and in the extracellular matrix of mammals. HS has been suggested to be an attachment receptor for EV71 on the cell surface.[2] To block the host-cell interaction with the virus, HS and HS mimetic compounds (polysulfated small molecules) were investigated and found to be active against EV71 infection. A recent study found that Suramin, an already approved drug with polysulfonated regions, also shows significant EV71 inhibition by binding the viral capsid protein at its anionic site.[3] These findings suggested that polyanionic, in particular polysulfated, carbohydrate-based, small molecules could have a strong potential to block the attachment of EV71 to the host cell surface.

In our work in this area, a number of polysulfated disaccharides with various functionalities at the anomeric position have been synthesized and their inhibitory activity against EV71 evaluated in a viral infection assay. Results show that some compounds have promising activity with improved inhibition compared to Suramin.

References

1) Pourianfar, H. R. et al., Development of antiviral agents toward enterovirus 71 infection. J. Microbiol. Immunol. Infect. 48, 1–8 (2015).
2) Tan, C. W. et al., Enterovirus 71 uses cell surface heparan sulfate glycosaminoglycan as an attachment receptor. J. Virol. 87, 611–620 (2013).
3) Ren, P. et al., The approved pediatric drug suramin identified as a clinical candidate for the treatment of EV71 infection—suramin inhibits EV71 infection in vitro and in vivo. Emerg. Microbes Infect. 3, e62 (2014).

STATINS AND PREECLAMPSIA

Petra Priščáková, Soňa Režnáková, Vanda Repiská, Helena Gbelcová

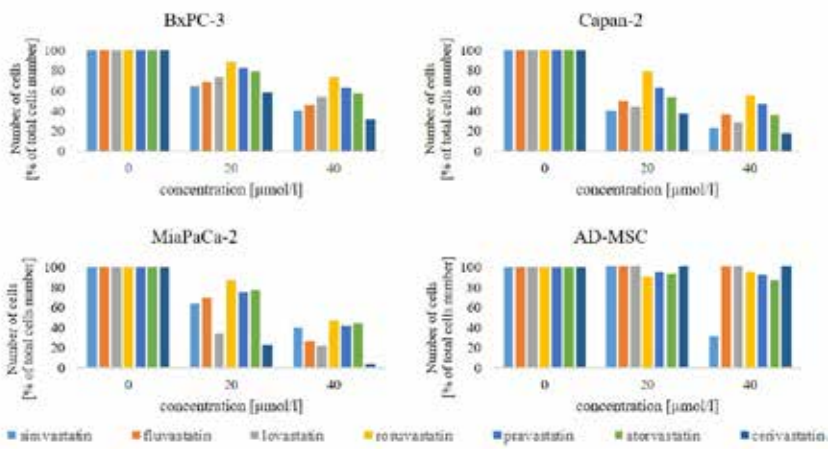
Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Preeclampsia is multifactorial disorder affecting pregnant women all-around the world. Main symptoms are hypertension (> 140/90 mmHg) and proteinuria (> 0.3 mg). Pathogenesis of preeclampsia has not been fully elucidated. There are numeral hypothesis about aetiology. Deficient spiral artery remodelling and abnormal production and differentiation of trophoblast are surely first steps in development of preeclampsia. Direct consequence of these failed processes is oxidative stress in placenta.

Placental oxidative stress has a major part in the pathophysiology of gestational syndromes. Oxidative stress leads to secretion of antiangiogenic factors that mediate maternal endothelial dysfunction and subsequent preeclampsia symptoms. Gestational hypertensive disorders share many similarities with cardiovascular disease (endothelial dysfunction and inflammation). Based on these observations, some drugs and treatments for cardiovascular diseases have been tested in preeclampsia.

Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors are the most frequently prescribed drugs for prevention of cardiovascular morbidity and mortality via inhibition of *de novo* cholesterol synthesis. Additionally statins have pleiotropic effects, including endothelial protection, antioxidant properties, anti-inflammatory, antithrombotic and proangiogenic. Pravastatin can restore angiogenic balance in rodent animal model. Usability of pravastatin to restore angiogenic balance (reduction of circulating antiangiogenic factors) in human and alleviate the severity of preeclampsia have been currently tested in the StAmP trial (Statins to ameliorate early onset preeclampsia) in the UK.

In our work we tested seven commercially available statins (concentrations 0, 20 and 40 μmol/l) on mesenchymal stem cells derivated from adipose tissue (AD-MSC) and on cancerous pancreatic cell lines (BxPC-3, Capan-2, MiaPaCa-2) with goal to set IC50 for tested cell lines (Figure 1). In subsequent experiments, we are going to test statins on BeWo cells with aim to assess effect of statins on oxidative stress in placenta during pathological conditions. BeWo cells are a placental cell line that has been widely used as an *in vitro* model for the placenta and for simulation of pathological gestational symptoms, like preeclampsia.



Statins with pleiotropic effects might be efficient therapy to prevent preeclampsia. Prescription of any medication in pregnancy is undoubtedly risky, hence any therapeutic pharmacological approaches have to be extensively tested.

This work was supported by the projects UK/95/2018, APVV-15-0217 and VEGA 1/0168/18.

INSIGHTS INTO BIOLOGICAL ACTIVITY OF SAHAQUINES, HYBRIDS BASED ON SAHA AND AMINOQUINOLINE MOTIFS

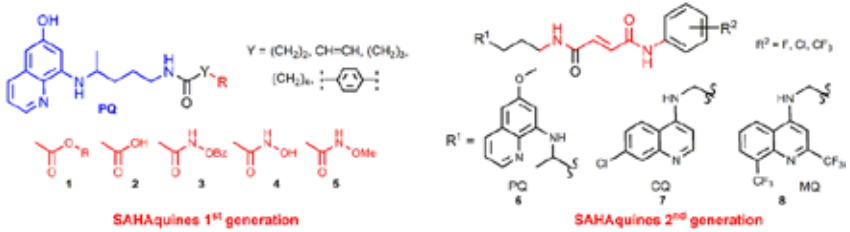
Maja Beus (1), Zrinka Rajic (1), Zvonimir Mlinaric (1), Maja Antunovic (2), Inga Marjanovic (1), Diana Fontinha (3), Miguel Prudêncio (3), Jana Held (4), Josipa Vlainic (5), Ivan Kosalec (1), Dominique Schols (6), Branka Zorc (1)

1) University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovacica 1, Zagreb, Croatia
2) University of Zagreb, Faculty of Science, Horvatovac 102A, 10 000 Zagreb, Croatia
3) Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028, Lisboa, Portugal
4) University of Tübingen, Institute of Tropical Medicine, Wilhelmstraße 27, 72074 Tübingen, Germany
5) Rudjer Boskovic Institute, Bijenicka cesta 54, 10 000 Zagreb, Croatia
6) Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

SAHAquines represent hybrid drugs, developed as a combination of SAHA, an anticancer drug with weak antiplasmodial activity, and an antiplasmodial agent bearing 8-aminoquinoline or 4-aminoquinoline motifs with low anticancer activity, i.e. primaquine (PQ), chloroquine (CQ) or mefloquine (MQ). Two generations of SAHAquines were designed, prepared and biologically evaluated.

In the 1st generation SAHA motif was combined with PQ. The designed hybrid drugs differ in linker length/type and/or functional groups: compounds **1** are esters, **2** are carboxylic acids, **4** are unsubstituted and **3** and **5** are *O* -benzyl and *O*-methyl substituted hydroxamic acids.

2nd generation of SAHAquines, fumaric acid diamides **6–8** were designed as Michael acceptors. One of the amide bonds was achieved with a terminal amino group of PQ, CQ or MQ, while the other amide bond was realized with halogen anilines.



To fully explore the biological potential of SAHAquines, an extensive screening was performed: antiproliferative (a panel of cancer cell lines), antiplasmodial (both erythrocytic and hepatic stages), antibacterial (G(+) and G(−) bacteria and several *Mycobacterium* species) and antiviral (a series of DNA and RNA viruses) activity was evaluated. So far, results have shown that SAHAquines of the 1st generation exert antiproliferative and antiplasmodial activity on both erythrocytic and hepatic stages, while the 2nd generation SAHAquines exhibit significant biofilm eradication capacity. Further testing is in progress.

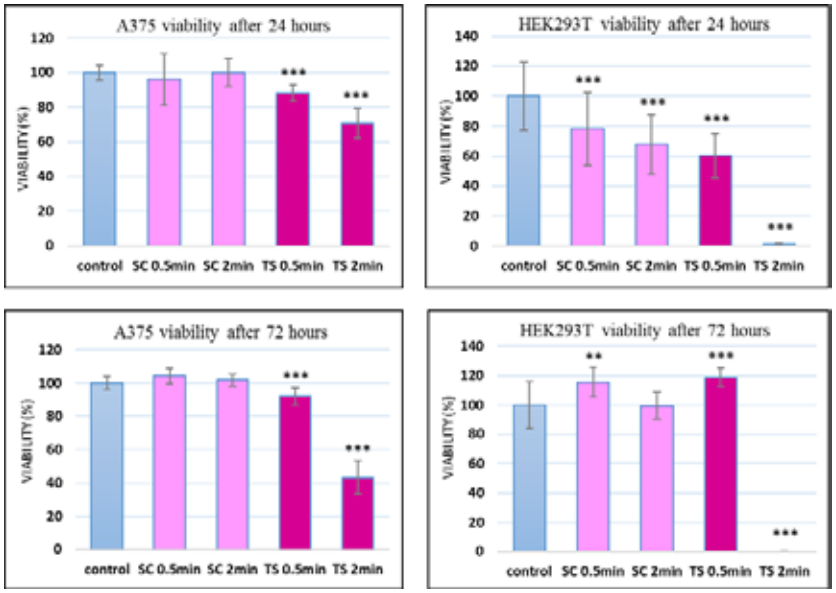
This work has been fully supported by the Croatian Science Foundation under the project number IP-09-2014-1501.

THE EFFECT OF THE PLASMA-ACTIVATED MEDIUM ON CANCEROUS AND NON-CANCEROUS CELLS

Vanda Repiská (1), Petra Priščáková (1), Dominika Sersenová (2), Dominka Miháliková (2), Zdenko Machala (2), Helena Gbelcová (1)

1) Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Bratislava, Slovakia
2) Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

Non-thermal plasma can be applied to live tissues and cells directly and indirectly, and its anticancer effect and potential selectivity are the subject of many current studies. We tested the effects of the plasma-activated medium (PAM) on non-cancer and cancer cells *in vitro*. We used two experimental models; human epithelial melanoma cells A375 and non cancer human embryonic epithelial kidney cells HEK293T. We studied the effect of two types of cold atmospheric plasma discharge; streamer corona and transient spark with two different exposure times (0.5 min/ml and 2 min/ml) to activate the medium. We applied the PAM on cells two hours after exposure of medium to plasma. We investigated the effects of PAM on cells by metabolic MTT assay and we studied microscopic changes of the cells confluence, size and shape of cells using light microscopy. The measurements were made 24 hours and 72 hours after the application of PAM on cells. The results are shown in figure.



Key words: non-thermal plasma, plasma-activated medium, cancerous cells, non-cancerous cells

This work was supported by the projects APVV-15-0217, APVV-0134-12 and VEGA1/0168/18.

References

1) J. Zirnheld, S. Zucker, T. DiSanto et al., IEEE Transactions on plasma science, 38, 948-52 (2010)
2) H. Tanaka, M. Mizuno, K. Ishikawa et al., IEEE Transactions on plasma science, 42, 3760 3764 (2014)
3) Polakovič, L. Strížencová, M. Kotrbancová et al.: Cold air plasma source for biomedical applications based on DC corona discharge, 6th ICPM, p. 300, Bratislava, Slovakia, 4-9 September 2016
4) K. Kučerová, A. Polakovič, H. Gbelcová et al.: Indirect treatment of cancer cells by air spark and corona discharge, 4th IWPCT, p. 57, Paris, 27-28 March 2017

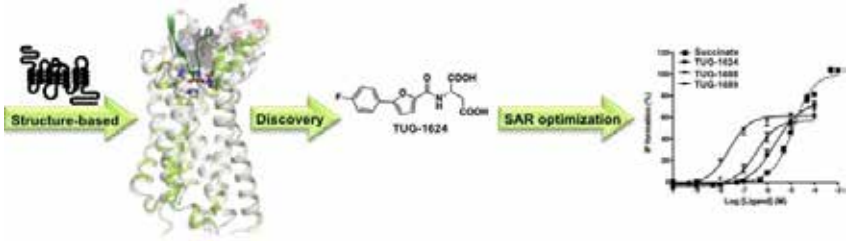
NOVEL AGONISTS FOR THE SUCCINATE RECEPTOR GPR91

Elisabeth Rexen Ulven (1), Mette Trauelsen (2), Matjaz Brvar (1), Michael Lückmann (3), Line Ø. Bielefeldt (1), Lisa K. I. Jensen (1), Thue W. Schwartz (2,3), Thomas M. Frimurer (2)

1) Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark
2) NNF Center for Basic Metabolic Research, Section for Metabolic Receptology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark
3) Laboratory for Molecular Pharmacology, Department of Biomedical Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen, Denmark

The succinate receptor GPR91 is a metabolite receptor activated by the citric acid intermediate succinate at micromolar concentrations[1]. GPR91 is highly expressed in liver, kidney and adipose tissue and mediates metabolic stress signaling[1-2], but selective and potent tool compounds are necessary for further investigations of GPR91 as a potential therapeutic target.

We have previously reported the discovery of non-metabolite GPR91 agonists with excellent selectivity and moderate activity[3]. We here report our structure-activity investigations and optimizations that have led to development of nanomolar potent GPR91 agonists.



References

1) He, W. et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature 429, 188-193, doi:10.1038/nature02488 (2004)
2) Regard, J. B., Sato, I. T. & Coughlin, S. R. Anatomical profiling of G protein-coupled receptor expression. Cell 135, 561-571, doi:10.1016/j.cell.2008.08.040 (2008)
3) Trauelsen, M. et al. Receptor structure-based discovery of non-metabolite agonists for the succinate receptor GPR91. Mol. Metab. 6, 1585-1596, doi:10.1016/j.molmet.2017.09.005 (2017)

MURINE KNOCKOUT STUDIES CONFIRM THE INVOLVEMENT OF THE MITOCHONDRIAL AMIDOXIME REDUCING COMPONENT (mARC) IN N-REDUCTIVE METABOLISM

Sophia Rixen (1), Antje Havemeyer (1), Jennifer Schneider (1), Kazimiera Psyniak (2), Ewa Ramotowska (2), Anita Tyl-Bielicka (2), Michal Mikula (2), Bernd Clement (1)

1) Christian-Albrechts-University, Pharmaceutical Institute, Department of Pharmaceutical and Medicinal Chemistry, Gutenbergstraße 76, 24118 Kiel. Germany
2) Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Genetics, Wilhelma Konrada Roentgena 5, 02-781 Warsaw, Poland

The mitochondrial amidoxime reducing component (mARC) is a recently discovered molybdenum-containing enzyme in mammals. In the presence of NADH and in conjunction with the two electron transport proteins cytochrome b5 type B (CYB5B) and NADH cytochrome b5 reductase (CYB5R), it catalyzes the reduction of various *N*-hydroxylated compounds such as amidoxime prodrugs[1],[2]. As the mARC-containing enzyme system is involved in *N*-reductive pathways, we expected significantly decreased reductive activity in mice, where the mARC gene is inactivated. Therefore mARC2 knockout (KO) mice (-/-), generated by the International Knockout Mice Consortium (IKMC), were characterized by *in vivo* and *in vitro* studies. For *in vivo* studies the established model substrate benzamidoxime (BAO) was given *i.v.* to KO and wildtype (WT) mice (+/+). BAO was proven to act very similar to all so far investigated amidoxime prodrugs (e.g. of pentamidine[3], melagatran [4]). For *in vitro* studies murine tissue homogenates were incubated with different *N*-hydroxylated and *N* -oxygenated compounds. Our *in vivo* results clearly show that murine mARC2 is mainly responsible for the *N* -reduction of BAO. Additionally, the *in vitro* studies revealed a significantly decreased reduction of BAO, the *N* -hydroxylated nucleoside *N*-hydroxycytidine, an endogenous metabolite, and the *N*-hydroxyaminohydrazone guanoxabenz in the murine KO tissues. Nevertheless, a remaining *N*-reductive activity of the KO tissues could be observed *in vivo* as well as *in vitro*. mARC1 might be responsible for these findings, indicating that one mARC protein can function as a backup enzyme if the dominant protein, which in mice is mARC2, should be inactive.

References

1) Havemeyer, A. et al., JB. 2006, 281(46), 34796–34802
2) Clement, B. et al., Drug Metabol. Rev. 2002, 34, 565–579
3) Clement, B. et al., Arzneimittel-Forschung. 1985, 35, 1009–1014
4) Gustafsson, D. et al., Thromb. Res. 2001, 101, 171–181

TOWARDS THE DISCOVERY OF BAY-850, A SELECTIVE AND CELL-ACTIVE ATAD2 CHEMICAL PROBE

Vincent RODESCHINI (1), Anne BRETHON (1), Frédéric FREBAULT (1), Didier ROCHE (1), Jean-Yves ORTHOLAND (1), Amaury E. FERNANDEZ-MONTALVAN (2), Markus BERGER (2), Benno KUROPKA (2), Seong Joo KOO (2), Volker BADOCK (2), Joerg WEISKE (2), Vera PUETTER (2), Simon J. HOLTON (2), Detlef STOCKIGT (2), Antonius TER LAAK (2), Jan HUBNER (2), Hilmar WEINMANN (2), Ingo HARTUNG (2), Matyas GORJANACZ (2), Matthew A. CLARK (3), John W. CUOZZO (3), Ying ZHANG (3), Anthony D. KEEFE (3)

1) Edelris, Lyon, France
2) Bayer AG, Pharmaceuticals, Drug Discovery, Berlin, Germany
3) X-Chem Pharmaceuticals, Waltham, Massachusetts, United States

ATAD2 is an epigenetic regulator that binds to chromatin through its bromodomain. It's overexpression has been associated with the progression of tumors and poor patient prognosis in various cancer types. However, ATAD2 has been considered as a difficult target, due to a predicted low druggability, and consequently, only a few inhibitors have been described to date.(1)

Here we report our medicinal chemistry approach that ultimately led to the discovery of BAY-850, a potent (IC_{50} = 166 nM), selective and cell active inhibitor of ATAD2.(2)Extensive SAR study allowed the identification of a cyclohexyl diamine substituent, leading to a substantial potency improvement. The introduction of MeO substituent on the central phenyl ring was found to improve permeability.

ATAD2 IC_{50} (nM)	166
Log(D (7.4)	2.9
LLE	4.8
Sw pH6.5 (mg/L)	> 3.10^3
Caco-2 (A-B, nm/s)	39
Efflux ratio	0.3
Cl_{int} (vitro, h liver microsomes) (L/h kg)	1.5

References

1) M. Hussain & al. Expert Opin. Ther. Targets, 2018, 22, 85-96. DOI:10.1080/14728222.2018.1406921
2) A. E. Fernandez-Montalvan & al. ACS Chem. Biol., 2017, 12 (11), 2730–2736. DOI:10.1021/acschembio.7b00708

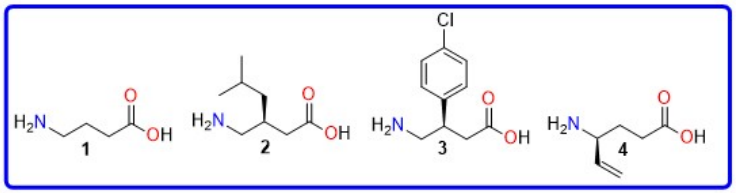
SYNTHESIS AND EVALUATION OF HETEROCYCLIC γ-AMINOBUTYRIC ACID ANALOGUES

Rodriguez Lozada Josue, Fernandez Zertuche Mario

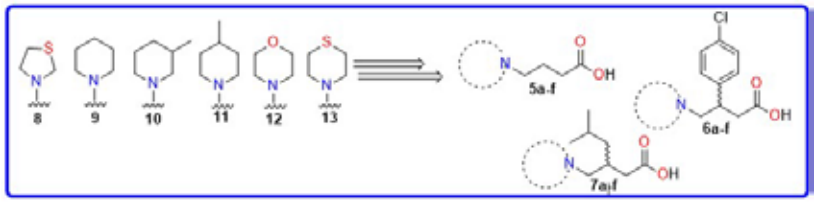
Instituto de Investigaciones en Ciencias Básicas y Aplicadas, Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Avenida Universidad No. 1001, Cuernavaca, Morelos, 62209, México. mfz@uaem.mx

The main inhibitory neurotransmitter in the mammalian brain is g-aminobutyric acid (GABA, **1**)[1]. GABA is synthesized via decarboxylation of L-glutamic acid by a process catalyzed by the glutamic acid decarboxylase (GAD)[2] and metabolized by the GABA-aminotransferase (GABA-AT) enzyme. A decrease in the concentration of GABA has been associated with several neurological disorders such as Alzheimer's disease[3], Parkinson's disease[4], Huntington´s chroea[5] and epilepsy[6].

Administration of GABA peripherally is not effective because GABA, cannot cross the blood-brain barrier[7], due to its low lipophilic character. Therefore, several research groups around the world, have been designed GABA analogues with improved lipophilic character to raise GABA concentration in the brain. (*S*)-Pregabalin **2**, (*R*)-Baclofen **3** and Vigabatrin **4** are examples of GABA analogues used in the clinic.



Here, we describe the design and synthesis of some new heterocyclic GABA analogs, where the nitrogen atom at the γ-position forms part of a heterocyclic ring system such as **5a-f, 6a-f**, and **7a-f**.



All synthesized compounds were evaluated *in vitro* against the GABA-aminotransferase (GABA-AT) enzyme**.** We found that compound **6b** and **7f** display 73% and 40% inhibition over the GABA-AT enzyme as compared with Vigabatrin **4** and sodium valproate **6**.

References

1) Krnjevic, K. Physiol. Rev. 1974, 54, 418-540.
2) Owens, D. F.; Kriegstein, A. R. Nat. Rev. Neurosci. 2002, 3 (9), 715–727.
3) Jo, S.; Yarishkin, O.; Hwang, Y. J.; Chun, Y. E.; Park, M.; Woo, D. H.; Bae, J. Y.; Kim, T.; Lee, J.; Chun, H.; Park, H. J.; Lee, D. Y.; Hong, J.; Kim, H. Y.; Oh, S.-J.; Park, S. J.; Lee, H.; Yoon, B.-E.; Kim, Y.; Jeong, Y.; Shim, I.; Bae, Y. C.; Cho, J.; Kowall, N. W.; Ryu, H.; Hwang, E.; Kim, D.; Lee, C. J. Nat. Med. 2014, 20, 886-896.
4) Gajcy, K.; Lochynski, S.; Librowski, T. Curr. Med. Chem. 2010, 17, 2338-2347.
5) a) Frank, S. S.; Jankovic, J.; Drugs. 2010, 70, 561–571. b) Gerlach, M.; Kuhn, W.; Muller, Th.; Winkel, R.; Eur. J. Neurol. 1996, 3, 467–470.
6) Treiman, D. M. Epilepsia, 2001, 42, 8–12. b) Bakay, R. A. E.; Harris, A. B. Brain Res. 1981, 206, 387. c) Ribak, C. E.; Harris, A. B.; Vaugh, J. E.; Roberts, E. Science 1979, 205, 211.
7) Shashoua, V. E.; Jacob, J. N.; Ridge, R.; Campbell, A.; Baldessarini, R. J. J. Med. Chem. 1984, 27 (5), 659–664.

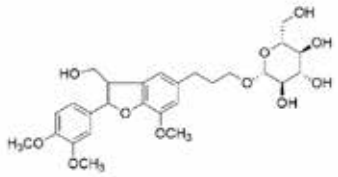
TYROSINASE INHIBITION BY A RARE NEOLIGNAN: AN IN VITRO AND IN SILICO STUDY

Didem Şöhretoğlu (1), Suat Sari (2), Burak Barut (3), Arzu Özel (3)

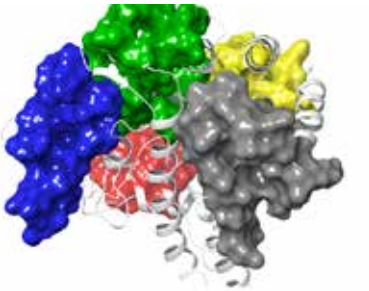
1) Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Sıhhiye, Ankara, TR-06100, Turkey
2) Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Sıhhiye, Ankara, TR-06100, Turkey
3) Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, Trabzon, Turkey

Backgrounds: Neolignans are a large group polyphenols found in plants and exhibit a wide-range of bioactivities including cytotoxic, apoptosis inducer, antimalarial, antifungal, acetylcholinesterase, tyrosinase, and α-glucosidase enzymes inhibitory effects [1].

Aims: In this study we tested acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), tyrosinase, and α-glucosidase enzymes inhibitory effect of a rare neolignan, (-)-4-*O*-methyldehydrodiconiyferyl alcohol 9´-*O*-β-glucopyranoside (**1**) in search for new pharmaceutical effects of **1**. Enzyme kinetics and molecular modelling were performed to understand its tyrosinase inhibition mechanism.



1 (IC50: 44.6 µg/mL)



Mushroom tyrosinase and possible binding sites

Methods: IC_{50} determination and enzyme kinetics studies of **1** were conducted according to the literature methods [2-4]. Galantamine, kojic acid, and acarbose were used as positive controls, respectively. Possible allosteric binding sites of mushroom tyrosinase (PDB ID: 2Y9X [5]) were identified using SiteMap and molecular docking was performed using Glide on extra precision mode (Schrödinger, LLC, NY, 2018) [6].

Results and Conclusions: 1 showed weak inhibition against acetylcholinesterase, butyrylcholinesterase, and α-glucosidase. However, its inhibitor effect on tyrosinase was as strong as kojic acid, the positive control. An enzyme kinetics analysis revealed that **1** inhibited tyrosinase in uncompetitive manner. Possible allosteric sites of mushroom tyrosinase and **1**'s binding mode were identified *in silico*.

References

1) W.M.N.H.W. Salleh et al. Phytochem. Lett. 15 (2016) 168–173.
2) E.N. Barut et al. Turk. J. Biochem. 42 (2017) 493–502.
3) D. Şöhretoğlu, S. Sari, A. Özel, B. Barut. Int. J. Biol. Macromol 105 (2017) 1062–1070.
4) T. Arslan, T. Keleş, B. Barut, A. Özel, Z. Biyiklioglu. Inorg. Chim. Acta. 471 (2018) 121–125.
5) W.T. Ismaya et al. Biochemistry 50 (2011) 5477–5486.
6) R.A. Friesner et al. J. Med. Chem. 49 (2006) 6177–6196.

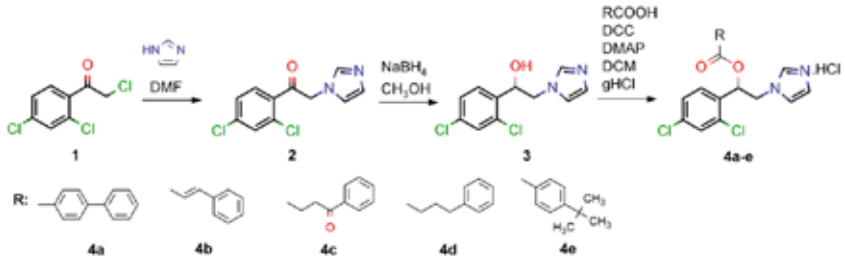
ANTICONVULSANT SCREENING OF NEW (ARYLALKYL)AZOLE ESTERS AND MOLECULAR MODELLING STUDIES THEREOF

Suat Sari (1), Arzu Karakurt (2), Selma Saraç (1), Sevim Dalkara (1)

1) Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Turkey
2) Inönü University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Malatya, Turkey

Backgrounds: Epilepsy is a common chronic neurological disorder. Currently available antiepileptic drugs fail to control one third of the seizures, cause toxicity, and side effects. (Arylalkyl)azoles emerged as a new class of anticonvulsants with nafimidone and denzimol [1].

Aims: In this study a set of (arylalkyl)azole derivatives in 1-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol ester structure were designed, synthesized, and their anticonvulsant activities were evaluated *in vivo* under the Epilepsy Therapy Screening Program (ETSP) of NIH. Their pharmacokinetic properties and possible anticonvulsant mechanisms were predicted *in silico*.



Methods: 4a-e were synthesized by Steglich esterification of **3** with various carboxylic acids in the presence of DCC and DMAP [2]. Their anticonvulsant identification was performed using 6 Hz and maximal electroshock (MES) tests in mice via ip route at two time points (0.5 and 2 h) and three doses (30, 100, and 300 mg/kg) according to the ETSP protocol [3]. Rotorod test was applied to identify neurotoxic effects. A number of physicochemical and pharmacokinetic properties and descriptors were calculated for **4a-o** using QikProp; molecular docking studies were conducted using $GABA_AR$ homology model and extra precision Glide (Schrödinger, LLC, NY, 2018) [4].

Results and Conclusions: All the compounds except **4a** were active in at least one of the models, time points, and doses. **4c** was the most promising among the series with protection at 100 mg/kg and 0.5 h against both 6 Hz and MES induced seizures. Neurotoxicity however was observed for **4b-e** at 300 mg/kg. The compounds showed druglikeness and favourable ADMET properties according to the Qikprop calculations. The active compounds showed high affinity binding to the benzodiazepine binding site of $GABA_AR$ model making interactions in line with the biological data.

Acknowledgements: This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK, grant number: 115S387).

References

1) S. Dalkara, A. Karakurt. Curr. Top. Med. Chem. 12 (2012) 1033-1071.
2) B. Neises, W. Steglich. Angew. Chem. Int. Ed. Engl. 17 (1978) 522-524.
3) J.P. Stables, H.J. Kupferberg. The NIH anticonvulsant drug development (ADD) program: preclinical anticonvulsant, in: G. Avanzini, G. Regesta, P. Tanganelli, M. Avoli (Eds.), Molecular and cellular targets for anti-epileptic drugs, John Libbey & Co., London, 1997, pp. 191-198.
4) R.A. Friesner et al. J. Med. Chem. 49 (2006) 6177–6196.

STRUCTURE-GUIDED DEVELOPMENT OF SUBTYPE-SELECTIVE MUSCARINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS

Hongtao Liu (1), Josefa Hofmann (2), Inbar Fish (3,4), Benjamin Schaake (2), Katrin Eitel (2), Amelie Bartuschat (2), Jonas Kaindl (2), Hannelore Rampp (2), Ashutosh Banerjee (2), Harald Hübner (2), Mary J. Clark (5), Sandra G. Vincent (6), John T. Fisher (6), Markus Heinrich (2), Kunio Hirata (7), Xiangyu Liu (1), Roger K. Sunahara (5), Brian K. Shoichet (3), Brian K. Kobilka (1,8), Peter Gmeiner (2)

1) Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, China
2) Department of Chemistry and Pharmacy, Friedrich Alexander University, Erlangen, Germany
3) Department of Pharmaceutical Chemistry, University of California, San Francisco, USA
4) Department of Biochemistry and Molecular Biology, Tel-Aviv University, Ramat Aviv, Israel
5) Department of Pharmacology, University of California San Diego School of Medicine, California, USA
6) Department of Biomedical & Molecular Sciences and Division of Respirology, Department of Medicine, Queen's University, Kingston, Canada
7) Advanced Photon Technology Division, SR Life Science Instrumentation Unit, Japan
8) Department of Molecular and Cellular Physiology, Stanford University School of Medicine, USA

Acetylcholine unfolds its diverse physiological effects by activating G-protein coupled muscarinic acetylcholine receptors. Five muscarinic subtypes of these class A GPCRs are involved in the regulation of numerous vital functions like heart rate reduction, smooth muscle contraction or glandula secretion [1]. The M3 muscarinic acetylcholine receptor (M3R) appears to be an attractive drug target for its modulating role in the autonomic nervous system. The concept of therapeutic use of M3R antagonism plays an important role in the treatment of chronic obstructive pulmonary disease (COPD) and overactive bladder. While successfully applying M3R antagonists for these disease patterns, almost all commonly used therapeutics suffer from the lack of subtype selectivity most significantly against the M2 muscarinic receptor subtype (M2R), which modulates heart rate [2,3].

The crystal structures of the M2R and M3R [2,3] provide the starting point of developing novel high affinity and subtype-selective ligands. Molecular docking and structure-based design were applied to develop antagonists revealing optimized ligand interactions in the M3R and repulsive interactions in the M2R. In fact, we took advantage of a single amino-acid difference in their orthosteric binding pockets. The resulting M3R antagonists show up to 100-fold selectivity towards M3R over M2R in binding assays while a selectivity over 1000-fold was observed *in vivo*. Using X-ray crystallography the structure of a novel high-affinity M3 receptor antagonist in complex with the M3R was determined. Presenting these results, this work underlines the potential of structure-based drug design to find more subtype-selective drugs with reduced off-target effects.

References

1) Wess, J., et al., Nat Rev Drug Discov, 2007, 6(9): 721-733.
2) Haga, K., et al., Nature, 2012, 482(7386): 547-551.
3) Kruse, A. C., et al., Nature, 2012, 482(7386): 552-556.

DISCOVERY OF NEW ATP-COMPETITIVE HUMAN DNA TOPOISOMERASE INHIBITORS THROUGH BIOCHEMICAL SCREENING OF BACTERIAL DNA GYRASE INHIBITORS LIBRARY

Žiga Skok, Martina Durcik, Nace Zidar, Janez Ilaš

University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana

Human DNA-topoisomerase II is an ATP-dependent enzyme that plays vital roles in processes of transcription, replication and chromosome segregation and therefore represents an attractive target in anticancer drug discovery.[1] Because of the presence of GHKL ATPase domain, DNA topoisomerase II belongs to the same protein superfamily as bacterial DNA Gyrase, Hsp90, histidine kinase and MutL proteins.[2] Based on this fact we used the biochemical screening of existing ATP-competitive bacterial DNA Gyrase inhibitors library, that is a product of an extensive research work of our group on discovery of new antibacterial agents,[3–5] as a starting point in discovery of new human DNA-topoisomerase inhibitors. Initial screening of approximately 100 bacterial DNA-gyrase inhibitors resulted in identification of 12 hit compounds, 9 of which contained a common N-phenylpyrrolamide scaffold that was later used in design and synthesis of new series of human DNA-topoisomerase II inhibitors. Structure-based optimisation of newly discovered hit compounds led to synthesis of new series of human DNA topoisomerase II inhibitors. New inhibitors posses significantly lower molecular weights than original hits which gives them an improved potential for hit-to-lead optimisation. Cytotoxic activity of novel inhibitors was tested on MCF-7 and HepG2 cancer cell lines and one of the compounds showed activity comparable to one of etoposide, a clinically successful DNA-topoisomerase II inhibitor.

References

1) Nitiss JL. DNA topoisomerase II and its growing repertoire of biological functions. Nat Rev Cancer. 2009; 9:327.
2) Dutta R, Inouye M. GHKL, an emergent ATPase/kinase superfamily. Trends Biochem Sci. 2000; 25(1):24-28.
3) Gjorgjieva M, Tomašič T, Barančokova M, et al. Discovery of Benzothiazole Scaffold-Based DNA Gyrase B Inhibitors. J Med Chem. 2016; 59(19):8941-8954.
4) Zidar N, Tomašič T, Macut H, et al. New N-phenyl-4,5-dibromopyrrolamides and N-Phenylindolamides as ATPase inhibitors of DNA gyrase. Eur J Med Chem. 2016; 117:197-211.
5) Durcik M, Tammela P, Barancokova M, et al. Synthesis and Evaluation of N-Phenylpyrrolamides as DNA Gyrase B Inhibitors. ChemMedChem. 2018; 13(2):186-198.

PREPARATION AND BIOLOGICAL EVALUATION OF 7-METHOXYTACRINE-AMANTADINE HYBRIDS AS MULTIPOTENT AGENTS IN THE ALZHEIMER'S DISEASE TREATMENT

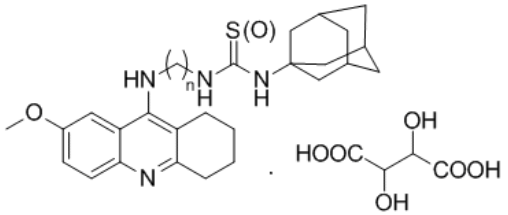
Katarina Spilovska (1,2), Jan Korabecny (3), Kamil Musilek (2), Eugenie Nepovimova (2), Ondrej Soukup (3), Zuzana Gazova (4), Katarina Siposova (4), Daniel Jun (5), Jana Hroudova (1), Eva Mezeiova (1), Kamil Kuca (2)

1) Department of Psychiatry, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic
2) University of Hradec Kralove, Faculty of Sciences, Department of Chemistry, Hradec Kralove, Czech Republic
3) University Hospital, Biomedical Research Centre, Hradec Kralove, Czech Republic
4) Institute of Experimental Physics, Slovak Academy of Sciences, Department of Biophysics, Kosice, Slovak Republic
5) University of Defence, Faculty of Military Health Sciences, Department of Toxicology and Military Pharmacy, Hradec Kralove, Czech Republic

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by a severe, progressive loss of memory. [1] Currently available drugs for AD are the cholinesterase inhibitors tacrine (THA), donepezil, rivastigmine, galantamine and the N-methyl-D-aspartate (NMDA) antagonist memantine. [2] Tacrine was the first inhibitor of acetylcholinesterase (AChE; E.C. 3.1.1.7) to be approved by Food and Drug Administration (FDA). It was withdraw for its hepatotoxicity. 7-methoxytacrine (7-MEOTA) was prepared as a pharmacologically equal active compound with lower toxicity compared to THA. [3]

Memantine (1-aminoadamantane derivate) is an uncompetitive, moderate affinity antagonist of NMDA receptors that inhibits the pathological functions of NMDA receptors while physiological processes in learning and memory are unaffected. It has beneficial effects also in other CNS disorders e.g. Parkinson's disease, stroke, epilepsy. Amantadine, a low-affinity NMDA-receptor blocker, is used in the treatment of Parkinson's disease and also has antiviral activity. [4]

The synthesis of novel class based on 7-MEOTA and amantadine was synthesized and evaluated for their ability to inhibit both cholinesterase, AChE and butyrylcholinesterase (BChE, E.C. 3.1.1.8), to counteract Aβ fibril formation and to act as NMDA receptor antagonists. These compounds could be effective in the treatment of complex disease such as AD with the respect of their ability to interact with the multiple targets. Within our contribution, synthesis, biological properties and molecular modeling studies of 7-MEOTA-amantadine series will be presented. [5,6,7]



K1010-K1023
n = 2-8

This study was supported by grant GA CR No. 17-07585Y given by Grant Agency CR. Authors declare no conflict of interest.

