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BOOK OF ABSTRACTS

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PLENARY LECTURES

SCIENCE, ART AND DRUG DISCOVERY, A PERSONAL PERSPECTIVE

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At the start of our research programme that lead to amlodipine, a once-daily calcium antagonist for the treatment of angina and hypertension, there were over 90 published patents around the parent dihydropyridine ring system which posed a significant challenge for innovative drug design. Moreover, all agents of the class suffered poor pharmacokinetics, and there was little information on how these might be improved. However, rational medicinal chemistry led to a novel series of dihydropyridines with potent calcium antagonist activity which displayed high, and uniform bioavailability, together with long plasma half-lives. After extensive pharmacological profiling, UK 48,340 (amlodipine) was selected for clinical development and subsequently received worldwide approval as Norvascä for the treatment of hypertension and angina. Norvascä became the world's leading antihypertensive agent and the fourth best selling drug, with some billions of patient days of therapy achieved since launch

Sildenafil, the first oral treatment for male erectile dysfunction, was the result of a cardiovascular research programme to block the action of PDE 5 and increase tissue levels of cGMP, even though the endogenous ligand that stimulated guanylate cyclase was unknown at the time. Starting from zaprinast, a weak and non-selective PDE 5 inhibitor, computer modelling guided rational medicinal chemistry to achieve significant increases in potency and selectivity within a novel series of pyrazolopyrimidones. Optimisation of SARs and pharmacokinetics led to UK 92,480 (sildenafil) that was essentially devoid of cardiovascular activity in clinical trials. However, the emerging role of nitric oxide and cGMP in controlling blood flow in the penis suggested that sildenafil would have a beneficial effect on erectile dysfunction. This hypothesis was confirmed by extensive clinical trials in nearly 5,000 patients and sildenafil was approved as Viagraä for the treatment of male erectile dysfunction. Viagraä became one of the most widely prescribed medicines, and has been used by 100s of millions of patients throughout the World.

These research programmes will be discussed from a personal perspective that will highlight the importance of multidisciplinary project teams, challenges that arose during discovery and development, and factors that influenced key decisions.

THE EUROPEAN RESEARCH COUNCIL (ERC) AND ITS SUPPORT FOR MEDICINAL CHEMISTRY

Klaus Bock

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Set up in 2007 by the European Union, the fundamental activity of the European Research Council (ERC) is to provide attractive, long-term funding to support excellent investigators and their research teams to pursue ground-breaking, high-gain/ high-risk research. Supporting best researchers in any field of research on the sole criterion of excellence is expected to have a direct impact through advances at the frontier of knowledge, opening the way to creating new scientific and technological results, which ultimately can lead to innovation. The ERC uses a typical panel-based peer-review system, in which panels of high-level scientists and/or scholars make recommendations for funding. The ERC panel structure consists of 25 panels across three domains: Social sciences and Humanities (SH), Life sciences (LS), Physical and Engineering Sciences (PE).

At ERC chemical research is funded mainly in two big panels: PE4 “Physical and Analytical Chemical Sciences” and PE5 “Synthetic Chemistry and Materials”. Around 580 Starting, Consolidator and Advanced grants were awarded between 2007 and 2015 in these two panels with a total value of 1 billion euro (or almost 10% of the entire ERC budget awarded to the three main types of grants in this period).

About 1 in 6 grants in panels PE4 and PE5 are working on medicinal chemistry or perform research with impact in medicine and on medical applications (including drugs). The areas where ERC grantees are most active are:

- the creation of new molecular entities and subsequent exploitation of their properties for drug design and synthesis. The problems and difficulties associated with chemical syntheses (e.g. more rapid and robust techniques in organic synthesis) are addressed in many of these grants. A few projects are in the area of bioinorganic chemistry and explore new therapeutic applications of metal complexes. Other projects tend to focus on mechanistic studies of biochemical processes and reactions, on proteomics and on new approaches in drug design and delivery. Projects dealing with drug design cover a broad spectrum of research questions, ranging from development and analysis of new drugs and their impact on specific diseases (cancer, Alzheimer's disease, HIV, etc.) to the development of improved methods for drug analysis (more selective and efficient screening methods, enhancing the existing libraries of spectra of compounds, etc.);

- research for improved diagnosis, early diagnosis and prognosis for preventive and personalized medicine;

- design and preparation of new materials that interact with components of living systems in view of therapeutic or diagnostic applications. Materials targeted by these projects are, for example, hydrogels with applications in tissue engineering and repair, optical metamaterials, supramolecular biomaterials etc. Nanoscale engineering is a promising road for the development of novel materials with tailor-made properties, achieved by precise control of the materials structure and composition;

- development of advanced imaging techniques (e.g. magnetic resonance, surgical imaging) or spectroscopic methods used in medicine.

Projects doing research in medicinal chemistry are found in other ERC panels as well, especially in some of the LS panels and, to a lesser degree, in the engineering panels (PE7 “Systems and communication engineering” and PE8 “Products and process engineering”).

My presentation will offer more details on the scope and objectives of ERC projects in medicinal chemistry as well as information about their results.

LATE-STAGE FLUORINATION

Tobias Ritter (1,2,3)

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The unnatural isotope fluorine–18 (^{18}F) is used as a positron emitter in molecular imaging. Currently, many potentially useful ^{18}F-labeled probe molecules are inaccessible for imaging, because no fluorination chemistry is available to make them. Syntheses must be rapid on account of the 110-minute half-life of ^{18}F and benefit from using [^{18}F]fluoride due to practical access and suitable isotope enrichment. But [^{18}F]fluoride chemistry has been limited in reaction and substrate scope. I will describe the development of novel, modern fluorination reactions and evaluate them based on their utility for F-19 and F-18 chemistry. Late-stage fluorination enables the synthesis of new drug candidates and conventionally unavailable positron emission tomography (PET) tracers for anticipated applications in pharmaceutical development as well as pre-clinical and clinical PET imaging.

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CROSS-COUPLING APPROACHES TO SATURATED N-HETEROCYCLES

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Chiral saturated N-heterocycles including morpholines, piperazines, piperadines, diazepanes – as well as bicyclic and spirocyclic variants – are currently the most important scaffolds for the design and development of new small molecule pharmaceuticals. To improve synthetic access to these structures, our group has developed new reagents for preparing substituted variants from readily available aldehydes and ketones. Successes to date include SnAP (tin amine protocol) and SLAP (silicon ligh amine protocol) reagents, both of which operate via radical pathways. They are characterized by simple reaction protocols and exceptional substrate scope – aromatic, heteroaromatic, aliphatic, and glyoxic aldehydes are all excellent reaction partners. In many cases, spirocycles can be accessed by using ketones as substrates. Importantly, the SnAP and SLAP reactions afford the unprotected amines as the product.

Many of these reagents are commercially available, allowing diverse saturated N-heterocycles to be accessed in one step. Selected exampled of products formed with these reagents are shown in Figure 1.

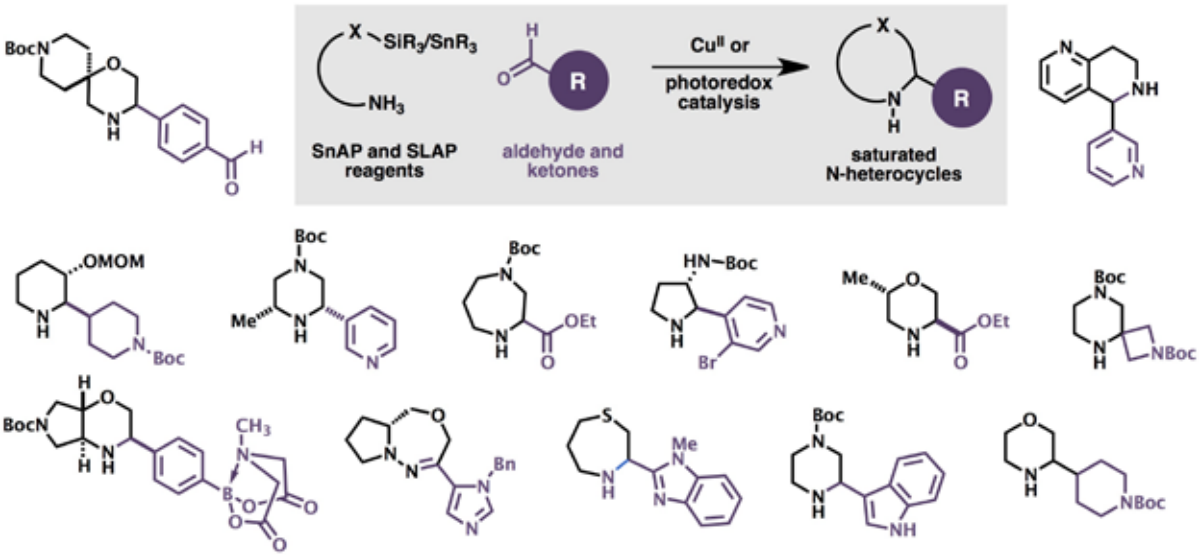

Figure 1. SnAP and SLAP reagents for the one-step synthesis of saturated N-heterocycles. Representative products of the various reagent types and diverse aldehydes are shown.

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ROBOTICS, ARTIFICIAL INTELLIGENCE, AND MEDICINAL CHEMISTRY: A LOOK INTO THE FUTURE

Derek Lowe

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Advances in robotic technology have already transformed several industries, and are making new inroads every year. Automated systems are common in analytical chemistry, and may soon be ready for wider use in synthetic organic and medicinal chemistry, fulfilling years of (perhaps premature) predictions. Past the physical manipulation of chemical samples, however, is the automation of some intellectual aspects of the field, which may be closer to real-world use than many chemists realize. This talk will look at the possible disruptive implications of these technologies for medicinal chemistry research and organic chemistry in general.

DNA-ENCODED CHEMICAL LIBRARIES

Dario Neri

ETH Zürich (Zürich, Switzerland) and Philochem AG (Otelfingen, Switzerland)

DNA-encoded chemical libraries (DECLs) are large collections of chemical compounds, individually encoded by distinctive DNA fragments serving as amplifiable identification barcodes. Innovative encoding procedures and synthetic strategies allow the construction and screenings of chemical libraries, containing hundreds of millions of compounds.

Two main types of DECLs can be constructed at used for screening purposes: (i) single-pharmacophore encoded chemical libraries (in which individual molecules, no matter how complex, are attached to one of the two strands of the DNA double helix), and (ii) dual-pharmacophore encoded chemical libraries (in which two sets of molecules are attached to the neighboring extremities of the two DNA strands, allowing a combinatorial self-assembly of the library).

In this lecture, I will first present basic concepts related to the implementation of DECL technology, and will then expand with experimental examples of ligands isolated from large chemical libraries, including "difficult" pharmaceutical targets.

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HETEROCYCLES AND MEDICINAL CHEMISTRY: THE IMPORTANCE OF INNOVATIVE SYNTHESIS

Anthony Wood

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Heterocyclic organic chemistry lies at the heart of modern medicinal chemistry design and synthesis. The lecture will use examples from recent projects at Pfizer to illustrate the importance of novel heterocycle synthesis as a means of accessing target molecules with the properties needed to meet demanding clinical candidate profiles. Examples will be described from Pfizer SGLT2, BACE and ALK projects.

AWARD & PRIZE LECTURES

MOLECULAR RECOGNITION STUDIES WITH CHEMICAL AND BIOLOGICAL SYSTEMS: A MULTIDIMENSIONAL APPROACH TO SUPPORT STRUCTURE-BASED DRUG DESIGN

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In collaborative networks of scientists from academia and industry, we pursue a multidimensional approach towards deciphering and quantifying weak intermolecular interactions in chemical and biological systems. Experimental study in this research, in close collaboration with scientists from Roche Basel, involves the investigation of protein-ligand interactions, synthetic host-guest complexation, and dynamic processes in designed unimolecular model systems, such as molecular torsional balances. These investigations are complemented by computational analysis and exhaustive data base mining in the Cambridge Crystallographic Database (CSD) and the Protein Data Bank (PDB). Examples of intermolecular interactions quantified by this approach are orthogonal dipolar interactions with a focus on organofluorine interactions, stacking of heteroarenes and fluorinated phenyl rings on peptide bonds in proteins, and halogen bonding. Halogen bonding interactions are found of similar strength to strong neutral hydrogen bonds. We also investigate the energetics of the replacement of conserved water molecules in protein co-crystal structures by ligand parts. Lessons learned from these fundamental studies are directly applicable to improve ligand design and optimization in drug discovery research.[1]

This multidimensional approach is illustrated in examples taken from three structure-based design projects. In collaboration with the group of G. Klebe at the Univ. Marburg, we explored the energetically favorable replacement of individual water molecules in water clusters by ligand parts in protein-ligand complexes of tRNA-guanine transglycosylase (TGT), a target against bacterial shigellosis dysenteriae. Together with the group of R. A. Engh at the Univ. of Tromsø, we developed a strategy how to address the glycine-rich loop at the ATP binding site of protein kinase A (PKA) by establishing a dense cooperative loop-ligand interaction network. This strategy is generally applicable to gain Gibbs energy from binding to the glycin-rich loop in protein kinases. Our search for new antimalarials is illustrated for serine hydroxymethyl transferase (SHMT), a key enzyme from the folate cycle for which ligands had surprisingly not been reported previously. This work is pursued in collaboration with, among many others, scientists from BASF, the Swiss Tropical and Public Health Institute (STPHI), and Mahidol University in Thailand.

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TRAVERSING THE VALLEY OF DEATH IN ANTICANCER DRUG DISCOVERY

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Anticancer drug discovery is a notoriously difficult enterprise, with only a fraction of programs advancing candidates to human clinical trials. And, once in the clinic, estimates suggest that less than 1 in 10 compounds becomes an approved drugs. The costs of these failures – in money, time, and patient lives – are enormous. Although the cause of these inefficiencies are myriad and complex, one major reason is the inability of rodent models of cancer to recapitulate the complexity of the human disease and predict response to therapeutics. At the University of Illinois we have been selecting candidate compounds based on their performance in the treatment of pets with cancer. These veterinary cancer patients (dogs and cats) have spontaneous disease, heterogeneous tumor populations, and metastases, and in many cases their cancers have remarkable similarities to the human cancer. This lecture will describe how the treatment of canine cancer patients facilitated the advancement of a procaspase-3 activator, called PAC-1, through the valley of death and into clinical trials in human cancer patients.

NANOBODY-ENABLED HTS FOR THE DISCOVERY OF GPCR AGONISTS

Jan Steyaert

Vrije Universiteit Brussel (VUB)
Belgium

Fragment-based drug discovery uses low-molecular-weight, moderately lipophilic, and highly soluble fragments as starting points for developing novel drugs. FBDD is particularly advantageous for its ability to more completely assess “compound space” for molecules that interact with the target of interest. Last years, our lab has shown that Nanobodies are emerging tools for GPCR drug discovery. Nanobodies, the recombinant antigen binding fragments of camelid heavy-chain only antibodies, have emerged as important research tools to lock GPCRs in particular conformational states. Active-state stabilizing nanobodies have elucidated several agonist-bound structures of hormone-activated GPCRs and have provided insight into the dynamic character of receptors. Nanobodies have also been used to stabilize transient GPCR transmembrane signaling complexes, yielding the first structural insights into GPCR signal transduction across the cellular membrane

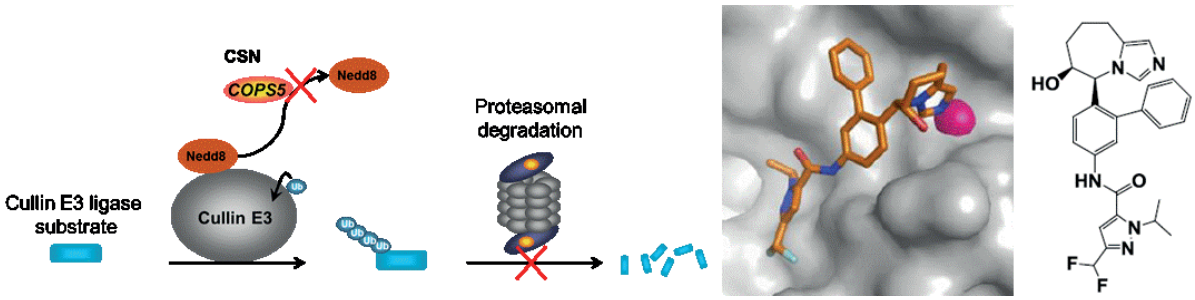
Building on our technology, we have developed a Nanobody-enabled fragment screening approach to explore new chemical space for the development of drugs targeting GPCRs. Our approach has the competitive advantage to other methods that we can screen fragments that exclusively bind to particular functional conformations of the receptor allowing us to triage our fragments according to efficacy profile and potency from a single biophysical assay. Nanobody-enabled screening of a moderate sized fragment library of 1000 compounds led to the discovery of several fragments with an agonist efficacy profile.

INHIBITION OF THE COP9 SIGNALOSOME AS A NOVEL APPROACH TO TREAT CANCER

Jean Quancard

Novartis Pharma
Switzerland

The COP9 signalosome (CSN) is the platform for assembly and disassembly of cullin-RING E3 ubiquitin ligases (CRL). Over 200 CRL complexes are implicated in the regulation of numerous cellular processes and aberrant CRL activity is frequently associated with cancer. Since CSN functions as the metallo-protease which cleaves the ubiquitin-like protein Nedd8 from CRLs and thereby initiates their disassembly, inhibitors of the catalytic subunit CSN5 may have therapeutic potential in cancers. We will describe the discovery and optimization of CSN5 inhibitors which led to potent, selective and orally available compounds. Hit finding on this complex of 8 proteins required a combination of biochemical assays and biophysical techniques such as NMR, SPR and affinity based MS on diverse components of the signalosome. The compounds have an unusual imidazole based Zinc binding motif and were optimized using structure based drug design. Initial optimization of biochemical potency led however to limited effects in cellular assays. We discovered that physico-chemical properties needed to be tuned to get better biochemical/cellular potency correlation. The optimized compounds trap CRLs in the active state but lead nevertheless to their inactivation by inducing degradation. As a result, corresponding CRL substrates, e.g. tumour suppressors p21 and p27, are stabilized and cell proliferation is inhibited. However, they exhibit a more differentiating effect than proteasome inhibitors which are used in the clinic, showing potential for better on-target safety. An advanced candidate suppressed growth of a human xenograft in mice and was well tolerated. These results provide insights into how CSN regulates CRLs and suggest that CSN5 inhibition has therapeutic potential for the treatment of cancer.



SIGMA HOLE BONDING IN KINASE DRUG DISCOVERY

Frank M. Boeckler

Eberhard Karls University, Tuebingen, Germany

Particularly in life sciences and drug discovery, halogen bonding has gained a lot of attention in recent years [1]. We extensively used QM model calculations on the MP2/TZVPP-level of theory to systematically map the relationship between strength and geometry of halogen bonds to different interaction partners (carbonyl backbone, sulfur contacts, nitrogen contacts, carboxylates, π-systems, …) [2-4]. We evaluated the potential for molecular design of additional halogen bonds in existing protein-ligand complexes of the PDB by applying XBScore, our first QM-derived scoring function for the recognition of contacts to the carbonyl backbone [5]. In addition, we used support vector regression to develop a QM-based scoring function for the recognition of halogen bonds targeting methionine. Still, application in molecular design can be limited by the high molecular precision needed for an appropriate prediction. We have recently demonstrated this, by studying the halogen bonding to the gatekeeper methionine in c-Jun N-terminal kinase 3 [6]. Surprisingly, exchange of a chlorine into bromine or iodine yielded a plateau of affinity, but showed effects on selectivity. The reason for this unexpected behavior was elucidated by solving the crystal structure of the iodinated ligand in complex with JNK3. Formation of a bivalent halogen/chalcogen bond toward the sulfur of the gatekeeper methionine rationalizes the observed behavior and may have an interesting impact on inducing kinase selectivity in the future.

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THE DISCOVERY OF SOFOSBUVIR: A BREAKTHROUGH CURATIVE THERAPY FOR HEPATITIS C

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Approximately 180 million individuals worldwide are infected with the hepatitis C virus (HCV). HCV infection ultimately leads to chronic liver disease, liver cirrhosis and eventually hepatocellular carcinoma. Sofosbuvir is a liver targeted nucleotide prodrug that acts upon the HCV RNA-dependent RNA polymerase (RdRp), an enzyme essential for viral replication. Sofosbuvir has become the backbone agent in combination regimens for HCV curative therapies. In a broad patient population and across all viral genotypes, sofosbuvir based regimens have demonstrated cure rates in excess of 90% with no observed resistance and a stellar safety profile. Since sofosbuvir's approval in the United States in 2013 and in the European Union in 2014, its use has led to the cure of many patients suffering from HCV. This presentation will discuss the discovery and development of sofosbuvir as a treatment for HCV. It will highlight the design and SAR around both the nucleoside and prodrug moieties, evaluation process used to ultimately select the optimal clinical candidate and the development of novel chemistry to prepare diastereomerically pure nucleoside phosphate prodrugs central to the development of sofosbuvir.

NOTES

INVITED LECTURES & ORAL COMMUNICATIONS

THE NEED OF IMPLEMENTING INTRAMOLECULAR HYDROGEN BONDING (IMHB) CONSIDERATIONS IN DRUG DISCOVERY AND HOW TO DO IT

Giulia Caron

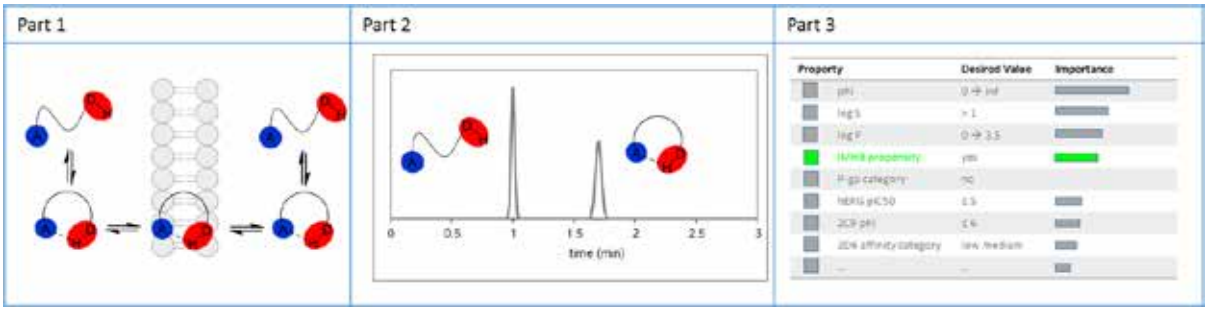
University of Torino, Molecular Biotechnology and Health Sciences Dept., Via Quarello, 15, 10135 Torino, Italy

The talk is divided into three parts.

Firstly, the propensity of compounds to form intramolecular hydrogen bonding (IMHB) is introduced as a molecular property relevant for solubility, permeability, and drug/receptor interaction. The impact of the environment is also discussed and the influence of ionization as well.

The second part of the talk focuses on new HT experimental and computational tools [1,2,3] in the determination of the tendency of candidates to form intramolecular interactions. Examples for both small molecules and macrocycles are provided.

Finally, some suggestions about the implementation of IMHB considerations in multiparameter optimization (MPO) strategies are given.



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INTRAMOLECULAR HYDROGEN BOND EXPECTATIONS IN MEDICINAL CHEMISTRY

Fabrizio Giordanetto (1), Christian Tyrchan (2), Johan Ulander (3)

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The design of molecular modifications that favor or disrupt the formation of intramolecular hydrogen bonds in a ligand structure is a well-known medicinal chemistry tactic. By perturbing the interaction of polar atoms in a given compound, a number of electronic and conformational effects can be postulated with potential positive effects on key optimization properties, such as biological activity, lipophilicity, solubility, metabolic stability, permeability and absorption among others. A survey of the current body of evidence for intramolecular hydrogen bonds in small molecules drug discovery projects is presented to assess its impact.

INTRAMOLECULAR HYDROGEN BONDING AS A DESIGN ELEMENT IN MEDICINAL CHEMISTRY

Bernd Kuhn, Peter Mohr, Martin Stahl

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The formation of intramolecular hydrogen bonds has a pronounced effect on molecular conformation and physicochemical properties. If hydrogen bonding functionalities and intramolecular ring size are suitably chosen receptor binding affinity as well as the ADMET profile of a compound can be improved. To allow for a more rational use of this interaction, we have derived propensities for internal hydrogen bond formation of different ring topologies and characterized specially designed model systems with respect to membrane permeability, water solubility, and lipophilicity.[1] In this presentation, we will highlight some of the more interesting ring systems with additional matched molecular pair analyses and examples from drug discovery projects.

References

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INTRAMOLECULAR HYDROGEN BONDS IN THE DESIGN OF CREBBP BROMODOMAIN LIGANDS

Stuart J. Conway

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During the development of a series of dihydroquinoxalinone-based CREBBP bromodomain ligands, an intramolecular hydrogen bond was identified as preorganising the ligand into a conformation that favours protein binding. Here the role played by this hydrogen bond is explored through the synthesis of a number of derivatives designed with computational guidance.

STUDYING CILIARY TRAFFICKING AND HOW IT LEADS TO RAS DRUG CANDIDATES

Alfred Wittinghofer

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Abstract not available at the time of printing!

CHEMICAL BIOLOGICAL MODULATION OF KRAS-SIGNALING

Herbert Waldmann

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The Ras-proteins are lipidated membrane bound GTPases that function as molecular switches translating growth factor-derived signals into cell growth and differentiation. Mutations in Ras are found in ca. 20-30 % of all human tumours making this oncogene product one of the most relevant targets for the development of anti-cancer drugs. However, despite intense world-wide efforts direct interference with signaling by the Ras proteins has not led to clinically useful drugs yet.

In the lecture the modulation of lipidation-dependent mechanisms which orchestrate localization and signalling of KRas, the most important mutated Ras isoform will be presented. The lecture will focus on the development of small molecule inhibitors of farnesylated KRas shuttling by the chaperone PDEdelta and describe three generations of KRas-PDEdelta interaction inhibitors.

The most potent compounds inhibit oncogenic signaling in cells by alteration of Ras localization and suppress proliferation of human pancreatic ductal adenocarcinoma cells. These findings may inspire novel drug discovery efforts aimed at the development of anti-cancer drugs targeting tumors with mutations in KRas.

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INHIBITION OF PRENYLATED KRAS: TOOLS TO DISCOVER AND CHARACTERIZE RAS LIGANDS

Johanna M. Jansen (1), Charles Wartchow (1), Wolfgang Jahnke (2), Susan Fong (3), Laura Tandeske (3), Keith Pfister (1), Tatiana Zavorotinskaya (3), Zhenhao Fang (4), Christopher B. Marshall (4), Mitsuhiko Ikura (4), Dirksen Bussiere (1), Jan Marie Cheng (3), Kenneth Crawford (3), Yumin Dai (3), Jeffrey Dove (3), Eric Fang (3), Jean-Michel Florent (2), John Fuller (3), Alvar Gossert (2), Mohammad Hekmat-Nejad (3), Christelle Henry (2), Julia Klopp (2), William Lenahan (3), Arndt Meyer (1), Jamie Narberes (3), Gwynn Pardee (3), Savithri Ramurthy (1), Sebastien Rieffel (2), Darrin Stuart (3), Sharadha Subramanian (1), Tiffany Tsang (3), Stephania Widger (3), Armin Widmer (2), Aurelie Winterhalter (2), Isabel Zaror (3), Stephen Hardy (3)

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Therapeutically relevant inhibitors of mutant KRAS should block activation of the downstream effector pathways. We designed a novel coupled biochemical assay that measures KRAS4B G12V activation of the effector BRAF, which is dependent on prenylation of KRAS4B and the presence of lipids. An iterative hit generation process driven by this novel assay resulted in compounds that block biochemical and cellular functions of mutant KRAS4B with low micromolar activity. NMR studies with truncated protein (amino acids 1-169) identified a site at which compound binding stabilizes the inactive conformation of KRAS G12V. This site is located adjacent to switch-II and is similar to sites described by others. The K_d determined for this binding event is almost 3 orders of magnitude weaker than the IC_{50} and EC_{50} values measured in biochemical and cellular assays. In order to understand this difference, we developed a biophysical assay using biolayer interferometry which enabled binding studies in a system with full-length prenylated protein in the presence of lipids, to match the context of the biochemical and cellular assays. These data are further complemented by NMR studies with a synthetically lipidated KRAS4B tethered to nanodiscs, which enabled us to probe the effect of compound binding on the conformational states of bilayer-associated lipidated KRAS4B. Our results underscore the importance of incorporating the lipid bilayer environment into drug discovery efforts for membrane-associated target proteins such as KRAS.

SMALL MOLECULE BINDING SITES ON THE RAS:SOS COMPLEX CAN BE EXPLOITED FOR INHIBITION OF RAS ACTIVATION

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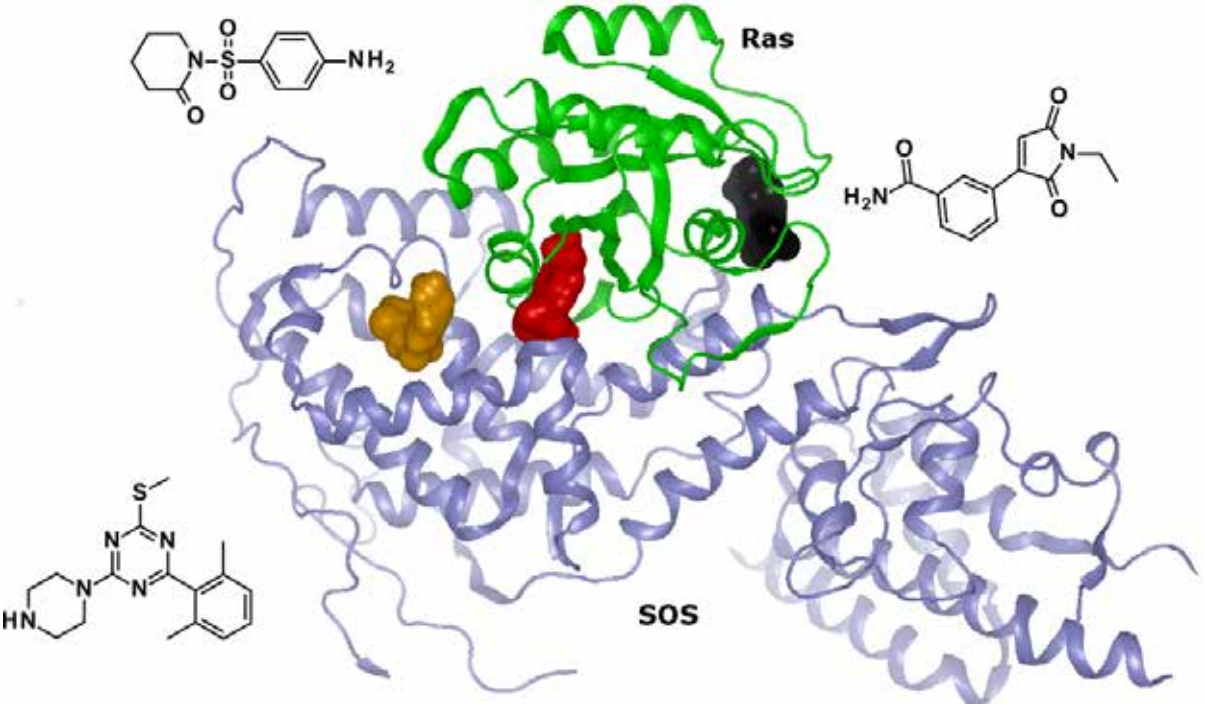
Constitutively active mutant KRas displays a reduced rate of GTP hydrolysis via both intrinsic and GTPase-activating protein-catalysed mechanisms, resulting in the perpetual activation of Ras signalling pathways. We performed a fragment screening campaign using X-ray crystallography that led to the discovery of three fragment binding sites on the Ras:SOS complex.



The identification of tool compounds binding at each of these sites allowed us to explore two new approaches to Ras pathway inhibition by either stabilising, or covalently modifying the Ras:SOS complex to prevent the reloading of Ras with GTP. We initially identified ligands that bound reversibly to the Ras:SOS complex in two distinct sites – one on SOS and the other at the Ras:SOS interface – but these compounds were not sufficiently potent inhibitors to validate our stabilisation hypothesis. We then performed a fragment screen of reactive ligands using LCMS detection to identify covalent modifiers of the Ras:SOS complex. This allowed us to demonstrate that covalent modification of Cys118 on the Ras subunit leads to a novel mechanism of inhibition of the SOS-mediated interaction between Ras and Raf, and is effective at inhibiting the exchange of labelled GDP in both mutant (G12C and G12V) and wild type Ras.

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NEW ANTIBACTERIAL AGENTS IN THE PIPELINE

Eric Desarbre

Basilea Pharmaceutica
Switzerland

Abstract not available at the time of printing!

MECHANISTIC AND INHIBITION STUDIES ON METALLO-BETA-LACTAMASES

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The metallo beta-lactamases (MBLs) are a growing clinical concern because they catalyse the hydrolysis of almost all beta-lactam antibiotics and serine beta-lactamase inhibitors. They are challenging targets from a medicinal chemistry perspective because of the variations in their structures and because they are structurally and mechanistically related to human MBL-fold proteins which have important roles, including in DNA repair and in resistance to chemotherapeutic agents. The lecture will describe work on the structures, mechanisms and inhibition of the MBLs. In addition to efforts towards enabling the development of broad-spectrum MBL inhibitors, work towards the identification of dual-action MBL and serine beta-lactamase inhibitors will be described.

We thank the MRC and EPSRC for funding our research on AMR.

ENTEROBACTIN-DEPENDENT IRON UPTAKE PATHWAY AS A GATE FOR ANTIBIOTIC TROJAN HORSE STRATEGIES AGAINST PSEUDOMONAS AERUGINOSA

Véronique Gasser (1), Etienne Baco (1), Olivier Cunrath (1,2), Quentin Perraud (1), Aurélie Paulen (1), Pamela Saint-August (2), Nicolas Zill (1), Christian Schleberger (2), Alexander Schmidt (2), Dirk Bumann (2), Isabelle Schalk (1), Gaëtan Mislin (1)

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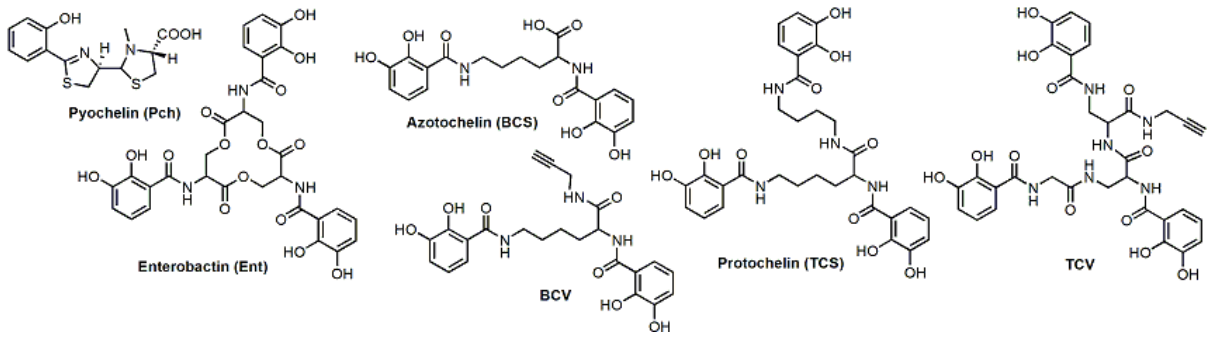
Pseudomonas aeruginosa is a pathogenic bacterium responsible of infections affecting cystic fibrosis patients but also immuno-compromised patients. This bacterium is naturally resistant to many antibiotics due to the low permeability of the bacterial envelope. In this context, transmembrane iron uptake systems could be used as gates to deliver efficiently antibiotics into the bacterial inner space using a Trojan Horse strategy (1). Iron is a crucial for the proliferation of pathogenic bacteria. Amongst mechanisms developed by bacteria to compete for iron, siderophore-dependent acquisition pathways are the more common. Siderophores are iron(III) chelators secreted by bacteria into the extracellular medium (2). The ferric complex is then transported into the periplasm by a specific outer membrane transporter (OMT) using the energy provided by the TonB machinery (3). Antibiotics can be attached to siderophores and resulting Trojan Horse conjugates will be delivered into the bacterial using siderophore-mediated iron uptake pathways. P. aeruginosa excrete two siderophores : pyochelin (Pch) and pyoverdin (Pvd), however this bacterium proceeds also to “iron piracy” by expressing OMTs able to transport siderophores produced by other bacteria. The expression of proteins involved in the uptake of an exogenous siderophore is induced by the presence of the corresponding siderophore in the bacterial environment. The genome of *P. aeruginosa* encodes for at least a dozen of such inducible iron uptake systems. Enterobactin (Ent) is a tris-catechol siderophore produced by *E. coli* but useable by *P. aeruginosa*. The Ent-dependent iron uptake pathway was well described in E. coli but was never exhaustively studied in *P. aeruginosa* so far. In *P. aeruginosa*, ferric-Ent is assimilated by the OMT PfeA. We reported recently the synthesis of catechol siderophores azotochelin (BCS) and protochelin (TCS), secreted by *A. vinelandii* and the development of synthetic siderophores BCV and TCV (4).



Using RT-qPCR and proteomic approaches, we showed that *P. aeruginosa* cells sensed the presence of Ent, BCS, BCV, TCS and TCV in the medium, leading to a strong activation of the transcription and expression of PfeA. ^{55}Fe uptake assays confirmed that Ent, BCS, BCV, TCS and TCV imported iron(III) into *P. aeruginosa* via PfeA. Uptake rates between 3.10^2 and 2.10^3 iron(III) atoms/bacterium/min were observed. We also demonstrate that this switching “ON” of the PfeA expression was associated with a repression of Pch pathway genes (5). This switch “ON” of PfeA expression and switch “OFF” of the endogenous Pch-dependent iron uptake pathway open new perspectives for antibiotic Trojan Horse strategies using catechol siderophores as vectors in *P. aeruginosa*. In this project, our contributions in organic chemistry, molecular biology and proteomic are also supported by structural and modeling data obtained by collaborations inside the WP3 of the ND4BB TRANSLOCATION European consortium.

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EXPLORING HIT-IDENTIFICATION STRATEGIES FOR ENERGY-COUPLING FACTOR TRANSPORTERS, A NOVEL TARGET FOR THE DEVELOPMENT OF ANTIBIOTICS

Leticia Monjas (1), Lotteke Swier (2), Friederike Reessing (1), Alrik de Voogd (1), Ruben Oudshoorn (2), Albert Guskov (2), Dirk Slotboom (2), Anna Hirsch (1)

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We have applied different hit-identification strategies – structure-based design, dynamic combinatorial chemistry, protein-templated click chemistry and virtual screening – in the search for new ligands for the energy-coupling factor (ECF) transporters. ECF transporters are a class of ATP-binding cassette (ABC) transporters that mediate the uptake of vitamins in prokaryotes. They consist of an energizing module and a substrate-binding protein (S-component). Different S-components can interact with the same energizing module.[1,2] ThiT is the thiamine-specific S-component.[3] Based on the co-crystal structure of ThiT-thiamine (**1**), we have designed and synthesized thiamine analogues to identify which residues are key for substrate binding and to elucidate the mechanism of transport. Ligand-binding assays have been performed and they showed that the new compounds bind with high affinity to ThiT (K_d = 4–660 nM). Co-crystallization studies of some of the compounds with ThiT confirmed the predicted binding mode and provide insight into the molecular recognition of thiamine by ThiT.

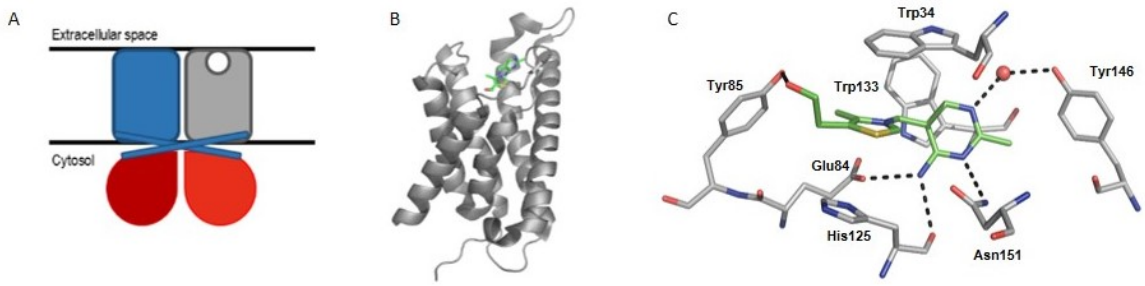

Figure 1. A: Schematic of target ECF-type ABC transporter. Multiple S-components (gray) interact with the same energizing module (red and blue). B: Co-crystal sctructure of ThiT in complex with thiamine. C: Binding of thiamine in the substrate-binding pocket of ThiT.

The synthesized molecules are the first reported ligands that bind to this transporter. These small-molecule will be used for the elucidation of the transport mechanism but also as a starting point for the development of novel antibiotics against pathogenic bacteria that depend on this class of transporters.[4,5]

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PEPTIDES AND PEPTIDOMIMETICS: OVERCOMING PROBLEMS OF ACTIVITY, SELECTIVITY AND BIOAVAILABILITY OF INTEGRIN LIGANDS

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Bidirectional integrin receptors are mediators of cell adhesion. There are 24 heterodimeric integrin subtypes which differ in their α and β subunits. The tripeptide sequence RGD (Arg-Gly-Asp) in extracellular matrix proteins is recognized by several of these integrin subtypes (αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, α5β1, αIIbβ3). The integrin pattern on cells strongly depends on many factors, such as tissue type, exposure of external stress and temporal conditions. For the elucidation of the distinct functions of the integrin subtypes, highly active and subtype selective peptidic and peptidomimetic ligands have been developed.[1] Functionalization under retention of their high activity and selectivity profiles allows biophysical studies to elucidate their function[2], coating of implant materials to improve osseointegration[3] and Molecular Imaging via PET (labelling with ^{18}F or ^{68}Ga)[4] - the varying integrin patterns of different cancers can be quantitatively elucidated *in vivo*, a prerequisite for personalized medicine.

In spite of their interesting biological profiles, peptides are considered as inferior drugs due to their metabolic instability and low bioavailability. However, cyclization, incorporation of D-amino acids and *N*-methylation of peptide bonds protect peptides completely from enzymatic cleavage. We have systematically investigated the dependence for Caco-2 permeability (as a measure of oral availability) from lipophilicity, rigidity as well as exposure of amide NH protons. We found that all these parameters cannot be used as single predictors for permeability. However, distinct peptidic backbone structures with good permeability have been identified[5,6] in a large library of cyclic *N*-methylated alanine peptides containing D-amino acids in some positions. Those are used as templates for the design of bioactive *N*-methylated cyclic peptides with oral availability.

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DISCOVERY & OPTIMZATION OF CLIPS-CONSTRAINED BICYCLIC PEPTIDES (2CLIPS) USING PEPSCAN PEPTIDE ARRAYS

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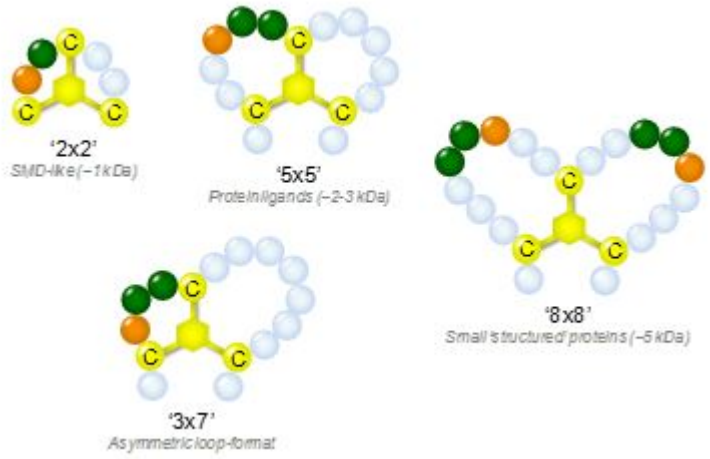
The majority of small and medium-size peptides (20-30 amino acids) derived from natural proteins are flexible and don't have a well-defined structure in solution. This may seriously limit the utility of peptides, either as protein mimics for epitope mapping purposes, or for therapeutic applications. PEPSCAN has developed a broadly applicable technology for fixation/constraining the two- and three-dimensional structure of short peptides. This platform technology, termed **CLIPS** (**C**hemical **LI**nkage of **P**eptides onto **S**caffolds) not only rigidifies the structure of the peptide, but also improves its binding activity and/or proteolytic stability to a significant extent. CLIPS technology is highly versatile and unique for its ease of application. The cyclization reaction can be applied under fully aqueous conditions at room temperature and neutral pH (7.5-8.0), and does not require any form of catalysis. Moreover, it is fully compatible with sensitive biological systems, like bacterial phage libraries.

The unique combination of CLIPS chemistry with PEPSCAN's peptide array technology creates an excellent technology platform for both epitope mapping and therapeutic peptide drug discovery. PEPSCAN has been one of the inventors of the combinatorial synthesis of large ensembles of overlapping peptides. The basic technology has been further optimized over the years into PEPSCAN's proprietary SIMPLIS (**S**urface **IM**mobilized **P**eptide **LI**brary **S**creening) platform. SIMPLIS allows high throughput (parallel) synthesis and screening of complete libraries of peptides (10,000-100,000). Its main strength involves the possibility to control diversity in a highly systematic manner. In addition, the use of non-natural amino acids (D-AA, b-AA, NMe-AA, aMe-AA, etc.) further extends the horizon for exploring new NCE's beyond the reach of phage-display type libraries that solely rely on the use of natural amino acids.



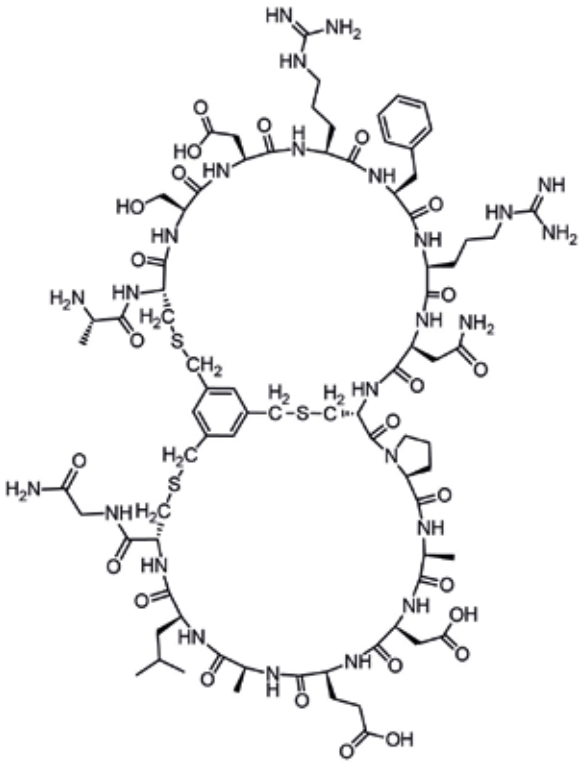
This lecture will present illustrative examples of Peptide Lead Discovery & Optimization using SIMPLIS, where constrained lead peptides were successfully affinity-matured starting at 50 nanomolar up to <100 picomolar binding affinities.

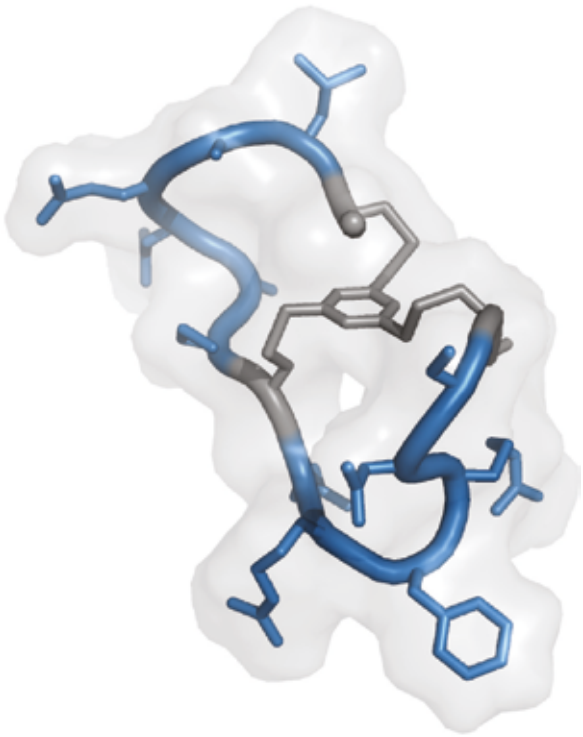
PHAGE-ENCODED COMBINATORIAL CHEMICAL LIBRARIES BASED ON BICYCLIC PEPTIDES

Christian Heinis

Ecole Polytechnique Fédérale de Lausanne (EPFL)
Switzerland

My laboratory is engaged in the discovery and development of peptide macrocycles for application as therapeutics and research tools. A major focus is the generation of ligands based on bicyclic peptides by phage display. The bicyclic peptides contain two macrocyclic rings that both can engage in binding interactions. The molecule format of bicyclic peptides combines key qualities of antibody therapeutics (high affinity and specificity) and advantages of small molecule drugs (access to chemical synthesis, diffusion into tissue, various administration options). By screening combinatorial libraries comprising billions of bicyclic peptides, we were able to identify ligands with nanomolar or even picomolar binding constants for a range of human disease targets. One of the targets is coagulation factor XII (FXII) which is implicated in several medical conditions including contact activation during extracorporeal circulation, thrombosis and the swelling disorder hereditary angioedema. In my talk, I will present the development of a picomolar bicyclic peptide FXII inhibitor and the assessment of its activity in vivo.





ATTENMPTS AT ORAL DELIVERY OF A SERIES OF SHORT UNNATURAL PEPTIDES: STILL LIKE PUSHING A CAMEL THROUGH THE EYE OF A NEEDLE?

Claudio Mapelli

Bristol-Myers Squibb
United States

Cell membrane permeability and oral bioavailability of peptides remain a challenge, in spite of recent advances in peptide drug design and formulation methods. While numerous biologically relevant peptides possess potencies and specificities that render them attractive therapeutic candidates, their lack of permeability and oral absorption still represents a barrier to their broader acceptance as clinical therapeutics. This presentation will focus on a number of approaches investigated to orally deliver a new class of re-designed GLP-1 peptides with improved chemical and enzymatic stability. Key findings will be presented on how structural modifications and formulation methodologies enabled bioavailability. Mechanistic hypotheses will also be provided to explain the observed enhancements of peptide uptake. Lessons learned will be reviewed and utilized to define some of the challenges in oral delivery of peptide therapeutics clearly outside the rule of 5.

MOLECULAR PHARMACODYNAMICS OF VISUAL CYCLE MODULATORS IN PROTECTION AGAINST RETINAL DEGENERATION

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Abstract not available at the time of printing!

THE BACK OF THE EYE: TREATMENT CHALLENGES AND OPPORTUNITIES

Muneto Mogi

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Age-related macular degeneration (AMD) is the leading cause of vision loss and blindness in the industrialized countries. Although significant progress has been made in recent years for the treatment of wet AMD using intravitreal injection of anti-VEGF antibody such as ranibizumab and aflibercept, there is still an unmet need for less invasive treatment options for wet AMD. Furthermore, unlike for wet AMD, there is currently no treatment available for dry AMD or geographic atrophy (GA). Target tissues for these diseases are located in the back of the eye such as retina and choroid, and it is critical for the therapeutic agents to possess adequate back of the eye exposure to maximize efficacy while having low systemic exposure to minimize on-target side effects. This presentation will cover drug design concepts, delivery challenges, and attempts made with different administration routes such as oral, topical, and intravitreal to safely and effectively deliver drugs to the back of the eye.

REGORAFENIB EYE DROPS FOR WET AMD - CHALLENGES IN TRANSLATABILITY OF PRECLINICAL DATA INTO CLINICAL EFFICACY

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Bayer Pharma
Germany

For the treatment of neovascular age-related macular degeneration (nAMD), intravitreal injections are clinically used to administer approved protein therapeutics directed against VEGF to the retina. In order to provide a potential non-invasive treatment alternative, we initiated a program using regorafenib, a multi-kinase inhibitor mainly targeting VEGFR2, administered topically as eye drops. In preclinical models, convincing efficacy in rodents and primates could be demonstrated, and tissue levels in different eye compartments were considered sufficient. The overall favorable safety profile observed in toxicology experiments was confirmed in a phase I study in healthy volunteers. To study the efficacy, safety, and tolerability of regorafenib eye drops in treatment-naïve patients with nAMD, a multicenter, single-arm, open-label, Phase 2a/b study was initiated. The primary endpoint was the mean change in best-corrected visual acuity ETDRS letter score (BCVA) from baseline to Weeks 4 and 12. Unfortunately, the predefined success criteria were not met, and the program was terminated. The translational challenges of this program will be presented.

SYSTEMIC ADMINISTRATION OF AN ALLOSTERIC GSK-3 INHIBITOR DELAYS PHOTORECEPTOR CELL DEATH AND PRESERVES VISUAL FUNCTION IN A RETINITIS PIGMENTOSA MOUSE MODEL

Ana Martinez (1), Josefa Zaldivar (1), Beatriz Villarejo (1), Miguel Marchena (1), Carmen Gil (1), Catalina Hernandez (1), Pedro de la Villa (2), Enrique de la Rosa (1)

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Retinitis pigmentosa (RP) comprises a group of degenerative retinal dystrophies affecting 1 in 3000-4000 people and being characterized by photoreceptor (rods and cones) missfunction and degeneration which leads to progressive visual loss.There is not any available treatment for RP diseases up to date [1].

Based on our experience in the development of innovative drugs for protection of central nervous system, we have designed a heterocyclic small molecule, named VP3.15, with an innovative mechanism of action for retinal diseases: It targets allosterically glycogen synthase kinase 3 (GSK-3), being a GSK-3 substrate competitive inhibitor [2].

The neuroretina is part of the central nervous system and can be considered as an easily accessible brain span. As in the brain, glia-neuron interactions are very important in physiology and pathology. Our innovative approach is to use our knowledge with neuroprotective and anti-inflammatory agents for CNS neurodegenerative diseases to attenuate retinal degeneration.

We have shown that VP3.15 is able to decrease by 50% photoreceptor cell death *ex vivo.* Moreover, intraperitoneal administration of VP3.15 to *rd10* mice, a mouse model of RP, preserved photoreceptor cell number and prevented microglial infiltration at P23, decreased pro-inflammatory TNF-α and IL-1β, as well as GFAP gene expression at P23, and improved visual function up to P46.

Our results show that GSK-3 inhibitors, and specifically VP3.15, may constitute a therapeutic strategy for Retinitis Pigmentosa, as well as other retinal dystrophies.

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LIGAND-TARGETED THERAPEUTIC AND IMAGING AGENTS FOR MULTIPLE HUMAN DISEASES

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We have been developing methods to target drugs specifically to pathologic cells, thereby avoiding collateral toxicity to healthy cells. In the case of cancer, we have exploited up-regulation of the folate receptor on cancers of the ovary, lung, kidney, endometrium and breast to target imaging and therapeutic agents to these cancers. Clinical trials of six folate-linked drugs demonstrate that the ligand-targeting strategy holds significant promise for increasing drug potency while reducing unwanted toxicity. Data on treatment of tumor-bearing mice, dogs, and humans will be presented.

We have also developed a targeting ligand (DUPA) that can selectively deliver attached drugs to PSMA on prostate cancer cells. Human imaging and therapeutic studies suggest that this targeting ligand can not only improve the diagnosis of prostate cancer, but also enhance treatment of the disease. Additional tumor-specific ligands that target cancers of the bladder, pancreas, stomach, brain, liver, colon, skin and esophagus have also been developed, and preclinical and clinical data on several of these will also be presented.

One application of our tumor targeting ligands that has attracted recent attention has been the use of these ligands to deliver bright fluorescent dyes selectively to tumor nodules. When injected intravenously shortly before cancer surgery, these near infrared dyes reveal both superficial and buried malignant lesions that would have otherwise gone undetected, thereby enabling the surgeon to resect significantly more diseased tissue than was previously possible. Videos of recent surgeries of ovarian, brain and lung cancers will be presented.

The newest application of our tumor-specific ligands has been the targeting of immune cells to tumors. In this application, the chimeric antigen receptor of a patient's T cells is engineered with an exoplasmic ScFv to recognize fluorescein and a cytoplasmic 4.1BB domain to assure T cell activation. The engineered T cell is then targeted to cancer cells with a tumor-specific ligand-fluorescein conjugate that can bridge between the CAR T cell and the malignant cell, thereby forcing the two cells to interact. Preclinical data in mice demonstrate that this modification of CAR T cell technology can allow sensitive control of the rate of tumor lysis, termination of target cell killing when desired, and elimination of antigenically heterogeneous tumors with a single "universal" CAR T cell.

Finally, ligand-targeted imaging and therapeutic agents for a number of autoimmune, inflammatory, and infectious diseases (e.g. malaria, rheumatoid arthritis, multiple sclerosis, psoriasis, atherosclerosis, osteoarthritis, etc.) have also been developed. Recent applications of this variation of the small molecule targeting approach will also be described.

PEPTIDE DRUGS TO TARGET GPCR - STATE OF THE ART AND INNOVATIVE APPLICATIONS

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Peptides hormones play an important role in the regulation of manifold activities in the body. Many of them transmit their activity through G-protein coupled receptors (GPCR), which are among the most promising drug targets nowadays. However, in addition to their direct activity, indirect mechanisms have been shown. This includes their use as drug shuttles, e. g. in tumour targeting. Accordingly, in addition to ligand binding, internalization has to be addressed and to be studied, including arrestin recruitment. The neuropeptide Y/pancreatic polypeptide family contains 36 amino acid peptides that bind in human to four different so-called Y-receptors [1]. We recently could demonstrate that chemical modification of the ligand, including fluorescence labelling, lipidisation and PEGylation significantly modifies the activity of the ligand [2]. Furthermore, by labelling of the receptor with a novel template-assisted ligation strategy [3], we can follow ligand/receptor complexes in living cells. As in breast cancer human Y_1 receptors have been shown by us to be addressable by peptide conjugates using ^{99m}Tc or ^{18}F PET-tracers [4,5] we now designed Y_1 receptor selective peptides linked to different toxophors [6-8] in different numbers [9]. We identified novel linkers that lead to a rapid and efficient release of the toxin inside of the cell [6,9] and subsequently to cell death. Furthermore, we characterized the mechanism of direct and peptide-mediated uptake of tubulysin-related toxins [8].

In the field of tumour therapy peptide-drug conjugates are already well accepted. However, the concept of receptor-mediated internalisation and subsequent tissue specific intracellular application is not limited to the selective addressing of tumours. This may open up a new field of targeted therapy by mid-sized drugs.

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THE DEVELOPMENT OF PYRROLOBENZODIAZEPINE ANTIBODY DRUG CONJUGATES

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The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antitumour antibiotics produced by various *Streptomyces* species. PBDs bind sequence selectively in the minor groove of DNA to purine-guanine-purine motifs. Synthetically prepared PBD dimers bind covalently to embedded Pu-GATC-Py sequences to produce non-distortive and persistent DNA interstrand cross-links. The resulting PBD-dimer/DNA adducts cause dividing cells to arrest at the G2/M boundary and ultimately enter apoptosis.

These highly potent PBD dimers are ideally suited to act as warheads in Antibody-Drug Conjugate (ADC) therapy. This novel approach to cancer therapy involves combining the potency of small molecule cytotoxic agents with the tumour target selectivity of antibodies. In order to construct an ADC, the cytotoxic warhead must be fitted with a linker to allow conjugation to the antibody.

The PBD-linker tesirine (SG3249) was designed to combine potent antitumour activity with desirable physico-chemical properties such as favourable hydrophobicity, and improved conjugation characteristics. One of the two reactive imines in the PBD dimer was capped with a cathepsin B-cleavable valine-alanine linker. A robust synthetic route was developed to allow the production of tesirine on clinical scale, employing a flexible, convergent strategy.

Tesirine was conjugated to anti-HER2 antibodies to afford both stochastic and site-specifically engineered ADC constructs. The resulting ADCs were evaluated in both human tumour cell lines *in vitro* and in xenograft models *in vivo* to afford proof of concept. Conjugation of tesirine to the anti-DLL3 antibody rovalpituzumab has resulted in rovalpituzumab-tesirine (Rova-T), currently under phase II clinical evaluation for the treatment of small cell lung cancer.

The presentation will give an overview of the design and synthesis of tesirine, its conjugation to tumour targeting antibodies as well as its preclinical and clinical evaluation.

PHOTOISOMERISABLE ALLOSTERIC MODULATORS ALLOW A FINE CONTROL OF mGLU RECEPTORS WITH LIGHT IN VIVO

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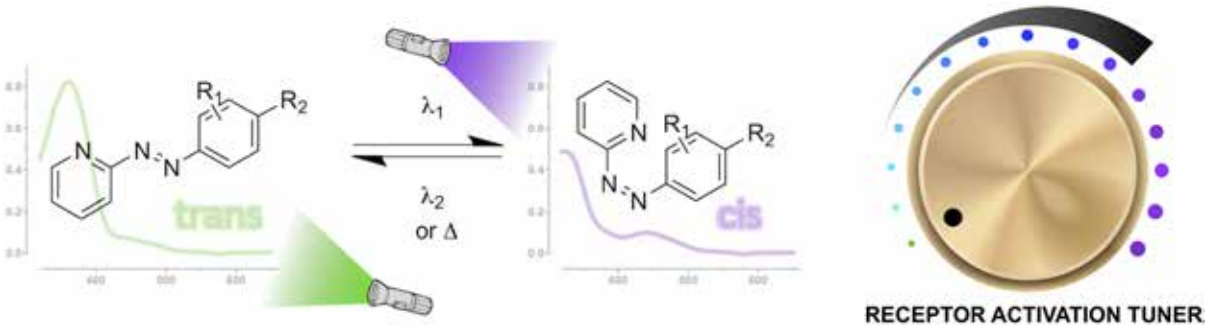
Metabotropic glutamate receptors (mGluRs), are class C GPCRs widely distributed through the CNS and considered pharmacological targets for neurologic disorders.

Recently, our group published alloswitch-1 as the first GPCR photoswitchable allosteric modulator with activity *in vivo*[1]. Alloswitch-1 is a structurally substituted phenylazopyridine and selectively performed NAM activity in $mGlu_5$ in the *trans*-isomer, while in the *cis* disposition it was inactive. This behaviour was easily controlled by illumination with 380 nm and 500 nm of wavelength and was consistent and reversible in transfected cells and native cultures. Moreover, it allowed the possibility to control the natatorial motility of native Zebrafish larvae or tadpoles with light, and it was also effective in the reduction of pain-like behaviour in rodents, showing a clear light-dependent activity. Additionally, these effects could be also reproduced with other photoswitchable allosteric modulators, acting on $mGlu_4$ subtype.

We next design and synthesise new related phenylazopyiridnes, not only to study the structure - pharmacological activity relationship, but also the efficiency of photoisomerisation form *trans* to *cis* isomers and the rate of thermal relaxing from *cis* to *trans* isomers. Thus, we obtained many compounds with a very similar potency to alloswitch-1, but other with an enhanced potency and improved photoswitching properties with biological-friendly wavelengths for *on/off* switching. Furthermore, we demonstrated that if we tune the wavelength of illumination, we are able to control the rate of activation of the receptor, which is something that can only be afforded with dose-adjustment with conventional pharmacology. Moreover, we were able to detect an atypical over-activation of the receptor upon "swiching- *off*" some compounds, both *in vitro* and *in vivo*.

Overall, we proved that optopharmacology might be advantageous for different reasons: We could perform assays with native tissues or *in vivo* with no genetic modification, we could adjust the activation of the receptor with light with a single dose of compound, instead of changing doses to find the optimal one and we could also allow the study of several processes that can only detected upon *on/off*-switching of a receptor activity.



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DISCOVERY AND DEVELOPMENT OF THE HIGHLY POTENT, HIGHLY SELECTIVE CATHEPSIN S INHIBITOR RG7625 FOR THE TREATMENT OF AUTOIMMUNE DISEASES

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The lysosomal cysteine protease cathepsin S plays an important role in antigen presentation by degrading the invariant chain fragment p10 to CLIP. This CLIP fragment is associated to the major histocompatibility complex MHCII. After exchange of CLIP by antigens the MHCII/antigen complex is transported to the surface of antigen presenting cells such as microglia, dendritic and B-cells. This complex may be recognised by e.g. T-cells which subsequently become activated. If this process is disturbed, occasional loading of MHCII by self antigens may occur followed by an autoimmune response. Therefore, inhibition of cathepsin S may be an effective treatment of autoimmune diseases.

This presentation will cover the medicinal chemistry optimization of a series of cathepsin S inhibitors culminating in the identification of RG7625 as a highly potent and highly selective cathepsin S inhibitor. Aspects of structure based design, enzyme kinetics and multi dimensional optimisation will be highlighted. The preclinical profiling of RG7625 and clinical Phase I data will be outlined as well.

FIRST TIME DISCLOSURE OF A DEVELOPMENT CANDIDATE TO TREAT SEVERE ACUTE PANCREATITIS THROUGH A DRUG DISCOVERY PARTNERSHIP BETWEEN GSK AND THE UNIVERSITY OF EDINBURGH

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Discovery Partnerships with Academia (DPAc) was established in late 2010 as a new mechanism to combine disease insight from the academic community with GSK's drug discovery engine in order to translate academic innovative ideas into medicines that meet the needs of patients. The collaboration with Mr Damian Mole, Clinical Senior Lecturer and Honorary Consultant Surgeon, at The University of Edinburgh was one of the first DPAc collaborations to be established. Damian's work uncovered a novel link between 3-hydroxykynurenine (3HK), a tryptophan metabolite, and the development of severe acute pancreatitis suggesting that inhibition of the enzyme responsible for the generation of 3HK, kynurenine monooxygenase (KMO), could represent a revolutionary new approach to the treatment of acute pancreatitis.

The presentation will describe the medicinal chemistry strategy which utilised substrate knowledge to discover KMO inhibitors with the required properties commensurate with intravenous dosing. Low molecular weight inhibitors with excellent aqueous solubility were rapidly identified and shown to have a clear protective effect in a rat model of acute pancreatitis when dosed therapeutically. Subsequent structural knowledge enabled the fine tuning of key interactions with the protein and ultimately led to the identification of the development candidate, a highly potent and selective KMO inhibitor with outstanding physicochemical properties.

DISCOVERY OF AZD3241, A POTENT AND SELECTIVE MYELOPEROXIDASE INHIBITOR FOR THE TREATMENT OF NEURODEGENERATIVE DISORDERS

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Myeloperoxidase (MPO) is a heme containing enzyme catalyzing the conversion of hydrogenperoxide (H_2O_2) to hypohalous acids like e.g. hypochlorous acid (HOCl) and is thought to play an important role in the human antimicrobial defense mechanisms. Excessive MPO activity has been shown to be a significant parameter in a number of oxidative stress mediated pathologies like e.g. in CNS diseases as Parkinson's disease (PD) or multiple system atrophy (MSA) as well as in peripheral conditions like chronic obstructive pulmonary disease, rheumatoid arthritis, or atherogenesis. An ideal MPO intervention therapy would leave its antimicrobial activity intact while reducing the associated pathologies.

This presentation will describe AstraZeneca Neuroscience's work pioneering the first truly selective, irreversible MPO inhibitors culminating in the discovery of AZD3241, which is currently in phase 2 clinical trials for PD and MSA.

DISCOVERY OF THE HCV NS5A INHIBITOR MK-8408 (RUZASVIR)

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Nonstructural Protein 5A (NS5A) inhibitors are potent direct-acting antiviral agents (DAA) useful for the treatment of chronic Hepatitis C virus (HCV) infection. While the first generation NS5A inhibitors displayed high in vitro potency, early clinical experience with these agents revealed that they suffered from a low genetic barrier to resistance against both gt1a and non-gt1a resistance-associated variants (RAVs). This made necessary the identification of additional NS5A inhibitors possessing broad, pan-genotypic profiles, and activity against a wider variety of NS5A RAVs. Modifications to the molecular scaffolds of our first generation NS5A inhibitors influenced their mutant profiles and enabled us to develop structure-activity relationships (SAR) that led to the discovery of the potent pan-genotypic NS5A inhibitor, MK-8408 (Ruzasvir). This NS5A inhibitor contains a unique tetracyclic indole-based core, while maintaining the imidazole-proline-valine Moc motifs of our previous NS5A inhibitors and is currently in clinical trials as part of an all-oral DAA regimen for the treatment of chronic HCV infection. The SAR studies that led to the discovery of MK-8408 and its antiviral effect in combination with the HCV protease inhibitor MK-5172 will be discussed in this presentation.

TOWARD A MORE GENERALIZED AND AUTOMATED APPROACH FOR SMALL MOLECULE SYNTHESIS

Martin Burke

University of Illinois at Urbana-Champaign

Small molecule natural products already represent or have inspired more than half of all modern medicines. Yet, largely due to limitations inherent in the highly customized processes by which such complex molecules are typically synthesized, much of the functional potential of natural products remains untapped. The common biosynthetic machinery and small number of building blocks from which most natural products are derived suggests that a more generalized building block-based approach for the synthesis of such compounds should be accessible. Harnessing this potential with iterative cross-coupling of MIDA boronates has emerged as an increasingly general and automated approach for the synthesis of a wide range of natural products and their derivatives. REVOLUTION Medicines is industrializing and further developing this technology into the REVBLOCKS™ platform, and this discovery engine is now being harnessed to drive the transformation of natural products into best-in-class medicines for treating serious human diseases.

ROBOT SCIENTISTS, THE REPLICATION CRISIS, AND CANCER

Ross King

University of Manchester

A Robot Scientist is a physically implemented robotic system that applies techniques from artificial intelligence to execute cycles of automated scientific experimentation. A Robot Scientist can automatically execute cycles of: hypothesis formation, selection of efficient experiments to discriminate between hypotheses, execution of experiments using laboratory automation equipment, and analysis of results. The motivation for developing Robot Scientists is to better understand science, and to make scientific research more efficient. The Robot Scientist 'Eve' was originally developed to automate early-stage drug development using synthetic biology yeast assays. We are now adapting Eve to work with and learn about human cancer cell lines. We are also teaching 'Eve' to autonomously extract information from the scientific literature. Specifically we are interested in statements about small-molecules changing the expression level of genes in cancer cells, as these can be experimentally verified by Eve. The information extracted by Eve from the scientific literature is integrated into a probabilistic knowledge, which is updated by the experimental evidence. There is currently a 'replication crisis' in biology, and many scientific results have been shown to difficult to replicate. The automation of replication is one approach to dealing with this. We have demonstrated that Eve can automatically extract information from the cancer literature and experimentally test whether it is confirmed under well-defined experimental conditions.

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IDENTIFYING SYNTHETIC OPPORTUNITIES USING CHEMPLANNER - HOW COMPUTERS ASSIST CHEMISTS IN COVERING A GREATER SYNTHETIC SPACE

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Organic synthesis is vital to the iterative discovery cycle in medicinal chemistry. It is a determining factor in how quickly new ideas can be put to the test and at what cost, and it is a facilitator of innovation as it provides access to novel molecular structures. Several papers in recent years have shown that a strikingly small set of reaction classes are used in the pharmaceutical industry to synthesize target molecules, suggesting that predictability and low-risk may be trumping simplicity and efficiency in synthesis design. Furthermore, confining oneself to a small synthetic space may inadvertently lead to confining the chemical space one explores in the discovery process. A comprehensive evaluation of synthetic opportunities available for making a target compound is therefore crucial for achieving greater productivity, efficiency and novelty. Wiley ChemPlanner, a Computer-Aided Synthesis Design (CASD) system, is designed to suggest to medicinal chemists a large spectrum of alternative synthetic strategies and methods by carrying out rule-based retrosynthetic analysis from the target molecule to available starting materials. In addition to the integrated reaction database, the system allows users to upload their own reactions and starting materials to the system in order to accelerate and provide further focus to the machine learning processes, consequently improving the quality of results over time.

ChemPlanner derives reaction rules automatically from large databases of reactions. Chemical perception algorithms cluster together reactions that share the same underpinning chemistry and generalize the rules. Manual editing tools provide means for cheminformatics experts to fine-tune the reaction rules. During the retrosynthetic analysis, the rules as well as the source reactions are used to find full synthetic routes using readily available starting materials. When deployed locally, proprietary reactions and starting materials can be loaded into ChemPlanner in order to generate rules and to carry out federated searches based on the supplied and the uploaded reactions and educts. The generated synthesis plans thus include synthetic approaches from both within and beyond the user's areas of expertise. Customizable ranking algorithms and other analysis tools provide means to prune the solution set and to access the most relevant experimental information.

In this talk we discuss the rule generation process, and in particular recently added capabilities in asymmetric synthesis. We describe the scoring criteria and how those can be modified by the users. We discuss the benefits of loading proprietary information into the system in the context of the retrosynthetic analysis as well as in terms of internally exposing and disseminating the organization's own knowledge, and suggest ways in which automated synthesis platforms can be linked to the system using feedback mechanisms. Via several case studies we demonstrate the ability of the system to generate viable and innovative synthetic routes.

THE evoSpace - A SYNTHESIS-DRIVEN ENVIRONMENT THAT FINDS ACCESSIBLE, WELL-BEHAVED COMPOUNDS BY DESIGN

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We present the evoSpace, a giant chemical space that is designed from both public and corporate chemical reactions. The EvoSpace can be searched very efficiently for interesting compounds using fuzzy similarity searches.

Searching large chemical compound spaces efficiently has always been on the agenda in drug research; the motivation being to obtain novel scaffolds and thus to gain novel intellectualproperty. However, associated challenges have also emerged already from the beginning in the 90ies:

a. Ensuring synthetic accessibility of the resulting proposals

b. Optimizing the physico-chemical property profiles, targeting the interesting regions in the search space.

Evotec, in collaboration with BioSolveIT have created a huge virtual molecular space, the genesis of which is driven by the most-used chemical reactions and apt building blocks. The reactions are added using a standard drawer, and comprise both public and in-house procedures. On the educt side, duplicate removal and related bookkeeping are taken care of. The resulting chemical space (evoSpace) which comprises more than 10^{15} virtual compounds can be mined for novel compounds using an efficient search engine that exploits the Feature Tree descriptor.[1] Property filtering to ensure attractive profiles of results is carried out both along the way during the searches as well as by post-filters.

The entire system including visualization and reporting is encoded as a KNIME[2]-based engine - it is envisioned that larger parts of the workflow setup including the publicly accessible compounds and reactions will be exposed to the general public.

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DISCOVERY OF A FIRST-IN-CLASS PAR4 ANTAGONIST AS A NOVEL ANTITHROMBOTIC

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The protease-activated receptors PAR1 and PAR4 are activated by the serine protease thrombin and transduce signals that trigger platelet activation, including platelet morphology changes, granule secretion, and integrin activation. Although both receptors are potential antithrombotic drug targets, research efforts have focused on PAR1, as it was characterized as the primary platelet thrombin receptor. We will present our validation of PAR4 as an antithrombotic drug target, using a PAR4 antibody and guinea pig thrombosis and bleeding models. We will also describe the discovery of BMS-986120, a potent, selective, and reversible small molecule PAR4 antagonist that has recently advanced into clinical trials. Importantly, BMS-986120 demonstrates strong antithrombotic efficacy and low bleeding liability in preclinical primate thrombosis and bleeding models.

DISCOVERY OF NOVEL LPA1 ANTAGONIST: DESIGN AND SAR STUDIES

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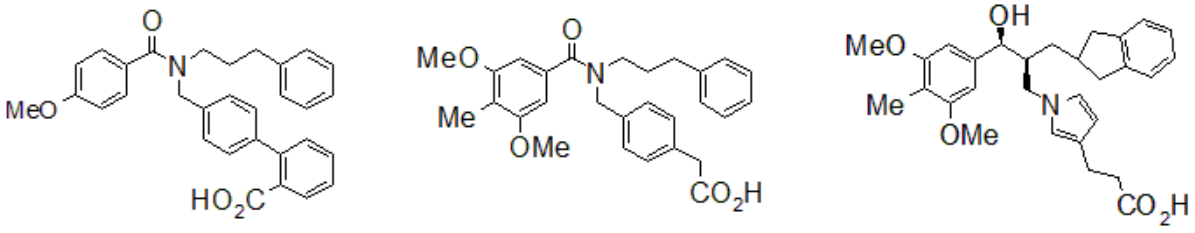
Lysophosphatidic acid (LPA) is a class of bioactive phospholipids which exerts a wide range of physiological and pathophysiological responses. These biological effects of LPA are mediated through G protein coupled receptors (GPCRs). Six LPA receptors (LPA_{1-6}) have been identified and characterized so far. Of which, we have reported that LPA induces the contraction of the urethra via LPA_1 receptor.[1] The potency of urethral contraction by LPA is almost the same as by phenylephrine.[2] Therefore LPA_1 antagonist is expected to be a remedy of benign prostatic hypertrophy (BPH). To obtain a good start point, high throughput screening campaign against LPA_1 receptor was conducted and identified hit compound. Lead optimization efforts to improve *in vitro* potency using LPA_1 receptor expressed CHO cells to give a lead compound ONO-7300243. This compound has reduced intraurethral pressure (IUP) in rat as the same efficacy as tamsulosin (α_1 adrenoceptor antagonist), but has not affected on mean blood pressure unlike tamsulosin. The improvement of *in vivo* efficacy against LPA-induced rat IUP model was achieved by scaffold hopping to alcohol template affording the optimized compound ONO-0300302. Binding experiment using [^{3}H]-ONO-0300302 has revealed that this compound possesses a slow binding property. This presentation will describe the SAR at lead optimization stage and *in vivo* efficacy of novel class of LPA_1 antagonist.[3] We will also describe the analysis based on MD calculations using the LPA_1 antagonist bound x-ray crystal structure.[4]



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DISCOVERY OF THE CLINICAL CANDIDATE RIBUVAPTAN, A DUAL ACTING VASOPRESSIN V1A/V2 RECEPTOR ANTAGONIST FOR THE TREATMENT OF HEART FAILURE

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Congestive heart failure (CHF) is a severe chronic disease which usually progresses steadily towards death, despite recent therapeutic advances. In CHF, the peptide hormone vasopressin is increased in plasma and the associated fluid retention has been shown to be a prognostic factor in this patient population [1,2,3]. Elevated vasopressin levels mediate deleterious effects via two different GPCRs: vascular V1a and renal V2 receptors. Selective V2 receptor antagonism has proven short-term beneficial effects (i.e. body weight loss due to aquaresis), but leads to compensatory increased vasopressin levels which might activate unprotected V1a receptors.

In our hypothesis, a dual acting V1a/V2 antagonist should blunt V1a-mediated effects expected with chronically elevated vasopressin levels (i.e. peripheral vasoconstriction and reduced cardiac output) while maintaining the favorable decongestive effects of V2 antagonism [4, 5].

A recombinant cell line expressing the human V1a receptor was used for high throughput screening delivering triazolones as a novel structural motif for vasopressin receptor blockers with IC_{50}s both on the V1a and the V2 receptor in a range of 100 - 300 nM. Initial optimization efforts were focused on increasing potency on both the V1a and the V2 receptor and on enhancing metabolic stability. Despite the potent inhibition of the V2 receptor and increased metabolic stability, only moderate activity was seen in vivo in the rat diuresis model after oral administration most likely due to limited absorption of the compounds. In addition, many compounds exhibited a strong drug-drug interaction potential. Significant improvements in potency on both receptors and oral absorption could be achieved by replacing a cyclopropyl substituent by a trifluoromethylhydroxyl substituent. The overall increase in polarity in the course of the following optimization rounds further improved metabolic stability. Finally, Ribuvaptan was identified as a novel potent, dual acting V1a/V2 receptor antagonist with excellent pharmacokinetic properties. Its characterization includes various in vivo diuresis models in rats as well as a heart failure model in paced dogs.

The prediction of human PK parameters based on an allometric scaling approach as well as the predicted minimal effective dose in humans based on rat diuresis data translate reasonably well to observed PK/PD data from single and multiple dose studies in healthy volunteers.

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CLASSIFICATION OF ADAMTS BINDING SITES: THE FIRST STEP TOWARD SELECTIVE ADAMTS7 INHIBITORS

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Genome-wide association studies identified the *ADAMTS7* gene as a risk locus for coronary artery disease (CAD) [1,2]. In rat carotid arteries, neointima formation goes along with enhanced Adamts7 expression after balloon-mediated injury [3]. Furthermore, *Adamts7*-knockout mice display reduced neointima formation following vascular injury [4]. Although a causal link between ADAMTS7 and CAD remains to be proven, inhibition of ADAMTS7 represents a promising new target for intervention in CAD [5]; however, neither inhibitors nor structural data for ADAMTS7 are currently available.

ADAMTS7 belongs to the "a disintegrin and metalloproteinase with thrombospondin motifs" (ADAMTS) protein family. The human ADAMTS family consists of 19 members, all of which are proteolytically active [6]. In their metalloproteinase domains ADAMTS proteins contain a conserved zinc-binding motif, HEXGHXXGXXH (where X represents any amino acid residue). The three histidine residues in this motif coordinate a catalytic zinc ion in the binding site of the metalloproteinase domain [7].

In our recently published paper [8] we used *in silico* methods, including homology modeling and pharmacophore modeling, to analyze the ADAMTS7 metalloproteinase domain, particularly its binding site and determine differences to other ADAMTS proteins. We revealed differences in structure and sequence in the ADAMTS binding pockets; these non-conserved regions represent potential binding regions for selective ADAMTS7 inhibitors. The main contribution of this study is the proposal of a pharmacophore for ADAMTS7 (see Figure 1). The characterization of the ADAMTS7 binding site and definition of a pharmacophore are the first step toward developing a novel therapeutic treatment for CAD.

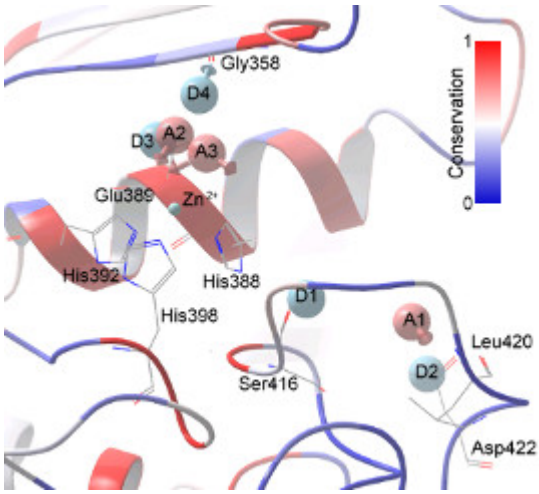

Figure 1. ADAMTS7 binding site with pharmacophore [8]. A1-A3 represent hydrogen bond acceptor features, D1-D4 hydrogen bond donor features.

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UTILIZATION OF NOVEL BENZOXABOROLES AS DRUG CANDIDATES TO TREAT NEGLECTED TROPICAL DISEASES

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Parasitic and bacterial infections in the developing world present a significant medical challenge, and have been largely ignored by the pharmaceutical industry due to the limited economic resources of those afflicted. Despite widespread prevalence of these diseases, particularly amongst children, no fundamentally new treatments have been delivered over the past several decades. Existing treatments for these diseases suffer from significant limitations including drug toxicity, complex dosing regimens and development of resistance – all of which lead to minimal effectiveness in the field. Anacor Pharmaceuticals, in collaboration with a number of philanthropic and private-public partnerships, have explored and developed a novel boron chemistry platform that shows great promise for delivery of new, effective and safe drugs for treatment of several of these parasitic diseases. In particular, discovery and development of benzoxaboroles for the treatment of human African trypanosomiasis, Chagas disease, malaria and tuberculosis will be highlighted.

THE DEVELOPMENT OF DDD853651; A POTENTIAL CANDIDATE FOR THE TREATMENT OF VISCERAL LEISHMANIASIS

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Visceral Leishmaniasis (VL) is a poverty associated parasitic infection responsible for around 40,000 deaths worldwide every year. Currently available treatments are hampered by issues such as toxicity, teratogenicity, cost and increasingly, resistance. There is therefore an urgent need for new treatments.

The Drug Discovery Unit, University of Dundee, and the GlaxoSmithKline Kinetoplastid Discovery Performance Unit, Tres Cantos, with support from the Wellcome Trust, have formed a five year partnership to conduct drug discovery within kinetoplastid diseases, with a particular focus on VL. Within this collaboration, a novel chemical series was identified with *in vitro* activity in an intra-cellular Leishmania assay. This presentation will concentrate on the lead optimisation and progression of this series, focusing on the identification of compounds with *in vivo* efficacy, utilizing X-ray crystallography for optimization of solubility, and finally, progress towards candidate selection.

PHARMACOLOGICAL REVERSION OF ANTIBIOTIC RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS : DESIGN OF SMART TROJAN MOLECULES TO REPROGRAM THE BACTERIA

Benoit Deprez

University of Lille 2
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Abstract not available at the time of printing!

THE DISCOVERY AND DEVELOPMENT OF NOVEL MACROFILARICIDAL AGENTS FOR THE TREATMENT OF ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

Thomas von Geldern (1,5), Dale Kempf (1), Kennan Marsh (1), Howard Morton (1), Marc Scanio (1), Louise Ford (2), Joe Turner (2), Stephen Ward (2), Mark Taylor (2), Achim Hoerauf (3), Marc Huebner (3), Ivan Scandale (4)

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2) Filariasis Research Laboratory, Liverpool School of Tropical Medicine, Liverpool, UK
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5) Franciscan Institute for World Health, Franciscan University, Steubenville, Ohio, USA

Onchocerciasis and lymphatic filariasis (LF), diseases resulting from filarial worm infections, together affect nearly 150 million people worldwide, with over 1.5 billion at risk. These diseases create an enormous burden of morbidity and lost productivity, in addition to the social stigma associated with their clinical manifestations. Current treatment options (ivermectin, albendazole, and diethylcarbamazine, alone or in combination) focus on eliminating microfilaria and sterilizing adult worms, but do not clear the infection. New agents with adulticidal activity are needed.

There are two distinct approaches to the discovery of novel macrofilaricidal agents. Direct screening of compounds libraries against adult worms is possible, though this strategy is labor-intensive and the number of compounds that can be tested is limited. Alternatively, it is possible to eliminate these worms by targeting the endosymbiont bacterium *Wolbachia*. We will describe progress on both of these fronts. Several novel classes of direct-acting macrofilaricides have been identified through screening of a high-value subset of the AbbVie compound collection. These series are in late lead-optimization; progress on this front will be summarized. Simultaneously, screening of a targeted subset of the AbbVie collection has identified the veterinary antibiotic Tylosin A as a powerful inhibitor of *Wolbachia*, with effects on worm fertility and health. Medicinal-chemistry optimization of this lead has led to an analog which has now been proposed as a clinical candidate.

EXPANDING THE MACROCYCLE CHEMICAL SPACE

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Iran

Artificial macrocycles recently became popular as a novel hunting ground for drug discovery. As opposed to their natural twin artificial macrocycles promise to have better control over synthesisability and physicochemical properties, eventually resulting in drug-like properties. Very few synthetic methods allow for the convergent, fast but diverse access to large macrocycles chemical space. We have devised several efficient synthetic methods which allow for the fast synthesis of macrocyclic libraries of ring size 10-25.[1-5] Moreover we show use of computationally enumerated libraries (3D conformer, pharmacophore) for the discovery of novel protein protein interaction antagonists.

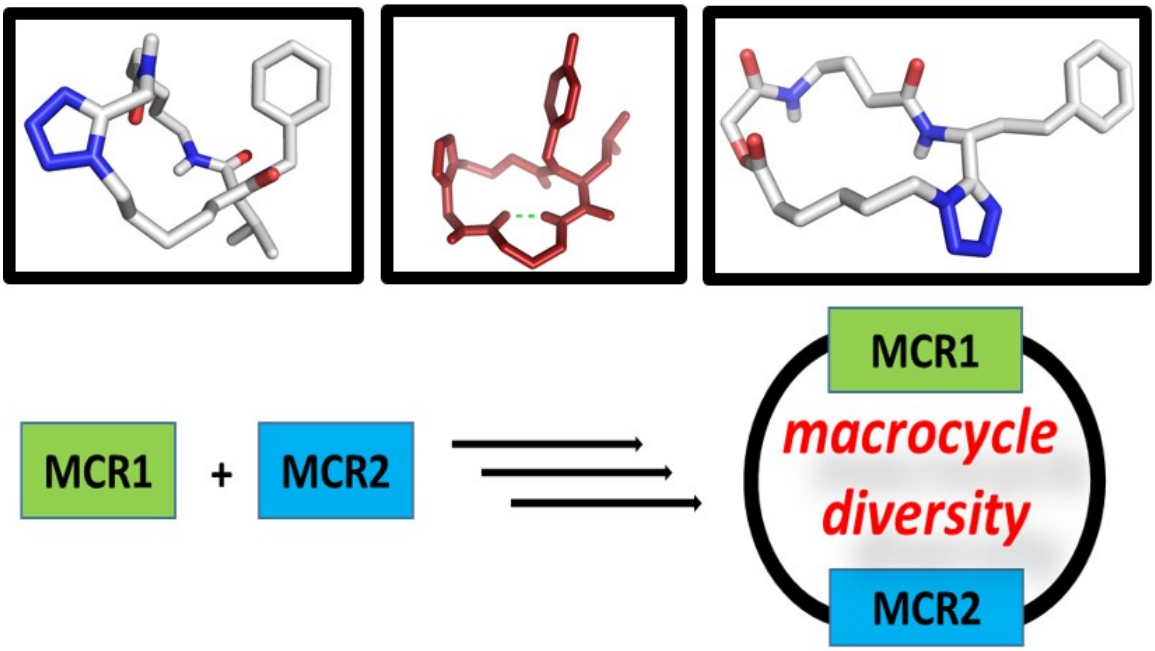

Figure: Above from left to right: X-ray structures of a 22-membered ring comprising a tetrazole and 4CR-Ugi reaction; an intramolecular hydrogem bond based on a g-aminoacid fragment; a 18-membered ring formed by a Passerini 3-CR. Below: A synthetic platform to rapidly access diverse macrocycles by the union of two MCRs.

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POTENT AND HIGHLY SELECTIVE BROMODOMAIN LIGANDS: A PLATFORM FOR REACTION DISCOVERY

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Bromodomains are considered an emerging topic in the field of drug discovery due to their involvement in the regulation of many genes.[1] Bromodomains are protein interaction modules, part of large protein architectures, which function as epigenetic readers able to specifically recognize the ε-N-acetylated lysine residues (KAc group) present in proteins (especially in histones) altering the process of chromatin remodelling.[2] A computer based high-throughput screening study followed by a structure-based medicinal chemistry optimization campaign, has led to the discovery of small-molecule, nM potent bromodomain ligands. These compounds, highly selective towards specific bromodomain proteins, can be used as chemical probes to dissect both the specific function as well as the biological implications of these protein targets.[3]

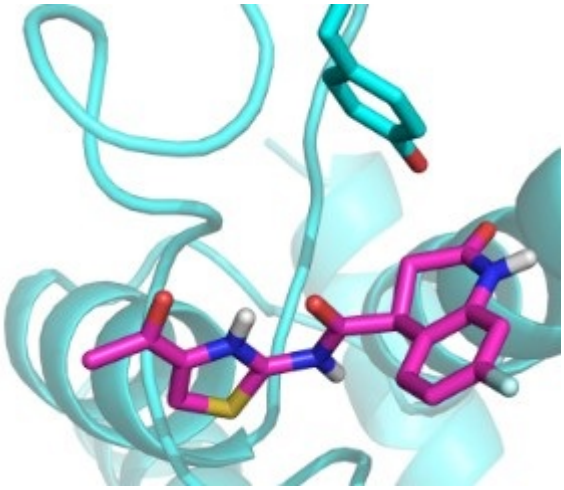

Figure 1. Docked pose of a nanomolar, highly selective, bromodomain ligand.

To produce some of these chemical probes, alkenes and alkynes have been revealed as privileged building blocks as they enable the simultaneous introduction of different functional groups across the π-system.[4] Late transition metals play a prominent role in these transformations. Here, we will also present Au, Ag and Cu-catalyzed reactions accomplishing the functionalization of these simple building blocks. Both, oxidative cross-coupling as well as, radical mediated reactions have been discovered as valuable tools to access densely functionalized structures.[5]

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NUCLEOPHILIC BORON FOR THE PREPARATION OF FUNCTIONALIZED SMALL RINGS

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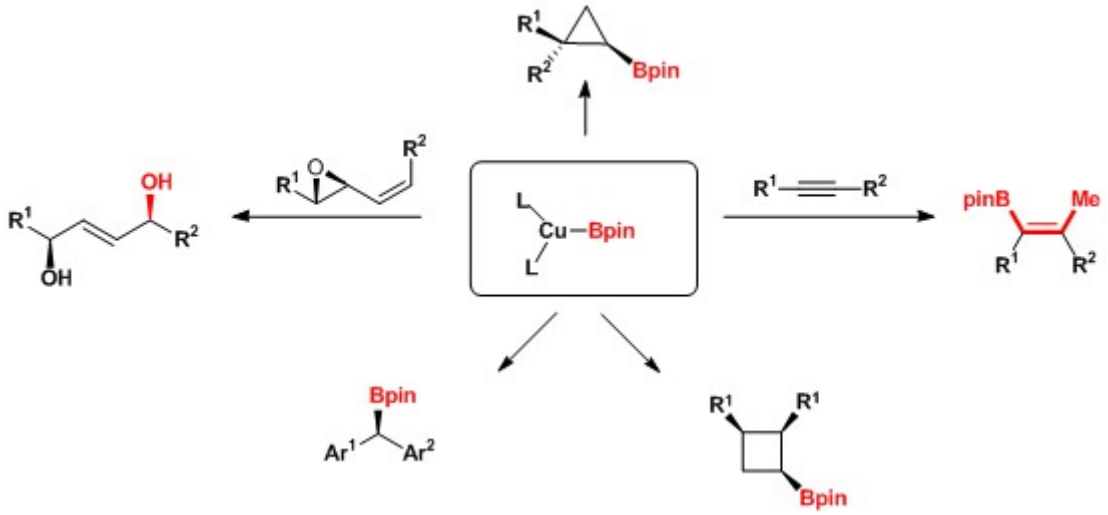
Boronic esters are versatile synthetic intermediates for the preparation of a wide range of organic molecules.[1] The development of new methods to create C-B bonds in an efficient, inexpensive, and environmentally friendly way is therefore an important challenge in organic chemistry. Traditionally, the methods to form C-B bonds have mostly been based on the electrophilic nature of boron. While this classical approach works well for reactions with nucleophilic partners, it naturally limits the types of boron compounds that can be prepared. Recently, copper-catalyzed borylations have emerged as a new source of nucleophilic boron. The lower price and toxicity of copper versus other transition metals and the unique reactivity of the boryl-copper intermediates make these processes particularly attractive. Inspired by unsolved problems found in the total synthesis of complex molecules, we have used boryl-copper species to synthesize useful synthetic intermediates such as 1,4-diols,[2] trisubstituted alkenes,[3] dibenzylic derivatives[4] and functionalized small rings.[5] Some of these results will be presented in this talk.



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RAPID COMPOSITION OF TRICYCLIC SPIRANOID LACTONES: ACCESS TO NATURAL FRAMES AND APPLICATION IN SELECTIVE SILENCING OF THE PAIN RECEPTOR TRPV1

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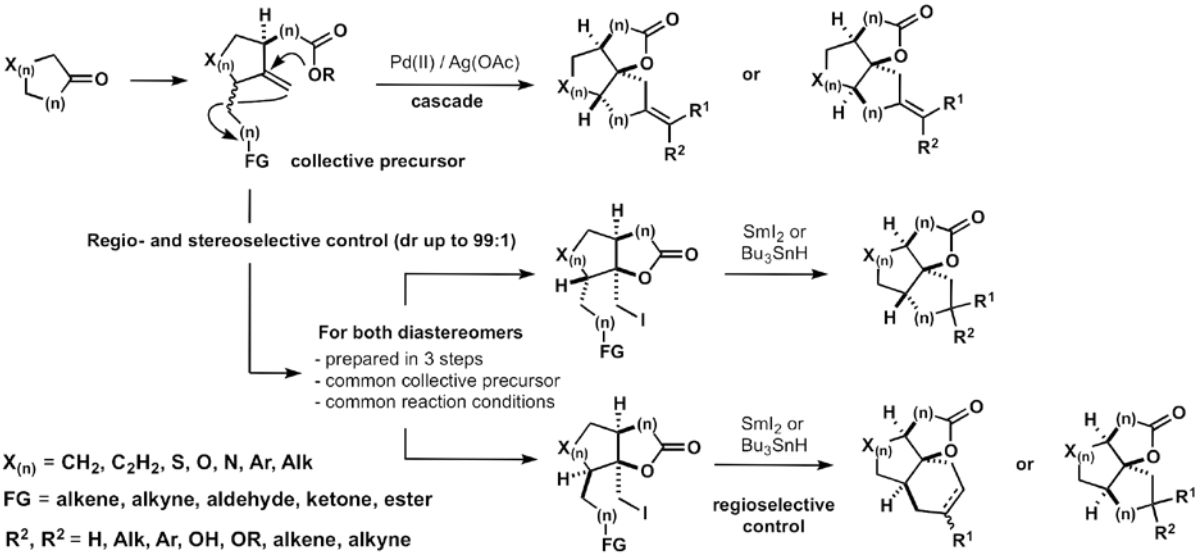
Tricyclic spiranoid lactones can be frequently observed as scaffold segments of various biochemical compounds of natural origin. Analysis of their molecular frames reveals a compact carbon skeleton with angularly fused tricycles of different oxidation states in each of the rings, which together present a substantial synthetic challenge. We designed a general and collective synthesis of topologically diverse tricyclic spirolactones via controlled cyclizations of simple and easily accessible key precursors. Our synthetic strategy is short, regioselective, and offers the possibility to access a broad spectrum of quaternary carbon-centered spiranoid scaffolds. The rapid composition of cycloalkylmethylene key precursors yields an assembly of bicyclic diastereomeric iodolactones, which are individually converted to form a wide range of tricyclic angularly fused spiranoid lactones of different topologies by simple diastereomeric differentiation in a regioselective and stereodirected fashion. The synthetic advantage of novel protocol is exemplified by the successful preparation of tricyclic topologies of tricyclic spirafuranone frames via the shortest sequence reported to date and through the use of easily accessible starting materials. Given high degree of similarity of such scaffolds to natural compounds and the chemical diversity, that is now accessible by fast and simple synthetic routes, it is believed that such compounds may pave the way to the design, synthesis, and biological evaluation of new materials with potential drug-like activity.

The distinguished group of substances drew our close attention – was the family of natural agonists of the Pain Receptor Transient Receptor Potential Vanilloid 1 (TRPV1). Analyzing the scaffolds of natural TRPV1 agonists, we realized that there is remarkable overlap in their molecular architectures and the structures of novel tricyclic spiranoid lactones. We hypothesized that our molecules could serve as operationally acceptable ligands for TRPV1. Notably, although the structures of spiranoid lactones and natural agonists of TRPV1 are closely related, no attempt to use these scaffolds as activator agents has been reported to date. Using calcium imaging and the neuronal activation profile in response to new scaffolds, we found that our compounds evoke TRPV1 activation.



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VALIDATING NEW EPIGENETIC TARGETS BY SELECTIVE PROTEIN INTERACTION INHIBITORS OF BROMODOMAINS

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Bromodomains (BRDs) are evolutionary conserved protein interaction modules that specifically recognize ε-N-lysine acetylation motifs, a key event in the reading process of epigenetic marks. The human proteome encodes 61 of these highly diverse domains present in 46 mainly nuclear proteins. The recent discovery of potent and highly specific inhibitors for the BET (bromodomain and extra-terminal) family of bromodomains has stimulated intensive research activity in diverse therapeutic areas, particularly in oncology, where BET proteins regulate the expression of key oncogenes and anti-apoptotic proteins. During the recent years we have established a family wide platform of reagents, assays and crystal structures enabling the rational design and comprehensive selectivity screening of bromodomain inhibitors. Using this platform we and our collaborators have developed highly selective chemical tool compounds for most bromodomain subfamilies. In this talk I will present recent data on the developed tool compounds that cover now most bromodomain subfamilies including their *in vitro* characterization and phenotypic responses observed in cellular model systems as well as their potential for the development of new targeted therapies.

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RELEASING THE BRAKE ON APOPTOSIS. DISCOVERY OF BCL-2 FAMILY PROTEIN INHIBITORS

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Abstract not available at the time of printing!

DNA-ENCODED CHEMICAL LIBRARY FOR PROTEIN-PROTEIN INTERACTIONS

Jin Li

HitGen Ltd
China

Abstract not available at the time of printing!

FROM FRAGMENT TO IN VIVO ACTIVITY FOR A CHALLENGING PPI TARGET:THE DISCOVERY OF POTENT INHIBITORS OF THE KEAP1-NRF2 INTERACTION

David Norton (1), Joseph E. Coyle (1), Thomas G. Davies (1), Charlotte Griffiths-Jones (1), Keisha Hearn (1), Tom D. Heightman (1), Rachel McMenamin (1), Sharna J. Rich (1), Caroline Richardson (1), Gordon Saxty (1), Henriëtte M. G. Willems (1), Alison J.-A. Woolford (1), Jeffrey K. Kerns (2), Jen-Pyng Kou (2), John G. Yonchuk (2), Heidi G. Feldser (2), Yolanda Sanchez (2), Joseph P. Foley (2), Brian J. Bolognese (2), Gregory Logan (2), Patricia L. Podolin (2), Hongxing Yan (2), James F. Callahan (2), William E. Wixted (2), Joshua E. Cottom (3)

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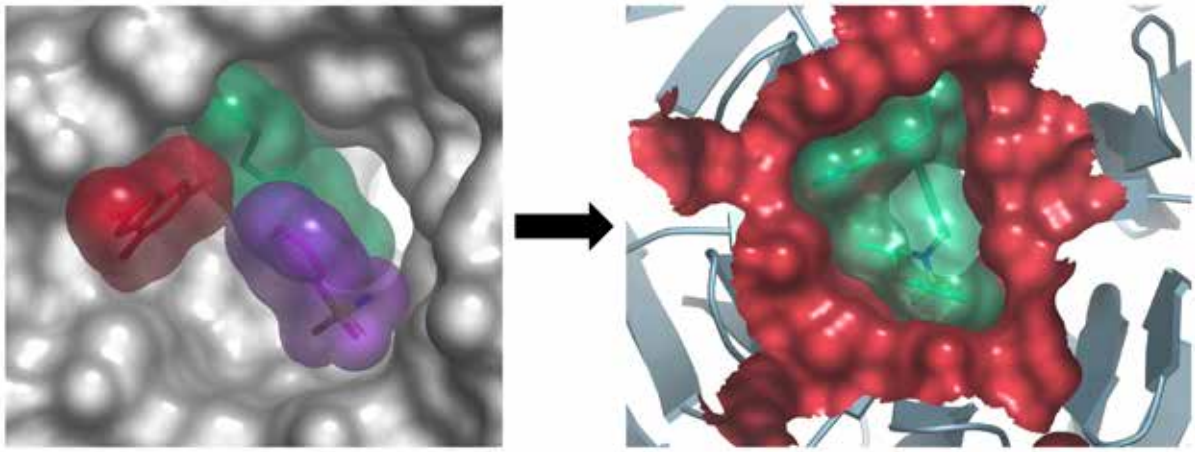
Here we describe the successful application of X-ray crystallographic fragment screening to deliver potent and selective inhibitors of the KEAP1-NRF2 PPI interaction. KEAP1 is the key negative regulator of the NRF2-mediated cytoprotective response and is a target for diseases involving excessive oxidative stress, such as COPD.

The initial hit fragments bound in multiple locations in the NRF2 pocket of the KEAP1 Kelch domain, providing a clear understanding of available binding features. Despite their weak (>1 mM) affinity, the hits possessed good vectors for elaboration. Structure-based fragment optimization and growing led to the discovery of the highly potent and selective small molecule, non-peptidic inhibitor KI-696. KI-696 potently activates NRF2 in cells and shows promising activity in *in vivo* models of oxidative stress, thereby providing a high quality chemical probe to explore the therapeutic potential of disrupting the Kelch-NRF2 interaction.



Fragment hits Kd >1 mM

KI-696 Kd 1.3 nM

POSSIBILITIES AND PITFALLS: DISEASE MODIFYING DRUGS FOR ALZHEIMER'S DISEASE

Eric Karran

Foundational Neuroscience Center, Abbvie
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There has been significant progress in our understanding of Alzheimer's disease (AD). In particular, the genetic architecture of the disease is being resolved leading to new insights into disease causation and pathogenesis. New imaging tools are allowing researchers to reveal the interplay of the key pathologies of AD – amyloid plaques and tau tangles – in living people. Despite this progress, the track record for testing new drugs for AD is very poor. Current hypotheses for AD causation and progress will be discussed as will the implications these have for clinical trials. Recent clinical trial failures will be interrogated for lessons learned, and the future clinical trial landscape will be reviewed.

TACKLING NEURODEGENERATIVE DISEASES THROUGH MULTI-TARGET AND THERANOSTIC SMALL MOLECULES

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Neurodegenerative diseases are major disorders with inadequate standards of treatment and a therapeutic area dogged by disappointing clinical results.

In drug discovery and development, there is a general conviction that the "single-drug-single-target" philosophy of the post-genomic era had inherent conceptual limitations in treating complex neurodegenerative diseases. These diseases may be tackled in a more rational and effective way using what we and others have dubbed multi target-directed ligands (MTDLs), i.e. drugs hitting multiple targets within the neurotoxic cascade underlying neurodegeneration.[1]

In parallel, the very recent literature signals a growing paradigm shift towards integrating therapeutics and diagnostics, rather than developing and deploying them separately. As a result "theranostics", i.e. single chemical entities able to deliver therapy and diagnosis simultaneously are in the process of taking center stage. This strategy has been successfully exploited in oncology and is now emerging as a possibility for Alzheimer's disease and related diseases, where its feasibility has caught the attention of researchers from industry and academia.[2] In this lecture, I will try to substantiate these concepts (at a conceptual and practical level) by providing selected examples taken from our recent research. Despite no one knows if MTDLs and theranostics will reach the clinics, there are already valid arguments that these unconventional tools might increase our overall understanding of neurodegenerative diseases and could be critical for both finding and personalizing their treatment.

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DISCOVERY OF BRAIN-PENETRABLE LpPLA2 INHIBITOR FOR ALZHEIMER DISEASE

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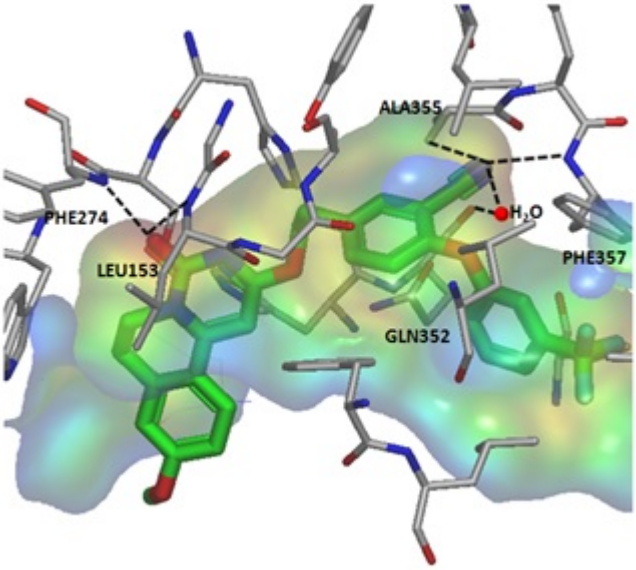
Alzheimer's disease is a progressive neurological disorder that manifests clinically as significant memory loss, cognitive and functional decline. AD is defined post mortem through the presence of the hallmark senile plaques and neurofibrillary tangles, but the etiological mechanism for sporadic AD is still unknown. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) Lp-PLA_2 uniquely cleaves oxidized phosphatidylcholines (oxPCs) generated during the oxidation of low density lipoproteins (LDL), producing pro-inflammatory and pro-apoptotic lyso-phsophatidylcholine (lysoPC) and oxidized nonesterified fatty acids (oxNEFA). Higher plasma Lp-PLA_2 activity has been demonstrated to be associated with increased risk of developing dementia[1] and higher plasma oxidized LDL (oxLDL) levels have been reported in AD patients.[2] Therefore, Lp-PLA_2 is a potential biotarget for the treatment of Alzheimer's disease. Rilapladib targeting Lp-PLA2 shows no cognition decline comparing with placebo in phase IIa study. Tricyclics (TRC) was identified as a lead series to inhibit LpPLA2 enzyme by a High-throughput screen of GSK molecular libraries. Here, we report the structure-activity relationship of TRC, and discuss how to reduce the CYP liability, TDI issue and improve the chemical stability of TRC by optimization of the lead compound. Based on extensive SAR study and soaking crystal structure, the first CNS-penetrable candidate molecule was designed from tricyclics and darapladib, which was demonstrated to be brain penetrable in clinical PET study.



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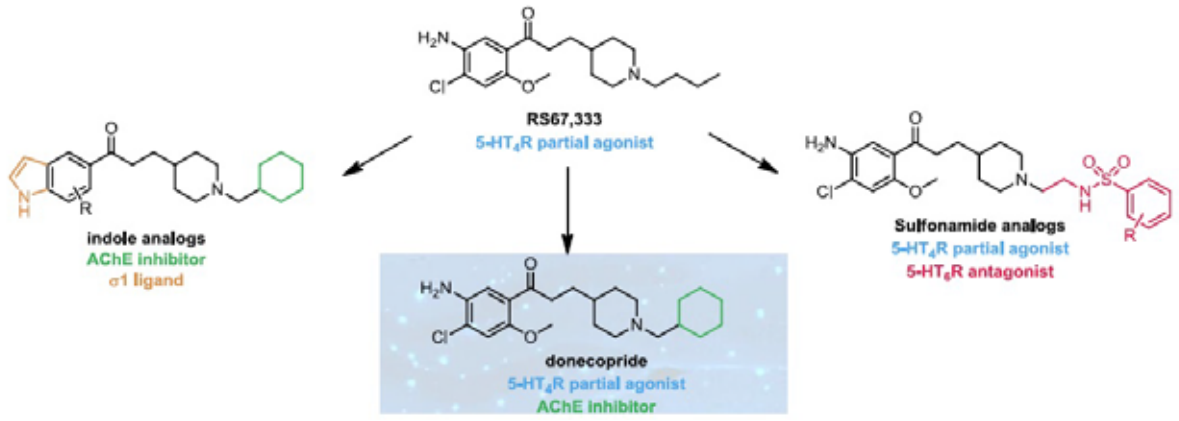
MODULATION OF RS67,333: FROM A 5-HT4R PARTIAL AGONIST TO THE IDENTIFICATION OF SEVERAL PROMISING MULTI-TARGET DIRECTED LIGANDS FOR ALZHEIMER'S DISEASE

Christophe Rochais

Centre d Etudes et de Recherche sur le Medicament de Normandie (CERMN) - UPRES EA 4258 - FR CNRS INC3M - SFICORE, Universite de Caen Normandie, UFR des Sciences Pharmaceutiques - Bd Becquerel F-14032 Caen, France

Targeting more than one molecular cause implied in the pathogenesis of Alzheimer s disease (AD) with a sole drug is considered a promising challenge, because it may address the numerous failures that recently occurred during clinical trials that were conducted in this area. A new strategy is now emerging on the basis of the assumption that a single compound may be able to hit multiple targets, more particularly for the treatment of diseases like neurodegenerative syndromes, which involve multiple pathogenic factors. This concept known as Multi-Target-Directed Ligands (MTDLs) can be used with a great potential benefit towards multiple targets implicated in the complex AD.[1] We will present in this communication our own contribution to this field obtained through the modulation of a RS67,333, a reference 5-HT$_4$R partial agonist, which possesses moderate acetylcholinesterase (AChE) inhibition properties.[2]



Among the different candidate that we have identified we firstly discuss the case of Donecopride, an original compound which associates AChE inhibition and 5-HT$_4$R activation. This compound was identified starting from an *in silico* high throughput screening and a rational drug design strategy followed by *in vitro* biological activities against both targets as well as drugability. These efforts allowed us to select donecopride as a valuable dual (h)5-HT$_4$R partial agonist (Ki = 10.4 nM)/(h)AChEI (IC_{50} = 16 nM) that further promotes sAPP-alpha release (EC_{50} = 11.3nM). The evaluation of donecopride in several animal models, including transgenic 5XFAD mouse model of AD, as well as its first preclinical evaluation will be presented. [3] Novel pharmacomodulations of RS67,333 were conducted and allowed us on one hand to introduce a sulfonamide substituent on the piperidine ring to obtain the first compound able to activate the 5-HT$_4$R and to block the 5-HT$_6$R with promising *in vivo* activities.[4] On the other hand the modulation of the aromatic region led to the identification of potent indolic AChE inhibitor, co-crystallized in the active site of the enzyme which presents additional sigma-1 binding properties. This two novel families will be for the first time disclosed in this communication.

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BIG DADA IN MEDICINAL CHEMISTRY?

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Good compounds are overlooked for various reasons. Computers can help by examining molecular features no chemist can see. Identifying promising candidates (positive design) is equally important as eliminating the bad apples to avoid undesired effects (negative design) as early as possible in the drug discovery process. While medicinal chemists excel in optimizing hits to eventually become lead structures and enter clinical trials, the computer's domain is to rapidly sift through many millions of molecules to discard the bulk before any screening assay is performed with the selected hits that remain after thorough *in silico* scrutiny. "Big Data" in this context means sifting through chemical space while considering available bioactivity data for navigation. "Deep learning" methods may help in this endeavor. In fact, recent technological advances in both computer hardware and software have enabled a renaissance of "de novo" design of molecules with desired pharmacological properties.

We will present our current perspective on the concept of automated molecule generation by highlighting chemocentric methods that may capture druglike chemical space, consider ligand promiscuity for hit and lead finding, and provide fresh ideas for the rational design of customized screening compound libraries. We will specifically focus on natural-product-inspired molecular design by computational means. Recent applications of automated *de novo* design methods will be presented that suggest innovative, synthetically accessible small compounds mimicking structurally more complex natural products.

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EXPLOITING LINKED OPEN DATA - TRANSPORTER PROFILING AND BEYOND

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With the public availability of large data sources such as ChEMBL and the Open PHACTS Discovery Platform, retrieval of data sets for certain protein targets of interest measured under consistent assay conditions is no longer a time consuming process. Furthermore, especially in case of Open PHACTS it is possible to query across different domains such as compounds - targets - pathways- diseases. This allows to target rather complex research questions [1]. In addition, the use of workflow engines such as KNIME or Pipeline Pilot enables to simultaneously search for several targets and filter the results according to e.g. assay type and scaffolds. Within this talk we will present case studies for the exploitation of linked open data for the development of ligand-transporter interaction models, as well as MDR1/BCRP and SERT/DAT selectivity profiling. The latter exemplifies a use case where we started with a search in the Open PHACTS Discovery Platform and ended up in a structure-based hypothesis for transporter selectivity of a set of cathinone analogs. Furthermore, we will demonstrate workflows for performing read across studies for safety assessment.

Acknowledgements:

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MINING STRUCTURAL PROTEIN-LIGAND INTERACTIONS TO NAVIGATE MEDICINAL CHEMISTRY SPACE

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A systematic analysis is presented of all structural protein-ligand interactions in kinase, phosphodiesterase, and G Protein-Coupled Receptor crystal structures present in the Protein Data Bank (PDB). The consistent structural alignment of ligand binding site residues enables the systematic analysis of protein−ligand interaction fi ngerprints (IFPs) within kinases, PDE, and GPCR protein families, the identification of subtype-specific protein−ligand interaction features, and the classification of ligands according to their binding modes. We illustrate how systematic mining of the constructed kinase (KLIFS[1-3], PDE (PDEStrIAn[4-5]), and GPCR[6-8] structure and ligand interaction annotated databases gives new insights into how conserved and protein selective interaction hot spots can accommodate the large diversity of chemical scaffolds in ligands for different protein targets.

The combination of protein-ligand interaction fingerprint analyses, protein site-directed mutagenesis, ligand SAR, and protein-ligand selectivity profiles via integrated chemoinformatics workflows provide three-dimensional interaction maps to predict protein-ligand complexes for which no experimental structures are available. A substructure analysis of the cocrystallized ligands in combination with those in bioactivity databases provides a toolbox for scaffold hopping and ligand design. The structural chemogenomics analyses lead to an improved understanding of the structural requirements of selective interactions with kinase, PDE, GPCRs that will be useful in structure-based ligand selectivity and polypharmacology prediction and drug discovery studies.

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NON CODING RNA AS A SMALL MOLECULE DRUGGABLE TARGET

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The human proteome consists of some 25,000 gene targets from which most current drug discovery efforts are based. However in recent years it has become clear that the human transcriptome contains a similar number of targets whose RNA does not code for any protein. The role of these transcribed non coding RNA (ncRNA) targets is the subject of much active research in the field of epigenetics. They have been shown to be involved in regulating a diverse range of cellular functions. Their genetic linkage to diseases and traits is both numerically and statistically the same as that for the protein coding RNA when using Single Nucleotide Polymorphisms (SNPs) and Genome Wide Association Studies (GWAS).

We asked ourselves if we could find small molecules leads which could function through binding to putative ncRNA targets by using an analogous approach to our high throughput small molecule – protein discovery platforms at Merck. We initially identified and prioritized over 100 sequences on which to build our experiment. We designed a pragmatic approach to triage these targets using our ALIS affinity selection HTS platform and parts of our small molecule screening collection. We used both chemically diverse screening collections and functionally annotated collections from previous phenotypic screens. To date we have generated millions of screening data points from which we are revealing new targets and mechanisms involving small molecule ncRNA interactions.

We will outline our approach and results so far, including validation experiments and discuss their implications for wider small molecule drug discovery efforts.

Contributors:

Julja Burchard, Peter Dandliker, Fiona Elwood, Joel Klappenbach, Dan Klein, Peter Kutchukian, Charles Lesburg, Ali Nahvi, Elliott Nickbarg, Jennifer O'Neil, Noreen Rizvi, Marija Tadin-Strapps and Graham F. Smith.

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IDENTIFICATION OF ALLOSTERIC INHIBITORS OF GPCRS USING SBDD

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G protein-coupled receptors (GPCRs) are an important and long-standing family of drug targets. Despite many historical success stories, today there are still a significant number of GPCRs with compelling pre-clinical validation that remain highly challenging for drug discovery. Over the last 8 years there has been significant progress in the structural biology of GPCRs facilitating Structure-Based Drug Design (SBDD) approaches. Heptares uses its proprietary StaR® technology to thermostabilise GPCRs by mutagenesis into a chosen conformational state. These purified proteins can then be used for biophysical screening techniques and crystallisation to yield X-ray structures with multiple ligands.

Using the StaR® approach, Heptares has solved structures of multiple GPCRs, leading in some cases to the identification of allosteric binding sites. In some GPCRs, notably Class B members that bind large peptides, the orthosteric binding sites are typically open, mostly occupied by bulk-like solvent, and are extremely challenging from a SBDD perspective. In contrast, allosteric ligands bind in smaller sites with both hydrophobic and hydrophilic regions, making them more tractable for small molecule drug discovery. These recently solved structures highlight the diversity of GPCR binding sites and reveal a much greater variety of ligand binding modes and positions than might have been expected.

A description of the allosteric binding sites on the CRF1 and glucagon receptors will be presented. Insights into their druggability, the implications for wider Class B targets and how this structural information gives insight for a SBDD campaign against the GLP-1 receptor will be discussed. Parallels will be drawn with the Class C GPCR metabotropic glutamate receptor sub-family, where discovery of allosteric ligands has proven more tractable than targeting the large extracellular orthosteric glutamate binding site. A brief case study of the discovery of an mGlu5 Negative Allosteric Modulator using fragment-based approaches will be outlined.

MEDICINAL CHEMISTRY OF PURINERGIC SIGNALLING: TARGETS AND DRUGS FOR THE IMMUNOTHERAPY OF CANCER

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Membrane receptors activated by purines are subdivided into two major families: (i) nucleotide or P2 receptors, further divided into G protein-coupled P2Y receptors and ATP-gated ion channel P2X receptors, and (ii) adenosine or P1 receptors (A_1, A_{2A}, A_{2B}, A_3).[1,2] Purine receptors are widely distributed in the body. Their important role in signal transduction is increasingly recognized and appreciated, and their potential as drug targets is explored and exploited with growing success. The physiological ligands of the two classes of receptors, ATP, ADP (and other nucleotides) and adenosine, are metabolically linked, and enzymes interconverting them, in particular ectonucleotidases, are fine-tuning purinergic signalling. Tool compounds for a wide range of membrane proteins involved in purinergic signalling have been developed in the past decades. Recent successful efforts of our group have focused on the development of novel assays[3-5] and structure-based approaches[6-8] to identify and optimize P1 and P2 receptor antagonists and ectonucleotidase inhibitors.[9-11] Potent and selective tool compounds have been shown to be crucial for elucidating the (patho)physiological roles of purinergic signalling.[e.g. 12] The nucleoside adenosine is strongly immunosuppressive and involved in the immune escape of cancer cells. The presentation will focus on the development of compounds that may be useful for the immunotherapy of cancer.

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TARGETING LONG-CHAIN FATTY ACID RECEPTORS FFA1 AND FFA4 FOR TREATMENT OF METABOLIC DISEASES

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Type 2 diabetes is a serious disease that has reached massive proportions following the global obesity epidemic over the last few decades, and the need for better therapeutics represents an urgent challenge for medicinal chemists. FFA1 and FFA4, also known as GPR40 and GPR120, are seven-transmembrane receptors that are activated by medium- and long-chain free fatty acids. Both receptors have been associated with various forms of activity against metabolic diseases and have in recent years received considerable interest as targets for treatment of type 2 diabetes and related metabolic disorders. FFA1 is highly expressed in the pancreas where it enhances glucose-stimulated insulin secretion and has been pursued as a target for development of safe oral insulin secretagogues, a concept that was proved in clinical trials with the now discontinued drug candidate fasiglifam. The receptor has also been associated with protection of pancreatic b-cells and with incretin secretion. FFA4 is more widely expressed, notably in adipose tissue, macrophages, lungs, pancreas and the intestinal tract, and has been connected to various effects to counteract metabolic disorders. These include insulin sensitization, anti-inflammatory effects, protection of pancreas and liver, and regulation of various hormones that are implicated in appetite and glucose control, such as incretins, glucagon, cholecystokinin and ghrelin. FFA1 and FFA4 therefore hold promise as targets for treatment of type 2 diabetes and related metabolic diseases. The talk with focus on development of specific agonists for the two receptors and discuss the possibility of dually targeting therapeutics.

OPTIMISING MEMBRANE INTERACTIONS TO ACHIEVE DURATION OF ACTION FOR INHALED DRUG CANDIDATES

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We will present the design concepts around optimising membrane affinity for inhaled GPCR targeted drug candidates, including for the first time, NMR evidence that demonstrates the specific interaction of molecules with phospholipids, and the structure activity relationship related to these interactions. This has resulted in the discovery of QCC374, the first selective and potent prostacyclin (IP) receptor agonist rationally designed for dry powder inhalation (DPI) for pulmonary arterial hypertension (PAH). Inhaled QCC374 offers high levels of lung exposure, a long duration of action (via optimised cell-membrane affinity) and low systemic exposure (high plasma protein binding, rapid clearance) and is due to begin Ph2 trials in 2016.

Pulmonary arterial hypertension (PAH) is an orphan disease characterised by chronic elevation in pulmonary arterial pressure, progressive pulmonary remodeling, right heart failure, and mortality. Despite major advances with the development of marketed therapies targeting three pathways: the endothelin, nitric oxide (PDE5 and guanylate cyclase), and prostacyclin pathways, PAH remains a fatal disease with a median survival from diagnosis of 7 years (Benza et al, 2012).

Prostacyclin (IP) analogues confer antiproliferative, vasodilatory, and anti-inflammatory effects. Despite the precedent within this class of approved therapies (epoprostenol, iloprost, treprostinil, beraprost & recently approved oral selexipag), all these agents have significant dose-limiting adverse events (AEs), ranging from headache and jaw pain to nausea/vomiting/diarrhea and hypotension, that significantly impact the safety, tolerability and efficacy of this class. The initiation of prostacyclin therapy is therefore delayed in many PAH patients, with a subsequent negative effect on prognosis (Badagliacca et al 2012).