References

1) Craig, L. A. et al. Neurosci. Biobehav. Rev. 2011, 35, 1397-1409.
2) Salloway, S. et al. Alzheimers. Dement. 2008, 4, 65-79.
3) Korabecny, J. et al. Bioorg. Med. Chem. Lett. 2011, 21, 6563-6566.
4) Sonkusare, S. K. et al. Pharm. Res. 2005, 51, 1-17.
5) Spilovska, K. et al. Molecules. 2013, 18, 2397-2418.
6) Spilovska, K. et al. Med. Chem. Res. 2015, 24, 2645-2655.
7) Gazova, Z. et al. Biochim. Biophys. Acta. 2017, 1863, 607-619.

DESIGN, SYNTHESIS AND TESTING OF THE PLASMODIUM FALCIPARUM DIHYDROOROTATE DEHYDROGENASE INHIBITORS

Nika Strašek (1), Jurij Svete (1), Marko Novinec (1), Jernej Wagger (2)

1) Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia
2) Lek Pharmaceuticals d.d., ChemOps Menges, Kolodvorska 27, 1234 Mengeš

Malaria is a third world disease that annually causes around 200 million infections and about half million deaths. The biggest issue is the rapid development of resistance to all newly approved medicines, which means that malaria is still an incurable disease. The main cause of infection is the *Plasmodium falciparum* parasite, which can be transmitted through mosquitoes of the *Anopheles* type.

Plasmodium falciparum dihydroorotate dehydrogenase (*Pf*DHODH), a fourth enzyme in the *de novo* pyrimidine biosynthesis pathway has emerged as a promising target for antimalarial drugs. *Pf*DHODH is located on the outer side of the inner mitochondrial membrane and catalyzes the conversion of dihydroorotate to orotate. The reaction requires two cofactors, a flavinmononucleotide (FMN), which is needed for the oxidation of dihydroorotate and ubiquinone, a terminal electron acceptor, necessary for the reoxidation the FMN.

In *Plasmodium falciparum* the path of *de novo* pyrimidine biosynthesis is the only source of pyrimidine production, whereas in humans this biosynthetic pathway is only one of the sources of pyrimidines, which makes *Pf*DHODH a promising target. Studies have shown that *Pf*DHODH inhibition leads to parasite death.

In an effort to discover new and potent *Pf*DHODH inhibitors, a number of different compounds were virtually tested using the Schrödinger Glide molecular docking program. The best results were obtained with two types of compounds, bicyclic 3-pyrazolidinones and theophylline-7-acetamides. Bicyclic 3-pyrazolidinones were prepared by a microwave-assisted three-component reaction between a 3-pyrazolidinone, an aldehyde, and an acrylate via formation of an azomethine imine, followed by 1,3-dipolar cycloaddition. Teophylline-7-acetamides were prepared by condensation of easily available theophylline 7-acetic acid with α-amino esters, followed by hydrolysis of the ester group.

The recombinant enzyme *Pf*DHODH was expressed by DH5α *E. coli* cells. After the expression the enzyme was isolated and purified with nickel affinity chromatography.

The assays of biological activity were performed by measuring the absorbance of the colorimetric substrate dichlorophenolindophenol (DCIP). Results show IC_{50} values for compounds **1–4** in the range of low μM concentrations (**Figure 1**).

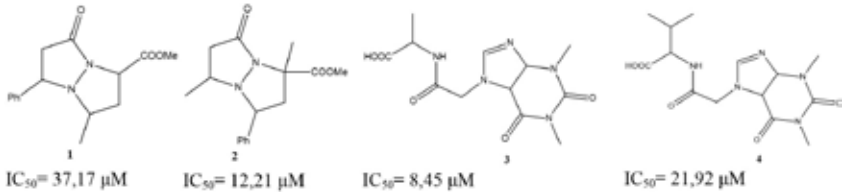


Figure 1. Compounds with the most promising results

The selectivity against *Homo sapiens* dihidroorotate dehydrogenase (*Hs*DHODH) was tested. Bicyclic 3-pyrazolidinones show better selectivity compared to teophylline-7-acetamides.

References

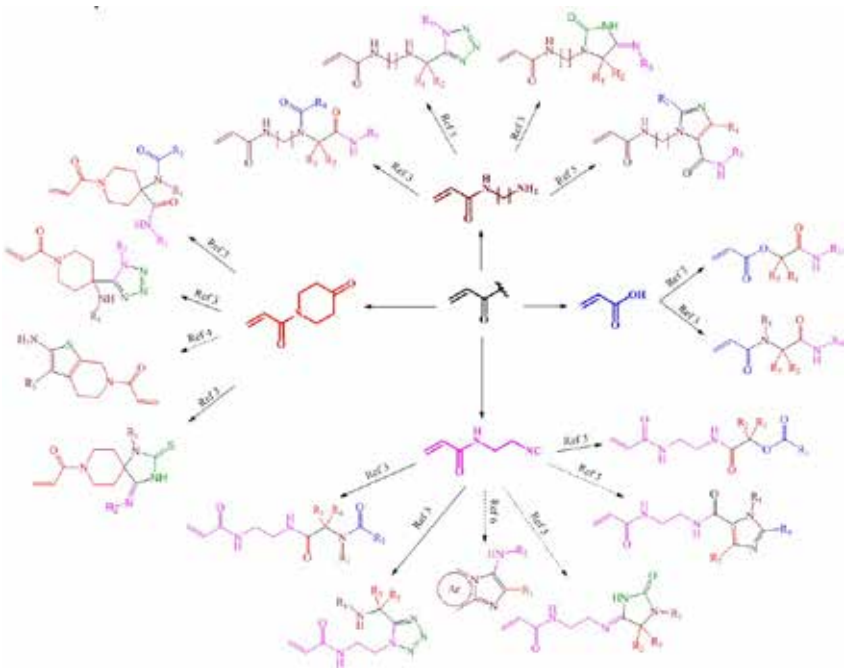
1) Baldwin, J.; Farajallah, A.M.; Malmquist, N.A. et al., Malarial Dihydroorotate Dehydrogenase. The Journal of Biological Chemistry 2002, 44, 41827–41834.
2) Grošelj, U.; Svete, J., Recent advances in the synthesis of polysubstituted 3-pyrazolidinones. ARKIVOC 2015, 6, 175–205.
3) Voynikova, Y.; Valchevab, V.; Momekova, G. et al., Theophylline-7-acetic acid derivatives with amino acids as anti-tuberculosis agents. Bioorganic & Medicinal Chemistry Letters 2014, 24, 3043–3045.

EXPLORING MULTI-COMPONENT REACTIONS TO SYNTHESIZE COVALENT INHIBITORS

Fandi Sutanto, Alexander Dömling

Department Drug Design, University of Groningen, Antonius Deusinglaan 1, 9713AV, Groningen, The Netherlands, f.sutanto@rug.nl, a.s.s.domling@rug.nl

Covalent inhibitors play important role in drug discovery and therapeutics. About 30% of marketed drugs are covalent inhibitors, ranging from obesity to cancer.[1] The toxicity of covalent inhibitors is a major concern, but the advantages provided by them offer a large opportunity of exploring them even further. There are different warheads that act as covalent inhibitors, for example α,β-unsaturated carbonyl, epoxide, β-lactam, β-lactone, halomethyl, α-keto derivatives, etc.[2] Multi-component reactions are powerful tools that can be used to synthesize covalent inhibitors. This work focused on synthesizing α,β-unsaturated carbonyl compounds, a Michael acceptor that binds covalently towards cysteine residue, through multi-component reactions.



References

1) A. R. Bauer, Drug Discov. Today. 2015, 20, 1061-1073.
2) S. D. Cesco, J. Kurian, C. Dufresne, A.K. Mittermaier, N. Moitessier, Eur. J. Med. Chem. 2017, 138, 96-114.
3) A. Dömling, I. Ugi, Angew. Chem. Int. Ed. 2000, 39, 3168-3210.
4) Y. Huang, A. Dömling. Mol Divers. 2011, 15, 3-33.
5) C. Zhang, E. J. Moran, T. F. Woiwode, K. M. Short, A. M. M. Mjalli. Tetrahedron Lett. 1996, 37, 751-754.
6) H. Bienaymé, K. Bouzid. Angew. Chem., Intl. Ed. 1998, 37, 2234-2237.

DESIGN, SYNTHESIS AND MOLECULAR MODELING OF POTENTIAL LIGANDS FOR IONOTROPIC GLUTAMATE RECEPTORS

Ewa Szymanska, Paulina Chalupnik

Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland

Fast excitatory synaptic transmission in the CNS relies almost entirely on the neurotransmitter glutamate and its family of ion ligand-gated channel receptors (*i*GluRs). The family of *i*GluRs is divided into three functionally distinct subclasses: NMDA, AMPA and kainate receptors. Structurally, AMPA-receptors are cation-selective tetrameric heterooligomers formed by combinations of the highly homologous subunits GluA1-4, while kainate receptors are tetrameric assemblies of GluK1-5 subunits.

The present project is a continuation of earlier studies on potent and selective competitive AMPA and/or KA receptors ligands among phenylalanine derivatives.[1-3] In the design process, a series of molecular docking experiments to recently published X-ray structures of the glutamate ionotropic receptors binding sites was performed for a set of compounds with a general structure based on the biphenylalanine scaffold and substituted with the aryl/alkyl amine group at the 5-position of the phenylalanine ring. The influence of the amine substituent structure (length of the alkyl chain, presence of branched chains or aromatic groups) on the observed docking scores as well as an expected affinity and subtype-selectivity of ligands was intensively studied. On the basis of docking results the most promising compounds, presenting the best docking score function values, were selected to further synthetic studies. A method of their synthesis was developed, using as the key step the Buchwald cross-coupling reaction. The optimization of chemical conditions (palladium catalyst, base, solvent as well as the temperature and time of the reaction) applied to the Buchwald reaction was performed. In the present work both the docking-based design and synthesis of selected amino acids is reported.

Acknowledgements

The financial support of the National Science Centre Poland (2014/15/B/NZ7/00908) is gratefully acknowledged.

References

1) E. Szymańska, K. Frydenvang, D.S. Pickering, C. Krintel, B. Nielsen, A. Kooshki, L.G. Zachariassen, L. Olsen, J.S. Kastrup, T.N. Johansen: Studies on Aryl-Substituted phenylalanines: Synthesis, activity, and different binding modes at AMPA receptors, J. Med. Chem. 2016, 59, 448-461.
2) E. Szymańska, B. Nielsen, T.N. Johansen, A.M. Cuñado Moral, D.S. Pickering, K. Szczepańska, A. Mickowska, K. Kieć-Kononowicz: Pharmacological characterization and binding modes of novel racemic and optically active phenylalanine-based antagonists of AMPA receptors, Eur. J. Med. Chem. 2017, 138, 874-883.
3) E. Szymańska, P. Chałupnik, K. Szczepańska, A.M.C. Moral, D.S. Pickering, B. Nielsen, T.N. Johansen, K. Kieć-Kononowicz: Design, synthesis and structure–activity relationships of novel phenylalanine-based amino acids as kainate receptors ligands, Bioorg. Med. Chem. Lett. 2016, 26, 5568-5572.

IRREVERSIBLE INHIBITION OF MONOAMINE OXIDASE B ENZYME. A COMPUTATIONAL INSIGHT

Tana Tandaric, Robert Vianello

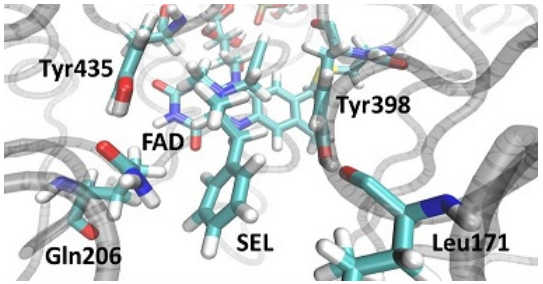
Ruder Boskovic Institute, Bijenicka cesta 54, 10000 Zagreb, Croatia

Monoamine oxidases are mammalian flavoenzymes responsable for regulation of amine neurotransmiter levels. This enzymes represent main pharmacological target for threatment of depression and neurodegenerative diseases. Two isoform of this enzyme are present in human body, MAO A and MAO B, which share about 70% of the identity in the primary sequence, but show significant differences in substrate selectivity and inhibitor specificity and in particular. [1] Focus of this work are selective irreversible inhibitors of MAO B, selegiline and rasagiline, widely used in treatment of symptoms of Parkinson and Alzheimer disease. Both inhibitors form covalent bond with organic cofactor flavin adenine dinucleotide (FAD). In that way they prevent MAO B enzyme's further catalytic activity.

Here, we used a molecular dynamics (MD) simulations, to simulate 300 ns of interaction of MAO B with both inhibitors. It is shown that Tyr398 and Tyr435 form aromatic cage responsable for interaction with aromatic part of inhibitor. Ile199 is characterized as structurally responsible for the selectivity of the inhibitor, which confirms the experimentally obtained results. [2] Aromatic interactions of the inhibitors with the aromatic cage amino acids as well as the hydrogen bonds between the inhibitors and the flavin cofactor carbonyl oxygen O8 orient the inhibitors in a favorable position for the reaction leading to covalent binding of the FAD inhibitor. Using MM-PBSA tools, free binding energy values were obtained. The results show that selegiline binds better than rasagiline by 1.4 kcal/mol which is consistent with experimental IC_{50} values. [3]

Quantum-chemical analysis within the enzyme cluster model showed that MAO inhibition proceeds troughtthe 4-step reaction, with the first step determining the total reaction rate, in which FAD cleaves the hydride ion from the α-methylene group of the substrate in complete analogy with the MAO catalytic mechanism. [4] The resulting reaction profiles and the final structure inhibited by the enzyme are in excellent agreement with the experimental data.

The results obtained are of great importance for the development of new and more effective MAO B inhibitors for clinical use.



References

1) R. R. Ramsay, Curr. Top. Med. Chem. 2012, 12, 2189–2209.
2) F. Hubálek, C. Binda, A. Khalil, M. Li , A. Mattevi, N. Castagnoli, D.E. Edmondson, J. Biol. Chem. 2005, 280, 15761-15766.
3) M. B. H.Youdim, A. Gross, J. P. M. Finberg, Br. J. Pharmacol., 2001, 132, 500–506.
4) R. Vianello, C. Domene, J. Mavri, Front. Neurosci. 2016, 10, 327-351.

NEW BRAIN PENETRANT COMPOUNDS IN ADVANCED STUDIES FOR CNS DISEASES

Nikolay T. Tzvetkov (1), Andrew Lowerson (2), Steve Brough (2)

1) NTZ Lab Ltd., Krasno Selo 198, 1618 Sofia, Bulgaria, ntzvetkov@ntzlab.com
2) Key Organics Ltd., Highfield Road Industrial Estate, Camelford, Cornwall, PL32 9RA, UK

A new collection of structurally optimized compounds addressing central nervous system (CNS) diseases, which have been recently licensed, is now available in the BIONET collection. This screening collection with experimentally-determined bioactivity, ADME and bioavailability will address the need of new, robust, and multipotent small molecules for the treatment and diagnosis of CNS diseases, such as Parkinson´s disease, Alzheimer´s disease, dementia and/or other neurodegenerative diseases [1–3].

The neuroprotective effects and the induction of the neurite network outgrowth of the most promising compounds have been investigated. These effects are associated with a good BBB penetration that was confirmed in several *in vitro* assays. To investigate the multiple mode of action of this set of compounds, we applied a combined X-ray/modelling platform, which is also discussed.

In addition, the compounds are easily accessible and offer the possibility of broad structural diversities in order to further explore the chemical space within further biological screening on relevant CNS targets. This poster will summarize the potential of this next generation compounds that are available for further screening by prospective licensees and elaboration for CNS disease treatment [4].

References

1) Tzvetkov et al. J. Med. Chem., 2014, 57(15), 6679–6703.
2) Tzevtkov et al. Eur. J. Med. Chem., 2017, 127, 470–492.
3) Tzvetkov PCT Int. Appl. 2014/107771.
4) www.keyorganics.net

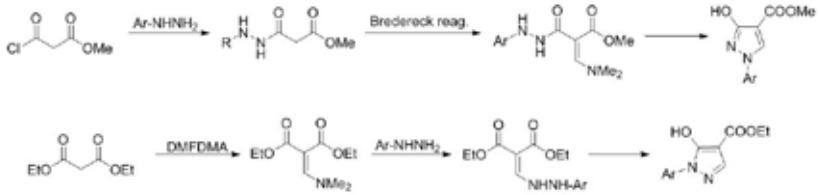
DESIGN, SYNTHESIS AND IN VITRO TESTING OF PYRAZOLE BASED INHIBITORS OF PLASMODIUM FALCIPARUM DIHYDROOROTATE DEHYDROGENASE

Luka Vah (1), Jernej Wagger (2), Marko Novinc (1), Jurij Svete (1)

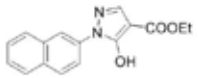
1) Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana
2) Lek Pharmaceuticals d.d., ChemOps Menges, Kolodvorska 27, 1234 Mengeš

Malaria is an everlasting problem in the tropical region. Although there are several drugs in practical use nowadays, there is an ongoing problem of developing resistance towards these therapies. Therefore a constant development of new therapheutic drugs is needed.[1]

Design of our molecules was based on methylene malonates that were already proven in the past as inhibititors of P*f*DHODH.[2] This is an enzyme essetnial for the *de novo* biosynthesis of pyrimidines used in the nucleic acid biosynthesis in *Plasmodium Falciparum.* The rationale behind our design was to prepare comformationally more rigid scaffolds with aromatic lipophilic moeties protruding in different directions of active site. Molecular docking was used as an assisting tool for the design of our compounds. Designed compounds were prepared using enaminone based synthesis.[3]



All compounds tested in vitro on isolated P*f*DHOD showed moderate to good inhibition. Compound with the biggest inhibition potential was ethyl 5-hydroxy-1-(naphthalen-2-yl)-1H-pyrazole-4-carboxylate. Inhibiton was above 70% with IC_{50}=200±30 µM.



References

1) M. Mushtaque, Shahjahan. Reemergence of chloroquine (CQ) analogs as multi-targeting antimalarial agents: a review. Eur. J. Med. Chem. 2015, 90, 280-295.
2) T. Heikkilä, C. Ramsey, M. Davies, C. Galtier, A. M. W. Stead, A. P. Johnson, C. W. G. Fishwick, A. N. Boa, G. A. McConkey. Design and Synthesis of Potent Inhibitors of the Malaria Parasite Dihydroorotate Dehydrogenase. J. Med. Chem. 2007, 50, 186-191.
3) B. Stanovnik, J. Svete. Synthesis of Heterocycles from Alkyl 3-(dimethyamino)propenoates and Related Enaminones. Chem. Rev. 2004, 104, 2433-2480.

P421

SMALL SCALE PURIFICATION OF FRACTIONS FROM A COMPLEX PHARMACEUTICAL FORMULATION USING AN ANALYTICAL FRACTION COLLECTOR AND A UHPLC-MS SYSTEM

Eric S.E. van Beelen (1), Fadi L. eb Alkhate (2), Ronan Cleary (2), Paul Rainville (2)

1) Waters Corporation, Paris, France
2) Waters Corporation, 34 Maple Street, Milford, MA 01757

Isolating active pharmaceutical ingredients from excipients can be challenging especially in complex pharmaceutical formulations. Researchers with such challenging samples frequently use multidimensional systems to separate analytes of interest from other components. While the use of such systems is normally sufficient, the setup may not always be available or practical. In this study we demonstrate the use of a microscale analytical fraction collector in conjunction with an ultra-high pressure liquid chromatography/mass spectrometry system to purify, separate and identify several components of a model complex pharmaceutical formulation.

An over-the-counter cold and cough syrup (DayQuil) was used as a model complex pharmaceutical formulation in this work. The challenges of performing fractionation in a complex matrix is highlighted in this study. Multiple modes of fraction collection will be demonstrated showing the benefits of using mass directed collection of fractions.

P422

EVALUATION OF DART (DIRECT ANALYSIS IN REAL TIME), COUPLED TO A PORTABLE MASS DETECTOR FOR RAPID CLEANING VALIDATION

Chris Henry (1), Mark Wrona (2), Jane Kirk (1), Jannet Hammond (1), Richard Ladd (1), Eric S.E. van Beelen (3)

1) Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, Cheshire SK9 4AX, UK
2) Waters Corporation, 34 Maple Street, Milford MA, USA
3) Waters Corporation, Paris, France

Cleaning validation is a vital part of Quality Control (QC) workflow and is defined as the process of providing documented evidence that the cleaning methods employed within a facility consistently controls potential carryover of product (including intermediates and impurities), cleaning agents and extraneous material into subsequent product to a level which is below predetermined levels.

Cleaning validation is a required activity within the pharmaceutical, biological, nutritional supplement and medical device industries. From both a regulatory and industry standpoint, cleaning validation is recognised as an important activity to establish that product cross-contamination is controlled to ensure patient safety and product quality.

Two analytical methods predominate cleaning validation analysis i.e HPLC/UV which is limited to chromophore containing compounds, and TOC (Total Organic Carbon) which will detect any source of carbon but is not specific and therefore anomalous results are required to be submitted for further testing i.e. LC/MS

Here we discuss MS based strategies for cleaning validation. The Waters Acquity UPLC H-Class coupled to a Waters Acquity QDa mass detector provides a robust, sensitive and specific methodology. A more rapid and convenient analysis methodology was also evaluated using DART (IonSense, Saugus, MA, USA) coupled to a QDa, which offers a direct ambient ionisation sampling technique with little or no sample prep required.

Both approaches will be evaluated for speed, efficiency, and also sensitivity to ensure sufficiently low levels of quantitation are being reached to ensure industry vessels etc are sufficiently clean.

IN-PROCESS CONTROL OF ERGOLINE PSYCHEDELICS DURING CHEMICAL SYNTHESIS BY HPTLC COUPLED WITH MASS DETECTION

Matthias Grill (1), Melanie Broszat (2), Eric S.E. van Beelen (3)

1) Lipomed AG, Fabrikmattenweg 4, 4144 Arlesheim, Switzerland
2) CAMAG, Sonnenmattstr. 11, 4132 Muttenz, Switzerland
3) Waters Corporation, Paris, France

TLC is a simple and convenient tool for monitoring classical organic syntheses. Standardized HPTLC may additionally provide reliable analytical endpoints and significantly improved separation. Using mass detection in combination with HPTLC allows for structure confirmation.

The CAMAG TLC-MS Interface 2 is used to directly elute target zones from the HPTLC plate into the Waters ACQUITY QDa® mass detector. A second confirmation can be achieved by recording the UV spectra with the TLC scanner. This work shows a generic method for the identification of synthetic products with HPTLC-MS, using the in-process synthesis and quality control s ergoline psychedelics as an example.

HYBRID MICROPARTICLES BASED ON ZERO-VALENT IRON FOR SIMULTANEOUS DRUG DELIVERY AND ULTRASONOGRAPHY VISUALIZATION

Sergei Vlasov (1,2), Mikhail Belousov (2), Mekhman Yusubov (1,2), Antonio Di Martino (1,3), Pavel Postnikov (1)

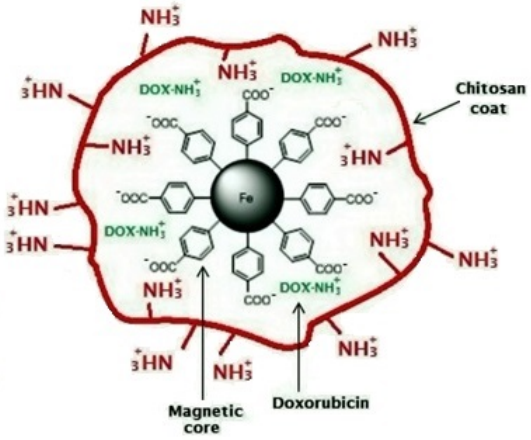
1) National Research Tomsk Polytechnic University, Lenin Avenue 30, 634050 Tomsk, Russian Federation
2) Siberian State Medical University, Moskovsky trakt 2, 634055, Tomsk, Russian Federation
3) Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Tr. T. Bati 5678, 760 01 Zlin, Czech Republic

The diagnostic ultrasound, or ultrasonography, represents a method that uses high-frequency sound waves to produce images of structures within the body. In the last decades, a great interest has been focused on the use of ultrasound irradiation not only as a diagnostic tool but also as external stimuli to trigger the release of bioactive compounds from specific carriers [1]. Herein, organic-inorganic microparticles able to carry a model drug, doxorubicin, control the release and be detectable upon ultrasound application were developed.

The particles with dimension in the range 50-100mm are based on iron zerovalent magnetic core stabilised by low molecular weight chitosan adsorbed on the surface. Amount of doxorubicin per 1 mg of zero-valent iron carriers is 0.179 mg.

Release studies demonstrate the capability to trigger and control the release of doxorubicin in simulated physiological conditions by varying the intensity of the ultrasonic irradiation. It is supposed to reduce the toxicity of doxorubicin and increase its concentration at the target site.

The possibility to detect the microparticles using ultrasound was investigated ex-vivo using Sus Domesticus liver. A stock solution of bare microparticles was injected in a designed site of the organ and subjected to ultrasound using a linear array transducer. As a result at the site of administration of the microparticles solution contrasting was observed on the echogram.



References

1) CHEN, M., XU, A., HE, W., MA, W., SHEN, S. Ultrasound triggered drug delivery for mitochondria targeted sonodynamic therapy. Journal of Drug Delivery Science and Technology, 2017,39, pp.501-507.

FRAGMENT BASED DESIGN OF O-GLCNAC TRANSFERASE INHIBITORS

Matjaž Weiss (1), Tihomir Tomašič (1), Hao Zhang (2), Shi Jie (2), Rob Ruijtenbeek (2,3), Roland Pieters (2), Marko Anderluh (1)

1) University of Ljubljana, Faculty of Pharmacy
2) Utrecht University, Medicinal Chemistry and Chemical Biology
3) PamGene International BV

O-GlcNAc transferase (OGT) is an essential mammalian enzyme involved in the dynamic O-GlcNAcylation of cytosolic and nuclear proteins. Through catalyzing the attachment of N-acetylglucosamine to specific serines and threonines of proteins, OGT is associated with numerous biological processes such as transcription, the cell cycle progression, the stress response and nutrient sensing.[1,2,3,4,5] In metabolic diseases like cancer and diabetes, increase of various metabolic products like glucose into the cell alters the production of UDP-GlcNAc through the hexosamine biosynthetic pathway. This promotes O-GlcNAcylation since OGT is highly sensitive to intracellular UDP-GlcNAc levels.[6,7,8,9]

To identify fragments targeting the donor UDP site, we have conducted a structure-based virtual screening in a fragment library containing more than 216,000 molecules. Among virtual hits, seven compounds contained the same scaffolds as they were all quinolone-4-carboxamides. A common feature of these molecules is that in the predicted binding mode the quinolone ring is anchored in the uridine binding site of OGT and the additional carboxamides point to the diphosphate binding site.

To further explore this finding, a series of 22 fragments carrying diverse carboxamides was prepared. The synthesis was conducted by coupling 2-hydroxyquinoline-4-carboxylic acid with various amines using EDC/HOBt to effect the coupling. The inhibitory potency of these compounds on OGT activity was measured using the UDP-Glo assay and several fragments were found to inhibit OGT activity. The most potent fragments were conjugated by short peptide with intent to reach improved synergy effect of the two component hybrid inhibitor.

References

1) J. A. Hanover, M. W. Krause and D. C. Love, Nat. Rev. Mol. Cell Biol., 2012, 13, 312–321.
2) S. Daou, N. Mashtalir, I. Hammond-Martel, H. Pak, H. Yu, G. Sui, J. L. Vogel, T. M. Kristie and E. B. Affar, Proc. Natl. Acad. Sci., 2011, 108, 2747–2752.
3) C. Butkinaree, K. Park and G. W. Hart, Biochim. Biophys. Acta - Gen. Subj., 2010, 1800, 96–106.
4) J. A. Groves, A. Lee, G. Yildirir and N. E. Zachara, Cell Stress Chaperones, 2013, 18, 535–558.
5) G. W. Hart, C. Slawson, G. Ramirez-Correa and O. Lagerlof, Annu. Rev. Biochem., 2011, 80, 825–858.
6) Y. Fardini, V. Dehennaut, T. Lefebvre and T. Issad, Front. Endocrinol., 2013, 4, 99.
7) G. W. Hart, M. P. Housley and C. Slawson, Nature, 2007, 446, 1017–1022.
8) R. M. de Queiroz, E. Carvalho and W. B. Dias, Front. Oncol., 2014, 4, 1–10.
9) C. M. Ferrer, T. P. Lynch, V. L. Sodi, J. N. Falcone, L. P. Schwab, D. L. Peacock, D. J. Vocadlo, T. N. Seagroves and M. J. Reginato, Mol. Cell, 2014, 54, 820–831.

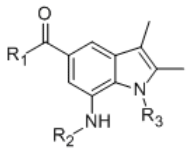
DISCOVERY OF NOVEL AND POTENT POTASSIUM COMPETITIVE ACID BLOCKERS: JP-1366

In-Hae Ye, Chang-Seok Lee, Joonwoo Nam, Jiseon Park, Taejeong Kim, Jungmee Lee, Jiyoon Kim, Jeongmin Kim

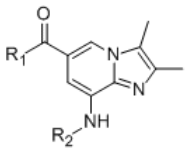
R&D Center, JEIL Pharmaceutical Co., Ltd., Cheongganggachang-ro 7, Yongin-si, 17172, Republic of Korea

Potassium-competitive acid blockers (P-CABs) inhibit H^+/K^+-ATPase of the stomach in a similar manner to that of proton pump inhibitors (PPIs). Unlike PPIs, P-CABs bind reversibly to the H^+/K^+-ATPase without a change of their chemical structure, and inhibit acid secretion even in the resting state of the proton pump. Therefore, P-CABs are superior to PPIs in the rapidity, persistence and convenience of treatment and are being developed as next-generation therapy for the gastrointestinal disease.

Herein, we synthesized 7-amino-1*H*-indole derivatives (I) and imidazo[1,2-*a*]pyridine derivatives (II) as novel and potent H^+/K^+-ATPase inhibitors, and evaluated their inhibitory activities against H^+/K^+-ATPase and other pharmacological properties. Modification with structure-activity relationship study yielded JP-1366, which is more potent than vonoprazan (TAK-438) and is best-in-class in the area of PCABs. It showed excellent activity at *in-vitro* enzymatic assay (IC_{50} = 16.7 nM) and *in-vivo* assay in a histamine-stimulated gastric acid secretion in pylorus-ligated rat (ED_{50} = 0.66 mg/kg) and a gastroesophageal reflux disease (GERD) animal model (ED_{50} = 0.53 mg/kg). Fast onset time and long-term activity were confirmed in a lumen perfused rate (LPR) model and heidenhain pouch dog (HPD) model. Pharmacokinetic studies of rat and dog showed good profiles (rat, C_{max}: 389 ng/ml, $t_{1/2}$: 2.6 hr; dog, C_{max}: 4,530 ng/ml, $t_{1/2}$: 5.6 hr). HepG2 cytotoxicity assay, hERG assay, and Ames tests showed no toxicity. Based on the efficacy, safety pharmacology and toxicity studies, JP-1366 is selected as a clinical candidate, and currently, its phase I clinical trial is underway.



(I)



(II)

COMPUTATIONAL STUDIES, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW AMINOACID 2,3-DICHLORONAPHTHALEN-1,4-DIONE DERIVATIVES

José Alejandro Lozada (1), Margarita Gutiérrez (2), Jorge Campusano (3), Flavia Zacconi (1,4)

1) Faculty of Chemistry, Pontificia Universidad Católica de Chile, Chile
2) Instituto de Química de Recursos Naturales, Universidad de Talca, Chile
3) Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Chile
4) Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile, Chile

Quinone and naphthoquinones moieties are present in many drugs used for the treatment of different pathologies like cancer and neurodegenerative diseases.[1] One of the most important synthetic precursors in the Medicinal Chemistry field is 2,3-dichloronaphthalen-1,4-dione (**1**, Dichlone). This is a small synthetic quinone molecule, which contains two chlorine atoms in position 2 and 3 of the quinone structure. The substitution of one of the chlorine atoms confer dichlone derivatives diverse biological activity.[1–3]

Dichlone has been used as an insecticide and larvicide for specific mosquito species, It is not know the molecular target underlying this action, but one possibility is that dichlone acts on insect cholinesterase.

We began studying the possibility that dichlone derivatives interact with acetyl and butyrylcholinesterase enzymes (AChE, BuChE), to assess differential activity and selectivity of these compounds. Here we report the synthesis of a new series of dichlone amino acid derivatives (**3**) as potential inhibitors of AChE, by a nucleophilic substitution reaction under mild reaction conditions. The synthesis was carried out considering that inhibitors must bear a central aromatic ring system that interacts in the active site to meet structural requirements.[4-6]. The isolated products were obtained with good yields (75 – 85 %) and were characterized by spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, IR, MS)

We also studied the potential cytotoxic activity of these compounds on Schneider 2 (S2) insect cells, which could be explained by an action on the cholinesterase enzyme. S2 is a cell line derived from fly *Drosophila melanogaster* (Dm) embryos. Dm is a worldwide insect model used to identify molecular targets of new chemicals with insecticide potential activity, manipulate insecticide resistance genes, and also to investigate the interactions between ligands and proteins.

Finally, to determine the possible interaction between the compounds and *Dr*osophila AChE (DmAChE) docking studies were performed (DmAChE PDB code 1DX4). The docked compounds were stabilized in the cavity through different types of interactions including hydrogens bonds and π-π stacking interactions. *In silico* assays were used to estimate the binding energy and inhibition constant of these compounds.

Our data suggest that some moieties enhance cytotoxic properties of the dichlone derivatives in the nano and micromolar range.

Acknowledgements: Financially supported by Proyecto FONDECYT Regular N° 1150822 to FCZ. AL is grateful to Beca VRI-UC.

References

1) Valderrama JA, Leiva H, Rodrıguez JA, Theoduloz C, Schmeda-Hirshmann G. Studies on quinones. Part 43:Synthesis and cytotoxic evaluation of polyoxyethylene-containing 1,4-naphthoquinones. Bioorg Med Chem. 2008;16:3687-3693.
2) Kim MG, Lee HS. Insecticidal toxicities of naphthoquinone and its structural derivatives. Appl Biol Chem. 2016;59;3-8.
3) Zacconi FC, Cabrera AL, Ordoñez-Retamales F, del Valle JM, de la Fuente JC. Isothermal solubility in supercritical carbon dioxide of solid derivatives of 2,3-dichloronaphthalene-1,4-dione(dichlone): 2-(Benzylamino)-3-chloronaphthalene-1,4-dione and 2-chloro-3-(phenethylamino)naphthalene-1,4-dione. J Supercrit Fluids. 2016;1-8.
4) Chaudhari D, Gejji SP, Lande DN, Chakravarty D, Salunke-Gawali S. Polymorphism in chloro derivatives of 1,4-naphthoquinone: Experiment and density functional theoretic investigations. J Mol Struct. 2016;1120:281-293.
5) Prachayasittikul V, Pingaew R, Worachartcheewan A. Anticancer activity and QSAR study of 1,4-naphthoquinone derivatives. Eur J Med Chem. 2014;84:247-263.
6) Lan J, Zhang T, Liu Y, Yang J, Xie S, Liu J. Synthesis and biological activity of novel donepezil derivatives bearing N-benzyl pyridinium moiety as potent and dual binding site acetylcholinesterase inhibitors. Eur J Med Chem. 2017;133:184-196.

COMPUTATIONAL DESIGN, SYNTHESIS AND ENZYMATIC ANALYSIS OF DISUBSTITUTED AMINES TRIAZOLES AS POTENTIAL FACTOR XA INHIBITORS

Fabián Santana-Romo (1), Yanina Moglie (2), Flavia Zacconi (1,3)

1) Faculty of Chemistry, Pontificia Universidad Católica de Chile, Chile.
2) Instituto de Química del Sur (INQUISUR-CONICET), Departamento de Química, Universidad Nacional del Sur (UNS), Argentina.
3) Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile

Factor Xa (FXa) plays a key role in haemostasis, it is a central part of the blood coagulation cascade which catalyzes the production of thrombin and leads to clot formation and wound closure. Clotting is a sequential process that involves the interaction of coagulation factors. Therefore, FXa is a focus target for the development anticoagulants due to its central position in the blood coagulation cascade.[1] FXa has emerged as an attractive target for developing safer anticoagulant drugs. Inhibition of FXa should prevent the production of new thrombin without affecting its basal level, ensuring primary haemostasis, unlike injectable heparins or the most commonly used oral anticoagulant in the US, such warfarin.

In this work novel arylazides were synthesized incorporating a lactamic ring (**1**) with different heteroatoms in position 4 as starting materials for the new derivatives. The pharmacophore fragment of these compounds was considered essential to achieve the FXa inhibitor activity.[2] From the arylazides synthesized in the first step, a series of triazoles (**3**) were prepared using copper nanoparticles as catalyst, to obtain 1,2,3-triazoles product of the dipolar cycloaddition[3] by using a variety of terminal alkynes with good yields (70-85%).

In our research using the computational tools allows us to develop new synthetic ligands to interact with high specificity with the S1 and S4 pockets enzyme. The aryl lactam core present favorable π- π interactions with the S4 pocket and hydrophobic interactions produce by the aliphatic chain with residues GLY193, GLN192, CYS191, ALA190, ASP189, VAL213, SER214 and TRP215 present in S1 pocket.

Moreover, FXa inhibition assays were performed in order to obtain the IC_{50} values of the corresponding new derivatives.

Acknowledgements: Financially supported by Proyecto FONDECYT Regular N° 1181408 to FCZ. FS is grateful to Beca VRI-UC.