We believe with optimised membrane affinity and lung duration of action, QCC374 has the attributes to demonstrate a class-leading profile, differentiating from other marketed prostacyclin analogues, with the potential for earlier and broader use across PAH.

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SMALL MOLECULE APPROACHES TO IMMUNE MODULATION IN CANCER

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The recent approvals of immune-modulatory agents for treatment of a wide range of cancers highlights the importance of understanding the role of the host immune system in cancer therapy. Monoclonal antibodies that relieve the T-cell co-inhibitory signals from cell surface receptors such as PD-1 or CTLA-4 exhibit broad activity in multiple cancers and treatment with these antibodies frequently results in durable responses that can continue beyond the cessation of treatment. The breadth and durability of responses to T-cell checkpoint inhibitors such as Pembrolizumab, Nivolumab and Ipilumimab has engendered a great deal of enthusiasm for immune-modulation as cancer therapy. It is possible that immune-modulation will become the foundation for oncology therapy. However, not all patients respond to current immunotherapies. To expand and enhance the effectiveness of such agents it will be necessary to combine them with additional agents that target complementary aspects of immune and cancer biology.

Currently approved immune-targeting therapies consist primarily of biologics-based approaches to target cell surface molecules on tumor or immune cells. For such targets a monoclonal antibody provides a number of advantages including specificity, and potency in inhibition of protein-protein interactions. However, to maximize the benefit of our emerging understanding of immune-oncology it will be necessary to develop agents that impact tumor cells in ways that facilitate immune recognition, agents that alter the tumor microenvironment to enable effective immune function, and agents that affect the immune system directly, including both innate and adaptive immune responses. For example, small molecules affecting the tumor microenvironment such as anti-angiogenics, and effectors of tumor metabolism pathways such as tryptophan metabolism have demonstrated clinical benefit in conjunction with immune-modulatory agents. Similarly, compounds affecting the epigenetic state of a cancer cell appear from pre-clinical data to alter immunogenicity and have shown clinical benefit in combination with T cell checkpoint inhibitors. In addition, traditional cytotoxic agents show combination benefit when used with immune targeting agents, although the specific mechanisms underlying this combinatorial activity are not well understood. Small molecule targets also play important roles in immune cell recruitment or function and may represent therapeutic opportunities.

In order to fully engage the immune system in a therapeutic response it will be necessary to understand the interactions between different cell populations within a tumor. To this end, a modality agnostic approach is required to generate the tools and drugs that will enable further dissection of these complex interactions. The inability of monoclonal antibodies to effectively access intra-cellular targets significantly limits the accessible target space. As a result, small molecule antagonists and agonists of pathways that either directly or indirectly affect a wide spectrum of immune and cancer cell biology will be critical to understanding the role of the immune system in tumor maintenance. Ultimately, the goal is to translate improved understanding into more effective therapies that enhance and expand the emerging paradigm of immune-modulation as a foundation for cancer therapy.

THE IDENTIFICATION OF GSK2879552, A MECHANISM BASED IRREVERSIBLE INHIBITOR OF THE HISTONE LYSINE DEMETHYLASE LSD1

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LSD1 (*lysine specific demethylase I*), a flavin-dependent histone demethylase that oxidatively removes methyl groups from mono- and di-methylated Lys-4 of histone H3 (H3K4), is a component of various transcriptional corepressor complexes that often include HDAC1/2 and CoREST. LSD1 is a key regulator of the epigenome, modulating gene transcription at both histone and DNA levels, making it an interesting target in oncology. High-throughput screening of the GSK compound collection identified two validated hit series, one based on tranylcypromine (Parnate®), a known irreversible LSD1 inhibitor, the other a reversible inhibitor series. Parallel lead optimization of the two series lead to the design of the clinical asset GSK2879552, a highly selective irreversible inhibitor of LSD1 .

DISCOVERY OF AG-120 – A FIRST-IN-CLASS INHIBITOR OF IDH1 MUTANT ENZYMES FOR THE TREATMENT OF CANCERS HARBORING IDH1 MUTATIONS

Janeta Popovici-Muller (1), René M. Lemieux (2), Jeffrey Saunders (3), Francesco G. Salituro (4), Katharine Yen (1), Kimberly Straley (5), Erica Tobin (1), Fang Wang (1), Stefan Gross (1), Erin Artin (1), Lenny Dang (1), Hua Yang (1), Luke Utley (6), Yue Chen (1), Andrew Olaharski (7), Lee Silverman (1), Sam Agresta (1), Michael Su (1), Scott Biller (1)

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Somatic point mutations at a key arginine residue (R132) within the active site of the metabolic enzyme isocitrate dehydrogenase 1 (IDH1) confers a novel gain-of-function in cancer cells resulting in the production, and accumulation, of high levels of D-2-hydroxyglutarate (2-HG), an oncometabolite. Elevated levels of 2-HG is implicated in epigenetics alterations and impaired cellular differentiation. IDH1 mutations have been described in an array of hematological malignancies and solid tumors. This presentation will recount the discovery of AG-120, a first-in-class, potent, reversible, selective, orally active inhibitor of the IDH1 mutant enzyme. AG-120 has an acceptable safety profile and exhibited early indication of antitumor activity in a Phase 1 clinical trial in patients with cancers harboring an IDH1 mutation.

TARGETING THE JANUS-FACED NATURE OF IDO1 IN IMMUNO-ONCOLOGY.

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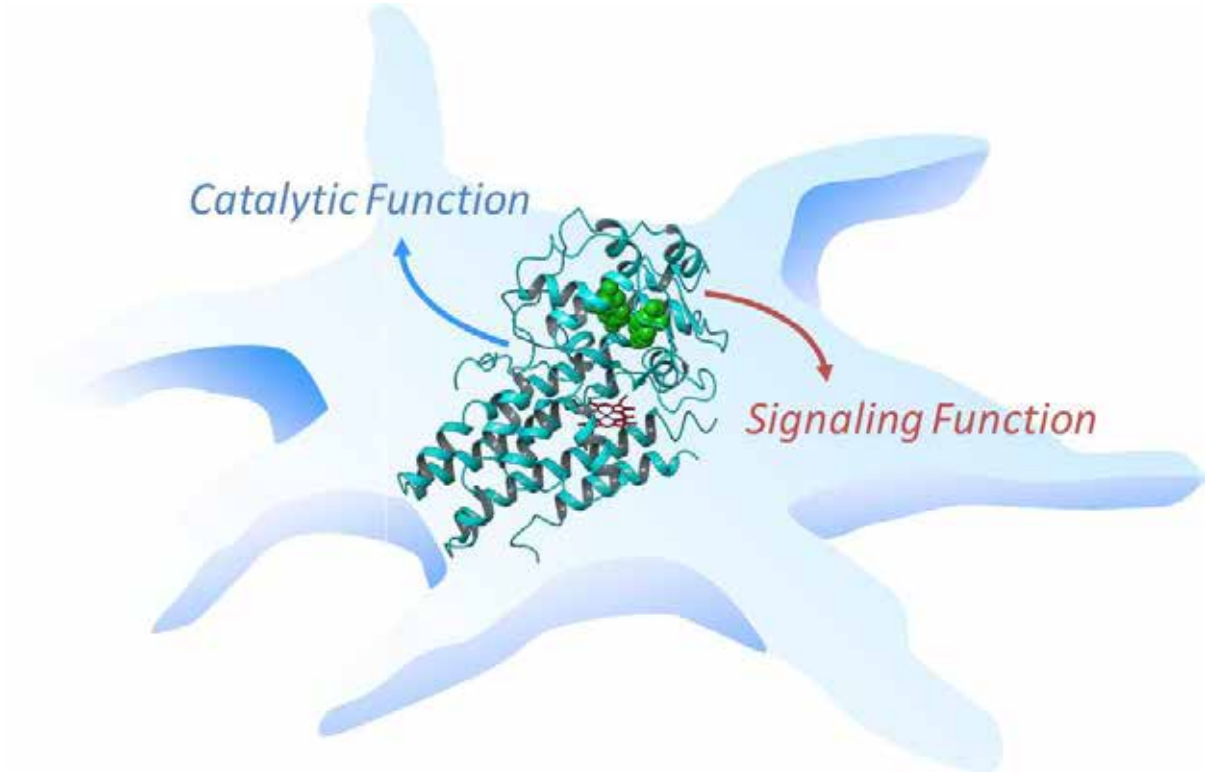
The immune-oncology landscape has evolved rapidly in recent years, with first-generation therapies entering into the market and next-generation combination therapies being seek to boost the efficacy of anticancer drugs by stimulating the immune system in detecting and destroying cancer cells.

In this framework, IDO1 has attracted a great deal of interest from pharmaceutical companies and academic research groups, being highly expressed in cancer cells and participating to the tumor immune-editing process which sets up peripheral tolerance to tumor antigens.[1-3] Nevertheless, only very few IDO1 inhibitors have hitherto progressed into clinical settings, despite the large variety of IDO1 inhibitors being reported in literature and patent applications.[4]



In this communication, an integrated approach to disclose novel ligands of IDO1 will be presented, highlighting challenges that make IDO1 a difficult target to deal with. Specifically, the approach is based on the combination of computational studies, biophysical methods and cell-based assays that allow disclosing small molecules with different pharmacological profiles of target modulation activity.

Acknowledgments

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PHENOTYPIC SCREENING FOR THE DISCOVERY OF NOVEL MOLECULES FOR THERAPEUTIC HEART REGENERATION

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Regeneration of heart tissue after a heart attack has the potential to improve heart function through generation of new contractile muscle instead of scar tissue. Multipotent progenitor cell populations have been reported to exist in the heart, some of which can play a role in normal turnover and/or repair after injury. However, these progenitor cells are rare and true functional repair after a myocardial infarction does not spontaneously occur. Identification of compounds and target mechanisms aimed at expanding the progenitor cell populations and generating new cardiomyocytes and vasculature is a promising approach to enable effective regeneration of cardiac tissue.

We have performed phenotypic screens for proliferation of epicardium-derived cells (EPDCs) isolated from adult human heart and Nkx2.5+ cardiac progenitor cells (CPCs) derived from human induced pluripotent stem cells. The screens were run as medium throughput assays using the same biologically annotated 10K compound set, with hits tested on human cardiac fibroblasts to remove non-specific cell proliferative agents. Developing and running these screens in parallel has allowed us to identify compounds that specifically proliferate CPCs and/or EPDCs without affecting proliferation of cardiac fibroblasts. In addition, a multi-lineage differentiation assay has led to the discovery of novel compounds and mechanisms, which can induce differentiation of the CPCs towards the cardiac or endothelial lineages as well as molecules that can induce differentiation to both cell types simultaneously.

Phenotypic screening is a multi-disciplinary activity and this talk will describe our progress and early findings using these relevant human cells combined with high content image analysis. The selection and testing of biologically annotated compound libraries, the use of appropriate computational and bioinformatic tools to help generate and test early target hypotheses and the subsequent build of chemical clusters and structure-activity relationships from the hit molecules will be discussed.

The identification of compounds that proliferate CPCs and EPDCs, but not cardiac fibroblasts, has allowed us to discover novel compounds and targets that have the potential to result in true regeneration of the heart.

HOW CAN ADVANCES IN CRYO-ELECTRON MICROSCOPY REVOLUTIONIZE MEDICINAL CHEMISTRY?

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The Astbury Centre for Structural Molecular Biology
University of Leeds

Recent advances in cryo-electron microscopy have led to a 'resolution revolution' whereby the 3D structures of macromolecular complexes of interest to medicinal chemistry can now be solved at near atomic resolution. Cryo-electron microscopy is now able to routinely achieve 3 – 4 Å resolution for challenging biomedical targets that have so far proved to be intractable for traditional methods of structural discovery. Suitable targets include membrane proteins such as ion channels and transporters, and a wide range of low-abundance, labile macromolecular complexes such as the ribosome and spliceosome. Crystals need not be grown and the technique typically requires 2-3 orders of magnitude less material than a crystal structure. Critically, the structures of complexes with bound ligands ranging from proteins to small molecules can be determined in solution at micromolar concentrations, heralding the beginning of a new age of rational design of drugs against the most challenging targets. My talk will introduce high resolution cryo-EM with a particular focus on what structures can be determined, and how medicinal chemists with an interest in structural biology can take advantage of the world-class facilities for cryo-EM (and ultra-high field NMR) within the Astbury Biostructure Laboratory at the University of Leeds.

INTERACTION OF 4,5,6,7-TETRAHYDROBENZO[1,2-D]THIAZOLES WITH THE CELLULAR CHAPERONE HSP90 AS A POTENTIAL PATHWAY TO INHIBIT HEPATITIS C VIRUS REPLICATION

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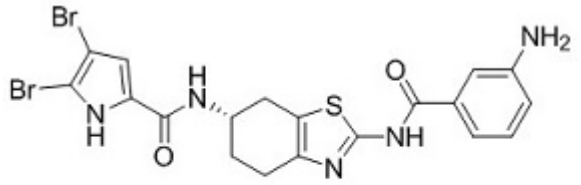
Hepatitis C virus (HCV; genus *Hepacivirus*) is a small, enveloped, positive-stranded (+)-RNA virus that causes severe liver disease, a global public health problem estimated to cause 500 000 deaths annually. HCV therapy relied for long on the use of interferons together with the guanosine analogue ribavirin. Despite recent therapeutic advances, the treatment costs are extremely high and novel therapies are therefore out of reach for most HCV patients even in high-income countries. A further concern is the swift development of resistance against direct-acting antivirals, notably if these drugs are given as monotherapy.[1]

A complementary route to combat resistance development is inhibition of host factors, which makes development of resistance unlikely. Chaperones are cellular proteins that ensure correct folding and assembly of other proteins. One of the most well-known members of this protein family is heat shock protein 90 (Hsp90), a chaperone with ATPase activity. Besides being vital for cellular protein processing, chaperones are a key factor enabling efficient virus replication. HCV, along with other positive-strand (+)-RNA viruses, is dependent on chaperones of the host cell. In addition to needing the chaperones for protein processing, viruses are also capable of optimizing the cellular microenvironment for virus replication through chaperone regulation.[2]

A library of 157 synthetic analogues of marine alkaloids clathrodin and oroidin were screened against replicon models of two RNA viruses, HCV and Chikungunya virus. Four compounds were found to selectively inhibit the HCV replicon (IC_{50} 2.0-11 μM), being more potent than drug ribavirin (IC_{50} 58 μM) and showing low cytotoxicity (CC_{50} 79-120 μM) in HCV replicon. These belong to the 4,5,6,7-tetrahydrobenzo[1,2-*d*]thiazole class of compounds originally designed to target the ATP-binding site of bacterial DNA gyrase [3], which has high structural similarity to the ATP-binding site of Hsp90, a host-cell chaperone universally required for viral replication. Compound binding to Hsp90 was evaluated through microscale thermophoresis and molecular modelling, which confirmed our hypothesis of compounds' interaction with Hsp90 (K_d 18-79 μM) as a basis for their antiviral activity. Structure-based optimization of initial hits resulted in compounds with improved Hsp90 (K_d 4.2-158 μM) and anti-HCV (IC_{50} 1.1-20 μM) activity. The presented novel structural class of small-molecule Hsp90 inhibitors has potential for development of antiviral agents.



IC_{50} (HCV replicon) = 1.1 μM
CC_{50} (HCV replicon) >100 μM
K_d (Hsp90) = 37 μM

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ADVANCED CHEMICAL GENETICS FOR EPIGENETICS: BUMP AND HOLE AND PROTACs

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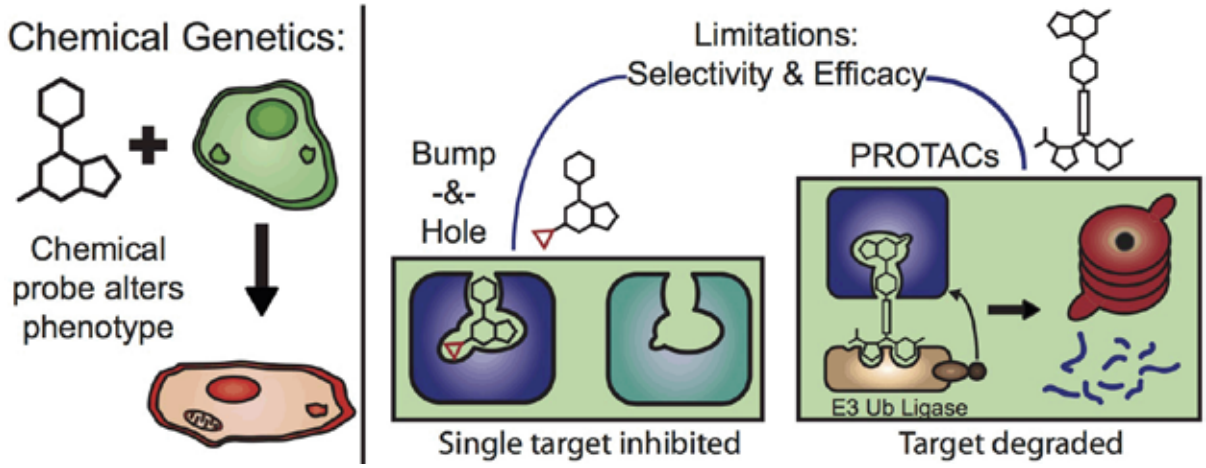
Chemical genetics is the use of biologically-active small molecules (chemical probes) to investigate the functions of gene products, through the modulation of protein activity. Recent years have seen significant progress in the application of chemical genetics to study epigenetics. The difficulty in generating single-target selectivity has long been a thorn in the side of chemical genetics, however recent developments in advanced forms of chemical genetics promise to bypass this, and other, limitations [1].

In my talk, I will first describe our development of a 'bump-and-hole' strategy with which we have engineered an allele-specific derivative (ET) of BET bromodomain inhibitors JQ1 that achieves up to 540-fold selectivity for a BET bromodomain Leu/Ala mutation [2]. Using this approach, we have showed that blockade of the first bromodomain of the BET protein Brd4 is sufficient to displace the protein from chromatin [2]. Second, I will describe how we could achieve for the first time selective intracellular targeting of Brd4 over the homologous BET family memebrs Brd2 and Brd3 by conjugating the pan-selective ligand JQ1 to a potent ligand that we had previously developed against a specific E3 ubiquitin ligase, VHL [3]. Our proteolysis-targeting chimeric (PROTAC) molecule MZ1 achieves rapid, time-dependent, long-lasting, and dose- dependent preferential removal of Brd4 over Brd2 and Brd3, and induces a more profound anti-proliferative effect than BET inhibition in cancer cells [4].

The bump-and-hole approach now demonstrated successfully with BET bromodomains, may be applicable to other epigenetic domains and has potential to enhance target validation of epigenetic cancer targets in future. Meanwhile targeted protein degradation by PROTACs has been shown to be significantly more efficacious than standard domain inhibition, and has the potential to enhance on-target selectivity, with attractive untapped therapeutic potential.



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BROAD MULTIPARAMETRIC PROFILING OF KINASE INHIBITORS

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The explored kinome was extended with broad compound activity profiling using the DiscoveRx and Millipore assay panels. The analysis of the profiling of 3368 selected inhibitors on 456 kinases in the DiscoveRx format delivered several insights. First, the coverage depended on the threshold of the selectivity parameter. Second, with single point profiling false positive results are an important factor, especially for the more selective compounds, and confirmation with dose response curves is essential. Third, comparing the coverage of a focused to a random library showed that the design based on a maximum number of scaffolds was superior to a limited number of scaffolds.
High content multiparametric cellular imaging of these inhibitors and subsequent clustering allows identification of phenotypic-similar compounds to known marketed kinase inhibitors and reference compounds. The cellular imaging data was also used to construct predictive models for compound activity on specific targets, by correlating the multiparametric readouts with known actives and inactives for those specific targets. These predictive models allow for identification of specific kinase inhibitors by using their cellular profiling data exclusively.
In conclusion, selective compounds can be used in target validation, enable the jumpstarting of new kinase drug discovery projects, and chart new biological space via phenotypic screening. Large scale multiparametric cellular imaging of kinase inhibitors can be used to identify compounds with desired target activity profiles.

NEW PSEUDOKINASE DRUG TARGETS IN THE HUMAN KINOME

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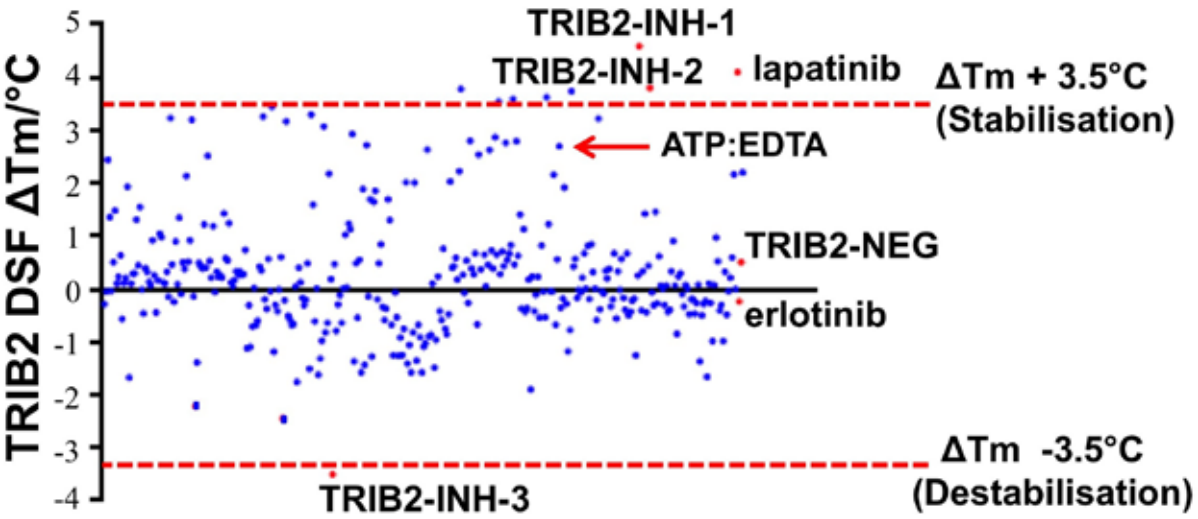
Protein kinases are often mutated or dysregulated in human diseases, making them valuable therapeutic targets. Most (but not all) kinases cycle reversibly between low and high activity catalytic states, and both active and inactive kinase conformations are targetable with small molecule ligands. In addtion, approximately 10% of human kinases are classified as pseudokinases, since they have evolved (and maintain) unusual amino acid signatures at key catalytic and regulatory loci that are likely critical for biology and disease [1,2]. There has been some debate as to the function of protein pseudokinases, but given the presence of pseudoenzyme-like variants throughout all major enzyme families, including phosphatases, proteases and ubiquitin-modifying enzymes, understanding cellular pseudoenzme biology is of prime importance [3]. We have focused our work on the study of protein kinases and pseudokinases that are of therapeutic interest. In this talk, we will discuss progress towards the medium throughput screening of pseudokinase domains with oriented small molecule libraries, and how hits from such screens can be used to evaluate pseudokinase signalling in human cells using on-target validation approaches. An emerging theme is that drug repurposing and drug re-optimisation has the potential to be useful for understanding both biology and therapeutic potential amongst pseudokinases.



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SWITCH CONTROL INHIBITORS: AN ADVANCE IN TYPE II KINASE INHIBITION

Daniel Flynn, Michael Kaufman, Bryan Smith, Wei-Ping Lu, Scott Wise, Yu Mi Ahn, Gary Brandt, Timothy Caldwell, Carol Ensinger, Molly Hood, Cynthia Leary, William Patt, Thomas Rutkoski, Thiwanka Samarakoon, H Telikepalli, Ben Turner, LN Vogeti, Karen Yates, Michael Clare

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Deciphera Pharmaceuticals has developed a platform for Type II kinase inhibition called Switch Control Inhibition. This advanced Type II inhibitor platform places an emphasis on control of switches that are embedded in kinase structures. An embedded kinase switch (activation loop) fluxes to engage its cognate switch control pocket: a process required for kinase conformational activation. Small molecule Switch Control inhibitors antagonize the embedded switch for occupancy of this critical control pocket. Additionally, and equally important, Switch Control inhibitors also stabilize the displaced switch of the kinase, thereby functioning as agonists of the Type II off-switch state. By optimizing inhibitor interactions with both the switch (activation loop) and the switch pocket, clinical stage drug candidates have been developed with improved and differentiated durability properties.

One application of the Switch Control Inhibitor platform is the the ability to inhibit a cancer-causing (oncogenic) kinase regardless of its expression or mutational state and to withstand *de novo* emergence of resistance mutations. This enhanced durability will be highlighted for the MET/TRK inhibitor altiratinib and the pan-KIT inhibitor DCC-2618.

Aliratinib (clinical Phase 1): Altiratinib inhibits wild type MET and TRK kinases as well as their oncogenic fusion protein variants. Significantly, altiratinib inhibits MET kinase regardless of the presence of activating mutations in the MET switch region: residues D1228, Y1230, M1250, and others. Long term exposure studies revealed no mutational outgrowth upon incubation with altiratinib, in contrast to mutational outgrowth, especially at residue D1228, upon incubation with other Type I MET inhibitors. Altiratinib also retains potency versus treatment-emergent NTRK1 resistance mutations that have recently been identified.

DCC-2618 (clinical Phase 1): KIT inhibitors were designed to both antagonize occupancy of the KIT switch control pocket by activating mutant switch variants and also agonize the Type II off state by stabilizing the KIT switch in its inactive conformation. Structure-based design led to identification of the KIT inhibitor DCC-2618, which inhibits wild type KIT and virtually all known activating KIT mutant forms found in GIST or systemic mastocytosis, including KIT V654 (IC50 9 nM), T670I (IC50 18 nM), and D816V (IC50 14 nM) mutations. DCC-2618 also exhibited durability in saturation mutagenesis studies.

Another application of the Switch Control Inhibitor platform is the design of ultra-selective kinase inhibitors. CSF1R kinase (a macrophage immunomodulatory checkpoint) is known to mediate immunosuppression by maintenance of pro-tumoral M2 macrophages in the tumor microenvironment. Leveraging the unique switch control pocket of CSF1R, structure-based design led to the development of the IND-ready specific inhibitor DCC-3014.

FIRST DISCLOSURE OF CDZ173 - DISCOVERY OF A NEW GENERATION OF POTENT AND SELECTIVE PI3K DELTA INHIBITORS FOR AUTOIMMUNE AND INFLAMMATORY DISEASES

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We disclose the discovery and characterization of CDZ173, a potent and selective inhibitor of Phosphoinositide 3-kinase delta (PI3Kdelta). We report how innovative medicinal chemistry efforts led to the identification of a novel and promising tetrahydro-pyrido-pyrimidine lead series that could be rapidly further optimized into a favorable physicochemical space and resulted in the identification of CDZ173, currently in clinical development as an anti-inflammatory therapeutic agent.

In vitro, CDZ173 shows the capacity to inhibit a large spectrum of immune cell functions, as demonstrated in B and T cells, neutrophils, monocytes, basophils, plasmocytoid dendritic cells and mast cells. In vivo, CDZ173 inhibits B cell activation (measured as inhibition of ex vivo -stimulated phosphorylated Akt levels in B cells) in rats and monkeys in a concentration- and time-dependent manner. In preclinical animal models, CDZ173 potently inhibited the antibody production in response to immunization and reduced clinical symptoms in a prophylactic as well as a therapeutic rat collagen-induced arthritis model. Structurally, CDZ173 differs significantly from the first generation of PI3Kdelta and/or PI3Kgamma/delta-selective clinical compounds and, therefore, could differentiate favorably in its safety profile.

First-in-human study indicated an excellent tolerability, favorable pharmacokinetic properties and a direct PK/PD relationship. CDZ173 is currently undergoing a clinical trial in patients suffering from APDS/PASLI, a disease caused by gain-of-function mutation of PI3Kdelta.

SOLUTE CARRIERS, METABOLISM AND DRUG RESPONSE: A MAGIC TRIANGLE

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All biological organisms have genetic material that is kept apart from the environment by lipid-containing membranes. Management of exchange across membranes is critical to ensure access to nutrients, riddance of waste and to safeguard integrity and identity of the organism, by counteracting hostile pathogen invasion pathogen or intrusion of toxic matter. Dedicated proteins are thought to be involved in the import of most chemical matter. Solute carriers proteins (SLCs) represent the largest group of transporters in the human genome and most are poorly annotated. Collectively, their regulated expression contributes to control of metabolism of the cell, as well as of the organs integrated in the organism. We reasoned that if we were to know the transport specificity and function of most SLCs, their dynamic expression pattern could act as proxy for the metabolic state of the associated cell/tissue. We have started to systematically dissect SLC functions by bioinformatics analysis of co-regulation, genetic and proteomic interactions and drug perturbations. To map the regulatory SLC genetic interaction among SLC genes, we are mutating one and scoring for increase or decrease of fitness by genetic altering the expression of the others, across varying environmental conditions. We also score for mutations that confer resistance to cytotoxic drugs. In parallel, we use AP-MS and BioID to characterize the proteomic environment of transporters. While we are only at the beginning of the process, we are already able to identify groups of SLCs that represent co-regulated and functionally interacting modules, integrated over ligands that are either common or related or of which they depend on. We confident that determining the network of SLC as a function of metabolism could lead to the pharmacological exploitation of obligate dependencies.

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THE DESIGN AND EVALUATION OF URAT1 INHIBITORS FOR THE TREATMENT OF HYPERURICEMIA AND GOUT

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Abstract not available at the time of printing!

THE CELLULAR UPTAKE OF PHARMACEUTICAL DRUGS IS TRANSPORTER-MEDIATED - A PROBLEM NOT OF BIOPHYSICS BUT OF SYSTEMS BIOLOGY

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A fundamental question remains as to whether xenobiotic drugs cross cellular membranes mainly (or exclusively) by transporter-independent diffusion across whatever bilayer lipoidal parts of cellular membranes may be present, or whether they normally (or exclusively) 'hitchhike' rides using the carriers normally involved in the metabolism of natural metabolites. The former (for which, astonishingly, there is in fact no actual experimental evidence) would involve a biophysical mechanism, based mainly on lipophilicity, while the latter requires a mechanistic understanding of which carriers are involved, and is thus a problem of network or systems biology. In other words [1], "is carrier-mediated transport of pharmaceutical drugs the exception or the rule?"

A huge amount of literature (e.g. [1-5] and references therein), that I shall summarise, indicates that there is no serious evidence against the view that trans-**p**hospho**b**ilayer-mediated transfer of pharmaceutical drugs across biological membranes **i**s **n**egligible ('**PBIN**'), while there is abundant and increasing evidence for the carrier-mediated route. A recent approach in yeast illustrates this experimentally [6], while the digital availability of principled metabolic network models [7-9] allows one to determine [10; 11], consistent with this, that successful pharmaceutical drugs are much more like metabolites than are the 'Lipinski-compliant' molecules typically available in drug discovery libraries. This suggests (or is at least consistent with the view) that cellular drug uptake is more or less exclusively transporter-mediated, and that knowledge of both the metabolome **and of the concentrations and activities of transporters** used by individual xenobiotics will be of much value in designing better drugs [12-15] and bioprocesses [16].

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DRUG TARGETS IN THE SOLUTE CARRIER CLASSIFICATION (SLC)

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Several solute carriers are reported targets of approved drugs[1]. Here, we use the SLC classification[2] as framework to give an overview on the diseases a transporter is connected to, known drug molecules which are directly targeting the transporter, and a count of bioactivity data and filed patents to give an estimate on the degree of interest on the target.

The data presented here was collected from different sources, where possible with automated KNIME workflows. Molecules targeting transporters were retrieved from the DrugBank xml. Gene/Disease associations from DisGeNET, bioactivity data counts from ChEMBL, and patent counts from SureChEMBL were accessed via the Open PHACTS Discovery Platform. Cladograms to view the data in a tree structure were generated with FigTree using SLC sequences retrieved from Uniprot. Multiple sequence alignments for the members of one Pfam clan (e.g. the major facilitator superfamily MFS) were generated with Clustal Omega using the default parameters on the EBI web server. Counts for each target were added manually.

A preliminary investigation of the counts for SLC members belonging to the amino acid-polyamine-organocation (APC) superfamily shows that seven of the families have reported drugs, with three of them being previously reported by Rask-Andersen et al.[1] to be targets of approved drugs, or under investigation (SLC5, SLC7, and SLC12). Closer investigation of the drugs for the remaining families of the APC superfamily shows that these are mostly vitamins or amino acids. Investigating the number of associated diseases for families without known drugs finds SLC4 and SLC26 as interesting families. Indeed, these are mentioned as potential new targets by Rask-Andersen et al.

Classifications allow (semi)automatic clustering of information. We used the SLC families to give an overview of interacting drugs and associated diseases. One disadvantage of an automated approach, however, is that false positive connections can be drawn. For example, the only human member of SLC32, the vesicular inhibitory amino acid transporter (VIAAT) seems to have a targeting drug according to Figure 3. On closer inspection, this is glycine, which is one of the natural substrates of this transporter. A more detailed investigation will therefore be necessary to draw valid conclusions from these investigations. Additional comparison of the retrieved information with data collections such as the Genetics Home Reference (https://ghr.nlm.nih.gov/geneFamily/slc) will allow to access the relevance of this analysis.

Acknowledgements

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TARGETED COVALENT INHIBITORS OF BRUTON'S TYROSINE KINASE – DESIGN, EVALUATION AND FOLLOW-UP

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Covalent drugs, once considered as a taboo by many people, have gained significant progress during past years. They form covalent bonds with target proteins, and achieve high potency and selectivity. Bruton's tyrosine kinase (Btk), a target for treating several B-cell lineage cancers, has served as an excellent example for targeted covalent drugs. Ibrutinib, a covalent irreversible Btk inhibitor, has demonstrated impressive benefits in multiple clinical trials and won market approvals in both EU and USA. Several new covalent inhibitors of Btk are undergoing clinical testing.

Here, we present the discovery of two generations of covalent irreversible inhibitors of Btk. From ibrutinib to a novel 2,5-diaminopyrimidine-based series of compounds, these inhibitors exhibit different selectivity profiles and modes of inhibition against Btk. Evolution of these compounds would be discussed with emphasis on the role of non-covalent interactions. Enabled with companion covalent probes, covalent inhibitors are advantageous in elucidating their properties in complex biological environment. Design and application of these novel evaluation methods will also be presented. Experiences in developing multiple Btk inhibitors should be useful in expanding the covalent-inhibitor approach for a broad range of targets even beyond kinases.

Keywords: Covalent Inhibitor, Chemical Probe, Bruton's Tyrosine Kinase

CHEMICAL BIOLOGY EFFORTS IN CANCER DRUG DISCOVERY

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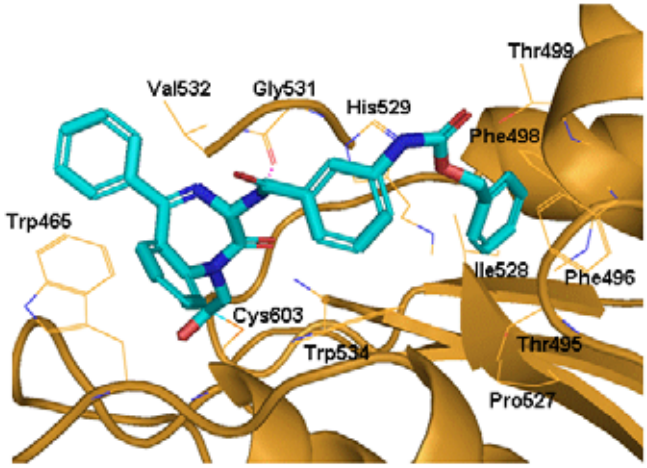
As the best-characterized ubiquitin-like protein (UBL), small ubiquitin-related modifier (SUMO) was found to conjugate with a number of proteins to regulate cellular functions including transcription, signal transduction, and cell cycle. While E1, E2 and E3 ligases are responsible for the forward SUMOylation reaction, SUMO-specific proteases (SENPs) reversibly remove SUMO from the SUMOylated proteins. Recently, SENP1 was found to be a potential therapeutic target for the treatment of prostate cancers. We designed and synthesized two series of SENP inhibitors, and they showed inhibitory activity as good as IC_{50} =0.76 uM. Their structure-activity relationship and selectivity among SENP isoforms will be discussed.



** We thank the National Science Foundation of China (81222042) for financial support.

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ADVANCES IN THE DEVELOPMENT OF COVALENT EGFR INHIBITORS: FROM EARLY HITS TO OSIMERTINIB

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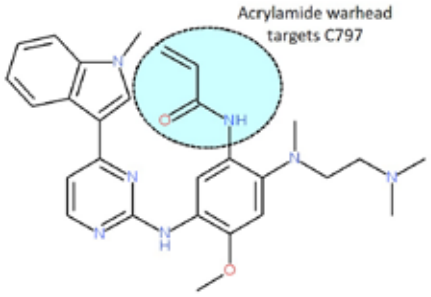
Non-small cell lung cancer (NSCLC) is a leading cause of death globally. Sensitising mutations of the epidermal growth factor receptor (EGFR) have been shown to be responsible for a significant number of cases of NCSLC. Treatment with small molecule inhibitors of the EGFR tyrosine kinase domain (gefitinib, erlotinib or afatinib) results in responses in approximately 70% of patients. However, progression invariably occurs, with most patients developing resistance to these therapies within 9-14 months. In approximately two thirds of cases, acquired-resistance has been shown to be due to the development of a second mutation in exon 20 of the EGFR kinase domain (T790M mutation).

This talk will review aspects of the medicinal chemistry program within AstraZeneca aimed at tackling the T790M resistance mechanism. One element leading ultimately to osimertinib was the development of an inhibitor that could covalently bind to the C797 residue in the T790M mutant form of EGFR. This culminated in the recent approval in the US, EU and Japan of osimertinib, for EGFR T790M mutation-positive NSCLC. Project progression from initial hit-finding activities, through to the profiling of shortlist compounds will be described.



AZD9291/osimertinib

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RAPID DISCOVERY OF POTENT AND SELECTIVE COVALENT INHIBITORS VIA COVALENT DOCKING

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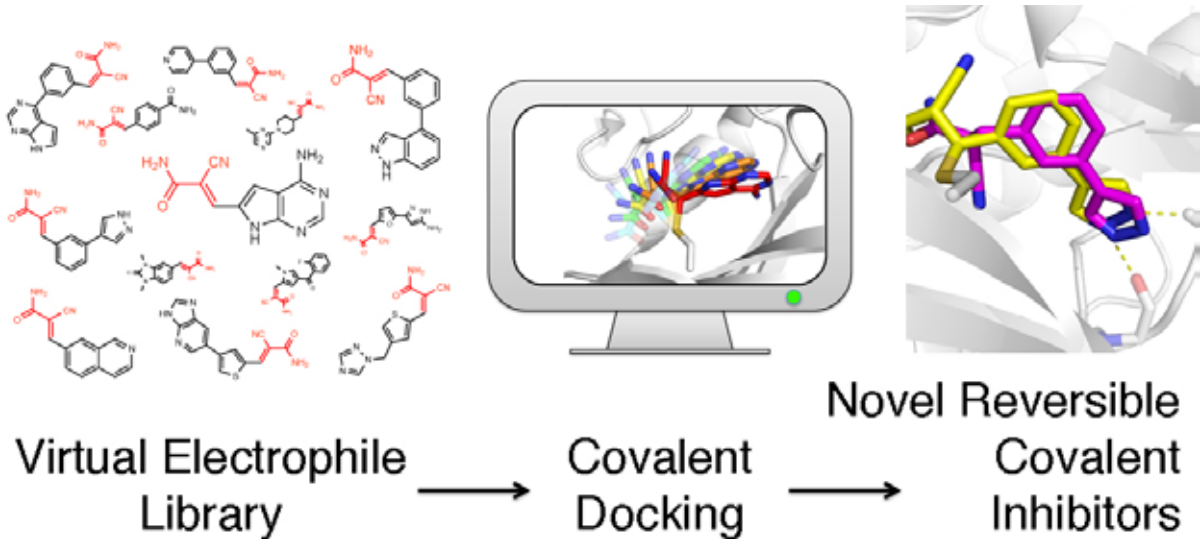
Molecules that are able to form a covalent bond with their target often show enhanced selectivity, potency and utility for biological studies, but are hard to discover, as they are typically expunged from high throughput screening libraries. Computational methods can help bridge this gap. We developed a covalent docking method for the discovery of covalent probes. Applying this method prospectively to several protein targets, and specifically to protein kinases, we were able to discover potent covalent inhibitors (typically with <50nM IC50), with chemotypes not previously explored. The docking predictions were confirmed by crystallography, with blind predictions showing less than 2Å RMSD to the experimental structure. The inhibitors displayed marked selectivity against closely related off-targets and were active in cellular assays. In some cases docking models alone successfully drove compound optimisation.

Here we will describe new results demonstrating how this method can also be applied to very challenging targets such as oncogenic K-Ras bearing a Glycine12->Cysteine mutation. Recently, Shokat and colleagues reported the first covalent compounds targeting a new pocket in K-Ras by forming a covalent bond with this mutant cysteine. By leveraging various available crystal structures of these compounds, and a new virtual acrylamide screening library, we were able to apply covalent docking to discover a novel series of chemically distinct covalent binders for this important oncogene. The compounds showed K-Ras(G12C) labeling in-vitro and exhibited mutant specific killing of patient derived cancer cell lines. On-going work aims to validate the predicted binding mode. Our approach should be applicable for a broad range of protein targets, including challenging targets as we show here, that have resisted drug-discovery efforts for decades.



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ANTIBODY IMAGING AND THERAPY USING IN VIVO CLICK AND CLICK-TO-RELEASE STRATEGIES

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Tagworks' technology enables the in vivo actuation of tagged antibodies through selective chemical manipulation in vivo. Based on a click reaction between a tag and a probe, this approach aims to improve the efficacy of several established antibody-based imaging and therapy applications. Tagged antibodies are allowed to circulate and accumulate at their target site. After clearance from non-target areas, a chemical probe is administered which binds to and actuates the tagged antibody. This talk will address our recent advances in two approaches: 1) click conjugation of radiolabeled probes to tumor-bound antibodies, boosting the target-to-background ratio in Radioimmunoimaging and –therapy, and 2) click release of drugs from tumor-bound Antibody-Drug Conjugates (ADCs), expanding the scope of ADC therapy to non-internalizing cancer receptors.

NOVEL ANTIBODY-TETRAZINE CONJUGATE FOR BREAST CANCER IMAGING

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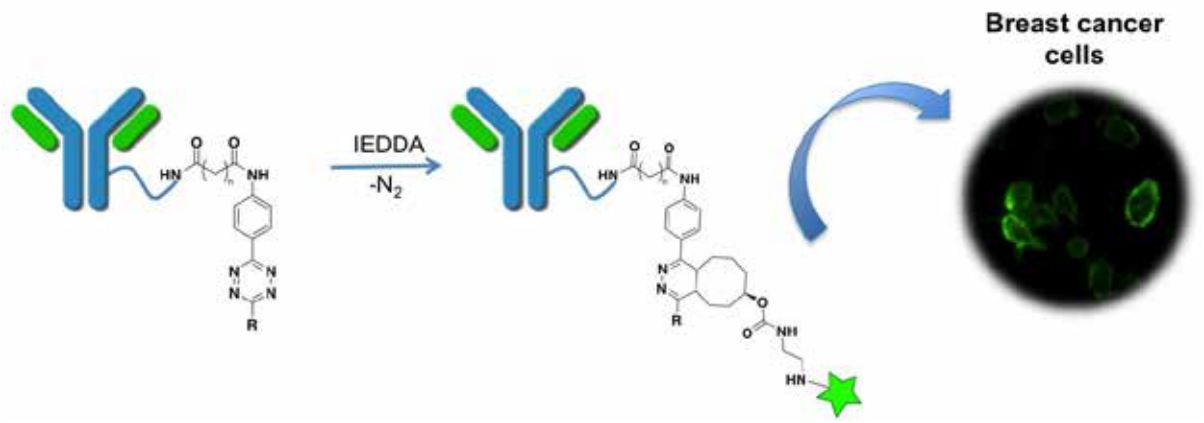

Bioorthogonal chemistry has found rapidly growing applications in the field of molecular imaging. The inverse-electron-demand Diels-Alder cycloaddition (IEDDA) between trans-cyclooctenes (TCOs) and tetrazines is not only biocompatible, but also provides exceptionally fast kinetics, enabling *in vitro* and *in vivo* pretargeting studies.[1]

The remarkable specificity and affinity of antibodies make them extremely attractive vectors for the delivery of diagnostic tools to biological targets, and represent an interesting platform for *in vitro* and *in vivo* application of bioorthogonal chemistry. [2] Considerable research has been devoted to the development of antibody-TCO conjugates, and their application for pretargeted tumor imaging has been reported under different modalities [3]. However, it has been shown that TCO has the tendency to isomerize to its isomer, cis-cyclooctene (CCO) that is orders of magnitude less reactive with tetrazine, after prolonged exposure to physiological conditions.[4] Given this, we envisaged a different approach where the use of tetrazine tags is investigated for antibody modification.

In the present study, we developed a library of tetrazines that were screened in parallel to identify the most suitable candidate for antibody conjugation. The selected tetrazine displayed the best profile regarding stability and reactivity with a half-life ($t_{1/2}$) of 82 h in FBS at 37 °C and a k_2 of 3083±352 M^{-1} s^{-1} in PBS at 37 °C. Further, antibody conjugation was performed using the anti-HER-2 monoclonal antibody trastuzumab (Herceptin) to obtain a novel Herceptin-tetrazine conjugate which showed long stability and fast kinetics towards TCO modified-fluorophores. Moreover, we proved the suitability of the Herceptin-tetrazine conjugate for pretargeted breast cancer cell labeling using fluorescence microscopy. [5]

These findings indicate that this approach is suitable for *in vitro* labeling experiments and suggests that it may be a useful strategy to be translated to *in vivo* pretargeted positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging.

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ATYPICAL BILE ACIDS AS CHEMICAL PROBES FOR DIAGNOSIS AND THERAPY MONITORING OF PROGRESSIVE CHOLESTATIC LIVER DISEASE IN HSD3B7 PATIENTS

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Inborn errors in bile acid synthesis are a well-recognized category of metabolic liver disease.[1] These autosomal recessive genetic defects manifest as a broad phenotype presenting with an overlapping spectrum of variable degrees of neonatal cholestasis, fat-soluble vitamin malabsorption, and neuropathies. 3β-Hydroxy-Δ^5-C27-steroid oxidoreductase (HSD3B7) deficiency is the most common of the 9 known defects in bile acid biosynthesis and is often the cause of idiopathic forms of late-onset chronic cholestasis in children and adolescents,[2] and even in adult.[3] Early diagnosis of these genetic defects is crucial to prognosis, because if undiagnosed or untreated, the liver disease, which is a progressive form of intrahepatic cholestasis, leads to fibrosis, cirrhosis, and end-stage disease. Treatment options include liver transplantation or preferably oral bile acid therapy with the primary bile acids, cholic or chenodeoxycholic acids.[1] Diagnosis of HSD3B7 deficiency is based on the detection of increased concentrations of atypical 3-hydroxy-Δ^5-bile acids that accumulate in urine because of the lack of enzyme activity caused by mutations in HSDB7 gene.[4] So far, direct and accurate measurement of these conjugated 3β-hydroxy-Δ^5-bile acids has not been possible for the lack of reference standards.

We describe here the synthesis and bio-chemical characterization of a series of Δ^5-cholenoic acid analogs, which are the signature metabolites of the HSD3B7 deficiency in humans.[5] Using these synthesized compounds as tools to gain insights into the mechanism(s) responsible of the cholestasis and liver damage in patients with the HSD3B7 deficiency, we report their cellular hepatocytotoxicity, their affinity towards a subset of bile acid-responsive nuclear receptors and the effects on genes and cytochromes involved in bile acid homeostasis and detoxification. Moreover, the availability of these reference compounds has allowed developing an electrospray ionization (ESI) LC-MS/MS method for the accurate measurement of their concentrations in clinical diagnosis and monitoring of response to therapy in patients with HSD3B7 deficiency.[6]

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CHEMICAL PHARMACOLOGY OF PROTEIN CONJUGATES AND NATURAL PRODUCTS

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Instituto de Medicina Molecular, Lisbon, Portugal & University of Cambridge

Our work centers on reaction engineering for site-selective chemical protein modification and its use to provide insight into biology and for the development of protein therapeutics.[1] This lecture will cover recent examples of emerging areas in our group in: (i) site-selective chemical modification of proteins at cysteine and lysine, (ii) bioorthogonal labeling of collagen in live cells,(iii) a new method for histidine-metallation of proteins with a $[Ru(CO)_2]^{2+}$ fragment that yields artificial metalloproteins that are able to deliver CO *in vivo* in a controlled manner,[2] (iv) CO-mediated immunomodulation for cancer therapy, and (v) the exploitation of natural product scaffolds for targeting of calcium channels overexpressed in cancer cells.[3]

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THE PAINFUL REALITY OF DRUG DISCOVERY AND DEVELOPMENT

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There is an indisputable and urgent need for new therapeutic agents to treat chronic pain [1]. People who are unfortunate enough to suffer from any of the various long-term pain disorders represent a vast population whose conditions are poorly managed due to a combination of inadequate efficacy and/or intolerable side effects. The front line standard of care for many of these conditions were developed for other indications, such as epilepsy or depression, which both highlights our failure in bringing through new, targeted analgesics but additionally complicates the translational path for bringing forward alternative, improved therapies [2,3].

Responding to this patient need, many institutions have invested heavily over the years in discovering and developing potential new medicines. Unfortunately, the end result of these investments has yielded disappointingly few new treatment options, and the translation of findings from pre-clinical research into clinical development has been notoriously unsuccessful. Considerable retrospective analysis has apportioned blame on the unreliability of animal models, the narrow and overlapping focus of the pharmaceutical industry and the underlying patient and disease heterogeneity, to name just a few. In line with this lack of success, and despite the patient need, many organisations have reduced or closed their drug discovery efforts in pain, judging the risks too high.

A newer generation of approaches, focussing on emerging understanding from human genetic studies into the pathophysiology of the disease ushered in a new dawn of hope, although it is far from clear that these approaches will be as transformative as initially hoped.

This presentation will review the landscape of therapeutic strategies for the development of new drugs to treat chronic pain disorders, including lessons learned from the various failed approaches of the past. Future perspectives will discuss the opportunities of improved target validation and alternative research models as well as improved confidence in target engagement and clinical read-out.

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3) Novel Translational Approaches for Improving Drug Discovery Efforts in Chronic Pain. Munro. CNS Neuroscience & Therapeutics, 2016 85-87

CHALLENGES IN THE DISCOVERY OF SIGMA-1 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF PAIN

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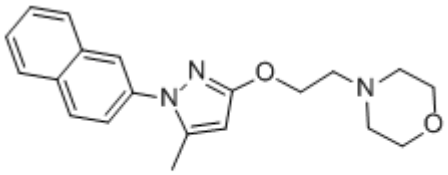
The sigma receptor presents two different subtypes, sigma-1 and sigma-2, which show distinct functions and are related to different potential therapeutic indications. The sigma-1 receptor is a molecular chaperone, mainly located in the endoplasmic reticulum, from where it translocates to the cellular membrane to exert a complex function, involving the modulation of different receptors and ion channels when they become challenged by disease-related stress or mutations. The structure of the sigma-1 receptor bound to two different ligands has been recently solved, showing a fairly unique structure with one transmembrane domain and trimeric organization, which is quite different from the previously most accepted views of the receptor. However, many questions still remain to be solved, such as how the ligands access a rather closed binding site, as well as the processes behind the observed ligand-mediated receptor oligomerization, which may be related to the difficult assessment of functional behaviour of sigma-1 receptor ligands.

The sigma-1 receptor is expressed in regions of the CNS key for pain control and studies in animal models support a role for its antagonists in the treatment of pain states where hypersensitivity develops as hyperalgesia and allodynia, two common symptoms encountered in neuropathic pain and other chronic pain conditions. Moreover, sigma-1 receptor antagonists potentiate the analgesia of opioids, but not the secondary effects associated to them. The progression of E-52862 (**1**), up to clinical trials for the treatment of different pain states, will soon reveal how the relevant preclinical evidence generated by us and other laboratories translates into man.

We summarize here the main challenges encountered in the identification of sigma-1 receptor antagonists for the treatment of pain. In spite of the simplicity of the receptor pharmacophore, which consists of a positive ionisable nitrogen atom and an aromatic group at a certain distance, the design of therapeutically useful sigma-1 receptor ligands, *ie* selective and drug-like, is complicated by the high lipophilicity of the receptor binding site and the overlap of its pharmacophore with that of a number of other targets, including the hERG channel. In addition, due to the intracellular localization of the receptor and its apparent plasticity there is a lack of truthful functional assays with adequate throughput to drive comprehensive medicinal chemistry programs, and hence the identification of sigma-1 receptor antagonists relies on *in vivo* testing.



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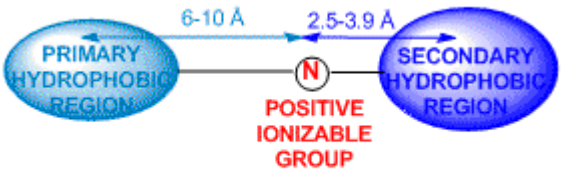

σ_1 Receptor pharmacophore

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FUNCTIONALLY IRREVERSIBLE NON-COVALENT INHIBITORS OF FAAH: A SAFER APPROACH?

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Inhibition of fatty acid amide hydrolyase (FAAH) may promote analgesia through accumulation of fatty acid amides primarily in the CNS and therefore represents an attractive approach for the treatment of pain. It has been postulated that near complete enzymatic inhibition is required. Covalent attachment of an inhibitor to the active site serine has typically been relied upon to achieve maximal inhibition. We chose to avoid the potential liabilities associated with covalent modification and focused our research efforts on non-covalent inhibitors of FAAH, leading to the discovery of our first clinical candidate MK-4409. During our back-up campaign, we developed an understanding of the mechanism of inhibition across five structurally and mechanistically distinct classes of FAAH inhibitors. Results from kinetics, biochemistry, and modeling indicate that potent inhibitors exhibit slow onset of inhibition and a two-step mechanism in which enzyme and inhibitor interact to form an encounter complex which then undergoes an isomerization to a stable form. The rates of isomerization fall within a narrow range across all inhibitor structural classes including those with the potential to form covalent interactions. We conclude that acylation is not a key driver of potency and instead differences in potency are driven by the stability of the encounter complex. This collaborative multi-disciplinary effort involving chemistry, molecular modeling, *in vitro*, and *in vivo* biology negated the need for a covalent interaction (even if transient) and resulted in the discovery of "functionally irreversible" non-covalent inhibitors of FAAH that may redefine the paradigm for best-in-class FAAH inhibitors.

TOWARDS LOW-RISK PAINKILLERS: HUMAN DIPEPTIDYL PEPTIDASE III (hDPPIII) AS A NOVEL TARGET FOR THERAPEUTIC PAIN INTERVENTION

Shalinee Jha (1), Jakov Ivkovic (2), Ulrike Taschler (3), Prashant Kumar (3), Robert Zimmermann (3), Karl Gruber (3), Rolf Breinbauer (2), Peter Macheroux (1)

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Dipeptidyl peptidases III (DPPIII) is a zinc-dependent metalloenzyme involved in degrading shorter peptides with 4–12 amino acid residues. It exhibits high affinity to opioid peptides and to some of the vasoconstrictor peptides from renin-angiotensin-aldosterone system. In view of this it has been associated with pain signalling, cardiovascular pressure regulation and enhancement of cancer cell defense against oxidative stress [1], but the precise function of DPPIII is still unknown. The availability of the first cocrystal structure of human DPPIII (hDPPIII) with a peptide substrate [2] provided the basis for structure-based design of selective inhibitors for this enzyme. The main objective of creating small-molecule inhibitors is to use them as tools for chemical probing of the role of DPPIII *in-vivo*. Peptidomimetic transition state inhibitors were developed where a hydroxyethylene isostere was used to replace the scissile peptide bond in the earlier reported peptide substrate mimetic inhibitors [3], thereby making the inhibitor stable against degradation by hDPPIII itself. Two epimers of hydroxyethylene based mimetics have been synthesized and they successfully inhibited recombinant hDPPIII at low micromolar concentrations. This is the first instance of efficient inhibition of a metalloprotease by a hydroxyethylene pseudopeptide.

Additionally, to better understand the physiological function of this peptidase, DPPIII knock-out mice have been generated. Using fluorescence based assays, the tissue-specific expression and *in-vivo* substrate specificities of the enzyme were investigated. The insights gained by the characterization of hDPPIII will provide a starting point for the design of molecular tools specific for inhibiting hDPPIII and pave the way to exploit this enzyme as a potential drug target for pain intervention strategies. These inhibitors might offer alternatives to conventional treatments in the nociceptive field, especially the use of new opioid receptor agonists to replace morphine and its derivatives, which possess severe side effects.

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HISTONE ACETYLTRANSFERASE INHIBITORS, FROM SCREENING TO OPTIMIZATION - A TRICKY TRACK

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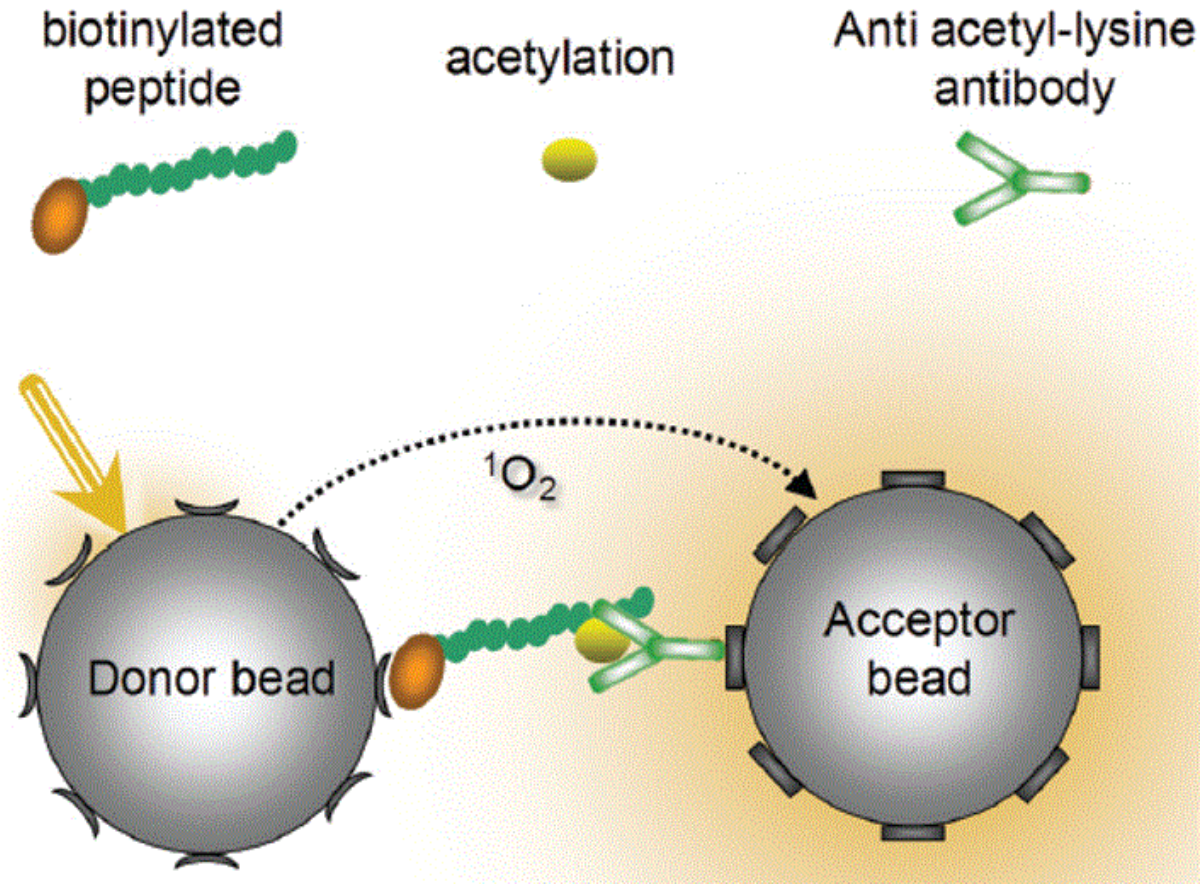
There is currently great interest in compounds that modulate epigenetics. With respect to some epigenetic targets, such as histone decacetylases (HDACs), many inhibitors have been successfully developed and are in clinical trials for a variety of indications. Similarly, bromodomains have been shown, somewhat unexpectedly by some, to be highly druggable. However, there is an elephant in the room, and that is the histone acetyltransferase (HAT) family, which is large but essentially "undrugged" and barely has any compounds that could be considered to be useful tools. Why is it so hard to find good tool compounds for these enzymes? Not so long ago we undertook HTS against a MYST HAT [1] and eventually discovered a genuine hit that we have recently just optimized to nanomolar levels of inhibition. However, we encountered many problems en route. In this talk we will discuss such issues and how these could help explain why there are so few, if any, useful tool compounds for these enzymes.



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DIRECT AND SYNERGISTIC INHIBITION OF THE HCV NS5A PROTEIN

Makonen Belema

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Hepatitis C virus (HCV) therapy has evolved rapidly over the past five years from interferon-based regimens to highly efficacious, small molecule combinations that inhibit at least two of the three non-structural viral proteins critical to replication (i.e., NS3, NS5A and NS5B). Whereas the respective enzymatic activities of the NS3 and NS5B proteins in the viral replication cycle as a protease and a polymerase were sorted out well before the identification of their marketed inhibitors, the exact function of the NS5A protein has, in many regards, remained a mystery. That noted, as part of our HCV drug discovery effort, we had uncovered from a phenotypic screening exercise an NS5A-targeting hit that was successfully optimized to daclatasvir (DCV) (Daklinza™), a potent HCV inhibitor that provided clinical proof of concept for the NS5A protein as a therapeutic target and which has secured regulatory approvals in over 60 countries. During the mode of action studies into DCV-associated molecules, we uncovered a synergistic interaction that led us to conduct a phenotypic co-screen in the presence of DCV that ultimately resulted in the identification of NS5A-targeting molecules that in and of themselves lack meaningful inhibitory activity but are able to restore the potency of DCV toward various clinically relevant NS5A resistant mutants. Key highlights from these two related efforts, with roots in phenotypic screens, will be discussed.

DISCOVERY OF IN VIVO ACTIVE AND SELECTIVE DIACYLGLYCEROL LIPASE-ALPHA INHIBITORS

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The endocannabinoid system is a clinically proven biological signaling system involved in human energy balance. Diacylglycerol lipases (DAGLα and DAGLβ) are responsible for the formation of the endocannabinoid 2-arachidonoylglycerol, a full agonist of the cannabinoid CB_1 receptor, which is also the main precursor for arachidonic acid and pro-inflammatory eicosanoids in the brain. Our understanding of DAGLα function, which belongs to the class of serine hydrolases, has been hindered by a lack of chemical probes that can selectively perturb DAGLα *in vivo*.

Here, we will publicly disclose the first structures from a lead discovery program executed in the framework of a public-private partnership with the European Lead Factory. The Joint European Compound Collection was successfully screened using a 1536-wells high throughput assay with recombinant human DAGLα. This resulted in a qualified hit list of 47 compounds. Activity-based protein profiling (ABPP) with mouse brain proteomes was employed as an orthogonal assay to prioritize the hits based on activity and selectivity over endogenously expressed serine hydrolases. We identified 1,2,4-triazole urea sulfonamides as an excellent starting point for a medicinal chemistry program. ABPP and detailed enzyme kinetic analyses were employed to efficiently guide the hit optimization process. This resulted in the discovery of a highly selective DAGLα inhibitor that demonstrated *in vivo* target engagement. This new inhibitor can be used for target validation and further lead optimization.

Acknowledgements:

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n' 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7 / 2007-2013) and EFPIA companies' in-kind contribution. We thank the Dutch Research Council–Chemical Sciences for the ECHO-grant to APAJ and MvdS.

ANTI-WOLBACHIA (A·WOL) DRUG DISCOVERY: NOVEL HIT FINDING VIA LIGAND BASED VIRTUAL SCREENING COMBINED WITH HTS

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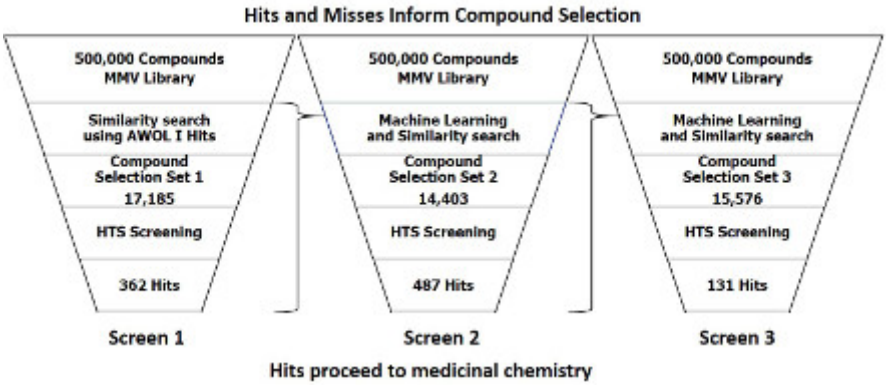
Filariasis inflicts serious health problems throughout tropical communities causing lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness).[1] These diseases infect 157 million people worldwide, ranking filariasis as one of the leading causes of global morbidity.[1] *Wolbachia* is a bacterium that lives inside the cells of the filarial worms. As the filaria are dependent on *Wolbachia* bacteria for survival, eliminating the bacteria with antibiotic drugs radically cures patients and delivers a new strategy for eradicating these debilitating diseases. Through targeting the *Wolbachia* bacteria, we aim to discover novel antibiotic anti-*Wolbachia* (A·WOL) therapy which will deliver safe macrofilaricidal activity with superior therapeutic outcomes compared with the current standard anti-filarial drugs.

Our present hit identification activities involve an iterative combination of HTS and ligand based virtual screening, with the results of each screen (active and inactive compounds) informing computational models which are utilised to select the next set of compounds for screening with the aim of improving both hit rate and diversity of the hits.

An initial screen of 10,000 compounds from the BioFocus diversity library yielded 50 actives (hit rate of 0.5%). These active compounds acted as queries in a range of similarity searches of the 500,000 compound Medicines for Malaria Venture (MMV) library to select a set of compounds to screen. As the compounds in the MMV library are arranged on plates, the aim was to identify the 'best' plates, i.e. those containing a high number of compounds predicted to be active. Methods to prioritise individual compounds would be insufficient as all compounds on a plate need to be taken into account. A range of data fusion methods (e.g. sum, rank, reciprocal rank, parallel, Z2) were used to assign a score or rank to each plate rather than to an individual compound. In total ~17,000 molecules were selected (Set 1) and screened successfully with a fourfold increase in increased hit rate (2.1%). The results from Set 1 (actives and inactives) were fed back into computational modelling to select the next set of compounds (Set 2, ~14,000 compounds). For this selection of compounds in Set 2, a range of machine learning tools (support vector machines, random forests, neural networks) were employed in addition to the similarity search used previously. The results of these screening rounds were fed back into computational modelling to select a third set of ~15,500 compounds for screening (Set 3).

We will present the background, methodology and successes of our approach in our iterative compound selection and screening approach. Through our iterative approach we will show i) an increased hit rate, ii) expansion of SAR around hits, iii) discovery of novel hit chemotypes, iv) achieveing scaffold hops, and v) probing new areas of chemical space. We will also present an analysis of the physicochemical properties of A·WOL active compounds. The hit compounds are now being progressed through medicinal chemistry optimisation.



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DISCOVERY OF PF-06840003, A NOVEL IDO1 INHIBITOR FOR CANCER IMMUNOTHERAPY

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Tumors use tryptophan-catabolizing enzymes such as Indoleamine 2-3 dioxygenase (IDO1) to induce an immunosuppressive environment. IDO1 is induced in response to inflammatory stimuli and promotes immune tolerance, effector T-cell anergy and enhanced Treg function. As such, IDO1 is a nexus for the induction of key immunosuppressive mechanisms and represents an important immunotherapeutic target in oncology. We have identified and characterized a new, highly selective, orally bioavailable IDO1 inhibitor, PF-06840003. The SAR around PF-06840003 will be presented, and rationalized using the X-ray crystal structure of PF-06840003 bound to human IDO1. The key pharmacology and ADME data of PF-06840003 will be discussed: PF-06840003 is capable of reversing human T-cell anergy *in vitro*, and is selective versus other targets. It also shows a very favorable ADME profile (solubility, human hepatocyte stability, low in vivo clearance in preclinical species, high permeability, and high fraction absorbed in preclinical species) leading to favorable predicted human pharmacokinetic properties, including a predicted $t_{1/2}$ of 16-19 hours. These studies highlight the strong potential of PF-06840003 as a clinical candidate in Immuno-Oncology.

DRIVING TUMORS INTO MITOTIC CATASTROPHE - LEAD GENERATION OF MPS1 INHIBITORS LEADING TO TWO CLINICAL CANDIDATES BAY 1161909 AND BAY 1217389

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Cell cycle deregulation represents one of the classical hallmarks of cancer and consequently induction of cell cycle arrest is the predominant mode of action of a number of antimitotic cancer drugs (e.g. taxanes and vinca alkaloids). Targeted disruption of the spindle assembly checkpoint offers a novel approach to cancer treatment. Mps1, a mitotic kinase that is overexpressed in several human cancers, has been shown to function as the key kinase which activates the spindle assembly checkpoint (SAC) to secure proper distribution of chromosomes to daughter cells. Treated with an Mps1 inhibitor, tumor cells will not arrest in mitosis despite DNA damage or unattached/misattached chromosomes resulting in chromosome missegregation, aneuploidy and cell-death.

Here, we report the discovery and characterization of BAY 1161909 and BAY 1217389, two novel, structurally unrelated, orally bioavailable, highly selective small molecule inhibitors of Mps1, which are currently in phase I clinical trials (NCT02138812 and NCT02366949). The two new lead structures of Mps1 inhibitors were discovered by HTS. Optimization of potency and pharmacokinetic properties of these lead structures as well as in vitro (including live-cell imaging) and in vivo characterizationof selected compounds that led to the identification of BAY 1161909 and BAY 1217389 will be presented.

DELIVERING FIRST-IN-CLASS IN VITRO CHEMICAL PROBES AGAINST POLY(ADP RIBOSE) GLYCOHYDROLASE (PARG)

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DNA repair is a critical process for the survival and normal proliferation of healthy cells. However, given the enhanced levels of cellular stress and genomic instability, these repair processes are even more critical to the survival of malignant cells, where rates of DNA damage are considerably increased. Given this, inhibitors of DNA damage repair have seen a resurgence of interest in recent years. One such example of this approach has resulted in the recent approval of the PARP inhibitor olaparib (Lynparza™) for women with advanced ovarian cancer associated with defective BRCA genes.

Olaparib acts against the poly(ADP-ribose)polymerase (PARP) enzymes. Whilst PARP is widely known to play critical and well-understood roles in DNA repair, the counterpart enzyme poly(ADP ribose) glycohydrolase (PARG) is less well known but equally essential for effective DNA repair, degrading PAR chains and facilitating effective DNA repair. Inhibition of PARG may offer several key advantages over PARP inhibition. Most critically, whilst there are 18 PARP isoforms, there exists only a single PARG protein, offering a specific, nodal point of therapeutic intervention. However, due to the open and polar nature of the PARG binding cleft, this protein has been considered to be difficult to inhibit with drug-like small molecules, particularly in the cellular context. As such, robust chemical probes to explore PARG pharmacology are absent from the literature.

Through our innovative collaboration with AstraZeneca, we have discovered a novel PARG-binding pharmacophore and have used this information to discover drug-like chemotypes, facilitating the development of first-in-class potent and selective inhibitors.

We will describe our work in this emerging area, optimising a two series of drug-like hemotypes to deliver molecules with the correct physicochemical and biochemical properties to function as in vitro cell probe compounds. These unprecedented agents display potent on-target biochemical (5 nM) and cell (10 nM) activity with a significant window to acute 3-day cytotoxicity. Moreover, these agents are selective against PARP family members, the close glycohydrolase homologue ARH3 and are highly selective when screened in a Eurofins safety panel. The medicinal chemistry optimisation of the scaffolds will be described, alongside the outline pharmacology demonstrating on-target, selective inhibition of PARG in cells. We believe that these tool compounds will be of value in revealing the detailed mechanisms of action of PARG in DNA repair and other PAR chain-mediated cellular processes, with the ultimate goal of delivering novel and clinically relevant therapeutic agents.

DISCOVERY OF UBROGEPANT (MK-1602): A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE CGRP RECEPTOR ANTAGONIST FOR THE ACUTE TREATMENT OF MIGRAINE

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Calcitonin gene-related peptide (CGRP) receptor is a clinically validated target for the treatment of migraine headache. While a number of small molecule CGRP receptor antagonists have demonstrated efficacy in the clinic, none has reached regulatory filing. Concerns for liver safety factored into the discontinuation of Merck's telcagepant in Phase III and MK-3207 in Phase II clinical studies. In our efforts to identify a development candidate with lower risk of liver injury, our strategy centered on the identification of a molecule with a differentiated structure and metabolism profile compared to telcagepant and MK-3207, and a low human dose projection. These criteria were met with the discovery of ubrogepant. The presentation will describe the origin of a new structural series of lactam amides and optimization that led to the identification of ubrogepant. An X-ray crystal structure of ubrogepant bound to the extracellular domain of the CGRP receptor will be utilized to illustrate the key binding interactions. The preclinical profile of the compound will be discussed in detail and compared to telcagepant and MK-3207.

MOLECULAR DESIGN, SYNTHESIS AND TRYPANOCIDAL ACTIVITY OF DIPEPTIDYL NITRILES AS CRUZAIN INHIBITORS

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Chagas disease is a parasitic infection with high morbidity and mortality that is endemic in much of Latin America where it remains a serious public health problem. With increased migration, Chagas disease represents an emerging worldwide challenge and there is an urgent, unmet need for safe and effective medication. The available drugs to treat Chagas disease maybe effective in the acute phase of the disease, but efficacy in the chronic phase remains controversial. They can cause serious side effects that lead sufferers to abandon treatment. Using a hypothesis-driven approach to molecular design and drawing on cysteine protease cruzain structural information, we have mapped structure-activity relationships for a dipeptidyl nitrile scaffold and demonstrated that compounds are competitive inhibitors, bind reversibly and bear trypanocidal activity. The binding mode revealed by the crystal structure of the protein-ligand complex for one of the inhibitors shows that binding involves the formation of a covalent bond between the catalytic cysteine and the nitrile carbon. Conversely, there is biochemical and structural evidence for the reversibility of the process. As such, we believe that our study represents a valuable step in the search for new drugs for the treatment of a neglected disease that continues to affect the lives of millions of people.

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RESEARCH, DEVELOPMENT & INNOVATION AT ACHÉ LABORATÓRIOS FARMACÊUTICOS

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After the regulation of the generic market in the 1990s, Brazilian pharmaceutical companies have demonstrated significant growth rates. The current scenario is one of great competition for market share and price reduction among generics. This pressure is creating a shift in paradigm where the Brazilian companies are seeking market differentiation through increasing investment in innovation. In this work, we will present Aché's current strategy and organization in Research, Development and Innovation.

EFFECTS OF NOVEL ACYLHYDRAZONES DERIVED FROM 4-QUINOLONE ON THE ACETYLCHOLINESTERASE ACTIVITY AND Aβ42 PEPTIDE FIBRILS FORMATION

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Introduction Acetylcholinesterase inhibitors and compounds that trigger Aβ amyloid oligomerization and fibrillization represent an opportunity to discover new drug candidates to treat Alzheimer's disease.We noted that 3-carboethoxy-4-quinolone inhibited acetylcholinesterase (AChE), an important target for drugs action. However, the effect was moderated (IC_{50} = 70 μM).[1] Acylhydrazones are extensively studied and most of them are bioactive compounds. In view of this, we studied some molecular hybrids based on the structures of the 3-carboxy-4-quinolone and hydrazones derived from aromatic aldehydes (Figure 1).

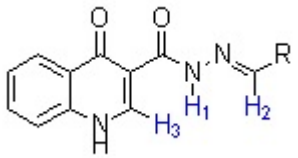

Figure 1. Structure of Acylhidrazones

Biology and docking molecular The AChE activity was evaluated by the quantitative Ellman´s colorimetric assay and Aβ42 fibrillization by the thioflavin-T (ThT) fluorescence emission. AutoDock Vina was used as molecular docking tool.

Results and discussion Quinolone was obtained through a condensation reaction involving diethyl (diethoxymethylene) malonate and aniline.[2] In order to accomplish our synthetic plan, hydrazide was coupled with the appropriate aromatic aldehyde to produce acylhydrazones 3–12 in 63 to 90% yields, as presented in Table 1.The ^{1}H-NMR spectra showed some duplicated signals. At room temperature, we observed a duplication of the peaks attributed to H-1, H-2 and H-3. The duplication is due to the presence of rotamers of the amide bond.

Table 1.Results of the inhibition acetylcholinesterase by acylhydrazones

Acylhydrazones **3-12**		
Compounds	R	IC_{50} ±SD (μM)
3	Phenyl	4.1±0.1
4	4-Chlorophenyl	13±0.2
5	**3-Chlorophenyl**	**1.3 (*)**
6	2,3-Dichlorophenyl	17±0.3
7	4-Nitrophenyl	9.8±0.2
8	**2-Nitrophenyl**	**1.2 (*)**
9	4-(Methylsulfonyl) phenyl	nd
10	4-(Methylthio) phenyl	8±0.1
11	4-(20-Thiophenyl)	3.4 (*)
12	4-Methoxyphenyl	5.9±0.1
Tacrine	-	0.04 (*)

We have found that acylhydrazones inhibited AChE, with IC_{50} values ranging from 1,2 to 17 μM. The compound 5 e 8 were the most effective *in vitro* inhibitor and also had the best energy interaction (ΔG=-9.1 e -8,2 kcal/mol, respectively) in the molecular docking study. The first showed better interaction at the molecular docking compared to tacrine standard (ΔG= -8,3 kcal/mol).

Conclusion We synthesized 10 acylhydrazones and nine of them are not reported in literature. We found that the ability of the majority of acylhydrazones in inhibiting AChE is interesting. With respect to the effect on the Aβ fibrils formation, except for acylhydrazone 9, the studied compounds have an impressive inhibitory effect on the formation of $A\beta_{42}$ fibrils.These findings encourage us to continue the search for more potent analogues.

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ANTIPLATELET AND ANTITHROMBOTIC ACTIVITY OF 1,2,5-OXADIAZOLE-2-N-OXIDE DERIVATIVES

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Atherosclerosis is one of the most common cardiovascular diseases worldwide. The disease is characterized by an inflammatory process, accumulation of white blood cells (WBC) and lipid contend into subintima layer of arteries. The raised diameter in arteries could be disrupted by metabolic activity leading to the recruitment of thrombotic contents including collagen and von willebrand factor. There are an activation on platelets aggregation that trigger atherothrombotic event and lead to myocardium infarction and stroke[1]. One of the major treatments to prevent atherothrombosis is use of antiplatelet drugs such as thromboxane A_2 (TXA_2) inhibitors (i.e. acetylsalicylic acid - ASA), P_2Y_{12} receptor antagonism (i.e. clopidogrel) and PAR-1 receptors inhibitors (i.e. ximelagatran). Despite of effectiveness, these drugs have exhibited some limitations during long-term therapy that includes increased bleeding time, lack of effectiveness, gastric ulceration gastric among others[2]. Therefore, the search for new safe and effective antiplatelet and antithrombotic drugs is urgent. Furoxans (1,2,5-oxadiazole 2-*N*-oxide) derivatives are known by its ability to release nitric oxide (NO) after biotransformation *in vivo*. NO is an important mediator that avoid platelet aggregation and can be useful during drug design[3]. Herein, in this work we designed new furoxan derivatives, containing *N*-acylhydrazone as a spacer, responsible for inhibition of both platelet aggregation pathway: TXA_2 and ADP[4] (Figure 1). The antiplatelet activity of all compounds (at 10μM) were performed using rat platelets and the platelet aggregation were measured by chronolog aggregometer[4].The agonists used were ADP (10μM) and collagen (10μM). All compounds were able to inhibit platelet aggregation induced by ADP and collagen pathway. Compounds LD1-LD6 have exhibited platelet aggregation ranging from 80-95% for ADP; while compounds LD-3, LD-5 and LD-6 inhibited aggregation induced by collagen at 39.0, 46.2 and 69.2%, respectively (Figure 1). It was observed that NO contributes to antiplatelet activity once derivatives without *N*-oxide function were less active (LD-7-12, Figure 1). For bleeding time assay, ASA (positive control) and compounds were administrated orally in mice (at 100 μmol/Kg). After one hour, a tail incision (2mm) was performed and the bleeding time was monitored[4]. All compounds have exhibited bleeding time inferior of that of ASA and demonstrated to be safer than this standard drug for long-term therapy. *In vivo* antithrombotic activity for the most active compound (LD-5) was evaluated after oral administration of compounds and ASA (100 μmol/20g in 0.5% of CMC). After one hour, a mixture containing collagen and epinephrine was injected into mice tail in order to induce the thrombus formation[4]. For this assay, we selected the most active compound (LD-5), which was able to protect against thrombosis by 90%; while ASA protected only 40%. In conclusion, we have identified furoxan derivatives with antiplatelet effect, low bleeding time useful to prevent antithrombotuc events.

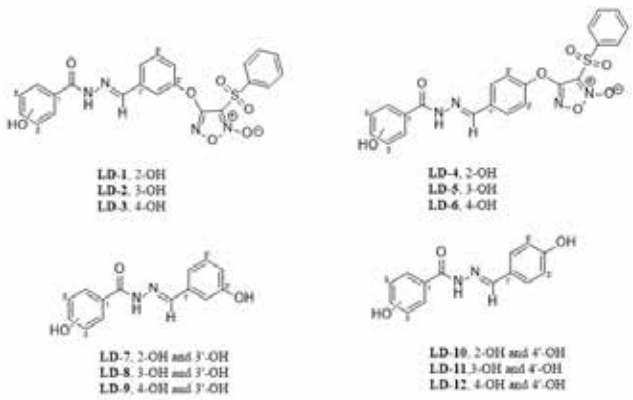

Figure 1. Structure of NO donors and non-donors compounds

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NOTES

POSTERS

Drug Discovery Approaches Toward Targeting Ras

BLOCKADE OF RAS ACTIVITY BY INHIBITORS OF THE ENZYME ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE (ICMT)

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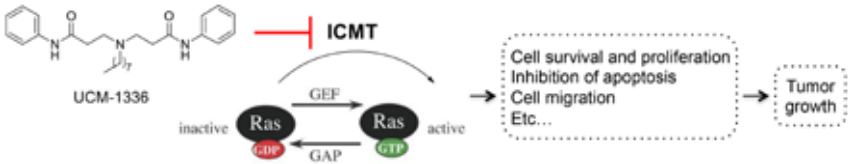
Activating mutations in Ras proteins have been found in almost 30% of all cancers, including 40% of colorectal and up to 90% of pancreatic tumors. In absence of its post-translational modifications, Ras losses its ability to induce tumor transformation. Therefore, the blockade of the enzymes involved in these modifications represents an attractive strategy to inhibit Ras activity. Among them, isoprenylcysteine carboxyl methyltransferase (ICMT), which catalyzes the last step of the post-translational modifications of Ras, is receiving an increasing attention as a new therapeutic target in oncology (1). Up to date, very few structurally distinct inhibitors have been disclosed and only one molecule (cysmethynil) and close derivatives have been characterized as ICMT inhibitors capable of interfering with Ras activity and endowed with in vivo efficacy (2). These findings provide a compelling rationale for the development of ICMT inhibitors as a promising approach to anticancer drug development.

Towards this aim, we built a homology model of the human ICMT (h-ICMT) using the reported *Methanosarcina acetivorans* ICMT (Ma-ICMT) structure (3) as template and used it to develop a structure-based pharmacophore model. After the identification of an initial hit, we carried out an extensive medicinal chemistry program, which led to compound UCM-1336, which inhibits more than the 90% of ICMT activity at 50 mM with an IC_{50} value of 2 mM and shows adequate pharmacokinetic properties. In addition, UCM-1336 (i) enhances programmed cell death, affecting specially those cell lines expressing oncogenic mutant K-Ras; (ii) induces mislocalization of all Ras isoforms; (iii) reduces Ras activity and blocks the activation of the downstream MEK/ERK and PI3K/AKT signaling pathways; and (iv) impairs the migratory capacity of tumor cells. Noteworthy, UCM-1336 is more potent than cysmethynil in all assays, suggesting that it could work as a new ICMT inhibitor that would help to definitively validate this enzyme as a therapeutic target of interest for the treatment of cancers characterized by high Ras overactivation, a current unmet clinical need (4). All these promising results have prompted us to study the in vivo efficacy of compound UCM-1336 in a xenograft mouse model of pancreatic cancer, experiments that are currently ongoing.



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STRUCTURE-ACTIVITY STUDY IN A SERIES OF ANALOGUES OF THE DIPEPTIDE MIMETIC OF BRAIN DERIVED NEUROTROPHIC FACTOR

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Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family that also includes nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), promotes neuronal survival, differentiation, and synaptic functions [1] through the signaling of its receptor tropomyosin-related kinase-B (TrkB). BDNF is of particular therapeutic interest because its expression level was reported to be reduced in Parkinson's disease, depression, and stress [2].

BDNF has been recognized to have potential for the treatment of a variety of human neurodegenerative diseases. However, clinical trials with recombinant BDNF have yet to yield success, leading to the suggestion that alternative drug with BDNF-like activity for therapeutic use may be required.

Thus, the use of molecules such as small peptides that could mimic or modulate the functions of BDNF, is an attractive alternative approach.

In previous work we have obtained a dimeric dipeptide mimetic of the BDNF loop 4 - *bis*-(*N*-monosuccinyl-*L*-seryl-*L*-lysine) hexamethylenediamide (GSB-106), having a BDNF-like neuroprotective activity *in vitro* in a concentration range of 10^{-5} – 10^{-8} M and antidepressant activity *in vivo* at doses of 0.1 and 1.0 mg / kg i.p. in rats [3].

In this work we have investigated the structural and functional relationships among analogues of GSB-106. Glycine scan was performed and a number of appropriate compounds were synthesized: GT-105 (lysine is replaced by glycine), GT-107 (serine is replaced by glycine), GT-106Ac (monosuccinic radical is replaced by acetyl group). We have studied the dependence of activity of the following compounds on the configuration of amino acid residues: GT-107D (*D*-enantiomer of the GT-107), GT-106DL (*L*-serine was replaced by *D*-serine), GT-106LD (*L*-lysine was replaced by *D*-lysine).

The investigation of these compounds using the HT22 cell culture in conditions of oxidative stress has approved only two analogues of GSB-106 to have a neuroprotective effect: in the cases of replacement of serine to glycine (GT-107) and of replacement of succinic radical to acetic group (GT-106Ac). A disappearance of this effect was observed in the cases of the replacement of lysine residue to glycine in GT-105, *L*-lysine residue to *D*- lysine (GT-106LD) and also by conversion of serine configuration (GT-106DL). These results showed that lysine residue is crucial for the neuroprotective activity of GSB-106. *L*-configuration of the lysine and serine residues required. Configuration of lysine residue becomes critical in the absence of serine side group. Thus, the following fragment is the minimum pharmacophore (highlighted in italics) of the BDNF loop 4 beta-turn:

(HOOC-CH_2-***CH_2-CO-NH-(S)-CH-***(CH_2OH)-***CO-NH-(S)-CH((CH_2)$_4$$NH_2$)-CO-NH-($CH_2$)$_3$-***)$_2$

Only one (GT-106Ac) from two analogues of GSB-106 possessing neuroprotective activity, exhibited antidepressant activity. This fact indicates a necessity of more stringent structural requirements for the manifestation of antidepressant activity. The results obtained can be useful for designing of new active mimetics of BDNF.

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POSTERS

New Antibacterials. An Update

SYNTHESIS AND IN VITRO LEISHMANICIDAL ACTIVITY OF NOVEL [1,2,3]TRIAZOLO[1,5-a]PYRIDINE SALTS

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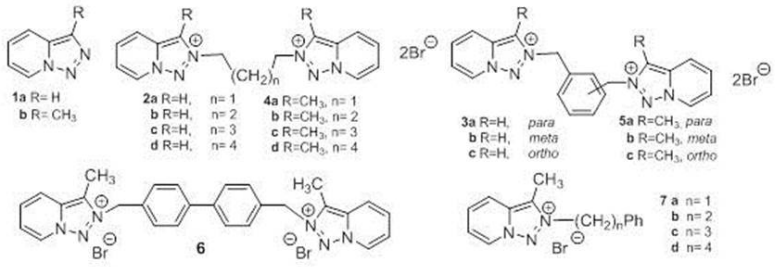
We have reported the first [1,2,3]triazolo[1,5-*a*]pyridine compounds with leishmanicidal activity, their interaction with DNA could be a mechanism to explain the activity found.[1] We have also studied some triazolopyridopyrimidines, a fluorescent family of compounds, that have demonstrated to present photoinduced DNA cleavage,and antiprotozoal activity against different types of *Leishmania* spp.[2] A disadvantage of these compounds is their poor solubility in water. A general method to improve this property is the formation of salts. In this communication we report the synthesis of new triazolopyridine salts **2-7** from triazolopiridines **1**, and the study of their *in vitro* leishmanicidal activity.



The activity was tested on *Leishmania infantum*, *Leishmania braziliensis* and *Leishmania donovani* parasites, using promastigotes and intracellular amastigotes forms. The cytotoxicity of the tested compounds on J774.2 macrophage cells was also measured. Six of the tested compounds (**1b**, **2b**, **4c**, **6**, **7b,c**) showed selectivity indexes higher than those of the reference drug Glucantime for the three Leishmania species. Moreover, the data on infection rates and on amastigotes showed that these compounds are the most active against the three Leishmania species. The changes in the excretion products profile of parasites treated with the six compounds (**1b**, **2b**, **4c**,**6**, **7b,c**) were also consistent with substantial cytoplasmic alterations. On the other hand, the most active compounds were potent inhibitors of Fe-SOD in the three parasite species considered whereas their impact on human CuZn-SOD was low. The high activity, low toxicity, stability, low cost of the starting materials and straightforward synthesis make these compounds appropriate molecules for the development of affordable antileishmanicidal agents.

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TOWARDS THE RAPID IDENTIFICATION OF NEW ANTI-MICROBIAL AGENTS USING SOLID PHASE SYNTHESIS.

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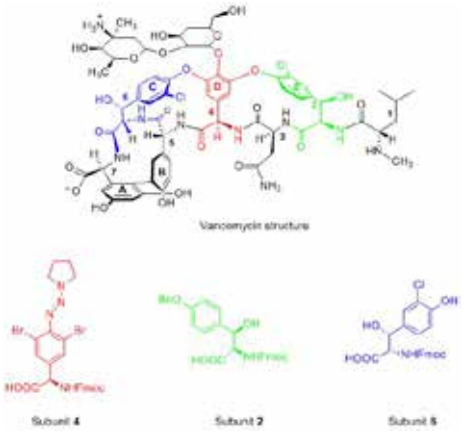
Vancomycin is a glycopeptidic antibiotic used mainly for treatment of infections caused by gram positive bacteria via inhibiting cell wall synthesis.[1] Vancomycin is considered as the last resort for the treatment of infections caused by methicillin resistant *S. aureus (MRSA)* [2]. The appearance in 1987 of vancomycin resistant enterococci has aroused much interest because the genes involved can be transferred to *S. aureus*, and thus result in a vancomycin resistant *strain.*[2]

The main objective of using solid phase methods for the synthesis of vancomycin is that it allows for more flexible and rapid modification of its structure. Thus, an extended library of vancomycin analogues will be generated and evaluated for their biological activity.

Our strategy starts firstly with the synthesis of each of the seven amino acids of vancomycin via solution phase chemistry, suitably substituted to attempt on-resin cyclisation of the linear peptide structure. We have adapted the Nicoloau approach[3-4] to the individual building blocks and the early steps of the synthesis have focussed on adapting this chemistry to generate Fmoc-protected amino acids and also to make these compounds on a large scale as individual monomers for solid phase synthesis.

With all seven building blocks in hand, we will evaluate different approaches to obtain and cyclise vancomycin's structure prior to cleavage from the resin. Furthermore, modifications will be introduced to produce novel analogues to be biologically assessed. The assessment of the biological activity of compounds will be carried out against both resistant and non-resistant bacterial strains.



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DEVELOPMENT AND DELIVERY OF CYCLIC PEPTIDE INHIBITORS OF SPSB2 AS POTENTIAL NEW ANTI-INFECTIVE AGENTS

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The SPRY domain-containing SOCS box protein 2 (SPSB2) is one of four proteins (SPSB1 to -4) consisting of a C-terminal SOCS box motif and a protein interaction domain known as the SPRY domain. We have shown that SPSB1 and 2 are key components in an E3 ubiquitin ligase complex and direct this complex to degrade the inducible form of nitric oxide synthase (iNOS) and reduce nitric oxide production in macrophages.[1] SPSB2-deficient macrophages showed prolonged iNOS expression, which resulted in enhanced nitric oxide levels following challenge with endotoxin (LPS), gram - positive *Listeria* and *Mycobacteria*, and *Leishmania* parasites, and resulted in enhanced killing of *Leishmania major* parasites.[1] Thus inhibitors of SPSB2 in macrophages may be anti-infective agents for bacterial and parasitic diseases.

iNOS contains a highly conserved DINNN sequence in its disordered N-terminus. We showed using isothermal titration calorimetry (ITC) that a peptides corresponding to these residues bound SPSB2 with high affinity (*K*D 13 nM).[1] High-resolution NMR was used to further characterize the SPSB2-iNOS peptide interaction and crystal structures were determined for SPSB2 bound to peptides containing the DINNN sequence.[1,2] The cyclic peptide Ac-c[CVDINNNC]-NH_2 was designed based on *in silico* modelling and was shown to bind with *K*D of 4.3 nM.[3] This peptide was resistant to proteolysis and stable in human plasma *in vitro*. Cyclic derivatives without the disulphide moiety were also found to be redox-stable and similarly exhibited low nanomolar affinities on SPSB2.[4]

Peptides with favourable binding properties were assessed for their ability to be taken up by live cells using fluorescently-labelled derivatives. This imaging was used to define the intracellular location of peptides within cells, and our preliminary results show accumulation of fluorescent peptide in the macrophage endosome with only minor accumulation in the cytoplasm. Our current work focuses on improving cytoplasmic uptake by modifying peptides to improve penetration of the endosomal membrane.

Delivery of peptide to cellular targets is very challenging due to the complex issues around pharmacokinetics, degradation and excretion, as well as cellular uptake. In order to address these issues we have employed polymeric delivery vehicles that are designed to allow macrophage selective uptake through the provision of a mannose-coated surface. These delivery vehicles also incorporate enzyme cleavable linkers between the polymer and the peptide payload. Thus, after receptor-mediated endocytosis of the polymer, linker cleavage by endosomal proteases may allow release of the active peptide and cytoplasmic delivery.

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MICROWAVE-ASSISTED SYNTHESIS IN WATER: FIRST ONE-POT SYNTHESIS OF A NOVEL CLASS OF POLYSUBSTITUTED BENZO[4,5]IMIDAZO[1,2-b]PYRIDAZINES VIA INTRAMOLECULAR SNAr AS ANTIMICROBIAL AGENTS

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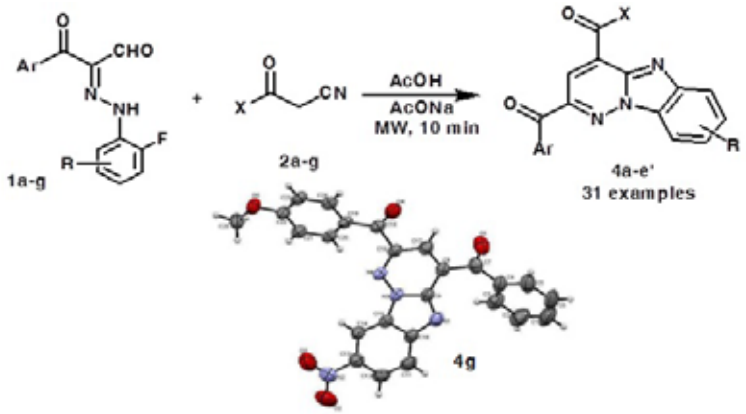
A novel and straightforward one-pot synthesis protocol has been developed for the synthesize of benzo[4,5]imidazo[1,2-*b*]pyridazines through intramolecular S_NAr, utilizing water as green solvent and microwaves as efficient green energy source. The entire strategy consist of just one step, reaction between 3-oxo-2-arylhydrazonopropanals which contain *o*-fluorine substituent on the *N*-aryl ring of the arylhydrazone moieties with active methylene compounds, including 3-oxo-3-phenylpropionitrile, 3-oxo-3-hetarylpropio-nitrile, ethyl cyanoacetate and 2-cyano- acetamide giving the target compounds in an overall yield of 89-99 %. The reaction is carried out under microwave irradiation as well as under conventional heating. The factors affecting the optimization of the reaction are examined in details. X-ray crystallographic analysis was used in the establishment of structures and regioselectivity of the reaction. Most of the synthesized compounds in this investigation were tested and evaluated as antimicrobial agents



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STRUCTURAL BASIS FOR SELECTIVE TARGETING OF LEISHMANIAL RIBOSOMES: AMINOGLYCOSIDE DERIVATIVES AS PROMISING THERAPEUTICS

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Leishmaniasis comprises an array of diseases caused by pathogenic species of Leishmania, resulting in a spectrum of mild to life-threatening pathologies. Currently available therapies for leishmaniasis include a limited selection of drugs. This coupled with the rather fast emergence of parasite resistance, presents a dire public health concern. Paromomycin (PAR), a broad-spectrum aminoglycoside antibiotic, has been shown in recent years to be highly efficient in treating visceral leishmaniasis (VL) - the life-threatening form of the disease. While much focus has been given to exploration of PAR activities in bacteria, its mechanism of action in Leishmania has received relatively little scrutiny and has yet to be fully deciphered. In the present study we present an X-ray structure of PAR bound to rRNA model mimicking its leishmanial binding target, the ribosomal A-site. We also evaluate PAR inhibitory actions on leishmanial growth and ribosome function, as well as effects on auditory sensory cells, by comparing several structurally related natural and synthetic aminoglycoside derivatives. The results provide insights into the structural elements important for aminoglycoside inhibitory activities and selectivity for leishmanial cytosolic ribosomes, highlighting a novel synthetic derivative, compound 3, as a prospective therapeutic candidate for the treatment of VL.

HTS AGAINST BACTERIAL METALLO-BETA-LACTAMASES, TOWARDS CLINICALLY USEFUL INHIBITORS

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The β-lactams remain the most important antibiotics in clinical use. The most important type of resistance to them is mediated via β-lactamases. The recent clinical introduction of avibactam as a serine-β-lactamase inhibitor is a major advance. However, the metallo-β-lactamases (MBLs) are of particular concern because they catalyse the hydrolysis of almost all types of β-lactam antibiotic and serine-β-lactamase inhibitors. MBLs are particularly challenging targets because of the need to obtain potency against the different subtypes of clinical relevance, which differ in the loops surrounding their active site. Moreover, human enzymes especially human MBL fold enzymes should not be inhibited by bacterial MBL inhibitors. Although non-selective MBL inhibitors have been reported, these compounds are unlikely to be clinically useful. Therefore, we developed a novel cephalosporin based fluorogenic substrate that enabled us to screen for potential inhibitors against the clinically relevant MBLs, including NDM, VIM and IMP. Using NDM-1 as a target, a high throughput screen against >300K compounds was conducted via the IMI European Lead Factory. We determined multiple inhibitor complex crystal structures of resulting hits and also performed a crystal-based fragment screen (>700 compounds) at Diamond's new XChem facility. This work has led to the identification of potent inhibitors active against all major types of clinically relevant MBLs (IC50 values50 fold increases in activity (IC50 low nanomolar range against NDM-1, VIM-2 and IMP-1), good physicochemical properties and promising in vitro DMPK profiles. Overall the work has revealed novel chemotypes and selective inhibitors for bacterial MBLs that are active in both isolated protein and in cells. These compounds are being developed further toward clinically useful MBL inhibitors.

Acknowledgment

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BORONATES AS BROAD-SPECTRUM INHIBITORS OF BETA-LACTAMASES

Samuel Cahill (1), David Y. Wang (1), Ricky Cain (2), Colin Fishwick (1), Juan-Carlos Jiménez-Castellanos (2), Matthew B. Avison (2), James Spencer (3), Michael A. McDonough (1), Jürgen Brem (1), Christopher J. Schofield (1)

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The growth and spread of β-lactamase mediated resistance continues to threaten our use of β-lactam antibiotics as clinical therapeutics for the treatment of bacterial infections. Co-administration of β-lactamase inhibitors including clavulanic acid, sulbactam, tazobactam and, more recently, avibactam with β-lactam antibiotics has helped to retain their efficacy against bacteria producing serine-β-lactamases of Ambler classes A, C & D. However, this approach has not been extended to organisms that produce metallo-β-lactamases (Ambler class B), since, to date, there are no clinically useful inhibitors of these enzymes. In addition, the sequence and structural diversity of the metallo-β-lactamases has lead to difficulties in the design of broad-spectrum inhibitors. Here we report the use of boronates as broad-spectrum inhibitors of both the serine- and metallo-β-lactamases. Inhibition studies of the boronates against all classes of β-lactamase reveal potent inhibition with IC_{50} values in the low μM to nM range. Further to this, incubation time courses show that there is no loss of potency after up to six hours of incubation with the enzymes and that there is no latency period of inhibition by these molecules. Structural characterisation of these inhibitors bound to both serine- and metallo-β-lactamases reveals a new inhibitor binding mode. These compounds potentiate β-lactam activity against laboratory strains and clinical Gram-negative bacteria that express multiple β-lactamases. Combined, our work demonstrates that broad-spectrum inhibitors of β-lactamases are achievable and further study of the boronates' mode of inhibition paves the way for the design of inhibitors that can potentiate the use of β-lactam antibiotics against infections exhibiting resistance mediated by a broad range of β-lactamases.

COMPUTATIONAL DESIGN, SYNTHESIS AND MOLECULAR PROPERTIES OF NOVEL MODIFIED FLUOROQUINOLONE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AND ANTITUBERCULOSIS AGENTS

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Fluoroquinolones (FQs) are commonly used antibacterial agents that have been shown to possess broad spectrum of antibacterial activity, great potency and good oral bioavailability, besides low side effects (1).

Despite the remarkable success of FQs in clinic, new fluoroquinolone containing medicinal agents are needed immediately owing to increasing resistance against commonly prescribed antibacterial agents (2), since resistance is a growing problem for treatment (3).

They are commonly prescribed as broad spectrum antibacterial agents that are used against respiratory tract infections, urinary tract infections, gastrointestinal infections and sexually transmitted diseases (4). On the other hand, World Health Organization (WHO) approves FQs as second-line antituberculosis agents (5). FQs were demonstrated to show activity against *M. tuberculosis* (6, 7); meanwhile, studies to generate new ones are going on globally (8, 9, 10).

Based on mentioned approaches, novel of fluoroquinolone derivatives were designed and synthesized as potential antibacterial and antituberculosis agents. The target compounds were synthesized by introduction of alkyl, acyl or sulphonyl moieties to basic secondary amine function of FQ. Purity of the synthesized compounds was checked by TLC and HPLC while their structures were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data besides elemental analysis.

Molecular docking studies concerning the synthesized compounds were performed to simulate potential inhibition of related bacterial and mycobacterial targets. Synthesized compounds as ligands revealed promising results according to docking calculations.

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JAMMING BACTERIAL COMMUNICATION SYSTEMS YIELDS POTENT BLOCKERS OF PSEUDOMONAS AERUGINOSA PATHOGENICITY

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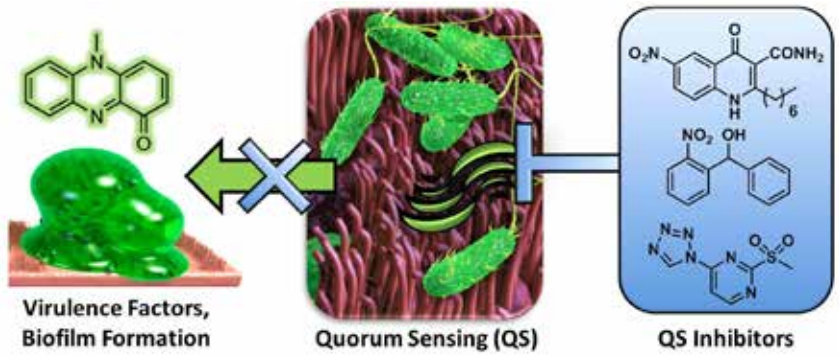
Multi-drug and even pan-drug resistant bacteria are on the rise and pose a huge threat toward modern health standards. Among the most feared "ESKAPE" pathogens resides *Pseudomonas aeruginosa*, which causes severe hospital-acquired infections that are often notoriously difficult to treat.[1)]

A novel strategy to combat this resilient bacterium is to interfere with its Quorum Sensing (QS) machinery. We and others have shown that disrupting this cell-to-cell communication system by small molecules results in attenuated pathogenicity.[1–5)]



Expanding from proof-of-concept studies on recently discovered antivirulence agents interfering with the Pseudomonas Quinolone Signal (PQS) QS system,[2,3)] we identified a novel dual-acting QS inhibitor with favorable physicochemical properties and strong effects on virulence/biofilm.[4)] Most strikingly, this compound increased the susceptibility of *P. aeruginosa* biofilms toward antibiotic treatment and showed *in vivo* efficacy in an arthropode infection model. Additionally, fragment-like inverse agonists[5)] of the signal molecule receptor PqsR were successfully grown into potent pathoblockers with nanomolar potency against the target pathogen. Our continuous efforts to optimize antivirulence efficacy and drug-likeness of these compounds may open new avenues for antiinfective treatment of *P. aeruginosa*-related diseases.

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TARGET-DRIVEN DYNAMIC COMBINATORIAL CHEMISTRY – POTENTIALS AND PITFALLS AS EXEMPLIFIED ON A BACTERIAL ADHESIN

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Dynamic combinatorial chemistry (DCC) generates substance libraries starting from reversibly reacting building blocks. These libraries are under thermodynamic control and their composition in equilibrium state is affected by the conditions applied. For instance the presence of a target protein can stabilize ligands bound to the protein, leading to a shift in the composition of a library. This target-driven DCC has the potential to rank library members according to their affinities. (1, 2)

We studied acylhydrazone libraries formed from aldehydes reacting reversibly with hydrazides. The goal was to identify inhibitors of FimH, a bacterial adhesin crucial for the development urinary tract infections (UTI). (3, 4) FimH can be inhibited with biaryl mannosides (5, 6), which we used as lead structure in our library design.

The acylhydrazones can be monitored by UV-HPLC due to their strong absorption at 310 nm. Alterations in the composition of a library equilibrated in presence of FimH can be determined by comparison with the library equilibrated in absence of the target protein. However, sample preparation prior to HPLC analysis and ratio of building blocks can substantially influence the outcome and thereby the information content of the experiment.

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INSERTION OF A TRIPHENYLPHOSPHONIUM MOIETY TO 1-PROPYLINDOLES IMPROVED ANTI-MYCOBACTERIAL ACTIVITY WITH INVOLVEMENT OF MEMBRANE DISRUPTION.

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There is an urgent need for new anti-tuberculosis (TB) drugs acting via novel mechanisms of action to address the growing threat of emerging resistant strains of the mycobacteria. The mycobacterial membrane has not been widely explored as an antimycobacterial target. There is however a legitimate case for targeting the bacterial membrane as opposed to specific macromolecules. [1] These are (i) the mandatory requirement of a functionally intact membrane for viability, regardless of whether organisms are actively replicating or non-replicating; (ii) The lethal pleiotropic effects of membrane damage because multiple critical targets involved in maintaining homeostatic functions reside within the mycobacterial membrane and (iii) The concurrent loss of a multitude of critical processes which would severely limit the ability of the mycobacteria to become resistant. The feasibility of targeting the bacterial membrane has been recently demonstrated for several small molecules like boromycin, xanthones and benzophenone-based teramides. [2] A common structural feature of these compounds is the presence of a cationic centre and a lipophilic moiety (ie cationic amphiphilicity). The incorporation of a triphenylphosphonium functionality (a cationic amphiphile) has been shown to improve the inhibitory properties of phenothiazine derivatives in *Mycobacterium tuberculosis*. [3] Although the phenothiazines possess the cationic amphiphilic motif, they displayed only moderate anti-mycobacterial activity. The activity enhancing effect of the alkyltriphenylphosphonium moiety was attributed to its lipophilicity which presumably promoted accumulation within the mycobacterial membrane, thus ensuring that bactericidal concentrations were achieved at the required site of action, which in the case of the phenothiazines, is the membrane protein Type II NADP dehydrogenase (NDH-2). [3] To further explore the anti-mycobacterial enhancing properties of the alkyltriphenylphosphonium moiety, we prepared a series of [3-(1H-indol-1-yl)propyl]triphenylphosphoniums which were substituted with fluoro or methoxy at positions 4,5,6 or 7 of the indole ring. All the compounds were antimycobacterial against *M bovis BCG* with MIC_{50} values ranging from 4-10μM. Triphenyl(n-propyl)phosphonium bromide and 1-propyl-5-fluoroindole (a representative substituted indole) were devoid of activity (MIC_{50} > 50 μM). We found that the indolyltriphenylphosphoniums had membrane disrupting properties as seen from their effects on the melting endotherms (transition temperature Tm, molar enthalpy DH) of dimyristoylphosphatidylglycerol (DMPG) vesicles when investigated by differential scanning calorimetry. These changes were significantly more pronounced than those observed with triphenyl(n-propyl)phosphonium bromide under similar conditions. Interestingly, the test compounds did not induce hemolysis of human erythrocytes at their MIC_{50} concentrations. Taken together, these results suggest that the lipophilic enhancement associated with the alkyltriphenylphosphonium moiety does not only lead to accumulation within the membrane but also induces a perturbative effect which may contribute to antimycobacterial activity.

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ANTIMYCOBACTERIAL AMPHIPHILIC MANNICH BASES OF 1-ALKYLINDOLES WITH SELECTIVE MEMBRANE DISRUPTING PROPERTIES

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Tuberculosis remains a major global health problem in spite of concerted efforts by the global community to contain the disease. 1 The main obstacles to TB eradication are resistance and persistence of mycobacteria to current TB chemotherapy, a problem that can only be alleviated by new drugs, directed preferably on novel targets.
We have identified Mannich bases of 1-substituted indoles as a promising chemotype for antimycobacterial activity. Structure activity investigations revealed a critical dependence on the cationic amphiphilic motif for activity, namely the lipophilic alkyl chain at position 1 and a positively charged aminomethyl ("Mannich base") at position 3 of the indole ring. Omission of either feature completely abolished activity. The optimal lipophilic side chain was n-octyl, followed by geranyl. Extension to n-decyl increased lipophilicity but not antimycobacterial activity. Various Mannich bases were permissible but there was a preference for tertiary amino groups although secondary amines were acceptable if the N-substituent was not excessively bulky, elongated or too small. Thus, structural requirements at this position were less prescriptive and unlike position 1, not entirely dictated by lipophilicity. Substitution at the benzenoid portion of the indole ring served a predominantly modulatory role. Fluoro and methoxy groups were found to be acceptable substituents. Contrasting regioisomeric preferences were observed for these groups, namely a preference for position 4 among the fluoro regioisomers, and positions 5 or 6 for the methoxy regioisomers. Attempts to replace the indole ring with either 7-azaindole or indazole failed to improve activity, suggesting some role for the scaffold. Six analogs with MIC50 < 5 uM (Mycobacterium tuberculosis, H37Rv) and 10-fold or more selective cytotoxicity (against mammalian Vero cells compared to M tuberculosis) were identified. As the methylindole fragment is a potential PAINS,2 a preliminary assessment of the thiol scavenging potential of the scaffold was conducted for some analogs but found lacking. As cationic amphiphilicity promotes membrane disruption, we prepared dimyristolylphosphatidyl-glycerol (DMPG) vesicles taken here as a proxy of the bacterial membrane and investigated the effects of the analogs on the DMPG melting endotherm. A striking correlation between antimycobacterial activity and membrane disruptive activity (as seen from analog induced changes in the melting endotherm of DMPG) was observed. This prompted further investigations on treated mycobacteria for changes in membrane potential, permeability, ATP levels and induction of a gene promoter linked to cell envelope disruption. These alterations which reflected perturbation of membrane structure and function were indeed observed. More importantly, none of these analogs induced significant hemolysis of red blood cells at their MIC50 concentrations. Taken together, we have found a potential anti-mycobacterial chemotype that may exert its activity by perturbing the mycobacterial membranes.

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BACTERIAL GROWTH INHIBITION BY TARGETING DAHP SYNTHASE WITH A RATIONALLY DESIGNED OXIME-BASED INHIBITOR

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Expanded bacterial resistance towards conventional antibiotics is creating an urgent need for new strategies against bacteria. DAHP (3-deoxy-D-arabinoheptulosonate-7-phosphate) oxime is a transition state inhibitor for DAHP synthase (DAHPS). To better understand inhibition, the pH profiles of catalysis and inhibition were investigated. While the enzyme's k_{cat}/K_M value decreased at high pH, inhibition ($1/K_i$) by DAHP oxime increased. Ionization of enzymatic residues could not be identified as responsible for stronger inhibition, so the ionization of the inhibitor itself was investigated. The K_i value of the derivative DAHP *O*-methyloxime showed little pH dependence. Therefore, we hypothesized that the anionic DAHP oximate (C=N–O$^-$) inhibits DAHPS more strongly than the neutral form, and that a molecule with a lower oxime pK_a would be a tighter inhibitor. The analysis of the pK_a and K_i values of pyruvate oxime, 3-fluoropyruvate oxime, and 3,3,3-trifluoropyruvate oxime confirmed that fluorine bonded to the oxime α-carbon lowers the inhibitor's pK_a and improves K_i. An enzymatic synthesis of 3-fluoro-DAHP oxime is being developed. Furthermore, the ethyl ester of 3,3,3-trifluoropyruvate oxime is the first oxime-based inhibitor to reduce *E. coli* growth in culture (IC_{50} = 0.2 mg/mL), and its effects were mitigated by DAHPS overexpression. This is evidence that this oxime-based inhibitor targets DAHPS.

NEW METAL CHELATING PYRAZOLONE BASED LIGANDS AND THEIR Co/Cu COMPLEXES OF THERAPEUTIC PROPERTIES

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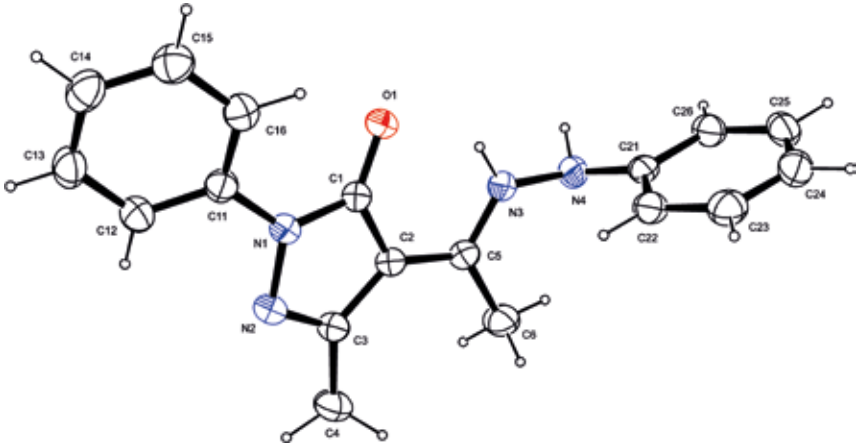
The need for the design and synthesis of new bioactive compounds with novel characteristics that may become alternative replacement to commonly used medicinal drugs due to the problems of disease resistance to the drugs and their toxicity effects is still a huge concern for synthetic as well as medicinal chemists. These compounds have further been coordinated with transition metal ions as this may increase their pharmacological activities. Acylpyrazolones are good biological, metal ion chelating and analytical reagents. Antipyrine, a derivative of pyrazolone has been employed as a clinical drugs and this work is based on its structural modifications towards a possible increase in its therapeutic applications. Via a condensation reaction with amines, acylpyrazolones form a more chelating and superior group of compounds known as azomethines.
4-acyl-3-methyl-1-phenyl-2-pyrazolin-5-ones were reacted with corresponding phenylhydrazine derivatives to get a new phenylhydrazone (azomethine), which were further reacted with aqueous solutions of cobalt and cuopper to afford their metal complexes. The compounds were characterized/identified by analytical, spectroscopic, TGA, as well as x-ray crystallography. The bidentate ON ligand formed stable octahedral geometry with metal ions. Using the disc diffusion technique to screen the synthesized compounds at 20 mg/ml against selected bacterial isolates in triplicates, potential bactericides were identified. Their bioactivity varies, with the metal complexes showing higher antibacterial activity at an MIC value of 0.63 mg/ml for Co(II). Similarly, ligands and complexes also showed antioxidant scavenging properties against 2, 2-diphenyl-1-picrylhydrazyl DPPH radical at 0.5mg/ml relative to Ascorbic acid. Anticancer studies of synthesized compounds are ongoing as possible antitumour leads are proposed.



Crystal structure of Phenylhydrazone

ENZYME INHIBITION AS A NEW STRATEGY TO OVERCOME MULTIDRUG RESISTANT BACTERIA

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The emergence of multidrug resistant (MDR) bacteria across the world is threatening the treatment of common infections and minor injuries both in the community and hospitals. Statistical data estimate that each year in the United States 2 million people are infected with a multidrug resistant bacteria and about 23,000 die due to the infection.[1,2]

De novo cysteine biosynthetic machinery, which is exclusive in prokaryotes, has been associated with the growth, survival and pathogenicity of several bacterial species.[3,4] Therefore, inhibition of the cysteine synthase complex which is formed by the enzymes catalysing the last two steps of cysteine biosynthesis, O-acetylserine sulfhydrylase (OASS) and serine acetyl transferase (SAT) may provide a new therapeutically relevant target against MDR strains.

Investigation of the interaction between OASS and SAT initially afforded peptide inhibitors[5] and to overcome peptides drawbacks as chemotherapeutical tools a campaign aimed to obtain the first small molecule inhibitors of OASS (**Figure 1**) [6,7] was started.

Herein we report the synthesis, the binding affinity and preliminary permeability studies of novel series of OASS inhibitors.

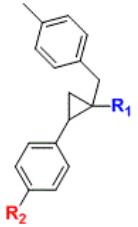

Figure 1 - OASS inhibitors general structure.

Acknowledgments

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TETRAMATES AS ANTIBACTERIAL AND ANTICANCER CORE SCAFFOLDS

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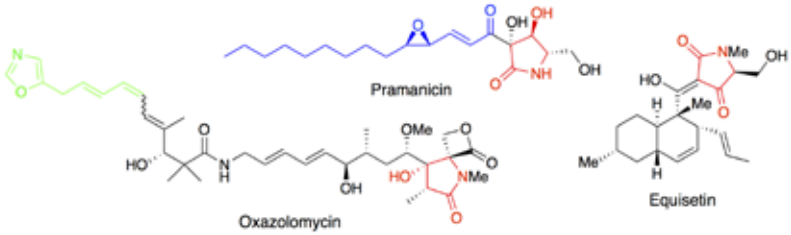
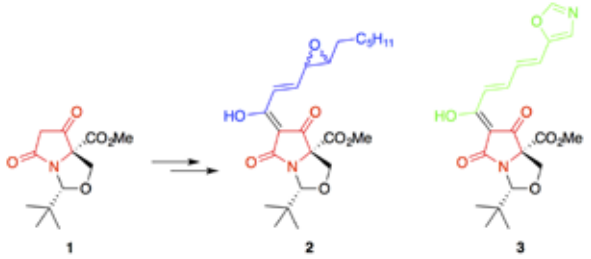
Several natural products with a tetramate or pyroglutamate core have proven to possess a wide range of biological activity including antibiotic, antiviral, antiulcerative and antitumours activity.[1] However, due to the complexity of their structures, their total syntheses are long, making it challenging to prepare analogues with optimised biological activities.



Our group has developed highly effective methodology which allows the construction of tetramates, and has shown that these compounds may be used to generate libraries of potent antibacterial agents.[2] This project focuses on equisetin,[3] pramanicin[4] and oxazolomycin,[5] three examples of such natural products; the main objective is to use tetramate **1** as a core scaffold to rapidly synthesise analogues which mimic key features of the natural product structures.



The poster describes highly regioselective and stereoselective routes to synthesise libraries of analogues which are hybrid mimics between equisetin and pramanicin (**2**) and equisetin and oxazolomycin (**3**). The biological properties against Gram-positive and Gram-negative bacteria have been studied, and some analogues have shown activity at MICμg/mL against *S. aureus*. These activities have also shown to be highly dependent on the physicochemical properties of the compounds, with PSA and logP having a big influence on the MIC values.

These results demonstrate that tetramates provide valuable 3D-templates suitable for drug discovery.

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DESIGN, SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF PROLINE HYDROXAMATES TARGETING ZINC-DEPENDENT ENZYMES

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Bacterial infections, complicated by the emergence of widespread multidrug resistance, are considered to be a serious threat to human population. The harmful effects of bacterial resistance result in 23,000 deaths in the USA and 25,000 deaths in Europe every year [1]. Nowadays, multidrug-resistant Gram-negative bacteria are a matter of great concern, as there is a lack of new effective classes of antibiotics on the market being active against these pathogens. The unique Zn^{2+}-dependent deacetylase LpxC (an essential enzyme in the biosynthesis of lipid A, **Fig.1, A**) can serve as an excellent antibacterial target as its inhibition is lethal to Gram-negative bacteria [2].

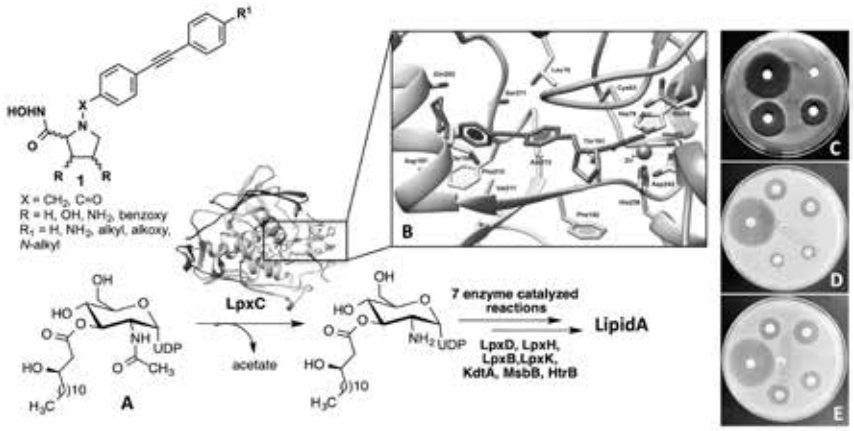

Fig.1. A. The deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine catalysed by LpxC. **B.** Binding pose of a synthesized LpxC inhibitor in the active site of *E. coli* LpxC. Antibacterial activity of the selected proline hydroxamates in disc diffusion assays against *E. coli* (**C**), *K. pneumonia* (**D**) and *E. aerogenes* (**E**).

To get access to selective and potent LpxC inhibitors, starting from L- and D-proline, (2*S*,4*R*)-4-hydroxyproline and D-mannose different proline derived hydraxamates **1** were synthesized. Some of the synthesized compounds demonstrated significant inhibitory activity in a fluorescence-based LpxC enzyme assay as well as in disc diffusion tests against clinically important Gram-negative bacteria. Molecular docking studies revealed essential interactions of the synthesized inhibitors with LpxC. Additionally, the selectivity of the synthesized compounds toward LpxC was studied by determining the inhibitory activity of the hydroxamic acids against the human Zn^{2+} -dependent enzymes MMP-2, -8, -9, and -13.