References

1) Núñez-Navarro, NE.; Santana FM.; Parra LP.; Zacconi FC. Curr. Med. Chem. 2018, 25, 42.
2) Núñez-Navarro, N. E.; Segovia, G. F.; Burgos, R. A.; Lagos, C. F.; Fuentes-Ibacache, N.; Faúndez, M. A.; Zacconi, F. C. J. Braz. Chem. Soc. 2017, 28, 203-207.
3) Alonso, F.; Moglie, Y.; Radivoy, G. Acc. Chem. Res. 2015, 48, 2516−2528.

NEW N-PHENYLPYRROLAMIDES AS DNA GYRASE B INHIBITORS

Nace Zidar (1), Martina Durcik (1), Skok Žiga (1), Cristina Durante Cruz (2), Päivi Tammela (2), Tihomir Tomašič (1), Gábor Draskovits (3), Ákos Nyerges (3), Csaba Pál (3), Janez Ilaš (1), Lucija Peterlin Mašič (1), Danijel Kikelj (1)

1) Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
2) Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), 00014 Helsinki, Finland
3) Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged H-6726, Hungary

Today, we are increasingly faced with life-threatening infections due to resistant Gram-positive and Gram-negative pathogens. The ATP binding site located on the subunit B of DNA gyrase is an attractive target for the development of new antibacterial agents. In recent decades, several small-molecule inhibitor classes have been discovered but none has so far reached the market.

Using structure-based design starting from the recently determined crystal structure of the *N* -phenyl-4,5-dibromopyrrolamide inhibitor-DNA gyrase B complex,[1] we have prepared a series of improved *N* -phenylpyrrolamides and evaluated them against DNA gyrase and topoisomerase IV from *Escherichia coli* and *Staphylococcus aureus*. IC_{50} values for the most potent compounds were in the low nanomolar range. Minimum inhibitory concentrations (MICs) against Gram-positive and selected Gram-negative bacteria were in the low micromolar range. The oxadiazolone derivative **11a**, with an IC_{50} value of 85 nM against *E. coli* DNA gyrase displayed MIC values of 1.56 μM against *Enterococcus faecalis*, and 3.13 μM against wild type *S. aureus*, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). The activity against wild type *E. coli* in the presence of efflux pump inhibitor phenylalanine-arginine β-naphthylamide (PAβN) was 4.6 μM.[2-4]

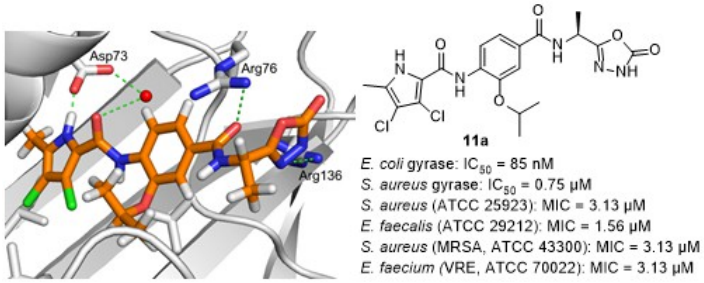


Figure. a) Docking binding mode of the representative *N*-phenylpyrrolamide **11a** coloured according to the atom chemical type (C, orange; N, blue; O, red; Cl, green) in the ATP binding site of *E. coli* GyrB (in grey, PDB code: 4DUH). The water molecule is presented as a red sphere.; b) Structure of **11a**, and its inhibitory activities on DNA gyrase and selected bacterial strains.

References

1) Zidar, N.; Macut, H.; Tomašič, T.; Brvar, M.; Montalvao, S.; Tammela, P.; Solmajer, T.; Peterlin Mašič, L.; Ilaš, J.; Kikelj, D., J Med Chem, 2015, 58, 6179-6194.
2) Zidar, N.; Tomašič, T.; Macut, H.; Sirc, A.; Brvar, M.; Montalvao, S.; Tammela, P.; Ilaš, J.; Kikelj, D., Eur J Med Chem, 2016, 117, 197-211.
3) Durcik, M.; Tammela, P.; Barančokova, M.; Tomašič, T.; Ilaš, J.; Kikelj, D.; Zidar, N. ChemMedChem, 2017, 13, 186-198.
4) Durcik, M.; Lovison, D.; Skok, Ž.; Durante Cruz, C.; Tammela, P.; Tomašič, T.; Benedetto Tiz, D.; Draskovits, G.; Nyerges, Á.; Pál, C.; Ilaš, J.; Peterlin Mašič, L.; Kikelj, D.; Zidar, N. Eur J Med Chem, 2018, submitted manuscript.

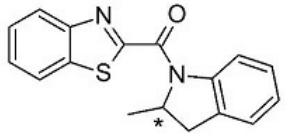
IN SILICO STUDIES OF INTERACTIONS OF ALLOSTERIC MODULATORS WITH DOPAMINE D2 RECEPTOR

Justyna Żuk (1), Damian Bartuzi (1), Dominika Patrzałek (1), Agnieszka A. Kaczor (1,2)

1) Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Laboratory, Faculty of Pharmacy with Division of Medical Analytics, Medical University, 4A Chodzki St., PL-20093 Lublin, Poland; E-mail: j.zuk86@gmail.com
2) School of Pharmacy, University of Eastern Finland, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland

Currently one of the hot topics in drug discovery is design of allosteric modulators of GPCRs instead of orthosteric ligands. The allosteric mode of action brings several advantages, e.g. ceiling effect preventing overdosage, high receptor selectivity, and even activation pathway selectivity which may in consequence lead to safer and more efficient drugs.

The aim of our studies was to construct homology models of human D_{2LONG} receptor (the isoform including long intracellular loop 3, IL3) in active conformation in complex with G_{i1} or G_{i2} protein and to use these models to investigate their interaction with dopamine and a recently reported D_2 receptor positive allosteric modulator, PAM (see below) [1]. The studied racemic compound acts as a PAM of the rat and human dopamine D_2 and D_3 receptors. The R isomer did not directly stimulate the dopamine D_2 receptor but potentiated the effects of dopamine. In contrast the S isomer attenuated the effects of the PAM and the effects of dopamine (displayed negative allosteric modulator, NAM properties) [1].



The homology models of D_{2LONG} receptor in complex with respective G proteins were built using Modeller applying the X-ray structures of β_2 adrenergic receptor in complex with G_s (PDB ID: 3SN6) as a template for helix bundle and G proteins, as well as X-ray structures of dopamine D_2, D_3 and D_4 receptors in inactive conformation (PDB ID: 6CM4, 3PBL and 5WIU, respectively) as additional templates. Yasara software was used to generate a long receptor IL3 loop, consisting of 139 residues which was refined using Modeller based on its predicted secondary structure. Dopamine was docked to the receptor models using induced-fit docking approach of Schrödinger software while enantiomers of a modulator were docked using Surflex incorporated in Sybyl.

Molecular dynamics simulations using Gromacs were performed to study the effect of the ligands on the receptor. To properly simulate subtle allosteric effects, emphasis on native-like conditions was put. For this purpose, the active-state models with G proteins were immersed in an asymmetrical membrane composed of 8 types of lipids in proportions appropriate to membrane rafts. Amber force field was used to describe the interactions of protein and ligands while the Slipids were used to describe the cell membrane. The trajectories were analyzed using the Principal Component Analysis and Mutual Information methods.

References

1) Wood M, Ates A, Andre VM, Michel A, Barnaby R, Gillard M. Mol Pharmacol. 2016; 89(2):303-12

3-D FRAGMENT-BASED LIBRARY

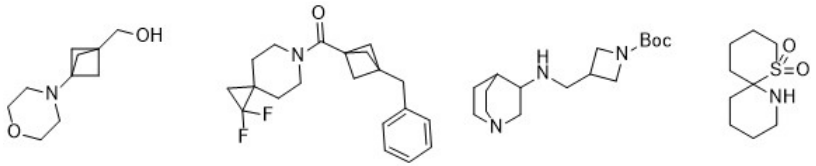
Thomas Fessard, Christophe Salome, Kristina Goncharenko

SpiroChem AG, Rosental area, Mattenstrasse 24, 4058 Basel, Switzerland

Fragment-based drug discovery (FBDD) is a widespread approach leading discovery for over 20 years in academia and industry.[1] This approach usually defines fragments that contain less than 20 non-hydrogen atoms, resulting in an efficient move from lead-like to drug-like compounds. A crucial aspect of FBDD is the design of a fragment library. Currently, the prominence of fragment libraries depends on the better coverage of the novel chemical space.

Properly designed libraries, with more complex fragments (typically 3D small molecules) can reduce the rate of false positives and provide excellent starting points for drug discovery programs. The rationale is that scaffolds with a three-dimensional character have better chances of binding to biological targets. This comes from the simple observation that all natural products, i.e. biologically relevant proteins and their ligands, are chiral and three-dimensional.

SpiroChem designs readily-available sp^3-enriched fragment libraries to support Life Sciences companies in exploring new chemical spaces and generating IP-protected starting points for drug design. This Library is unique and freely accessible to other chemical spaces. All the compounds were specifically designed using chemical informatics tools to optimize the properties (lop P, sp3 , pKa, ….).



References

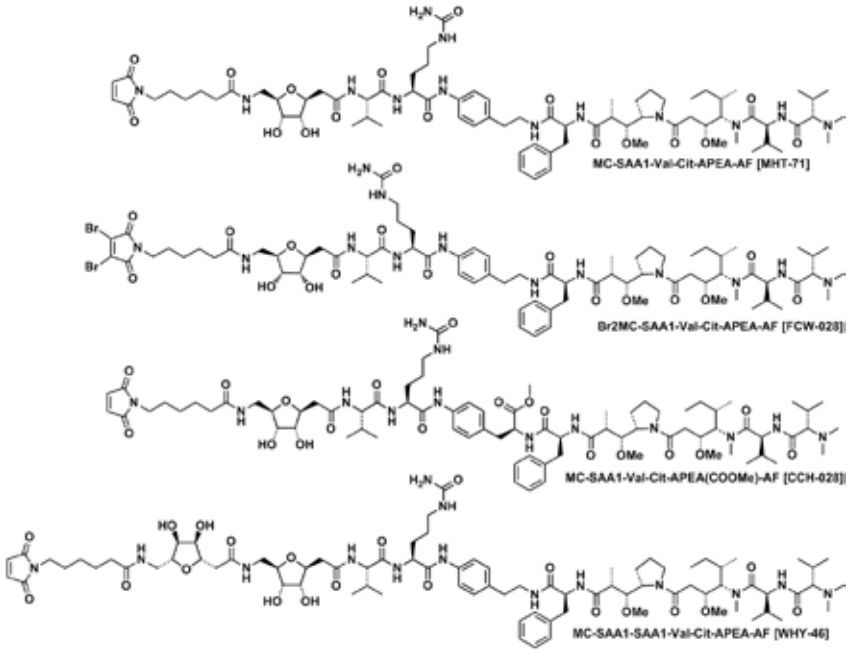
1) Erlanson D.A. et al. Nat Rev Drug Discov. 2016 Sep;15(9):605-19.

INCORPORATING SUGAR AMINO ACIDS INTO LINKERS FOR PREPARING HIGHLY HYDROPHILIC ANTIBODY-DRUG CONJUGATES

On Lee, Wan-Hsu Yang, Chien-Chin Huang, Hsiao-Chun Wang, Chih-Wei Fu, Yi-Ju Ko, Jheng-Sian Li, Yu-Cheng Wang, Chun-Min Liu, Ming-His Wu, Ming-Jin Jheng, Chia-Mu Tu Tu, Yuan-Jang Tsai, Maggie Lu

Drug Discovery Chemistry, Industrial Technology Research Institute, No. 321, Guang-Fu Road 2nd Sec, 300 Hsin-Chu, Taiwan

Antibody-drug conjugates (ADCs) have been developed to deliver cytotoxic agents to tumors and have the potential for increased clinical benefit to cancer patients. One of the major drawbacks of ACDs is the tendency to form aggregates which is due to the inherent property of high molecule weight antibodies. The coupling of hydrophobic toxins onto the monoclonal antibodies renders the ADCs more easily to form aggregates. To overcome this issue, we incorporated highly hydrophilic sugar amino acids into linkers and MHT-71 was generated as one of the promising linker-toxins and used to conjugate to various monoclonal antibodies. The EGFR-targeting Erbitux-MHT-71 was prepared and characterized with D-ribose derived sugar amino acid to enhance the hydrophilicity and cathepsin B cleavable Val-Cit linkage to release the auristatins payload. The average DAR of Erbitux-MHT-71 was within 3.5~4. The in vitro cytotoxicity assay results showed that the general IC[50] of Erbitux-MHT-71 is below 0.1 nM in FaDu (HNSCC cell line) and several esophageal squamous cancer cell lines such as KYSE510, KYSE150 and KYSE30. Erbitux-MHT-71 was stable in rat and human plasma and less than 5% of toxin leaking after incubation for 3 days. The PK profile of Erbitux-MHT-71 was similar to Erbitux in rat. In mouse xenograft tumor models (FaDu and KYSE 30), Erbitux-MHT-71 showed impressive efficacy in tumor growth inhibition after a single intravenous dose of 5 mg/kg. In conclusion, Erbitux-MHT-71 having highly hydrophilic sugar amino acid moiety could enhance several properties including solubility, conjugation efficiency, stability, and efficacy.



INTRACELLULAR IRREVERSIBLE PROBES FOR GPCRS: A COVALENT, NEGATIVE ALLOSTERIC MODULATOR FOR CC CHEMOKINE RECEPTOR 2 (CCR2)

Natalia V. Ortiz Zacarias (1), Julien Louvel (1), Irina Kufareva (2), Tereza Šimková (1), Arwin Hsiao (1), Emy Theunissen (1), Tracy Handel (2), Adriaan P. IJzerman (1), Laura H. Heitman (1)

1) Division of Drug Discovery and Safety, Leiden Academic Centre for Drug Research (LACDR), Leiden University, Leiden, The Netherlands
2) University of California, San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences, La Jolla, CA 92093, USA

CC chemokine receptor 2 (CCR2) is a class A G protein-coupled receptor (GPCR) that plays a key role in the migration of leukocytes to sites of inflammation. As such, CCR2 represents a potential drug target in many inflammatory and immune diseases, such as atherosclerosis, multiple sclerosis and cancer. Yet all CCR2 antagonists developed so far have failed in clinical trials due to lack of efficacy. This makes the development of novel tools and concepts necessary to better study drug receptor pharmacology in early drug discovery phases.

In this regard, the recent crystal structure of CCR2 has suggested a new manner of pharmaceutical intervention, i.e. using intracellular allosteric modulators.[1] In addition, irreversible or covalent probes represent important pharmacological tools that allow a variety of applications: study of drug-target binding kinetics, assist in target crystallization or study of *in vivo*target localization, among others. Thus, we aimed to develop and characterize an intracellular covalent probe for CCR2, as this might lead to the development of a new pharmacological tool for this receptor.

Based on the structure of a known CCR2 intracellular ligand, SD-24, we designed and synthesized several potential covalent ligands by incorporating different electrophilic groups as reactive warheads. Next, a combination of radioligand binding and functional assays allowed us to identify compound LUF7591 as an intracellular covalent binder for CCR2. In addition, *in silico* modeling followed by site-directed mutagenesis of CCR2 confirmed that LUF7591 binds to the intracellular pocket of CCR2, where a cysteine residue appears to be the target amino acid for the irreversible interaction.

To conclude, we report the design, pharmacological characterization and binding mode of LUF7591, a first covalent probe for CCR2. This tool compound might represent a promising approach to further study CCR2, both *in vitro* and *in vivo*.

References

1) Zheng, Y.; Qin, L.; Zacarias, N. V.; de Vries, H.; Han, G. W.; Gustavsson, M.; Dabros, M.; Zhao, C.; Cherney, R. J.; Carter, P.; Stamos, D.; Abagyan, R.; Cherezov, V.; Stevens, R. C.; AP, I. J.; Heitman, L. H.; Tebben, A.; Kufareva, I.; Handel, T. M. Structure of CC chemokine receptor 2 with orthosteric and allosteric antagonists. Nature 2016, 540, 458-461.

SILENT AGONISM MEDIATED BY THE α7 NICOTINIC ACETYLCHOLINE RECEPTOR: THE ROLE OF TRIFLUOROMETHYL GROUP IN THE NS6740 MOLECULAR SKELETON

Maria Chiara Pismataro (1,2), Clare Stokes (3), Roger L. Papke (3), Nicole A. Horenstein (2), Clelia Dallanoce (1)

1) Pharmaceutical Sciences, University of Milan, Milan, Milan, Italy
2) Dept of Chem Box 117200, Univ of Florida, Gainesville, FL, United States
3) Pharmacology and Therapeutics, University of Florida, Gainesville, FL, United States

The hypothesis that states a relationship between non-ionotropic activity of the α7 nicotinic acetylcholine receptor (nAChR) and its desensitized states is an emerging research topic. The ability of a new class of compounds, defined as "silent agonists", to stabilize the desensitized states of the α7 nicotinic acetylcholine receptor engendering anti-inflammatory responses, most likely via a metabotropic mechanism, would seem to confirm that hypothesis (1). In this study, we dissected the exemplary compound NS6740 (2) ((1,4-Diazabicyclo[3.2.2]-non-4-yl[5-[3-(trifluoromethyl)phenyl]-2-furanyl]methanone)), the most potent desensitizing agent for the α7 nAChR, characterized by both profound desensitization and relatively long term binding to the receptor. NS6740 shows promising anti-inflammatory activity, both *in vitro* and *in vivo*, in a mouse model of chronic pain and inflammation (3,4). In particular, we explored the role of the meta trifluoromethyl substituent of the phenyl ring in inducing the silent agonist binding mode. Compounds MCP5, MCP6, MCP7, MCP8 were prepared by introducing halogen atoms, i.e. fluorine, chlorine, bromine, iodine with increasing size in the meta position of the phenyl ring; MCP18, instead, showed the original trifluromethyl group moved on para position (Figure 1).

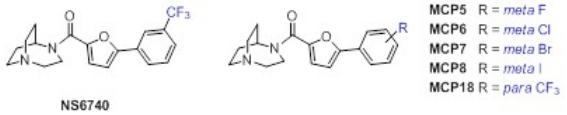


Figure 1. Structures of NS6740 and the newly synthesized derivatives.

Two-electrode voltage clamping was employed to assess the electrophysiological profile of the newly synthesized compounds. Each experiment was conducted with 10 μM of drug, 60μM ACh pre- and post-control, and an application of 10 μM of the type-II positive allosteric modulator, PNU-120596 to evaluate the induction of PAM-sensitive desensitization (5). Taken together, our data suggest the *meta* trifluoromethyl group has a crucial role in minimizing the partial agonist behavior. Moreover, we found that the ability to stabilize the desensitized states of the α7 nAChR is preserved when trifluoromethyl is replaced by halogen atoms. When the CF_3 group is moved on *para* position, the desensitizing activity is compromised, suggesting the *meta* substitution is strictly required.

References

1) Horenstein, N.A. and Papke, R.L., ACS Med. Chem. Lett. 2017, 8, 989-991
2) Peters, D. et al., Novel diazabicyclic aryl derivatives, WO 2004/076453
3) Thomsen, M. S. et al., Journal of Neuroimmunology 2012, 251, 65-72
4) Papke, R.L. et al., Neuropharmacology 2015, 91, 34-42
5) Papke, R.L. et al., Methods, 2010, 51, 121-133

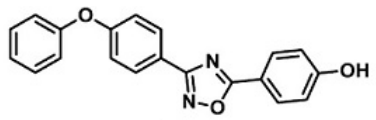
OXADIAZOLE ANTIBIOTICS DISPLAY ACTIVITY AGAINST MULTIDRUG RESISTANT ENTEROCOCCUS FAECIUM

Glen P. Carter (1,2), Jitendra R. Harjani (3), Lucy Li (2), Noel P. Pitcher (3), Yi Nong (2), Thomas V. Riley (4,6), Deborah A. Williamson (1,2), Timothy P. Stinear (2), Jonathan B. Baell (3,7), Benjamin P. Howden (1,2)

1) Antimicrobial Reference and Research Unit, Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology & Immunology, Doherty Institute, The University of Melbourne
2) Department of Microbiology & Immunology, Doherty Institute, The University of Melbourne
3) Monash Institute of Pharmaceutical Sciences, Monash University
4) School of Medical and Health Sciences, Edith Cowan University
5) School of Veterinary and Life Sciences, Murdoch University
6) Department of Microbiology, PathWest Laboratory Medicine
7) School of Pharmaceutical Sciences, Nanjing Tech University
Email: noel.pitcher@monash.edu

Enterococcus faecium is an important nosocmial pathogen. The emergence of multidrug-resistant strains has resulted in *E. faecium* infections that are increasingly difficult to treat. The most serious of these is vancomycin-resistant *E. faecium* and the treatment for these infections usually relies on last-line antibiotics linezolid and daptomycin. However resistance to these last-line treatments has been reported[1,2] and as such there is an urgent need for new antibacterial agents to combat the increasing prevalence of multidrug-resistant enterococcal infections.

Recently, a novel antibiotic drug class of 1,2,4-oxadiazole compounds has been discovered that exhibit considerable activity against several clinically important pathogens.[3] Here we show that compound Oxd from the novel antibiotic class is active against a range of *E. faecium* strains, including against isolates that display nonsusceptibility to vancomycin and daptomycin.[4] The oxadiazole compound showed rapid bactericidal activity in time-kill assays and is superior to daptomycin in its ability to kill *E. faecium*. The 1,2,4-oxadiazole also worked synergistically with daptomycin to improve both its MIC and killing efficiency against non-susceptible isolates. The 1,2,4-oxadiazole antibiotics hold promise in the development of effective treatments for multidrug-resistant *E. faecium* infections.



Oxd

Figure 1. Compound Oxd of the 1,2,4-oxadiazole class.

References

Deshpande, L. M. et al. Antimicrobial agents and chemotherapy 2015, 59, 6256-61.
Lellek, H. et al. Int. international Journal of Medical Microbiology 2015, 305, 902-909.
O'Daniel, P. I. et al. Journal of the American Chemical Society 2014, 136, 3664-72.
Carter, G. P. et al. The Journal of antimicrobial chemotherapy 2018, 73, 1562-1569.

DISCOVERY AND CHARACTERIZATION OF NOVEL SELECTIVE NKCC1 INHIBITORS FOR THE TREATMENT OF DOWN SYNDROME AND BRAIN DISORDERS WITH DELPOLARIZING GABAERGIC TRANSMISSION

Marco Borgogno (1), Annalisa Savardi (2,3), Giuseppina La Sala (1), José Antonio Ortega (1), Maria Summa (4), Rosalia Bertorelli (4), Sine Mandrup B. (5), Andrea Armirotti (5), Andrea Contestabile (2), Laura Cancedda (2,6), Marco De Vivo (1)

1) Molecular Modeling and Drug Discovery Laboratory, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy
2) Local Micro-environment and Brain Development Laboratory, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy
3) Università degli Studi di Genova, Via Balbi, 5, 16126 Genova, Italy
4) In Vivo Pharmacology Facility, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy
5) Analytical Chemistry Facility, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy
6) Dulbecco Telethon Institute, Varese Street 16b, 00185 Rome, Italy

Proper GABAergic transmission trough Cl^-permeable $GABA_A$ receptors is fundamental for physiological brain development and function. Indeed, defective GABAergic signaling, due to a high ratio of the expression of the Cl^- importer NKCC1 and Cl^- exporter KCC2, has been implicated in several neurodevelopmental disorders including Down Syndrome (DS). Interestingly, NKCC1 inhibition by the FDA-approved diuretic bumetanide reverts cognitive deficits in DS mice[1] as well other core symptoms in several neurodevelopmental disorders[2]. However, the required chronic treatment with bumetanide is burdened by its diuretic side effects caused by the antagonization of the kidney Cl^- importer NKCC2, which jeopardizes drug compliance and leads to hypokalemia. In order to solve these drawbacks, we seek to find novel potent and selective NKCC1 inhibitors, devoid of diuretic activity. Starting from bumetanide's structure, we applied a ligand-based approach to design new molecular entities that we tested *in vitro* for their capacity to selectively block NKCC1. Extensive synthetic efforts as well as structure-activity analyses aided to improve *in vitro* potency, efficacy, and drug-like properties of the initially identified chemical scaffolds. As a result, a few compounds emerged for their activity to inhibit NKCC1 in cultured neurons. In particular, one showed excellent solubility and metabolic stability *in vitro*. This lead compound proved to be effective also on NKCC1 inhibition *in vivo*, showing the recovery of cognitive deficits in a mouse model of DS. Moreover, mice systemically treated with this NKCC1 inhibitor revealed no significant diuretic effect. Herein, the main pharmacological features of this new molecular entity will be discussed. Our results indicate that a selective NKCC1 inhibitor devoid of the diuretic effect could represent a suitable and solid therapeutic strategy for the treatment of Down syndrome and all the brain disorders with depolarizing GABAergic transmission.

References

1) Deidda, G.; Parrini, M.; Naskar, S.; Bozarth, I. F.; Contestabile, A.; Cancedda, L., Reversing excitatory GABAAR signaling restores synaptic plasticity and memory in a mouse model of Down syndrome. Nat Med 2015, 21 (4), 318-26.
2) Ben-Ari, Y., NKCC1 Chloride Importer Antagonists Attenuate Many Neurological and Psychiatric Disorders. Trends Neurosci 2017, 40 (9), 536-554.

NOTES

INDEX OF ABSTRACTS

ABLASSER Andrea	INTRACELLULAR DNA SENSING IN HEALTH AND DISEASE	LE037
ADLERE Ilze	STRUCTURE-BASED DISCOVERY OF CXCR4 CHEMOKINE RECEPTOR ANTAGONISTS	P195
AGNEW-FRANCIS Kylie	REPURPOSING COMMERCIAL AHAS-INHIBITING HERBICIDES AS ANTIFUNGALS TO TARGET THE EMERGING PATHOGEN, CANDIDA AURIS.	P305
AHONEN Tiina	SYNTHESIS AND EVALUATION OF 7B-HYDROXY-8-KETONE OPIOID DERIVATIVES	P340
AIGUABELLA FONT Nuria	SHAPING THE PROSPECTS OF LABORATORY WORK: THE LAB OF THE FUTURE INITIATIVE	P306
AJAMIAN Alain	TARGETING SPECIFIC INTERACTIONS TO IMPROVE EGFR-LIGAND BINDING	P261
ALDANA Ignacio	STRUCTURE-ACTIVITY RELATIONSHIP OF NEW ANTIMALARIAL 1-ARYL-3-SUSBTITUTED PROPANOL DERIVATIVES: SYNTHESIS, PRELIMINARY TOXICITY PROFILING AND PARASITE LIFE CYCLE STAGE STUDIES	P170
ALI Amjad	DISCOVERY AND CLINICAL EVALUATION OF MK-8150, A NOVEL NITRIC OXIDE DONOR WITH A UNIQUE MECHANISM OF NITRIC OXIDE RELEASE	LE101
AMBLER Martin	THE DEVELOPMENT OF MICROTUBULE AFFINITY REGULATING KINASES (MARK) INHIBITORS AND THEIR IN-VIVO PROFILE	P262
ANDREWS Steve	BRAIN-PENETRANT AUTOPHAGY MODULATORS FOR TREATING NEURODEGENERATIVE DISEASES	LE036
ARDIGO Diego	THE THERAPEUTIC CHALLENGE OF THE NEW ERA: DEVELOPING AND MAKING AVAILABLE LIFE CHANGING TREATMENT TO PATIENTS WITH RARE DISEASES	PL004
ARNAUDOVA Ralica	SYNTHESIS OF BENZAZULENES AS POTENT PIM-1 AND PIM-3 INHIBITORS	P263
ATKINSON Ben	DEVELOPMENT OF AN ENANTIOSPECIFIC SYNTHETIC ROUTE TO HSP CO-INDUCER ARIMOCLOMOL AND IT'S ANALOGUES	P060
ATTON Holly	SYNTHESIS AND CHARACTERISATION OF SMALL MOLECULES FOR THE TREATMENT OF AUTOIMMUNE DISEASES	(P196)
AVSIC-ZUPANC Tatjana	ZIKA VIRUS: AN OLD VIRUS WITH A NEW FACE	PL001
BARBARO Lisa	THE DEVELOPMENT OF NEW TREATMENTS FOR MULTI-DRUG RESISTANT TUBERCULOSIS	P171
BARLAAM Bernard	DISCOVERY OF THE CLINICAL CANDIDATE AZD1390: A HIGH QUALITY, POTENT AND SELECTIVE INHIBITOR OF ATM KINASE WITH THE ABILITY TO CROSS THE BLOOD BRAIN BARRIER	P264
BARLAAM Bernard	DISCOVERY OF AZD4573, A POTENT AND SELECTIVE INHIBITOR OF CDK9 THAT ENABLES TRANSIENT TARGET ENGAGEMENT FOR THE TREATMENT OF HAEMATOLOGICAL MALIGNANCIES	LE096
BARLOW Nicholas	PEPTIDE MACROCYCLES THAT PERMEATE MEMBRANE BARRIERS. COMBINING N-METHYLATION AND PRODRUG APPROACHES	P061
BARNIOL-XICOTA Marta	RHOMBOID PROTEASES: DOES THE ENVIROMENT MATTER?	P236

COLLIER Philip	A ROADMAP FOR PI3KΓ SELECTIVITY DESIGN: DISCOVERY OF CNS-PENETRANT PI3KΓ INHIBITORS FOR THE POTENTIAL TREATMENT OF MULTIPLE SCLEROSIS	P267
CONTINO Marialessandra	DEVELOPMENT OF FLUORESCENT LIGANDS TO DETECT CB2 RECEPTORS IN CANCER AND NEURODEGENERATIVE DISEASES	P334
CONTRERAS Jean-Marie	IDENTIFICATION OF SELECTIVE NF-KB KINASE (NIK) INHIBITORS	P268
COSTANTINO Gabriele	TARGETING NON ESSENTIAL BACTERIAL TARGETS AS A NOVEL ROUTE TO COUNTERACT BACTERIAL RESISTANCE	LE020
COURTENS Charlotte	FOSMIDOMYCIN ANALOGS AS ANTIMALARIAL AND ANTITUBERCULAR AGENTS - A PRODRUG APPROACH	P175
CRAVATT Benjamin	ACTIVITY-BASED PROTEOMICS - PROTEIN AND LIGAND DISCOVERY ON A GLOBAL SCALE	AL003
CRAWFORD James	ADDRESSING METABOLISM THROUGH STRUCTURE-BASED DESIGN: IDENTIFICATION OF POTENT AND SELECTIVE INHIBITORS OF NF-KB INDUCING KINASE (NIK)	P197
CSEKEI Marton	THE CHALLENGES OF RESTORING PROGRAMMED CELL DEATH THROUGH MCL1 INHIBITION	PR001
CVIJANOVIC Danijela	ENAMINONES AND THEIR MOLYBDENUM(VI) COMPLEXES AS NON-CYTOTOXIC COMPOUNDS WITH ANTIBACTERIAL ACTIVITY	P345
DANICA Agbaba	COMBINED MOLECULAR DYNAMICS AND VIRTUAL SCREENING STUDIES TO IDENTIFY NOVEL SIRTUIN 2 INHIBITORS	P347
DANICA Agbaba	RATIONAL DRUG DESIGN OF HISTONE DEACETYLASE 6 INHIBITORS	P346
DARMENCY Vincent	DISCOVERY OF CANDIDATES FOR PET MOLECULAR IMAGING OF PATHOLOGICAL TDP-43 AGGREGATES IN FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS PATIENTS	LE092
DAVIES Nichola	ENHANCING STRUCTURE-BASED DRUG DESIGN WITH MEASURED FREE LIGAND CONFORMATIONS FOR CLASSICAL AND EMERGING DRUG CLASSES	P309
DAVIES Nichola	DISCOVERY OF AZD0364, A POTENT AND SELECTIVE ORAL INHIBITOR OF ERK1/2 THAT IS EFFICACIOUS IN BOTH MONOTHERAPY AND COMBINATION THERAPY IN MODELS OF NSCLC	P270
DAVIS Benjamin	SUGARS & PROTEINS: GLYCOMIMETICS TO TARGET INFECTIOUS DISEASE	PL005
DE CARVALHO ACURCIO Ana Rita	NEW SMALL-MOLECULE IMMUNE CHECKPOINT INHIBITORS: A STEP FORWARD IN CANCER IMMUNOTHERAPY	LE008
DE LANGE Liesbeth	TARGET BINDING KINETICS AND ITS RELEVANCE IN THE IN VIVO CONTEXT	LE015
DEMONT Dennis	DISCOVERY OF POTENT AND SELECTIVE COVALENT ITK INHIBITORS	P271
DEPREZ-POULAIN Rebecca	MEDCHEM STRUCTURE GENIUS : FREE MOBILE APPS TO LEARN DRUG STRUCTURES	P348
DEPREZ-POULAIN Rebecca	CONTROLLING PLASMA STABILITY OF HYDROXAMIC ACIDS: A MEDCHEM TOOLBOX	P025

DEPREZ-POULAIN Rebecca	ULLMAN-DERIVED INHIBITORS OF ER-AMINOPEPTIDASES (ERAPS)	P151
DI MARTINO Rita Maria Concetta	DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF THE FIRST DUAL MODULATORS OF DOPAMINE D3 RECEPTOR AND GSK-3B AS PROMISING AND INNOVATIVE TOOLS FOR BIPOLAR DISORDER TREATMENT	P166
DIAMANTI Eleonora	EXPLORING HIT-IDENTIFICATION STRATEGIES FOR ECF TRANSPORTERS, A NOVEL ANTI-INFECTIVE TARGET	P063
DOBIE Chris	INHIBITORS OF HUMAN ST8SIA AS NOVEL ANTI-METASTATIC AGENTS	P137
DOLEZAL Martin	TOWARD A BETTER PYRAZINAMIDE; NEW STRUCTURAL MODIFICATIONS, NEW BIOLOGICAL ACTIVITIES	P181
DOLSAK Ana	DESIGN, SYNTHESIS AND EVALUATION OF TOLL-LIKE RECEPTOR 7 AGONISTS WITH 2-(TRIFLUOROMETHYL)QUINOLINE-4-AMINE AND 2-(TRIFLUOROMETHYL)QUINAZOLINE-4-AMINE SCAFFOLDS	P152
DOMINGOS Sofia	EXPANDING PARKIN TOOLBOX - NOVEL CHEMICAL PROBES TO EXPLORE PARKIN ACTIVATION	P026
DÖMLING Alexander	DRUG DISCOVERY AT THE SPEED OF SOUND	P064
DONCKELE Etienne	ENCODED SELF-ASSEMBLING CHEMICAL (ESAC) LIBRARIES: A POWERFUL TECHNOLOGY FOR LIGANDS DISCOVERY AND AFFINITY MATURATION	P239
DORMAN György	DESIGN, SELECTION AND IN VITRO EVALUATION OF POTENTIAL, SMALL-MOLECULE COMPLEMENT C1S INHIBITORS	P198
DORMAN György	DEVELOPMENT OF MATRIX METALLOPROTEINASE-2 INHIBITORS FOR CARDIOPROTECTION	P001
DOROCKA BOBKOWSKA Barbara	IN VITRO ACTIVITY OF MICONAZOLE AGAINST CANDIDA BIOFILM	P199
DOSSETTER Al	ACCELERATING MULTIPLE MEDICINAL CHEMISTRY PROJECTS USING MATCHED MOLECULAR PAIR ANALYSIS FOR KNOWLEDGE BASED DESIGN – A REVIEW FROM THE PAST 8 YEARS OF USE AT THE FRONT LINE	P027
DOSSETTER Al	POTENCY AND PATENTS, NEW ARENAS FOR MATCHED MOLECULAR PAIR ANALYSIS IN THE AI WORLD	LE004
DUBURS Gunars	SYNTHESIS AND EVALUATION OF 1,4-DIHYDROPYRIMIDINONE DERIVATIVES - HEPATITIS B VIRUS CAPSID SELF-ASSEMBLY INHIBITORS	P176
DUCA Maria	SYNTHETIC SMALL-MOLECULE RNA LIGANDS: SCOPE AND THERAPEUTIC APPLICATIONS	LE066
DUDUTIENE Virginija	FUNCTIONALIZATION OF FLUORINATED BENZENESULFONAMIDES - AN APPROACH TOWARDS CARBONIC ANHYDRASE IX SELECTIVITY	P104
DUFFY James	PLASMODIUM PI4K. THE DISCOVERY AND DEVELOPMENT OF KINASE INHIBITORS AS ANTIMALARIAL DRUGS	P272
DURCIK Martina	DISCOVERY OF BENZOTHIAZOLE-BASED DNA GYRASE AND TOPOISOMERASE IV INHIBITORS WITH BROAD SPECTRUM ANTIBACTERIAL ACTIVITY	P349
ETTMAYER Peter	CHEMICAL PROBES FOR NEW THERAPEUTIC CONCEPT DISCOVERY	LE063

GARCIA Sara	TARGETING GLUCOSE METABOLISM FOR CANCER TREATMENT: A STRUCTURE-BASED VIRTUAL SCREENING TOWARD HEXOKINASE 2 INHIBITORS	P274
GASTREICH Marcus	SIGMA HOLES — REALLY THAT INFLUENTIAL?	P311
GBELCOVA Helena	EFFECT OF STATINS ON THE GENES´ EXPRESSION	P352
GEE Antony	PET MOLECULAR IMAGING - AN OVERVIEW	LE089
GEHRINGER Matthias	BURULI ULCER AND THE MTOR PATHWAY: TOTAL SYNTHESIS, STRUCTURE–ACTIVITY AND TARGET ELUCIDATION STUDIES OF MYCOLACTONES	LE052
GEORGI Victoria	LARGE-SCALE ANALYSIS OF KINASE INHIBITORS' TARGET BINDING KINETICS AND ITS IMPLICATIONS FOR DRUG DISCOVERY	LE016
GERSTENBERGER Brian	DISCOVERY OF THE TYK2 SELECTIVE INHIBITOR PF-6826647 FOR THE TREATMENT OF CROHN'S DISEASE, AND OTHER AUTOIMMUNE CONDITIONS	LE064
GIANNAKOPOULOU Erofili	METAL-CHELATING ACETOHYDROXAMIC ACIDS AGAINST HEPATITIS C VIRUS AND FLAVIVIRUSES	P177
GIGANTE Alba	IDENTIFICATION OF IN VIVO ACTIVE HITS FROM THE GSK TRES-CANTOS ANTI-KINETOPLASTID SET (TCAKS) AGAINST CHAGAS DISEASE	P147
GIOIELLO Antimo	INTEGRATED SYSTEM FOR THE EXPEDITED GENERATION AND CHARACTERIZATION OF DRUG-LIKE LIBRARIES FOR HIT-TO-LEAD EXPLORATIONS	LE044
GIOVANNINI Riccardo	FIRST TIME DISCLOSURE OF BI 409306, A FIRST IN CLASS PDE9 INHIBITOR FOR THE TREATMENT OF CNS DISEASES	LE028
GIZYNSKA Malgorzata	TUNING HUMAN 20S PROTEASSOME ACTIVITY BY PROLINE- AND ARGININE-RICH PEPTIDES	P292
GOBEC Stanislav	NONPEPTIDIC SELECTIVE INHIBITORS OF IMMUNOPROTEASOME	LE081
GODDARD Zoe	TARGETED PHOTODYNAMIC THERAPY OF LUNG CANCER	P312
GÖKHAN-KELEKCI Nesrin	ANTIMICROBIAL ACTIVITIES OF CHIRAL THIOUREAS AND THEIR CYCLIZED DERIVATIVES THIAZOLIDINONES	P242
GÖKHAN-KELEKCI Nesrin	MONOAMINE OXIDASE A/B INHIBITING EFFECT AND MOLECULAR MODELING OF SOME SYNTHESIZED HYDRAZONE DERIVATIVES	P241
GOLIC GRDADOLNIK Simona	INSIGHT INTO THE SELECTIVE BINDING OF NOVEL INHIBITORS OF FUNGAL CYP51	P353
GOLOJUCH Sebastian	SYNTHESIS OF NOVEL PHOSPHORAMIDATE AND THIOPHOSPHORAMIDATE CAP ANALOGS - POTENTIAL PRONUCLEOTIDE TRANSLATION INHIBITORS	P145
GONCHARENKO Kristina	3-D FRAGMENT-BASED LIBRARY	P432
GONCHARENKO Kristina	N-ARYLATION OF OXETANYLAMINE FOR THE PREPARTION OF N-ARYL-AMIDE ISOSTERES	P096

PLANO Daniel	AS-10, A UNIQUE SE-ASPIRIN: PRE-CLINICAL EVIDENCES OF A POTENT AND SELECTIVE CANCER DRUG CANDIDATE	P040
PRASHANTKUMAR Khirsariya	ENANTIOSELECTIVE SYNTHESIS AND PROFILING OF A NOVEL POTENT SELECTIVE INHIBITOR OF CHK1 KINASE	P281
PRATI Federica	DISCOVERY OF NOVEL INDAZOLE-3-CARBOXAMIDE GSK-3B INHIBITORS FOR THE TREATMENT OF MOOD DISORDERS \R\N	P282
PRAVEEN KUMAR Chinthakindi	SYNTHESIS OF SULFONIMIDAMIDE (SIA) BASED AMINO ACID BUILDING BLOCKS	P045
PRINCIC Grisa	NON-SYMMETRIC 1,2,4,5-TETRAOXANES: SYNTHESIS WITH HYDROGEN PEROXIDE AND THEIR BIOLOGICAL ACTIVITY	P081
PRISCAKOVA Petra	STATINS AND PREECLAMPSIA	P402
PROVENZANI Riccardo	SCAFFOLD HOPPING FROM ISOPHTHALATE TO MULTISUBSTITUTED PYRIMIDINE DIMINISHES BINDING AFFINITY OF LIGANDS TARGETED TO THE C1 DOMAIN OF PKC	P296
PULZ Robert	DISCOVERY OF LOU064, A COVALENT BTK INHIBITOR WITH BEST IN CLASS SELECTIVITY	LE026
QUARANTA Laura	SYNTHESIS AND FUNGICIDAL ACTIVITY OF A NEW FAMILY OF OXYSTEROL BINDING PROTEIN INHIBITORS	LE019
RAJIC Zrinka	INSIGHTS INTO BIOLOGICAL ACTIVITY OF SAHAQUINES, HYBRIDS BASED ON SAHA AND AMINOQUINOLINE MOTIFS	P403
RAMACHANDRA Murali	SMALL MOLECULE IMMUNE CHECKPOINT ANTAGONISTS FOR CANCER THERAPY	LE005
RAMILO-GOMES Filipa	PHARMACOPHORE-BASED VIRTUAL SCREENING TOWARD THE DISCOVERY OF EZH2 INHIBITORS	P328
RAMURTHY Savithri	DISCOVERY OF A NEXT GENEARTION B/C RAF KINASE INHIBITOR TO TREAT RAS/RAF MUTANT SOLID TUMORS: THE PATH TO THE CLINIC	P283
RATNI Hasane	DISCOVERY OF RG7916, A SELECTIVE SMN2 SPLICING MODIFIER FOR THE TREATMENT OF SPINAL MUSCULAR ATROPHY	LE025
REDDAVIDE Francesco	A DNA-ENCODED LIBRARY TECHNOLOGY BASED PLATFORM FOR HIT DISCOVERY, OPTIMIZATION AND ANALYSIS ACROSS DIVERSE TARGET FAMILIES	P329
REKKA Eleni	CONJUGATION OF NSAIDS WITH ACTIVE ALCOHOLS AND THEIR EFFECT ON INFLAMMATION	P212
REKKA Eleni	NITRIC OXIDE RELEASING NSAID DERIVATIVES AS ANTIATHEROGENIC COMPOUNDS	P228
RENN Christian	HIGH-THROUGHPUT-SCREENING TO IDENTIFY ECTO-5'-NUCLEOTIDASE (CD73) INHIBITORS WITH POTENTIAL FOR THE IMMUNOTHERAPY OF CANCER	P154
REPISKA Vanda	THE EFFECT OF THE PLASMA-ACTIVATED MEDIUM ON CANCEROUS AND NON-CANCEROUS CELLS	P404
RESSURREICAO Ana	REPURPOSING HUMAN MTOR INHIBITORS FOR NEGLECTED TROPICAL DISEASES: TOWARD OPTIMIZATION OF A SINGLE CHEMOTYPE WITH ACTIVITY AGAINST MULTIPLE PROTOZOAN PARASITES	P184

REVESZ Tamas	CHARTING THE STRUCTURE-RESISTANCE LANDSCAPE OF NOVEL ANTIBIOTICS	LE051
REXEN ULVEN Elisabeth	NOVEL AGONISTS FOR THE SUCCINATE RECEPTOR GPR91	P405
RHEINHEIMER Joachim	NATURAL PRODUCTS AS LEADS IN AGROCHEMISTRY	LE018
RIVERA SANCHEZ Gildardo	DESIGN AND SYNTHESIS DE NEW PHTHALOYL DERIVATIVES AS TRANS-SIALIDASE INHIBITORS	P185
RIXEN Sophia	MURINE KNOCKOUT STUDIES CONFIRM THE INVOLVEMENT OF THE MITOCHONDRIAL AMIDOXIME REDUCING COMPONENT (MARC) IN N-REDUCTIVE METABOLISM	P406
ROCHE Didier	PREDICTING FRAGMENT BINDING BY MOLECULAR DYNAMICS : TOWARDS NEW INHIBITORS OF CYCLOPHILIN D	P082
RODESCHINI Vincent	TOWARDS THE DISCOVERY OF BAY-850, A SELECTIVE AND CELL-ACTIVE ATAD2 CHEMICAL PROBE	P407
RODRIGUEZ Raphaël	AN IRON HAND OVER CANCER STEM CELLS	LE050
RODRIGUEZ LOZADA Josue	SYNTHESIS AND EVALUATION OF HETEROCYCLIC Γ-AMINOBUTYRIC ACID ANALOGUES	P408
RODRIGUEZ-GIMENO Alejandra	TARGETING BASIC DEFECT IN CYSTIC FIBROSIS: DISCOVERY AND DEVELOPMENT OF NOVEL NANOMOLAR F508-DEL CFTR CORRECTORS	P330
ROONEY Tim	INHIBITION OF PI5P4 KINASES TO UPREGULATE AUTOPHAGY FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES	P162
RUBIO RUIZ Belen	DEVELOPMENT OF PRECURSORS OF COMBINATION THERAPY THAT ARE SPECIFICALLY ACTIVATED BY GOLD-MEDIATED BIOORTHOGONAL CHEMISTRY	P331
RÜBSAMEN-SCHAEFF Helga	DEVELOPMENT OF NON-NUCLEOSIDIC COMPOUNDS AGAINST DNA VIRUSES OF THE HERPES GROUP. THE ERA AFTER NUCLEOSIDES: LETERMOVIR AND PRITELIVIR	PL006
RYU Jae-Sang	GOLD(I)-CATALYZED SYNTHESIS OF 4-ISOXAZOLINES AND ITS SYNTHETIC APPLICATION	P125
SAARBACH Jacques	KINASE TEMPLATED ABIOTIC REACTION	P288
SAGUD Ivana	BIOLOGICAL, ANTIOXIDANT AND CHOLINESTERASE INHIBITION ACTIVITY STUDIES ON NAPHTHO[1,2-D]/[2,1-D]OXAZOLES	P126
SANDOVAL-RAMIREZ Jesus	NOVEL ANTIINFLAMMATORY STEROIDAL COMPOUNDS.	P213
SANNINO Alessandro	THE RECOVERY OF DNA-TAGGED LIGANDS: IMPACT OF DIFFERENT EXPERIMENTAL PARAMETERS THROUGH AFFINITY SELECTIONS	P249
SANTOS Webster L.	CONTROLLING SPHINGOSINE-1-PHOSPHATE LEVELS AS A THERAPEUTIC STRATEGY	LE076
SANTOS Maria M. M.	TRYPTOPHANOL-DERIVED OXAZOLOISOINDOLINONES: PROMISING SMALL MOLECULES FOR ANTICANCER THERAPY	P046

SARI Suat	ANTICONVULSANT SCREENING OF NEW (ARYLALKYL)AZOLE ESTERS AND MOLECULAR MODELLING STUDIES THEREOF	P410
SARI Suat	TYROSINASE INHIBITION BY A RARE NEOLIGNAN: AN IN VITRO AND IN SILICO STUDY	P409
SAVIC Jelena	DESIGN OF NOVEL B-HYDROXY-B-ARYLALKANOIC ACIDS WITH IMPROVED GASTROINTESTINAL ABSORPTION BASED ON QSRR STUDIES	P215
SCAFFIDI Salvatore	DRUGGING THE FBW7 E3 LIGASE WITH A FRAGMENT-BASED APPROACH	P250
SCHAAKE Benjamin	STRUCTURE-GUIDED DEVELOPMENT OF SUBTYPE-SELECTIVE MUSCARINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS	P411
SCHEFFLER Frank	EXPANDING CHEMICAL SPACE VIA MICROBIAL BIOCATALYSIS LATE STAGE FUNCTIONALIZATION	P084
SCHIFFRER Eva Shannon	SYNTHESIS AND CHARACTERISATION OF PSORALEN DERIVATES AS INHIBITORS OF THE B5I SUBUNIT OF THE IMMUNOPROTEASOME	P216
SCHIO Laurent	DESIGN AND RATIONALE FOR EXQUISITE SELECTIVITY OF PRECLINICAL AND CLINICAL KINASE INHIBITORS	LE062
SCHMIES Constanze	SYNTHESIS OF NUCLEOTIDE DERIVATIVES AS POTENT AND SELECTIVE NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE 1 (CD39) INHIBITORS	P140
SCHOU Magnus	PET IN NEUROSCIENCE DRUG DISCOVERY AND DEVELOPMENT	LE090
SEBASTIAN PÉREZ Víctor	ARTIFICIAL INTELLIGENCE IN MEDICINAL CHEMISTRY: A REAL AVENUE FOR SPEEDING UP NEURODRUGS DISCOVERY PROCESS	P006
SEEBERGER Peter H.	SYNTHETIC GLYCOCONJUGATE VACCINES AGAINST BACTERIAL INFECTIONS	PL003
SEGLER Marwin	COMPUTER-AIDED SYNTHESIS PLANNING	LE003
SELWOOD David	C-NATRUIRETIC PEPTIDE AGONISTS FOR CARDIOVASCULAR DISEASE	LE098
SERDA Maciej	BIOTRANPORT AND BIODISTRYBUTION OF [60]FULLERENE DERIVATIVE IN MURINE ORTHOTROPIC MODEL OF BREAST ADENOCARCINOMA MODULATED BY NON-INVASIVE HYPERTHERMIA	P243
SERDA Maciej	THE WATER-SOLUBLE GLYCOFULLERENES FOR ANTI-CANCER THEREPIES	P085
SERRA Patricia	FOCUSING ON VIRAL SURFACE GLYCOPROTEINS AS TARGET AGAINST HIV-2 INFECTIONS - STRUCTURAL ELUCIDATION AND MOLECULAR DYNAMICS STUDY	P186
SHAABANI Shabnam	SMALL MOLECULES INHIBITING PD1-PDL1 IMMUNE CHECKPOINT	LE007
SHEN Hong	ANTI-HBV DRUG DISCOVERY ENABLED BY STRUCTURE-BASED DRUG DESIGN AND PHENOTYPIC SCREENING	LE095
SHUTO Satoshi	DESIGN AND SYNTHESIS OF CONGERNERS OF CYCLIC ADP-RIBOSE, A CA2+-MOBILIZING SECOND MESSENGER, TOWARD IDENTIFICATION OF THE TARGET PROTEIN	LE079

TARNOVSKIY Andrey	AMINOSULFONYL FLUORIDES – NOVEL SCAFFOLDS FOR PROTECTING-GROUP FREE SYNTHESIS OF SULFONAMIDES	P078
TARNOVSKIY Andrey	DIFLUOROCYCLOPROPANES FOR DRUG DISCOVERY	P077
TARNOVSKIY Andrey	SYNTHESIS OF NOVEL BICYCLIC AMINES FOR DRUG DESIGN	P076
TERRETT Nick	CELL PERMEABILITY WITH BEYOND 'RULE OF 5' MODALITIES – DO WE UNDERSTAND HOW THIS WORKS?	LE030
THI ANH NGUYEN Ngoc	DESIGN AND SYNTHESIS OF POTENTIAL ALLOSTERIC INHIBITORS OF TISSUE TRANSGLUTAMINASE	P127
THOMÄ Nicolas	THE ZINC-DEGROME	LE023
THOMSEN Martin K.	IN VIVO ANTI-VIRAL AND -TUMOR EFFECT OF 3'3'-CAIMP STING ACTIVATION	LE040
THURING Jan Willem	DISCOVERY OF JNJ-64619178 AS A POTENT AND SELECTIVE PRMT5 INHIBITOR FOR THE TREATMENT OF LUNG AND HEMATOLOGIC CANCERS	LE078
TIBAUT Tjasa	STRUCTURE BASED DESIGN OF GLYCOMIMETIC LIGANDS OF BACTERIAL N-ACETYLGLUCOSAMINIDASE ATLE	P189
TICHY Michal	THIENO-FUSED 7-DEAZAPURINE RIBONUCLEOSIDES: SYNTHESIS AND BIOLOGICAL ACTIVITIES	P141
TIMUR Savchenko	CONFORMATIONALLY-RESTRICTED PYRROLIDINES FOR DRUG DISCOVERY	P048
TIMUR Savchenko	RAPID ACCESS TO NOVEL MULTIFUNCTIONAL SPIROCYCLIC CORES FOR DRUG DISCOVERY	P047
TIMUR Savchenko	TOWARDS UNEXPLORED REGIONS OF DRUG-LIKE CHEMICAL SPACE – NOVEL FUNCTIONAL SCAFFOLDS BASED ON SPIROCYCLIC AND FUSED CYCLOPROPANES	P088
TITZ Alexander	CARBOHYDRATE-BINDING PROTEINS AS TARGETS FOR ANTI-INFECTIVES AND DIAGNOSTICS: ESKAPE PATHOGEN PSEUDOMONAS AERUGINOSA AND ITS LECTINS	PR002
TODD Matthew	ALL BUGS ARE SHALLOW: OPEN SOURCE DRUG DISCOVERY	LE069
TOMASIC Tihomir	3D-PHARMACOPHORE MODELS FOR IDENTIFICATION AND OPTIMIZATION OF ATP-COMPETITIVE BACTERIAL DNA GYRASE AND TOPOISOMERASE IV INHIBITORS	P190
TOMASKOVIC Linda	FIDELTAMACRO™: MACROLIDE INSPIRED MACROCYCLIC LIBRARY	P217
TOMICH Carlos	DRUG DESIGN AND BIOLOGICAL EVALUATION OF NOVEL ARYL HYDROCARBON RECEPTOR (AHR) ANTAGONISTS	P218
TOMOVIC Katarina	BENEFICIAL EFFECTS OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS ON VASCULAR DYSFUNCTION	P253
TOTH Gergely	IDENTIFICATION OF NOVEL DJ-1 TARGETING SMALL MOLECULES WITH PROTECTIVE ACTIVITY IN CELLULAR AND IN VIVO MODELS OF PARKINSON'S DISEASE	P169

TOTH Gergely	TARGETING THE MONOMERIC INTRINSICALLY DISORDERED STRUCTURAL STATE OF TAU AND ALPHA-SYNUCLEIN BY SMALL MOLECULES AS A POTENTIAL THERAPEUTIC STRATEGY FOR ALZHEIMER'S AND PARKINSON'S DISEASE	LE035
TOZKOPARAN Birsen	NOVEL TRIAZOLE BASED MANNICH BASES AS ANALGESIC AND ANTIINFLAMMATORY AGENTS	P219
TROTTER Wes	CYCLIC DINUCLEOTIDE STING AGONISTS AS ANTI-TUMOR AGENTS	LE077
TSCHAMMER Nuska	IN SITU LABELING OF POLYHISTIDINE-TAGGED PROTEINS FOR QUANTITATIVE PROTEIN INTERACTION ANALYSIS BY MICROSCALE THERMOPHORESIS	P254
TULLY David	DISCOVERY OF LJN452 (TROPIFEXOR), A HIGHLY POTENT, NON-BILE ACID FXR AGONIST FOR THE TREATMENT OF CHOLESTATIC LIVER DISEASES AND NASH	LE058
TURNER Sean C.	POSITIVE ALLOSTERIC MODULATORS OF THE GABA-B RECEPTOR	LE086
TWIGG David	FRAGMENT PROPERTIES AND APPLICATION OF FBDD TO BORON-CONTAINING INHIBITORS OF LP-PLA2	P089
TZVETKOV Nikolay	NEW BRAIN PENETRANT COMPOUNDS IN ADVANCED STUDIES FOR CNS DISEASES	P419
TZVETKOV Nikolay	BENZAMIDES: A NEW SERIES OF POTENT, BBB PERMEABLE AND REVERSIBLE MAO-B INHIBITORS WITH NEUROPROTECTIVE EFFECT ON CORTICAL NEURONS	P301
VAH Luka	DESIGN, SYNTHESIS AND IN VITRO TESTING OF PYRAZOLE BASED INHIBITORS OF PLASMODIUM FALCIPARUM DIHYDROOROTATE DEHYDROGENASE	P420
VALADE Anne	THE VALUE OF ORTHOGONAL TECHNIQUES FOR ELUCIDATING BINDING SITE(S) OF GPCR ALLOSTERIC MODULATORS: A CASE STUDY WITH POSITIVE ALLOSTERIC MODULATORS OF DOPAMINE RECEPTORS	LE087
VALENTI Dario	FRAGMENT-BASED APPROACH APPLIED TO THE DISCOVERY OF PROTEIN-PROTEIN INTERACTION STABILISERS	P049
VALENTINA Straniero	HOW TO FIGHT ANTIMICROBIAL RESISTANCE: DESIGN AND SYNTHESIS OF FTSZ INHIBITORS AS NOVEL POTENT GRAM-POSITIVE ANTIBIOTICS	P194
VALEUR Eric	NEW MODALITIES FOR COMPLEX AND UNPRECEDENTED BIOLOGICAL TARGETS	LE029
VALLIN Karl	DEVELOPMENT OF INHIBITORS OF THE NUDIX HYDROLASE NUDT22	P332
VAN AERSCHOT Arthur	AMINOACYLATED SULFAMOYL-3-DEAZAADENOSINE ANALOGUES: DETAILED ANALYSIS OF THE AASA SCAFFOLD FOR AMINOACYL TRNA SYNTHETASE INHIBITION	P142
VAN BEELEN Eric	IN-PROCESS CONTROL OF ERGOLINE PSYCHEDELICS DURING CHEMICAL SYNTHESIS BY HPTLC COUPLED WITH MASS DETECTION	P423
VAN BEELEN Eric	EVALUATION OF DART (DIRECT ANALYSIS IN REAL TIME), COUPLED TO A PORTABLE MASS DETECTOR FOR RAPID CLEANING VALIDATION	P422
VAN BEELEN Eric	SMALL SCALE PURIFICATION OF FRACTIONS FROM A COMPLEX PHARMACEUTICAL FORMULATION USING AN ANALYTICAL FRACTION COLLECTOR AND A UHPLC-MS SYSTEM	P421

VAN DE GEVEL Iris	KNOCKING ON THE BACKDOOR: SEARCHING FOR ALLOSTERIC POCKETS IN NUCLEAR RECEPTORS	P255
VAN DER VLAG Ramon	ACYLHYDRAZONES AS POTENT INHIBITORS OF HUMAN 15-LIPOXYGENASE-1	P128
VAN DIJK Evert	TRICYCLIC PEPTIDES VIA TEMPLATED TANDEM CLIPS/CUAAC CYCLIZATIONS	P129
VAN DIJK Evert	RGD BICYCLES: HIGH-AFFINITY LIGANDS FOR SELECTIVE INTEGRIN-TARGETING FOR CANCER THERAPY	P050
VAN HOORN Willem	RE-ENERGISING SMALL MOLECULE DRUG DISCOVERY	LE002
VASSILIOU Stamatia	NOVEL CATECHOLIC UREASE INHIBITORS	P092
VASSILIOU Stamatia	SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL LIPOYL HYBRIDS	P091
VASSILIOU Stamatia	NOVEL AND POTENT ORGANOPHOSPHOROUS UREASE INHIBITORS	P090
VEGA ALANIS Blanca Angelica	SELECTIVITY OF PYRAZOLOQUINOLINONE DERIVATIVES TOWARDS THE ALPHA+/BETA- INTERFACE OF THE GABAA RECEPTOR	P093
VELCICKY Juraj	DISCOVERY OF SPL-707: A POTENT, SELECTIVE, AND ORALLY BIOAVAILABLE SPPL2A INHIBITOR	LE084
VESELOVSKA Lucia	SYNTHESIS OF NOVEL HETERO-FUSED 7-DEAZAPURINE RIBONUCLEOSIDES	P143
VLASOV Sergei	HYBRID MICROPARTICLES BASED ON ZERO-VALENT IRON FOR SIMULTANEOUS DRUG DELIVERY AND ULTRASONOGRAPHY VISUALIZATION	P424
VOLOCHNYUK Dmytro	SYNTHESIS OF GEM-DIFLUOROCYCLOPENTANE/HEXANE BUILDING BLOCKS – USEFUL REAGENT FOR DRUG DISCOVERY	P124
VOLOCHNYUK Dmytro	AN EFFICIENT APPROACH TO NOVEL TETRAHYDROPYRIDOAZEPINES. EXPANSION OF AZEPINES' DRUG-LIKE CHEMICAL SPACE.	P123
VOLOCHNYUK Dmytro	COMMERCIALLY AVAILABLE CHEMICAL SPACE: DOES IT MEET MODERN REQUIREMENTS?	P095
VOLOCHNYUK Dmytro	VIRTUAL CHEMICAL SPACE WITH HIGH PREDICTABLE SYNTHETIC FEASIBILITY. IS IT ACHIEVABLE?	P083
VOLOCHNYUK Dmytro	RUPPERT-PRAKASH REAGENT AS A DIFLUOROCARBENE SYNTHETIC EQUIVALENT FOR THE SYNTHESIS OF MEDCHEM-RELEVANT BUILDING BLOCKS	P122
VOLOCHNYUK Dmytro	CYCLOBUTANE RING AS A CONFORMATIONAL RESTRICTION TOOL	P094
VON NUSSBAUM Franz	A NEW TARGET IN FUNGAL PROTEIN BIOSYNTHESIS: SHARED LEARNINGS FOR AGCHEM AND MEDCHEM	LE017
VUORINEN Aini	TOWARDS THE ELUCIDATION OF THE MECHANISM OF ACTION OF SMALL MOLECULE UPREGULATORS OF UTROPHIN USING CHEMICAL PROTEOMICS	P256
WAHALA Kristiina	CHARACTERIZATION OF SEX HORMONE BINDING GLOBULIN BOUND TO 3,4-DIVANILLYLTETRAHYDROFURAN: POTENTIAL FOR THE DESIGN OF HIGHER ACTIVITY SHBG COMPETITIVE INHIBITORS	P258

INDEX OF AUTHORS

Last Name and First Name Initial		Pub id
ABATE	C.	P334
ABDUL-RIDHA	A.	P032
ABLASSER	A.	LE037
ÁBRÁNYI-BALOGH	P.	P316, LE081
ACEVES-LUQUERO	C.	P351
ACÚRCIO	R. C.	LE008
ADLERE	I.	P195
ADLURI	B.	P161
ADRIO	J.	P006
AERTSSEN	S.	P350
AFROZ	T.	LE092
AGBABA	D.	P346, P347
AGNEW-FRANCIS	K. A.	P305
AGUSTI	R.	P185
AHMADIAN MOGHADDAM	M.	P064
AHONEN	T. J.	P340
AICHHOLZ	R.	LE084
AIGBIRHIO	F. I.	P149
AIGUABELLA FONT	N.	P306
AIRAVAARA	M.	P340
AITKEN	L.	P342
AKAVARAM	N.	P107
AKIMOTO	M.	P388
ALANCAY	E.	P363
ALDANA	I.	P170
ALEIXO	M. A. A.	P017
ALEXANDER	M.	P377
ALI	A.	LE101
ALIAGA	C.	P041
AL-KARADAGHI	S.	P268
ALKHATE	F. L. E.	P421
ALPERT	G.	P200
ALTAMURA	S.	P182
ALTMANN	K.-H.	LE052
AMBLER	M.	P262
AMESTY	Á.	P385
AMETAMEY	S. M.	P148
AMIN	S.	P040, P041, P042
AMIRI	P.	P283
ANDERLUH	M.	P204, P245, P425, P189, P326, P253
ANDERSEN	C.	P074
ANDERTON	M. J.	P270
ANDREA	C.	P194
ANDREW	F.	LE064
ANDREWS	D. M.	P270
ANDREWS	S.	LE036
ANDRYIANAU	G.	P209, P211
ANGELUCCI	F.	P206
ANGST	D.	LE026
ANNA	H.	P063
ANNALA	S.	P072
ANNUNZIATO	G.	LE020
ANTONENKO	T.	P119
ANTONIOLI	L.	P206
ANTONOV	L.	P301
ANTUNES	A. M. M.	P046
ANTUNOVIC	M.	P403
ARANDA-TAVÍO	H.	P385
ARAUJO	N.	P256
ARDIGÓ	D.	PL004
ARIMONT	M.	P195
ARISTA	L.	LE075
ARMIROTTI	A.	P330, P437
ARNAUDOVA	R.	P263
ARONOV	A.	P267
ARSOV	Z.	P245
ARTES	S.	P296
ARVIDSSON	P. I.	P045
ASHALL	L.	P357
ATKINSON	B. N.	LE065, P060
ATMANENE	C.	P390
AUBERSON	Y. P.	P148
AUMÜLLER	I.	P263
AUNO	S.	P319
AUTAR	R.	P210
AVCI	A.	P219
AVERINA	E. B.	LE088
AVERSA	R. J.	P283
AVŠIČ ŽUPANC	T.	PL001
AZEVEDO	C.	P074
AZZALI	E.	P193
BACHOVCHIN	D. A.	P252
BACSA	I.	P021
BADOCK	V.	P407
BADOVINAC	M.	P251
BAELL	J.	P180, P172, P171
BAELL	J. B.	P436
BAGGIO	C.	P029, P030
BAILLY	B.	P400

BAJIĆ	M.	P035
BAKER	J.	P037
BAKER	J. G.	P399
BALAZS	A.	P309
BALLONE	A.	P049
BALOGH	D.	LE051
BAMPALI	K.	P093
BANASZAK	K.	P031
BANDIERA	T.	P330, P166
BANERJEE	A.	P411
BANJANAC	M.	P314
BANKER	M. E.	LE064
BANOGLU	E.	P201
BAPTISTA	S. J.	P387
BARANČOKOVÁ	M.	P349
BARANOWSKI	M. R.	P146
BARBARO	L.	P171
BARCHERINI	V.	P046
BARF	T.	P271
BARGE	A.	P268
BARLAAM	B.	LE096, P264
BARLOW	N.	P061
BARNIOL-XICOTA	M.	P236
BARRATT	D.	P084
BARRETEAU	H.	P316
BARRETO	A. M. S.	P356
BARRIL	X.	P250, LE024
BARSANTI	P. A.	P283
BARTENSCHLAGER	R.	P192, P177
BARTOSZEWICZ	A.	P209, P211
BARTUSCHAT	A.	P411
BARTUZI	D.	P431, P323, P341
BARTYZEL	A.	P034
BARUT	B.	P409
BASTA	A.	P182
BASTA-KAIM	A.	P207
BATISTA	P. H. J.	P174
BATISTE	L.	PL007
BAUDRU	A. S.	LE039
BAUER	M.	P086
BÄURLE	S.	LE093
BAYLE	E.	LE065
BEDNAR	D.	P010
BEDNARCZYK	M.	P138
BEDNAREK	S.	P145bis
BEEKMAN	A. M.	P100

BEERLI	C.	LE075, LE026
BEERLI	R.	LE075
BEGAN	J.	P252
BEHRENS	M.	P333
BEISNER	D. R.	LE084
BEKE	L.	LE078
BELLIS	S.	P137
BELOGLAZKINA	E.	P384
BELOUSOV	M.	P424
BENCSIK	P.	P001
BENDER	A.	LE046
BENDERITTER	P.	P016
BENEDIKTSDOTTIR	A.	P013, P045
BENEK	O.	P342
BENKEL	T.	P072
BENNETT	J.	P276
BENOHOUD	M.	P158
BER	A.	P384
BERARDI	F.	P334
BERG	A.	P041
BERG	M.	P014
BERGANT	K.	P343
BERGER	B.-T.	LE016
BERGER	M.	P407
BERGNER	A.	LE063
BERGSDORF	C.	LE075
BERLICKI	L.	P090, P092
BERNHAGEN	D.	P050
BERNIER	D.	LE017
BERNSMEIER	N.	P306
BERST	F.	P359
BERTHELOT	D.	LE078
BERTI	F.	P330
BERTÓK	B.	P015
BERTOLINI	S.	P287
BERTORELLI	R.	P330, P437
BERTOŠA	B.	P314
BERTOZZI	F.	P330
BEUS	M.	P403
BEVERIDGE	J.	P172
BIAGETTI	M.	P287
BIAŁAS	A.	P031
BIANCOFIORE	I.	P182
BICTASH	M.	LE065
BIE	J.	P051
BIELA	A.	P025

BIELA	A.	P313
BIELEFELDT	L. Ø.	P405
BIEŃ	M.	P031
BIERI	R.	LE052
BIGATTI	M.	P239
BIGAUD	M.	LE026
BILIĆ	L.	P345
BILLICH	A.	LE075
BILSLAND	J.	LE065
BINDA	C.	P338
BISCHOFF	M.	P368
BITRA	A.	P202
BITTNER	A.	P382
BLACKBURN	R. S.	P158
BLAKSKJÆR	P.	P074
BLANDIZZI	C.	P206
BLANK	J.	P359
BLOM	P.	P016
BODENDORF	U.	LE084
BOECHAT	N.	P022, P017, P279
BOECKX	A.	LE078
BOIJE AF GENNÄS	G.	P296, P319, P179
BOLD	P.	P273
BOLLENBACH	M.	P096
BON	D. J.	P113
BONILLA-RAMIREZ	L.	P170
BOREK	B.	P209
BORGOGNO	M.	P437
BOSC	D.	P151, P025
BOSCC	D.	P348
BOSCHI	D.	P268
BOSSERT	M.	P070
BOSTRÖM	J.	P069, LE042
BOTTEGONI	G.	P166, LE047
BOUDOU	C.	LE092
BOULARAN	C.	LE039
BOURGUIGNON	M.-P.	P005
BOUTARD	N.	P031
BOUZ	G.	P181
BOUZANNE DES MAZERY	R.	P210
BOVINA	E. M.	LE088
BOZDAG	M.	P248
BRADFIELD	P.	P200
BRAENDLIN	N.	LE075
BRANCALE	A.	P376
BRANDL	T.	LE084

BRANDOLI	G.	P296
BRANDS	M.	LE016
BRANDT	P.	P013
BRAUN	M.-G.	P265, LE094
BRBORIĆ	J.	P215
BREED	J.	P270
BREHMER	D.	LE078
BREINBAUER	R.	LE060
BREITENSTEIN	W.	P359
BRESCIANI	A.	P182
BRETHON	A.	P407
BREZOVSKY	J.	P010
BRIEM	H.	LE016
BRIGHTBILL	H.	P266
BRINDANI	N.	P330
BRITTON	R.	P161
BROMHEAD	L.	P324
BROOKES	S.	P153
BROSZAT	M.	P423
BROUGH	D.	P019
BROUGH	S.	P419, P020, P062
BROWN	D.	P187
BROWN	D.	P069
BROWN	M. F.	LE064
BROWNLEES	J.	P262
BRUGAL	G.	P391
BRUNSCHWEIGER	A.	P101
BRUNSVELD	L.	P295, P255
BRUS	B.	P396
BRUTSCH	M.	PL007
BRÜTSCH	M.	P039
BRUYERE	D.	P235
BRVAR	M.	P405
BRYAN	M. C.	P266
BRYANT	S. D.	P190, P328
BRZEZINSKA	A.	P145bis
BRZÓZKA	K.	P313, P031
BUA	S.	P248
BUCKI	A.	P164
BUDA	A.	P031
BÜDEFELD	T.	P021
BUKVIĆ	M.	P314
BUREAU	E.	P262
BURGER	M. T.	P283
BURLEY	G.	P087
BURN-HEATH	I.	P310

BURTOLOSO	A. C. B.	P174
BYSTROWSKA	B.	P320
CABASSI	C. S.	LE020
CABRERA	M.	P173
CACI	E.	P330
CADOGAN	E.	P264
CAFLISCH	A.	P039, PL007
CAI	J.	P376
CALIENDO	G.	P167, LE072
ÇALIŞKAN	B.	P201
CALLEGARI	D.	P287
CALLEJA	J.	P153
CAMBERLEYN	V.	P151
CAMPANINI	B.	LE020
CAMPILLO	N. E.	P006
CAMPUSANO	J.	P427
CAN	Ö.	P219
CANCEDDA	L.	P437
CAÑEQUE	T.	LE050
CANTRELLE	F.-X.	P049
CAO	S.	P013
CAPACCHI	S.	P287
CAPELLI	A. M.	P287
CAPOBIANCO	A. J.	P277
CAPPEL	D.	P308
CAPUANO	B.	P293, P302
CARBAIN	B.	P102, P107
CARBAJO MARTINEZ	R. J.	P309, LE031
CAROTTI	A.	LE044
CARRARO	C.	LE072, P136
CARREIRA	E.	P096
CARRERAS-PUIG	J.	P332
CARTER	G. P.	P436
CARTER	M.	P332
CARTER	T.	P324
CARTWRIGHT	O.	P023
CARVALHO	L.	P022
CARZANIGA	L.	P287
CASALE	E.	P080
CASTRO	M.	P034
CATHERINE	P.	P025
CAVALIERE	A.	P149
CAVALLI	A.	P166
CENNI	B.	LE026
CENTORRINO	F.	P049
CERRA	B.	LE044

CEUSTERS	M.	LE048
CHACON-VARGAS	K. F.	P185
CHAI	C. L.	LE070
CHALMERS	D.	P061
CHALUPNIK	P.	P416
CHALYK	B.	P047
CHAMBERLAIN	P.	LE022
CHAMBRIER	I.	P312
CHAMPNESS	E.	P066, P002
CHAN	C.	LE094
CHAN	K.	P264
CHANG	C.-W.	P400
CHAPMAN	K.	P153
CHARLTON	S. J.	LE013
CHARTON	J.	P348
CHATZOPOULOU	M.	P256
CHAUDHARY	S.	P081
CHEN	H.	P051
CHEN	Y.	P045
CHERNYKH	A.	P047
CHIARPARIN	E.	P309, P084
CHIHA	S.	P238
CHING	K. C.	LE070
CHINTHAKINDI	P. K.	P045
CHISHOLM	J.	P066
CHŁON-RZEPA	G.	P220
CHMIELINSKI	S.	P145bis
CHO	H. M.	P103
CHO	M. C.	P380
CHO	Y. S.	P103
CHOI	K. I.	P372
CHOI	M. J.	P286
CHOO	E.	P266
CHOY	K.	P293
CHRISTOPOULOS	A.	P032, P293
CHUGANI	R.	LE012
CIAMMAICHELLA	A.	P182
CIANNI	L.	P174
CID	J.	LE048
CIELUCH	E.	P031
CIERPICH	A.	P031
CIESIELSKI	F.	P390
CINDRIĆ	M.	P345
CINTULOVA	D.	P344
CIOMEI	M.	P080
CIPRIANI	F.	P050

CIRLA	A.	P080
CLARK	J. D.	LE064
CLARK	M. A.	P407
CLARK	M. J.	P411
CLAUSEN	S.	LE094
CLAYDEN	N.	P235
CLEARY	R.	P421
CLEMENT	B.	P406
CLERICI	F.	P382
COBAN	G.	P363
CODONY	S.	P335
COE	D. M.	LE038
COGSWELL	T.	P318
COIMBRA	J. R. M.	P387
COLABUFO	N. A.	P334
COLCLOUGH	N.	P264
COLETTIS	N.	P338
COLLET	C.	P392
COLLET	T.	P392
COLLIER	P.	P267
COLLINS	P.	P230
COME	J.	P267
COMPANYS-ALEMANY	J.	P335
CONOLE	D.	LE098
CONSTANTINOU	S.	LE065
CONTESTABILE	A.	P437
CONTINO	M.	P334
CONTRERAS	J.-M.	P367, P268
COOKE	A.	P066
COOKE	V. G.	P283
COOPER	T.	P041
ČORAK	N.	P314
CORPAS	R.	P335
CORPET	M.	P049
CORR	S. J.	P085, P243
CORSI	M.	P287
CORVINO	A.	P167
CORVO	I.	P173
COSTA	A.	LE065
COSTANTINO	G.	P193, LE020
COURTENS	C.	P175
COUTTET	P.	LE084
COVEY	T.	P271
COVIELLO	V.	P206
CRAVATT	B.	AL003
CRAVERO	F.	P006