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BIOACTIVE M. TUBERCULOSIS THIOREDOXIN REDUCTASE INHIBITORS: AN UPDATE

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The resurgence of tuberculosis, caused primarily by *Mycobacterium tuberculosis*, and the appearance of multi-drug resistant and extensively drug resistant *M. tuberculosis*. strengthen the need for new drugs with alternative modes of action.[1] The interaction between the mycobacterial thioredoxin reductase (TrxR) and its substrate thioredoxin (Trx) is a promising new drug target for the treatment of tuberculosis, since *M. tuberculosis* lacks the common glutathione system and the TrxR of *M. tuberculosis* shows a substantial difference in sequence, mechanism and structure to human TrxRs. In *M. tuberculosis*, TrxR is part of the antioxidant system that reduces hydroperoxides, contributes to ribonucleotide reduction, and thus guarantees the survival within macrophages.[2] Although the target mechanism is a protein-protein interaction, the first known inhibitors with different scaffolds could be identified using an exhaustive high-throughput docking based on the available TrxR X-ray structures.[3] By means of structure-based design, the activity of the most promising candidate could be increased to an IC_{50} up to the low nanomolar range that also showed a strong influence on the growth of *M. tuberculosis*.[4]

In order to further improve the bioactivity of the promising compounds we focused on optimizing the physicochemical properties that are important for permeability, since *M. tuberculosis*shows an unusual thick and impermeable cell wall. An analysis of calculated polar surface area and logP indicated an influence of these properties on the minimum inhibitory concentration. Based on computational molecular design, compounds with improved psychochemical properties were designed and synthesized. In fact, these compounds showed an improved activity on mycobacterial growth which underlines the assumption regarding optimized properties. Permeability measurements using a PAMPA-assay also proved an increased permeability and showed the usability as a model system for mycobacterial cell wall permeability. In addition, the most promising compound do not show any cytotoxic effect on human macrophages in the range of minimum inhibitory concentration on *M. tuberculosis* growth.

We will present and discuss the design and the results of our improved compounds. In addition, further experiments towards a detailed biochemical characterisation and tests on infected macrophages are in progress.

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DESIGN AND SYNTHESIS OF NOVEL TRICYCLIC TOPOISOMERASE INHIBITORS (NTTI'S) TO TACKLE THE THREAT OF MULTI-DRUG RESISTANT BACTERIAL INFECTIONS

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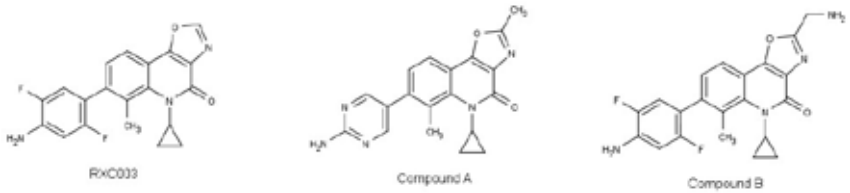
Since the discovery of nalidixic acid, the essential bacterial topoisomerase enzymes DNA gyrase and topoisomerase IV have been extensively exploited by the quinolone class of antibiotics. Resistance to antibiotics, including fluoroquinolone class agents, is on the increase. It is estimated that 700,000 deaths occur every year from drug-resistant infections with this figure predicted to rise to 10 million by 2050.[1]



In response, Redx Pharma has developed a novel series of DNA gyrase and topoisomerase IV inhibitors that are chemically distinct from quinolones and demonstrate novel binding interactions. Redx's novel tricyclic topoisomerase inhibitors (NTTIs) demonstrate potent activity against a range of Gram-positive and some Gram-negative organisms including fluoroquinolone-resistant and methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria gonorrhoeae.*

RXC003, Compound A and Compound B demonstrate excellent biological properties, including superior frequency of spontaneous resistance at 4 × MIC compared to the fluoroquinolone ciprofloxacin and efficacy in murine models of sepsis and thigh infection.

This poster will showcase the synthesis, SAR, and *in vivo* efficacy of these novel tricyclic topoisomerase inhibitors.

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DESIGN AND BIOCHEMICAL STUDIES OF NEW AZOLE DERIVATIVES TARGETING MYCOBACTERIUM TUBERCULOSIS CYTOCHROME P450 CYP121

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The cytochrome P450s (CYP450s) are a large haem-containing enzyme superfamily found in all prokaryotic and eukaryotic organisms. The genome sequence of Mycobacterium tuberculosis (Mtb) revealed the existence of 20 putative CYP450s within this organism[1]. In Mtb, the Rv2276 gene encodes a CYP450-CYP121 that catalyzes an unusual reaction by forming a C-C bond between the carbons ortho to the phenolic hydroxyl of the two tyrosyl side chains of the cyclodipeptide cyclo(L-Tyr-L-Tyr); cYY, resulting in a novel chemical entity called mycocyclosin[2]. Studies suggested that either mycocyclosin is essential for Mtb, or the overproduction of cYY is toxic[3]. Azole drugs have been shown to coordinate tightly with high affinities (submicromolar) to the haem iron in CYP121[4].
Based on docking studies with the crystal structure of CYP121 (PDB; 2IJ7) using molecular operating environment (MOE) 2014.0901[5], two new libraries of fourteen pyrazole derivatives and eight piperazine derivatives were designed and synthesized.

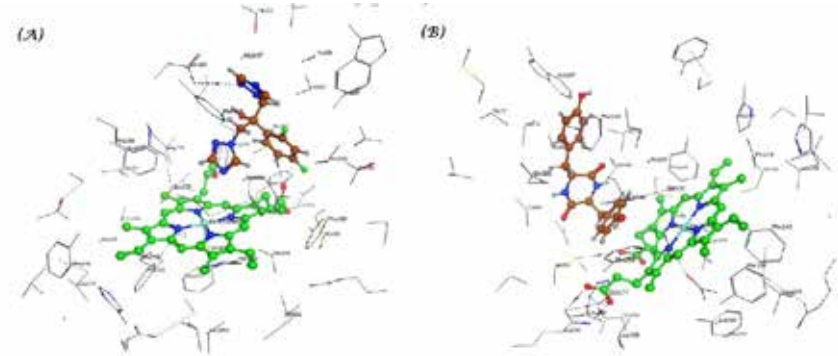

Figure (1): Three dimensional docking pose of (A) fluconazole; (B) cYY, with CYP121 using MOE software.
The synthetic pathway for the first series involved the formation of ethylidene phenylhydrazine derivatives, which were cyclized to form pyrazole carbaldehyde via a Vilsmeier–Haack reaction. The formed carbaldehydes were reduced, chlorinated and alkylated. For the second series, diimines were first synthesized, then reduced and cyclized to form piperazine derivatives which were chlorinated and alkylated. Both series gave final pure novel imidazole and triazole compounds confirmed by ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HRMS and/or microanalysis, to be evaluated for biological activity.

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NOVEL ANTIMICROBIAL AGENTS AND BIOFILM INHIBITORS DERIVED FROM N-ARYL AND N-ACYLISATINS

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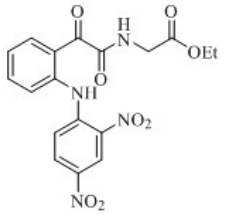
Antibiotic resistance has become an increasing problem in recent years due to the slackening rate of discovery of novel antibiotics, while at the same time antibiotic use is on the rise. Bacteria cooperatively regulate the expression of many phenotypes such as biofilm formation and virulence factor expression through a mechanism called quorum sensing (QS). Whereas, antimicrobial peptides (AMPs) are a key component of the mammalian immune system that provides protection against infections caused by various pathogens. Thus, the development of novel quorum-sensing inhibitors and AMP mimics are important strategies to counteract the increasing incidence of antimicrobial resistance. In the present study, we report the design and synthesis of novel glyoxylamides *via* the ring-opening reaction of *N*-arylisatins with amines and amino acid esters, and *N* -acylglyoxylamide peptide mimics *via* the reaction of *N*-acylisatins with *N*,*N*-dimethylethane-1,2-diamine and *N*, *N*-dimethylpropane-1,3-diamine. The QSI activity of the *N*-arylglyoxylamide derivatives was determined in the *P. aeruginosa* MH602 and *E. coli* MT102 reporter strains. These compounds exhibited significant QSI activity with compound **1** showing a 49% and 74% reduction in PAMH602 and *E. coli* MT102 respectively. The antibacterial and biofilm disruption activity of *N*-acylglyoxylamide peptide mimics was investigated against *Staphylococcus aureus.* The HCl salt **2** exhibited the lowest MIC of 16 μg mL^{-1}, wheras the corresponding quaternary ammonium iodide salt **3** had a MIC of 38.9 μg mL^{-1}. The glyoxylamide based QS inhibitors and AMP mimics represent a new avenue for the development of novel, cost-effective antimicrobial agents.



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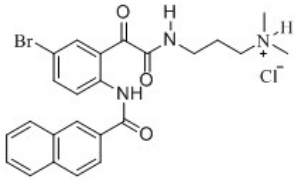

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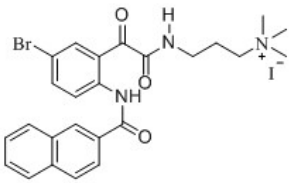

3

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF ACHYROFURAN ANALOGS

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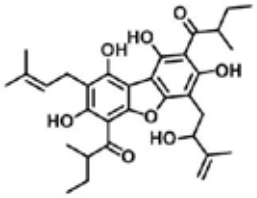
Bacterial resistance to drugs is a serious and growing public health problem. Thus, the discovery of new and improved antimicrobial agents is one of the most urgent needs.[1] Recently our research group has identified achyrofuran (1), a prenilated polioxygenated dibenzofuran from Achyroclyne satureoides, as an antibacterial agent against some clinically relevant Gram-positive bacteria, including methicillin-resistant (MRSA) and vancomycin-intermediate Staphylococcus aureus (VISA) in the nanomolar range.[2] Taking this into consideration we decided to synthesize structural analogs in order to evaluate their antibacterial activity.

In this communication we will report the results obtained in the synthesis of symmetric simpler achyrofuran analogs, their antibacterial evaluation and structure-activity relationship studies.



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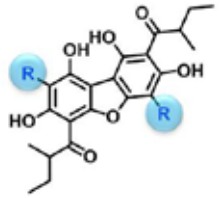

R = H
R = $CH_2CH_2CH_3$
R = $CH_2CH(CH_3)_2$

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HIGH-THROUGHPUT SCREENING FOR INHIBITORS OF PSEUDOMONAS AERUGINOSA ADP-RIBOSYLATING EXOENZYME S

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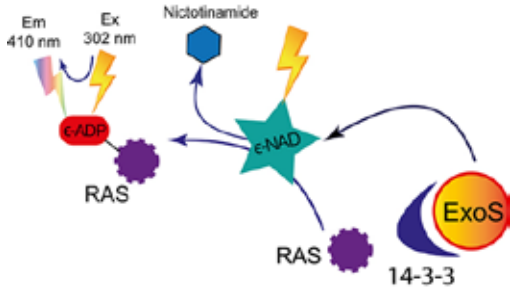
Pseudomonas aeruginosa is a gram-negative pathogenic bacterium and one of the top lethal hospital-acquired infections. It causes a wide variety of life-threating infections including blood, pneumonia, and wounds infections, especially in immunocompromised patients such as cystic fibrosis and cancer patients. Due to the remarkable antibiotic resistance of *P. aeruginosa* there is an immense need to develop novel antipseudomonal agents that can be used in combination therapy to ensure treatment of resistant strains. Targeting bacterial virulence, the ability of the bacteria to promote disease, is an attractive approach to combat antibiotic resistance by 'disarming' the bacteria instead of killing it. The type III secretion system (T3SS) is an essential virulence factor and a valid therapeutic target in many gram-negative bacteria including *P. aeruginosa*. It is a syringe-like apparatus located on the bacterial surface and responsible for transporting effectors proteins into eukaryotic cells.

Exotoxins S and T (ExoS and ExoT) are toxins that are secreted by the *P. aeruginosa* T3SS. They are ADP-ribosyltransferase (ADPRT) enzymes that modify various eukaryotic proteins, such as small GTPase proteins (e.g. Ras, Rho, Rac), which leads to signal-transduction malfunction and eventually cell death. Mutation of *exoS*at the ADPRT domain attenuates the infection [1, 2] and makes ExoS-ADPRT a putative therapeutic target.



For that, we developed and optimized an *in vitro* enzymatic assay (Figure 1) and identified an inhibitor of ExoS-ADPRT activity [3]. Recently, we optimized and employed the assay for a high-throughput screen (HTS), and a library of 30,000 diverse small-molecules was screened at 10 µM against ExoS ADPRT enzymatic activity. As a result 80 primary hits was identified as potential ExoS inhibitors. A follow-up hit validation has shown 6 inhibitors of ExoS-ADPRT with an IC_{50} of 3 – 27 µM. An ongoing enzyme kinetics and medicinal chemistry programs with the goal to reach compounds with efficacy *in vivo* will eventually allow us to support the scientific community with a novel chemical probe(s), which can be used to study bacterial virulence *in vitro* and *in vivo.*

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TOWARDS NONNATURAL CARBOHYDRATE-BASED INHIBITORS OF PSEUDOMONAS AERUGINOSA VIRULENCE FACTOR LECTIN LECB

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The rise of resistance against antibiotics in bacteria is a major threat and demands the development of novel antibacterial therapies. Infections with *Pseudomonas aeruginosa*, an opportunistic pathogen, are a severe problem for hospitalized patients and for patients suffering from cystic fibrosis. These bacteria can form biofilms and thereby increase their resistance towards antibiotics through the physical barrier of the biofilm matrix. The *Pseudomonas* virulence factor lectin LecB is a carbohydrate-binding adhesin and plays an important role in biofilm formation.[1] The natural ligands for LecB are glycosides of D-mannose (e.g., methyl α-D-mannoside (**1**)) and L-fucose (e.g., methyl α-L- fucoside (**2**)), the latter displaying an unusual strong affinity to its lectin receptor. This fact was explained by the interaction of the carbohydrate with two calcium ions bound by the receptor and an additional lipophilic interaction of the terminal methyl group in fucose.[2] Interestingly, although mannosides are much weaker ligands for LecB, their inhibitory potency was remarkably improved upon modification of the equatorial C-6 substituent.[3]

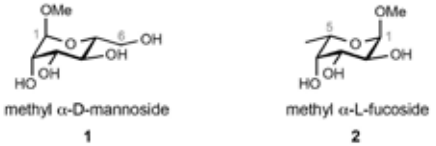

Figure 1: Methyl α-D-mannoside (**1**) and methyl α-L-fucoside (**2**) are the carbohydrate epitopes recognized by the *Pseudomonas aeruginosa* lectin LecB.

Several aspects should be taken into account in development of carbohydrate-derived drugs. These are unspecific binding to the human pathogen-recognition receptors (PRRs), low oral bioavailability and metabolic instability in human and pathogen. In order to mask the natural carbohydrate character of LecB inhibitors and to increase their affinities, a detailed structure-activity relationship study was performed.[4, 5, 6] Compared to unmodified carbohydrates, methyl α-D-mannoside (**1**) binding properties of LecB ligands were improved regarding their potency and complex stability. Herein discovered LecB inhibitors are supposed to overcome drawbacks of the natural carbohydrates as a drug and are a good base for future drug development in the anti-virulence approach.

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SEMISYNTHETIC GLYCOPEPTIDE ANTIBIOTICS WITH PROMISING ANTIBACTERIAL AND ANTIVIRAL ACTIVITY

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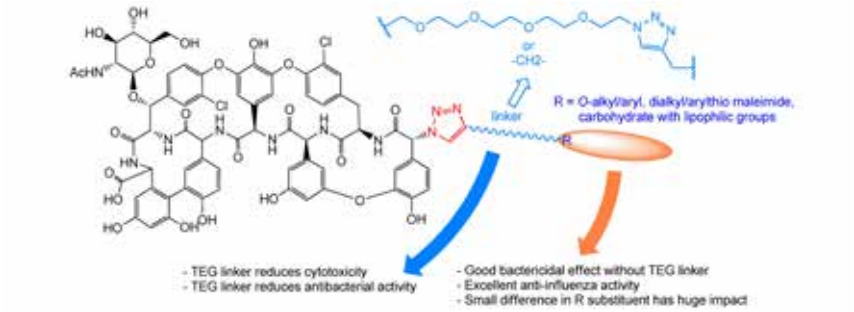
Over the past decades antibiotic resistance has become one of the most serious threats to human health worldwide. The most hard-to-treat infections are caused by Gram-negative bacteria e.g. *Klebsiella pneumoniae.* Among Gram-positive bacteria vancomycin resistant *Enterococcus faecalis* and *E. faecium* (VRE) can cause severe infections (besides MRSA), which makes it necessary to develop antibiotics which can effectively eradicate such pathogens.

The development of modern semisynthetic glycopeptide antibiotics like oritavancin, which are highly capable of eliminating VRE, encouraged our group to synthesize numerous derivatives from members of the dalbaheptide group of glycopeptides by various chemical methods.[1]

Recently we utilized a Nefkens-type reaction between *N*-ethoxycarbonyl maleimide derivatives and teicoplanin pseudoaglycon[2] and the copper-catalysed azide-alkyne click reaction to obtain systematic series of compounds which possess different lipophilic substituents. This way we could identify some of the structural determinants that are needed for impressive biological activity. In many cases, minimal changes in lipophilicity and structure produced great differences in biological activity.



The antibacterial tests by broth microdilution showed that a high portion of compounds with good activity are primarily bacteriostatic, but a smaller portion of them also displayed bactericidal properties when tested against vanB and vanA positive *E. faecalis*. Our most promising molecules were tested against larger collections of clinical isolates of *E. faecalis* and *E. faecium*, and several strains were susceptible to the compounds.

In vitro experiments showed that many of these compounds are also able to prevent the influenza virus infection of cell cultures in very low concentrations by an unknown mechanism. Several of them were highly cytotoxic at the same time but some of them displayed a reasonable selectivity index. The antiviral properties of the derivatives may not be restricted to influenza viruses, as e.g. the anti-HIV activity of dalbaheptide antibiotics have been reported by other research groups.[3] This could potentially lead to the development of antiviral drugs which possess the key structural elements that are needed for the antiviral activity, while not having those which provide antibacterial effect. Structure-activity relationships to this extent have not been investigated by us yet, but would be an interesting area to fully discover.

Our future plans include the further optimization of the most promising substances. Ex vivo/in vivo experiments are planned to evaluate the possible usefulness of these molecules in real-life situations, primarily in bacterial infections. In conclusion, these findings present a great opportunity for the development of both antimicrobial and antiviral compounds, which could certainly contribute to the improvement of global health.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF DUAL ACTION ANTIBACTERIAL ANTIBIOTICS

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The growing resistance of microorganisms to currently available antibiotics calls for the development of new strategies that can solve the problem of antibacterial resistance. One of such strategies is the development of dual-acting hybrid antibiotics – structures that contain two covalently linked antimicrobial drugs that interact with different targets in a bacterial cell [1].

We developed method of synthesis of hybrid antibiotics which contain a glycopeptide antibiotic (vancomycin or eremomycin or teicoplanin aglycon) covalently linked via spacer to a 4”- or 11-position of azithromycin (Fig. 1). The structures of the obtained hybrid antibiotics were confirmed using NMR spectroscopy and HR mass spectrometry methods, including MS/MS data.

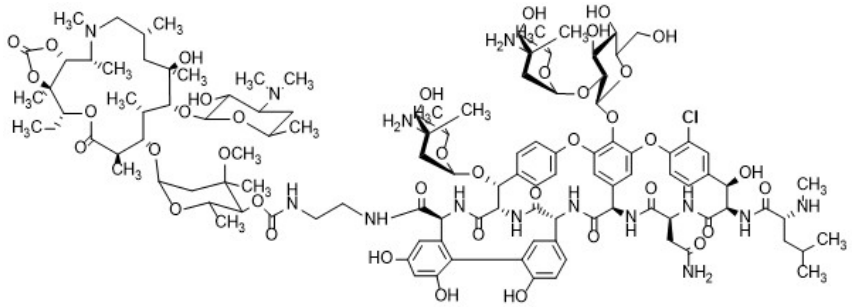

Fig. 1. Dual aciting antibiotics on the basis of azithromycin and glycopeptide antibiotics (on the example of eremomycin).

It has been demonstrated that all novel dual-acting antibiotics are as active as azithromycin and vancomycin against different *Staphylococcus aureus* strains and have superior activity than azithromycin and vancomycin against *Streptococcus pneumoniae* strains. Synthesized hybrid antibiotics were active against *Enterococcus faecium* and *Enterococcus faecalis* strains resistant both to azithromycin and vancomycin. Most of the obtained dual action antibiotics demonstrated an ability to cause translation arrest although their activity was lower than that of azithromycin. Investigation of antibacterial activity of azithromycin-eremomycin conjugate (Fig. 1) on model of the *Staphylococcus aureus* sepsis of mice revealed that the new antibiotic is equivalent by efficiency to vancomycin. Thus, synthesis of the dual-acting antibiotics on the basis of azithromycin and glycopeptides lead to highly-active compounds that in some cases overcome bacterial resistance. Further investigations including some SARs are under way.

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MECHANISTIC STUDIES ON THE INHIBITION OF OXA ENZYMES BY LACTIVICINS

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The increasing emergence of clinically relevant bacterial strains resistant to antibiotics is a major threat to human health. The most widely used antibiotics are the β-lactams, such as penicillins, cephalosporins and carbapenems, which account for over 50% of global antibiotic consumption. These antibiotics, however, are hydrolysed by β-lactamases, acquired enzymes which result in antibiotic resistance. Several clinically useful β-lactamase inhibitors have been developed, i.e. clavulanic acid, sulbactam, tazobactam and avibactam. These inhibitors are effective against β-lactamases, i.e. avibactam is effective against Class A, C and, some, D β-lactamases. Class D β-lactamases, otherwise known as oxacillinases (OXA), are of particular interest, not only due to their clinical significance, but also from a mechanistic perspective – their enzymatic activity requires carbamylation of a key active site lysine residue, and consequently differs from all other β-lactamases. In this study, we screened several different types of (potential) β-lactamase inhibitors and their derivatives, including the non β-lactam (γ-lactam) containing lactivicins, against the clinically relevant Class D enzymes including OXA-10, -23 and -48. A novel mode of action of lactivicins against Class D enzymes was revealed and confirmed by kinetic analyses using UV-vis and various NMR spectroscopic methods. A crystal structure of an OXA-10-lactivicin complex validated the proposed mechanism of action. Furthermore, cell-based susceptibility studies indicate bactericidal activity and support that lactivicin derivatives are a promising line of investigation for combating clinical challenges introduced by Class D β-lactamases. In particular, lactivicins display activity against multidrug resistant *Acinetobacter baumannii*, a major source of hospital-acquired infections.

VIRTUAL SCREENING FOR LIGANDS OF THE FMN RIBOSWITCH, A TARGET FOR ANTIBIOTICS

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The increasing number of infections with multi-resistant bacteria is a significant public health threat and therefore new antibiotics with new targets are required. Attractive drug target are the so-called "riboswitches" - bacterial mRNA capable of specifically binding small molecules.[1] The flavin mononucleotide (FMN) riboswitch is exclusively found in bacterial species and binding of its natural ligand FMN controls expression of genes involved in biosynthesis and/or transport of riboflavin (vitamin B2) on either transcriptional or translational level. Recently, the FMN riboswitch was chemically validated as a target for antibiotics.[2] We performed a virtual screening of our digital in-house library of about 5M purchasable compounds with the aim of identifying new chemotypes binding to the FMN riboswitch.[3] After screening the whole database, a substructure search to exploit privileged structures was conducted. RNA-ligand docking was carried out following our previously established protocols.[4,5] The most promising candidates resulting from docking studies were purchased and subjected to binding affinity studies on the *in vitro* transcribed FMN riboswitch RNA. Due to the inherent fluorescent properties of FMN fluorescence quenching experiments were carried out in a competitive manner to obtain K_D -values. Since RNA-ligand interactions are most often enthalpy-driven processes, isothermal titration calorimetry (ITC) was also employed in a displacement method. ITC experiments revealed one promising candidate. X-ray crystallography studies are ongoing to determine the binding mode of the new ligand.

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IN SILICO AND ANTIMYCOBACTERIAL STUDIES OF 4,6-DIAMINO-1,2-DIHYDRO-1,3,5-TRIAZINES

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Tuberculosis (TB) as a leading cause of death worldwide accounts for the global health problem among millions of people each year. In 2014, WHO estimated 9.6 million new TB cases and reported 1.5 million TB death. [1] In recent years, the emergence of multi- and extensively-drug-resistant (MDR-TB and XDR-TB) strains has exacerbated TB epidemic. [2] Therefore, more efforts need to be focused on the discovery of new anti-TB agents. Dihydrofolate Reductase (DHFR), a key enzyme in the folate pathway, has been identified as a clinical drug target for the discovery of anticancer, antibacterial, antimalarial, and antifungal agents. [3] However, no DHFR inhibitors is reported to be used in clinical treatment against TB. In this study, as 2,4-diamino-1,3-diaza fragment has been identified as the pharmacophore present in the chemical structure of almost all potent DHFR inhibitors [3], 5 series of compounds were designed on the basis of DHFR pharmacophore via side chain modification, which involved the attachment of a few antimycobacterial scaffolds. The designed derivatives were docked into *Mtb* DHFR active site to investigate the *in silico* binding interaction. The amino group of all the designed triazine ring exhibited favourable H-bonding interaction with Asp27, Ile5 and Ile94 of the anchoring active site of *Mtb* DHFR. The synthesis of 5 selected compounds was carried out via multi-step reactions. The preliminary antimycobacterial screening was conducted by using paper disc diffusion assay at the loading of 50 µg per disc against *M. smegmatis*, a surrogate model of *M. tuberculosis*. It was found that 2 compounds displayed comparable activity to Trimethoprim (TMP). In conclusion, molecular modelling was used for virtual screening and prediction of potential ligand-receptor interaction. 5 selected compounds were synthesized for antimycobacterial screening and 2 compounds showed comparable activity to TMP.

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NEW N-PHENYL-4,5-DIBROMOPYRROLAMIDES AND N-PHENYL-3,4-DICHLORO-5-METHYLPYRROLAMIDES AS ATPASE INHIBITORS OF DNA GYRASE

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DNA gyrase is a member of type IIA bacterial topoisomerases and is a well-known and clinically validated pharmacological target for antibacterial drugs. The function of DNA gyrase is to catalyse the transient break and reunion of the DNA double strand, a process crucial for negative supercoiling or relaxation of positive supercoils in the DNA molecule during its replication. After several decades of research in the field of ATPase inhibitors of gyrase/topo IV, novobiocin, a natural coumarin antibiotic, remains the only GyrB inhibitor that has ever progressed to the clinic. To bypass the increasing resistance problems, new research, including a contribution from the academia, is urgently needed.[1]

Using structure-based design and starting from the recently determined crystal structure of the *N* -phenyl-4,5-dibromopyrrolamide inhibitor-DNA gyrase B complex,[2] we have prepared a series of new *N* -phenyl-4,5-dibromopyrrolamides and *N*-phenyl-3,4-dichloro-5-methylpyrrolamides and evaluated them against DNA gyrase from *Escherichia coli*. The most potent compounds had a nanomolar IC_{50} value against *E. coli* gyrase. A selected set of compounds was evaluated against DNA gyrase from *Staphylococcus aureus* and against topoisomerase IV from *E. coli* and *S. aureus*. The binding affinities of selected compounds to *E. coli* gyrase were studied using surface plasmon resonance. In the design of the present series, the focus was on the optimisation of biological activities of compounds – especially by varying their size, the position and orientation of key functional groups, and their acid-base properties. The structure-activity relationship (SAR) was examined and the results were rationalised with molecular docking.[3-4]

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POSTERS

Peptides: Pushing Permeability and Bioavailability Beyond the Rule of 5

DISRUPTION OF G PROTEIN-COUPLED RECEPTOR DIMERS BY CELL-PENETRATING INTERFERENCE PEPTIDES: BREAKING THROUGH TO THE OTHER SIDE

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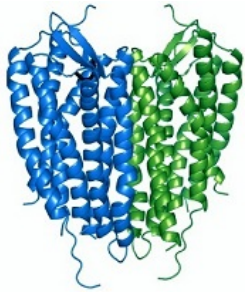

G protein-coupled receptors (GPCRs) form the leading class of membrane proteins in the human genome and account for the majority of signal transduction across the cell membrane.[1] Until recently, it was generally accepted that GPCRs existed as monomers. However, with our evolved understanding of GPCR function and with a few technological advances, this dogma was gradually overturned by the concept of GPCR dimerization.[1,2] A vast number of open questions about the functional mechanisms of GPCRs focus on the existence of GPCR dimers, which exhibit novel biochemical properties and could be appreciated as large therapeutic target resources.Targeting GPCR dimers with their distinctive signaling and functional properties when compared to homomers, is expected to broaden the therapeutic potential of drugs targeting GPCRs.[2] The nature of the interaction interface specifies which GPCR displays significant protein-protein interactions (PPIs) and although some efforts have been made to explore contributions of the extracellular N-terminus and intracellular C-terminal tail as an exclusive PPI domain, data now supports direct interactions between helical transmembrane (TM) residues.[3] One approach to probe GPCR dimerization interfaces is to use synthetic peptides, derived from an interface where the PPIs are likely probable. To mediate the TM peptide's uptake into the cell, fusion with cell permeable cargoes make TM peptides tangible for cell penetration and proper orientation in the membrane.[4,5] Furthermore, helical TM cell-penetrating interference peptides derived from GPCR receptors can elicit behavior and physiological effects *in vivo* by selectively disrupting the hydrophobic core architecture and function of the receptor which they were derived from.[5] Therefore, construction of such cell penetrating peptides that can specifically disrupt the physical interaction between receptors in the plasma membrane and dissect the function of particular motifs throughout signaling is not only a tool for the functional investigation of PPIs *in vitro* or/and *in vivo,* but might also constitute a new molecular strategy for the development of therapeutics targeted to the dimeric interface.

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THE EFFECT OF GLUTATHIONE ANALOGUES UPF1 AND UPF17 ON NA,K-ATPASE ACTIVITY IN THE KIDNEYS OF C57BL/6 MICE IN VITRO AND IN VIVO

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Glutathione is low-molecular weight tripeptide, which has an important role in the antioxidative defense system of human body. Goal of the antioxidative treatment is to avoid or diminish the oxidative stress. The aim of this study was to investigate the effect of the GSH analogues UPF1 (O-methyl-L-tyrosinyl-g-L-glutamyl-L-cysteinyl-glycine) and UPF17 (O-methyl-L-tyrosinyl-L-glutamyl-L-cysteinyl-glycine)[1] on Na,K-ATPase activity in two months old C57BL/6 mice kidney *in vitro* and *in vivo.* Based on GSH and the ability of antioxidants to relieve oxidative stress, creation of agents with better bioavailability is under investigation.

Na,K-ATPase is a crucial enzyme of plasma membrane. Kidney Na,K-ATPase is composed of α subunit, β subunits and a regulatory subunit belonging to the FXYD protein family, which all contain free cysteine residues. The glutathionylation of the Na,K-ATPase causes a decrease in the Na,K-ATPase activity[2].

The experimental results of the study *in vitro* showed that UPF17 decreased the activity of Na,K-ATPase in the kidneys of mice and was dependent on the concentration of the UPF17 (10^{-3},10^{-5} and 10^{-7} M was observed). The time-dependent inhibitory effect of UPF17 became apparent (10 and 45 minutes were observed). UPF1 did not have any effect in the given experimental conditions.

The experimental results *in vivo* showed the significant decrease of Na,K-ATPase activity in C57BL/6 mice after intraperitoneal administration of UPF1 and UPF17 (1 mg/kg, 5 days). UPF17 showed stronger effect on enzyme activity than UPF1 *vs* control (fold change 1.3 and 1.2, respectively).

The present study was supported by Institutional Research Funding No IUT20-42.

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HOW TO DESIGN CELL PERMEABLE NON-PEPTIDIC MACROCYCLES

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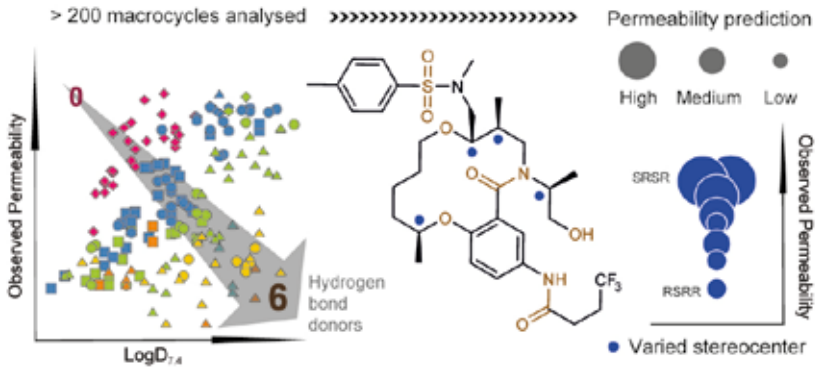
Macrocycles, drug design, cell permeability, efflux, stereospecific permeability prediction



BACKGROUND: Macrocycles are of increasing interest as chemical probes and drugs for intractable targets like protein-protein-interactions, but determinants of their cell permeability and oral bioavailability are poorly understood.[1]

OBJECTIVE: We aim to create an understanding of the properties that govern cell permeability, efflux and solubility of macrocycles, to incorporate our learnings into guidelines describing an orally bioavailable property space for macrocycles.[2]

METHODS: We generated an extensive dataset by measuring cell permeability (Caco-2) and efflux ratios as well as aqueous solubility, $LogD_{7.4}$ and pKa values under consistent experimental conditions for >200, non-peptidic, *de novo*-designed macrocycles from the Broad Institute's diversity-oriented screening collection, including structurally diverse sets of matched pairs of stereo- and regioisomers.

RESULTS: This analysis revealed how specific functional groups, substituents and molecular properties impact cell permeability. Analysis of energy minimized structures for stereo- and regioisomeric sets, combined with NMR structure verification, provided fundamental insight into how, sometimes dynamic, intramolecular interactions in the 3D conformations of macrocycles are linked to physicochemical properties and permeability.

IMPACT: Combined use of quantitative structure-permeability modeling and the procedure for conformational analysis now, for the first time, provides chemists with a rational approach to design cell-permeable and orally bioavailable non-peptidic macrocycles.[3]

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SP1-7 AMIDE RELATED PEPTIDES WITH ANTI-ALLODYNIC EFFECT IN SPARED NERVE INJURY MICE AFTER SYSTEMIC ADMINISTRATION

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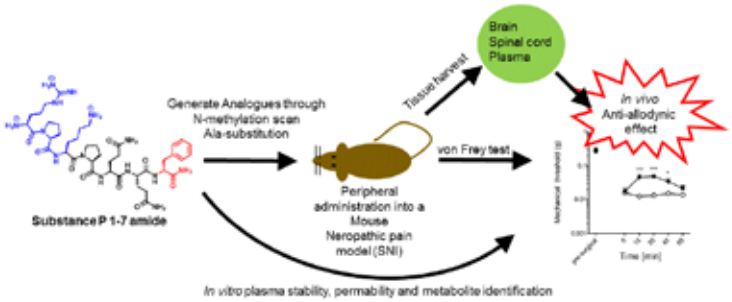

The renewal of interest in peptides as potential drug candidates in recent years has brought several peptide based drugs, beyond the rule-of-five, to clinical trials and to the market.[1-2] Furthermore, since the current treatment of neuropathic pain is unsatisfactory, new and better alternatives are needed. In our recent study we confirmed that the full-length amidated N-terminal fragment of substance P, SP_{1-7} amide, more effectively attenuated mechanical allodynia as compared to the parent peptide SP_{1-7} and truncated fragments (SP_{2-7} amide and SP_{3-7} amide).[3]

In the study presented here we analyzed a set of twelve new SP_{1-7} amide analogs in an attempt to explain structure-activity relationships between peptides with subtle amino acid modifications, such as Ala-substitution and N-methylation of the backbone, and their anti-allodynic effect in SNI mice after intraperitoneal administration. We also evaluated the peptides regarding stability in both mouse and human plasma as well as their passage through the Caco-2 monolayer in order to explain their effect *in vivo*. Additionally, we have observed possible degradation path for the SP_{1-7} amide. The observations described here will add new knowledge and guidelines to the design of SP_{1-7} analogs destined for future neuropathic pain management.

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DEVELOPMENT OF HEXAPEPTIDE-GEMCITABINE CONJUGATES FOR PANCREATIC CANCER THERAPY

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Gemcitabine (Gem, Figure 1) is a nucleoside analogue used in the treatment of various cancers, being a reference treatment for advanced pancreatic cancer. The major impediment related to gemcitabine efficacy is its rapid inactivation. Another important drawback regarding gemcitabine therapy is that, after initial tumour regression, tumour cells may develop different forms of drug resistance, namely resistance related to nucleoside transporter deficiency [1]. These efficacy problems, together with the known toxicity and secondary effects of chemotherapeutical drugs, highlight the need to improve the delivery of Gem specifically to tumour cells.

As part of our effort to identify orally active prodrugs, we were interested in conjugating hexapeptides to the 4-*N*-amine of Gem. These new conjugates were developed using cell-penetrating peptides (CPP) to facilitate intracellular delivery of Gem, based on the common virtue of all CPP: i) ability to efficiently pass through cell membranes; ii) non-cytotoxicity and iii) ability to carry a wide variety of cargos inside cells [2]. Our conjugates were prepared by solid phase peptide synthesis (SPPS), purified and characterized by HPLC and LC-MS. The evaluation of the cytotoxicity of the formulated conjugates (versus gemcitabine alone or CPP6 alone) was analysed with the Resazurin assay and concentration-dependent response curves were determined.

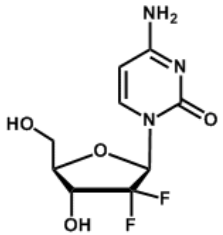

Figure 1. Structure of Gemcitabine (Gem).

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POSTERS

Molecular Tissue Targeting

SITE-SELECTIVE ROS SENSITIVE PRODRUGS FOR IMPROVED TREATMENT OF INFLAMMATORY DISORDERS AND CANCER

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Chronic inflammation is associated with a wide range of diseases, including cardiovascular diseases, cancer, diabetes, and autoimmune diseases.[1] Inflammation is normally a defensive strategy in response to infection or tissue injury that subsides once the potential danger has been eliminated. However, in chronic inflammation the inflammatory response continues and can cause extensive damage to host tissue.[2]

A major issue of treating many inflammation-related diseases is the lack of drug selectivity.[3] Consequently, treatment is often associated with severe adverse effects. Prodrugs, that are inactive forms of drugs to be chemically and/or enzymatically activated *in vivo*, are typically introduced to improve poor ADME properties.[4] However, one strategy to increase efficacy of drugs and reduce toxic side effects is the development of site-selective targeting prodrugs.

A distinctive feature of inflammation, to be targeted with this prodrug strategy, is the significant increase in the concentration of reactive oxygen species (ROS) that are produced by immune cells to combat invading pathogens.[2] In this work, we propose the use of ROS sensitive prodrugs for improved treatment of inflammatory disorders (see Figure 1). Based on a ROS labile promoiety, ROS sensitive prodrugs of existing drugs were designed and synthesized. *In vitro* testing of ROS activation and activity in cell-based assays have shown promising results. Furthermore, the prodrugs have shown good *in vitro* stability under various physiological conditions. Based on these preliminary results, this proposed ROS prodrug strategy shows great potential in improving the treatment of various inflammatory disorders including cancer.

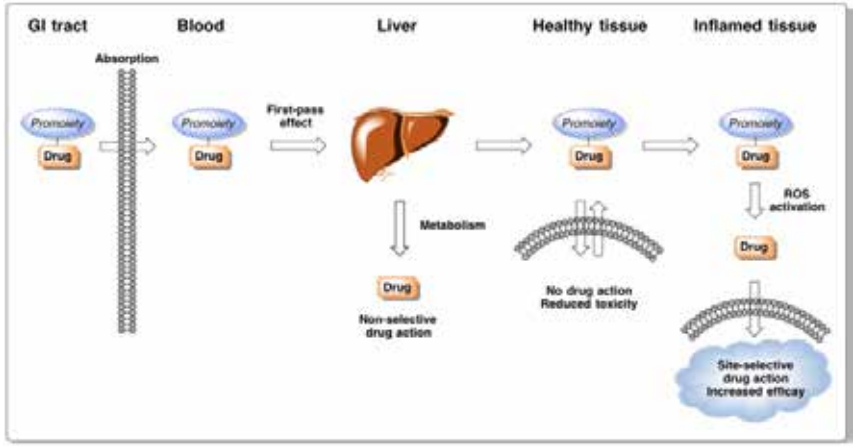

Figure 1: Proposed site-selective drug delivery to inflamed tissue via oral administration.

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NEW QUINAZOLINE DERIVATIVES FOR TUMOR HYPOXIA IMAGING

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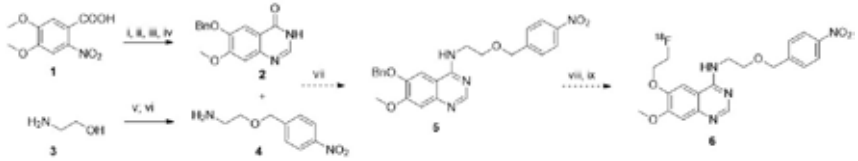
Tumour hypoxia represents an aggressive tumour phenotype with therapeutic resistance to conventional chemotherapy and radiotherapy procedures, requiring different treatments, which involve cell radiosensitiser compounds, hypoxic cell cytotoxins or oxygen delivery therapy[1].

Detection and monitoring of changes in hypoxia tumours are extremely important to establish specific cancer treatment. Positron emission tomography (PET) imaging is the preferred method since it provides information about intracellular oxygenation levels and shows high specificity and sensitivity to probe *in vivo* physiological processes.

Despite the availability of probes for the tumor hypoxia diagnosis by PET, all radiotracers have limitations that stimulate the research for obtaining new radiopharmaceuticals with better hypoxia tracer properties[2].

Thus, we envisage the synthesis of quinazoline derivatives as a common scaffold in antitumor drugs as new radiotracers for diagnosis of hypoxia tumour. The target structure **6**, depicted in **Scheme 1**, comprises two different functions related to the bioreductive nitrophenyl-oxymethylene group, preferentially at C-4, and the radionuclide ^{18}F at C-6.

The synthetic strategy was pursued a convergent synthesis with the preparation of 7-methoxy-6-benzyloxyquinazolin-4-one ring (**2**) for subsequent functionalization with bioreductive group (**4**) and ethylene fluorine moieties to give product **6**.



Scheme 1 – Synthetic sequence for the synthesis of radiotracer **6**. Conditions: i: NaOH (aq),100°C; ii: MeOH, H_2SO_4, reflux; iii: BnBr, DMF, Cs_2CO_3; iv: $InCl_3$, 150 °C, formamide; v NaOH, THF, Boc_2O; vi: NaH, THF; vii: PyBOP, DBU, DMF; viii: TFA, ACN; ix: ACN, K_2CO_3, 2-fluoro-ethyltosylate.

The synthesis of quinazolinone nucleus (**2**) was achieved by the demethylation of the 4,5-dimethoxy-2-nitrobenzoic acid (**1**) (position 5) by treatment with NaOH, followed by protection of the carboxylic acid and fenol groups with methyl and benzyl group, respectively, and one-pot reductive cyclization with $InCl_3$. In parallel, the bioreductive unit (**4**) was obtained from ethanolamine (**3**) which was *N*-protected with Boc, followed by ether formation with 4-nitrobenzyl bromide, and deprotection of the amino group. The complete synthesis will be achieved by linking compound **4** with quinazolinone ring **2** for the synthesis of functionalized quinazoline **5** followed by the debenzylation at C-6 position to give free phenolic derivative for further addition of 2-fluoro-ethyltosylate, containing a cold or hot fluorine on the quinazoline ring.

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DESIGN, SYNTHESIS, RADIOSYNTHESIS, AND BIOLOGICAL EVALUATION OF RADIOFLUORINATED QUINOLINE DERIVATIVES FOR PET IMAGING OF PDE5A IN BRAIN

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Cognitive decline due to age-associated neurodegenerative disorders is the leading cause of disability in the elderly. Only few treatment options currently exist which at best alleviate part of the symptoms. Therefore, identification of novel biological targets for treatment of cognitive impairments becomes urgent. Among the drug targets under investigation, the cyclic nucleotide phosphodiesterase enzymes (PDEs) and in particular PDE5 appear very promising because of their regulatory function in the signaling cascade of the cyclic nucleotides cAMP and/or cGMP. Several interesting neurological benefits such as improvement of learning and memory have been demonstrated in different mouse models using inhibitors of PDE5, especially sildenafil. Encouraged by these results, we proposed to develop a specific ^{18}F-labelled radiotracer for imaging of PDE5 in brain using positron emission tomography (PET). With this functional and dynamic imaging technology, a suitable PDE5 radiotracer should provide new insights into the cerebral physiology of this enzyme under normal and pathological conditions.

Based on a large bibliographic study on inhibitors of PDE5, we selected the quinoline scaffold (Compound **1**)[1] as lead compound. Indeed, it presents a sub-nanomolar inhibitory potency (Figure 1), a great selectivity over other PDEs, and is described to cross the blood-brain barrier.[2]

Figure 1: Development of fluorinated quinoline derivatives for PET imaging of PDE5 in brain

We designed a series of fluorinated derivatives with fluorine containing groups introduced either at position 3 or 4 of the quinoline. The organic syntheses of 12 novel fluorinated compounds were successfully accomplished. Then, their inhibitory potency at PDE5 and their selectivity towards other PDE families were determined *in vitro* . The most promising candidate **ICF24027** with an IC_{50} value of 1.86 nM was selected for further ^{18}F-radiolabelling which was performed *via* a one-step nucleophilic substitution reaction on the corresponding tosylate precursor using [^{18}F]TBAF in high radiochemical purities (≥99%) and specific activities (70-126 GBq/µmol). *In vitro* autoradiographic studies of **[^{18}F]ICF24027** on slices of different PDE5-expressing organs of mouse as well as of porcine brain indicated a moderate specific binding to PDE5. *In vivo* studies in mice revealed that **[^{18}F]ICF24027** was metabolized under formation of brain penetrable radiometabolites, thus hampering the applicability of **[^{18}F]ICF24027** for PET imaging of PDE5 in brain.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SMALL-MOLECULE PSMA-TARGETED CONJUGATES FOR PROSTATE CANCER TREATMENT

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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 the current work we present design, synthesis and preliminary biological evaluation of novel high-affinity small-molecule carriers equipped by Doxorubicin, with degradable hydrazone linker, able to release doxorubicin in physiological conditions after conjugate delivery . The most active compound showed a CC_{50} value of 95 nM vs. PSMA positive LNCaP cells while PC-3 culture remained intact. Moreover, the best carrier molecule effectively released the active substance in LNCaP cells instead of PC-3 line providing an excellent nuclei targeting and good selectivity. In contrast, a reference covalent Dox conjugate was drastically less effective. The best carrier molecule effectively released the active substance in LNCaP cells instead of PC-3 line providing an excellent nuclei targeting and good selectivity.

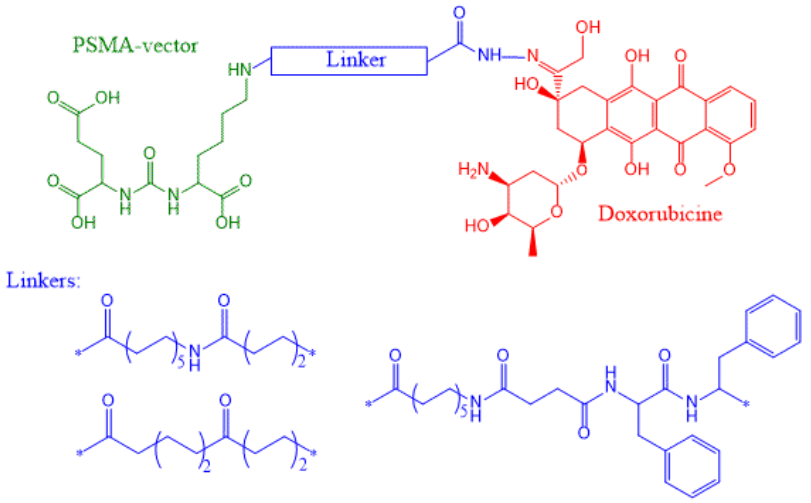

Figure 1. Structure of doxorubicin conjugates with PSMA-vector Glu-urea-Lys

Synthetic approaches and biological evaluation of synthesized structures would be minutely discussed in the report.

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CONJUGATION OF LIGANDS OF THE ASIALOGLYCOPROTEIN RECEPTOR WITH ANTICANCER DRUGS, FLUORESCENT DYES AND siRNA

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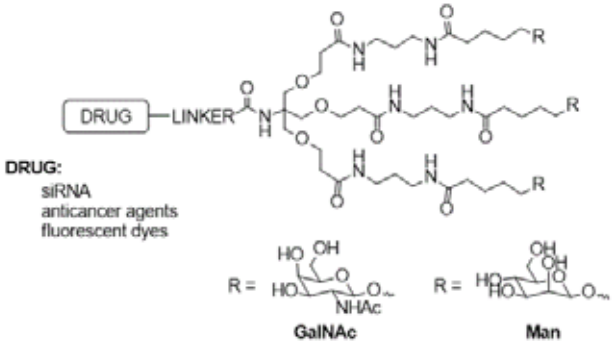
Targeted delivery of biologically active compounds to specified tissues is a rapidly developing field of organic medicinal and pharmaceutical chemistry. This approach allows to improve efficiency of a drug and to diminish its toxicity.

The asialoglycoprotein receptor (ASGPr) is highly expressed predominantly in hepatocytes. It facilitates uptake and clearance of glycoproteins, containing terminal D-galactose and N-acetylgalactosamine residues[1]. In view of its abundant presence on parenchymal liver cells, selective binding with aforementioned carbohydrate moieties and ability to transport molecules through cell membrane, the ASGPr is widely used as a target in drug delivery studies[2].



We investigated synthetic approaches to conjugates of ligands for the ASGPr with oligonucleotides, anticancer therapeutics and fluorescent dyes. Biological testing of obtained compounds were evaluated on cell lines HepG2 and HuH7 with induced by biotin ASGPr.

We would like to thank the Russian scientific fund, grant №14-34-00017.

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POSTERS

First Time Disclosures

DESIGN AND SYNTHESIS OF 3-DIMENSIONAL FRAGMENTS TO EXPLORE PHARMACEUTICAL SPACE

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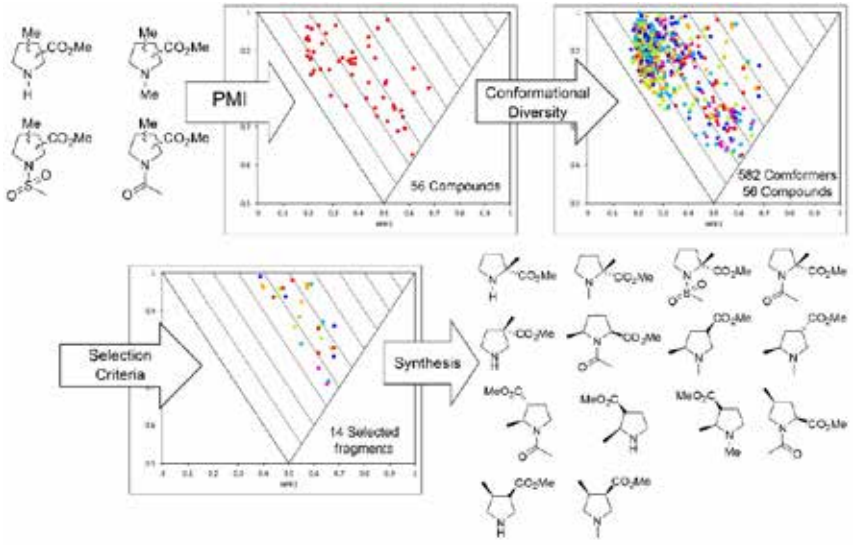
Fragment Based Drug Discovery (FBDD) has had significant success: there are small-molecule drugs in Phase I/II clinical trials (range of disease areas) and one approved drug (Vemurafenib) for late-stage skin cancer. These successes have mostly been for conventional targets (e.g. kinases and ATPases), where the aromatic fragments in current libraries have provided suitable starting points. However, most compounds in current libraries are highly 2-D in character. This is limiting since binding pockets on many proteins are highly 3-dimensional in shape, particularly for some of the new target classes.[1] There is thus recent growth in interest in more 3-D fragments, which could exhibit alternative binding modes and improve success rates through the drug discovery process.[2,3] The aim of this project is to design and synthesise novel 3-D fragments.

Our approach to the design and synthesis of novel 3-D fragments is shown schematically below. To identify novel 3-D fragments, all possible isomers are considered and 3-D shape analysis using principal moments of inertia (PMI) triangular plots[4] are used to select compounds for synthesis. For example, for a pyrrolidine with four different nitrogen groups (H, Me, Ac, SO_2Me) and two substituents (MeO_2C, Me), there are 56 possible isomers and 582 conformers with energy ≤ 1.5 kcal mol^{-1}above the lowest energy. Selecting the compounds with greater 3-D character (towards the top right corner of the PMI plot) led to the identification of 14 compounds for synthesis. In this poster, the selection process will be described in more detail and our efforts so far on the synthesis of the selected compounds will be presented.



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OVERCOMING INFLUENZA A DRUG RESISTANCE PROBLEM. IS MONOTHERAPY FEASIBLE?

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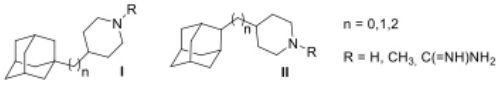
Influenza A (*Orthomyxoviridae*) is a highly contagious virus, best-known for the name of its surrogate illness, *the flu*. Even its importance tends to be belittlered, every year the seasonal flu outbreak causes arround 0.5 M deaths worldwide and together with the non-mortal infections it translates in 87.1 billion US$ burden. Worse, is its singular capacity to spontaneously mutate giving mortal periodic pandemics. Despite all this, an effective treatment has not been discovered, turning it into a major threat to human health and a serious biowarfare pathogen.

The M2 proton channel protein, essential for virus viability, contains a single transmembrane domain that forms a tetrameric pore[1] targeted by Amantadine and Rimantadine (FDA approved drugs). Despite, this pathogen has overcomed its inhibition by point mutations in the M2 protein, finding among the circulating strain isolates almost a 98% of drug-resistant M2 mutants (mainly S31N, V27A and L26F), rendering the approved drugs obsolete. During the last years several new potential M2 inhibitors have appeared[2], however none is able to simultaneously inhibit the wild type (wt) and the three main mutants, remaing a challenge to date.



Using medicinal chemistry as the main tool, we have rationally designed, synthesized and exhaustivelly analized a familly of amantadine analogues (Fig. 1), that has allowed both, gain information about the mechanism of action and to develop triple M2 inhibitors. The bioactive molecules prepared, have been pharmacologically evaluated by means of three assays: cytophatic effect reduction (CPE), virus yield reduction (yield) and virus plaque reduction (PRA); submited to target validation assays as NMR and Patch Clamp and theoretical calculation as dockings, molecular dynamics and metadynamics.

[image]

This multidisciplinary project has rendered the most potent triple (wt, V27A, L26F) inhibitor to date and shown differences in the binding mode of structural related compounds (Fig.1). This unexpected behaviour, which was first theoretically predicted (Fig. 2), has translated in an experimental difference in the inhibition mechanism in the Patch Clamp and CPE tests and was finally confirmed by NMR experiments, solving the first 3D structure with a guanidine group bind in the channel.

Overall our research results[3] provide new insights into the M2 inhibition mechanism and robust new SAR information (optimal lipophilicity, pKa, bulkiness) which will lay the ground work for the design of new inhibitors of this set of resistant mutants that ideally, will provide a single drug to combat multiple circulating influenza virus strains, thereby alleviating the need for combination therapy.

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INSIGHT INTO THE STRUCTURE OF HUMAN 17β-HYDROXYSTEROID DEHYDROGENASE TYPE 14

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Human 17β-hydroxysteroid dehydrogenase type 14 (17β-HSD14) is the latest 17β-HSD, which has been identified [1, 2]. This cytosolic enzyme is a member of the SDR super family (Short-chain Dehydrogenase Reductase), 17β-HSD14 catalyses *in vitro* the oxidation of estradiol (E2), 5-androstene-3β,17β-diol (5-diol) and testosterone to estrone, dehydroepiandrosterone and androstenedione, respectively, using NAD^+ as cofactor. This protein has not yet been deeply investigated and its physiological role remains unknown. Two variants of this enzyme were found in two different tissues with only a single mutation in position 205, where a Ser is replaced by a Thr, however, the two variants show *in vitro* the same efficiency. The crystal structure of a target protein can provide important structural insights into the binding site and supports the understanding of the chemical biology of this enzyme. We will present the crystal structures of the human 17β-HSD14 in apo form, in binary complex with NAD^+ and in ternary complex with estrone. Potent and selective inhibitors are also useful tools to study the role of enzymes *in vivo*. No inhibitors of this enzyme are known. We discovered the first potent non-steroidal inhibitor of 17β-HSD14 as measured in a fluorimetric assay and we determined its crystal structure in complex with the protein.

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17β-HYDROXYSTEROID DEHYDROGENASE TYPE 14: SCAFFOLD IDENTIFICATION OF THE FIRST INHIBITORS, STRUCTURE OPTIMISATION AND 3D-CHARACTERISATION IN COMPLEX WITH THE PROTEIN

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17β-Hydroxysteroid dehydrogenase type 14 (17β-HSD14) is a recently characterized enzyme[1]. Its physiological role as well as its localisation remains unclear. 17β-HSD14 catalyses the oxidation of estradiol and 5-androstene-3β,17β-diol into estrone and dehydroepiandrosterone, respectively using NAD^+ as cofactor.

Potent and selective inhibitors are useful tools to study the role of an enzyme in vivo. As no inhibitor of this enzyme has been reported to date, the goal of this study was to identify the first inhibitors of 17β-HSD14.

In a preliminary study a library of 17β-HSD1 and 17β-HSD2 inhibitorsselected with respect to scaffold diversity was tested for 17β-HSD14 inhibition. The most interesting hit was taken as starting point for chemical modifications of the initial lead. The designed compounds were synthesised and tested for 17β-HSD14 inhibitory activity. A fluorescence-based assay was established using the recombinant purified protein and estradiol as substrate. The synthetic strategy and the biological results of the first potent inhibitors of 17β-HSD14 will be presented together with the first crystal structures in complex with the protein.

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DISCOVERY OF HIGHLY POTENT AND SELECTIVE ORALLY BIOAVAILABLE COMPLEMENT ALTERNATIVE PATHWAY INHIBITORS FOR TREATMENT OF PNH

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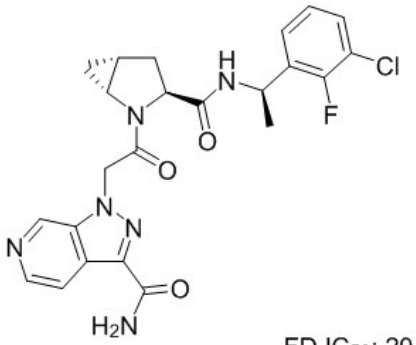
The complement system is one of the major defense mechanisms of the innate immune system composed of the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP). There is strong scientific evidence for AP involvement in Paroxysmal Nocturnal Hemoglobinuria (PNH) and other immune disorders. The serine proteases Factor B (FB) and Factor D (FD) are part of the central amplification loop of the AP.

We report on the discovery and preclinical evaluation of highly potent and selective low-molecular weight FD inhibitors which were identified using structure guided optimization.



FD IC_{50}: 20 nM

Oral administration of these inhibitors blocked systemic and ocular lipopolysaccharide (LPS)-induced activation of the AP in mice. In vitro inhibition of FD is shown to prevent both hemolysis and erythrocyte C3 deposition on human PNH erythrocytes ex vivo differentiating it from the standard of care, eculizumab.

EFFECTS OF A NEW PPAR DELTA AGONIST NCP-1046 ON THE WOUND HEALING IN ANIMAL MODELS

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Peroxisome proliferator-activated receptor δ (PPARδ) is ubiquitously expressed in a variety of cell lineages, including keratinocytes, and contributes to inflammatory responses and lipid metabolism. Recent reports demonstrated that PPARδ plays an important role in the keratinocyte responses to inflammation produced immediately after a skin injury and in skin wound healing. In this study, we investigated whether a new PPARδ agonist NCP-1046 we discovered, shows an effect of the recovery promotion to *in vivo* wound models. In the rat pressure ulcer model, NCP-1046 was absorbed into the rat skin with topical treatment of the ointment and time to complete wound closure was significantly reduced in 0.005% NCP-1046-treated rats compared with vehicle-treated rats. Furthermore, 0.05% NCP-1046 ointment accelerated wound healing in *db/db* mice, a mice model of type 2 diabetes mellitus. These results suggested that NCP-1046 promoted wound healing with topical treatment. Therefore, targeting PPARδ could be a novel therapeutic strategy for the treatment of skin wound.

DISCOVERY OF TAK-272: A NOVEL, POTENT AND ORALLY ACTIVE RENIN INHIBITOR

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The action of the aspartic proteinase renin is the rate-limiting initial step of the renin-angiotensin-aldosterone system; therefore, renin is a particularly promising target for blood pressure as well as onset and progression of cardiovascular and renal diseases. New pyrimidine derivatives were designed in an attempt to enhance the renin inhibitory activity of pyridine **1** identified by our previous fragment-based drug design approach. Introduction of a basic amine essential for interaction with the two aspartic acids in the catalytic site and optimization of the S1/S3 binding elements including an induced-fit structural change of Leu114 (“Leu-in” to “Leu-out”) by a rational structure-based drug design (SBDD) approach led to the discovery of *N* -piperidin-3-ylpyrimidine-5-carboxamide **2,** a 65,000-fold more potent renin inhibitor than compound **1**. Surprisingly, this remarkable enhancement in the inhibitory activity of compound **2** has been achieved by the overall addition of only seven heavy atoms to compound **1**. Next, introduction of an S1’ site binder into compound **2** in order to enhance the potency and further optimization of physicochemical properties for improvement of the PK profile led to the discovery of benzimidazole derivative **3** (TAK-272) as a highly potent and orally active renin inhibitor. Compound **3** demonstrated good bioavailability and long-lasting efficacy in rats. Compound **3** is currently in clinical trials.