CRAWFORD	J.	P197
CRIBIU	R.	PL007
CRUCES COLADO	J.	P322
CRUZ	T.	P184
CSEH	S.	P001, P198
CSÉKEI	M.	PR001
CSUK	R.	P369, P383
CUI	M.	P329
CULLY	D.	LE101
CUNNINGHAM	T.	P191
CUOZZO	J. W.	P407
CURLEY	S. A.	P085, P243
CURTOLO POIANI	J. G.	P218
CVIJANOVIĆ	D.	P345
CZESTKOWSKI	W.	P209, P211
CZOPEK	A.	P393
DA COSTA	S.	P174
DA COSTA PEREIRA	D.	P315
DAHL	G.	LE031
ĐAKOVIĆ	M.	P345
DALIANI	T.	P092
DALKARA	S.	P410
DALLANOCE	C.	P435
DAMBORSKY	J.	P010
DAMEN	E.	P350
DAMIANIDOU	C.	P168
DAMJANOVIĆ	V.	P345
DANHOF	M.	LE015
DANIEL-HENRY	C.	P105
DARDENNE	L. E.	P356
DARFEUILLE	F.	LE066
DARMENCY	V.	LE092
DAS	S.	P258
DAVENPORT	A.	LE093
DAVIES	E.	P270
DAVIES	G.	P161
DAVIES	J.	P324
DAVIES	K. E.	P256
DAVIES	N. L.	P309
DAVIES	S.	P318, P324
DAVIES	S. G.	P256
DAVIS	A.	P273
DAVIS	B.	PL005
DAWSON	J.	LE026
DE ALBURQUEQUE	S.	P174
DE BOER	B.	P315

DE BRUIN	G.	P271
DE CARVALHO	A. S.	P017
DE CASTRO BAZAN	S.	P279
DE ESCH	I.	P187, P195, P315
DE GRAAF	C.	P195, P315
DE GRAEF	S.	P142
DE JONG	A.	P271
DE JONGHE	S.	P285
DE LANGE	E. C.	LE015
DE MAGALHÃES	C. S.	P356
DE NAZARE CORREIA SOEIRO	M.	P187, P017
DE OLIVEIRA	A. P.	P279
DE SAVI	C.	LE096
DE SIMONE	A.	P166
DE VITA	D.	P174
DE VIVO	M.	P437
DE WITTE	W. E.	LE015
DE ZWART	E.	P271
DEBRECZENI	J. E.	P270
DECRUY	T.	P202
DEHARO	E.	P170
DEKKER	F. J.	P128
DELLSÉN	A.	LE031
DEMETER	O.	P073
DEMONT	D.	P271
DENIS	A.	P016
DEPREZ	B.	P151, P025
DEPREZ-POULAIN	R.	P348, P151, P025
DESPERATI	V.	P080
DEVINE	S.	P302
DHEERE	A. K. H.	P149
DI GIORGIO	A.	LE066
DI MARCO	A.	P182
DI MARTINO	A.	P424
DI MARTINO	R. M. C.	P166
DI MICHELE	A.	LE044
DI PIZIO	A.	P333
DIAS	A.	P178
DIAZ	M. F.	P006
DIAZ	R.	P184
DÍAZ-GAVILÁN	M.	P325
DIDIER	B.	P367
DIELS	G.	LE078
DILLIARD	S.	P243
DILLON	M. P.	P283

DIMITRA	H.-L.	P248
DINIS	T. C. P.	P387
DIRK	S.	P063
DISTEFANO	M.	P357
DIVOKY	V.	P275
DIX	I.	LE084
DJOKOVIC	N.	P347
DMITRIY	S.	P377
DO	S.	P266
DO HUU	N.	P005
DOBIE	C.	LE080, P137
DOBRIČIĆ	V.	P215
DOEMLING	A.	LE007
DOGAN	İ.	P242
DOLBOIS	A.	P039, PL007
DOLER	C. J.	LE060
DOLEZAL	M.	P181
DOLEZAL	R.	P389
DOLGA	A.	LE007
DOLJAK	B.	P396
DOLŠAK	A.	P152, P155
DOMINGOS	S.	P026
DOMLING	A.	P064
DÖMLING	A.	P071, P415
DONATI	D.	P080
DONCKELE	E.	P239
DORE	T.	P357
DÖRING	E.	LE061
DORMÁN	G.	P001, P015, P198
DOROCKA-BOBKOWSKA	B.	P199
DOSSETTER	A.	LE004, P027
DOSSOU	S.	LE078
DOSTALEK	J.	P050
DOZIER	J.	P357
DRABEK	M.	P108
DRASKOVITS	G.	P429, LE051
DRENICHEV	M.	P139
DREW	L.	LE096
DRINKWATER	N.	P379
DROBNICK	J.	P266
DRURY	W.	P079
DUARTE	T.	P026
DUBIN	G.	LE007
DUBOVA	U. N.	P176
DUBURS	G.	P176
DUCA	M.	LE066

DUCASSE	L.	LE039
DUCIME	A.	P268
DUCROT	P.	P005
DUDDA	A.	LE100
DUDEK	Ł.	P313, P031
DUDUTIENĖ	V.	P104
DUFFY	J.	P272
DUGDALE	H.	P256
DUMONT	J.	P151
DUNLOP	J.	P360
DUQUE	C.	P174
DURANDO	L.	P282
DURANT	S.	P264
DURANTE CRUZ	C.	P429
DURCIK	M.	P429, P349, P412
DUZGUNES	N.	P199
DYMEK	B.	P209, P211
DZIENIA	A.	P313
DZWONEK	K.	P209, P211
EATHERTON	A.	P264
EBERT	B.	LE023
ECKER	G. F.	P344
EDGAR	K.	LE094
EDINK	E.	P187
EDMAN	K.	P246
EDWARDS	J. P.	LE078
EGGENWEILER	H.-M.	P313, P031
EIGENBROT	C.	LE094
EINAV	S.	P285
EITEL	K.	P411
EKIZOGLU	M.	P242
ELEFTHERIADIS	N.	P128
ELENA	B.	P377
ELEONORA	D.	P063
ELEWAUT	D.	P202
ELKINS	J.	P276
ELLIOTT	R.	LE094
ELLSON	R.	P064
ELSINGA	P.	LE007
EL-TAYEB	A.	P140
EMER	E.	P256
EMMELOT - VAN HOEK	M.	P271
EMSON	C.	P266
ENCÍO	I.	P043, P044
ENDRES	N.	LE094
ENGEL	C. K.	LE100

ENGEL	J.	LE061
ENGELHARDT	H.	LE063
ENRIC	L.	P105
EPPLE	R.	LE084
ERDELYI	M.	P013
ERMANNO	V.	P194
ERNST	M.	P093
EROL GUNAY	S.	P242
ERVER	F.	P306
ERŽEN	N.	P375
ESCOLANO	C.	P335
ESPADINHA	M.	P046
ESSIGMANN	B.	LE017
ESTÉVEZ-BRAUN	A.	P385
ETTMAYER	P.	LE063
EVANS	L.	P084
EVERTSSON	E.	P273
F. DANTAS	R.	P279
FABRIS	D.	LE072
FABRITIUS	C.-H.	P031
FALCAO	A.	P003
FALLARERO	A.	P358
FALLARERO	F.	P327
FAUCHER	N.	P016
FEDERICO	A.	P268
FEIFEL	R.	LE084
FELDER	E. R.	P080
FELIX	R.	P235
FENG	F.	P283
FERDINANDY	P.	P001
FERGUSON	A. D.	LE096
FERGUSON	D.	LE096
FERNANDES	M. X.	P325
FERNANDEZ	E.	LE071
FERNÁNDEZ DE MATTOS	S.	P351
FERNÁNDEZ ZERTUCHE	M.	P386
FERNÁNDEZ-HERRERA	M. A.	P213
FERNANDEZ-MONTALVAN	A. E.	P407
FERNÁNDEZ-MONTALVÁN	A.	LE016
FERNÁNDEZ-PÉREZ	L.	P385
FERNANDEZ-RUIZ	J.	P391
FERRARO	F.	P173
FERRERA	L.	P330
FERRIGNO	F.	P182
FESSARD	T.	P132, P096, P432
FIANDOR	J. M.	P147

GBELCOVÁ	H.	P352, P404, P402
GEALAGEAS	R.	P151
GEBREMEDHIN	S.	P199
GEE	A.	LE089
GEHRINGER	M.	LE052
GELLIBERT	F.	P005
GELMI	M. L.	P294
GENIX	P.	LE017
GENNE	P.	P016
GEORGEON	S.	P288
GEORGI	V.	LE016
GERARDY-SCHAHN	R.	P137
GERASIMIDOU	A.	P168
GERLAND	K.	LE094
GERSBACH	P.	LE052
GERSTENBERGER	B. S.	LE064
GERSTMEIER	J.	P201
GESSIER	F.	LE026
GETTER	T.	P200
GIAMPIETRO	R.	P334
GIANNAKOPOULOU	E.	P192, P177
GIBBONS	F. D.	P270
GIGANTE	A.	P147
GIL	C.	P006
GILARDONE	M.	P082
GILARDONI	E.	P294
GILISSEN	R.	LE078
GILLEN	K.	P262
GIOIELLO	A.	LE044
GIOVANNINI*	R.	LE028
GIRARDINI	M.	P193
GISBERT QUILIS	N.	P050
GIZYNSKA	M.	P292
GLAVAŠ-OBROVAC	L.	P035
GLINCA	S.	P075
GLOANEC	P.	P005
GLOMBIK	H.	LE100
GLUKHOVA	A.	P032
GMEINER	P.	P108, P333, P411
GOBBI	A.	P266
GOBEC	M.	P216, LE081
GOBEC	S.	P188, P321, P216, P338, P152, P155, P004, P316, P106, P164, P183, P396, LE081

GODDARD	Z. R.	P312
GODOI	P.	P276
GODYN	J.	P164
GOETTLICH	R.	LE072, P136
GOHIER	A.	P005
GÖKHAN KELEKÇI	N.	P241, P219, P242
GOLAB	J.	P209
GOLDBERG	K.	P264
GOLDMAN	A.	P179
GOŁĘBIOWSKI	A.	P209, P211
GOLIČ GRDADOLNIK	S.	P353
GOLLNER	A.	LE063
GOLOJUCH	S.	P145
GOMES	C.	P046
GOMES	J. C.	P174
GOMES	S.	P046
GÓMEZ ARRAYÁS	R.	P006
GOMEZ-CAÑAS	M.	P391
GÓMEZ-VIDAL	J. A.	P325
GONÇALVES	P.	P178
GONCHARENKO	K.	P432
GONDELA	A.	P313, P031
GONZALEZ	S.	P147
GONZÁLEZ SISO	M. I.	P322
GONZÁLEZ-ROSENDE	M. E.	P351
GOPALAKRISHNAN	R.	P079
GOPKO	V. V.	P128
GÓRA	M.	P393
GÖRBE	A.	P001
GORIČAN	T.	P354
GORJANACZ	M.	P407
GOULIOU	I.	P168
GOURGUES	M.	LE017
GOYA	P.	P391
GRABNER	G.	LE060
GRACHEVA	Y.	P119
GRAEDLER	U.	P082
GRAHAM	M. A.	P270
GRANDJEAN	C.	P390
GRAY	H.	P355
GRAZIANI	R.	P182
GREVE	G.	P300
GREVOT	A.	LE075
GRGIČEVIĆ	I.	P314
GRICE	D.	P230
GRIFFANTE	C.	P287

GRIFFEN	E.	LE004, P027
GRIFFITHS	R.	P087
GRIGORIEV	V. V.	LE088
GRILL	M.	P423
GRILO	J.	P178, P184
GRIÑAN-FERRÉ	C.	P335
GROSCHE	P.	P359
GROŠELJ	U.	P397
GROSICKI	M.	P320
GRUDNIK	P.	LE007
GRUTSCHREIBER	P.	P340
GRUZA	M.	P209, P211
GRUZMAN	A.	P200
GRYGORENKO	O.	P094, P122, P083, P123, P124
GU	C.	LE096
GU	X.-H.	LE094
GUCKY	T.	P275
GUCKÝ	T.	P280
GUDDAT	L. W.	P305
GUEDES	I. A.	P356
GUEDES	R. A.	P003
GUEDES	R. C.	LE008, P026, P003, P186, P274, P328
GUÉRET	S. M.	LE031
GUERINI	D.	LE084
GUERRA	B.	P385
GUERRA-RODRÍGUEZ	M.	P385
GUILLAUME	V.	P151
GUNNARSSON	A.	P246
GUNN-MOORE	F.	P342
GÜNTHER	M.	LE061
GUO	H.	P128
GÜR	Z. T.	P201
GÜSSREGEN	S.	LE100
GUTIÉRREZ	M.	P427
GUTMANN	S.	LE026
GÜTSCHOW	M.	P234, P072
GUZIK	P.	P031
HA	M. W.	P121
HABINOVEC	I.	P314
HAHAM	D.	P115
HAHN	M. G.	LE027
HAIDER	A.	P148
HAJDÚ	I.	P001, P198
HALING	J. R.	P283

HALLAM	L.	LE080
HAMILTON	P.	LE094
HAMMOCK	B. D.	P335
HAMMOND	G.	P258
HAMMOND	J.	P422
HAMPTON	S.	P357
HAN	C.	LE094
HANAN	E.	LE094
HANDEL	T.	P434
HANDZLIK	J.	P368
HANNAH	D.	P153
HANSEN	N.	P074
HANTSCHEL	O.	P288
HARJANI	J.	P172
HARJANI	J. R.	P436
HARLFINGER	S.	LE026
HARPER	S.	P182
HARTMAN	A. M.	P068
HARTUNG	I.	P407
HASHIMOTO	T.	P388
HASHIMOTO	Y.	P067
HASSAN	A. H.	P380
HASSAN	G.	P358
HAU	M.	P300
HAUSTEDT	L.	P075
HAUTALA	L.	P014
HAUWERT	N.	P315
HAVEL	S.	P102, P107
HAVEMEYER	A.	P406
HAWKINS	J.	LE096
HAYWARD	M. M.	LE064
HEALD	R.	LE094
HEDSTRÖM	U.	P273
HEGEN	M.	LE064
HEIDEN	S.	P329
HEINIS	C.	P208
HEINRICH	M.	P411
HEITMAN	L. H.	LE014, P011, P434
HEKKING	K.	P065
HEKMAT-NIJJAD	M.	P283
HELD	J.	P403
HELLEDAY	T.	P332
HELLER	L.	P369
HELLMANN	J.	P108
HELLSBERG	E.	P344
HELM	M.	P371

HENDERSON	S.	P276
HENDERSON	T.	LE101
HENDRYCHOVÁ	D.	P365
HENRIQUE TOMICH DE PAULA DA SILVA	C.	P218
HENRY	A.	P109
HENRY	C.	P422
HERBERTH	B.	P169
HERDEWIJN	P.	P285
HERLIHY	K.	P359
HERMANT	P.	P025
HERR	P.	P332
HERRLINGER	E.-M.	P300
HERSPERGER	R.	LE026
HIBBING	M.	LE012
HIEMSTRA	H.	P129
HILDERBRANDT	E.	P357
HINNIGER	A.	LE026
HINRICHS	J.	P359
HIRATA	K.	P411
HIRD	A. W.	LE096
HIRONO	S.	P394
HIROSE	Y.	P117
HIRSCH	A. K. H.	P071, P068, P128
HOBBS	A.	LE098
HOBBS	H.	P284
HOCEK	M.	P141, P143
HODNIK	V.	P189
HODOŠČEK	M.	P353
HODZIC	A.	P239
HOERRNER	R. S.	LE101
HOFLACK	J.	P016
HOFMANN	J.	P411
HOLAK	T.	LE007
HOLLINGSHEAD	B. D.	LE064
HOLMGAARD KASPERSEN	M.	P360
HOLTON	S. J.	P407
HOMAN	E. J.	P332
HONG	R.	LE094
HONKISZ-ORZECHOWSKA	E.	P320
HOOGENBOOM	N.	P271
HOPCROFT	P.	P270
HORENSTEIN	N. A.	P435
HOWARD	S.	LE068
HOWARD	T.	P270
HOWDEN	B. P.	P436

HRAST	M.	P316, P106, P183
HRENAR	T.	P345
HRIBERNIK	N.	P189
HRISTEVA	S.	P049
HROCH	L.	P342
HROUDOVA	J.	P413
HSIAO	A.	P434
HU	X.	P294
HUANG	C.-C.	P433
HUANG	S.	P283
HUANG	Z.	P266
HUBER	K. V. M.	P256
HUBNER	J.	P407
HÜBNER	H.	P108, P411
HUCK	B.	PL002
HUCZYŃSKI	A.	P112
HUDSON	J.	P270
HUET	Y.	LE017
HULTGREN	S.	LE012
HUNT	P.	P066, P002
HUNT	T.	P264
HUO	P.	LE101
HURLEY	B.	P359
HYLSE	O.	P281
ICHIKAWA	S.	P033
IJZERMAN	A. P.	P011, P434
ILAŠ	J.	P429, P349, P412, P317
IMHOF	B.	P200
IMINOV	R.	P088
IMRAMOVSKÝ	A.	P365, P361
INDA	S.	P063
INOUE	K.	P388
IRIE	T.	P388
ISACCHI	A.	P080
ISHIKAWA	T.	P144
ISHIKAWA	Y.	P388
ISKRA	J.	P081
ISLAM	R.	P388
ISTANBULLU	H.	P363
JACKMAN	M.	P153
JACKSON	P.	LE094
JACKSON	T.	P318
JACOB	C.	P368
JAGEROVIC	N.	P391
JAIME	R.	P105

JAKOS	T.	P396
JAKUBIEC	K.	P031
JALINK	K.	P050
JAMIESON	C.	P284
JANETKA	J.	LE012
JANEZIC	D.	P321
JANEŽIČ	M.	P343
JANG	C.	P110
JANG	D. S.	P364
JANG	S. K.	P372
JANIN	C.	P132
JANKOWSKA	E.	P292
JANOCKOVA	J.	P389
JANSEN	I.	P038
JANSEN	J. M.	P283
JANSER	P.	LE026
JANSSENS	J.	P202
JÄNTTI	M.	P296
JARED	D.	P240
JEGANATHAN	F.	LE065
JEMIELITY	J.	P138, P259, P145, P145bis, P146, P146bis
JEMTH	A.-S.	P332
JENKINS	L.	P360
JENNIFER	H.	P063
JENSEN	L. K. I.	P405
JEONG	Y.	P372
JHENG	M.-J.	P433
JIAN	X.	P082
JIAO	Y.	P180
JIE	S.	P425
JIMENEZ-BARBERO	J.	LE009
JIN	C.	P364
JINA	K.	P373
JOAO GAMA	M.	P003
JOHANSSON	M.	P079
JOHANSSON	N. G.	P179
JOHNSON	D. C.	P252
JOHNSON	T.	P082
JOHNSTRÖM	P.	P264
JOLLY	S.	LE065
JONCZYK	J.	P164
JONES	C.	P270
JONES	C. D.	P270
JONES	E. Y.	LE065

JORAND LEBRUN	C.	P082
JORDA	R.	P365, P275, P280, P361
JORDAN	A.	LE067
JÖRG	H.	P063
JÖRG	M.	P032, P293
JORGE	G.-G.	P240
JOSA CULLERE	L.	P318, P324
JOSUE	R. L.	P408
JUCHUM	M.	LE061
JUKIČ	M.	P004, P183
JUMDE	V. R.	P071
JUMPPANEN	M.	P319
JUN	D.	P413
JUNG	L.	P367
JUNG	M.	P300
JUNG	M.-L.	P367
JUNG	D. W.	P110
JUNGBLUTH	V.	P050
JUNGWOOK	C.	P373
JUNIOR	P. A. S.	P017
JURCIK	A.	P010
JURZAK	M.	P031
KÄCK	H.	P273
KACZOR	A.	P368
KACZOR	A. A.	P034, P431, P323, P341
KAHNT	M.	P369
KAHREMANY	S.	P200
KAINDL	J.	P411
KAISER	M.	P182
KALAS	V.	LE012
KALETA	M.	P320
KAMIŃSKI	K.	P393
KANG	D. H.	P364
KANNT	A.	LE100
KANTÚN-MORENO	N.	P213
KAPTEIN	A.	P271
KARAKURT	A.	P410
KARASCH	J.	P383
KARCZ	T.	P320
KARELIA	D.	P040
KARLÉN	A.	P013
KARLOV	D. S.	LE088
KARPOWICZ	P.	P292
KASAKIN	M.	P203
KASHIF	M.	P185

KASPRZYK	R.	P259
KATILA	P.	P378
KATTHAB	A.	P179
KAZOKAITĖ	J.	P104
KEDZIERSKA	E.	P167
KEEFE	A. D.	P407
KELLEY	T. T.	P277
KELLY	J. M.	P192
KELLY	S.	LE094
KELTER	G.	LE061
KENNEL	P.	LE017
KENNY	P.	P174
KERSTEN	C.	P371
KESERŰ	G. M.	P316, P069
KESSLER	A.	P172, P147
KEUL	M.	LE061
KEUM	G.	P372
KEURULAINEN	L.	P358
KHAJEHALI	E.	P293
KHARITONASHVILI	E.	P119
KHIRSARIYA	P.	P281, P102
KIEĆ-KONONOWICZ	K.	P368, P320
KIEFER	J.	P266
KIKELJ	D.	P429, P190, P349
KIM	D.	P131
KIM	D. M.	P111, P125
KIM	H. I.	P380
KIM	H. J.	P364
KIM	H. S.	P372
KIM	J.	P426
KIM	J.	P426
KIM	J.	P286
KIM	J. S.	P286
KIM	S.	P374
KIM	S.	P374
KIM	S. Y.	P120
KIM	S.-Y.	P041
KIM	T.	P426
KINNUNEN	S.	P319
KIRIAZIS	A.	P263, P179
KIRICHOK	A.	P047
KIRK	J.	P422
KIRPOTINA	L. N.	P207
KITAHATA	S.	P033
KITAMURA	Y.	P394
KITLINSKA	A.	P031

KIURU	P. S.	P179
KIZRAK	U.	P363
KLEBE	G.	P071, P075
KLEIN	E.	P390
KLEJBOROWSKA	G.	P112
KLIKA-SKOPIC	M.	P101
KLINGLER	F.-M.	P070
KNAFELS	J. D.	LE064
KNAPP	S.	LE016
KNEHANS	T.	PL007
KNERR	L.	P079
KNEZ	D.	P338, P155, P164, P396
KNIGHT	J.	LE094
KNÖPFEL	T.	LE075
KO	Y.-J.	P433
KOBILKA	B. K.	P411
KOCH	G.	LE026
KOCHER	J.	LE092
KOCIĆ	G.	P253
KOCJAN	D.	P353
KŐHIDAI	L.	P015
KOHL	F.	P136
KOIZUMI	F.	P388
KOKKONEN	P.	P010
KOLACZKOWSKI	M.	P164
KOLÁŘ	M.	P352
KOLARIČ	A.	P204
KOLB	P.	P108
KOLENC PEITL	P.	P326
KOMAROV	I.	P094
KONC	J.	P321, P152, P155, P375
KONDEJ	M.	P034
KONDRATOV	I.	P094
KONG	W.	P087
KONOPKA	K.	P199
KONRÁDOVÁ	D.	P113
KONSTANTINIDOU	M.	P071
KOO	S. J.	P407
KOOISTRA	A.	P315
KOOLMEISTER	T.	P332
KOPCIAL	M.	P145
KORABECNY	J.	P413, P389
KORALEWSKI	R.	P209, P211
KOS	J.	P338, P396
KOSALEC	I.	P403

KÖSE	M.	P398
KOSKINEN	P.	P263
KOSSENJANS	M.	P064
KOSSNER	M.	P261
KOSTENIS	E.	P072
KOTAR	A.	P245
KOTELYANSKY	V.	P384
KOTLINSKA	J. H.	P167
KOUROUNAKIS	A.	P227
KOUROUNAKIS	P. N.	P212
KOURTI	M.	P376
KOVACS	L.	P073
KOVAL	V.	P203
KOWALCZYK	P.	P031
KOWALSKA	J.	P138, P259, P145, P145bis, P146, P146bis
KOWALSKI	M.	P209, P211
KOZARSKI	M.	P138
KOZLIKOVA	B.	P010
KOZLOVSKA	T.	P176
KRAEMER	S. D.	P148
KRAINC	D.	LE085
KRÄMER	L.	P329
KRASAVIN	M. Y.	P080
KRAUS	F.	P108
KRAUSS	S.	P350, LE006
KREJČÍ	L.	P281
KREMPSER	E.	P356
KRISTENSEN	O.	P074
KRÓL	M.	P313
KRÖNKE	J.	P234
KROŠELJ	M.	P326
KROTH	H.	LE092
KRSTULOVIĆ	L.	P035
KRYŠTOF	V.	P365, P275, P280, P361
KRZYKAWSKA-SERDA	M.	P085, P243
KUBACKA	D.	P138
KUBBUTAT	M.	P268
KUBOVČIAK	J.	P352
KUCA	K.	P413
KUDER	K.	P320
KUFAREVA	I.	P434
KÜHNE	R.	P238
KUIPER	B. J.	P011
KUJAWA	M.	P031

KUKKONEN	J. P.	P381
KULATHILA	R.	P359
KUMAR	A. V.	P200
KUNIG	V.	P101
KÜPPERS	J.	P072
KUROCHKIN	N.	P139
KUROPKA	B.	P407
KUTLIK	R.	P357
KWIECIŃSKA	K.	P031
KWON	Y.	P378
KYSIL	V.	P114
LA MOTTA	C.	P206
LA SALA	G.	P437
LABER	B.	LE017
LABONTE	L.	P283
LACIVITA	E.	P207
LADD	R.	P422
LAHAV	R.	P200
LAHIANI	A.	P115
LAHTELA-KAKKONEN	M.	P347
LAM	B. V.	P151
LAMB	M. L.	LE096
LAMBERT	A.	P283
LAMBERTH	C.	LE019
LAMERS	C.	P208
LAMOUREUX	A.	LE039
LANCELIN	J.-M.	P082
LANDRIEU	I.	P049
LANG	H.	P340
LÁNG	O.	P015
LANGE	G.	LE017
LANIŠNIK RIŽNER	T.	P021
LANKRI	D.	P115
LAPIN	N. A.	P243
LAPLANTE	S.	P062
LAQUERRE	S.	LE078
LARSSON	N. H.	LE031
LATACZ	G.	P320
LATEGANH	J.	LE061
LAU	J.	LE099
LAUFER	R.	P182
LAUFER	S.	LE061
LAVREYSEN	H.	LE048
LAVROV	M. I.	LE088
LAWSON	E. C.	LE078
LAZAROVICI*	P.	P115

LE	T.	P180
LEACH	A.	LE004, P027
LEAHY	D.	LE001
LECROQ	W.	P096
LEDIEU	D.	LE075
LEE	B. H.	P036
LEE	C.-S.	P426
LEE	H.	P364
LEE	J.	P426
LEE	J.	P379
LEE	J. K.	P103, P120
LEE	J. T.	P372
LEE	J. Y.	P120, P110
LEE	K. W.	P380
LEE	K.-T.	P120, P110
LEE	M.-L.	LE094
LEE	O.	P433
LEE	S.	P116
LEE	S.-Y.	P140
LEE	T.	P374
LEE	Y. S.	P364, P380
LEE	E.-S.	P378
LEENDERS	R.	P350, LE006
LEES	E.	P283
LEHMANN	J.	P161
LEHTIÖ	L.	P350, LE006
LEHTONEN	S.	P014
LEINO	T. O.	P381, P179
LEITĀNS	J.	P104
LEITAO	A.	P174
LEITE	D. I.	P017
LEIVA	R.	P335
LEMOINE	H.	P082
LEMPEREUR	V.	LE017
LEMPINEN	A.	P296
LEMURELL	M.	LE031
LENSELINK	E. B.	P011
LENTINI SANTO	D.	P049
LENZ	E.	P084
LEONARDO SOUSA	C.	P003
LEONETTI	F.	P334
LEOPOLDO	M.	P207
LEPŠÍK	M.	P252, P275
LEŚ	M.	P031
LESNIK	S.	P321
LEŠNIK	S.	P152, P155, P375

LETICIA	M.	P063
LEURS	R.	P187, P195, P315
LEUTHNER	B.	P082
LEVENETS	O.	P313
LEVER	S.	P273
LEVTEROV	V.	P048
LEVY	A.	P262
LEWIS	S.	P262
LEYSSEN	P.	P139
LI	H.	P372
LI	J.-S.	P433
LI	L.	P436
LI	R.	P051
LI	Y.	LE101
LIM	C. J.	P036
LIN	D. W.	LE064
LIN	T. H.	LE064
LIN	W.	P329
LINDBERG	E.	P288
LINDEMANN	R.	P031
LINDNER	S.	P234
LINDSAY	N.	P270
LINES	G.	LE065
LIOUX	T.	LE039
LISZKA	K.	P323
LITKA	M.	P323
LITTLEWOOD-EVANS	A.	LE026
LIU	B.	LE084
LIU	C.-M.	P433
LIU	H.	P411
LIU	S.	P051
LIU	X.	P411
LIU	Z.	P262
LLINARES	J. M.	P351
LO	M. M.	LE101
LOCARNO	S.	P382
LODOLA	A.	P287
LOESCHE	A.	P383
LOETSCHER	E.	LE075
LOLKJE	S.	P063
LOLLI	M. L.	P268
LÓPEZ DE CERAIN	A.	P170
LORENZI	M.	LE078
LŐRINCZ	Z.	P198
LOUREIRO	J.	P046
LOUVEL	J.	P434

LOVRIĆ	J.	P345
LOWERSON	A.	P419, P062
LOZADA	J. A.	P427
LU	A.	LE094
LU	J.	P040, P041
LU	L.	P013
LU	M.	P433
LÜCKMANN	M.	P405
LUEBBERT	M.	P300
LUMB	E.	P037
LUO	X.	P140
LUPARDUS	P.	P266
LUPINO	E.	P268
LUTHMAN	K.	P327
M. KESERŰ	G.	LE081
MACCHIARULO	A.	LE044
MACEDO BASTOS	M.	P279
MACEGONIUK	K.	P090, P092
MACHADO	D.	P193
MACHALA	Z.	P404
MACHAUER	R.	LE097
MACHULKIN	A.	P384
MACIUSZEK	M.	P031
MACKAY	A.	LE075
MACKEY	M.	P086
MACKEYEV	Y.	P243
MACLEOD	C.	LE094
MACUT	H.	P294
MADDEN	K.	P324
MADDIRALA	A.	LE012
MÄDER	P.	LE052
MAEGAWA	T.	P117
MAES	L.	P187
MAGALHAES	J.	LE020
MAGAR	P.	P365, P361
MAGARI	F.	P071
MAGIERA	K.	LE007
MAGLI	E.	P167
MAGYAR	C.	P015
MAHARDHIKA	A.	P320
MAHY	W.	LE065
MAJ	E.	P112
MAJER	P.	P252
MAJOUGA	A.	P384
MAKARA	G.	P073
MALANCONA	S.	P182

MALARZ	K.	P085
MALAWSKA	B.	P164
MALES	A.	P161
MALÍNKOVÁ	V.	P280
MALULEKA	M.	P118
MALUZYNSKA-HOFFMAN	M.	PL007
MAMO	M.	P283
MAMOT	A.	P138
MANANDHAR	S.	P357
MANCINI	F.	P282
MANDRUP B.	S.	P437
MANDRUP BERTOZZI	S.	P330
MANEL	R.	P105
MANNENS	G.	LE078
MANNER	S.	P358
MANSLEY	T.	P066, P002
MAPLE	H.	P235
MARCINKOWSKA	M.	P164
MARDER	N. M.	P338
MARGALIT	R.	P200
MARGAROLI	N.	P330
MARIA DOLORS	P.	P105
MARIN	M. J.	P312
MARIO	F. Z.	P408
MARJANOVIC	I.	P403
MARQUES	M. M.	P274
MARQUES	S.	P010
MARQUES M.	M.	P328
MARSH	G.	P235
MARTA	V.	P105
MARTARELLO	L.	LE092
MARTIN	D.	LE064
MARTIN	S.	P084
MARTÍN-ACOSTA	P.	P385
MARTINEZ	M.	P250
MARTINEZ	M. J.	P006
MARTÍNEZ	A.	P006, P247
MARTÍNEZ CAMPOS	Z.	P386
MARTINEZ CARRANZA	M.	P332
MARTÍNEZ-CARTRÓ	M.	LE024
MARTÍNEZ-CRISTÓBAL	P.	P310
MARTÍN-HIGUERAS	C.	P325
MARTINI	R.	P190, P328
MARTÍN-RODRÍGUEZ	P.	P385
MARTINS	A.	P186
MARX	A.	P082

MARZINZIK	A.	P359
MASLIAH	E.	LE034
MASSARELLI	P.	P167
MASSE	J.	P322
MASTROMARINO	M.	P207
MASUTANI	M.	P388
MATHEEUSSEN	A.	P187
MATHEW GRINER	L.	P283
MATHUR	M.	P081
MÄTLIK	K.	P340
MATOSIUK	D.	P034, P323, P341
MATRALIS	A.	P227
MATSUNO	K.	P388
MATTHES	T.	P200
MATULIS	D.	P104
MATYSZEWSKI	K.	P209, P211
MAUNY	M. A.	LE039
MAXIM	K.	P377
MAY	L.	P032
MAYER	F.	P344
MAYER	N.	LE060
MAZUR	M.	P209, P211
MAZURKIEWICZ	M.	P211
MCCARREN	P.	P062
MCCORMICK	P. J.	P334
MCGONAGLE	G.	P233
MCGOWAN	S.	P379
MCGUIGAN	C.	P149
MCGUIRE	T.	P084
MCIVER	E.	P262
MCKEEVER	U.	LE026
MCKENZIE	A.	LE094
MCLEOD	M.	P306
MCNAIR	A.	P153
MEERPOEL	L.	LE078
MEIJER	F.	P295
MELNYKOV	K.	P124
MENZ	S.	LE016
MERI	S.	P179
MERK	D.	LE083, P007
MERKL	S.	P075
MERLINO	A.	P173
MERNYÁK	E.	P021
MERRITT	H.	P283
MEXIA	N.	P158
MEZEIOVA	E.	P413, P389

MEZŐ	G.	P015
MICCO	I.	P074
MIETHING	C.	P300
MIGGLAUTSCH	A.	LE060
MIHÁLIKOVÁ	D.	P404
MIHOVILOVIC	M. D.	P344, P093
MIKHAILOV	S.	P139
MIKI	Y.	P117
MIKLES	D. C.	P252
MIKROS	E.	P192, P177
MIKULA	M.	P406
MIKULSKI	M.	P031
MILAEVA	E.	P119
MILEO	V.	P287
MILLAN	M. J.	LE045
MILLAR	H.	LE078
MILLIGAN	G.	P360
MILNE	T.	P318
MILNER	J.	P256
MILROY	L.-G.	P295
MIN	S.-J.	P103
MINOVSKI	N.	P204
MIQUEL	V.	P105
MIRABELLI	C.	P139
MIRGUET	O.	P005
MISTRY	N.	P262
MISTRY	S. N.	P399
MITRA	K.	LE101
MITROVIC	A.	P396
MITTAG	D.	P271
MLINARIC	Z.	P403
MLINARIČ RAŠČAN	I.	LE081
MLINARIC-RASCAN	I.	P216
MOCKING	T.	P315
MOFFAT	D.	P153
MOGEMARK	M.	P273
MOGLIE	Y.	P428
MOHAMMED	I.	P357
MOLA SOLA	L.	P318, P324
MOLENVELD	P.	P210
MONECKE	P.	LE100
MONJAS	L.	P128
MONNEE	M.	P210
MONS	E.	P038
MONTAGNOLI	A.	P080
MONTAGUE	S.	LE004, P027

MONTANARI	C. A.	P174
MONTEAGUDO	E.	P182
MONTEL	F.	LE063
MONTGOMERY	A.	LE080, P137
MOON	Y.	P120
MOR	M.	P287
MORAES	C.	P174
MORALES	P.	P391
MORAVCSIK	E.	P169
MOREIRA	R.	P026, P184
MOREIRA	V.	P358, P340
MORGILLO	C. M.	P082
MORICE	C.	P367, P268
MORISSEAU	C.	P335
MOROZ	Y.	P070, P083, P095
MOSHARI	M.	P112
MOSRIN	M.	LE017
MOULIN	P.	LE075
MOWBRAY	S. L.	P013
MOYA-GARZÓN	M. D.	P325
MOZZARELLI	A.	LE020
MPAMHANGA	C.	P262
MPHAHLELE	J.	P118
MRAVLJAK	J.	P245
MU	L.	P148
MUCHA	A.	P090, P092
MUELLER	J.	P075
MUHS	A.	LE092
MULARD	L.	LE011
MULLER	C.	P390
MÜLLER	C.	P320
MÜLLER	C. E.	P140, P154, P398, AL001
MÜLLER	S.	LE050
MÜLLER HERDE	A.	P148
MUNCK AF ROSENSCHÖLD	M.	P273
MUNIER	C.	P246
MUSAELYAN	K.	P324
MUSILEK	K.	P413, P342
MUSIOŁ	R.	P085
MYERS	S.	LE098
MYKHAILIUK	P.	P076, P048
MYKHAILIUK	P.	P077, P078, P088, P047
MYRIANTHOPOULOS	V.	P192, P177
NAAMS	J.-B.	P014
NAGALINGAM	G.	P171

NAITO	M.	LE021
NAKAMURA	A.	P117
NAM	J.	P426
NANNINI	M.	LE094
NARDI	V.	P182
NARUKULLA	R.	LE094
NATALIA	V.	P377
NAUTIYAL	M.	P142
NAVARRO	M.	P184
NAZARÉ	M.	P350, LE006
NAZAROVA	A. A.	LE088
NEGAHBAN	M.	P392
NEGRONI	J.	LE017
NELSON	A.	AL002
NEMEC	S.	P188, P183
NEOCHORITIS	C.	LE007, P064
NEPOVIMOVA	E.	P413, P389
NERI	D.	P249, P239
NEUBAUER	T.	P306
NEUMANN	T.	P390, P169
NEVADO	C.	P039, PL007
NEVES	J.	P049
NEVES CARVALHO	A.	P003
NEWMAN	M.	P262
NEWTON	G.	P153
NEWTON	J.	P085
NEYTS	J.	P139
NG	L. F.	LE070
NGUYEN	A.	LE094
NGUYEN	A.	P032
NGUYEN	M. T.	P252
NGUYEN	N.	P180
NGUYEN	N. T. A.	P127
NIECZYPOR	P.	P350, LE006
NIEDZIEJKO	P.	P031
NIEDZIEJKO	P.	P209, P211
NIELLO	M.	P344
NIETO-ACOA	D. A.	P170
NIKOLAY	Z.	P377
NIKOLIC	K.	P346, P347, P215
NISHIGUCHI	G.	P283
NISSINK	W.	P264, P028
NITEK	W.	P368
NIV	M. Y.	P333
NOGATA	M.	P117
NOGUEDA-TORRES	B.	P185

NOH	Y.	P374
NOISIER	A.	P079
NONATO	M. C.	P017
NONG	Y.	P436
NORBERG	M.	P273
NORLEY	M.	P235
NORTON	R.	P061
NOSIK	P.	P122
NOVAK	D.	P326
NOVAK	P.	P314
NOVIČ	M.	P189
NOVINC	M.	P420
NOVINEC	M.	P397, P414, P354
NOWAK	M.	P031
NOWICKA	A.	P146, P146bis
NOZAL-GARCIA	V.	P247
NTATSOPOULOS	V.	P090
NYERGES	Á.	P429, LE051, P349
O'CONNELL	N.	LE096
OBARA	A.	P313, P031
OBERHAUSER	B.	LE075
OBNISKA	J.	P393
OBŠIL	T.	P049
OFFRINGA	J.	P011
OH	K.-S.	P036
OH	W.-K.	P206
OKAWARA	T.	P388
OLANDERS	G.	P013
OLCZAK	J.	P209, P211
OLECHNO	J.	P064
OLEJARZ-MACIEJ	A.	P320
OLEJKO	T.	P146bis
OLEJNICZAK	S.	P209, P211
OLGAÇ	A.	P201
OLIOSI	B.	P287
OLIVERA-CASTILLO	L.	P213
OMBRATO	R.	P282
ONODERA	T.	P388
ONTORIA	J. M.	P182
OPAČAK-BERNARDI	T.	P035
ORR	D.	P147
ORRLING	K. M.	P187
ORSINI	P.	P080
ORTEGA	J. A.	P437
ORTHOLAND	J.-Y.	P407

ORTIZ ZACARIAS	N. V.	P434
ORZELSKA-GORKA	J.	P167
OSBORNE	J.	P262
OSLOVSKY	V.	P139
OSMULSKI	P.	P292
OTTL	J.	P359
OTTMANN	C.	P246, P049, LE032
OTTONELLO	G.	P330
OUDE ALINK	P.	P065
OVAA	H.	P038
OVERKLEEFT	H. S.	LE049
OWEN	D.	LE064
OZAWA	S.-I.	P394
ÖZEL	A.	P409
OZKAY	Y.	P241
P. S. JUNIOR	F.	P279
PABÓN	A.	P170
PACANOWSKA	D. G.	P184
PACHL	P.	P252
PACKER	M.	P309
PADILLA	A. M.	P147
PÁEZ	J. A.	P006
PAGONI	A.	P091, P092
PAJK	S.	P188, P245, P183
PAL	S.	P284
PÁL	C.	P429, LE051, P349
PALA	D.	P287
PALLÀS	M.	P335
PALOMO	V.	P247
PALOP	J. A.	P043
PALYULIN	V. A.	LE088
PAMPÍN CASAL	B.	P322
PANDE	V.	LE078
PANEK	D.	P164
PANG	J.	LE094
PANG	L.	P142
PAONESSA	G.	P182
PAPAGIOUVANNIS	G.	P212, P168
PAPEO	G.	P080
PAPKE	R. L.	P435
PARADE	M.	LE078
PARDALI	K.	P273
PARDALI	V.	P192, P177
PARIS	G.	P359
PARK	E.	P121, P131

PARK	H.-G.	P121, P131
PARK	J.	P426
PARK	K. D.	P380
PARUCH	K.	P102, P107
PARUCH*	K.	P281
PASCANU	V.	P039, PL007
PASS	M.	P264
PASSERI	D.	LE044
PATEL	J.	P327
PATERSON	D.	LE092
PATRZAŁEK	D.	P431
PAUK	K.	P361
PAVIĆ	K.	P395
PAWLIK	H.	P031
PAWŁOWSKI	M.	P220
PAZ-GONZALEZ	A. D.	P185
PAZOS	R.	P391
PEACE	S.	P284
PEAK	I.	P230
PEASE	J. E.	P270
PECAR FONOVIC	U.	P396
PEDEMONTE	N.	P330
PELLECCHIA	M.	P029, P030
PELLEGRINI	C.	P206
PELLEGRINO	S.	P294
PELLICCIARI	R.	LE044, LE057
PENNA	I.	P330, P166
PENNICOTT	L.	P191
PEPERIDOU	K.	P248
PERDIH	A.	P343
PÉREZ	C.	P247
PÉREZ-LÓPEZ	A. M.	P331
PÉREZ-TERNERO	C.	LE098
PERISSUTTI	E.	P167
PERKINS	K.	P256
PERKOVIĆ	I.	P395
PERNOMIAN	L.	P218
PEROUZEL	E.	LE039
PERRON	Q.	P005
PERRY	M.	P153
PERRY	M.	P246
PESCE	E.	P330
PETEK	N.	P397, P354
PETERLIN MAŠIČ	L.	P429, P349
PETERSEN	L.	P074
PETKOVIC	M.	P346

PETZOLD	G.	LE023
PEYRARD	S.	LE017
PFEIFER	A.	LE092
PHIPPS	R.	P084
PIAIA	A.	LE075
PIANTANIDA	I.	P251
PICCININI	M.	P268
PICKERING	A. M.	P292
PIERONI	M.	P193, LE020
PIETERS	R.	P425
PIKE	K.	LE096, P264
PILLAIYAR	T.	P398
PINHEIRO	L.	P022
PINKNER	J.	LE012
PINTAR	S.	P189
PINTO	G.	P010
PIOTROWICZ	M.	P209, P211
PIPA	G.	P400
PIPPIONE	A. C.	P268
PISK	J.	P345
PIŠLAR	A.	P338, P396
PISMATARO	M. C.	P435
PITCHER	N. P.	P436
PITON	N.	P153
PITUCHA	M.	P034
PLANO	D.	P040, P041, P042, P043, P044
PLOTNIKOVA	E.	P384
PLUSCHKE	G.	LE052
PLUTA	E.	P209, P211
POCIECHA	K.	P220
POCZE	G.	P073
POCZKAJ	A.	P313
PODLEWSKA	S.	P368
POKROVSKY	A.	P203
POLYAKOV	V.	P283
PONZI	S.	P182
PONZONI	I.	P006
PORTER	D.	P359
POSPÍŠIL	J.	P113
POSTERI	H.	P080
POSTNIKOV	P.	P424
POTOWSKI	M.	P101, LE031
POULIOT	M.	LE019
POUTON	C.	P302
POŽGAN	F.	P397

PRATI	F.	P282
PREDTECHENSKAYA	E.	P203
PREIBBENOW	D.	P171
PRESTON	S.	P180
PREUSSE	C.	LE016
PRICE	D.	LE034
PRIDMORE	S.	P235
PRINČIČ	G.	P081
PRIŠČÁKOVÁ	P.	P352, P404, P402
PROBST	K.	P149
PROIA	T.	LE096
PROJ	M.	P321, P155
PROVENZANI	R.	P296
PRUDÊNCIO	M.	P403, P178
PSYNIAK	K.	P406
PU	S.-Y.	P285
PUETTER	V.	P407
PUGH	K.	P256
PUIGORIOL-ILLAMOLA	D.	P335
PUJOL	E.	P335
PULZ	R.	LE026
PURKEY	H.	LE094
PUROHIT	A.	LE092
QUARANTA	L.	LE019
QUATTRINI	L.	P206
QUILIANO	M.	P170
QUINN	M. T.	P207
RADCHENKO	D.	P094
RADCHENKO	E. V.	LE088
RADOVANOVIĆ	V.	P314
RAINVILLE	P.	P421
RAJIC	Z.	P403
RAJIĆ DŽOLIĆ	Z.	P395
RAMACHANDRA	M.	LE005
RAMILO-GOMES	F.	P328
RAMIS	G.	P351
RAMISETTI	S. R.	P041
RAMOTOWSKA	E.	P406
RAMPP	H.	P411
RAMURTHY	S.	P283
RAO	S.	P191
RAO ANUMALA	U.	P350, LE006
RAPACZ	A.	P393
RATHBONE	D.	P127
RATNI	H.	LE025
RAUH	D.	LE061

RAWLINS	P.	P270
RAYNER	C. M.	P158
RAZO HERNÁNDEZ	R. S.	P386
REDDAVIDE	F.	P329
REDHABER	D.	P300
REDONDO-GALLEGO	L.	P391
REGAZZONI	L. G.	P294
REGGIANI	A.	P282
REIS	F.	P046
REISS	D.	P340
REKKA	E.	P228, P212, P168
REMPEL	V.	P320
RENN	C.	P154
REPISKÁ	V.	P352, P404, P402
REQUENA	C.	P006
RESSURREIÇÃO	A. S.	P178, P184
REVESZ	L.	LE026
RÉVÉSZ	T.	LE051, P349
REXEN ULVEN	E.	P405
REY	J.	LE017
REYES-ARELLANO	A.	P185
REZEKIN	I.	P114
REZENDE	M. C. S.	P017
REŽNÁKOVÁ	S.	P352, P402
REZNICKOVA	E.	P275
ŘEZNÍČKOVÁ	E.	P280, P361
RHEINHEIMER	J.	LE018
RHENIUS	M.	P306
RIBEIRO	J.	P174
RICCARDI-SIRTORI	F.	P080
RICHELLE	G.	P129
RICKLIN	D.	P208
RICO	A.	P283
RIERA	A.	P310
RIGANTI	C.	P334
RIGOLLIER	P.	LE084
RILEY	T. V.	P436
RIMPELOVÁ	S.	P352
RINNE	M.	P340
RISSEEUW	M.	P175
RITCHIE	T.	P355
RIVARA	S.	P287
RIVERA	G.	P185
RIXEN	S.	P406
RIZZI	A.	P287

ROBERS	M. B.	LE016
ROBERTS	K.	P270
ROCA	C.	P006, P247
ROCHE	D.	P407, P082
ROCHET	J.-C.	P169
RODESCHINI	V.	P407, P082
RODRIGUEZ	R.	LE050
RODRÍGUEZ-GIMENO	A.	P330
ROECKLIN	D.	P390
ROGACHEV	A.	P203
ROGERIO	K.	P022
ROJAS	A.	P005
ROLF	M.	P063
ROMAUCH	M.	LE060
RONIN	C.	P390
ROONEY	T.	P162
ROSENBROCK**	H.	LE028
ROUND	P.	P258
ROWLEY	J.	P256
ROZMAN	D.	P353
ROŽMAN	K.	P188, P183
RÚA RODRÍGUEZ	M. L.	P322
RUAN	B.	P180
RUBČIĆ	M.	P345
RUBERTE	A. C.	P044
RUBIO-RUIZ	B.	P331
RÜBSAMEN-SCHAEFF	H.	PL006
RUČILOVÁ	V.	P220
RUF	M.-T.	LE052
RUF	S.	LE100
RUFFONI	A.	P310
RUIJTENBEEK	R.	P425
RUSKOAHO	H.	P319
RUSSELL	A.	P318, P256, P324
RUSSELL	D.	P312, P023
RUSSO	D.	P330, P166
RUZA	R.	LE065
RUZIC	D.	P346
RYABUKHIN	S.	P122, P083, P095, P123, P124
RYU	J.-S.	P111, P116, P125
RYU	K. D.	P110
RYZSHOVA	E.	P114
RZYMSKI	T.	P313, P031
SAARBACH	J.	P288
SABINIARZ	A.	P031

SACCONE	I.	P167
SACHDEVA	S.	P085
SADEK	P.	P187
SAGLIK	B. N.	P241
ŠAGUD	I.	P126
SAIAH	E.	LE064
SAINAS	S.	P268
SAKAMOTO	T.	P144
SALAMON	M.	P209, P211
SALEM	A.	P029, P030
SALIDO-RUIZ	E.	P325
SALINAS	P.	LE065
SALOME	C.	P132, P096, P432
SALPHATI	L.	LE094
SALVADOR	J. A. R.	LE008, P387, P003
SALVATELLA	X.	P310
SAMADDER	P.	P281
SAMAIN	F.	P249, P239
SAMPATH	D.	LE094
SAN MARTIN	M.	LE096
SANDOVAL-RAMÍREZ	J.	P213
SANDSTRÖM	A.	P045
SANFELIU	C.	P335
SANMARTIN	C.	P040, P041, P042, P043, P044
SANNINO	A.	P249
SANSILVESTRI	P.	P005
SANTAGADA	V.	P167, LE072
SANTAMARIA	J.	P331
SANTANA-ROMO	F.	P428
SANTOS	A. E.	P387
SANTOS	M. M. M.	P046
SANTOS	W.	LE076
SARAÇ	S.	P410
SARAIVA	L.	P046, P026
SARDA	S.	P084
SARDELLA	R.	LE044
SARI	S.	P409, P410
SASAKI	Y.	P388
SAVARDI	A.	P437
SAVCHENKO	T.	P088, P047, P048
SAVCHIUK	T.	P088
SAVIĆ	J.	P215
SAVIJOKI	K.	P327
SAWADA	T.	P388

SCAFFIDI	S.	P250, LE024
SCAMMELLS	P.	P032, P293, P302, P037, P399, P379
SCHAAKE	B.	P411
SCHAARSCHMIDT	D.	P340
SCHAEFER	M.	P359
SCHÄNZLE***	G.	LE028
SCHEEPSTRA	M.	P065
SCHEFFLER	F.	P084
SCHEMBRI	L. S.	P379
SCHEPENS	W.	LE078
SCHEPETKIN	I. A.	P207
SCHERR	N.	LE052
SCHEUERMANN	J.	P239
SCHEUFLER	C.	LE026
SCHIBLI	R.	P148
SCHIELE	F.	LE016
SCHIFFRER	E. S.	P216
SCHIO	L.	LE062
SCHIRMEISTER	T.	P371
SCHMALZ	H.-G.	P238
SCHMIDT	M.	P342
SCHMIDT	S.	LE094
SCHMIDT	W.	P357
SCHMIES	C.	P140
SCHMITT	M.	P132, P096
SCHMOLL	D.	LE100
SCHNEIDER	G.	P007
SCHNEIDER	J.	P406
SCHNITZER	R.	LE063
SCHOEDER	C.	P320
SCHOLS	D.	P403
SCHOU	M.	LE090
SCHROEDER	S.	P187
SCHUBART	A.	LE075
SCHUELE	R.	P300
SCHUHMACHER	L.	LE065
SCHÜMANN	J.	LE075
SCHÜRER	S. C.	P277
SCHUTT	L.	LE094
SCHWARTZ	T. W.	P405
SCHWARZ	D.	P082
SCHWEIGER	M.	LE060
SCOLARO	A.	P080
SCUDIERI	P.	P330
SEARCEY	M.	P100, P312, P023

SEBASTIAN	V.	P331
SEBASTIÁN PÉREZ	V.	P006
SEDENKOVA	K. N.	LE088
SEEBERGER	P. H.	PL003
SEGALL	M.	P066, P002
SEGLER	M.	LE003
SEIFERT	T.	P327
SELLERS	W. R.	P283
SELWOOD	D.	LE098
SENADI	G. C.	P130
SENDEROWITZ	H.	P200
SERDA	M.	P085, P243
SEREDENINA	T.	LE092
SERRA	P.	P186
SERRANO-WU	M.	P062
SERSENOVÁ	D.	P404
SETHURAMAN	V.	P283
SETTI	L.	P283
SEVERINO	B.	P167
SEVIN	G.	P363
SEXTON	P.	P032
SHAABANI	S.	LE007, P064
SHAH	N.	P256
SHANU-WILSON	J.	P084
SHAO	W.	LE096, P283
SHARMA	A. K.	P040, P041, P042
SHARMA	R.	LE064
SHEN	H.	LE095
SHEN	Z.	P051
SHEPHERD	E.	P324
SHILOVA	O.	P114
SHIMOYAMA	T.	P388
SHOICHET	B. K.	P411
SHOOK	B.	LE078
SHPAKOVSKY	D.	P119
SHRESTHA	A.	P378
SHRESTHA	R.	P378
SHUTO	S.	LE079
SHYAMLAL	B. R. K.	P081
SIBGATULIN	D.	P124
SICILIANO DE ARAUJO	J.	P187
SIDERIUS	M.	P187
SIEBERT	D. C.	P093
SIEPRAWSKA-LUPA	M.	P031
SIEVERS	Q.	LE023
SIJM	M.	P187

SIKORSKI	P. J.	P146
SILVA	A. G.	P034
SILVA	M.	P174
SILVA	M. M. C.	P387
SILVEIRA	F.	P022
SILVERMAN	R. B.	LE085
SIMEONE	X.	P093
ŠIMKOVÁ	T.	P434
SIMPSON	I.	P270
SINDIKUBWABO	F.	LE050
SINGH	M.	P283
SINGH	S.	P230
SĪPOLA	A.	P176
SIPOSOVA	K.	P413
SIPTHORP	J.	LE065, P060
SITARZ	K.	P313
SITTE	H. H.	P344
SIVARAMAN	K. K.	P379
ŠKERLE	J.	P252
SKLEPKIEWICZ	P.	P209
SKOGMAN	M.	P358, P327
SKOK	Ž.	P349, P412
SKONE	P.	P153
ŠKORIĆ	I.	P126
ŠKRLJ	B.	P375
SKROPETA	D.	LE080, P137
SKVORTSOV	D.	P384
SLATER	M.	P086
SLEDZ	P.	PL007
SLØK	F.	P074
SMAGIN	G.	P169
ŠMELCEROVIĆ	A.	P253
ŠMIDLEHNER	T.	P251
SMIT	M.	P195, P315
SMITH	A.	P283
SMITH	H.	P357
SMITH	P.	LE084
SMITH	T. K.	P342
SMITS	Y.	P065
SOARES	J.	P046
SOBRAL	L.	P328
SOEBERDT	M.	P210
ŞÖHRETOĞLU	D.	P409
SONDO	E.	P330
SONG	K.	LE094
SORANA	F.	P330

SORIANO	C.	P351
SORRELL	F.	P276
SOSIC	A.	LE072, P136
SOSIČ	I.	P188, P216, P183, LE081
SOUČEK	K.	P281
SOUKUP	O.	P413, P389
SOURAL	M.	P220
SOUSA	E. P.	P399
SOVA	M.	P321, P338, P152, P155
SOWA	S.	P350, LE006
SOWIŃSKA	M.	P313, P031
SPANÒ	R.	P330
SPILIOTOPOULOS	D.	PL007
SPILOVSKA	K.	P413, P389
SPINELLI	F.	P334
SPRING	D. R.	P028
SPUNDE	K.	P176
SPYRIDON	B.	P063
SQUIRE	S. E.	P256
SRINIVAS	H.	LE075
STABEN	S.	LE094
STACHOWICZ	A.	P031
STAEDEL	C.	LE066
STAMMLER	H.-G.	P301
STANCHEV	S.	P252
STÄNDER	S.	P210
STARČEVIĆ	K.	P035
STAREK	B.	P259
STARKL RENAR	K.	P081
STEADMAN	D.	LE065
STECHMANN	B.	P237
STEELE	J.	P084
STEFANACHI	A.	P334
ŠTEFANE	B.	P397
STEFANIAK	F.	P211
STEFFEN	A.	LE016
STEINEBACH	C.	P234
STEINSOULT	P.	P132
STENMARK	P.	P332
STERK	G. J.	P187, P210
ŠTERMAN	A.	P188, P183
STEWARD	O.	P264
ST-GALLAY	S.	P270
STINEAR	T. P.	P436
STOCKIGT	D.	P407

STOCKS	M.	P037
STOEHR	J.	LE092
STOJAN	J.	P396
STOKES	C.	P435
STORCKEN	R.	P210
ŠTRANCAR	J.	P245
STRAŠEK	N.	P414
STRELKOV	S.	P142
STŘÍŠOVSKÝ	K.	P252
STRZELECKA	D.	P145, P145bis
STUART	D. D.	P283
SUBOTA	A.	P123
SUBRAMANIAN	S.	P283
SUCHÁNKOVÁ	T.	P281
SUH	J.	P286
SUJATHA-BHASKAR	S.	P266
SULZER	S.	LE019
SUMMA	M.	P330, P437
SUMMA	V.	P182
SUMMERS	D.	P284
SUN	L.	P139
SUN	S.	P195
SUN	S.-Y.	LE101
SUN	W.	LE078
SUNAHARA	R. K.	P411
SUNG JIN	C.	P373
SUPURAN	C.	P248
SUTANTO	F.	P071, P415
ŠVAJGER	U.	P152, P204, P245
ŠVEHLOVÁ	K.	P252
SVETE	J.	LE043, P397, P420, P414, P354
SVIRIDOVA	O.	P114
SWALLOW	S.	P270
SWAMI	A. K.	P081
ŚWIERCZEK	A.	P220
SZABO	R.	P137
SZABO	R.	LE080
SZALAJ	N.	P013
SZELE	F.	P324
SZEREMETA-SPISAK	J.	P313, P031
SZILÁGYI	K.	P198
SZILI	P.	LE051
SZILLAT	H.	LE100
SZULC	E.	P310
SZYMANSKA	E.	P416

TAE	J. S.	P103
TAFT	B. A.	P283
TAJMOUATI	H.	P005
TAKAMURA	T.	P388
TALBOT	E.	P087
TALMAN	V.	P296, P319
TAMEZ	V.	P283
TAMMELA	P.	P429, P349
TANDARIC	T.	P417
TANNER	H.	P283
TARANTINO	D.	P294
TARLETON	R. L.	P147
TĀRS	K.	P104
TART	J.	P028
TARVAINEN	I.	P296
TASCI	H.	P241
TAŞÇI	H.	P219
TASKE	A.	P306
TAVEIRA	N.	P186
TAYLOR	D.	P262
TAYLOR	M. C.	P192
TEBBUTT	C.	P153
TEDESCO	G.	P086
TELLEW	J. E.	P283
TEMNYAKOVA	N. S.	LE088
TER LAAK	A.	P407
TERRETT	N.	LE030
THAL	D.	P032
THALER	T.	P306
THAVAM	S.	LE031
THEODOSIS-NOBELOS	P.	P228, P212, P168
THEUNISSEN	E.	P434
THOMA	N.	LE023
THOMAS	A. P.	P028
THOMPSON	M.	P329
THOMPSON	P.	P061
THOMSEN	M. K.	LE040
THOMSON	R. J.	P400
THROUP	A.	P332
THURING	J. W.	LE078
TIBAUT	T.	P189
TICHÁ	A.	P252
TICHÝ	M.	P141
TIEBES	J.	LE017
TIMMERMAN	P.	P050, P129
TIRABY	M.	LE039

TITZ	A.	PR002
TIWARI	M. K.	P081
TKACHENKO	S.	P114
TODD	M.	LE069
TOLNAI	F.	P169
TOMÁS	A. M.	P184
TOMAŠIČ	T.	P429, P190, P204, P349, P425, P326
TOMAŠKOVIĆ	L.	P217
TOMATI	V.	P330
TOMCZYK	M. M.	P031
TOMOVIĆ	K.	P253
TONGE	M.	P270
TONGIANI	S.	P282
TORROBA	M. B.	P324
TÓTH	G.	LE035, P169
TOYAMA	H.	P067
TOZKOPARAN	B.	P241, P219
TRABANCO	A. A.	LE048
TRAN	Q. T.	LE070
TRAN	T. P. A.	LE066
TRAUELSEN	M.	P405
TRICCAS	J.	P171
TROJAN	E.	P207
TRONTELJ	J.	P338, P396
TROTTER	B. W.	LE077
TROXLER	T.	LE075
TSAGRIS	D.	P262
TSAI	Y.-J.	P433
TSCHAMMER	N.	P204
TSCHAMMER	N.	P254
TSIGELNY	I.	LE034
TSVELIKHOVSKY*	D.	P115
TU	C.-M.	P433
TULLY	D.	LE058
TUMBRINK	H.	LE061
TUNCEL	S. T.	P242
TUOMINEN	R. K.	P296
TURK	B.	P038
TURK	D.	P189
TURKU	A.	P381, P296, P179
TURNER	S. C.	LE086
TUSZYNSKI	J. A.	P112
TWIGG	D.	P089
TWYMAN	R.	LE048
TYL-BIELICKA	A.	P406

TZALIS	D.	P049
TZIONA	P.	P228, P212
TZVETKOV	N. T.	P301, P419, P020
UDOMPHOLKUL	P.	P029, P030
UHRIG	M. L.	P185
ULTSCH	M.	LE094
ULVEN	T.	P360
UNCITI-BROCETA	A.	P331
ÜNVER	M. Y.	P071
UNZUE	A.	PL007
URBANČIČ	I.	P245
VADIM	F.	P377
VAH	L.	P420
VAHERMO	M.	P358
VAJDOS	F.	LE064
VALADE	A.	LE087
VALANT	C.	P293
VALENTI	D.	P049
VALENTINA	S.	P194
VALERIE	G.	P240
VALEUR	E.	LE031, LE029, P079
VÄLIMÄKI	M.	P319
VALLIN	K. S.	P332
VAN AERSCHOT	A.	P142
VAN BEELEN	E. S.	P421, P422, P423
VAN BOECKEL	S.	P038
VAN CALENBERGH	S.	P202, P175
VAN DE GEVEL	I.	P255
VAN DE KAR	B.	P271
VAN DE MEER	T.	P187
VAN DEN BERGH	T.	P065
VAN DER ES	D.	P011
VAN DER EYCKEN	J.	P202
VAN DER GRAAF	P. H.	LE015
VAN DER VLAG	R.	P128
VAN DER WESTHUIZEN	E.	P293
VAN DOODEWAERD	B.	P038
VAN HEERDEN	E.	LE078
VAN HOORN	W.	LE002
VAN MAARSEVEEN	J.	P129
VAN OOSTERWIJK	N.	LE007
VAN PETEGEM	F.	P258
VAN VELDHOVEN	J. P.	P011
VAN-ABBEMA	A.	P283
VARNES	J.	LE096

VASBINDER	M. M.	LE096
VASILENKO	D. A.	LE088
VASSILAKI	N.	P192, P177
VASSILIOU	S.	P090, P091, P092
VASTA	J. D.	LE016
VAVRA	O.	P010
VAZIRI	S.	P283
VÁZQUEZ	S.	P335
VECCHIA	L.	LE065
VECCHIO	E.	P032
VEGA-ALANIS	B. A.	P093
VÉGH	D.	P352
VELCICKY	J.	LE084
VENKEN	K.	P202
VEREMEEVA	P. N.	LE088
VERHELST	S. H. L.	P252, P236
VERHULST	T.	LE078
VERKAIK	S.	P271
VERNEJOUL	F.	LE039
VESELOVSKÁ	L.	P143
VETTORAZZI	A.	P170
VIANELLO	R.	P417
VIDILASERIS	K.	P179
VIELLEVOYE	M.	LE078
VĪGANTE	B.	P176
VILLALBA	F.	LE017
VILLALONGA	P.	P351
VILLALONGA-PLANELLS	R.	P351
VINCENT	F.	LE064
VINCENT	J.	LE065
VINCENT	S. G.	P411
VINH	N. B.	P379
VINKEN	P.	LE078
VINKLAROVA	L.	P342
VINOTHKUMAR	K. R.	P252
VISCHER	H.	P195, P315
VISENTINI	F.	P287
VIVAT	V.	P390
VIVEIROS	M.	P193
VIVIANI	F.	P016
VLADIMIR	N.	P377
VLAINIC	J.	P403
VLASOV	S.	P424
VO	D. D.	LE066
VOCADLO	D.	P161

VOLOCHNYUK	D.	P094, P122, P083, P095, P123, P124
VON ITZSTEIN	M.	P400
VON NUSSBAUM	F.	LE017
VRDOLJAK	V.	P345
VULPETTI	A.	LE075, LE026
VUORELA	P.	P358
VUORINEN	A.	P256
VYAS	P.	P318
VYBORNYI	M.	P088
WAALER	J.	P350, LE006
WAELCHLI	R.	LE026
WAGGER	J.	P420, P414
WAGNER	P.	P132, P096
WÄHÄLÄ	K.	P258
WÄHÄLÄ	K.	P014
WALCZAK	S.	P146bis
WALDMANN	H.	LE031
WALLÉN	E. A. A.	P381
WAN	L.	P283
WANDALL	H. H.	LE010
WANG	H.-C.	P433
WANG	J.	LE096
WANG	J.	P202
WANG	J.-J.	P130
WANG	X.	P039, PL007
WANG	Y.	P283
WANG	Y.-C.	P433
WANG	Z.	P262
WARD	R. A.	P270
WARD	S.	P191, P276
WARE	M. J.	P085
WARENGHEM	S.	P151
WARING	M. J.	P019
WATERLOO	L. A. W.	P333
WECKBECKER	G.	LE026
WEEKS	S.	P142
WEGERT	A.	P210, P350, LE006
WEIDNER	N.	P390
WEILER	S.	LE075
WEINMANN	H.	P407
WEISEL	M.	LE101
WEISKE	J.	P407
WEISS	M.	P425
WEN	B.	LE084

WERZ	O.	P201
WEST	R.	P191
WESTWELL	A. D.	P376, P149
WHITE	P.	P032
WHITEHEAD	B.	LE101
WHITING	P.	LE065
WICHUR	T.	P164
WIDMER	T.	LE084
WIECKOWSKA	A.	P164
WIECKOWSKI	K.	P164
WIEMANN	J.	P383
WIETRZYK	J.	P112
WIJTMANS	M.	P195, P315
WIKLIK	K.	P031
WILLIAMS	C. M.	P305
WILLIAMS	H.	P084
WILLIAMS-NOONAN	B.	P061
WILLIAMSON	D. A.	P436
WILLIS	M.	P087
WILLIS	N.	LE065
WILSON	F. X.	P256
WILSON	J.	P230
WILSON	L. J.	P085, P243
WIMMER	L.	P344, P093
WINDHORST	A. D.	LE091
WINFIELD	N.	P153
WINKEL	R.	P065
WINSSINGER	N.	P288
WIPFLI	P.	LE084
WISSLER	L.	P246
WITEK	K.	P368
WITKOWSKA	J.	P292
WŁOSZCZAK	Ł.	P031
WOJCIECHOWSKI	J.	P313
WÓJCIK	R.	P146
WOJTCZAK	B.	P138
WOLTER	M.	P049
WOODWARD	H.	LE065, P060
WOUTERS	R.	P285
WRASIDLO	W.	LE034
WRIGHT	M.	P257
WRIGHT	S.	LE064
WRÓBEL	T. M.	P034
WRONA	M.	P422
WU	M.-H.	P433
WU	T.	LE078

WÜNSCH	B.	P148, P210
WYDRA	K.	P320
WYNNE	G.	P318, P256, P324
WYSKA	E.	P220
XHAARD	H.	P381, P340, P319, P179
XIA	H.	P114
XIE	X.	P359
XIN	J.	LE094
XING	L. H.	LE064
XU	B.	P051
XUE	R.	P064
YAKUBOVSKAYA	R.	P384
YAKUSHIJI	F.	P033
YAMAGISHI	K.	P144
YAMAOTSU	N.	P394
YAN	L.	LE101
YAN	Y.	P264
YANG	B.	P121, P131
YANG	J.	P335
YANG	J. S.	P380
YANG	L.	LE101
YANG	W.-H.	P433
YANG	X.	LE064
YANG	X.	LE064
YANG	X.	P011
YANOVICH	C.	LE078
YE	I.-H.	P426
YEAP	K.	LE094
YETIK-ANACAK	G.	P363
YI	K. Y.	P036
YIN	H.	LE082
YLI-KAUHALUOMA	J.	P381, P358, P340, P296, P263, P327, P319, P179
YONG	C.	P302
YONG HYUN	J.	P373
YOO	K. H.	P286
YOO	S. Y.	P380
YOON	H. B.	P120
YOON	Y. M.	P380
YOSHIDA	H.	P144
YOUNG	A.	LE094
YOUNG	R.	P069
YU	H.	LE080, P137
YUAN	J.	P283
YUSUBOV	M.	P424

ZACCONI	F.	P427, P428
ZAGOŻDŻON	A.	P209, P211
ZAJDEL	P.	P220
ZAJONC	D. M.	P202
ZAK	K. M.	LE007
ŽAKELJ	S.	P338, P396
ZAMARATSKI	E.	P013, P045
ZAMOYSKI	V. L.	LE088
ZAMPONI	G. W.	P110
ZANG	R.	P283
ZANIEWSKA	M.	P320
ZAPATA	P. M.	LE016
ZARCA	A.	P195
ZARĘBSKI	A.	P031
ZARGANES-TZITZIKAS	T.	LE007
ZARGANES-TZITZIKAS	T.	P064
ZÁVODSZKY	P.	P198
ZECHNER	R.	LE060
ZEFERINO-DÍAZ	R.	P213
ZEGA	A.	P349
ZELENKO	U.	P353
ŻESŁAWSKA	E.	P368
ZETTERBERG	F.	LE059
ZHANG	B.	P142
ZHANG	H.	P425
ZHANG	L.	LE064
ZHANG	X.	LE055
ZHANG	Y.	P407
ZHANG	Z.	P329
ZHAO	Y.	LE065
ZHENG	J.	LE085
ZHENG	L.	P264
ZHONG	Y.-L.	LE101
ZHOU	J.	P051
ZHU	X.	P277
ZIDAR	N.	P429, P349, P412
ŽIGA	S.	P429
ZILBER	S.	P200
ZIMMERLIN	A.	LE026
ZIMMERMANN	H.	P154
ZIMMERMANN	R.	LE060
ZITKO	J.	P181
ZOIDIS	G.	P192, P177
ZORC	B.	P403, P395
ZUBRIENĖ	A.	P104
ŻUK	J.	P431, P341
ŻURAWSKA	M.	P313
ZWECKSTETTER	M.	LE033
ZYK	N.	P384

EFMC
International Symposium
on Medicinal Chemistry
Ljubljana, Slovenia September 2-6, 2018

INDEX OF PARTICIPANTS

ABEYSENA Induka	SP SCIENTIFIC GENEVAC, IPSWICH, GB
ABLASSER Andrea	EPFL, LAUSANNE, CH
ABRANYI-BALOGH Peter	HUNGARIAN ACADEMY OF SCIENCES, RESEARCH CENTRE FOR NATURAL SCIENCES, BUDAPEST, HU
ADLERE Ilze	GRIFFIN DISCOVERIES BV, AMSTERDAM, NL
AGNEW-FRANCIS Kylie	UNIVERSITY OF QUEENSLAND, ST LUCIA, AU
AHONEN Tiina	UNIVERSITY OF HELSINKI, HELSINKI, FI
AIGUABELLA FONT Nuria	BAYER AG, BERLIN, DE
AJAMIAN Alain	CHEMICAL COMPUTING GROUP, MONTREAL, CA
AKKARI Rhalid	GALAPAGOS, ROMAINVILLE, FR
ALANINE Alexander	BACTEVO, CAMBRIDGE, GB
ALCOUFFE Chantal	EVOTEC FRANCE SAS, TOULOUSE, FR
ALDANA Ignacio	UNIVERSITY OF NAVARRA, PAMPLONA, ES
ALEN Jo	GRÜNENTHAL GMBH, AACHEN, DE
ALI Amjad	MERCK, SHARP & DOHME, KENILWORTH, US
ALTMANN Karl-Heinz	SCS DMCCB & SWISS FEDERAL INSTITUTE OF TECHNOLOGY, ZURICH, CH
ALZA Esther	FUNDACIO INSTITUT CATALA INVESTIGACIO QUIMICA, TARRAGONA, ES
AMBLER Martin	LIFEARC, STEVENAGE, GB
ANDERLUH Marko	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
ANDREWS Steve	UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, GB
ANSON James	ROYAL SOCIETY OF CHEMISTRY, CAMBRIDGE, GB
ARDIGO Diego	CHIESI GROUP, PARMA, IT
ARNAUDOVA Ralica	UNIVERSITY OF HELSINKI, HELSINKI, FI
ARVELA Riina	ORION PHARMA, TURKU, FI
ATKINSON Ben	UNIVERSITY COLLEGE LONDON, LONDON, GB
ATTON Holly	EVOTEC UK LTD, ABINGDON, GB
AUBERSON Yves P.	NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH, BASEL, CH
AUGUSTYNS Koen	UNIVERSITY OF ANTWERP, ANTWERP, BE
AUSTIN Wesley	MACROLIDE PHARMACEUTICALS, CAMBRIDGE, US
AVKIRAN Timur	LIFEARC, STEVENAGE, GB
AVSIC-ZUPANC Tatjana	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
BAELL Jonathan	MONASH UNIVERSITY, PARKVILLE, AU