STRUCTURE-BASED DRUG DESIGN AND DISCOVERY OF BENZYLPIPERAZINE DERIVATIVES AS ORALLY ACTIVE RENIN INHIBITORS

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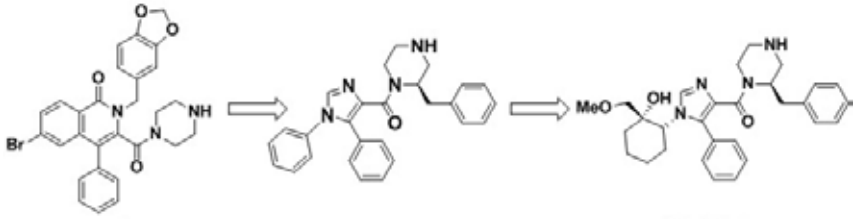
ABSTRACT: Renin, an aspartyl protease, is the rate-determining enzyme in the renin-angiotensin system (RAS) that is the key regulator of blood pressure and homeostasis of body fluid volume. Historically, peptidomimetic inhibitors of renin have dominated the research area. These efforts resulted in inhibitors with excellent renin inhibition potency, however, the peptide-like inhibitors showed low oral bioavailability and poor PK properties. In order to develop novel orally active renin inhibitors, a high-throughput screening (HTS) of our in-house library was carried out, and compound **1** which exhibited a moderate renin activity was discovered. Based on SAR study of **1**, compounds having tri-substituted azole scaffolds, constructed by parallel synthesis using amide formation with benzylpiperazine, were found to be potent renin inhibitors. By additional high-throughput synthesis, we identified imidazole derivatives **5** (hPRA IC_{50}=33 nM). While the imidazole as the optimal core in hand, we next turned to the extensive variation of S3-site binders to identify new compounds with improved both renin inhibitory activity and PK properties. These efforts led to the identification of **(1S,2R)-15** exhibiting potent renin inhibitory activity (hPRA IC_{50}=1.5 nM) with excellent ADME-Tox profile and demonstrating increased reduction in SBP compared with aliskiren in hAOGEN-hREN dTG rats.



1 **5** **(1S,2R)-15**

STUDIES WITH (THIO)XANTHONES AS ACTIVATORS OF P-GLYCOPROTEIN TO PREVENT CYTOTOXICITY INDUCED BY XENOBIOTICS

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ATP-binding cassette (ABC) transporters (P-glycoprotein (P-gp), among others) are efflux pumps present in the membrane of all cells that limit the absorption and distribution of endobiotics and harmful xenobiotics, by decreasing their intracellular accumulation. Over the past years we have performed studies evaluating the ability of P-gp inhibitors to overcome drug resistance [1].

If this field of research is fairly well explored, less is known about the effect of small molecule as P-gp activators. Hence, in our group we decided to explore this area for application against the toxicity induced by P-gp substrates, using the herbicide paraquat as model. Herein, *in silico* and *in vitro* studies concerning the characterization of newly synthetized (thio)xanthonic derivatives as inducers of the pump´s expression and/or activity, will be presented.

Several (thio)xanthones (20 μM) caused a significant increase in both P-gp expression (up to 208% for the hit compound, 1-(propan-2-ylamino)-4-propoxy-9*H*-thioxanthen-9-one, when compared to control cells) and activity (up to 156% for 1-(propan-2-ylamino)-4-propoxy-9*H*-thioxanthen-9-one, when compared to control cells) as evaluated by flow cytometry using the UIC2 antibody and rhodamine 123, respectively. Additionally, it was demonstrated that some of the tested compounds, when present only during the efflux of rhodamine 123, rapidly induced an activation of P-gp (up to 198% for 1-(propan-2-ylamino)-4-propoxy-9*H*-thioxanthen-9-one, when compared to control cells). The tested compounds also increased P-gp ATPase activity in MDR1-Sf9 membrane vesicles, indicating that derivatives acted as P-gp substrates. Moreover, when simultaneously incubated with paraquat, several (thio)xanthones significantly reduced the cytotoxicity of the herbicide, and these protective effects were completely reversed upon incubation with a specific P-gp inhibitor.

In silico studies showed that the tested thioxanthones fitted onto a previously described three-feature P-gp induction pharmacophore. Moreover, *in silico* interactions between (thio)xanthones and P-gp in the presence of paraquat suggested that a co-transport mechanism may be operating. A QSAR model was developed and validated, and the maximal partial charge for an oxygen atom was the descriptor predicted as being implicated in P-gp activation by the dihydroxylated xanthones. Based on the *in vitro* activation results, a pharmacophore model for P-gp activation was built, which will be of further use in the screening for new P-gp activators.

In conclusion, the study demonstrated the potential of (thio)xanthonic derivatives in protecting against toxic effects induced by P-gp substrates through P-gp induction and activation and opens news perspectives in the application of P-gp modulators as potential therapeutic agents.

Acknowledgments: ERDF, COMPETE, and FCT under the projects PTDC/SAU-OSM/101437/2008, PTDC/MAR-BIO/4694/2014, and INNOVMAR - reference NORTE-01-0145-FEDER-000035, Research Line NOVELMAR, to FCT and FEDER under Program PT2020 (project 007265 -UID/QUI/50006/2013).

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POSTERS

Making Small Molecule Synthesis Simpler, General, and Automatic

INTRODUCTION OF AN AMIDE ISOSTERE BY C-H FUNCTIONALIZATION OF HETEROCYLES

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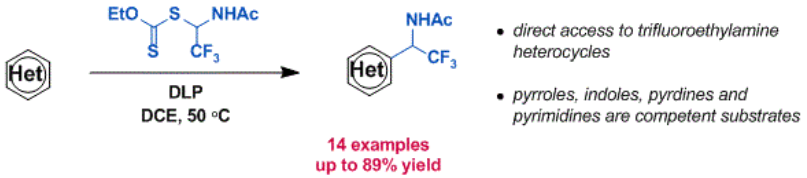
Trifluoroethylamines have been proposed to be amide isosteres capable of modulating ADME properties. For example, amide-to- trifluoroethylamine isosterism in clinical cathepsin K inhibitor (Odanacatib) improved metabolic stability at a good level of potency. Except for this successful example, further applications of amide-bond substitution by trifluoroethylamine surrogates are scarce, most likely owing to the lack of convenient synthetic methods. Current approaches toward trifluoroethylamine surrogates require multi-steps synthesis and heterocycle prefunctionalization. Herein, we report a direct access to trifluoroethylamine substituted heterocycles using an intermolecular oxidative radical addition of xanthates. This method represents a metal free equivalent of the Minisci reaction. The reaction is tolerant of electron-rich and electron poor heterocycles such as pyroles, indoles, pyridines and pyrimidines. Optimal yields were obtained using DCE at 50 ºC and dilauroylperoxide (DLP) as radical initiator. We also demonstrate that CSA is required for a good conversion in the case of electron-poor heterocycles.



- *direct access to trifluoroethylamine heterocycles*
- *pyrroles, indoles, pyrdines and pyrimidines are competent substrates*

SYNTHESIS OF NOVEL HETEROAROMATIC SULFONAMIDES AS VLCFA INHIBITORS

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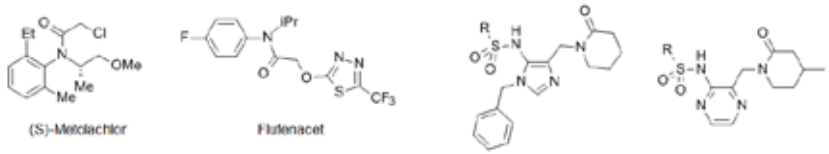
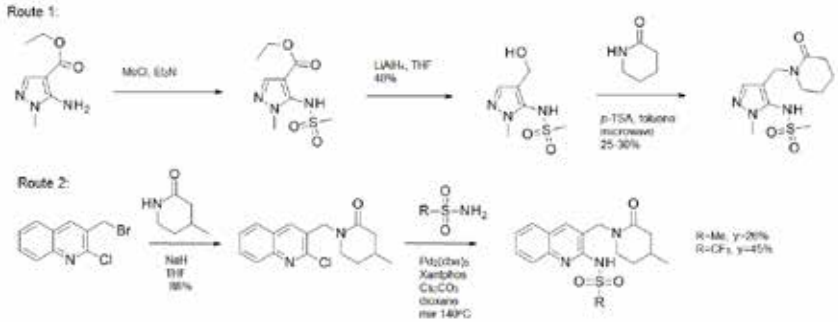
Compounds which inhibit the biosynthesis of Very Long Chain Fatty Acids (VLCFA) are an important class of herbicides. They typically display broad spectrum pre-emergence grass control with selectivity in a range of crops, including corn and cereals. The class includes established products such as S-Metolachlor (Syngenta) and Flufenacet (Bayer). We prepared some novel heteroaromatic sulfonamides that showed herbicidal activity with symptomology consistent with a VLCFA inhibiting mode of action.



Two routes were mainly used in the preparation of these targets. The first one started by the preparation of the sulfonamide followed by reduction of the ester and coupling with a lactam. The second route involved a newly developed palladium coupling between a sulfonamide and a heteroaromatic halide. This presentation will describe the scope and limitations of both routes and alternatives that were also considered for the preparation of numerous analogues.



SEAMLESS INTEGRATION OF 2D AND 3D SAR TO GUIDE MEDICINAL CHEMISTRY

Matthew Segall (1), Marcus Gastreich (2), Ed Champness (1), Peter Hunt (1), Christian Lemmen (2), Carsten Detering (2)

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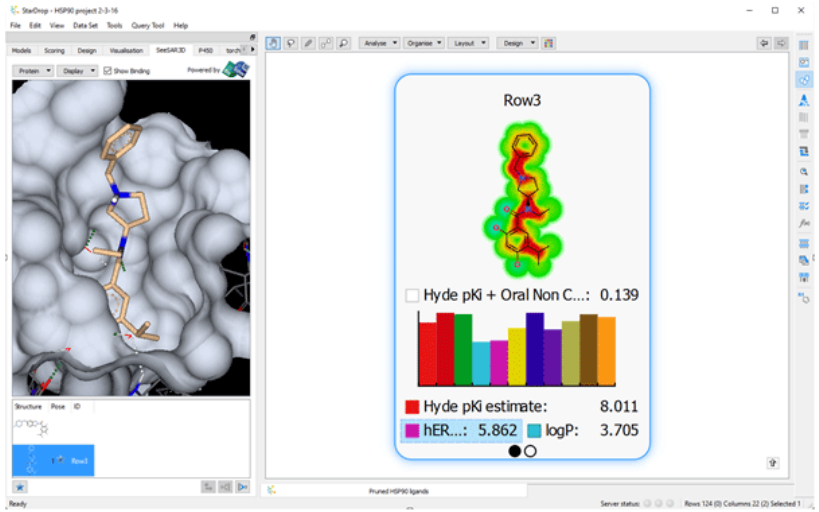
Both 2-dimensional (2D) and 3-dimensional (3D) analysis of compound structures and their relationships with biological activities are important to guide optimisation of high quality, potent compounds. However, 2D and 3D methods are typically available in separate, specialised software. In particular, access to 3D structure-based design is often restricted to expert computational scientists due to the complexity of the corresponding tools.

Nonetheless, these different views of structure-activity relationships (SAR) are most valuable when used together. A seamless, highly visual link between 2D and 3D information helps chemists to better understand the SAR within their project chemistry and guide the design of improved compounds. It also supports collaboration between computational and synthetic chemists, helping to share the results of 3D modelling studies with all decision makers. We will present such an integrated approach and some illustrative examples of its application.

Cheminformatics methods based on 2D compound structures, such as matched molecular pair analysis and activity cliff detection, highlight important SAR in data. A link with 3D structural information from crystal structures or docking experiments helps to rationalise these results and identify key interactions that drive target affinity. Furthermore, quantitative SAR models may be used to predict important physicochemical, absorption, distribution, metabolism, excretion and toxicity (ADMET) properties based on 2D compound structures. Using the SAR captured by these models, the influence of each atom or functional group on these properties can be highlighted. Coupled with an understanding of the 3D binding conformation, this enables the efficient design of compounds with an improved balance of target affinity and other critical properties in a truly multi-parameter optimisation environment.



AVOIDING MISSED OPPORTUNITIES BY ANALYSING THE SENSITIVITY OF OUR DECISIONS

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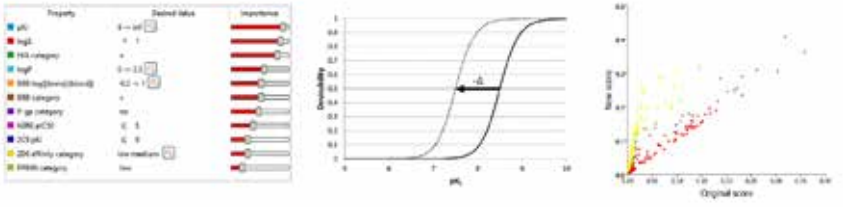
Optibrium Ltd, 7221 Cambridge Research Park, Cambridge, UK

Drug discovery is a multi-parameter optimisation process, in which the goal of a project is to identify compounds that meet multiple property criteria required to achieve a therapeutic objective. However, having chosen a profile of property criteria, their impact on the decisions made regarding progression of compounds or chemical series should be carefully considered. In some cases, the decision will be very sensitive to a specific property criterion and such a criterion may be artificially distorting the direction of the project; any uncertainty in the 'correct' value or the importance of this criterion may lead to valuable opportunities being missed. We will describe a method for analysing the sensitivity of the prioritisation of compounds to a multi-parameter profile of property criteria [1]. We show how the results can be easily interpreted and illustrate how this analysis can highlight new avenues for exploration.



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A CONVENIENT ONE-POT PROTOCOL FOR N-ACYLATION MEDIATED BY CYANURIC CHLORIDE UNDER MICROWAVE CONDITIONS

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FWF Project P 28243-B27

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Due to our interested in the synthesis of new compounds based on the substituted benzimidazolones scaffold, such as GSK1702934A,[1] as possible inhibitors or activators of TPCR3/6 channels,[2] we have developed a convenient N-acylationprocedure *via* cyanuric chloride under microwave conditions.

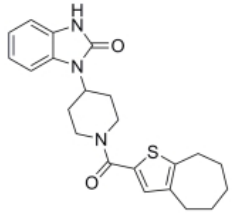
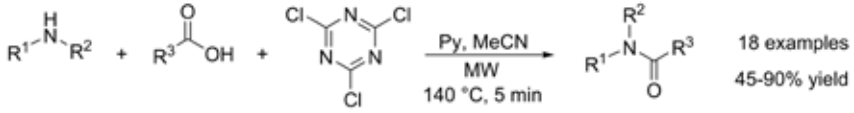


Figure 1. GSK1702934A - a TRPC3/6 channel activator.

Various amides were easily prepared in a very short reaction time with moderate to good yields using cyanuric chloride[3] as the coupling reagent and under microwaves conditions. In a direct comparison with a similar procedure employing phosphorus trichloride,[4] the freshly developed protocol *via* cyanuric chloride provided the desired substituted benzimidazolone derivatives in superior yields.



Scheme 1. One-pot procedure for N-acylation.

Acknowledgments:

We thank for the financial support to the Austrian Science Fund (FWF), Project *P 28243-B27*. We thank also to Chiara Mayer for the help.

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TOTAL SYNTHESIS AND EVALUATION OF BIOACTIVE NATURAL PRODUCTS

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Recently, natural products obtained from the nature exhibit a lot of biological activities and it sometimes become to the lead compound for drugs. For example, natural products and its derivatives such as taxol, vincristine, morphine and codeine are already used as anticancer drugs and anodynia, and so on. Especially, these natural products are suitable in terms of safety for human, therefore, drug development from natural products has received much attentions in the world. Focusing on new drug development, we have been continuing to synthesize pharmacophores of natural products including biological activities and further modifying them to functionalized derivatives. Also, to further enhance effective methods to find natural product derivatives, our research has applied to the concept of *Naturomimetic Approach* developed by our group. Biologically active pharmacophore of natural product is synthesized and efficient research to find further moiety is conducted through Pd-catalyzed C-C/C-N coupling and aldol coupling reactions utilizing general procedures. In the poster presentation, we will introduce our recent research results in *Naturomimetic Approach* derived from amorphstilbol, justicidin A, guggulsterone, nocarbenzoxazole F and shogaol.

ENAMINE READILY ACCESIBLE (REAL) ARRAYS - A TOOL FOR MAPPING SYNTHESIZABLE REGIONS IN A CHEMICAL FRAGMENT SPACE

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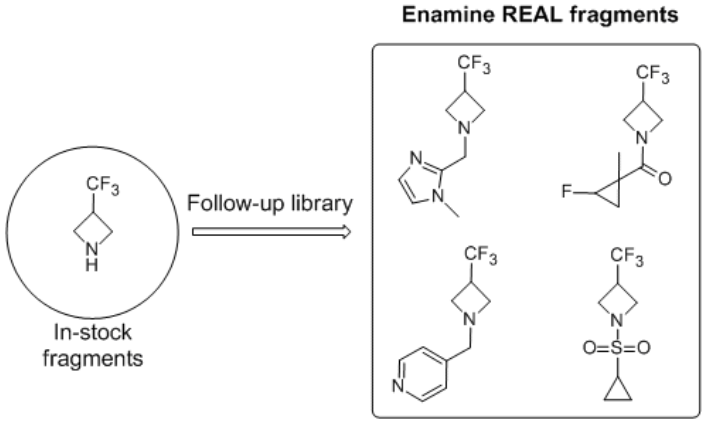
Fragment-based drug discovery (FBDD) has been a proven technology for providing new leads in many drug discovery projects. The success of FBDD strongly depends on quality of the starting compound library. The differences between fragments and regular screening compounds are the physicochemical profile and synthetic tractability of the screening library. These features are main challenges in the area because the accessible commercial chemical space does not meet FBDD purposes: on the market screening compounds collections fail the physicochemical criteria (molecular weight, lipophilicity, solubility) and novelty, building blocks are not always synthetically tractable thus expensive. Those issues are especially critical when designing a follow-up library.

In response to the challenges, research team of synthetic and medicinal chemists along with cheminformatics professionals from National Taras Shevchenko University of Kyiv and Enamine Company has created a REadily AccesibLe arrays. The REAL arrays cover drug-like (140M) and chemical fragment (2.5M) space and pursue three main goals:

1) Cost effective production of large and diverse sets within commercially acceptable periods of time.

2) Continuous expansion and optimization of chemical space of synthesizable compounds with potential biological activity.

3) Structural (core) and functional diversity of the compounds for the successful screening against new therapeutic targets.



The REAL arrays represent a feasible chemical space and comprise structures that are accessible in a single step synthesis. The arrays are a product of the validated synthetic technologies on the available reagents which based on a 25 years' experience in parallel synthesis of compound libraries for biological screening. This validation resulted in a 65K off-the-shelf collection of chemical fragments available for initial screening. Having the world's largest stock of novel building blocks makes the REAL arrays a valuable tool for generating inexpensive and diverse follow-up libraries for FBBD.

DESIGN, NEW SYNTHETIC APPROACHES AND BIOLOGICAL EVALUATION OF FLAVOPIRIDOL ANALOGUES

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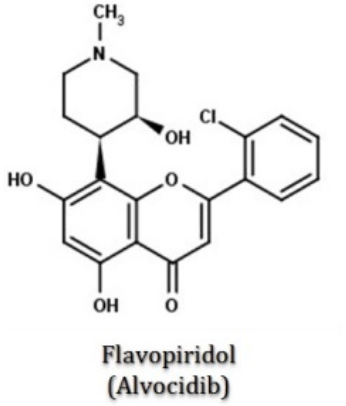
Flavopiridol, a hemisynthetic flavone inspired from the natural product rohitukine, is the first CDK inhibitor to enter clinical trials and has recently been granted an Orphan Drug designation for the treatment of patients with Acute Myeloid Leukemia [1,2,3].



The unique scaffold of flavopiridol combines the structural characteristics of flavonoids with some alkaloid properties and it has been submitted to several modifications the past decades, aiming to ameliorate the compound's biological properties. However, the synthesized derivatives had more than one features altered simultaneously and they were less active than flavopiridol itself. These data, along with the demanding synthetic path proposed, led us to design a series of analogues with only one structural alteration each time and also, to investigate a simpler route for their synthesis. Based on this rational, we synthesized derivatives with differentiated either the alkaloid moiety or the substitution of the flavone's aromatic ring, while we achieved to establish a more efficient and less expensive synthetic pathway. All the analogues, as well as some of the key compounds for this synthesis, have been evaluated for their ability to inhibit Cyclin Dependent Kinases and a panel of protein kinases. Additionally, they are under evaluation for their cytotoxic activity on several cancer cell lines, especially for Leukemia.

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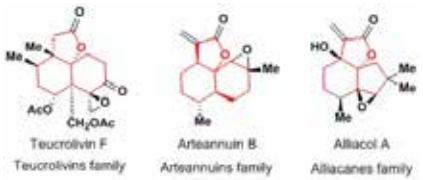
SYNTHETIC STRATEGY FOR THE CONSTRUCTION OF ANGULARLY FUSED TRICYCLIC NATURAL AND NATURAL-LIKE PRODUCTS

Yelena Mostinski, Viviana Valerio, Ramesh Kotikalapudi, David Lankri, Dmitry Tsvelikhovsky*

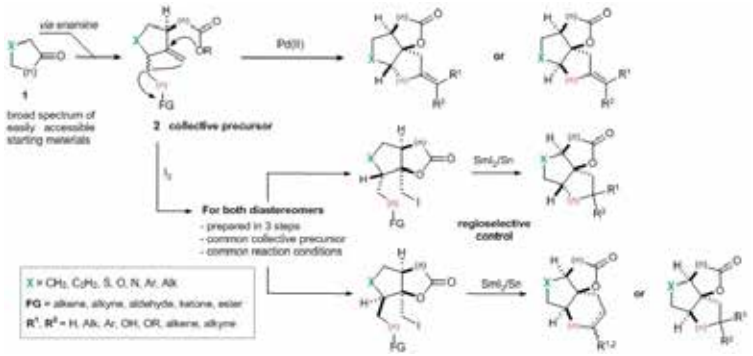
The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem

A wide variety of bioactive molecules of natural origin contain tricyclic angularly fused ring backbones (Scheme 1).[i] The presence of this pattern in phylogenetically diverse families of natural products reveals the significant effect such structures exert on physiological activities. Since these molecules are naturally produced only in small quantities and their synthetic preparation is hindered by multistep sequences,[ii] there is a need for a general, efficient protocol that would provide rapid access to a wide range of natural product families.



Scheme 1. Natural products sharing tricyclic angularly fused ring systems.

Our work is focused on the development of a *general synthetic strategy for the construction of the angularly fused tricyclic skeleton common to numerous classes of natural compounds of different biological origin*. Most of these tricyclic compounds might be derived from simple and structurally related precursors via stereo- and regio- controlled intramolecular transformations (Scheme 2).[iii]



Scheme 2. Common synthetic strategy for the construction of angularly fused tricyclic natural products.

The new synthesized compounds closely resemble common natural scaffolds and carry potential for becoming valuable drugs/therapeutic agents. They were submitted to various biological activity assays and showed promising bioactivity.

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GLUCOFURANOSE AS A SUBSTRATE FOR MOLECULAR SCAFFOLD SYNTHESIS

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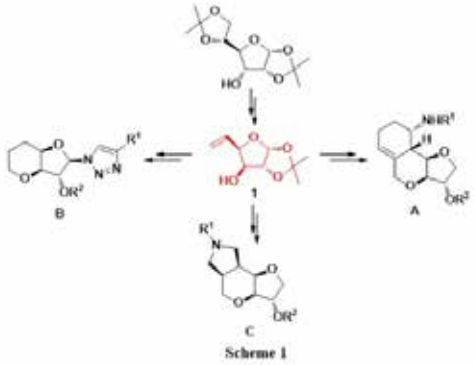
Carbohydrates are well established, readily available chiral building blocks rich in functional, conformational and stereochemical information.[1] At European Lead Factory our goal is to design and synthesise sp^3 carbon rich molecular scaffolds for molecular libraries of biological importance.[2] We use 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose, as a substrate for the design and synthesis of molecular scaffolds A-C (Scheme 1). Intermediate alkenol 1 was readily synthesized from 1,2:5,6-di-*O* -isopropylidene-α-D-glucofuranose.[3] The intermediate 1 was then transformed into three different scaffolds using ring closing metathesis[4], Diels-Alder reaction, 1,3-dipolar cycloaddition reaction and diastereoselective azide formation as key steps.



Scheme 1

Acknowledgement

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FSP3-RICH MONOSPIROCYCLES TO ACCESS NOVEL COMPOUND LIBRARIES

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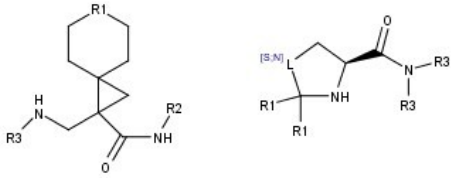
The European Lead Factory, a collaborative public private partnership established in 2013, provides high-quality compound libraries and the opportunity to screen those compounds against potential drug targets to a broader community[i]. Taros Chemicals, a privately owned CRO company, is leading the consortium and have contributed with more than 20.000 compounds into the Public Compound Collection (PCC).

All the compounds are novel and have drug-like properties. To ensure the variability at least three diversification points were explored. Moreover the synthetic routes were optimized to minimize the number of steps and certify a well understood, robust and efficient synthetic process both in the scale up of the intermediates synthesis (5-10g) and in the parallelization of the final diversification point (5µM). The purification of the final compounds by preparative HPLC-MS was also optimized allowing the successful isolation of compounds even in challenging cases like low yielding reactions or compounds devoid of UV absorbance with an average final purity of 97%. Finally a well understood, integrated and efficient workflow was implemented.

Taros contribution consists in scaffolds that are unique unsaturated ring systems with an overall high percentage of sp3 hybridized carbon. The three dimensional core structures are further expanded by the decoration of a diverse in-house collection of final diversification reagents. The substitution pattern and unsaturated character provide versatile starting points and ample opportunities for further chemical exploration and growth during the hit-to-lead phase.

We are illustrating the collection of scaffolds designed by Taros with two libraries enclosing 3 and 5 membered ring-containing spirocycles. We are describing the process of design of globular shaped structures, the validation of the chemistry as well as the production of the library and purification process.



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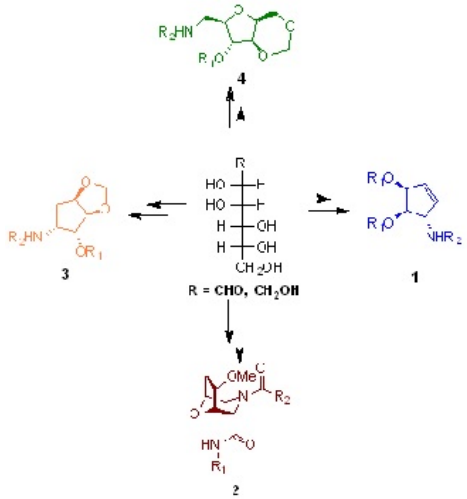
ii) The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

SUGARS ARE CONVENIENT STARTING MATERIAL FOR SP3 RICH CHIRAL SCAFFOLDS FOR DRUG DISCOVERIES

Jalindar Padwal, Dmitri Filippov, Bharat Narhe, Gijsbert A. van der Marel, Herman Overkleeft, Mario van der Stelt

Leiden Institute of Chemistry, Gorlaeus Laboratories, P. O. Box 9502, Universiteit Leiden, 2300 RA, Leiden; The Netherlands

The European Lead Factory (ELF) is a collaborative public-private partnership aiming to deliver innovative drug discovery starting points. Having established the first European Compound Library and the first European Screening Centre the EU Lead Factory gives free access to up to 500,000 novel compounds.[1]



Sugars are cheap and easily available starting material for the synthesis of 3-dimentional sp3 rich scaffolds for ELF drug discovery. Herein, a very concise and efficient syntheses of three-dimensional chiral scaffolds for ELF drug discovery is described. Starting from D-mannose and D-mannitol, we have many steps in common for the synthesis of different scaffolds that leads to very efficient and smart synthesis. The key steps in the syntheses are Wittig olefination, ring-closing metathesis (RCM), [3,3]-sigmatropic Overmann rearrangement, Staudinger/aza-Wittig/Ugi multicomponent reaction (SAWU-3CR) to form a highly functionalised, sp^3-rich, natural product-like scaffolds for library synthesis.

Acknowledgement:

"The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution."

References

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2.) 2. Jalindar D. Padwal1, Dmitri V. Filippov1, Bharat D. Narhe1, Sjoerd Aertssen2, Remmelt Jan Beuving2, Jorg C.J. Benningshof2, Gijsbert A. van der Marel1, Herman S. Overkleeft1 and Mario van der Stelt1* Bioorg. Med. Chem. 23 (2015) 2650–2655

OPEN INNOVATION DRUG DISCOVERY PROGRAM: COLLABORATION BETWEEN THE FRANTZ LAB AT UTSA AND LILLY

Ana Cristina Parra Rivera (1), Hector Aguilar (1), Doug E. Frantz (1), Christopher D. Beadle (2)

1) The University of Texas at San Antonio, Department of Chemistry, One UTSA Circle, San Antonio, TX 78249
2) Eli Lilly and Company Limited, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK

Our research program has been an active participant and collaborator with the Open Innovation Drug Discovery (OIDD) program at Lilly for the past few years. To date, we have submitted ~500 novel small molecules derived from the synthetic methodology developed in our labs to the OIDD program for high-throughput screening (HTS) in various biochemical and phenotypic biological assays in therapeutic areas of interest to Lilly. One of these compounds was identified as a hit in assays to identify negative allosteric modulators (NAMs or allosteric antagonists) of one of the group II subclass of metabotropic glutamate receptors, mGluR2, which is a potential therapeutic target for the treatment of cognitive impairment. What will be discussed are our unique industrial/academic collaborative efforts to synthesize new analogues through the Automated Synthesis Lab (ASL) at Lilly remotely from our laboratories in San Antonio, Texas. In particular, initial challenges translating reactions into an automated platform and the solutions developed to overcome them will be highlighted.

NOVEL F18 CHEMISTRY AND APPLICATION TO THE AUTOMATED SYNTHESIS OF PET RADIOPHARMACEUTICALS

Katarina Makaravage (1), Allen Brooks (2), So-Jeong Lee (2), Stephen Thompson (2), Naoko Ichiishi (1), Andrew Mossine (2), Matthew McCammant (1,2), Melanie Sanford (1), Peter Scott (2)

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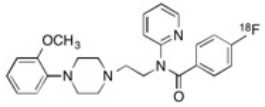
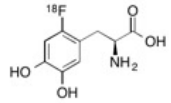
The development of new approaches for the late-stage incorporation of fluorine-18 into bioactive molecules with the goal of simplifying manufacture of radiopharmaceuticals for positron emission tomography (PET) imaging is of enormous current interest. One exciting new strategy employs transition metal catalysts to promote new nucleophilic radiofluorination reactions [1]. Our efforts to develop new reactions using $Ag^{18}F$ (e.g. C-H fluorination of 8-methylquinoline derivatives) will be communicated, as well as our recent progress developing and optimizing the copper-mediated radiofluorination of organoborons (boronic acids and pinacol boronate esters) [2], (mesityl)(aryl)iodonium salts [3], iodonium ylides and organostannanes with $K^{18}F$. The latter Cu-mediated radiofluoriantion reactions have greatly simplified the process of synthesizing PET radiotracers containing an [^{18}F]arene component as they are compatible with a wide range of functional groups as well as electronically diverse aryl groups, and uniformly afford excellent selectivity for a single ^{18}F-containing product. Optimized methods have subsequently been fully-automated using TRACERLab radiochemical synthesis modules and validated for production of clinical doses of PET radiopharmaceuticals according to the principles of current Good Manufacturing Practice (cGMP) [4]. Proof-of-concept has been demonstrated through development of fully-automated methods for the manufacture of radiopharmaceuticals of interest to our physician collaborators (e.g. [^{18}F]MPPF, [^{18}F]FDOPA) and, in addition to our progress optimizing/simplifying the radiochemistry for use in the synthesis modules, details of automation and process verification will also be presented.



[^{18}F]MPPF



[^{18}F]FDOPA

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EXPANDING THE TOOLS OF ORGANOPHOSPHORUS CHEMISTRY. MICROWAVE-ASSISTED HYDROPHOSPHINYLATION OF UNACTVATED ALKENES WITHOUT METAL OR RADICAL INITIATOR

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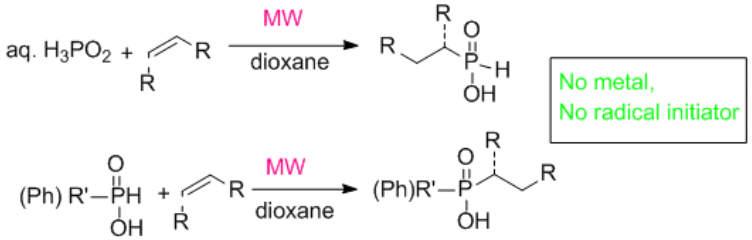
University of Athens, Department of Chemistry, Laboratory of Organic Chemistry, Panepistimiopolis Zografou, 15701, Athens, Greece

Phosphinic acids are a large class of important and valuable building blocks with applications ranging from pharmaceuticals to agrochemicals and materials.[1] Several potent and selective metalloproteases' inhibitors belong to this class of compounds.[2] Constructing the C-P bond has gained considerable amount of research the past few decadeswith the direct hydrophosphinylation of alkenes being the most attractive and atom economical. [3] Hydrophosphinylation of unactivated alkenes is usually performed using peroxide, AIBN, Et_3B/O_2, organic dye/photoirradiation, silver or palladium.

In this work, microwave-assisted hydrophosphinylation of unactivated alkenes with phosphinic acid and its derivatives was performed under metal-free and initiator-free conditions. Of special interest is the synthesis with our protocol of i) (4-phenylbutyl)phosphinic acid, an intermediate in the synthesis of the commercial heart drug Monopril(Bristol-Myers Squibb)[4] without any chromatography ii) Cbz-aminopropyl phosphinic acid, the precursor of 3-aminopropylphosphinic acid (APPA),[5] a potent and selective $GABA_B$ agonist (IC_{50} = 5 nM) introduced by Novartis iii) Hexadecylphosphinic acid, an intermediate in the synthesis of a phosphinate analogue of the anti-tumor phosphate di-ester Mitefosine.[6]



This new synthetic methodology aims to add to the arsenal of organophosphorus chemistry.

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TRIFLUOROMETHOXYLATED BUILDING BLOCKS FOR DRUG DISCOVERY

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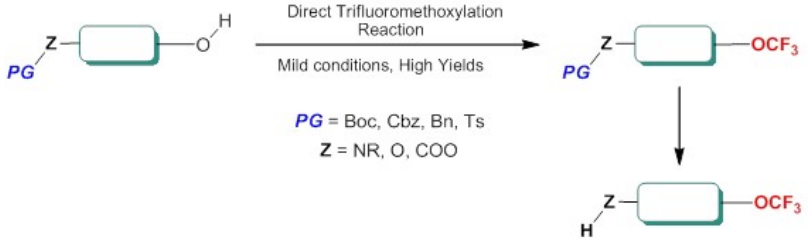
Fluorine plays a key role in the design of organic compounds with clearly known properties [1]. It's commonly accepted that the introduction of fluorine into organic compounds could dramatic enhance their metabolic stability, lipophilicity and acidity [2, 3]. Around a fifth of all drugs on the market today contain at least one fluorine atom. Among the fluorine containing groups, the trifluoromethoxy group (OCF3) turned out to be of special interest in biochemistry and medicinal chemistry due to its unique structural and electronic properties [4-6].

Here, we present a novel protocol for the introduction of a trifluoromethoxy group. This process includes one-pot procedure of direct conversion of functionalized alcohols to the corresponding trifluoromethyl ethers derivatives. The key-benefits of this novel approach are mild reaction conditions, chemoselectivity, high yields and easy work-up procedure [7].



The developed method is applicable for the preparation of trifluoromethoxylated amino and hydroxy acids, which have extremely important applications in biochemisty, medicinal chemistry and drug design.

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SPEEDING UP EARLY PHASES OF DRUG DISCOVERY: DoE-DRIVEN SYNTHETIC OPTIMIZATION OF CHEMICAL PROCESSES IN FLOW SYSTEMS

Antimo Gioiello (1), Serena Mostarda (1), Bruno Cerra (1,2), Valentina Mancino (1), Paolo Filipponi (1), Roberto Pellicciari (2)

1) Laboratory of Medicinal and Advanced Synthetic Chemistry, Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy.
2) TES Pharma, Corciano (Perugia), Italy.

In recent years, medicinal and synthetic chemistry have experienced a significant evolution in the approaches used and general thinking.[1] Several research groups from both academia and within pharmaceutical companies have adopted more technological solutions to help the delivery of compounds from early phases of discovery to (pre)clinical investigations and drug production.[2] Modern chemical manufacturing companies and academic laboratories are investing resources to drive a radical innovation in synthetic technologies, which would enable the conduction of chemical transformations that are difficult or impossible to be realized because of safety restriction, timing, costs, and environmental impact. Within this changing landscape, flow chemistry has rapidly emerged as a key technique that can significantly support these efforts leading to a radical change on how chemists think, perform and optimize chemical synthesis.[3]

In this communication, we have combined the potentiality of flow systems with automated equipment and software for the generation of compounds library and lead candidates advanced in preclinical settings of ischemia and cancer.[4] In particular, the profitable use of statistical design of experiments (DoE) will be demonstrated as a valuable tool to understand the benefits and risks associated with the chemical process and to speed up the synthetic optimization through a careful selection of experiments and the interpretation and validation of the results obtained.

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POSTERS

Hot Topics in Cardiovascular Diseases Research

DEVELOPING HIGH THROUGHPUT SCREENS TO IDENTIFY SMALL MOLECULE ACTIVATORS OF THE ADULT EPICARDIUM

Caitlin Clunie-O'Connor (1,4), Alison Howarth (2), Anke Smits (3), Angela Russell (4,5), Paul Riley (1)

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The epicardium plays an essential role during heart development. Cells derived from the epicardium (EPDCs) contribute essential cardiovascular cell types including vascular smooth muscle, interstitial fibroblasts, endothelium and cardiomyocytes via the process of epithelial to mesenchymal transition (EMT).[1] In addition to a physical contribution, EPDC paracrine signalling is critical for cardiomyocyte differentiation, myocardial compaction and proper organ formation.[2]

Whilst dormant in the adult heart, the epicardium becomes reactivated in response to injury in both mouse and zebrafish. Activation is characterised by epicardial expansion, EMT and re-expression of embryonic transcription factors including *Tbx18* and *Wt1*.[3] Moreover priming the mouse heart with thymosin β4 (Tβ4) increases the number of *Wt1+* EPDCs *in vivo* following myocardial infarction. Subsequently small numbers of Tβ4 activated *Wt1+* cells migrate into the wound forming functional cardiomyocytes.[4] The number of functional EPDC-derived cardiomyocytes is suboptimal to restore the lost heart muscle, therefore, we seek to augment the process using chemical or genetic approaches to fully exploit the epicardium as a source of resident adult stem cells for endogenous regenerative therapy.

Using both primary human patient-derived epicardial cells[5] and an immortalised murine epicardial cell line[6] we have established *in vitro* models of epicardial EMT. Subsequently we have developed two high throughput screening assays for the identification of small molecules that stimulate epicardial activation and studies are ongoing to validate primary hits that promote EMT and subsequent EPDC differentiation.

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KINETIC TARGET-GUIDED SYNTHESIS TO EXPLORE THE ROLE OF INSULIN-DEGRADING ENZYME IN THE CONTROL OF GLUCOSE INTOLERANCE

Jouda Jakhlal (1), Paul Hermant (1), Nathalie Hennuyer (2), Damien Bosc (1), Julie Charton (1), Julie Dumont (1), Sandrine Dassonneville (1), Florence Leroux (1), Wei-Jen Tang (3), Bart Staels (2), Peter Van Endert (4), Benoit Deprez (1), Rebecca Deprez-Poulain (1)

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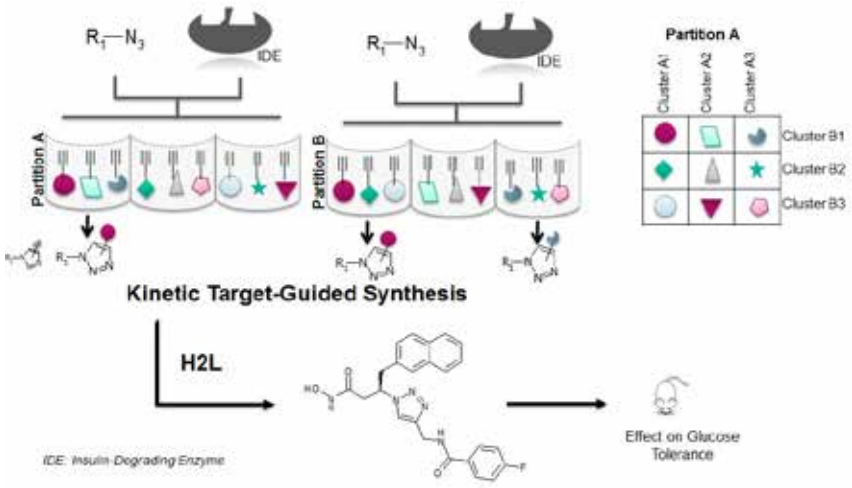
Target-Guided Syntheses are strategies used to discover ligands that are assembled by the protein itself from biocompatible-reactive reagents. Two main protein-templated strategies have emerged *in situ* Dynamic Combinatorial chemistry (DCC) and Kinetic Target-Guided Synthesis (KTGS). In KTGS, the biological target accelerates an irreversible reaction and led to the identification of hit(s). In our study, we have used kinetic target-guided synthesis to design the first catalytic site inhibitor of Insulin Degrading Enzyme (IDE) suitable for *in vivo* studies (BDM44768).IDE is a Zinc metalloprotease responsible for the inactivation of numerous bioactive peptides among which insulin and amyloid-β, thus IDE has been proposed as a putative drug target in diabetes. X-ray crystallography shows that the IDE inhibitor (BDM44768) binds to the catalytic site and locks the enzyme in a closed conformation. Amongst a panel of metalloproteases, BDM44768 selectively inhibits IDE. Moreover, the *vivo* result confirm that IDE is involved both in the clearance of insulin and pathway(s) that modulate short-term glucose homeostasis. It also suggests that the glucose intolerance observed in Ide-/- mice is not solely due to lifelong elevated insulinemia.



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NOVEL SMALL MOLECULE INHIBITORS OF ACTIVATED THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFIa) FROM NATURAL PRODUKT ANABAENOPEPTIN

Nis Halland, Mark Brönstrup, Jörg Czech, Werngard Czechtizky, Andreas Evers, Markus Follmann, Markus Kohlmann, Matthias Schiell, Michael Kurz, Herman Schreuder, Christopher Kallus

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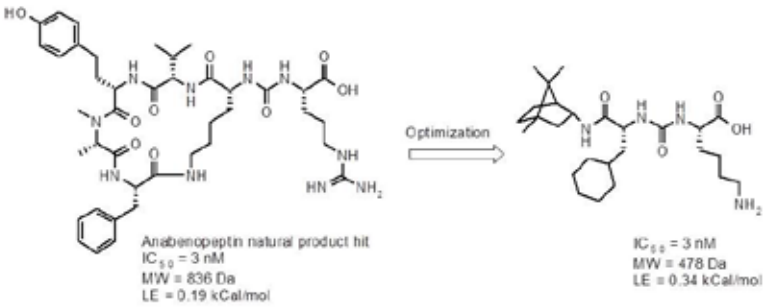

Anabaenopeptins isolated from cyanobacteria were identified as inhibitors of carboxypeptidase TAFIa. Co-crystal structures of these macrocyclic natural product inhibitors in a modified porcine carboxypeptidase B revealed their binding mode and provided the basis for the rational design of small molecule inhibitors with a previously unknown central urea motif. Optimization based on these design concepts allowed for a rapid evaluation of the SAR and delivered potent small molecule inhibitors of TAFIa with a promising overall profile.

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SYNTHESIS OF AN INHIBITOR OF SOLUBLE EPOXIDE HYDROLASE AND ITS BINDING MODE

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The enzyme soluble epoxide hydrolase (sEH) is involved in the metabolic cascade of arachidonic acid and converts epoxyeicosatrienoic acids (EETs) into dihydroxyeicosatrienoic acids (DHETs).[1] EETs exhibit cardioprotective properties but they are short-lived due to sEH activity.[2] To avoid the metabolism of the EETs an inhibition of sEH could be useful.[3] Talinolol, an approved drug, inhibits the hydrophobic active site of the hydrolase with an IC_{50} value of 3 µM. To improve the potency we focused on the design of a derivative of Talinolol with a slightly more hydrophobic character than Talinolol. Furthermore, to get more information about the binding mode we co-crystallized the protein in complex with this new inhibitor. Here we present a crystal structure which shows the binding mode of this new potent inhibitor.

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NEPRILYSIN INHIBITORS FOR THE TREATMENT OF CARDIOVASCULAR DISEASES: STRUCTURE KINETIC RELATIONSHIPS AND PRODRUG DESIGN

Anne-Marie Beausoleil, David L. Bourdet, Kevin DeMent, Erik Fenster, Melissa Fleury, L.G. Hegde, Sharath S. Hegde, Craig M. Hill, Uwe Klein, Donavon McConn, Alexander McNamara, William Martin, Donna A. A. Wilton, Yayan Zhou, Adam D. Hughes

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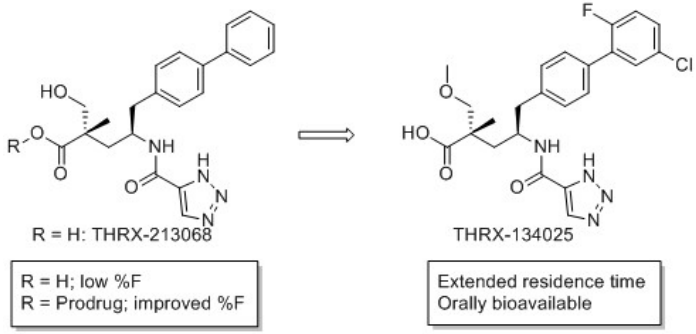
Neprilysin (NEP), a membrane bound endopeptidase, is the principal enzyme responsible for hydrolysis and inactivation of the natriuretic peptides ANP, BNP and CNP. Inhibitors of neprilysin have been targeted since the 1980s, but have been ineffective in the clinic as monotherapies due to concomitant increases in levels of the vasoconstrictor (and neprilysin substrate) angiotensin 2. A dual angiotensin converting enzyme (ACE)/NEP inhibitor, omapatrilat demonstrated greater blood pressure reductions than enalapril, an ACE inhibitor, in hypertensive patients, but had a high incidence of angioedema.[1] Our program sought to design a best-in-class NEP inhibitor (NEPi) and combine it with an angiotensin receptor blocker (ARB) which would avoid augmented bradykinin levels that have since been linked to the angioedema adverse effect. This approach has recently been validated by LCZ696 in both hypertensive and heart failure patients.

This presentation describes our early discovery efforts, using structure based design to guide and build structure activity relationships. We targeted high oral bioavailability and explored several prodrug strategies to improve the permeability and systemic exposure of a parent NEPi, THRX-213068. Structure kinetic relationships were established resulting in the identification of THRX-134025, which exhibits an extended enzyme residence time and attractive pharmacokinetic and pharmacodynamic properties.



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DESIGNED MULTIPLE LIGANDS - NOVEL ANTITHROMBOTIC COMPOUNDS WITH DUAL ACTIVITY TARGETING GPIIb/IIIa RECEPTOR AND THROMBIN

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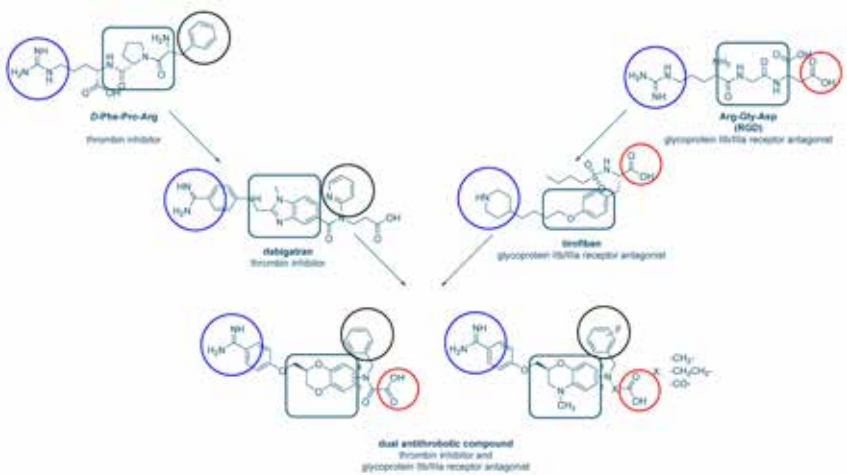
The development of effective and patient friendly antithrombotic agents, new anticoagulants as well antiplatelet drugs remains a permanent challenge to medicinal chemists. The rational design of compounds with designed multiple mode of action towards multiple targets is a widely used approach in drug 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. RGD tripeptide 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.



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DESIGN AND SYNTHESIS OF REGENERATIVE COMPOUNDS

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Ischemic heart disease, stroke, traumatic brain injury and degenerative brain diseases, such as Parkinson's disease, all lead to irreversible cell loss and are today major global causes of death and characterized by significant unmet medical needs. Vision of the 3iRegeneration project is to create new cardiac and neural cells locally in the heart and brain in order to treat associated diseases. This could be accomplished by the induction of cell proliferation; differentiation of stem cells; or cellular reprogramming, through the use of novel drugs.

Basis for adult heart and brain regeneration lies on processes and regulatory mechanisms involved in their growth and development.[1,2] Pathways active in embryogenesis can be "reawakened" by using gene therapy or small molecules.[1] Advantages of using small molecules over gene therapy are: often reversible binding, cell permeability, non-immunogenicity, cost-efficiency. In addition, small molecular compounds are more easily synthesized, preserved and standardized than the biomolecules used in gene therapy.[3] Novel compounds are synthesized based on biological and computational studies.

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SYNTHESIS AND CHARACTERIZATION OF THE AUTOREGULATORY DOMAIN OF PFKFB3

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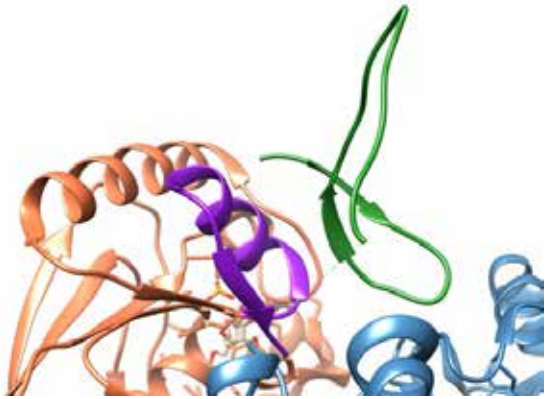
Artery and cerebrovascular diseases are the two most common causes of illness and death worldwide and their primary cause is atherosclerosis, which is characterized by the thickening of the arterial wall. Although beneficial, recent therapeutic strategies have limited efficacy[1]. Current research has shown that targeting misregulated endothelial cell (EC) metabolism could be a new therapeutic strategy[2].

6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) enzyme plays a crucial role in the regulation of the EC glycolytic flux and it is up-regulated during angiogenesis[3], thus representing an innovative target for atherosclerosis therapy. PFKFB3 is a dimeric bifunctional enzyme and possesses a very high kinase to phosphatase activity ratio. Its activity is controlled by the N-terminus autoregulatory domain (AD) in the kinase region. In the crystal structure this domain adopts a β-hairpin structure[4] (Figure 1: the autoregulatory domain is shown in green).



Here we present the chemical synthesis of the wild-type AD sequence as well as mutated ones and their characterization in solution by circular dichroism and DLS.

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TUNING PARTIAL FARNESOID X RECEPTOR (FXR) AGONISM TO AVOID SIDE EFFECTS OF FULL FXR ACTIVATION

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The nuclear farnesoid X receptor (FXR, NR1H4) acts as intracellular bile acid sensor and controls the expression of numerous genes involved in bile acid, lipid and glucose homeostasis[1-4]. Furthermore, current results indicate a promising role of FXR in treating inflammatory disorders such as multiple sclerosis[5]. FXR has therefore gained significant attention as novel drug target for the treatment of metabolic disorders, i.e. steatosis, diabetes mellitus and adipositas. Notably, recent data indicates that FXR is crucial for the beneficial metabolic effects of bariatric surgery[4,6]. The clinical development of obeticholic acid (OCA) has reported very promising effects of pharmacological FXR activation and has validated FXR as future drug target in human[7-8]. However, OCA has also revealed unfavourable effects on cholesterol metabolism that might be due to full FXR activation which inhibits bile acid synthesis from cholesterol via CYP7A1 and thereby blocks the major route of metabolic cholesterol elimination. Partial FXR agonism might therefore be a strategy to exploit the very beneficial effects of FXR activation without disrupting cholesterol metabolism. Similarly, partial agonism on the peroxisome proliferator-activated receptor γ (PPARγ) exhibited anti-diabetic effects without causing weight gain known as common side effect of full PPARγ agonists[9].

To evaluate partial FXR activation as therapeutic concept we have conducted a sequential virtual and in vitro screening where we identified an acylanthranilic amide derivative as promising hit with two-digit micromolar potency in partial FXR activation. In systematic structure-activity relationship (SAR) studies, this screening hit was optimized to highly potent partial FXR agonists with low nanomolar EC_{50} values. We also focused on the compounds' activity on related off-targets and identified determinants to preserve selectivity over cognate nuclear receptors (PPARs, LXRs and RXRs) as well as the membrane bile acid receptor TGR5. On the other hand, intensive SAR studies also revealed the opportunity to specifically design dual nuclear receptor modulators that might exhibit synergistic efficacy in certain metabolic disorders. Finally, aqueous solubility of the optimized agents could be maintained in a favourable range by identification of specific positions where polar residues were tolerated[10-13].

Intensive in vitro characterization of the most potent partial FXR agonists revealed low nanomolar potency and partial FXR activation of around 40% compared to the natural FXR agonist chenodeoxycholic acid. The agents displayed favourable toxicity profiles and exhibited partial induction of FXR target genes in various cell lines in a concentration independent manner. This pharmacodynamic profile might offer a valuable strategy to exploit beneficial effects of FXR activation without blockade of cholesterol metabolism. Pilot in vivo data indicated a favourable pharmacokinetic profile with effective plasma concentrations over 4 hours after a single oral dose of 10 mg/kg. In vivo evaluations also confirmed partial FXR agonistic activity with moderate induction of important FXR target genes and only a moderate repression of CYP7A1 in mice. Based on the promising in vitro and in vivo data, further development of partial FXR agonists as well as further exploration of partial FXR activation as therapeutic concept is warranted[10-13].

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13) unpublished data

HYBRIDS OF CINNAMIC ACIDS TARGETING COAGULATION AND CANCER.

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Hybrid molecules, which are taken as a combination of two different active pharmacophores, have shown enhanced biological activity and can be applied with better biological response in pleiotropic pathological conditions. Recently, the combination of appropriate pharmacophores into one compound has been developed to find out promising drug candidates. A hybrid may overcome the pharmacokinetic problems in the pharmaceutical field as well as to be potential therapeutic agent in newly appearing diseases and multiple pathogenic factors and drug resistant organisms.

It is widely known that cinnamic acid derivatives are interesting from the point of biological activities and present particular synthetic interest. Substituted cinnamic acid hybrids possess a wide range of biological activities, such as antimicrobial [1], anti-inflammatory [2], antioxidant [3], antimutagenic [4] and anti-HIV [5]. Besides, it also displays potential antitumor activities.

Bearing all these in mind and continuing our effort in the design of cinnamic acid hybrids [7] an attempt has been made to utilize the *α,β*-unsaturated acid scaffold and well known adrenergic drugs (e.g. propranolol, atenolol), in the design of new chemical entities more medically effective than their individual components and capable of interacting simultaneously with multiple targets implicated in inflammation, as pleiotropic agents.

Inflammation is a complex process which involves extensive changes at a cellular and molecular level and it is the physiological response of the organism in several tissues damage. It is involved in the pathogenesis and pathophysiology of many chronic diseases such as cardiovascular diseases, cancer via the formation of free radicals.

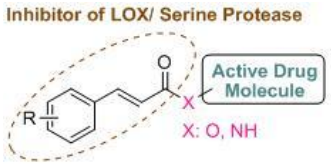

Fig. 1 Design of hybrids of cinnamic acids

For the synthesis of the novel hybrid compounds we applied known synthetic procedure 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. of lipoxygenase as well as of trypsin and thrombin. Finally, an attempt is made to correlate the biological results with principle physiochemical properties.

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THE MOLYBDOENZYME MARC DETOXIFIES TRIMETHYLAMINE N-OXIDE, A RISK FACTOR FOR CARDIOVASCULAR DISEASE

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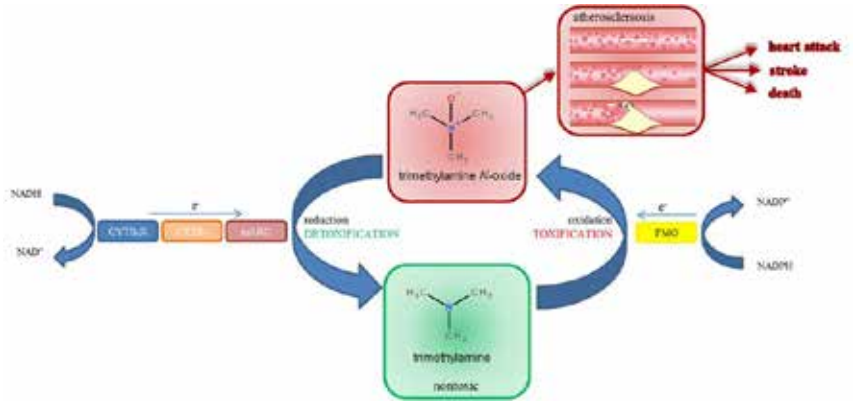
Cardiovascular disease (CVD) is the leading cause of morbidity worldwide. Therefore, it is of the utmost importance to learn more about its genesis. Within this context, trimethylamine *N*-oxide (TMAO), the physiological metabolite of dietary phosphatidylcholine, is in the center of interest. As the metabolic profile of TMAO in plasma correlates with the risk for CVD due to a pro-atherosclerotic mechanism [1], any process that leads to decreased TMAO plasma levels could possibly reduce the risk for CVD. TMAO is an oxidation product of hepatic flavin monooxygenase (FMO), thus its reduction to the precursor trimethylamine (TMA) is an obvious option to diminish its plasma concentration. So far, no enzyme catalyzing this reaction was identified.

The recently in our lab discovered mitochondrial amidoxime reducing component mARC, the fourth molybdenum containing enzyme in mammals [2], could potentially perform this reduction. Along with the heme-containing cytochrome b5 (CYB_5) and its flavin-containing cytochrome b5 reductase (CYB_5R), mARC forms an *N*-reductive enzyme system which is able to reduce a variety of *N*-hydroxylated compounds. The human genome encodes for two mARC proteins, hmARC1 and hmARC2. So far, the physiological role of mARC remains unknown.[2-4]

Biotransformation assays including the reconstituted recombinant *N*-reductive enzyme system and TMAO as substrate can easily reveal if a reduction to the metabolite TMA is of significance. The volatile character as well as the lack of a chromophore of TMA poses particular challenges for this task. Hence, we developed an LC/MS/MS-method involving a dedicated and reliable sample preparation comprising the derivatization of TMA to a non-volatile compound by quaternization of the amine.

With this newly developed analytical tool we investigated the *in vitro* formation of TMA through the reconstituted *N*-reductive enzyme system. We found that hmARC1 but not hmARC2 reduces TMAO. Moreover, we show that murine liver homogenates of wild type mice and mARC2(-/-) knock-out mice reduce TMAO without significant difference in specific activity. These data suggest that only one mARC isoform participates in the detoxification of TMAO. Our results prove that mARC reduces TMAO, thus represents the counterpart to FMO and plays a role in the prevention of CVD. Furthermore, these findings also propose a physiological function of the molybdoenzyme mARC.



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NOTES

POSTERS

Neglected Diseases

EXPLORING 7-NITROQUINOXALINE-2-ONE DERIVATIVES AS SUBSTRATES OF TYPE-I NITROREDUCTASES

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Oxygen-insensitive type-I nitroreductases (NTRs) have recently been disclosed as the responsible for activation of Nifurtimox (Nfx) and Benznidazole (Bnz),[1] classical nitro-substituted prodrugs used in treatments for different types of trypanosomiasis, including human African trypanosomiasis (sleeping sickness) and American trypanosomiasis (Chagas' disease), both, parasitic diseases caused by the protozoan parasites *T. brucei* and *T. cruzi*, respectively. Early screenings have shown that quinoxalines bearing nitro group (nitroquinoxaqlines, NI) are able to decrease parasite viability in *T. cruzi* cultivations, while on the other hand, activity of NI against *T. brucei* has not been explored yet. To date, first approaches suggest the involvement of putative type-II (oxygen-sensitive) NTRs in activation of NI as it follows from the increment in ROS production coupled to the decrease in parasite viability.[2] However, current reports contradict the early evidence, relegating to the background a putative type-II NTR-mediated ROS-based mechanism.[3] Herein, we will study a series of 1,4-disubstituted-7-NI-2-one derivatives as growth inhibitors of *T. cruzi* and *T. brucei*. In order to gain new insights into the action mechanism, kinetic studies on recombinant *T. brucei* and *T. cruzi* type-I NTRs will be conducted. Additionally, to confirm/reject the involvement of a type-I NTR in 7-NI activation, additional drug assays in *T. brucei* cultivations overexpressing type-I NTRs (by means of a *T. brucei* BSF derivative [2T1] engineered to express the tetracycline repressor protein) will be conducted for the best derivatives in order to reveal the actual role of a type-I NTR in 7-NI activation and cell toxicity.

ACKNOWLEDGEMENTS

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DISCOVERY, STRUCTURAL EXPLORATION AND BIOLOGICAL PROFILING OF A NOVEL CLASS OF ANTIMYCOBACTERIAL DPRE1 INHIBITORS

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Tuberculosis (TB) remains a global health threat, accounting for more than 9 million new cases per year and 1,5 million deaths.[1] The emergence of multi- and extensively-drug resistant *Mycobacterium tuberculosis* (Mtb) strains as well as HIV comorbidity fuel the TB epidemic resurgence. A pressing need for the discovery and development of new antitubercular agents that target new biochemical pathways and treat drug resistant forms of the disease is undeniable.

The presented work was performed in the frame of the OpenMedChem EID-ITN project (FP7) between the University of Antwerp and GlaxoSmithKline (GSK) with focus on early hit-to-lead anti-tubercular drug development. Here, we report a novel chemical series of antimycobacterials, discovered during a compound collection screening at GSK. Compounds in the series act through non-covalent inhibition of the essential and vulnerable flavo-enzyme deca-prenylphosphoryl-beta-D-ribose 2-epimerase (DprE1).

We present Structure-Activity-Relationship (SAR) data based on a set of analogues around the initial hit. Evaluation data for the obtained compounds include whole cell MIC-values for the Mtb strain H37Rv and inhibitory potencies on *Mtb*DprE1. Moreover, physicochemical profile, cytotoxicity (HepG2) and cardiotoxicity (hERG) are reported for these molecules. Activity against DprE1 was validated by the observed increase in MIC after testing the hit against an overexpressor strain. Overall, this novel series of DprE1 inhibitors contains highly active inhibitors with very good cellular potencies and balanced physicochemical profiles. Although no cytotoxic effects were found so far, appreciable hERG affinity is present for some analogues. Finally, the primary hit was tested against a panel of Gram-negative and Gram-positive pathogens indicating very selective antimycobacterial properties.

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FLUORINE WALK: THE ROLE OF FLUORINE IN QUINOLONE AMIDES ACTIVE AGAINST T.B.BRUCEI

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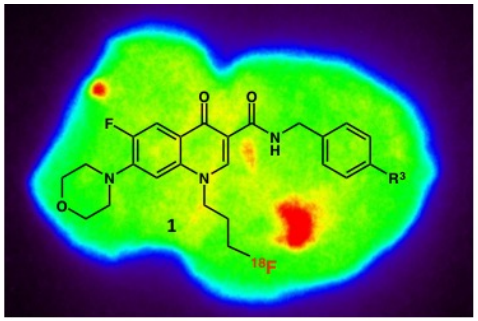
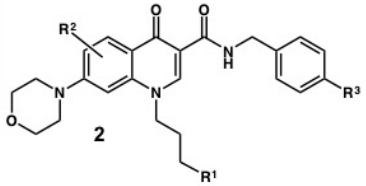
Human African Trypanosomiasis (HAT) is caused by an infection with *Trypanosoma brucei*, a vector-borne parasite, which is transmitted by the bite of infected tsetse flies. Two clinically relevant stages can be differentiated, i.e. stage I which is characterized by unspecific headache, fever and joint pains, and stage II in which the parasites cross the blood brain barrier (BBB) and affect the central nervous system.[1]

Previous investigations identified novel 4-quinolone-3-carboxamides as a promising scaffold having a submicromolar activity[2] and a confirmed *in vivo* efficacy against *T. b. brucei*.[3] As the ability of the quinolone amides to pass the BBB should be investigated, ^{18}F-labeled derivative of our most potent substance was synthesized and subjected to autoradiography studies applying positron emission tomography (PET). Experiments using murine brain confirmed the ability of the respective derivative **1** to pass the BBB 60 min after p.i. application.

Besides utilizing fluorine for PET, we explored its impact on toxicity, pharmacokinetic and pharmacodynamics properties when being added to the molecular scaffold **2**. A small library of differently substituted compounds was established. Antitrypanosomal testing designate them as promising drug candidates for the treatment of HAT.



in vitro PET autoradiography of murine brain



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ORALLY BIOAVAILABLE ANTIMALARIAL 4(1H)-QUINOLONE PRODRUGS WITH SINGLE-DOSE CURES

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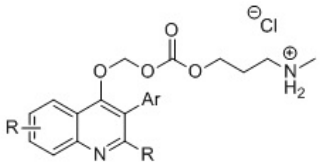
Malaria is estimated to have caused 438,000 deaths and 214 million cases of the disease globally in 2015. Four strains of *Plasmodium* parasite cause malaria in humans and the disease is transferred by *Anopheles* mosquitos. Though mortality rates are down 47% globally since 2000 and significant progress has been made in the quest for eradication, reported occurrences of resistance against current therapeutics threaten to reverse that progress. Longstanding treatment chloroquine has seen resistance since the 1950's, with resistance becoming widespread in the 70's and 80's. Artemisinin, the current main line of defense against malaria, is used in artemisinin combination therapies (ACTs) in order to curtail resistance, though at last count, artemisinin resistant parasites have been reported in 5 countries of the Greater Mekong sub region. In order to curb further resistance, it is essential that new antimalarial compounds be brought through the pipeline.

For approximately half a century, 4(1*H*)-quinolones such as endochin or ICI 56,780 were known to be causal prophylactic and potent erythrocytic stage agents in avian but not in mammalian malaria models. Hit-to-lead optimization of endochin lead to 4(1*H*)-quinolones ELQ-300 and P4Q-391, which target the liver, the blood as well as the transmitting stages of the parasite. Despite entering preclinical development, ELQ-300 did not enter phase I trials due to limited aqueous solubility and high crystallinity.[1]

To overcome these limitations, we designed and developed a prodrug approach containing an amino group linked to the parent 4(1*H*)-quinolone by an acetal carbonate group. Different reaction conditions were found to attach the prodrug moiety selectively onto the oxygen or the nitrogen of the 4(1*H*)-quinolone scaffold. The resulting O-alkylated prodrugs P4Q-1290 and P4Q-1291 were profiled for physicochemical properties such as chemical stability and aqueous solubility. The prodrugs are stable at low pHs and start releasing the parent drug independently of any enzyme activity at a pH level of about 7. Furthermore, prodrugs P4Q-1290 and P4Q-1291 were highly efficacious in *in vivo* efficacy assays displaying single-dose cures at low doses.



general structure of 4(1*H*)-quinolone prodrugs

The new discoveries are significant as mitochondrial inhibitors have the potential to advance the malaria elimination campaign by blocking parasite development in the blood and liver, as well as preventing transmission to mosquitoes.

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DEVELOPMENT OF NEW QUINONE DERIVATIVES AGAINST LEISHMANIA

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From a human health perspective, leishmaniasis is the second protozoan disease in importance, only superseded by malaria. The disease encompasses the wide range of clinical pathologies produced by the infection with different protozoan species of the genus *Leishmania*. Globally, the disease accounts for 10-12 million people infected worldwide, with an incidence of 1.5 million new cases per year. Despite the considerable advances carried out in the last years, nowadays none human vaccine is currently available, and the efforts to curtail dissemination by vector and reservoir control are far less than satisfactory. This leaves chemotherapy as the sole method to combat efficiently the disease. The chemotherapeutic arsenal is quite limited and its efficacy is increasingly eroded by growing resistance, aside from the severe side effects associated to many of them. Furthermore, the high cost for their implementation is unaffordable for the bulk of the patients, belonging to low-income countries. Thus, development of new drugs is urgently required.[1]

In this work the search of new drugs for leishmaniasis was based on a phenotypic-based approach using as source of new hits our in-house chemical library. A new class of quinone derivatives has emerged as potential hits for this disease and a medicinal chemistry optimization program is ongoing. Furthermore, initial results on their mechanism of action supported the importance of the bioenergetic collapse of the parasite induced by the quinones, with a rapid drop of intracellular ATP levels in the parasites and decrease of the respiration rate in *Leishmania donovani* promastigotes. Its role in the lethal mechanism of these quinones and SAR studies with a limited set of compounds will be discussed.

Acknowledgements: This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO, project no. SAF2015-65740), Redes de Investigación Cooperativa Instituto de Salud Carlos III RICET (RD12/0018/0007) and FEDER funds.

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DRUG REPURPOSING OF HUMAN KINASE INHIBITORS AS NEW HITS AGAINST LEISHMANIA

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Treatment of leishmaniasis, the second human protozoan disease in importance, relies almost exclusively on chemotherapy, on its turn reduced to a scarce number of drugs. Their efficacy is threatened by rising resistance, and pipeline for new leads is scarcely populated. Due to the low economical level of the bulk of affected population, investment for development of new drugs received a poor investment. Drug repurposing resulted as a fast and low cost approach to add new drugs into leishmaniasis treatment, in fact most of drugs in current use against this disease were formerly developed for other applications.[1]

The inhibition of protein kinases of *Leishmania* constitutes an appealing approach to tackle infections by this parasite, nowadays under a shortage of new leads, and decreasing efficacy due to the rise of resistance. Globally, protein kinases constitute a substantial target of the drug discovery efforts, accounting for nearly a third of the “druggable genome”. In fact, the inhibition of specific protein kinases by small molecules inhibitors has been pharmacologically validated for the treatment of a wide range of therapeutic indications. This fact, together with the growing number of parasite kinases validated as targets for parasitic disease allowed us to surmise that protein kinases are druggable targets for *Leishmania*.[2]

Based on our previous experience on human GSK-3 and CK1 inhibitors, we have focused our attention on these two kinases also present in *Leishmania* (GSK3 and CK1.2) as targets for the development of antileishmania drugs.

A number of specific human kinase inhibitors with different chemical structures have been tested in a phenotypic assay at low micromolar concentrations, some of them with an adequate specificity index. Their ability to inhibit *Leishmania* kinases will be further elucidated by experimental and molecular modelling techniques.

Acknowledgements: This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO, project no. SAF2015-65740), Redes de Investigación Cooperativa Instituto de Salud Carlos III RICET (RD12/0018/0007) and FEDER funds. P. M. acknowledges the contract from the Fondo de Garantía Juvenil (European Social Fund, Youth Employement Initiative) and FEDER funds.

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SEARCHING FOR NOVEL DRUGS TO TREAT SCHISTOSOMIASIS: PHENOTYPIC AND TARGET-BASED APPROACHES

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Schistosomiasis is a parasitic disease caused by the blood trematode of the genus *Schistosoma*. It is a serious health problem, endemic in 70 countries of the tropics and subtropics, with at least 230 million people in need for treatment per year. An effective vaccine is lacking and all emphasis is placed on one drug ”praziquantel” with no new therapeutic options, despite the fear of possible upcoming resistance.

In this work, the search for new drugs to treat schistosomiasis was based on both target- and phenotypic-based approaches. The target-based approach was focused on phosphodiesterases (PDEs) as potential new and innovative targets. Based on the published genome of the worm[1] and the potential PDE genes, a sequence analysis with different human and parasite PDEs has been done. In order to develop the most accurate model for *Sm*PDE and anticipating that *Sm*PDEs have a characteristic parasite-pocket that is missing in human PDEs, four homology models were developed using as template pdb X-ray structures from *Leishmania major*, *Trypanosoma brucei*, *T. cruzi* and human. Following a comparative analysis of the four models, the model performed using *Lmj*PDEB1 (2RQ8) as template was chosen as the best to perform a virtual screening using our in-house chemical library with the aim to discover new hits.

The second approach was a phenotypic screening against *S. mansoni* using as source of new hits our library selecting PDE inhibitors and analogues thereof. Those compounds that do not show toxicity to human cells were selected for the phenotypic screening.

Acknowledgement: This work was supported by the European Commission seventh Framework Programme FP7-HEALTH-2013-INNOVATION-1, PDE4NPD (no. 602666).

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DISCOVERY AND DEVELOPMENT OF PYRAZOLOPYRIDINES AND INDAZOLES TARGETING WOLBACHIA SYMBIONT OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS

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Lymphatic filariasis (LF) and onchocerciasis are parasitic diseases caused by filarial nematodes which are widespread in tropical regions affecting more than 150 million people. The major disease-causing species include *Wuchereria bancrofti* and *Brugia malayi* responsible for LF and *Onchocerca volvulus* for onchiocerciasis. Current treatments including diethylcarbamazine, albendazole, and ivermectin are principally effective against microfilariae (mf, juvenile worm) and so require prolonged delivery in order to break the transmission cycle of the long-lived adult worms (5-8 yrs for *W. bancroftil B. malayi*).

The Anti-*Wolbachia* Consortium (A•WOL) aims to provide a novel chemotherapy with macrofilarial activity by targeting at the essential endosymbiotic bacteria, *Wolbachia*, which are proven to be vital for nematode's survival and fertility. As exemplified by previous work on antibiotic doxycycline, anti-*Wolbachia* thepary delivers safe and substantial macrofilaricidal activity with superior therapeutic outcomes compared to all standard anti-filarial drug (SAFD). In addition, this treatment has been shown to improve clinical pathology. Our primary goal is to find drugs and regimens that reduce the period of treatment from weeks to days and are safe for vulnerable populations (pregnance and children).

In our on-going A•WOL drug discovery programme, a cell-based assay screen of compounds from MMV library reveals hits that show significant activity against *Wolbachia*. One of the series is identified as pyrazolopyridine analogues. Retesting of these hits confirmed their *in vitro* EC_{50}s ranging from 48 nM to 1.2 µM. The chemical synthesis of a library has been developed using reliable and robust chemistry for most target compounds. Hit-to-lead optimisation led to the identification of several lead compounds with an *in vitro* EC_{50} of less than 50 nM and an improved DMPK profile. *In vivo* pharmacokinetics studies were conducted in mouse models for selected leads and they showed only modest oral exposure and half-life. Further lead development and *in vivo* efficacy are still ongoing and will be presented elsewhere.

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NEW CO-CRYSTAL STRUCTURES OF INHIBITORS OF TRYPANOSOMA BRUCEI TRYPANOTHIONE REDUCTASE

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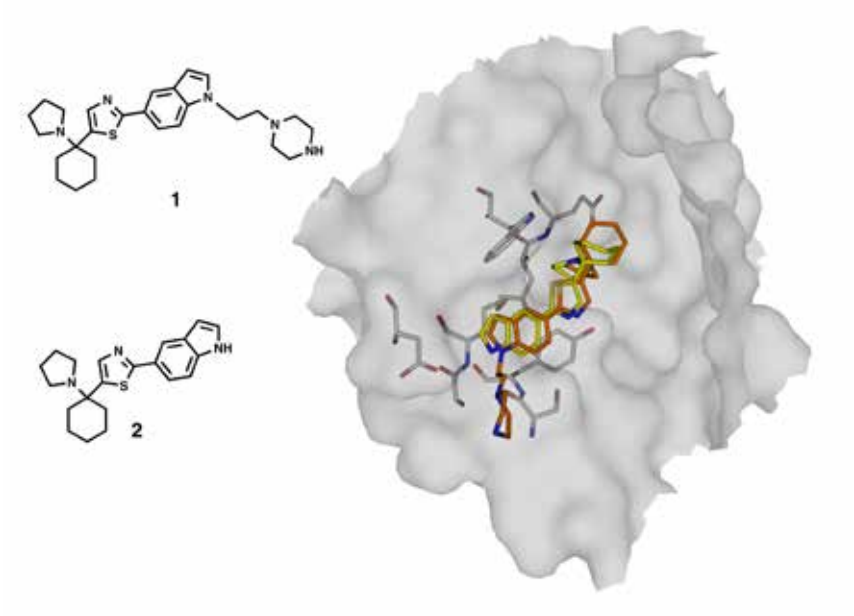
In 2014, we reported new trypanothione reductase inhibitor analogues of BTCP (1-[1-(benzo[*b*]thien-2-yl) cyclohexyl]piperidine), which was reported to be a low-molecular-weight inhibitor of trypanothione reductase by Fairlamb and co-workers.[1][2] Inhibition of the enzyme trypanothione reductase has proved to be effective against the parasite *Trypanosoma brucei*, the causative agent of african sleeping sickness. We were recently able to obtain co-crystal structures of inhibitor **1** with *Trypanosoma brucei* trypanothione reductase. Compound **1** is an extended version of our previously reported inhibitor **2** and shows a similar binding mode.[2]



The extended inhibitor **1** shows a three-fold increase in binding affinity with respect to parent compound **2** with an inhibition constant of K_{ic} = 3.9 ± 0.3 μM.

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DESIGN OF NOVEL TbrPDEB1/B2 INHIBITORS FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) is a fatal neglected tropical disease that is caused by the parasitic protozoan *Trypanosoma brucei* (*T.b.*) [1]. In the last 30 years, no new drug against HAT passed the clinical stage and current treatments suffer from lack of efficacy, complicated treatment regimes and/or severe side effects [2].

Trypanosoma phosphodiesterase B1 and B2 (TbrPDEB1/B2) have both been genetically and pharmacologically validated as drug targets [3-5]. This was further validated by studies in our lab, as inhibitors have been developed that block parasite proliferation at nanomolar concentrations. Being part of an EU-sponsored consortium (PDE4NPD) [6], our lab is currently involved in the structure-based optimization of TbrPDEB1/B2 inhibitors towards novel potential HAT treatments. Here, we will present the disovery of novel inhibitors with more than 10-fold selectivity over human PDE4, for which inhibition is associated with undesired side-effects such as nausea and emesis [7].

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QUINAZOLINDIONE SERIES IDENTIFIED FROM TCAMS: A NEW ANTIMALARIAL SERIES WITH POTENTIAL FOR BLOCKING TRANSMISSION OF THE DISEASE

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Nowadays, Malaria is still one of the major global health problems. *Plasmodium* has been able to adapt to the different treatments developed by humans along history. However there has not been such a wide knowledge of the illness as we currently have. This fact, joined to the urgent need for novel antimalarial drugs that can replace ACTs and the awareness of governments/health systems/funding agencies, makes the current time a unique opportunity to change the course of this disease and achieve the control and finally the eradication.

Using a Phenotypic screening approach, in 2010 GSK published the Tres Cantos antimalarial set (TCAMS) which comprises over 13,533 hits derived from whole cell screening of 2M compounds from the GSK corporate collection against *Plasmodium falciparum*.[1] A clear strategy was required to rapidly identify those molecules that have both the best chance of being converted into differentiated antimalarial drugs and that are also likely to have the lowest risk of attrition in development. Identification of a new class of anti-malarial agents that possess dual activity and are able to inhibit the asexual blood-stage (schizonticidal, responsible of disease symptoms) as well as block transmission was initiated in our group. As a result, a new assay to screen compounds for their potential to inhibit late stage gametocytes was developed and used successfully to screen the output from TCAMS.[2]

Quinazolindione series was identified as a very promising family with dual activity (both schizonticidal and gametocytocidal). Initial weaknesses of the series were modest *in vitro* and *in vivo* potency as well as poor pharmacokinetic profile. After a Lead Optimisation program, Late Leads have been identified having excellent *in vitro* and *in vivo* potency, a very good developability profile and potential for targeting two different TCPs (Target Compound Profiles). Medicinal Chemistry strategy followed during the Lead Optimisation program was focused on improving the physicochemical and developability properties. A detailed description will be provided in this communication.

"All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents"

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COLLABORATIVE STRUCTURE GUIDED DRUG DISCOVERY FOR MALARIA

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In this poster we present initial results of a project supported by an international consortium called the Structure-guided Drug Discovery Coalition (SDDC). The SDDC primarily supports structure-guided medicinal chemistry, with a focus on diseases of the developing world: tuberculosis, malaria, cryptosporidiosis and filarial infections. Our aim is to develop compounds to "early lead" status, with Proof-of-Concept in animal models of infection. We achieve this using structure-guided drug discovery on molecular targets for these diseases. The molecular targets selected have to be thoroughly validated; typically this is through association of a phenotypically (whole cell) active compound with the molecular target. These targets are then paired with crystal structures from a number of Structural Genomic Centres, giving rise to structure-guided hit to lead projects. The Drug Discovery Unit (DDU) based at the University of Dundee, is responsible for the medicinal chemistry development of the SDDC projects devoted to finding potential new start points for antimalarial drug discovery. Taking advantage of structural information, we develop chemical series to selectively target Plasmodium falciparum *(Pf)* enzymes compared to Homo sapiens *(Hs)* enzymes, with the aim of identifying compounds which meet Medicines for Malaria Venture (MMV) early Lead criteria for the development of new antimalarials.

DESIGN AND SYNTHESIS OF HETEROARYL-SUBSTITUTED ETHER PHOSPHOLIPIDS POSSESSING ANTIPARASITIC ACTIVITY

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Ether phospholipid derivatives possess a broad pharmacological spectrum including anticancer, antifungal and antiprotozoal activity. Miltefosine (hexadecylphosphocholine) is an alkylphosphocholine with demonstrated activity against various parasite species and is currently the only oral drug available for the treatment of visceral (VL) and cutaneous leishmaniasis (CL), a neglected tropical infection caused by unicellular parasites. Miltefosine is administered as first-line treatment for VL in India (28 day regimen, 2.5 mg/kg/day) and has been adopted in several national VL elimination programmes (e.g. in India, Bangladesh and Nepal). However, at the therapeutically effective doses, severe gastrointestinal side effects and serious weight loss were observed while teratogenicity restricts its use in female patients.

As a continuation of our studies on ring-substituted ether phospholipid derivatives[1-3] we investigated the presence of various heteroaromatic rings in the lipid portion of alkylphosphocholines. Heteroaromatic rings represent privileged scaffolds in drug discovery. They are considered as amide bioisosteres, while the presence of heteroatoms increases the interactions with biological targets. Thus, we introduced 1,2,3-triazolyl, isoxazolyl, 1,2,4-oxadiazolyl and 1,3,4-oxadiazolyl moieties in ether phospholipids and studied the effect on the antiparasitic activity against *T. brucei* (blood stream form) as well as *L. infantum*, *L. donovani and T. cruzi* amastigotes. Furthermore, a wide range of *in vitro* ADME-Tox studies releaved that the resulting derivatives were less toxic than Miltefosine.

Acknowledgement

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 603240 (NMTrypI - New Medicine for Trypanosomatidic Infections). http://www.nmtrypi.eu/ and by COST CM1307 (STSM for Chiara Borsari).

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NOVEL 6-OXOPURINE NUCLEOTIDE ANALOGUES AS HYPOXANTHINE-GUANINE-(XANTHINE) PHOSPHORIBOSYLTRANSFERASE INHIBITORS

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A series of novel acyclic nucleoside phosphonates (ANPs) bearing 6-oxopurine base and their bis-amidate prodrugs has been designed and synthesized as potential inhibitors of plasmodial hypoxanthine-guanine-(xanthine) phosphoribosyltransferase [HG(X)PRT]. HG(X)PRT represents a key enzyme in the purine salvage pathway. Parasites of plasmodium genus are unable to produce purine nucleoside monophosphates *de novo* and depend completely on the salvage pathway, unlike mammals that have both *de novo* and salvage pathways. This fact makes HG(X)PRT a valuable target for development of antimalarial agents. Novel ANPs are branched at the α-position of the acyclic moiety next to the nucleobase (structure **2**, Fig.1). This type of branching represents a new approach to plasmodial HG(X)PRT inhibitors[1,2]. The compounds are structural derivatives of ANPs with PEE (compounds **1**) and PME moieties, that have previously been shown to inhibit HG(X)PRT[3]. The compounds bear various substituents at the secondary R^2 linker in order to increase the binding affinity to the enzymes. These ANPs have highly polar phosphonate group(s) and are unable to cross cell membrane. To overcome this obstacle the corresponding amidate prodrugs **3** have been synthesized[4]. All phosphonates are currently being evaluated *in vitro* as potential inhibitors of plasmodial [HG(X)PRT]. Their prodrugs are being evaluated in *plasmodium* infected human erythrocytes assay.

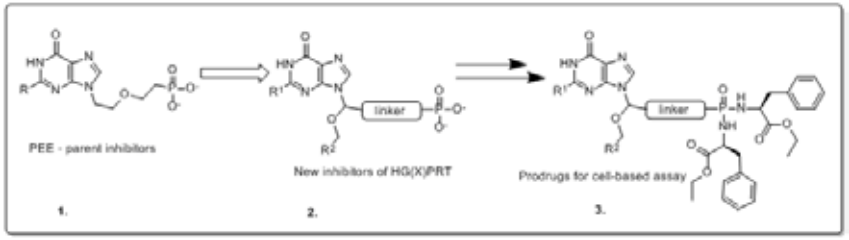

Figure 1. Design of new HG(X)PRT inhibitors

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CHLOROQUINE- AND PRIMAQUINE-QUINOXALINE HYBRIDS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF A NEW CLASS OF ANTIMALARIALS DRUGS

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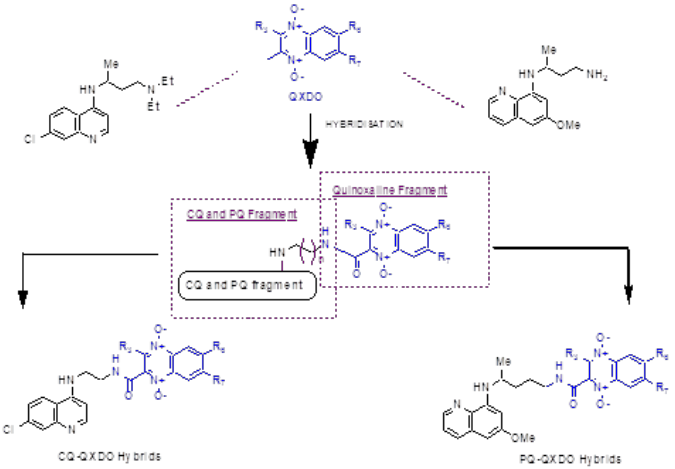
Despite of the last years efforts in the antimalarial drug discovery, malaria is still one the most important devastating parasitic disease caused by *Plasmodium* parasites, among these *Plasmodium falciparum* is the most deadly. According to World Malaria Report 2015, 214 million new cases and 438,000 deaths were reported in 96 endemic countries[1].

To avoid cross-resistances to the existing antimalarial drugs, artemisin-based combination therapies (ACTs) have been the first-line treatment in the last decades. The appearance and spreading of artemisin-resistant parasites in recent years have led to the concept of hybrids molecules as a new combination therapy strategy to tackle this alarming problem[2].

On the basis of this strategy and in continuation with our project for developing new antimalarial drugs, we proposed to combine quinoxaline 1,4-di-*N*-oxide derivatives with known classical antimalarial drugs in one single molecule. Two series of new antimalarial hybrids were designed, synthesized and evaluated against the FCR-3 cloroquine-resistant *Plasmodium falciparum* strain (Fig. 1).



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DESIGN AND SYNTHESIS OF NOVEL TRIAZINE DIMERS AS INHIBITORS OF ANTITRYPANOSOMAL ACTIVITY

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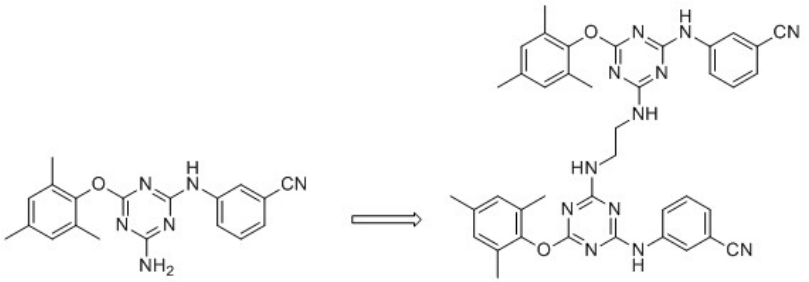
Human African trypanosomiasis (HAT, sleeping sickness) remains one of the most neglected life threatening diseases.[1] Over 60 million people living in 36 sub-Saharan countries are threatened with sleeping sickness and the estimated number of cases is thought to be between 300 000 and 500 000, with approximately 48 000 annual deaths.[2]

Phenotypic screening of triazine non-nucleoside HIV-1 reverse transcriptase inhibitors resulted in potent and selective antitrypanosomal compounds.[3] The importance of dimers in neglected tropical diseases prompted us to investigate antitrypanosomal activity of triazine dimers.[2,4]

We synthesized around 70 compounds and screened them using a phenotypic panel. Optimization of the triazine dimers resulted in compound 66; a compound with nanomolar *in vitro* antitrypanosomal inhibition and non cytotoxic profile.



Monomer

Antitrypanosomal activity
T. b. bruc. (IC_{50}) = 0.21 µM
Cytotoxicity
MRC-5 (IC_{50}) = 25.92 µM

Dimer

Antitrypanosomal activity
T. b. bruc. (IC_{50}) = 0.001 µM
Cytotoxicity
MRC-5 (IC_{50}) > 64 µM

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STRUCTURAL MODIFICATIONS OF NPD-226 TO ACHIEVE AN IN VIVO PROOF OF CONCEPT IN TRYPANOSOMA BRUCEI

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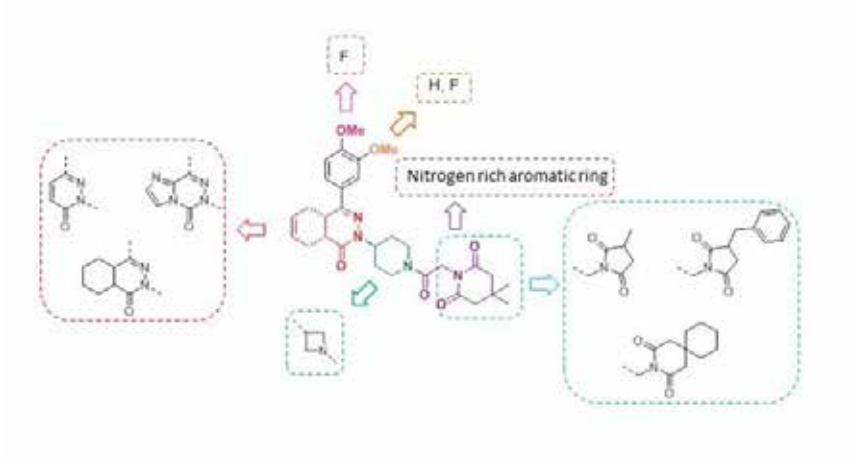
Sleeping sickness or Human African Trypanosomiasis (HAT) caused by the kinetoplastid protozoa *trypanosoma brucei* is a lethal disease. HAT is a neglected disease due to deficient investment comparing to those affecting the developed countries.

Two phosphodiesterases, TbrPDEB1 and TbrPDEB2 were recently validated as targets of HAT. With the aim of finding new phosphodiesterase inhibitors as a novel treatment for parasitic diseases, the European FP7 funded project 'Phosphodiesterases for Neglected Parasitic Diseases' (PDE4NPD)[1] was launched in 2014.

Metabolic stability is one of the challenges that medicinal chemists have to deal with. It is reported that approximately three quarters of the top 200 prescribed drugs in the US in 2002 were cleared by drug metabolism. [2]

As part of the PDE4NPD an *in vivo* proof of concept is being developed. Compound NPD-226 showed a good *in vitro* potency, but a lack of activity was found in its *in vivo* evaluation. Metabolic stability was postulated to be the cause.

Trying to improve this property, the structure was modified, obtaining around 20 compounds, most of them non-cytotoxic with submicromolar IC_{50} values.



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TARGETS IN ORPHAN DISEASES AND THEIR REPURPOSING CANDIDATES - ANALYSIS WITH A KNIME WORKFLOW USING OPEN PHACTS

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Worldwide an estimated number of 400 million people are affected by orphan diseases[1]. An orphan disease is defined as affecting less than 1 in 2000 citizens[2]. Such a low prevalence coupled with the sheer number of orphan diseases, estimated to be about 5000-8000, is the main reason for the small number of marketing approvals, amounting to treatments for roughly 200 conditions in the US and only about 45 in the European Union[1]. Drug repurposing therefore may prove to be the future of drug discovery for orphan diseases because it is an attractive option of reaching many patients with treatments that have already been deemed safe. This work aims at providing an overview of targets linked to orphan diseases as well as relevant compounds, consisting of possible repurposing candidates for these targets as well as experimental compounds as a starting point for drug discovery.

The biggest European platform for orphan diseases is Orphanet[3], with comprehensive information for patients as well as for healthcare professionals, providing also identifiers (UMLS, MeSH, OMIM etc.) for 5345 of its 9235 listed diseases, as well as for associated genes. These identifiers are perfectly suitable for data integration across different databases and thus also for data enrichment with the aim of understanding and visualizing the role of diverse protein classes such as ion channels or transporters in orphan diseases. The Open PHACTS Discovery Platform contains the linked datasets for this kind of data integration, given that the included databases (such as DisGeNET, UniProt, DrugBank, SureChEMBL and ChEMBL) enable the user to go from the disease to its targets and ultimately to approved and experimental compounds connected to these targets. The approved compounds may include candidates for drug repositioning, whereas the experimental compounds may provide a foundation for drug discovery.

The workflow was established by using the KNIME Analytics Platform and starts with extracting data related to orphan diseases from different sources. Primarily it extracts identifiers provided by Orphanet and links them to respective targets through the use of DisGeNET and UniProt, additionally target references provided by Orphanet are also linked to the Open PHACTS Discovery Platform for further data integration, resulting in a combined dataset of specific targets for orphan diseases. The workflow then links this dataset to DrugBank, SureChEMBL and ChEMBL, with the results consisting of approved drugs as well as experimental compounds for the targets involved in orphan diseases.

It is remarkable that solely by using the identifiers from Orphanet the workflow already results in 1269 approved compounds that may be examined for suitable drug repurposing candidates.

Acknowledgements

The work has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement no. [115191], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and in-kind contribution of EFPIA companies. We further wish to acknowledge the Open PHACTS Foundation, the charitable organisation responsible for the Open PHACTS Discovery Platform, without which this work would not have been possible.

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MEMBRANE-BOUND PYROPHOSPHATASES – A NOVEL APPROACH TO TARGET PATHOGENIC PROTOZOAN PARASITES

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Pathogenic protozoan parasites cause diseases like malaria (*Plasmodium* species) and leishmaniasis (*Leishmania* species), which can result in fatal consequences if not treated correctly in time.[1, 2] These diseases have a huge negative impact on human health across the globe, since almost half of the world's population were at risk of malaria and leishmaniasis in 2015. Even though there are medicines on the market, malaria and leishmaniasis are together responsible for close to 500 000 deaths annually due to resistance and side-effects.

An interesting feature of many protozoan parasites is that they have a unique integral membrane protein, a membrane-bound pyrophosphatase (mPPase).[3] These 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.[4] H^+-pumping pyrophosphatases, which are participating in life maintaining functions[5] in various parasites[6], have been found in e.g. *Plasmodium*[7] and *Leishmania*[8] species. Although mPPases can be readily found in many pathogenic protozoan parasites they do not exist in humans, which thereby make them ideal and novel drug targets.[3] 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. A compound library has been built to aid the biological and computational studies that later on will advise us in future synthesis work of more active compounds.

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WORMS, CAMERA, ACTION: DISCOVERING NEW CHEMICAL LEADS FOR THE CONTROL OF WHIPWORM, A NEGLECTED HUMAN PATHOGEN FOR WHICH CURRENT TREATMENTS ARE INADEQUATE

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The parasitic nematode *Trichuris trichiura* (whipworm) affects ~500 million people, resulting in disability and poor child development. The World Health Organisation currently identifies 112 countries in need of preventative chemotherapy, primarily in South and Central America, Asia and Africa (http://apps.who.int/iris/bitstream/10665/44804/1/9789241503129_eng.pdf). Current anthelmintic treatments show poor efficacy with low cure rates, thereby restricting the success of Mass Drug Administration programmes. The limited anthelmintic drug pipeline, the growth of resistance to existing chemicals and the absence of a vaccine means that new drugs are urgently needed.

We have screened a focused library of novel drug-like molecules in order to identify new hit classes of compound which demonstrate activity on the *ex vivo* model mouse parasite *Trichuris muris. T. muris* and *T. trichiura* are similar genetically, morphologically, antigenically and physiologically and provoke similar immune responses in their respective host species. *T. muris* is easily maintained in the laboratory mouse and thus provides us with a highly relevant model for our screening pipeline. The work has involved high-throughput chemical screens using Wormwatcher, a device developed for automated nematode phenotyping. Using this approach we have identified two chemical classes which show exciting levels of activity, and importantly have encouraging profiles in initial cell-based toxicity and host tolerance tests. A programme of medicinal chemistry for hit-to-lead optimisation of both sets of molecule is currently being pursued. The screening process for compound optimisation will utilise both *in vitro* assays on *ex vivo T. muris* worms and *in vivo* assays to demonstrate effective parasite clearance from the host, coupled with a clear safety profile and good host tolerance. Our goals are to develop new chemical leads, new biological tools and to identify new drug targets, all of which are essential in developing new drug candidates for the unmet clinical need of an effective treatment for Trichuriasis.

SEEKING A SINGLE DOSE TREATMENT FOR MALARIA: A NEW STRATEGY TO IDENTIFY LONG ACTING COMPOUNDS

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In recent years a new and ambitious paradigm to defeat malaria has appeared in the field. It seeks molecules able to treat the clinical symptoms and clear the parasite upon a single dose administration.[1] In our laboratories we have applied a "reverse" (non classical) approach (Figure 1) to identify compounds with potential long half life in humans. In this "reverse" approach, compounds will be first filtered by their pharmacokinetic profiles, and then by their activities against *Plasmodium Falciparum*. The outcome of this endeavour will be detailed.

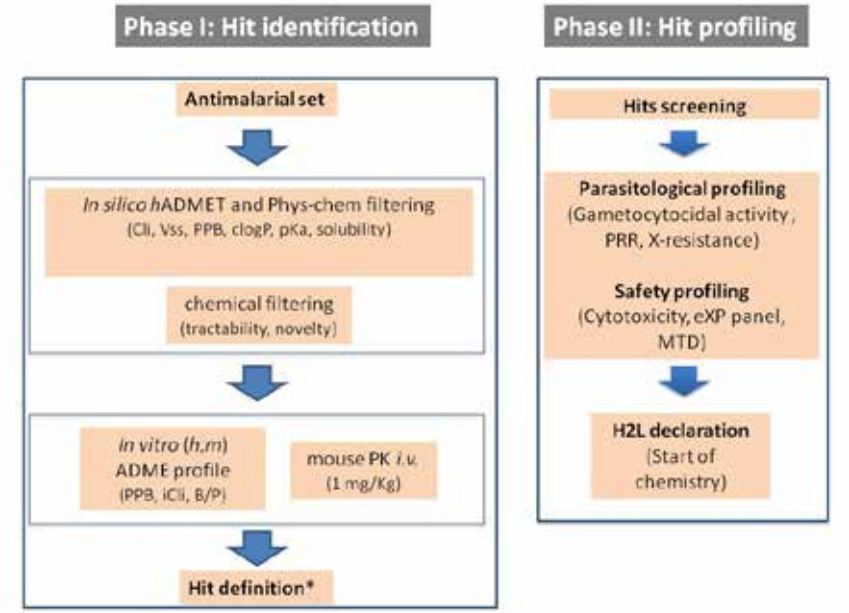

Figure 1. A "reverse" approach for the identification of long acting compounds.

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TARGETING TUBERCULOSIS BY INHIBITING THE Mtb CHOLESTEROL OXIDASE CYP125

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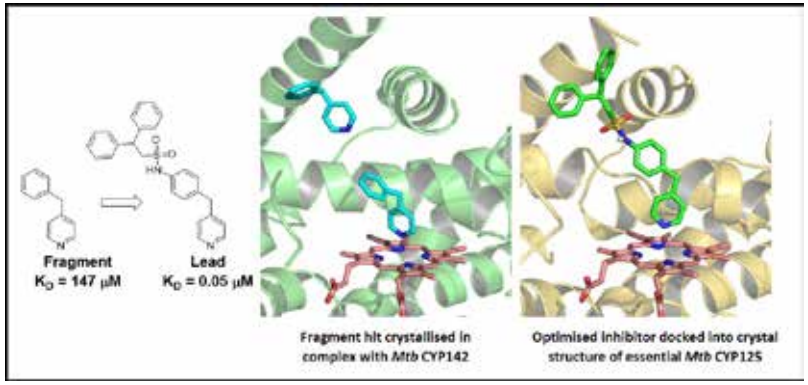
Tuberculosis (TB) is a chronic, infectious disease that is responsible for the deaths of more than 1.5 million people per annum.[1] The emergence of antibiotic resistant strains of *Mycobacterium tuberculosis (Mtb)* and co-morbidity of the disease with HIV/AIDS, means that there is an urgent need for new drugs to treat TB. The family of 20 cytochrome P450 enzymes (P450s) expressed by *Mtb* represent a series of novel drug targets for TB. Several of the P450 isoforms are essential for *Mtb* viability, and others are required for the production of virulence factors, the establishment of host infection, stress response and drug resistance mechanisms.[2,3]

We have been using fragment-based methods in our lab to study the *Mtb* P450s and to develop small-molecule P450 inhibitors that could provide new drug leads for tuberculosis. A range of biophysical techniques, including ITC, NMR, EPR, X-ray crystallography and native mass spectrometry have been used to identify ligands, assess the selectivity and druggability of different *Mtb* P450 isoforms and to guide the optimisation of inhibitors.

Our efforts in this area have recently delivered potent inhibitors of the essential *Mtb* P450s CYP121[4,5] and CYP125.[6,7] CYP125 is a cholesterol oxidase, that catalyses the prerequisite steps in production of energy and carbon precursors that are required for chronic TB infection.[8] Knockdown of the *CYP125* gene prevents *Mtb* from infecting murine macrophages[6] and causes the accumulation of toxic metabolites in the bacteria.[9]

This presentation will detail our use of comparative fragment-screening campaigns against CYP125, and the related isoforms CYP124 and CYP142, to identify a common fragment hit and to elucidate the SARs governing isoform selectivity. Crystallisation of fragment hits with CYP142, a structural proxy of CYP125, was used to guide the synthetic optimisation of fragments. The result was potent inhibitors that have low nanomolar binding affinities for CYP125 (K_D = 50 nM). These compounds effectively inhibit substrate oxidation with IC_{50} values of 1-4 mM and are currently being evaluated in cellular models. Our use of non-azole binding groups and the functional inhibition of CYP125 activity by these compounds, provides them with promise for further development as therapeutic agents for drug-resistant TB.



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DISCOVERY AND OPTIMISATION OF 5-AMINO-1,2,3-TRIAZOLE-4-CARBOXAMIDES; A NOVEL TRYPANOCIDAL SERIES WITH ORAL EFFICACY IN A MOUSE MODEL OF CHAGAS DISEASE

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is endemic to South America and presents itself in two stages, an acute and a chronic stage. Current standard therapeutic treatments suffer from significant side effects and are only proven to work in the acute phase of the infection, highlighting the need for new drugs to treat this disease.

A drug discovery program for Chagas disease carried out as part of a collaboration between the Drug Discovery Unit (DDU) at the University of Dundee and the Diseases of the Developing World team in Tres Cantos, GSK, has allowed the development of a screening cascade that has led to the identification of a novel 5-amino-1,2,3-triazole-4-carboxamides (ATC) hit series. Optimisation of the ATC series, gave improvements in potency, aqueous solubility and microsomal stability, which combined to give significant improvements in oral exposure. Mitigation of a potential Ames liability ultimately lead to a compound which demonstrated oral efficacy in an acute bioluminescent murine model of Chagas comparable to the standard therapeutic benznidazole.

SAR DEVELOPMENT OF IMIDAZOPYRIDINES AS POTENT AND SELECTIVE INHIBITORS OF PLASMODIUM FALCIPARUM PROTEIN KINASE G (PfPKG)

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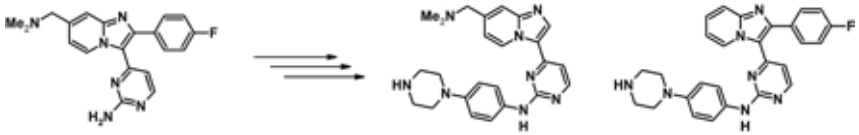
Malaria is one of the most prevalent infectious diseases of the developing world, whose primary causative agent in humans is the protozoan parasite *Plasmodium falciparum*. It is currently responsible for almost 0.5 million deaths per year, with both young children and pregnant women in sub-Saharan Africa particularly at risk.[1] There is significant concern about widespread and rapidly growing resistance to current standard malaria drugs; hence the development of structurally and mechanistically novel malaria treatments is urgently required to maintain control and advance eradication of the disease.

We are developing a class of inhibitors of *Pf*PKG, starting from previously reported compounds (such as **1**) with activity against the *Eimeria tenella* PKG homologue.[2] New analogues possessed potent enzyme affinity and *in vitro* anti-parasite activity, coupled with excellent selectivity against human kinases. This poster will describe our initial efforts to examine key structural motifs in the original starting point **1**, with the aim of maintaining or improving cell potency and binding efficiency in structurally diverse analogues such as **2** or **3**. The work has also confirmed and expanded SAR and highlighted important considerations for achieving good kinase selectivity and ADME profiles.



1 *Pf*PKG IC_{50} 2 nM
Pf EC_{50} 361 nM
LLE 6.2

2 *Pf*PKG IC_{50} 11 nM
Pf EC_{50} 290 nM
LLE 6.0

3 *Pf*PKG IC_{50} 2 nM
Pf EC_{50} 197 nM
LLE 5.8

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POTENT, SELECTIVE AND ORALLY EFFICACIOUS INHIBITORS OF PLASMODIUM FALCIPARUM PROTEIN KINASE G (PfPKG)

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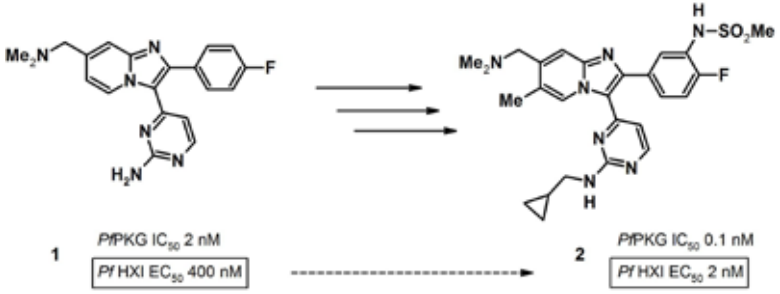
Malaria is one of the most prevalent infectious diseases of the developing world, whose primary causative agent in humans is the protozoan parasite *Plasmodium falciparum*. It is currently responsible for almost 0.5 million deaths per year, with both young children and pregnant women in sub-Saharan Africa particularly at risk.[1] There is significant concern about widespread and rapidly growing resistance to current standard malaria drugs; hence the development of structurally and mechanistically novel malaria treatments is urgently required to maintain control and advance eradication of the disease.

We have developed a class of inhibitors of *Pf*PKG, using known compounds (eg. **1**) with activity against the *Eimeria tenella* PKG homologue[2] as chemical starting points. Our new compounds show excellent *Pf*PKG enzyme affinity together with potent cell inhibition against the parasite, are highly selective against a human kinase panel and possess ADME profiles which translate to good levels of *in vivo* efficacy in rodent models of malaria. Development of both structure activity relationships and compounds which overcame pharmacokinetic and toxicity issues will be shown, together with some interesting aspects of *in vivo* protocol design. Key program compounds have been assessed in a range of malaria life cycle assay platforms, output from which continues to inform areas of PKG target biology as an approach to anti-malarial drug design. Some of the newest analogues such as **2** have shown very high levels of cell potency, improving on those of some known malarial drugs.



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2) Bioorg Med Chem Lett 2006, 16, 2479

DISCOVERY OF A SMALL MOLECULE THAT MITIGATES HEARING LOSS IN A MODEL OF USHER SYNDROME III

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Usher syndrome type III (USH3) characterized by progressive deafness, variable balance disorder, and blindness is caused by destabilizing mutations in the gene encoding the clarin-1 protein (CLRN1). Here we describe the development of a cell based screening assay to identify small molecules capable of stabilizing $CLRN^{N48K}$ and the subsequent discovery and optimization of a series of novel compounds to improve the potency and bioavailability which gave rise to a compound (BF844) which demonstrated efficacy in a mouse model that mimicked the progressive hearing loss of USH3.

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KINASE SCAFFOLD REPURPOSING FOR NEGLECTED DISEASE DRUG DISCOVERY: IN VIVO EFFICACIOUS ANTILEISHMANIAL COMPOUNDS BASED ON THE 3-AMINO-1H-PYRAZOLE SCAFFOLD.

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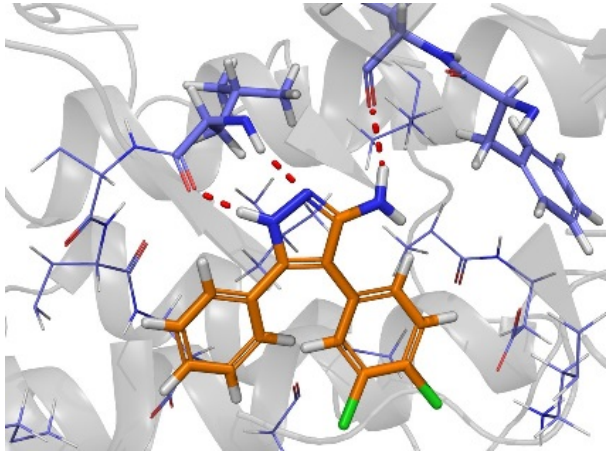
Estimates of as many as one in six in the world population (over 1 billion people) are infected by one or more of them, *Neglected tropical diseases* (NTDs) represent a significant global health burden, particularly in developing regions of the world. Because the limited investment in treating or preventing them, cost-effective approaches for identification of drug leads, as the "*repurpose*" of classes of proven molecular targets, are needed in order to spawn the discovery of new drugs. Specifically, *Kinase inhibitors* have received high attention as one of the principal enzyme target classes in drug discovery for a wide variety of indications.

Funded by EU inside 7FP framework, inside the TAKTIC project three kinases involved in the NFkB cascade (IKKalfa, IKKbeta and NIK) have been deeply targeted. In this occasion, a repurpose approach, based on the result of that experience allowed us to quickly identify a small library of 4-phenyl-3-amino-1H-pyrazols analogues presenting a potent, similar to Miltefosine itself, *anti-leishmanial* activity profile. The compounds selection was ruled by an *in silico* evaluation of the interaction with **Cdc2-related protein kinase 3** (CRK3). As in human, where *cyclin dependent kinases* (CDKs) are know to play important roles in cell division, transcription, etc., also in Leishmania, the Cdc2-related kinase family have attracted attention as potential drug targets. In particular, the CRK3 isoform is postulated to be an essential enzyme for transition through the G2/M phase checkpoint of the Leishmania cell cycle, responsible for parasite growth and survival[1]. Based from a homology model of Leishmania Mexicana CRK3 recently reported,[2] our *in-house* library of about 30 aminopyrazoles was therefore docked against the template protein (PDB: 1VYZ) selecting five compounds, based on best scores and valuable poses, for the next biological evaluation. In this occasion, *in silico* general strategy, specific binding modes, synthesis and biological evaluation of active compounds against *Leishmania Braziliensis* and *Leishmania Amazonensis*are presented and fully discussed.



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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 7-AMINO PYRAZOLOPYRIMIDINE COMPOUNDS POSSESSING POTENT ANTI-WOLBACHIA ACTIVITY FOR THE TREATMENT OF ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

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Filarial nematodes are a significant group of human pathogens that affect more than 157 million people worldwide, contributing to serious public health and socio-economic challenges within endemic regions. These parasites are responsible for the Neglected Tropical Diseases lymphatic filariasis (LF) and onchocerciasis, which is the second leading infectious cause of blindness. The main causative agents of these diseases are the nematodes, *Wuchereria bancrofti* and *Onchocerca volvulus* respectively.[1] These nematode infections are currently managed using mass drug administration (MDA) of drugs donated by large pharmaceutical companies, however, elimination is hampered by a number of challenges. The recommended treatment for LF in areas which are none co-endemic for onchocerciasis is a combination of diethylcarbamazine plus albendazole,[2] whereas a single dose of ivermectin can be given annually for the treatment of LF in area's which are co-endemic with onchocerciasis.[3] Ivermectin is the recommended treatment for onchocherciasis[2]and works to deplete microfilariae, the immature worm stage, thus preventing disease progression and transmission. However, ivermectin cannot be used in areas co-endemic with Loa loa. In addition, these standard anti-filarial drugs do not kill the long-lived adult (macrofilariae) worms.

The nematodes responsible for causing filarial diseases, have an essential endosymbiotic relationship with the bacterium, Wolbachia.[4] Although the exact nature of this relationship is not understood, anti-*Wolbachia* therapy has been identified as a viable treatment for filarial diseases which delivers safe macrofilaricidal activity with superior therapeutic outcomes, compared to current standard anti-filarial drugs.[5] The association of current anthelmintic agents (anti-parasitic drugs efficacious against roundworms) with undesirable adverse effects and concerns of resistance development to drugs within MDA programmes is driving current research efforts into the identification and generation of safe, anti-Wolbachia driven therapeutic alternatives. The broad-spectrum tetracycline antibiotic, doxycycline is the current gold standard for anti-Wolbachia activity and is macrofilaricidal, but requires a treatment regimen of at least four weeks and is contraindicated in children under 9 years and pregnant women. The Anti-*Wolbachia* drug discovery and development programme aims to identify alternative drugs which are suitable for a wider patient range and shorter treatment plan.[2]

A phenotypic screen of 10000 compounds from the BioFocus library identified 50 compounds, spanning 10 chemotypes, with good anti-*Wolbachia* activity. Chemoinformatic analysis of these 50 hits has been used to identify the most promising chemical scaffolds to be taken forward into a hit-to-lead optimisation program. This work aims to develop small molecule anti-*Wolbachia* agents of the pryazolopyrimidine chemotype which possess sufficient efficacy to provide greater than 90% *Wolbachia* reduction in less than 14 days of treatment within an *in vivo* model.[6] Molecules were tested for in vitro EC_{50} activity and DMPK properties to develop SAR around our template. Despite high potency demonstrated by the original hit, DMPK experiments highlighted poor metabolic stability. A phenyl ring, allyl functionality and methylene linker within the template highlight positions of our analogues that may be susceptible to metabolism. Organic synthesis has therefore enabled functionalization at these key positions within out template, generating a broad library of compounds of which many analogues possess nanomolar activity against *Wolbachia in vitro* as well as display significantly improved DMPK parameters.

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BUILDING BLOCKS FOR SYNTHESIS OF TRYPANOSOMA CRUZI GLYCOPHOSPHATIDYLINOSITOL ANCHORS -PART III.

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Chagas disease affects about 8 million people worldwide, being cause of morbidity and mortality of thousands ones, especially in developing countries. Therapy for Chagas disease is scarce and frequently ineffective, especially in chronic phase, drawing attention of searching for new compounds and selective parasite molecular targets. Several studies have shown that glycophosphatidylinositol (GPI) anchors are important molecules for parasite invasion and survival in host cells. They are responsible for anchoring important *T. cruz*i proteins and glycoproteins, such as mucins and *trans*-sialidase. In this context, this work aims to synthesize carbohydrates able to mimic *T. cruzi* GPI anchors as an strategy to construct a chemical probe to understand its molecular function and also prepare potential GPI inhibitors. Parallel synthesis of glucosamine and *myo* inositol derivatives (**3** and **6**) was performed using orthogonal protection/deprotection strategy. Ferrier rearrangement is being used to obtain the *myo* inositol derivative. *O*-glycosilation using TMSOTf as promoter will be performed between blocks **3** and **6** to obtain compound **7**. A protected ceramide derivative, previously prepared (compound **8**) will be linked to position 1 of *myo*-inositol moiety, after removing allyl group, giving compound **9** (Scheme 1).

Scheme 1: Proposed synthesis to obtain synthetic GPI anchor derivative **9**.

In conclusion, glucosamine building block were obtained in good yields in 5 steps. *Myo*-inositol derivatives are also being obtained with good yields by *Ferrier* rearrangement.

AMINOQUINAZOLINONES H2L PROGRAM FOR TUBERCULOSIS

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2-Aminoquinazolinones were prepared and tested for anti-malaria activity but proved to be inactive. Additional screening showed that these readily accessible compounds have low micromolar activity in assays for Tuberculosis. The goal was to identify compounds with “drug like “ physicochemical properties in this series. The solubility of an early active hit was not sufficient to generate plasma levels in vivo that exceeded the MIC level for TB. It was possible to overcome this problem by administering the more soluble sulfoxide as a pro-drug. The in vivo PK data show, that a ~ 10 fold higher concentration of the sparingly soluble sulfone could be achieved. This elevated the drug level high enough in vivo, so that the free fraction exceeded the MIC level for several hours. The compound was therefore found suitable for in vivo efficacy studies and a POC study in a mouse model for TB was conducted.

LEAD OPTIMISATION OF A NOVEL CLASS OF TRYPANOSOMACIDAL AGENTS

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	IC_{50} (μM)	SI
T. b. brucei	0.80	>96
T. cruzi	2.3	27

	IC_{50} (μM)	SI
T. b. brucei	0.028	2966
T. cruzi	0.04	>1258

SI = selectivity index vs. mammalian cells

Human African Trypanosomiasis (HAT) and Chagas Disease, caused by the protozoan parasites *Trypanosoma brucei* and *T. cruzi*, respectively, cause significant suffering and mortality in some of the poorest regions of the world. Current treatment regimens are not suited to third world conditions, have low efficacy and high toxicity. New, orally available and safe drugs for these parasitic infections are urgently required.

In this presentation, the hit to lead optimization of a novel class of potent, selective and broad spectrum trypansomacidal agents,[1] based on the high-throughput screening hit **1**,[2] will be reported. Pharmacokinetic characteristics and mode of action studies of the most potent compounds will also be discussed.

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REPRODUCTION INHIBITION OF TICK-BORNE FLAVIVIRUSES BY SMALL MOLECULES

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Human diseases transmitted by ticks present a great health concern in Russia and Europe. Severe central nervous system pathologies are caused by Flavivirus genus members such as the most known tick-borne encephalitis virus (TBEV) and closely related Powassan virus (POWV). Once introduced into the human organism, they can cause lifelong disabilities or death. Although vaccines were developed against some flaviviruses, the search for treatments that could interfere with the infection remains an important and challenging task. Given the high similarity between the tick-borne and mosquito-borne flaviviruses, such molecules can be further repurposed for the treatment of dengue fever,West Nilefever, and diseases caused by Zika virus.

A number of POWV and TBEV reproduction inhibitors belonging to the series of 1,4-dihydropyridines, 1,3,5-thiadiazolines [1], and 4-aminotetrahydroquinazolines [2] were identified during the docking-based virtual screening against hydrophobic pocket of open envelop protein homology models. The time-of-addition assay confirmed the compounds to be involved in disrupting virion – cell surface interactions prior to or at the stage of the virion entry into the host cell. These compounds showed no inhibitory effect against non-enveloped poliovirus, thus supporting the hypothesis about the specificity of their interaction with TBEV envelope.

Flaviviral RNA-dependent RNA-polymerase NS5 is an attractive target for the development of broad-spectrum antivirals because it is the most conservative flavivirus protein. We investigated pockets on the NS5 surface that could be used for inhibitors design via molecular dynamics simulations and revealed similarities and differences in the shape and volume of the pockets across *Flavivirus* genus representatives. The chemical space of known flaviviral NS5 inhibitors was analyzed and perspective unexplored regions were found.

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THE NTD DRUG DISCOVERY BOOSTER: A NOVEL APPROACH FOR HIT TO LEAD CHEMISTRY

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The NTD Drug Discovery Booster is an innovative collaboration designed to swiftly and efficiently maximise the Structure-Activity Relationship (SAR) space around new chemical hits and leads targeting *Leishmania donovani* and *Trypanosoma cruzi*, the Kinetoplastid parasites responsible for visceral leishmaniasis and Chagas disease respectively. Screening for new leads against these parasites has evolved with increased throughput over the past decade, yet valid starting points remain scarce. It is essential that the existing hits and leads that are identified are investigated thoroughly and efficiently.

In partnership with our consortium partners, we have implemented a novel *in silico* screening process that allows us to advance various chemical series through the hit-to-lead process via the mining of each pharmaceutical company's vast chemical library in an iterative and collaborative manner. The innovation of the NTD Booster lies in the companies simultaneously accepting to share with DNDi upfront structural and biological information about a promising chemical series that is essential for its rapid development. Thus, by exploring the combined libraries containing several million compounds, the chances of pulling out the best possible hit series with a pooled collection of valuable information are dramatically increased.

We demonstrate that this original, cooperative approach allows us to rapidly expand the SAR around hits, including examples of progressing hit IC_{50} potencies from >10 µM to in silico screening approaches.

The NTD Drug Discovery Booster consortium currently consists of AstraZeneca plc., Celgene Corporation, Eisai Co, Ltd., Shionogi & Co, Ltd., Takeda Pharmaceutical Ltd., Institut Pasteur Korea and the Drugs for Neglected Diseases *initiative*.

EXTENDED SAR EXPLORATION AND STRUCTURAL OPTIMIZATION OF A NOVEL CLASS OF QUINOLOXYACETAMIDE ANTIMYCOBACTERIAL COMPOUNDS

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a disease of antiquity with extraordinary endurance and complex clinical pathology. Within the last few decades, an increasing prevalence of Multi-Drug-Resistant tuberculosis (MDR-TB) and the often fatal comorbidity between TB and human immunodeficiency virus (HIV) has been observed. Therefore, the discovery of new chemical entities with novel modes of action is a high priority in the global health agenda.

We present a novel series of quinoloxyacetamide derivatives with antimycobacterial activity that was identified through a whole cell High Throughput Screening (HTS) performed by GSK. SAR exploration of the primary hit led to micromolar potencies and improved physicochemical profiles. A metabolic stability study of the series was performed and an amide bond was found to be responsible for the blood instability. Further medicinal chemistry efforts to replace this labile group, led to a derivative which opened new opportunities for the series. This compound did not exhibit any cytotoxic effects or hERG inhibition.

Alkylation of 4-hydroxy azaheterocycle precursors was used as a key synthetic step in the preparation of most presented compounds. The *N*- versus *O*- chemoselectivity of this reaction was strongly dependent on the nature of the azaheterocycle and its substitution pattern, highlighting the need for full structural assignment. Further optimization of this promising series of novel antimycobacterial compounds together with target identification, could provide a strong lead compound in the drug discovery arena.

MOLECULAR DESCRIPTORS CALCULATION AS A TOOL IN THE ANALYSIS OF THE ANTILEISHMANIAL ACTIVITY ACHIEVED BY A SERIES OF DISELENOSULFONAMIDE DERIVATIVES. AN INSIGHT INTO ITS POTENTIAL ACTION MECHANISM.