BANOGLU Erden	GAZI UNIVERSITY FACULTY OF PHARMACY, ANKARA, TR
BARANCOKOVA Michaela	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
BARBARO Lisa	MONASH UNIVERSITY, RESERVOIR, AU
BARLAAM Bernard	ASTRAZENECA, CAMBRIDGE, GB
BARLOW Nicholas	MONASH UNIVERSITY, PARKVILLE, AU
BARNIOL-XICOTA Marta	KU LEUVEN, LEUVEN, BE
BARON Anne	TOCRIS (BIO-TECHNE), BRISTOL, GB
BARRETT Tim	GSK, BRENTWOOD, GB
BARTUZI Damian	MEDICAL UNIVERSITY OF LUBLIN, LUBLIN, PL
BATOOL Maria	AJOU UNIVERSITY, SUWON, KR
BÄURLE Stefan	BAYER AG, BERLIN, DE
BAXTER Andrew	GSK, STEVENAGE, GB
BECK Hartmut	BAYER PHARMA, WUPPERTAL, DE
BEEKMAN Andrew	UNIVERSITY OF EAST ANGLIA, NORWICH, GB
BELIAEV Alexander	LABORATORIOS BIAL, CORONADO, PT
BENDER Andreas	UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, GB
BENEDIKTSDOTTIR Andrea	UPPSALA UNIVERSITY, UPPSALA, SE
BENEK Ondrej	UNIVERSITY HOSPITAL IN HRADEC KRALOVE, HRADEC KRALOVE, CZ
BENNETT Jonathan	MERCK, SHARP & DOHME, BOSTON, US
BERG Mika	UNIVERSITY OF HELSINKI, HELSINKI, FI
BERGANT Kaja	NATIONAL INSTITUTE OF CHEMISTRY SLOVENIA, LJUBLJANA, SI
BERMEO Rafael	UNIVERSITY OF GRENOBLE, GRENOBLE, FR
BERNIER David	BAYER S.A.S. - CROPSCIENCE DIVISION, LYON, FR
BERTANI Barbara	APTUIT (VERONA) SRL, AN EVOTEC COMPANY, VERONA, IT
BERTOK Béla	COMINNEX INC., BUDAPEST, HU
BERTRAND Sophie	GLAXOSMITHKLINE, STEVENAGE, GB
BESSHO Yuki	JAPAN TOBACCO INC., OSAKA, JP
BESTEDER Marteen	REACTION BIOLOGY CORP., WOODBRIDGE, US
BEVERIDGE Julia	MONASH INSTITUTE OF PHARMACEUTICAL SCIENCES, PARKVILLE, AU
BHAGWAT Shripad	BIOMARIN, NOVATO, US

BINCH Hayley	F. HOFFMANN-LA ROCHE, BASEL, CH
BINDER Florian	BOEHRINGER INGELHEIM PHARMA GMBH & CO. KG, BIBERACH AN DER RISS, DE
BIRCH Emma	FLUOROCHEM LTD, HADFIELD, GB
BLANC Sylvain	CHARNWOOD MOLECULAR LTD, LOUGHBOROUGH, GB
BLOM Petra	ONCODESIGN, VILLEBON SUR YVETTE, FR
BOECHAT Nubia	FARMANGUINHOS- FUNDACAO OSWALDO CRUZ, RIO DE JANEIRO, BR
BOEHLER Christophe	ACTIVATE SCIENTIFIC, PRIEN, DE
BOGDOS Michael	UNIVERSITY OF STRATHCLYDE, GLASGOW, GB
BOLOGNESI Maria Laura	UNIVERSITY OF BOLOGNA, BOLOGNA, IT
BONDARENKO Anna	LIFE CHEMICALS EUROPE GMBH, MUNICH, DE
BORGOGNO Marco	FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA, GENOVA, IT
BORSOS Klara	ACCOMPANYING PERSON, BUDAPEST, HU
BOSS Christoph	IDORSIA PHARMACEUTICALS LTD, ALLSCHWIL, CH
BOSTRÖM Jonas	ASTRAZENECA, MÖLNDAL, SE
BOTTEGONI Giovanni	HEPTARES THERAPEUTICS, WELWYN GARDEN CITY, GB
BOUBIA Benaissa	INVENTIVA, DAIX, FR
BOULARAN Cedric	INVIVOGEN, TOULOUSE, FR
BOULTADAKIS-ARAPINIS Mélissa	BAYER AG, DÜSSELDORF, DE
BOZO Éva	GEDEON RICHTER PLC, BUDAPEST, HU
BRAGA Claudia	FACULDADE DE FARMÁCIA DA UNIVERSIDADE DE LISBOA, LISBON, PT
BRAUN Marie-Gabrielle	GENENTECH, SAN FRANCISCO, US
BREINBAUER Rolf	GRAZ UNIVERSITY OF TECHNOLOGY, GRAZ, AT
BRENNECKE Philip	EU-OPENSCREEN ERIC, BERLIN, DE
BRINER Karin	NOVARTIS, CAMBRIDGE, US
BROUGH Daniel	NEWCASTLE UNIVERSITY, MATLOCK, GB
BROUGH Steve	KEY ORGANICS LTD, CORNWALL, GB
BROVC Ema Valentina	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
BRUNSCHWEIGER Andreas	TU DORTMUND, DORTMUND, DE
BRVAR Matjaz	UNIVERSITY OF SOUTHERN DENMARK, ODENSE, DK
BRYAN Callie	GENENTECH, SOUTH SAN FRANCISCO, US
BUDEFELD Tomaz	INSTITUTE OF BIOCHEMISTRY, LJUBLJANA, SI

BUNCH Lennart	UNIVERSITY OF COPENHAGEN, COPENHAGEN, DK
BUONOMO Nadia	ANALYTICON DISCOVERY GMBH, POTSDAM, DE
BURGAR Irena	LABTIM, LJUBLJANA, SI
BUSCHMANN Helmut	AICURIS ANTI-INFECTIVE CURES GMBH, WUPPERTAL, DE
CABRERA Mauricio	UNIVERSIDAD DE LA REPÚBLICA, PAYSANDÚ, UY
CALIENDO Giuseppe	UNIVERSITY OF NAPLES FEDERICO II, NAPLES, IT
CALISKAN Burcu	GAZI UNIVERSITY FACULTY OF PHARMACY, ANKARA, TR
CAPPEL Daniel	SCHRÖDINGER, MANNHEIM, DE
CAPUANO Ben	MONASH INSTITUTE OF PHARMACEUTICAL SCIENCES (MONASH UNIVERSITY), MELBOURNE, AU
CARBAIN Benoit	MASARYK UNIVERSITY, BRNO, CZ
CARLOS DA SILVA PINHEIRO Luiz	FARMANGUINHOS - FUNDACAO OSWALDO CRUZ, RIO DE JANEIRO, BR
CARRARO Caterina	UNIVERSITY OF PADOVA, PADOVA, IT
CARTA Fabrizio	UNIVERSITY OF FLORENCE, SESTO FIORENTINO, IT
CARTWRIGHT Oliver	UNIVERSITY OF EAST ANGLIA, NORWICH, GB
CERNA Igor	ZENTIVA, K.S., PRAGUE, CZ
CHA Subong	SK BIOPHARMACEUTICALS, SEONGNAM-SI, GYEONGGI-DO, KR
CHAI Christina	NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE, SG
CHAKRABARTI Anjan	SYNGENE INTERNATIONAL LTD, BANGALORE, IN
CHAMBERLAIN Philip	CELGENE, ALAMEDA, US
CHARLTON Steven	UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, GB
CHASSAING Christophe	MSD ANIMAL HEALTH INNOVATION GMBH, SCHWABENHEIM, DE
CHATTERJEE Swati	SYNGENE INTERNATIONAL LTD, BANGALORE, IN
CHEN Kaixian	SHANGHAI INSTITUTE OF MATERIA MEDICA, SHANGHAI, CN
CHEN Fener	FUDAN UNIVERSITY, , CN
CHIHA Slim	UNIVERSITY OF COLOGNE, COLOGNE, DE
CHING Lai Ming	UNIVERSITY OF AUCKLAND, AUCKLAND, NZ
CHO Yong Seo	KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, SEOUL, KR
CHOI Kyung Il	KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, SEOUL, KR
CHOMICKI Karol	ELSEVIER, WARSZAWA, PL
CIANNI Lorenzo	UNIVERSIDADE DE SAO PAULO, IQSC, SAO CARLOS, BR
CID Jose	JANSSEN R&D, TOLEDO, ES

CINTULOVA Daniela	TU WIEN, VIENNA, AT
CLEVE Arwed	BAYER AG, BERLIN, DE
CLEWLOW Paul	SYGNATURE DISCOVERY, NOTTINGHAM, GB
COE Diane	GLAXOSMITHKLINE, STEVENAGE, GB
COLBON Paul	LIVERPOOL CHIROCHEM LTD., LIVERPOOL, GB
COLLIER Philip	VERTEX PHARMACEUTICALS INC, BOSTON, US
COLLINS Karl	BAYER AG, DÜSSELDORF, DE
CONDE-KNAPE Karin	NOVO NORDISK, BAGSVAERD, DK
CONTINO Marialessandra	UNIVERSITÀ DEGLI STUDI DI BARI, BARI, IT
CONTRERAS Jean-Marie	PRESTWICK CHEMICAL SAS, ILLKIRCH-GRAFFENSTADEN, FR
CORVINO Angela	UNIVERSITY OF NAPLES, NAPLES, IT
COSCONATI Sandro	UNIVERSITY OF CAMPANIA LUIGI VANVITELLI, CASERTA, IT
COSTA Sofia	SPRINGER NATURE, HEIDELBERG, DE
COSTANTINO Gabriele	UNIVERSITY OF PARMA, PARMA, IT
COULTHARD Victoria	REACH SEPARATIONS, NOTTINGHAM, GB
COURTENS Charlotte	UGENT, GENT, BE
CRAVATT Benjamin	THE SCRIPPS RESEARCH INSTITUTE, LA JOLLA, US
CRAWFORD James	GENENTECH, SOUTH SAN FRANCISCO, US
CREBBIN Mike	ACTIVATE SCIENTIFIC, PRIEN, DE
CRUCES Jacobo	GALCHIMIA, S.A., O PINO, A CORUÑA, ES
CSEKEI Marton	SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, BUDAPEST, HU
CUFFE Sandra	TOCRIS (BIO-TECHNE), BRISTOL, BE
CUMMING Jared	MSD, BOSTON, US
CVIJANOVIC Danijela	SCHOOL OF MEDICINE, UNIVERSITY OF ZAGREB, ZAGREB, HR
D A Jeyaraj	JUBILANT BIOSYS LTD., BANGALORE, IN
DACIER Bernard	LD ORGANISATION, LOUVAIN-LA-NEUVE, BE
DAMJANOVIC Vladimir	SCHOOL OF MEDICINE, UNIVERSITY OF ZAGREB, ZAGREB, HR
DANICA Agbaba	FACULTY OF PHARMACY, BELGRADE, YU
DARMENCY Vincent	AC IMMUNE SA, LAUSANNE, CH
DAVIES Nichola	ASTRAZENECA, CAMBRIDGE, GB
DAVIS Benjamin	OXFORD UNIVERSITY, OXFORD, GB

DE BOECK Benoit	JANSSEN RESEARCH & DEVELOPMENT, BEERSE, BE
DE CARVALHO ACURCIO Ana Rita	UNIVERSITY OF LISBON, LISBON, PT
DE JONGHE Steven	REGA INSTITUUT, LEUVEN, BE
DE LANGE Liesbeth	UNIVERSITY OF LEIDEN, LEIDEN, NL
DE SIMONE Alessio	UNIVERSITY OF EDINBURGH, EDINBURGH, GB
DE VRIES Rens	EINDHOVEN UNIVERSITY OF TECHNOLOGY, EINDHOVEN, NL
DEMONT Dennis	ACERTA PHARMA BV, OSS, NL
DEPREZ-POULAIN Rebecca	UNIVERSITY OF LILLE, LILLE, FR
DERNOVSEK Jaka	FACULTY OF PHARMACY, LJUBLJANA, MEDVODE, SI
DESHPANDE Pratima	JUBILANT BIOSYS LTD., BANGALORE, IN
DESHPANDE Anil	EUROFINS ADVINUS LIMITED, BANGALORE, IN
DETTA Elena	AICURIS ANTI-INFECTIVE CURES GMBH, WUPPERTAL, DE
DI MARTINO Rita Maria Concetta	ISTITUTO ITALIANO DI TECNOLOGIA, GENOVA, IT
DI SANTO Roberto	SAPIENZA UNIVERSITY OF ROME, ROME, IT
DIAMANTI Eleonora	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
DIEDERICH François	ETH ZURICH, ZÜRICH, CH
DIFFERDING Ly	LD ORGANISATION, LOUVAIN-LA-NEUVE, BE
DIFFERDING Edmond	DIFFERDING CONSULTING, LOUVAIN-LA-NEUVE, BE
DILLON Michael	IDEAYA BIOSCIENCES, SOUTH SAN FRANCISCO, US
DOBIE Chris	UNIVERSITY OF WOLLONGONG, GWYNNEVILLE, AU
DOLLINGER Horst	BOEHRINGER INGELHEIM INTERNATIONAL GMBH, BIBERACH, DE
DOLSAK Ana	FACULTY OF PHARMACY, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
DOMINGOS Sofia	FACULDADE DE FARMÁCIA DA UNIVERSIDADE DE LISBOA, LISBON, PT
DÖMLING Alexander	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
DONCKELE Etienne	PHILOCHEM AG, OTELFINGEN, CH
DONGKEUN Song	ILDONG PHARMACEUTICAL CO., LTD., HWASEONG, KR
DORMAN György	FACULTY OF PHARMACY, UNIVERSITY OF SZEGED, SZEGED, HU
DOROCKA BOBKOWSKA Barbara	UNIVERSITY OF MEDICAL SCIENCES, POZNAN, PL
DOSSETTER Al	MEDCHEMICA LIMITED, MACCLESFIELD, GB
DUBURS Gunars	LATVIAN INSTITUTE OF ORGANIC SYNTHESIS, RIGA, LV
DUCA Maria	UNIVERSITÉ CÔTE D'AZUR, NICE, FR

DUDA-SEIMAN Corina	WEST UNIVERSITY OF TIMISOARA, TIMISOARA, RO
DUDUTIENE Virginija	VILNIUS UNIVERSITY, VILNIUS, LT
DUFFY James	MEDICINES FOR MALARIA VENTURE (MMV), GENÈVE, CH
DURCIK Martina	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
EBBERS Eelco	MERCACHEM-SYNCOM, NIJMEGEN, NL
EL AHMAD Youssef	SANOFI, VITRY SUR SEINE, FR
EL BKASSINY Sandy	GALAPAGOS NV, MECHELEN, BE
ELGIE Kerry	ASYNT, ISLEHAM, CAMBS, GB
ENRIC Lizano	UNIVERISTY OF BARCELONA, BARCELONA, ES
ETTMAYER Peter	BOEHRINGER-INGELHEIM, VIENNA, AT
EVERITT Simon	VERTEX PHARMACEUTICALS, OXON, GB
EVERTSSON Emma	ASTRAZENECA, MÖLNDAL, SE
FABRITIUS Charles Henry	SELVITA S.A., KRAKOW, PL
FANG Weishuo	CHINESE ACADEMY OF MEDICAL SCIENCES, BEIJING, CN
FAULKNER Adele	EVOTEC UK LTD, ABINGDON, GB
FERENCZY György G.	RESEARCH CENTRE FOR NATURAL SCIENCES, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HU
FERNANDEZ Joan-Carles	PARC CIENTIFIC DE BARCELONA, BARCELONA, ES
FERNANDEZ Esther	GLAXOSMITHKLINE, TRES CANTOS, ES
FESSARD Thomas C.	SPIROCHEM AG, ZURICH, CH
FIORELLI Claudio	CHIESI FARMACEUTICI SPA, PARMA, IT
FIORENTINO Francesco Paolo	KITOS BIOTECH, ALGHERO (SS), IT
FIORINO Fernando	UNIVERSITY OF NAPLES, NAPLES, IT
FISCHER Janos	EMERITUS MEMBER OF IUPAC, BUDAPEST, HU
FISCHER Christian	MERCK, SHARP & DOHME, BOSTON, US
FISH Paul	ARUK UCL DRUG DISCOVERY INSTITUTE, LONDON, GB
FLYNN Bernard	MONASH UNIVERSITY, PARKVILLE, AU
FOLMER Rutger	MERCACHEM, NIJMEGEN, NL
FOSTER Nick	OPTIBRIUM, CAMBRIDGE, GB
FRECENTESE Francesco	UNIVERSITY OF NAPLES, NAPLES, IT
FRIEDRICH Lukas	ETH ZURICH, WINTERTHUR, CH
FRIGOLE-VIVAS Marta	INSTITUTE FOR RESEARCH IN BIOMEDICINE (IRB BARCELONA), BARCELONA, ES

FRLAN Rok	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
FROLUND Bente	UNIVERSITY OF COPENHAGEN, COPENHAGEN, DK
FRORMANN Sven	GRUNENTHAL GMBH, AACHEN, DE
FUJII Shinya	UNIVERSITY OF TOKYO, TOKYO, JP
FUKUZAKI Takehiro	DAIICHI SANKYO CO., LTD., TOKYO, JP
FUMAGALLI Gabriele	ASTRAZENECA, CAMBRIDGE, GB
FYFE Matthew	SITRYX THERAPEUTICS, OXFORD, GB
GADEMANN Karl	UNIVERSITY OF ZURICH, ZURICH, CH
GALDEANO Carles	UNIVERSITY OF BARCELONA, BARCELONA, ES
GALIANA-ROSELLO Cristina	UNIVERSITY OF VALENCIA, PATERNA, ES
GAMBINI Luca	UC RIVERSIDE, RIVERSIDE, US
GAO Fang	BIOGEN, CAMBRIDGE, US
GARCIA Sara	IST-ID, LISBOA, PT
GASTON-MATHE Yann	IKTOS, PARIS, FR
GASTREICH Marcus	BIOSOLVEIT, ST. AUGUSTIN, DE
GBELCOVA Helena	FACULTY OF MEDICINE, COMENIUS UNIVERSITY, BRATISLAVA, SK
GEE Antony	KING'S COLLEGE LONDON, LONDON, GB
GEHRINGER Matthias	UNIVERSITY OF TUEBINGEN, TUEBINGEN, DE
GELLIBERT Françoise	INSTITUT DE RECHERCHES SERVIER, SURESNES CEDEX, FR
GENEVOIS-BORELLA Arielle	SANOFI, CHILLY-MAZARIN, FR
GENSKI Thorsten	TAROS CHEMICALS, DORTMUND, DE
GEORGE Colin	GENEVAC LTD, IPSWICH SUFFOLK, GB
GEORGE Pascal	SCT & INDEPENDENT SCIENTIFIC EXPERT & ADVISER, LONGVILLIERS, FR
GEORGI Victoria	BAYER AG, BERLIN, DE
GERSTENBERGER Brian	PFIZER, CAMBRIDGE, US
GIANNAKOPOULOU Erofili	FACULTY OF PHARMACY, ATHENS, GR
GIGANTE Alba	UNIVERSITY OF GEORGIA, TRES CANTOS-MADRID, ES
GIOIELLO Antimo	UNIVERSITY OF PERUGIA, PERUGIA, IT
GIORDANETTO Fabrizio	D.E. SHAW RESEARCH LLC, NEW YORK, US
GIOVANNINI Riccardo	BOEHRINGER INGELHEIM, BIBERACH, DE
GIZYNSKA Malgorzata	UNIVERSITY OF GDANSK, GDANSK, PL

GLOANEC Philippe	INSTITUT DE RECHERCHES SERVIER, SURESNES, FR
GLYNN Daniel	SELVITA S.A., KRAKOW, PL
GOBEC Stanislav	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
GODDARD Zoe	UNIVERSITY OF EAST ANGLIA, NORWICH, GB
GÖKHAN-KELEKCI Nesrin	HACETTEPE UNIVERSITY, ANKARA, TR
GOLIC GRDADOLNIK Simona	NATIONAL INSTITUTE OF CHEMISTRY, LJUBLJANA, SI
GOLKIEWICZ Piotr	ELSEVIER, WARSZAWA, PL
GOLOJUCH Sebastian	UNIVERSITY OF WARSAW, WARSAW, PL
GOMEZ Elena	ALMIRALL, BARCELONA, ES
GONCHARENKO Kristina	SPIROCHEM AG, BASEL, CH
GONDELA Andrzej	SELVITA S.A., KRAKOW, PL
GONZALEZ Jorge	UNIVERSITY OF BORDEAUX, PESSAC, FR
GORICAN Tjasa	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
GRAHAM Christopher	VERNALIS, CAMBRIDGE, GB
GRAHAM Keith	BAYER AG, BERLIN, DE
GRAY Janine	UNIVERSITY OF OXFORD, OXFORD, GB
GRAY Heather	GLAXOSMITHKLINE, BALDOCK, GB
GREINER Istvan	GEDEON RICHTER PLC., BUDAPEST, HU
GRGICEVIC Ivan	FACULTY OF SCIENCE, ZAGREB, HR
GRICE Irwin Darren	GRIFFITH UNIVERSITY, GOLD COAST, QUEENSLAND, AU
GRIFFITH Renate	UNIVERSITY OF NEW SOUTH WALES, UNSW SYDNEY, AU
GRILO Jorge	FACULDADE DE FARMÁCIA DA UNIVRSIDADE DE LISBOA, LISBON, PT
GROOTENHUIS Peter	VERTEX PHARMACEUTICALS, SAN DIEGO, US
GRUZMAN Arie-Lev	BAR ILAN UNIVERSITY, RAMAT GAN, IL
GUCKY Tomas	PALACKY UNIVERSITY, FACULTY OF SCIENCE, OLOMOUC, CZ
GUEDES Isabella	LABORATÓRIO NACIONAL DE COMPUTAÇÃO CIENTÍFICA, PETROPOLIS, BR
GUEDES Romina	IMED.ULISBOA - FACULTY OF PHARMACY, UNIVERSITY OF LISBON, LISBOA, PT
GUEDES Rita	UNIVERSITY OF LISBON, LISBON, PT
GUERET Stephanie	ASTRAZENECA-MAX PLANCK INSTITUTE SATELLITE UNIT, DORTMUND, DE
GUR Zehra Tugce	GAZI UNIVERSITY, ANKARA, TR
GWILLIAMS Drew	ROYAL SOCIETY OF CHEMISTRY, CAMBRIDGE, GB

HAAVIKKO Raisa	-, VANTAA, FI
HAHN Michael G.	BAYER HEALTHCARE, WUPPERTAL, DE
HAJDARI Teuta	SPIROCHEM AG, ZÜRICH, CH
HALKINA Toma	BIOGEN, CAMBRIDGE, US
HALL Adrian	UCB PHARMA S.A., BRAINE-L'ALLEUD, BE
HALLAND Nis	SANOFI, FRANKFURT AM MAIN, DE
HAMPTON Shahienaz	NEW YORK UNIVERSITY ABU DHABI, ABU DHABI, AE
HAMZA Daniel	SYGNATURE DISCOVERY, NOTTINGHAM, GB
HANCOCK Ashley	GLAXOSMITHKLINE, STEVENAGE, GB
HARADA Takaaki	EISAI CO., LTD., TSUKUBA, JP
HARTMAN Alwin	RIJKSUNIVERSITEIT GRONINGEN, GRONINGEN, NL
HASLETT Greg	UCB, SLOUGH, GB
HASSAN Ghada	UNIVERSITY OF HELSINKI, HELSINKI, FI
HAUSTEDT Lars Ole	ANALYTICON DISCOVERY GMBH, POTSDAM, DE
HAUWERT Niels	VRIJE UNIVERSITEIT AMSTERDAM, AMSTERDAM, NL
HAVEL Stepan	ST.ANNE'S UNIVERSITY HOSPITAL BRNO, BRNO, CZ
HEIMANN Annekatrin	BOEHRINGER-INGELHEIM PHARMA GMBH & CO KG, BIBERACH, DE
HEITMAN Laura	UNIVERSITY OF LEIDEN, LEIDEN, NL
HELLMANN Jan	FAU ERLANGEN-NUREMBERG, ERLANGEN, DE
HENDERSON Scott	UNIVERSITY OF SUSSEX, BRIGHTON, GB
HENRY Andrew	CHEMICAL COMPUTING GROUP, CAMBRIDGE, GB
HERRLINGER Eva-Maria	UNIVERSITY OF FREIBURG, FREIBURG, DE
HETZER Florian	FAU ERLANGEN-NUREMBERG, ERLANGEN, DE
HINRICHS Jürgen	NOVARTIS, BASEL, CH
HIRONO Shuichi	KITASATO UNIVERSITY, SCHOOL OF PHARMACY, TOKYO, JP
HIRST Gavin	JANSSEN PHARMACEUTICALS, SAN DIEGO, US
HITCHIN James	CHARNWOOD MOLECULAR, LOUGHBOROUGH, LEICS, GB
HOFFMANN Torsten	BIOSOLVEIT, SANKT AUGUSTIN, DE
HOFFMANN Remy	SAI LIFE SCIENCES, HYDERABAD, IN
HOFMANN Andrea	WATERS CORPORATION, VIENNA, AT
HOGG John	APOLLO SCIENTIFIC LTD, CHESHIRE SK6 2QR, GB

HOLLINGWORTH Gregory	NOVARTIS PHARMA AG, BASEL, CH
HOLMGAARD KASPERSEN Mads	UNIVERSITY OF SOUTHERN DENMARK, ODENSE, DK
HONDA Takeshi	DAIICHISANKYO, CO., LTD., TOKYO, JP
HORSLEY Helen	UCB, SLOUGH, GB
HOWARD Steven	ASTEX THERAPEUTICS, CAMBRIDGE, GB
HRAST Martina	FAKULTETA ZA FARMACIJO, LJUBLJANA, SI
HRIBERNIK Nives	UNIVERSITY OF MILAN, MILANO, IT
HUCK Bayard	MERCK BIOPHARMA, DARMSTADT, DE
HUZJAK Tilen	UL FACULTY OF PHARMACY, LJUBLJANA, SI
ICHIKAWA Satoshi	HOKKAIDO UNIVERSITY, SAPPORO, JP
IJZERMAN Ad P.	UNIVERSITY OF LEIDEN, LEIDEN, NL
ILAS Janez	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
IMRAMOVSKY Ales	UNIVERSITY OF PARDUBICE, PARDUBICE, CZ
INABA Yusuke	NISSAN CHEMICAL CORPORATION, FUNABASHI, JP
INDORF Patrick	CHRISTIAN-ALBRECHTS UNIVERSITÄT ZU KIEL, KIEL, DE
ISTANBULLU Huseyin	IZMIR KATIP CELEBI UNIVERSITY FACULTY OF PHARMACY, IZMIR, TR
JAEKYUN Lee	KIST, SEOUL, KR
JAGEROVIC Nadine	INSTITUTO DE QUIMICA MEDICA, C.S.I.C., MADRID, ES
JAKOPIN Ziga	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
JANETKA James	WASHINGTON UNIVERSITY IN SAINT LOUIS, SAINT LOUIS, US
JANG Changyoung	KYUNG HEE UNIVERSITY, SEOUL, KR
JANSSENS Jonas	GHENT UNIVERSITY, GENT, BE
JANTOS Katja	ABBVIE DEUTSCHLAND GMBH&CO KG, LUDWIGSHAFEN, DE
JHA Shalinee	GRAZ UNIVERSITY OF TECHNOLOGY, GRAZ, AT
JIMENEZ-BARBERO Jesus	CIC BIOGUNE, DERIO, ES
JIN Changbae	KOREA INSTITUTE OF SCIENCE & TECHNOLOGY, SEOUL, KR
JOANNESSE Caroline	GALAPAGOS NV, MECHELEN, BE
JOHANSSON Niklas	UNIVERSITY OF HELSINKI, HELSINKI, FI
JOHNSEN Lisa	MDPI, BASEL, CH
JONES Phil	BIOASCENT, GLASGOW, GB

JONES Geraint	SYGNATURE DISCOVERY, NOTTINGHAM, GB
JORDA Radek	PALACKY UNIVERSITY, OLOMOUC, CZ
JORDAN Allan	CANCER RESEARCH UK MANCHESTER INSTITUTE, MANCHESTER, GB
JORG Manuela	MONASH UNIVERSITY, PARKVILLE, AU
JOSA CULLERE Laia	UNIVERSITY OF OXFORD, OXFORD, GB
JUAREZ SALDIVAR Alfredo	INSTITUTO POLITECNICO NACIONAL, REYNOSA, MX
JUKIC Marko	FAKULTETA ZA FARMACIJO, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
JUMPPANEN Mikael	UNIVERSITY OF HELSINKI, HELSINKI, FI
JUNG Juyoung	ILDONG PHARMACEUTICAL, HWASEONG-SI, GYEONGGI-DO, KR
JUNG Myeong	PUSAN NATIONAL UNIVERSITY, YANGSAN-SI, KR
JUNG Marie-Louise	PRESTWICK CHEMICAL, ILLKIRCH, FR
JUNG Laurence	PRESTWICK CHEMICAL, ILLKIRCH, FR
KACZOR Aneta	JAGIELLONIAN UNIVERSITY COLLEGIUM MEDICUM, CRACOW, PL
KAHNT Michael	MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, HALLE (SAALE), DE
KAMINENI Pujitha Reddy	GVK BIOSCIENCES PVT. LTD., AMSTERDAM SCHIPHOL, NL
KARAWAJCZYK Anna	SELVITA, KRAKOW, PL
KARELOU Maria	FACULTY OF PHARMACY, ATHENS, GR
KARLÉN Anders	UPPSALA UNIVERSITY, UPPSALA, SE
KASAKIN Marat	INSTITUTE OF CHEMICAL BIOLOGY AND FUNDAMENTAL MEDICINE, NOVOSIBIRSK, RU
KAWAMURA Shuhei	MSD, CAMBRIDGE, US
KELLAM Barrie	UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, GB
KELLER Thomas	EXPERIMENTAL THERAPEUTICS CENTER, SINGAPORE, SG
KELLEY Tanya	THE UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE, MIAMI, US
KERSTEN Christian	JOHANNES GUTENBERG UNIVERSITY, MAINZ, DE
KESERU Gyorgy	RESEARCH CENTRE FOR NATURAL SCIENCES, HUNGARIAN ACADEMY OF SCIENCES, BUDAPEST, HU
KEUM Gyochang	KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, SEOUL, KR
KIEC-KONONOWICZ Katarzyna	JAGIELLONIAN UNIVERSITY, KRAKOW, PL
KIKELJ Danijel	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
KIM Shinae	DGMIF, DAEGU, KR
KIM Jina	DGMIF, DAEGU, KR
KIM Da Mi	EWHA WOMANS UNIVERSITY, SEOUL, KR

KIM Key-Sun	KOREA INSTITUTE OF SCIENCE AND TECHNOLOY, SEOUL, KR
KISO Ute	ABCR GMBH, KARLSRUHE, DE
KISS Arpad	SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, BUDAPEST, HU
KITAHATA Shun	HOKKAIDO UNIVERSITY, SAPPORO, JP
KLEIN Markus	MERCK KGAA, DARMSTADT, DE
KLEJBOROWSKA Greta	ADAM MICKIEWICZ UNIVERSITY IN POZNAN, POZNAN, PL
KLEMENCIC Marusa	STUDENT, DOL PRI LJUBLJANI, SI
KLENKE Burkhard	AICURIS ANTI-INFECTIVE CURES GMBH, WUPPERTAL, DE
KLINGLER Franca	BIOSOLVEIT, ST. AUGUSTIN, DE
KNEZ Damijan	FACULTY OF PHARMACY, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
KNIGHT Jamie	CHARLES RIVER, HARLOW, GB
KOKKONEN Piia	MASARYK UNIVERSITY, BRNO, CZ
KOLARIC Anja	NATIONAL INSTITUTE OF CHEMISTRY, LJUBLJANA, SI
KOLOCOURIS Antonios	UNIVERSITY OF ATHENS, ATHENS, GR
KOMIYA Masafumi	DAINIPPON SUMITOMO PHARMA CO., LTD., OSAKA, JP
KONC Janez	NATIONAL INSITUTE OF CHEMISTRY, LJUBLJANA, SI
KONDEJ Magda	MEDICAL UNIVERSITY OF LUBLIN, LUBLIN, PL
KONRADOVA Daniela	PALACKY UNIVERSITY OLOMOUC, OLOMOUC, CZ
KONSTANTINIDOU Markella	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
KOSAR Miroslav	UNIVERSITY OF EDINBURGH, , GB
KOTSCHY Andras	SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, BUDAPEST, HU
KOTTRAYIL Jafar	GVK BIOSCIENCES PVT. LTD., UTRECHT, NL
KOUKNI Mohamed	CISTIM, LEUVEN, BE
KOUROUNAKIS Angeliki	UNIVERSITY OF ATHENS, ATHENS, GR
KOUROUNAKIS Panos	UNIVERSITY OF THESSALONIKI, THESSALONIKI, GR
KOURTI Malamati	CARDIFF UNIVERSITY, CARDIFF, GB
KRAUSS Stefan	UNIVERSITY OF OSLO, OSLO, NO
KREIS Lukas	F. HOFFMANN-LA ROCHE, BASEL, CH
KRSTULOVIC Luka	FACULTY OF VETERINARY MEDICINE, ZAGREB, HR
KRZYKAWSKA-SERDA Martyna	FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY, JAGIELLONIAN UNIVERSITY, KRAKOW, PL
KUEPPERS Patrick Jim	UNIVERSITY OF BONN, BONN, DE

KUKUSHKIN Maxim	MOSCOW STATE UNIVERSITY, MOSCOW, RU
KULKARNI Santosh	SYNGENE INTERNATIONAL LTD, BENGALURU, IN
KUMAR Sneha	CRELUX - A WUXI APPTEC COMPANY, MARTINSRIED, DE
KUMAR Ashok	INDIAN INSTITUTE OF TECHNOLOGY (BHU), VARANASI, IN
KWON Sunoh	KRICT, DAEJEON, KR
KYSIL Volodymyr	CHEMDIV, INC., SAN DIEGO, US
LA MOTTA Concettina	UNIVERSITY OF PISA, PISA, IT
LABEEUW Olivier	BIOPROJET BIOTECH, SAINT GRÉGOIRE, FR
LACIVITA Enza	UNIVERSITÀ DEGLI STUDI DI BARI, BARI, IT
LACOSTE Nathalie	SCHRÖDINGER GMBH, MÜNCHEN, DE
LAM Kelvin	REACTION BIOLOGY CORP., WOODBRIDGE, US
LAMBERT Heather	FLUOROCHEM LTD, HADFIELD, GB
LAMBIN Dominique	CISTIM LEUVEN, LEUVEN, BE
LAMERS Christina	UNIVERSITY OF BASEL, BASEL, CH
LANKRI David	HEBREW UNIVERSITY, JERUSALEM, IL
LANZ Jan	THE INSTITUTE OF CANCER RESEARCH, SUTTON, GB
LARSEN Jens	LEO PHARMA, BALLERUP, DK
LAU Jesper	NOVO NORDISK, MAALOEV, DK
LAUFER Stefan	UNIVERSITY OF TÜBINGEN, TÜBINGEN, DE
LE Thuy	MONASH UNIVERSITY, PARKVILLE, AU
LEAHY David E.	THE DISCOVERY BUS LTD, MACCLESFIELD, GB
LEE On	INDUSTRIAL TECHNOLOGY RESEARCH INSTITUTE, HSIN-CHU, TW
LEE Chang-Seok	JEIL PHARMACEUTICALS, YONGIN-SI, KR
LEE Eung-Seok	YEUNGNAM UNIVERSITY, KYONGSAN, KR
LEE Yong Sup	KYUNG HEE UNIVERSITY, SEOUL, KR
LEE Soyun	EWHA WOMANS UNIVERSITY, SEOUL, KR
LEE Tony	GILEAD SCIENCES, SEATTLE, US
LEE Jisook	MONASH UNIVERSITY, PARKVILLE, AU
LEE FLEMING Cassandra	UNIVERSITY OF GOTHENBURG, GÖTEBORG, SE
LEEMHUIS Frank	MERCACHEM-SYNCOM, NIJMEGEN, NL
LEHMANN Johannes	SIMON FRASER UNIVERSITY, BURNABY, CA

LEINO Teppo	UNIVERSITY OF HELSINKI, HELSINKI, FI
LETH-PETERSEN Sebastian	NUEVOLUTION, COPENHAGEN, DK
LIM Chae Jo	KOREA RESEARCH INSTITUTE OF CHEMICAL TECHNOLOGY, DAEJEON, KR
LINDSTROM Johan	SPRINT BIOSCIENCE, HUDDINGE, SE
LIOUX Thierry	INVIVOGEN, TOULOUSE, FR
LIU Hong	SHANGHAI INSTITUTE OF MATERIA MEDICA, SHANGHAI, CN
LIU Kevin	NOVARTIS, SHANGHAI, CN
LOCARNO Silvia Alice	UNIVERSITÀ DEGLI STUDI DI MILANO, MILANO, IT
LOESCHE Anne	MARTIN-LUTHER-UNIVERSITÄT HALLE-WITTENBERG, HALLE (SAALE), DE
LOGIE Gavin	APOLLO SCIENTIFIC LTD, CHESHIRE SK6 2QR, GB
LOI Elena	UNIVERSITY OF LJUBLJANA, FACULTY OF PHARMACY, LJUBLJANA, SI
LOPEZ Irakusne	MESTRELAB RESEARCH, SANTIAGO DE COMPOSTELA, ES
LORENTE Adriana	LAB. DR. ESTEVE, BARCELONA, ES
LORENTE MACIAS Alvaro	UNIVERSIDAD DE GRANADA, GRANADA, ES
LUMB Elliott	UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, GB
LUMERAS Wenceslao	ELI LILLY AND COMPANY, MADRID, ES
LUPALA Cecylia Severin	BEIJING COMPUTATIONAL SCIENCE RESEARCH CENTER, BEIJING, CN
MAANSSON Kristoffer	LEO PHARMA AS, BALLERUP, DK
MACEDO BASTOS Monica	FUNDAÇÃO OSWALDO CRUZ - FIOCRUZ/FARMANGUINHOS, RIO DE JANEIRO, BR
MACHAUER Rainer	NOVARTIS PHARMA AG, BASEL, CH
MACHULKIN Aleksei	LOMONOSOV MOSCOW STATE UNIVERSITY, MOSCOW, RU
MACK Jürgen	BOEHRINGER INGELHEIM, BIBERACH AN DER RISS, DE
MACKAY Angela	NOVARTIS, BASEL, CH
MACUT Helena	UNIVERSITY OF MILANO, MILANO, IT
MADAPPA Veena	SYNGENE INTERNATIONAL LTD, BANGALORE, IN
MADGE Dave	WUXI APPTEC, HADDENHAM, GB
MAEGAWA Tomohiro	KINDAI UNIVERSITY, HIGASHI-OSAKA, JP
MAGLI Elisa	UNIVERSITY OF NAPLES, NAPLES, IT
MAHAJAN Yogesh	SAI LIFE SCIENCES, HYDERABAD, IN
MAKARA Gergely	CHEMPASS, BUDAPEST, HU
MALAWSKA Barbara	JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE, KRAKOW, PL

MALCOLM Noj	SCHRÖDINGER LTD, CAMBRIDGE, GB
MALINKOVA Veronika	PALACKY UNIVERSITY OLOMOUC, OLOMOUC, CZ
MALLINGER Aurélie	PHENEX PHARMACEUTICALS, HEIDELBERG, DE
MALOSE JACK Mphahlele	UNIVERSITY OF SOUTH AFRICA, PRETORIA, ZA
MAMIDI Srinivas Reddy	GVK BIOSCIENCES, AMSTERDAM SCHIPHOL, NL
MAMOT Adam	UNIVERSITY OF WARSAW, WARSAW, PL
MARCHESI Irene	KITOS BIOTECH SRLS, SASSARI, IT
MAROVIC Nina	/, MARIBOR, SI
MARQUES Matilde	INSTITUTO SUPERIOR TÉCNICO, LISBOA, PT
MARTIN Dolezal	FACULTY OF PHARMACY IN HRADEC KRALOVE, CHARLES UNIVERSITY, HRADEC KRALOVE, CZ
MARTIN ACOSTA Pedro	UNIVERSITY OF LA LAGUNA (ULL), IUBO-AG, LA LAGUNA, TENERIFE, ES
MARTINEZ Zuleyma	UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MORELOS, CUERNAVACA, MX
MARTINS COIMBRA Judite Raquel	FACULTY OF PHARMACY, UNIVERSITY OF COIMBRA, COIMBRA, PT
MARTIN-SANTAMARIA Sonsoles	BIOLOGICAL RESEARCH CENTER, MADRID, ES
MASSA Delphine	LD ORGANISATION, LOUVAIN-LA-NEUVE, BE
MASSE Julie	GALCHIMIA, O PINO A CORUÑA, ES
MATAGNE Brieuc	LD ORGANISATION, LOUVAIN-LA-NEUVE, BE
MATOSIUK Dariusz	MEDICAL UNIVERSITY, LUBLIN, PL
MATSUNO Kenji	KOGAKUIN UNIVERSITY (INSTITUTE OF TECHNOLOGY), TOKYO, JP
MAZUR Marzena	ONCOARENDI THERAPEUTICS S.A., WARSAW, PL
MCCLUSKEY Adam	UNIVERITY OF NEWCASTLE, CALLAGHAN, AU
MCGONAGLE Kate	DUNDEE DRUG DISCOVERY UNIT, DUNDEE, GB
MCGONAGLE Grant	GLAXOSMITHKLINE, STEVENAGE, GB
MCKENNA Sean	VERNALIS RESEARCH, CAMBRIDGE, GB
MEDEN Anze	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
MEIJER Femke	EINDHOVEN UNIVERSITY OF TECHNOLOGY, EINDHOVEN, NL
MEISSNER Hans	MERCACHEM-SYNCOM, GRONINGEN, NL
MELDRUM Eric	ENYO PHARMA, LYON, FR
MERAYO Nuria	GALAPAGOS SASU, ROMAINVILLE, FR
MERK Daniel	ETH ZÜRICH, ZÜRICH, CH
MERKERT Christine	ADVION, HARLOW, GB

MESSINGER Josef	ORION PHARMA, ESPOO, FI
MEXIA Nikitia	UNIVERSITY OF LEEDS, LEEDS, GB
MEZEIOVA Eva	UNIVERSITY OF HOSPITAL, HRADEC KRALOVE, CZ
MICCO Iolanda	VIPERGEN, COPENHAGEN, DK
MICHAELIDES Michael	ABBVIE INC., LIBERTYVILLE, US
MIGGLAUTSCH Anna	TU GRAZ, GRAZ, AT
MILAEVA Elena	MOSCOW STATE LOMONOSOV UNIVERSITY, MOSCOW, RU
MILLAN Mark J.	INSTITUT DE RECHERCHES SERVIER, CROISSY-SUR-SEINE, FR
MISSBACH Martin	NOVARTIS, BASEL, CH
MISTRY Shailesh	UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, GB
MITTENDORF Joachim	BAYER AG, WUPPERTAL, DE
MIURA Yusuke	KYOWA KIRIN, SHIZUOKA, JP
MIYAMOTO Shuichi	SOJO UNIVERSITY, KUMAMOTO, JP
MIYAZAKI Masaki	DAIICHI SANKYO CO., LTD., TOKYO, JP
MOFFATT Frank	NOVALIX, ILLKIRCH, FR
MOLA Laura	UNIVERSITY OF OXFORD, OXFORD, GB
MOMMART Nathalie	LD ORGANISATION, LOUVAIN-LA-NEUVE, BE
MONNEE Menno	MERCACHEM-SYNCOM, NIJMEGEN, NL
MONS Elma	LEIDEN UNIVERSITY MEDICAL CENTER, LEIDEN, NL
MONTGOMERY Andrew	UNIVERSITY OF WOLLONGONG, WOLLONGONG, AU
MOON Yoonhyoung	KYUNG HEE UNIVERSITY, SEOUL, KR
MORALES Paula	CSIC, MADRID, ES
MOREIRA Rui	UNIVERSITY OF LISBON, LISBON, PT
MORITA Norimasa	MERCACHEM, NIJMEGEN, NL
MORRISON Angus	BIOASCENT, MOTHERWELL, GB
MOYA-GARZON Maria Dolores	FACULTY OF PHARMACY (UNIVERSITY OF GRANADA), GRANADA, ES
MRAVLJAK Janez	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
MU Linjing	ETHZ, ZURICH, CH
MUELLER Bernd	BASF SE, LUDWIGSHAFEN, DE
MUELLER Janis	CRYSTALSFIRST GMBH, MARBURG, DE
MULARD Laurence	INSTITUT PASTEUR, PARIS, FR

MÜLLER Christa	UNIVERSITY OF BONN, BONN, DE
MUNIER Claire	ASTRAZENECA, MÖLNDAL, SE
MUTTENTHALER Markus	UNIVERSITY OF VIENNA, VIENNA, AT
MYKHAILIUK Pavel	ENAMINE, KYIV, UA
N.V. ORTIZ ZACARIAS Natalia	LACDR, LEIDEN UNIVERSITY, LEIDEN, NL
NAITO Mikihiko	NATIONAL INSTITUTE OF HEALTH SCIENCES, TOKYO, JP
NAUTA Andele	PROF. DR. W. TH NAUTA STICHTING, , NL
NEGAHBAN Marzieh	QUEENSLAND UNIVERSITY OF TECHNOLOGY (QUT), BRISBANE, AU
NELSON Adam	UNIVERSITY OF LEEDS, LEEDS, GB
NERI Dario	ETH ZÜRICH, ZÜRICH, CH
NEVADO Cristina	UNIVERSITY OF ZURICH, ZURICH, CH
NEVSKAYA Alisa	RUDN UNIVERSITY, MOSCOW, RU
NEWTON Gary	DOMAINEX, LITTLE CHESTERFORD, GB
NICHOLS Paula	SYNPLE CHEM AG, ZURICH, CH
NIEDZIEJKO Piotr	ONCOARENDI THERAPEUTICS, WARSAW, PL
NISHIDA Tomoaki	SUMITOMO DAINIPPON PHARMA CO., LTD., OSAKA, JP
NOISIER Anaïs	ASTRAZENECA, MÖLNDAL, SE
NORDHOFF Sonja	GRÜNENTHAL GMBH, AACHEN, DE
NORTH Kiri	VERTEX PHARMACEUTICALS, OXON, GB
NOVAK Doroteja	UKC LJUBLJANA, RAKA, SI
NOVERGES Barbara	FAES FARMA, S.A., LEIOA, ES
NTIE-KANG Fidele	MARTIN-LUTHER UNIVERSITY, HALLE-WITTENBERG, HALLE (SAALE), DE
NUSDORFER Nika	FAKULTETA ZA FARMACIJO, PORTOROZ, SI
OBNISKA Jolanta	MEDICAL COLLEGE UJ, KRAKOW, PL
OBST-SANDER Ulrike	F.HOFFMANN - LA ROCHE, BASEL, CH
OHKI Hitoshi	DAIICHISANKYO CO., LTD., TOKYO, JP
OLEJNICZAK Sylwia	ONCOARENDI THERAPEUTICS SA, WARSAW, PL
ONTORIA Jesus Maria	IRBM SCIENCE PARK, POMEZIA, IT
OOST Thorsten	BOEHRINGER INGELHEIM, BIBERACH, DE
OPPONG Emmanuel	ABCR GMBH, KARLSRUHE, DE
ORGOVAN Zoltan	RCNS HAS, BUDAPEST, HU

ORTHOLAND Jean-Yves	EDELRIS, LYON, FR
OSLOVSKY Vladimir	ENGELHARDT INSTITUTE OF MOLECULAR BIOLOGY, RUSSIAN ACADEMY OF SCIENCES, MOSCOW, RU
OTSUKA Eri	KISHIDA CHEMICAL, OSAKA, JP
OTTMANN Christian	EINDHOVEN UNIVERSITY OF TECHNOLOGY, EINDHOVEN, NL
OVERKLEEFT Hermen	LEIDEN UNIVERSITY, LEIDEN, NL
OZAWA Shin-Ichiro	KITASATO UNIVERSITY, TOKYO, JP
OZYAZICI Tugce	YEDITEPE UNIVERSITY, ISTANBUL, TR
PACIFICI Pier Giorgio	IRBM SCIENCE PARK, POMEZIA, IT
PAJK Stane	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
PALISSE Adeline	GALAPAGOS NV, MECHELEN, BE
PALMER Brian	UNIVERSITY OF AUCKLAND, AUCKLAND, NZ
PALOMO Valle	CENTRO DE INVESTIGACIONES BIOLÓGICAS-CSIC, MADRID, ES
PALYULIN Vladimir A.	LOMONOSOV MOSCOW STATE UNIVERSITY, MOSCOW, RU
PAPAGIOUVANNIS Georgios	ARISTOTLE UNIVERSITY OF THESSALONIKI, THESSALONIKI, GR
PAPEO Gianluca	NERVIANO MEDICAL SCIENCES SRL, NERVIANO (MILANO), IT
PARDALI Vasiliki	NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS, ATHENS, GR
PARK Eunhye	SEOUL NATIONAL UNIVERSITY, SEOUL, KR
PARK Hyeung-Geun	COLLEGE OF PHARMACY, SEOUL NATIONAL UNIVERSITY, SEOUL, KR
PASCANU Vlad	UNIVERSITY OF ZURICH, ZURICH, CH
PATEL Jayendra	UNIVERSITY OF HELSINKI, HELSINKI, FI
PATTABHI Sunil	SYNGENE INTERNATIONAL LIMITED, BANGALORE, IN
PAVIC Kristina	FACULTY OF PHARMACY AND BIOCHEMISTRY, ZAGREB, HR
PEARTREE Philip	MANCHESTER ORGANICS LIMITED, RUNCORN, GB
PECAR FONOVIC Ursa	UNIVERSITY OF LJUBLJANA, FACULTY OF PHARMACY, LJUBLJANA, SI
PELLICCIARI Roberto	TES PHARMA SRL, CORCIANO, IT
PEPERIDOU Katerina	ARISTOTLE UNIVERSITY OF THESSALONIKI, THESSALONIKI, GR
PEREZ PACHECO Manuel	MESTRELAB RESEARCH, SANTIAGO DE COMPOSTELA, ES
PERKOVIC Ivana	FACULTY OF PHARMACY AND BIOCHEMISTRY, ZAGREB, HR
PERRON Quentin	IKTOS, PARIS, FR
PERUN Thomas J.	ACS/IUPAC, HEMPSTEAD, US
PETEK Nejc	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, ZAGORJE OB SAVI, SI

PETERLIN MASIC Lucija	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
PETRYK Vitalii	ABCR GMBH, BREMEN, DE
PETTERSSON Lars	IMMUNAHR AB, LUND, SE
PIERONI Marco	UNIVERSITA' DEGLI STUDI DI PARMA, PARMA, IT
PIETERS Roland	UTRECHT UNIVERSITY, UTRECHT, NL
PILLAIYAR Thanigaimalai	UNIVERSITY OF BONN, BONN, DE
PINTO DE SOUSA Emanuel	UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, GB
PIPA Gergely	INSTITUTE FOR GLYCOMICS, GOLD COAST, AU
PIPER Mike	BIOASCENT DISCOVERY LTD, , GB
PISMATARO Maria Chiara	UNIVERSITY OF MILAN, MILANO, IT
PITCHER Noel	MONASH INSTITUTE OF PHARMACEUTICAL SCIENCES, MELBOURNE, AU
PITON Nelly	DOMAINEX LTD, SAFFRON WALDEN, GB
PLANO Daniel	UNIVERSITY OF NAVARRA, PAMPLONA, ES
PLOWRIGHT Alleyn T.	SANOFI, FRANKFURT-AM-MAIN, DE
PORTER David	UNIVERSITY OF DUNDEE, DUNDEE, GB
PRASHANTKUMAR Khirsariya	ST.ANNE´S UNIVERSITY HOSPITAL BRNO, BRNO, CZ
PRATI Federica	ACRAF S.P.A., S. PALOMBA - POMEZIA, IT
PRAVEEN KUMAR Chinthakindi	UPPSALA UNIVERSITY, UPPSALA, SE
PRICE Alexander	DIG WORLDWIDE, KENT, GB
PRINCIC Grisa	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, PESNICA PRI MARIBORU, SI
PRISCAKOVA Petra	FACULTY OF MEDICINE, COMENIUS UNIVERSITY, BRATISLAVA, SK
PROJ Matic	FAKULTETA ZA FARMACIJO, POLJANE, SI
PROSZENYAK Agnes	SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, BUDAPEST, HU
PROUS Josep Jr.	PROUS INSTITUTE FOR BIOMEDICAL RESEARCH, BARCELONA, ES
PROVENZANI Riccardo	UNIVERSITY OF HELSINKI, HELSINKI, FI
PROVINS Laurent	UCB, BRAINE-L'ALLEUD, BE
PUDELKO Maciej	SELVITA S.A., KRAKOW, PL
PULZ Robert	NOVARTIS PHARMA, BASEL, CH
QUARANTA Laura	SYNGENTA AG, BASEL, CH
RACKHAM Mark	BENEVOLENTAI, LONDON, GB
RADZIMIERSKI Adam	SELVITA S.A., KRAKOW, PL

RAJCOM Olivier	FIDELTA D.O.O., ROMAINVILLE, FR
RAJIC Zrinka	UNIVERSITY OF ZAGREB, ZAGREB, HR
RAMACHANDRA Murali	AURIGENE DISCOVERY TECHNOLOGIES LTD, BANGALORE, IN
RAMILO-GOMES Filipa	INSTITUTO SUPERIOR TÉCNICO -UNIVERSIDADE DE LISBOA, LISBON, PT
RAMURTHY Savithri	NOVARTIS, EMERYVILLE, US
RATNI Hasane	F. HOFFMANN-LA ROCHE LTD, BASEL, CH
RAUTIO Jarkko	UNIVERSITY OF EASTERN FINLAND, KUOPIO, FI
RAVINA Enrique	UNIVERSIDADE DE SANTIAGO DE COMPOSTELA, SANTIAGO DE COMPOSTELA, ES
RAYMOND Justine	FUNDACIÓ INSTITUT CATALA INVESTIGACIO QUIMICA, TARRAGONA, ES
REDDAVIDE Francesco	DYNABIND GMBH, DRESDEN, DE
REKKA Eleni	ARISTOTELIAN UNIVERSITY OF THESSALONIKI, THESSALONIKI, GR
RENAUD Jean-Paul	RIBOSTRUCT, OSTWALD, FR
RENN Christian	UNIVERSITY OF BONN, BORNHEIM, DE
REPISKA Vanda	FACULTY OF MEDICINE, COMENIUS UNIVERSITY, BRATISLAVA, SK
RESSURREICAO Ana	IMED.ULISBOA (INSTITUTO DE INVESTIGAÇÃO DO MEDICAMENTO), LISBOA, PT
REVESZ Tamas	BIOLOGICAL RESEARCH CENTRE, HUNGARIAN ACADEMY OF SCIENCES, SZEGED, HU
REXEN ULVEN Elisabeth	UNIVERSITY OF SOUTHERN DENMARK, ODENSE M, DK
RHEINHEIMER Joachim	BASF, LUDWIGSHAFEN AM RHEIN, DE
RICCI Antonio	F. HOFFMANN-LA ROCHE AG, BASEL, CH
RICHARDSON Reg	CHEMBRIDGE CORPORATION EUROPE, WORCESTERSHIRE, GB
RIDGWAY Peter	REACH SEPARATIONS, NOTTINGHAM, GB
RISSEEUW Martijn	UGENT, GHENT, BE
RIVERA SANCHEZ Gildardo	INSTITUTO POLITECNICO NACIONAL, REYNOSA, MX
RIXEN Sophia	CHRISTIAN-ALBRECHTS-UNIVERSITY, KIEL, DE
ROCHE Didier	EDELRIS, LYON, FR
RODESCHINI Vincent	EDELRIS, LYON, FR
RODRIGUEZ Raphaël	INSTITUT CURIE, PARIS, FR
RODRIGUEZ LOZADA Josue	UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MORELOS, CUERNAVACA, MX
RODRÍGUEZ-GIMENO Alejandra	ISTITUTO ITALIANO DI TECNOLOGIA, GENOVA, IT
ROGER Moses	CHEMICAL CONSULTANCY LTD., CORNWALL, GB
RÖHN Ulrike	BAYER AG, BERLIN, DE

RONAN Baptiste	SANOFI, VITRY SUR SEINE, FR
ROONEY Tim	UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, GB
ROZOT Roger	L'ORÉAL RECHERCHE & INNOVATION, AULNAY-SOUS-BOIS, FR
RUBIO RUIZ Belen	UNIVERSITY OF EDINBURGH, EDINBURGH, GB
RÜBSAMEN-SCHAEFF Helga	AICURIS GMBH & CO. KG, WUPPERTAL, DE
RYABUKHIN Sergey	ENAMINE LTD, KYIV, UA
RYU Jae-Sang	EWHA WOMANS UNIVERSITY, SEOUL, KR
SAARBACH Jacques	UNIVERSITÉ DE GENÈVE, GENEVE 4, CH
SACCONE Irene	UNIVERSITY OF NAPLES, NAPLES, IT
SAGUD Ivana	FACULTY OF CHEMICAL ENGINEERING AND TECH., ZAGREB, HR
SAITO Tadataka	JAPAN TOBACCO, TAKATSUKI, JP
SALO Harri	ORION PHARMA, TURKU, FI
SALVADOR ODEN Lourdes	MEDIVIR AB, HUDDINGE, SE
SAMAIN Florent	PHILOCHEM AG, OTELFINGEN, CH
SANDOVAL-RAMIREZ Jesus	UNIVERSIDAD AUTONOMA DE PUEBLA, PUEBLA, MX
SANNINO Alessandro	PHILOCHEM AG, OTELFINGEN, CH
SANTANA Fabian	PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE, SANTIAGO, CL
SANTANGELO Ellen	SPRINTBIOSCIENCE, HUDDINGE, SE
SANTOS Maria M. M.	FACULTY OF PHARMACY, UNIVERSIDADE DE LISBOA, LISBOA, PT
SANTOS Webster L.	VIRGINIA TECH, BLACKSBURG, US
SARI Suat	HACETTEPE UNIVERSITY, ANKARA, TR
SAVATEEV Konstantin	URAL FEDERAL UNIVERSITY, EKATERINBURG, RU
SAVIC Jelena	UNIVERSITY OF BELGRADE-FACULTY OF PHARMACY, BELGRADE, YU
SAXTY Gordon	FIDELTA, ZAGREB, HR
SBARDELLA Gianluca	UNIVERSITY OF SALERNO, FISCIANO (SA), IT
SCAFFIDI Salvatore	UNIVERSITAT DE BARCELONA, BARCELONA, ES
SCAMMELLS Peter	MONASH UNIVERSITY, MELBOURNE, AU
SCHAAKE Benjamin	FAU ERLANGEN-NUREMBERG, ERLANGEN, DE
SCHEFFLER Frank	HYPHA DISCOVERY LTD, SLOUGH, GB
SCHEPENS Wim	JANSSEN PHARMACEUTICA NV, BEERSE, BE
SCHEUERMANN Jörg	ETH ZURICH, ZÜRICH, CH

SCHIEMANN Kai	MERCK KGAA, DARMSTADT, DE
SCHIFFRER Eva Shannon	UNIVERSITY OF LJUBLJANA, FACULTY OF PHARMACY, ZIROVNICA, SI
SCHIO Laurent	SANOFI, VITRY SUR SEINE, FR
SCHMIES Constanze	UNIVERSITY OF BONN, BONN, DE
SCHOU Magnus	KAROLINSKA INSTITUTE, STOCKHOLM, SE
SCOFFIN Robert	CRESSET, CAMBRIDGESHIRE, GB
SEAL Jon	GLAXOSMITHKLINE, STEVENAGE, GB
SEBASTIAN PÉREZ Víctor	SPANISH NATIONAL RESEARCH COUNCIL (CSIC), MADRID, ES
SEBOK Ferenc	GEDEON RICHTER PLC., BUDAPEST, HU
SEEBERGER Peter H.	MAX PLANCK INSTITUTE OF COLLOIDS AND INTERFACES, BERLIN, DE
SEGLER Marwin	BENEVOLENTAI, LONDON, GB
SEITZBERG Jimmi	LEO PHARMA A/S, BALLERUP, DK
SELWOOD David	UNIVERSITY COLLEGE LONDON, LONDON, GB
SERDA Maciej	UNIVERSITY OF SILESIA IN KATOWICE, KATOWICE, PL
SERRA Patricia	IMED.ULISBOA - RESEARCH INSTITUTE FOR MEDICINES, LISBOA, PT
SETO Ryoko	KISHIDA CHEMICAL CO., LTD, OSAKA, JP
SHAABANI Shabnam	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
SHEN Jingkang	SHANGHAI INSTITUTE OF MATERIA MEDICA, SHANGHAI, CN
SHEN Hong	ROCHE INNOVATION CENTER, SHANGHAI, CN
SHEN Qilong	SHANGHAI INSTITUTE OF ORGANIC CHEMISTRY, SHANGHAI, CN
SHETH Jay	EUROFINS ADVINUS LIMITED, BANGALORE, IN
SHI Zhang-Jie	FUDAN UNIVERSITY, SHANGHAI, CN
SHIRINZADEH Hanif	ERZINCAN UNIVERSITY, ERZINCAN, TR
SHUTO Satoshi	HOKKAIDO UNIVERSITY, SAPPORO, JP
SIJM Maarten	VRIJE UNIVERSITEIT AMSTERDAM, AMSTERDAM, NL
SILVERMAN Richard B.	NORTHWESTERN UNIVERSITY, EVANSTON, US
SKOK Ziga	UNIVERSITY OF LJUBLJANA, FACULTY OF PHARMACY, LJUBLJANA, SI
SKOLC David	UCB PHARMA, BRAINE-L'ALLEUD, BE
SLATER Martin	CRESSET, LITLINGTON CAMBRIDGESHIRE, GB
SLOWINSKI Franck	SANOFI, CHILLY MAZARIN, FR
SMALLEY Adam	UCB, SLOUGH, GB
SMIDLEHNER Tamara	RUDER BOSKOVIC INSTITUTE, ZAGREB, HR

SMON Spela	UNIVERSITY OF LJUBLJANA, SLOVENJ GRADEC, SI
SMYTH Lynette	ABBVIE DEUTSCHLAND GMBH & CO.KG, LUDWIGSHAFEN, DE
SOLOMONSZ Arran	ASYNT, ISLEHAM, CAMBS, GB
SOSIC Alice	UNIVERSITY OF PADOVA, PADOVA, IT
SOSIC Izidor	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
SOVA Matej	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
SPILOVSKA Katarina	CHARLES UNIVERSITY, PRAGUE, CZ
SPÖRI Bodo	DOMAINEX, SAFFRON WALDEN, GB
STANCHEV Stancho	INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY, PRAGUE, CZ
STAREK Beata	UNIVERSITY OF WARSAW, WARSZAWA, PL
STAWUJAK Krzysztof	SELVITA S.A., KRAKOW, PL
STEFANACHI Angela	UNIVERSITÀ DEGLI STUDI DI BARI, BARI, IT
STEINEBACH Christian	UNIVERSITY OF BONN, BONN, DE
STEWART Alison	BENEVOLENTAI, CAMBRIDGE, GB
STILZ Hans Ulrich	NOVO NORDISK, MALOV, DK
STOLL Theodor	F. HOFFMANN-LA ROCHE AG, BASEL, CH
STRASEK Nika	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, POSTOJNA, SI
SUMIKAWA Yoshitake	KAKEN PHARMACEUTICAL CO., LTD., KYOTO, JP
SUMMERS Declan	GLAXOSMITHKLINE, STEVENAGE, GB
SUTANTO Fandi	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
SVETE Jurij	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
SZANTO Gabor	GEDEON RICHTER PLC., BUDAPEST, HU
SZILLAT Hauke	SANOFI, FRANKFURT, DE
SZLAVIK Zoltan	SERVIER, BUDAPEST, HU
SZYMANSKA Ewa	JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE, KRAKOW, PL
TAL Daniel	WEIZMANN INSTITUTE, REHOVOT, IL
TALBOT Eric	PHARMARON, HODDESDON, GB
TANDARIC Tana	RUDER BOSKOVIC INSTITUTE, ZAGREB, HR
TERRETT Nick	MERCK SHARP & DOHME RESEARCH GMBH (MSD), KRIENS, CH
THI ANH NGUYEN Ngoc	ASTON UNIVERSITY, BIRMINGHAM, GB
THOM Steve	SYGNATURE DISCOVERY, NOTTINGHAM, GB

THOMÄ Nicolas	FRIEDRICH MIESCHER INSTITUTE FOR BIOMEDICAL RESEARCH, BASEL, CH
THOMAS Russell John	AXXAM SPA, BRESSO, IT
THOMSEN Martin K.	AARHUS UNIVERSITY, AARHUS C, DK
THURING Jan Willem	JANSSEN PHARMACEUTICA NV, BEERSE, BE
TIAN Frank	WUXI APPTEC, MINNESOTA, US
TIBAUT Tjasa	NATIONAL INSTITUTE OF CHEMISTRY, LJUBLJANA, SI
TICHY Michal	IOCB AS CR, PRAGUE, CZ
TIEDT Stephanie	FAU ERLANGEN-NUREMBERG, ERLANGEN, DE
TIMMERMAN Henk	VU UNIVERSITY AMSTERDAM, OEGSTGEEST, NL
TIMUR Savchenko	ENAMINE LTD, KYIV, UA
TITZ Alexander	HELMHOLTZ INSTITUTE FOR PHARMACEUTICAL SCIENCES, SAARBRÜCKEN, DE
TIWARI Atul	SYNGENE INTERNATIONAL LTD, BANGALORE, IN
TODD Matthew	UNIVERSITY OF SYDNEY, SYDNEY, AU
TOMASIC Tihomir	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
TOMASKOVIC Linda	FIDELTA D.O.O., ZAGREB, HR
TOMICH Carlos	SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO, UNIVERSITY OF SÃO PAULO, RIBEIRAO PRETO-SP, BR
TOMOVIC Katarina	FACULTY OF MEDICINE, UNIVERSITY OF NIŠ, SERBIA, NIS, YU
TOPLAK Zan	STUDENT OF FFA, PTUJ, SI
TORRENS Antoni	ESTEVE, BARCELONA, ES
TOTH Gergely	CANTABIO PHARMACEUTICALS INC., SUNNYVALE, US
TOURWE Dirk	VUB, BRUSSELS, BE
TOZER Matthew	CONSULTANT, CAMBRIDGE, GB
TOZKOPARAN Birsen	HACETTEPE UNIVERSITY, ANKARA, TR
TROTTER Wes	MSD, BOSTON, US
TSCHAMMER Nuska	NANOTEMPER TECHNOLOGIES GMBH, MUNICH, DE
TSUKUDA Takuo	CHUGAI PHARMACEUTICAL, KAMAKURA, JP
TULLIN Soren	NOVO NORDISK A/S, BAGSVAERD, DK
TULLY David	NOVARTIS, EMERYVILLE, US
TURGUTALP Bengisu	YEDITEPE UNIVERSITY, ISTANBUL, TR
TURNER Sean C.	ABBVIE, LUDWIGSHAFEN AM RHEIN, DE
TWIGG David	ASTEX PHARMACEUTICALS, CAMBRIDGE, GB

TWIN Heather	VERTEX PHARMACEUTICALS, OXON, GB
TZALIS Dimitrios	TAROS CHEMICALS, DORTMUND, DE
TZVETKOV Nikolay	NTZ LAB LTD., SOFIA, BG
ULVEN Trond	UNIVERSITY OF COPENHAGEN, COPENHAGEN, DK
VADIVELU Saravanan	JUBILANTBIOSYS, BANGALORE, IN
VAH Luka	FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY, LJUBLJANA, SI
VAISANEN Emilia	ORION PHARMA, ESPOO, FI
VAKALOPOULOS Alexandros	BAYER AG, WUPPERTAL, DE
VALADE Anne	UCB, BRAINE-L'ALLEUD, BE
VALENTI Dario	TAROS CHEMICALS GMBH & CO. KG, DORTMUND, DE
VALENTINA Straniero	UNIVERSITÀ DEGLI STUDI DI MILANO, MILANO, IT
VALEUR Eric	ASTRAZENECA, IMED BIOTECH UNIT, GOTHENBURG, SE
VALLIN Karl	KAROLINSKA INSTITUTET, STOCKHOLM, SE
VAN AERSCHOT Arthur	KU LEUVEN, LEUVEN, BE
VAN BEELEN Eric	WATERS, SAINT-QUENTIN EN YVELINES, FR
VAN CALENBERGH Serge	UGENT, GHENT, BE
VAN DE GEVEL Iris	EINDHOVEN UNIVERSITY OF TECHNOLOGY, EINDHOVEN, NL
VAN DER VLAG Ramon	UNIVERSITY OF GRONINGEN, GRONINGEN, NL
VAN DER WAL Steffen	PEPSCAN, LELYSTAD, NL
VAN DIJK Evert	PEPSCAN, LELYSTAD, NL
VAN DONGEN Maria	JANSSEN PHARMACEUTICAL COMPANIES OF JOHNSON&JOHNSON, BEERSE, BE
VAN HIJFTE Luc	SCT & MERCACHEM-SYNCOM, NIJMEGEN, NL
VAN HOORN Willem	EXSCIENTIA, DUNDEE, GB
VAN WILTENBURG Jim	SYNCOM B.V., GRONINGEN, NL
VASSILIOU Stamatia	UNIVERSITY OF ATHENS-ELKE, ATHENS, GR
VEBER Katja	FAKULTETA ZA FARMACIJO, KRANJ, SI
VEGA ALANIS Blanca Angelica	TU WIEN, VIENNA, AT
VELCICKY Juraj	NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH, BASEL, CH
VELTHORST Nel H.	VRIJE UNIVERSITEIT AMSTERDAM, AMSTERDAM, NL
VERKAIK Saskia	ACERTA PHARMA BV, OSS, NL
VERMES Tamas	AICURIS ANTI-INFECTIVE CURES GMBH, WUPPERTAL, DE

VESELOVSKA Lucia	IOCB AS CR, PRAGUE, CZ
VINTER Adrijana	FIDELTA LTD, ZAGREB, HR
VIRGILI Marina	ENANTIA S.L., BARCELONA, ES
VISHWANATH Swamy	SAI LIFE SCIENCES, HYDERABAD, IN
VLASOV Sergei	TOMSK POLYTECHNIC UNIVERSITY, TOMSK, RU
VOLOCHNYUK Dmytro	ENAMINE LTD, KYIV, UA
VON NUSSBAUM Franz	BAYER, MONHEIM, DE
VRIES Ton	MERCACHEMSYNCOM, GRONINGEN, NL
VUORINEN Aini	UNIVERSITY OF OXFORD, OXFORD, GB
WADA Yasuhiro	ASAHI KASEI PHARMA CORPORATION, IZUNOKUNI, JP
WAHALA Kristiina	UNIVERSITY OF HELSINKI, HELSINKI, FI
WALDMANN Herbert	MAX PLANCK INSTITUTE OF MOLECULAR PHYSIOLOGY, DORTMUND, DE
WANDALL Hans	UNIVERSITY OF COPENHAGEN, COPENHAGEN, DK
WANG Jeh-Jeng	KAOHSIUNG MEDICAL UNIVERSITY, KAOHSIUNG, TW
WANG Bing	BIOMARIN PHARMACEUTICAL, INC., NOVATO, US
WANNER Benedikt	SYNPLE CHEM AG, ZÜRICH, CH
WATANABE Keiichi	KISHIDA CHEMICAL, OSAKA, JP
WATERLOO Lukas	FAU ERLANGEN-NUREMBERG, ERLANGEN, DE
WATSON Stephen	HEPTARES THERAPEUTICS, WELWYN GARDEN CITY, GB
WEBER Csaba	SERVIER RESEARCH INSTITUTE OF MEDICINAL CHEMISTRY, BUDAPEST, HU
WEIGAND Klaus	NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH, BASEL, CH
WEILER Sven	BASILEA PHARMACEUTICA INTERNATIONAL LTD., BASEL, CH
WEINREICH Frank	WILEY-VCH VERLAG GMBH & CO. KGAA, WEINHEIM, DE
WEISS Matjaz	UNIVERSITY OF LJUBLJANA, FACULTY OF PHARMACY, LJUBLJANA, SI
WENGLOWSKY Steve	BLUEPRINT MEDICINES, CAMBRIDGE, MA, US
WEST Ryan	UNIVERSITY OF SUSSEX, BRIGHTON, GB
WESTWELL Andrew D.	CARDIFF UNIVERSITY, CARDIFF, GB
WICHUR Tomasz	UNIWERSYTET JAGIELLONSKI COLLEGIUM MEDICUM, KRAKOW, PL
WIECKOWSKA Anna	JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE, KRAKOW, PL
WILKES Robin	CHARNWOOD MOLECULAR LTD, LOUGHBOROUGH, LEICS, GB
WILLIS Paul	MEDICINES FOR MALARIA VENTURE (MMV), GENEVA 15, CH

WINDHORST Bert	VU UNIVERSITY MEDICAL CENTER, AMSTERDAM, NL
WOJCIK Radoslaw	UNIVERSITY OF WARSAW, WARSAW, PL
WOUTERS Randy	KULEUVEN, LEUVEN, BE
WRASIDLO Wolfgang	UNIVERSITY OF CALIFORNIA SAN DIEGO, LA JOLLA, US
WRIGHT Miranda	UNIVERSITY OF OXFORD, OXFORD, GB
XIONG Bing	SHANGHAI INSTITUTE OF MATERIA MEDICA, SHANGHAI, CN
XU Bailing	INSTITUTE OF MATERIA MEDICA, BEIJING, CN
YAHIAOUI Mourad	MANCHESTER ORGANICS LIMITED, RUNCORN, GB
YAMAGISHI Kenji	NIHON UNIVERSITY, FUKUSHIMA, JP
YANG Xue	LEIDEN UNIVERSITY, LEIDEN, NL
YANG Byunggil	SEOUL NATIONAL UNIVERSITY, SEOUL, KR
YE In-Hae	JEIL PHARMACEUTICALS, YONGIN-SI, KR
YIN Hang	TSINGHUA UNIVERSITY, BEIJING, CN
YONG Cassandra	MONASH INSTITUTE OF PHARMACEUTICAL SCIENCE, SOUTH MELBOURNE, AU
YOO Kyung Ho	KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, SEOUL, KR
YOSHIDA Hisae	NIHON UNIVERSITY, FUKUSHIMA, JP
YOSHIKAWA Kenji	DAIICHI-SANKYO, TOKYO, JP
YOSUKE Kaburagi	EISAI, TSUKUBA-SHI, JP
YOUNG Rob	GLAXOSMITHKLINE, HERTFORDSHIRE, GB
ZACCONI Flavia	PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE, SANTIAGO DE CHILE, CL
ZAITSEVA Anna	LIFE CHEMICALS, KYIV, UA
ZAJDEL Pawel	JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE, KRAKOW, PL
ZAJEC Ziva	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
ZAWADZKA Magdalena	SELVITA S.A., KRAKOW, PL
ZEGA Anamarija	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
ZETTERBERG Fredrik	GALECTO BIOTECH, GOTHENBURG, SE
ZHANG Jiahua	MDPI, BEIJING, CN
ZHANG Xumu	SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY, GUANGDONG, CN
ZHANG Ming-Qiang	AMGEN, SHANGHAI CITY, CN
ZIDAR Nace	UNIVERSITY OF LJUBLJANA, LJUBLJANA, SI
ZITKO Jan	FACULTY OF PHARMACY HRADEC KRALOVE, HRADEC KRALOVE, CZ

ZOIDIS Grigorios	NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS, ATHENS, GR
ZORC Branka	FACULTY OF PHARMACY AND BIOCHEMISTRY, ZAGREB, HR
ZORN Ludwig	BAYER AG, BERLIN, DE
ZUK Justyna	UNIVERSITY OF MEDICINE, LUBLIN, PL
ZUMBRUNN Cornelia	IDORSIA PHARMACEUTICALS LTD, ALLSCHWIL, CH
ZWECKSTETTER Markus	MAX PLANCK INSTITUTE FOR BIOPHYSICAL CHEMISTRY, GÖTTINGEN, DE

EFMC-ISMC 2018
SYMPOSIUM SECRETARIAT
LD Organisation s.p.r.l.
Scientific Conference Producers
Rue Michel de Ghelderode 33/2
1348 Louvain-la-Neuve, Belgium

tel. office: +32 10 45 47 77
tel. onsite in Ljubljana: +32 472 651460
mail: secretariat@efmc-ismc.org
website: www.efmc-ismc.org

All information in this programme book is accurate at the time of printing