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A molecular modeling study has been carried out on a previously reported series of symmetric diselenide derivatives [1] that show remarkable antileishmanial in vitro activity against L. infantum intracellular amastigotes and in infected macrophages (THP-1 cells), in addition to showing favorable selectivity indices. The analyzed compounds can be considered as constructed over a diaryl diselenide central nucleus, decorated in 4 and 4' positions with an aryl or heteroaryl sulfonamide fragment, thus forming the diselenosulfonamide derivatives.

The activity can be related, as a first approximation, with (a) the ability to release bis(4-aminophenyl)diselenide, the common fragment which can be ultimately responsible for the activity of the compounds. (b) the anti-parasitic activity achieved by the sulfonamide pharmacophore present in the analyzed derivatives.

[dplano_01]

The data that support this connection include the topography of the molecules, the conformational behavior of the compounds, which influences the bond order, as well as the accessibility of the hydrolysis point, and possibly the hydrophobicity and polarizability of the compounds [2]

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IN VITRO TRYPANOCIDAL ACTIVITY AND METABOLISM STUDIES FOR NEW SELENOCOMPOUNDS

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There is an urgent need for new, safer, and effective treatments for the diseases caused by the protozoan parasites. Selenium and its derivatives stand out as having promising leishmanicidal activity. In fact, some parasites express selenoproteins and metabolize selenium. Taking this into account during the last years our research group has been working on the synthesis of new selenocompounds with trypanocidal activity.

In this work, we present a serie of 48 selenocyanate and diselenide derivatives that were used in screens of *in vitro* growth inhibition assays of promastigote and amastigote forms of *L. braziliensis* and *L. infantum*, as well as epimastigotes, amastigotes and trypomastigotes of *T. cruzi*. The cytotoxicity of all molecules for peritoneal murine macrophages or vero cells was also tested, allowing the determination of the selectivity index (SI) parameter. The maximum effects were for derivatives **PP08**, **PP10** and **PP15**.

To gain information concerning the effect of the most active derivatives on glucose metabolism in the parasites, the final excretion products were identified. These data were obtained by recording the ^{1}H NMR spectra of cultures of promastigotes of *L. braziliensis* after treatment with the studied compounds at their IC_{25} concentrations and separation of the parasite cells by centrifugation. The results were compared with a control of promastigotes maintained in a cell-free medium for four days after inoculation with the parasite, showing the characteristic signals of the CH_3 groups of acetate, alanine, lactate and pyruvate, and CH_2 signals of succinate. We observed different behavior among them and new studies *in vivo* models are performing in order to further known their potential therapeutic.

IDENTIFICATION, DESIGN AND BIOLOGICAL EVALUATION OF BENZISOTHIAZOLINONES AND BENZISOSELENAZOLINONES TARGETTING PLASMODIUM FALCIPARUM ISPD

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Malaria remains one of the world's most widespread parasitic diseases and is estimated to affect approximately 207 million people worldwide, causing ~438,000 deaths per year, most of which are children under the age of five.[1] The development of resistance to current frontline therapies is reducing the efficacy of current treatments, preventing eradication of the disease.[2] To combat these virulent, strains novel alternative chemotherapeutic options, with new mechanisms of action are required.

In order to deliver a compound with high selectivity over the human host, we have targeted a parasite specific pathway. The methyl erythritol phosphate pathway (MEP) is responsible for the biosynthesis of two isoprenoid precursors' dimethylallyl diphosphate and isopentyl diphosphate in malaria parasites, and has been validated as a potential target for antimalarial drug development. Crucially the MEP pathway is absent within mammalian cells which rely on the alternate mevalonate pathway for isoprenoid biosynthesis; this provides a unique opportunity to selectively target the MEP pathway exclusively. The pathway comprises of 7 steps and multiple enzymes that can be modulated by small molecule inhibition.[3]

The MEP pathway has been previously targeted in *P. falciparum* for the treatment of malaria using fosimidomycin, currently used in combination with clindamycin, which has demonstrated efficacy *in-vitro* and *in-vivo* against various malaria strains. Fosmidomycin's primary target is the second enzyme in the MEP pathway IspC, which is responsible for the reduct-isomerisation of 1-deoxy-D-xylulose 5-phosphate (DOXP) to MEP.[4] Here we present our findings for small molecule inhibition of an essential enzyme within this pathway, MEP cytidyl transferase (IspD), responsible for catalysing the cytidylation of MEP to cytidine diphosphate methylerythritol.

An enzymatic screen of 10000 compounds from the Biofocus Library identified 91 compounds with IC_{50} values below 10 µM. Analysis of these compounds identified the benzisothiazolinone chemotype to possess good enzymatic activity against *Pf*IspD. The work described here reports the SAR development around the benzisothiazolinone scaffold to develop small-molecule inhibitors of *Pf*IspD, which could ultimately be considered for candidate selection. Compounds have been screened using a *Pf*IspD enzymatic assay before selecting priority compounds for measurement of their *Pf*3D7 whole cell growth inhibition. We also detail synthesis and biological activity of the closely related benzoisoselenazolone chemotypes which have exhibited sub-micro molar IC_{50} activity against *Pf*IspD. The discovery of inhibitors against *Pf*IspD exhibiting both potent enzymatic and phenotypic activity demonstrates IspD as a promising new target for the potential treatment of malaria.

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EXPLORING THE SCOPE OF NEW ARYLAMINO ALCOHOL DERIVATIVES: SYNTHESIS, ANTIMALARIAL EVALUATION, TOXICOLOGICAL STUDIES, AND TARGET EXPLORATION

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Synthesis of new arylamino alcohol derivatives followed by SAR, *in silico* drug-likeness, toxicity, genotoxicity, *in silico* metabolism, and *in vivo* studies led to the identification of compounds with significant *in vitro* antiplasmodial activity against drug sensitive (D6 $IC_{50} \leq 0.19$ µM) and multidrug resistant (FCR-3 $IC_{50} \leq 0.40$ µM and C235 $IC_{50} \leq 0.28$ µM) strains of *P. falciparum*. Adequate selectivity index ($36 \leq SI \leq 245$) and no genotoxic behavior are also observed. Notably, one compound (22) displayed excellent parasitemia reduction (98 ± 1%), and complete cure with all treated mice surviving through the entire period without any signs of toxicity. One important factor is the match between *in vitro* potency and *in vivo* studies. Target exploration was performed in order to establish a possible mechanism of action, however, plasmepsin 2 enzyme and hemozoin inhibition pathway was discarded. Therefore, this amino alcohol series exhibits a non-classical antimalarial mechanism to its chemotype.

NEW HYDRAZINE AND HYDRAZIDE QUINOXALINE 1,4-DI-N-OXIDE DERIVATIVES AS POTENTIAL ANTIMALARIALS

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Malaria is one of the world's most important tropical parasitic diseases. Mortality due to malaria is estimated to be over 400,000 deaths annually and this situation is worsened by the spread of drug-resistant strains of the parasite. In the present study, a series of 19 quinoxaline 1,4-di-*N*-oxide derivatives were designed, synthesized and evaluated in *vitro* against infectious pathogens *Plasmodium falciparum* (3D7 chloroquine-sensitive and FCR-3 chloroquine-resistant strain*)*. Among them, 14 novel compounds correspond to hydrazine and hydrazide derivatives. Their cytotoxicity and selectivity were also evaluated against HEGP-2 cells. The *in silico* ADMET properties were calculated for all compounds. Hydrazine and hydrazide quinoxaline 1,4-di-*N*-oxide derivatives constitute a new class of antimalarial compounds. It can potentially serve as template for future drug-optimization and drug-development efforts to be use as therapeutic agents in developing countries.

NEW POTENTIAL TRANS-SIALIDASE INHIBITORS FROM Trypanosoma cruzi PREDICTED BY CHEMOINFORMATIC STUDIES

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The Chagas disease (CD) is a neglected parasitic disease caused by the protozoan parasite *Trypanosoma cruzi*. The current pharmacological treatment is based on the use of two drugs, nifurtimox and benznidazole; nevertheless, they have severe adverse effects and are not effective in the chronic stages of the disease, therefore, the need for developing new drugs is latent. *Trans*-sialidase is an essential enzyme of *T. cruzi* that lacks of human homologous protein, which is playing a relevant role in the host-parasite invasion during their maturing stages; therefore, the enzyme is a safe drug target (1-3).

In this study, we performed a computational cheminformatics protocol to screen 3180 compounds to be used as potential *trans*-sialidase inhibitors. From a first molecular docking, a set of 38 top compounds that showed higher predicted binding affinity were selected for further analysis. The subsequent ligand aminoacid clustering method analysis and the ranked position based on the consensus scoring binding affinity was used to choose seven known pharmacological compounds for the *in vitro* experiments. Blood samples infected with trypomastigotes from INC-5 and NINOA strains were used to prove the direct inhibitory effects of the selected compounds. Four compounds were more active on INC-5 strain and six on NINOA strain. Among those drugs, two compounds showed a higher rate of cellular lysis on both strains. As *trans*-sialidase is expressed on the trypomastigote stage this study suggests that two known drugs could be used for the pharmacological treatment of Chagas disease.

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INVESTIGATION OF THE BINDING MODE OF A NOVEL CLASS OF ANTIMYCOBACTERIAL DPRE1 INHIBITORS

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. With almost 9 million new cases diagnosed each year and a TB-related death occurring every 20 seconds, the disease is considered a significant threat to global health. In spite of decades of research, the first-line antitubercular therapy still consists of medicines developed more than 40 years ago. With the increasing prevalence of the drug resistant strains of *M. tuberculosis*, there is a growing and acute need for new, improved antitubercular agents with novel modes of action.

Over the past few years increasing interest has been drawn to a new mycobacterial protein - decaprenylphospho-beta-D-ribofuranose 2-oxidase (DprE1). This periplasmic enzyme catalyzes a key step in the biosynthesis of the cell wall components and is potentially an attractive target for future antitubercular drugs. In search of DprE1 inhibitors, a high-throughput screening campaign was performed by GlaxoSmithKline, leading to identification of a novel compound family, based on the imidazolidine-2,4-dione scaffold.

Within the framework of the OpenMedChem project – a collaboration between GlaxoSmithKline and the University of Antwerp, structure-activity relationship (SAR) exploration and compound profiling of the discovered hits was initiated. Based on the initial SAR information, the binding mode of the studied compounds to DprE1 was investigated by molecular docking, with the use of the MOE and the Autodock/Vina software. Herein we present the results of this analysis and its preliminary confirmation through subsequent design, synthesis and biological evaluation of new analogues of the active hits.

ANTILEISHMANIAL DINITROANILINE-ETHER PHOSPHOLIPID HYBRIDS

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Protozoa are unicellular eukaryotes and are the most important causes of neglected tropical diseases. Every year, more than one million people die from complications from protozoal infections worldwide. The Trypanosomatidae familycomprise the causative agents of several human diseases such as Chagas disease (*Trypanosoma cruzi*), sleeping sickness (*Trypanosoma brucei*) and leishmaniasis (*Leishmania* sp). which have been classified by the WHO as neglected diseases, affecting people living in poverty in developing countries and lackingefficient and affordable therapies.[2,3] Miltefosine (hexadecylphosphocholine) is an alkylphosphocholine with demonstrated activity against various parasite species and cancer cells, as well as some pathogenic bacteria and fungi. Moreover, Miltefosine is currently the only oral drug available for the treatment of visceral (VL) and cutaneous leishmaniasis (CL). However, at the therapeutically effective doses, severe gastrointestinal side effects and serious weight loss were observed while teratogenicity issues restrict their use.

During the last decade our group has been investigating ring-substituted alkylphosphocholines and we have indicated that introduction of cycloalkane rings in the lipid portion provides compounds with enhanced activity and reduced toxicity.[1-3]

In the context of more in depth SAR studies we designed and synthesised hybrid molecules which combine in one molecular scaffold two pharmacophores, the dinitroaniline moiety and the ether phospholipid structure.[4] This approach would enable us to address two different mechanisms of action, namely the inhibition of the alpha-tubulin of the parasite, targeted by dinitroaniline herbicides such as trifluraline, and the putative molecular targets of alkylphosphocholines.

The new trifluraline-substituted ether phospholipids encompass analogues active against *L. infantum, L. donovani* and *T. cruzi* amastigotes as well as *T. b. brucei* (blood stream form). Extensive ADME-Tox studies demonstrated that the toxicity of the majority of the compounds is very low and much lower than Miltefosine, especially against THP-1 macrophages.

Acknowledgement

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SHMT INHIBITION: A NEW HOPE TO DEFEAT MALARIA

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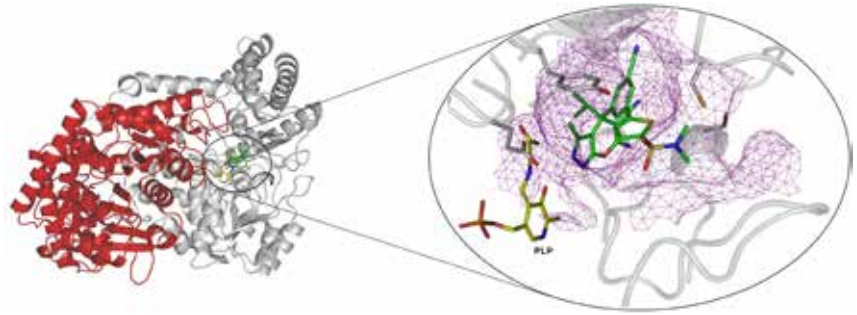

Malaria is mainly caused by the parasite *Plasmodium falciparum*. Due to the emergence of drug-resistant strains, there is an urgent need of novel treatment. The folate cycle contains several enzymes, and was identified as a promising target. Indeed few antimalarials already address this pathway.[1] However, inhibition of serine hydroxymethyl transferase (SHMT), a key enzyme of the folate cycle, has not been investigated so far. *A. Thaliana* SHMT inhibitors, based on a pyrazolopyran core, from an herbicide optimization program at BASF-SE demonstrated promising antimalarial activity on *P. falciparum* and *P. vivax*.[2] Our pioneering work on the inhibition of SHMT shed the light on this novel antimalarial target.[3] The binding mode was resolved by several X-ray crystal structures of *Pv*SHMT-ligand complexes.[3] Based on the high similarity of *P. vivax* and *P. falciparum* SHMT, the X-ray co-crystal structures can be utilized for 3D modeling to design small drug-like molecules against *Pf*SHMT. Nevertheless pharmacokinetic limitations of our lead compound prevented any *in vivo* activity in the *P. berghei* mouse model. In this work the development of novel inhibitors is focused on improving liver microsomal stability while keeping high *in vitro* potency. In that perspective, subtle modification of the scaffold and derivatization of the exit-vector led to promising candidates for further *in vivo* efficacy evaluation.

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PHENOTYPIC SCREENING IDENTIFIES HUMAN PDE4 INHIBITORS WITH SUBMICROMOLAR TRYPANOCIDAL ACTIVITY AGAINST THE INTRACELLULAR FORM OF TRYPANOSOMA CRUZI, THE CAUSATIVE AGENT OF CHAGAS? DISEASE

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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.[1]

The medicinal chemistry department of the VU University Amsterdam (VUA) is part of a European consortium (PDE4NPD) that aims to combat parasitic disease by targeting phosphodiesterases (PDEs) of these parasites. Phenotypic screening revealed human PDE4 inhibitors with submicromolar trypanocidal activity against *Trypanosoma cruzi*. Optimization of these phenotypic hits is ongoing and the Tcr PDE mediated mechanism of action is under investigation.[2]

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IONIC LIQUIDS BASED ON AMINOQUINOLINES AS NEW ANTIMALARIAL HITS

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Recently, ionic liquids (ILs) have attracted pharmaceutical and life sciences. ILs are organic salts that have gained popularity as solvents in organic synthesis, but quickly they spread through analytical chemistry, separation chemistry and material science. Due to significant developments in their chemical and biological properties and applications, ionic liquids are now bringing new opportunities at the interface of chemistry with the life sciences. The use of ILs is new in the field of antimalarials. This innovation is most likely to be converted into safe, effective and affordable medicines, given that they are based on well-known anti-infective agents and on simple ionic combination of two building blocks, one cationic (the aminoquinolines), and one anionic, to form the final ILs; hence, no elaborate or expensive chemistry is required, which may be translated into medicines with real application in the field.

With this in mind, the purpose of this work was the synthesis and study of ILs based on classical basic antimalarials, primaquine [PQ] and chloroquine [CQ], whose protonated forms were combined with several acids, some of them also interesting from a therapeutic standpoint. Several ILs were produced and screened in vitro regarding their activity against 3 developmental stages of malaria parasites. Comparable or improved in vitro activities were obtained, which will be presented and discussed in this communication.

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POSTERS

Synthesis Driven Innovation

DEVELOPMENT OF STEREOSELECTIVE SYNTHESES OF BROMODOMAIN INHIBITORS AS ANTI CANCER DRUG AND SYNTHESES OF OTHER PROBES FOR FINDING NEW BROMODOMAIN TARGETS

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"Epigenetics" refers to heritable changes in gene transcription that occur without changing the underlying DNA sequence. Post translational modifications (PTMs) of histones such as acetylation of histone lysine are central to epigenetic modulations of gene transcriptions. Bromodomain containing proteins (BCPs), co-ordinate transcription factor assembly by recognizing the lysine acetylation state and act as "readers" of histone. Bromodomains are ~ 110 amino acid 4-helix modules that exist as part of larger protein architectures. Inhibition of BCPs has potential therapeutic applications in a range of diseases including cancer. Inhibition of BET bromodomains also allows potent anti-proliferative activity in a number of hematological cancer models partly through suppression of Myc oncogene and downstream Myc-driven pathways.

(*S*)-*N*-acetyl tetrahydroquinaldine ((*S*)-*N*-Ac-THQ) has been identified as a fragment-style inhibitor of bromodomains, such as the BET bromodomain. One clinical candidate I-BET726, a BET inhibitor was developed at GSK for cancer treatment incorporates the (*S*)-*N*-Ac-THQ scaffold. The core moiety of (*S*)-*N* -Ac-THQ has been shown to mimicks the binding interactions of acetyl lysine (PDB: 4A9H). As well as the potential elaboration of the scaffold in a medicinal chemistry sense, derivatives of (*S*)-*N*-Ac-THQ could be used as probes for identification of BRD targets. Application of biotinylated probes in a pull down assay or fluorescent probes can allow the analysis of cellular extracts to examine bromodomain localization and interactions.

The importance of the enantiomeric configuration of this moiety to activity has encouraged us to examine enantiocontrolled syntheses of (*S*)-*N*-Ac-THQ analogues and to explore the structural elaboration of this compound. Our initial approach involved kinetic resolution (KR) using phthaloyl-leucine derivatisation to give optically pure (*S*)-*N*-Ac-THQ in >98% *ee*. In a second approach we used fluorenylethyl chloroformate (FLEC) as a chiral resolving agent for diastereomeric resolution. This involved the coupling of (*S*)-FLEC to a racemic mixture of THQ. The resulting diastereomers were resolved by silica gel chromatography, deprotected and acetylated to provide pure samples of both enantiomers in >98% *ee*. An improved cheap, high yielding and reproducible enantioselective synthesis of FLEC was also developed and carried out on multigram scale. A final approach, allowing access to 6-substituted derivatives, involved chiral reduction of 6-bromoquinoline to 6-bromoquinaldine. This bromodomain inhibitor core was then derivatised to create a biotinylated probe. The synthetic methods developed in this investigation provide a platform for the provision of further derivatives and the investigation of them as potential inhibitors of BCPs.

SYNTHESIS OF TWO NATURAL PRODUCT-LIKE SMALL MOLECULES LIBRARIES FOR THE PUBLIC COMPOUND COLLECTION

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High throughtput screening (HTS) of large organic molecules libraries is a valuable tool in the identification of new hits, and has led to the development of several successful drugs.[1] In spite of HTS potential, most "hits" never became a drug, and in reality, the attrition rate of drug candidates has been high for the last three decades [2,3]. The prevalence of lipophilic, heavy and flat molecules within drug candidates and commercial available small libraries can help to explain the low succesful rate[2,4,5]. Therefore, designing compound collections more natural product-like (spatially complex with several stereocenters and high Fsp^3) might increase the chances of success and create libraries that occupy an area of chemical space that is underepresented.

European Lead Factory (ELF) is a public-private partnership that aims the establishment of a joint european compound library (JECL) of half million molecules and a European screening center, which eventually will lead to innovative drug discovery starting points.[6] The collaboration between European universities and small and medium enterprises (SME) will contribute with 200.000 compounds wherein, academia is responsible for innovative library design and validation of the synthetic chemistry, and SME´s for the synthesis of large libraries. Our group has already contributed to this Public Compound Collection (PCC) with 14 scaffolds which gave origin to 5548 compounds synthesized by EDELRIS. Herein, we present the design and validation of two more natural product-like libraries for the PCC. The first library comprises a fused hexahydropyrrolidione with 4 points of diversification, 4 to 5 stereocenters and was synthesized in two steps using an Ugi reaction followed by a Diels-Alder and an acid rearrangement. The second library comprises a fused bycyclic and tetracyclic caged scaffold with 3 points of diversity and 4 to 5 stereocentes, and was obtained from carvone in 6 and 7 steps respectively. Both libraries are rich in hydridized sp^3 carbons and stereocenters and are relevant contributers to the PCC.

Acknowledgments:

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution

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DIALKYL(DIARYL)AMIDOPHOSPHATE DERIVATIVES OF LUCENSOMYCIN: DESIGN, SYNTHESIS, ANTIFUNGAL ACTIVITY AND DEVELOPMENT OF INTELLECTUAL COMPUTER SYSTEM

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Lucensomycin, antifungal antibiotic first obtained by Italian investigators, refers to a group of tetraene macrolide antibiotics. Lucensomycin is a product of microbiological synthesis with the help of *Streptomyces lucensis* producer, its structure, physicochemical, and biomedical properties were studied in detail. However, due to the high toxicity lucensomycin not find application in drug therapy of mycoses in contrast to other tetraene macrolide antibiotics: nystatin and pimaricin, which are widely used in a mycological practice for the treatment of many clinical forms of candidiasis.

The chemical modification of the tetraene macrolide antibiotic lucensomycin with dialkyl(diaryl)phosphites was carried out in the conditions of Todd-Atherton reaction. It was shown that reactions of lucensomycin with different dialkyl(diaryl)phosphites resulted in the formation of its corresponding dialkyl(diaryl)amidophosphate derivatives. Physical and chemical properties of dialkyl(diaryl)amidophosphate derivatives of lucensomycin, toxicity and their antifungal activity against a series of the test cultures of *Candida* yeast-like fungi were studied. Biological investigations showed that synthesized derivatives of lucensomycin were low toxic agents and possessed high antifungal activity.

The Intellectual Computer System (ICS) for optimal choice of the conditions for rational design and synthesis of novel derivatives of polyene macrolide antibiotics (PMA) was developed. ICS structure includes all specific data of pathogenic fungi, chemical and physical properties of semisynthetic derivatives of PMA, recommendations for their synthesis and perspectives for the treatment of various fungal infections. Thus, application of ICS allows: 1) to improve methods of the preparation of new derivatives of PMA, 2) to increase chemotherapeutic efficiency of their application in a mycological practice, and 3) to create new derivatives of PMA with more expanded spectrum of biological activity.

ACCESSING CHEMICAL SPACE WITH THREE TRIAZOLE SCAFFOLDS

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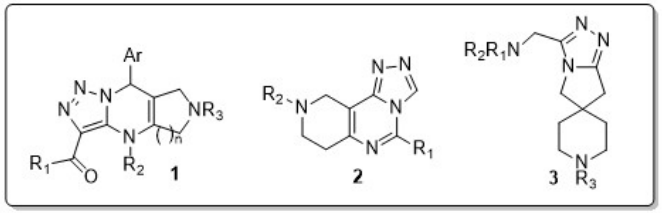
Syncom BV, Kadijk 3, 9747 AT Groningen, The Netherlands

Triazoles show a broad range of pharmalogical activities. In the course of our work for the European Lead Factory we designed three novel tricyclic triazole-based scaffolds.

Key-step of scaffold **1** is the multi-component reaction between an aminotriazole, an aldehyde and a ketone, a reaction with precedent in the literature.[2] This MCR results in the desired tricyclic structure, which is alkylated and further functionalized to synthesize a library of compounds.

Synthesis of both triazole **2** and **3** finds its start in N-Boc-piperidone. After condensation with urea the resulting bis-hydroxypyrimidine is converted in 6 steps into the required building-block **2** for library synthesis. Likewise for scaffold **3**, after construction of a lactam ring,[3] the amide functionality is converted into a triazole in high yield. Further standard manipulations lead to the desired building-block for library synthesis.



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BICYCLIC LACTAMS WITH HIGH SP3-CHARACTER AS SCAFFOLDS FOR LIBRARY PRODUCTION

Peter van den Boogaard, Alessandro Cecchi, Mark Borst, Kees Pouwer

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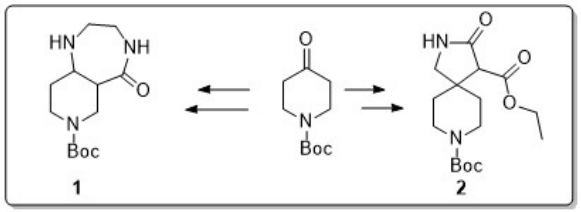
Flatland is the term coined for the area of chemical space with flat aromatic molecules.[1] One of the objectives of the Joint European Compound Library (European Lead Factory) is the synthesis of compounds with a high sp3-character, so escaping flatland. We designed two bicyclic scaffolds containing a lactam functionality, with a high sp^3-character (Fsp^3 = 0.78-0.87), both starting from N-Boc-piperidone.

In a first approach reductive amination of the piperidone with a diaminoethyl equivalent, followed by ring-closure afforded oxodecahydro-7H-pyrido[4,3-e][1,4]diazepine **1**. Further manipulations, including amine functionalization and amide alkylation resulted in the required building-blocks for the library synthesis.

A second successful idea concerned the transformation the piperidone into ethyl 3-oxo-2,8-diazaspiro[4.5]decane-4-carboxylate **2** according to literature procedures.[2] This versatile building-block was functionalized in various ways (arylation, saponification, Curtius rearrangement) to provide access to a large amount of compounds with highly spatial character.



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THE DEVELOPMENT OF A NEW PROTECTING GROUP STRATEGY FOR THE FORMATION OF ANALOGUES OF AN ULTRAPOTENT ANTITUMOUR ANTIBIOTIC VIA SOLID PHASE SYNTHESIS

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CC-1065 and the duocarmycins, including duocarmycin SA (DSA) (Fig 1), are 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] The high potency and broad spectrum of these natural products has demonstrated the potential of these agents as clinical candidates. However, these agents have been found to be too toxic for systemic use meaning they have never fulfilled their potential as possible chemotherapeutic agents.

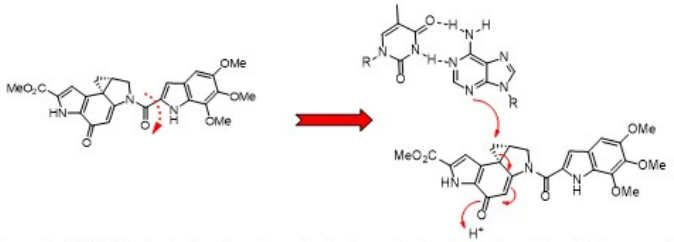

Figure 1: A DNA binding induced conformational change leads to disruption of the vinylogous amide and consequent activation of the cyclopropane ring. This leads to a SN2 reaction involving attack by the N-3 position of adenine

Research now focuses on ways in which these compounds can be developed to reduce the side effects which have prevented progression within clinical trials. Our group has previously published a synthesis of the alkylating subunit of DSA which is substituted for solid phase synthesis.[2] This has allowed the formation of a large number of analogues of the DSA structure at a greater ease. The previous synthesis of the alkylating unit involved benzyl protection of the phenolic oxygen allowing for the formation of large amounts of the protected, inactive alkylating unit. The deprotection of the benzyl group was then completed subsequent to cleavage from the solid phase resin.

We now present current investigations into a new protecting group strategy for the synthesis of the DSA alkylating subunit which allows simultaneous cleavage from the resin and deprotection to from the active DSA analogue (Fig. 2). The new strategy utilises a *p*-methoxybenzyl protecting group whose acid sensitivity allows for deprotection under cleavage conditions. The development of this strategy will allow for the removal of the sometimes difficult benzyl deprotection step and will further enhance the synthesis of DSA analogues at ease.

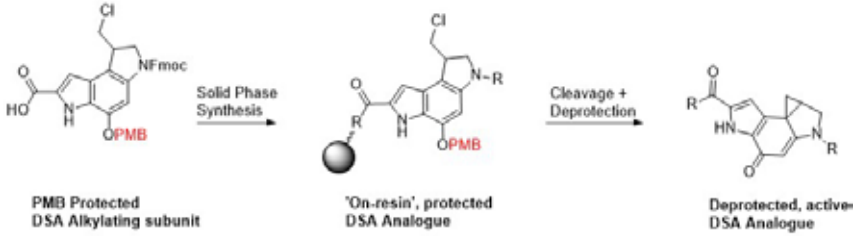

Figure 2: PMB protected DSA alkylating subunit can be introduced onto the solid phase for analogue synthesis. Subsequent cleavage from the resin and PMB deprotection allow for the formation of the active drug in one step

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POLYCYCLIC SULFOXIMINES AS SCAFFOLDS FOR LIBRARY PRODUCTION

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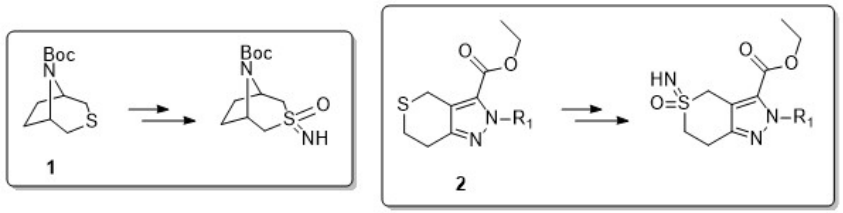
Sulfoximines are a relatively underexplored class of functional groups which only recently has received some attention. Up to now, the main biological applications of sulfoximines are as "suicide substrates" (for example against glutamine and glutamylcysteine synthase) or as transition state analogues. Biologically active sulfoximines were found as antiviral agents, radiation induced drugs, antifungal agent and antitumor drugs -malignant melanoma.[1] Despite this, sulfoximines can be considered a neglected opportunity in medicinal chemistry.[2]

In our efforts for the European Lead Factory we designed two sulfoximine scaffolds, starting with the corresponding sulfides. We explored their synthesis and showed the chosen approach to be amenable to library production.

Sulfide scaffolds known from the literature[3-4] were converted into sulfoximines in a three-step sequence, including mono-oxidation, Ru-catalyzed trifluoroacetamide addition and hydrolysis. Subsequent standard manipulations resulted in the building-blocks required for library synthesis.



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SYNTHESIS AND ANTIPLASMODIAL EVALUATION OF IMIDAZOPYRIDAZINE ANALOGUES

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According to the World Health Organisation (WHO), 198 million cases and 584,000 malaria-related deaths were reported in 2013. Children in Africa are the most affected with estimates indicating that a child dies from malaria every minute. The malaria eradication efforts have been hampered by the emergence of drug-resistant strains of malaria parasites. Recent reports have also documented signs of artemisinin resistance, a very important first-line antimalarial drug in current clinical use (Dondorp *et al*., **2010**; Rueangweerayut *et al*., **2012**; and Phyo *et al*., **2012**). Thus, there is an urgent need to develop structurally diverse antimalarial drugs to target the drug-resistant strains of the malaria parasites.

Imidazopyridazine derivatives have recently been explored as antimalarial agents. Recent reports on this class of molecules have demonstrated promising *in vitro* activity and *in vivo* efficacy albeit undesirable physico-chemical and pharmacological properties have been noted (Le Manach *et al*., **2014**).

In an effort to expand the scope of structure activity relationship (SAR) studies, and counter undesirable physico-chemical as well as pharmacological properties initially associated with this class of molecules, we report the synthesis and pharmacological evaluation of new analogues.

DEVELOPMENT AND SCOPE OF A NOVEL METHOD FOR THE SYNTHESIS OF 5-MEMBERED AROMATIC AZA-HETEROCYCLES

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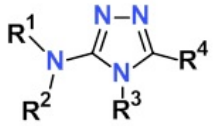
Abstract: 3-Amino-substituted 1,2,4-triazoles are structures of great interest in the field of medicinal chemistry. Since they offer an elegant way to introduce polarity in a molecule without a basic center due to their large dipole moment and strong hydrogen-bond accepting capacity, they have been widely used in drug discovery. Examples include oxytocin and vasopressin receptor, ACAT, GlyT1 and 2, IL-2 production, 11beta-HSD1 and dipeptidyl peptidase IV inhibitor programs. The oxytocin antagonist PF-3274167 had been in early clinical trials at Pfizer for the treatment of urinary incontinence and the treatment of disorders of sexual function and reproduction. The most common route to synthesize 1,2,4-triazoles includes activated S-methylthioimidate intermediates that have to be synthesized using hazardous reagents by a thionation reaction with Lawesson's reagent or phosphorus pentasulfide followed by S-alkylation with iodomethane. Another route is the activation as chloroimine-intermediates, which has very limited scope due to low stability and moisture sensitivity of these intermediates. We will present a practical and novel two-step procedure for the synthesis of 3-amino-substituted 1,2,4-triazoles featuring a shelf stable intermediate and avoidance of toxic reagents and waste products . The scope of our method is broad allowing the preparation of 1,2,4-triazoles substituted with aromatic as well as primary or secondary alkyl groups in the 4- and 5-positions and with aliphatic or aromatic secondary amine residues in the 3-position.



R^1, R^2, R^3, R^4 = 1° or 2° alkyl, (hetero)aryl

NOVEL CONCEPTS FOR BIOISOSTERIC SWITCH AND SCAFFOLD-HOPPING

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Isosteric replacement and scaffold hopping are powerful strategies to improve ADME properties and enter into new chemical space. The design of novel synthetic methods and molecular classes, as well as their evaluation in direct comparison with more classical fragments is a necessity to expand the toolbox of medicinal chemists. Understanding the advantages of such new motifs is of particular relevance to support rational design.

Our continuous efforts to expand chemical diversity and provide useful tools for drug discovery has led to the identification of several classes of molecules with potential impact in the field of isosterism. We will present recent work done to expand the repertoire of isosteric switches and scaffold hoping motifs and novel methods to facilitate their introduction. Finally, we will present results aiming at understanding their properties in order to accelerate their adoption in drug discovery programs.

STRUCTURE-BASED EVOLUTION OF AN ALLOSTERIC MMP-13 INHIBITOR TO DUAL BINDING MODE INHIBITORS WITH IMPROVED LIPOPHILIC LIGAND EFFICIENCIES

Thomas Fischer, Rainer Riedl

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Matrix Metalloprotease-13 (MMP-13) or collagenase-3 is one of the enzymes belonging to the zinc-depending endopeptidase family and is involved in angiogenesis as well as in tissue remodeling. An over activity of MMPs can lead to various pathological processes such as rheumatoid arthritis or tumor growth and metastasis for example [1].

In our present work, we evolved one of our previously identified allosteric inhibitors **1** [2] of MMP-13 using structure-based design and organic synthesis. All newly synthesized compounds showed elevated activities compared to the initial inhibitor. By targeting the catalytic zinc (II) ion with a zinc binding group (ZBG), we were able to decrease the IC_{50}-value from 9800 nM to 134 nM [3].

By variation of the ZBG we obtained compounds displaying lower clogP values while maintaining their potency. This led to a raise of the lipophilic ligand efficiencies from 1.07 to 2.91 which is in the range of lead like compounds.

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CARBONYLATIVE CYCLIZATION OF ALIPHATIC AMINES VIA C–H ACTIVATION: A POWERFUL TOOL FOR LATE STAGE DIVERSIFICATION OF DRUG CANDIDATES

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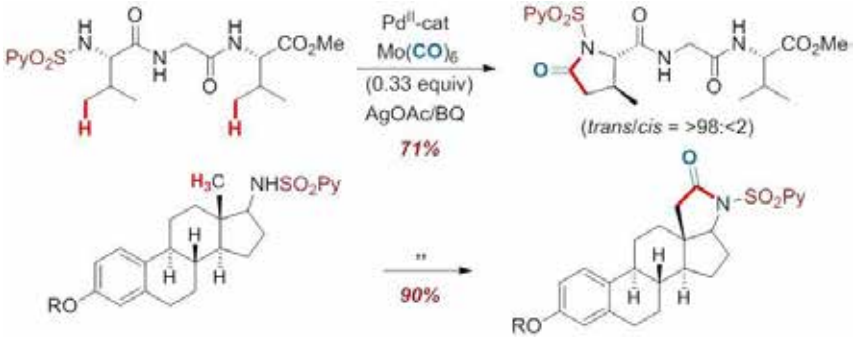
Aliphatic amine derivatives, especially amino acids and peptides, are an important class of compound in medicinal chemistry, as evidenced by their presence in a number of bioactive products and their use as lead compounds in drug discovery. Therefore, new methodologies for the straightforward chemical modification of such structural motifs could fuel lead discovery and optimization via a rapid library development. Although the benefits of direct functionalization of inert C–H bonds for rapidly introducing complexity and diversity on a core molecule are obvious, few methods have demonstrated to be amenable to late-stage diversification of complex multifunctional molecules.[1] On the other hand, compared to the landmark development of direct functionalization of (hetero)arenes, the activation of inert C(sp^3)–H bonds, especially methods involving carbonylation, is much more challenging and remains underdeveloped.[2]

Here we report the development of a practical and reliable Pd-catalyzed γ-selective C(sp^3)–H carbonylation/cyclization of *N*-(2-pyridyl)sulfonyl-protected[3] aliphatic amines leading to densely functionalized γ-lactams. The use of a substoichiometric amount of $Mo(CO)_6$ (0.33 equiv) as a nonhazardous, air-stable solid source of CO not only avoids the difficulties in handling toxic CO gas, but also enables slow in situ release of CO, thus preventing catalyst deactivation. Importantly, this carbonylation protocol is amenable to the late-stage diversification of complex multifunctional molecules.



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MICROWAVE HEATED CONTINUOUS FLOW SYNTHESIS OF ANGIOTENSIN II TYPE 2 RECEPTOR [AT2R] LIGANDS

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2) Science for Life Laboratory, Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden.
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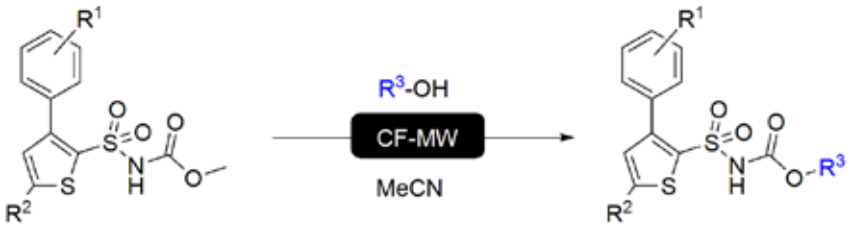
During the past two decades, continuous-flow (CF) synthesis has attracted significant interest due to the advantages in regards to safety, handling and scale-up. The predominant mode of heating in CF synthesis has previously been conductive heating, suffering from drawbacks such as time requirements regarding both heating and cooling. Combining CF with microwave (MW) heating has attracted much attention in recent years as it allows fast reaction optimization with quick temperature control. We have previously reported on a purpose built CF system utilizing a non-resonant MW applicator for heating allowing uniform heating due to an axial MW field.[1]

Using the CF-MW system, variability can be introduced to a structure in a swift and straightforward fashion, allowing for an exploration of the structure-activity relationship (SAR) of biologically active compounds. Our group has previously reported agonists and antagonists of the angiotensin II type 2 receptor (AT2R).[2, 3] AT2R is a GPCR of the renin-angiotensin system (RAS), well-known for its blood-pressure regulation and fluid/electrolyte balance. The role of AT2R has long been debated and is still today not fully understood; it has been suggested to act opposing AT1R, exert wound healing and anti-inflammatory properties, as well as promoting neuroprotection and neuronal regeneration.[4] We here present AT2R ligands produced using the CF-MW system via the key reaction described in scheme 1 below.[5]



Scheme 1: Key transformation utilizing the CF-MW system.

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SYNTHESIS OF CONFORMATIONALLY RESTRICTED SCAFFOLDS BY DOUBLE-MANNICH REACTION OF CYCLIC KETONES

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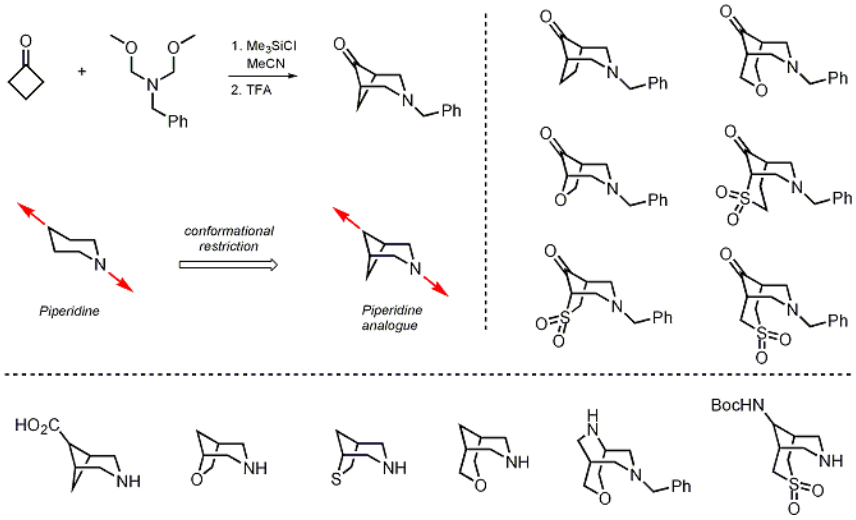
Conformational restriction is an effective tool used in medicinal chemistry to improve/modify pharmacological profiles of lead compounds. Due to a fixation of the functional groups in a biologically active conformation, the sterically restricted compounds are often more efficient and selective ligands for various targets, thus displaying pronounced biological activity.[1]



In this work, we synthesized a library of novel conformationally restricted bicyclic scaffolds by Double-Mannich reaction of cyclic ketones. Details of the synthesis and application of the obtained compounds will be discussed.

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DESIGN, SYNTHESIS AND APPLICATION OF NOVEL FLUORINATED AMINES

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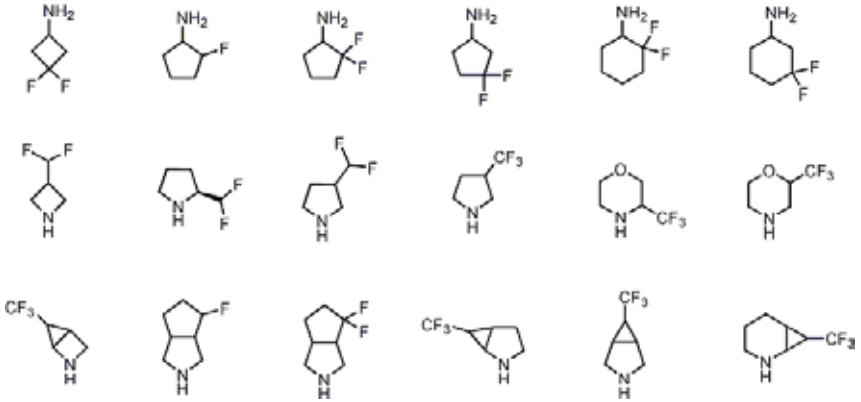
Modern drug discovery is hard to imagine without fluorine: ca. 20% of all pharmaceuticals contain this element. To date, however, only a tiny part of the theoretically possible building block structures are synthesized. Many simple combinations of fluorine with carbon and nitrogen atoms are still unknown.

Commercially accessible fluorinated alicyclic amines are mostly limited to pyrrolidines and piperidines. The latter are quite popular in medicinal chemistry (Figure a). In this work, we synthesized a library of novel aliphatic saturated amines (Figure b).[1-9] Details of their design and synthesis will be reported.



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SYNTHESIS OF NOVEL UNIQUE PYRROLIDINES BY [3+2]-CYCLOADDITION OF AZOMETHINE YLIDES WITH ELECTRON-DEFICIENT ALKENES.

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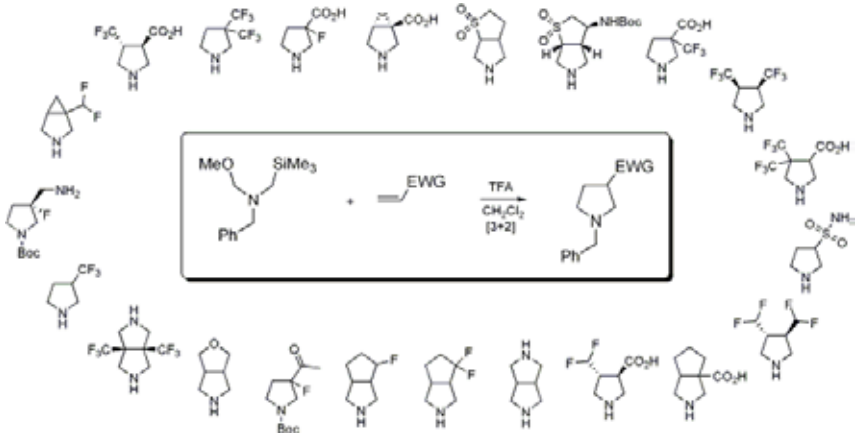
Pyrrolidine is one of the most frequently used aliphatic amines in medicinal chemistry: its residue can be found in at least ten FDA-approved drugs. Therefore, elaboration of practical synthetic approaches to novel substituted pyrrolidines is important.



In this work, we synthesized a library of novel unique pyrrolidine building blocks using [3+2] cycloaddition between electron-deficient alkenes and azomethine ylides.[1-4] Some synthesized structures are mimics of morpholine, β-proline derivatives, and fluorinated pyrrolidines.

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FROM NEW CHEMICAL ENTITIES TO ANTIVIRALS, TO HUMAN DIHYDROOROTATE DEHYDROGENASE INHIBITORS

Marianne Lucas-Hourani [1], Hélène Munier-Lehmann [2], Farah El Mazouni [3], Nicholas A. Malmquist [4], Jane Harpon [4], Eloi P. Coutant [2], Sandrine Guillou [2], Olivier Helynck [2], Gigliola Zanghi [2], Anne Noel [2], Artur Scherf [4], Margaret A. Phillips [3], Frédéric Tangy [1], Pierre-Olivier Vidalain [1], Yves L. Janin [2]

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2) Département de Biologie Structurale et Chimie, Institut Pasteur, Paris, France
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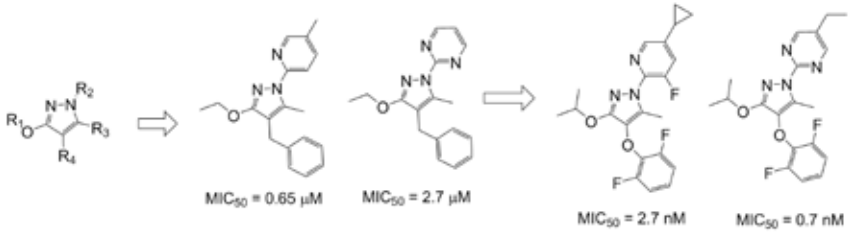
4) Département de Parasitologie et Mycologie, Institut Pasteur, Paris, France

From a research program based on the design of new chemical entities followed by extensive screening on various models of infectious diseases, an original series of 2-(3-alkoxy-1*H*-pyrazol-1-yl)azines endowed with notable antiviral properties were found. Using a whole cell measles virus replication assay, we describe here some aspects of the iterative process which led to a thousand fold improvement of antiviral activity with nanomolar level of inhibition as well as our attempts to improve their microsomal stability.[1-3]



MIC_{50} on measles virus replication

Moreover, recent precedents in the literature[4, 5] describing antiviral derivatives acting at the level of the de novo pyrimidine biosynthetic pathway led us to determine that the mode of antiviral action of this series is due to the inhibition of the cellular dihydroorotate dehydrogenase (DHODH), the fourth enzyme of this pathway.[6]

Since these compounds are more active than teriflunomide, an inhibitor of human DHODH used for the treatment of autoimmune diseases, our work is opening at least perspectives for their uses as tools or for the design of an original series of immunosuppressive agent.

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NOVEL ISOCYANIDE-BASED MULTICOMPONENT REACTION AND ITS APPLICATION FOR FOCUSED LIBRARIES DESIGN

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Isocyanide-based multicomponent reactions (IMCR) have become a valuable resource-economy synthetic tool of medicinal chemistry since they make easily accessible drug- and nature-like small molecules with privileged heterocyclic cores. Recently, we have developed a novel IMCR that leads to 2-amino-1,4-diazaheterocycles starting from diamines and carbonyl compounds in one simple synthetic step. The IMCR has been shown to be applicable for a wide range of diamines, carbonyl compounds, and isocyanides, whose structural variety defines diversity of obtainable amino-azaheterocycles including fused and spiro-heterocycles.

Applicability of the IMCR as an efficient tool for focused libraries design, such as toll-like receptors TLR-7, beta-secretase (BASE), nitric oxide synthetase (NOS-2) ligands, orexin receptor and cannabinoid receptor CB2 antagonists, mGlur5, TGR5 and RAR-related orphan receptor gamma (RORγ) modulators, and others, will be discussed.

NOVEL CHEMICAL REAGENTS - SUBSTITUTED DIAZOALKANES - FOR THE SYNTHESIS OF BIOACTIVE COMPOUNDS

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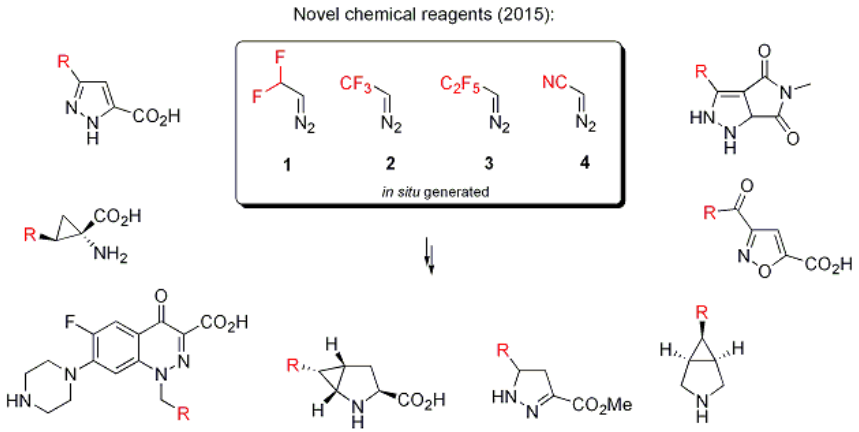
Modern medicinal chemistry requires novel chemical reagents to prepare bioaktive compounds. Quite recently, we developed several novel chemical reagents - **1**-**4** - and studied their reactions: cyclopropanation, carbene insertion, [3+2]-cycloaddition, Japp-Klingemann reaction, *etc*. [5-7]

The obtained reagents were used to prepare novel analogues of the antibacterial drug – Ciprofloxacine.



Analogues of Ciprofloxacine

We believe that novel reagents **1-4** will make a huge impact in organic and medicinal chemistry very soon.

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DESIGN, SYNTHESIS AND APPLICATION OF NOVEL BUILDING BLOCKS TO "ESCAPE THE FLATLAND" IN MEDICINAL CHEMISTRY

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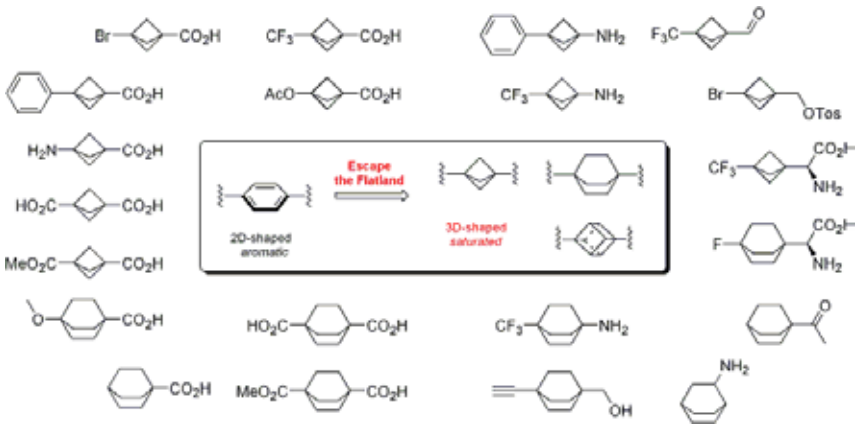
Given the modern trend in medicinal chemistry – "Escape the Flatland"[1] – saturated 3D-shaped building blocks do play an important role.[2] Compared to their aromatic 2D-shaped counterparts, the saturated analogues usually possess higher water solubility, higher activity and lower toxicity.



In this work, therefore, we have rationally designed and synthesized a library of novel saturated bioisosters of benzene. Details of the synthesis and application of the obtained compounds will be discussed.[3-5]

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RAPID ACCESS TO NOVEL MULTIFUNCTIONAL SPIROCYCLIC CORES FOR DRUG DISCOVERY

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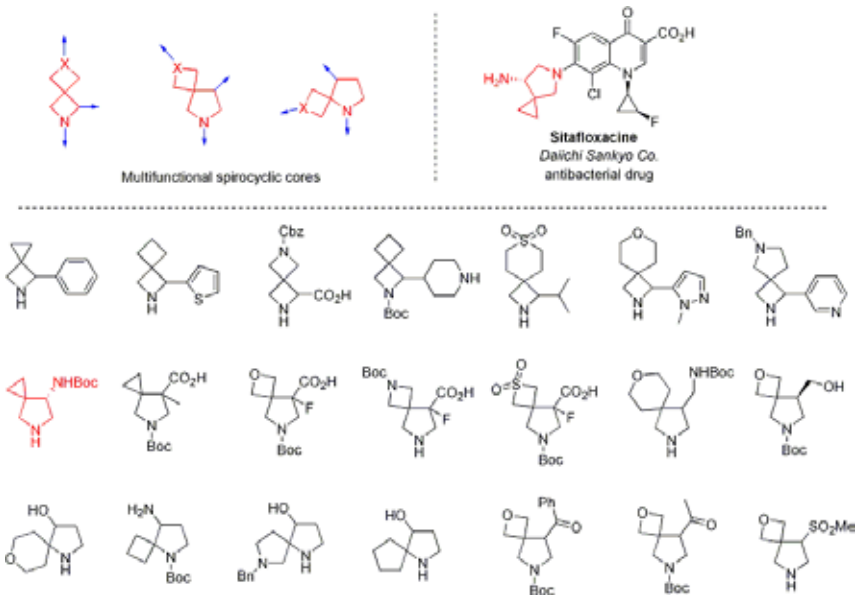
Trends in drug discovery are changing rapidly. During the past decade, terms "*Scaffold hopping*," "*Escape the Flatland*" and "*Conformational restriction*" have been introduced, and have already found huge practical application. Spiro compounds are especially interesting, because they are intrinsically 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]

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SYNTHESIS OF NEW CARBOCYCLIC C-NUCLEOSIDE ANALOGS

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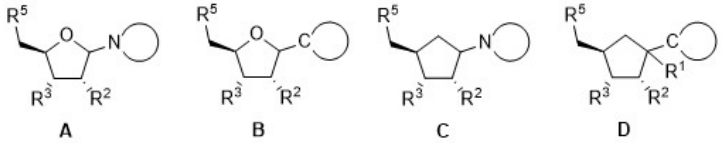
Development of nucleoside analogs has been a major research area in medicinal chemistry. Classical nucleosides (**A**) constitute an important class of biologically active compounds with attractive antiviral and anticancer properties.[1] Since they possess the labile hemiaminal motif, extensive effort has been invested into the identification of more stable substances, e.g. C-nucleosides (**B**) or carbocyclic N-nucleosides (**C**), while preserving the biological activity.



It is conceivable that, at least in some cases, carbocyclic C-nucleosides (**D**) might be even more robust versions of nucleoside analogs **(B)** and **(C).** In addition, installation of certain substituents (e.g. R^1 = OH) is meaningful only in this class as this would lead to chemically unstable ketals and aminals in the series **(A)**, **(B)** and **(C)**. However, analogs **(D)** are quite rare and most published syntheses produce only single target compounds.[2]

Our recently developed flexible synthesis of compounds **(D)** enables selective manipulation of individual positions around the cyclopentane ring, including highly diastereoselective installation of carbo - and heterocyclic substituents at position 1, orthogonal functionalization of position 5, and efficient inversion of stereochemistry at position 2. Some of the newly prepared carbocyclic C-nucleosides were found to inhibit certain human DNA glycosylases.

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6-NITROTETRAZOLO[1,5-A]PYRIMIDINES: STRUCTURES WITH ITS OWN VALUE AND SYNTHETIC PERSPECTIVES

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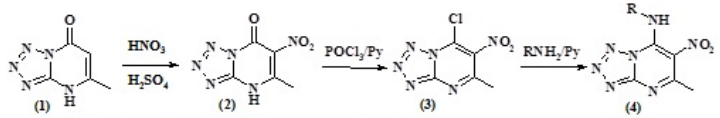
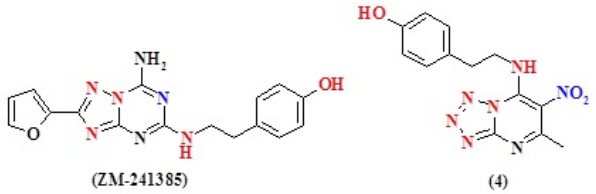
Tetrazolo[1,5-a]pyrimidines are relatively little studied class of compounds, but these compounds are very interesting because of their structural similarity to biologically active heterocycles. Furthermore, the possibility of the tetrazole ring destruction provides wide opportunities for the synthesis of different biological active structures. However, the synthetic approaches for nitro derivatives and their further modification remains a challenge today in this class of compounds.

We have developed a scheme for the preparation 6-nitro-7-alkylaminotetrazolo[1,5-a]pyrimidines (4). The nitration reaction by sulfuric and nitric acids nitrating mixture, based on readily available heterocycle (1), gives undescribed nitrocompound (2) with good yield. Also we have designed a method of chlorodeoxygenation for compound (2) with isolation of halogen-heterocycle (3) which proved to be stable compound with a low melting point. Further reaction of (3) with alkylamines gives a 7-alkylaminoheterocycles (4).



R = *n*-Pr; *i*-Pr; *i*-Bu; t-Bu; *n*-Bu; $(CH_2)_{1-4}OAc$; $(CH_2)_2Ph$-4-Cl; $(CH_2)_2Ph$-4-OH

First of all, these 6-nitro-7-alkylaminotetrazolo[1,5-a]pyrimidines (4) are perspective precursors of biological active purines as further reduction of the nitro group and the subsequent construction of a carbon bridge between vicinal amino groups leads to the tetrazolopurines structure. Further destruction of the tetrazole ring provides access to 7-substituted purines and this approach is regiospecific, in contrast to existing ones. On the other hand, compounds (4) have an independent value since they model structure of synthetic inhibitors triazolotriazines series of adenosine receptors.



(ZM-241385) (4)

6-Nitro-7-alkylaminotetrazolo[1,5-a]pyrimidines model key features of the ZM-241385 structure, such as an alkylamino group, which is responsible for the activity of compound in the extracellular space, as well as the π-system of 1,2,4-triazole ring, which is responsible for binding to the receptor. The main know-how of the proposed approach is to simulate one of the heterocyclic nitrogen atoms in the triazine ring of the original structure by electron-equivalent nitrogroup Despite the widespread toxicity of the nitro-derivatives it was shown that 6-nitrotriazole[1,5-a]pyrimidones possess high antiviral and other biological activity combined with low toxicity [1].

Thus, the synthesis method of 6-nitro-7-alkylaminotetrazolo[1,5-a]pyrimidine via nitration, amination and chlorodeoxygenation reactions was developed in this work. The synthesized compounds are valuable as precursors of natural heterocycles with biological activity, as well as they have an independent value as structural analogues of adenosine receptor inhibitors.

We thank the Russian Foundation for Basic Research grant № 16-33-00159 mol_a.

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DESIGN, SYNHTEIS AND APPLICATION OF NOVEL MORPHOLINE ANALOGUES

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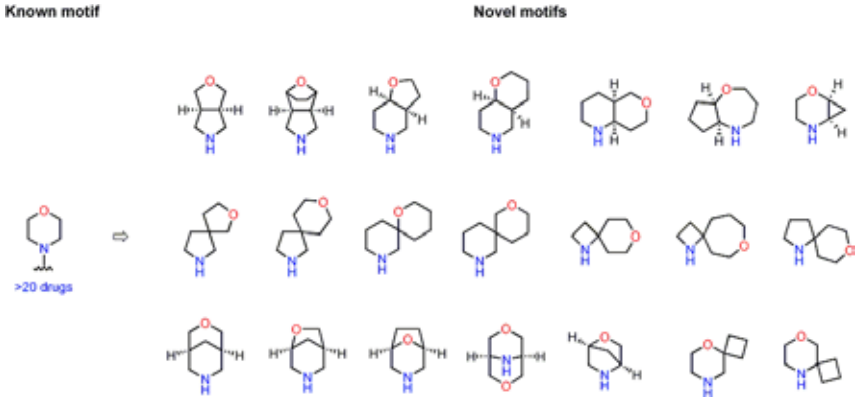
Morpholine is an established building block in drug discovery – more than 20 FDA-approved drugs contain its residue. In this work, therefore, we have rationally designed and synthesized a library of novel/previously scarcely available bicyclic morpholine surrogates. Details of the synthesis and application of this library will be discussed.[1,2]



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DEVELOPMENT OF NOVEL BUILDING BLOCKS TO ACCELERATE DRUG DISCOVERY

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>96% of medicinal chemistry projects fail. Often, medicinal chemists can not fine-tune the chemical structure of lead-candidates, because of low availability of the corresponding building blocks: many tiny molecules with 4-6 carbon atoms still remain unknown.

Therefore, recently we started a project on developing novel structures for drug discovery. We first rationally designed each compound following the principles of "Conformational restriction," "Escape the Flatland"[2] and "Scaffold hopping."[3] Then, we synthesized diverse libraries of novel *morpholine surrogates* (Figure 1a), *unusual scaffolds*, and *fluorinated amines* (>500 structures).[4]

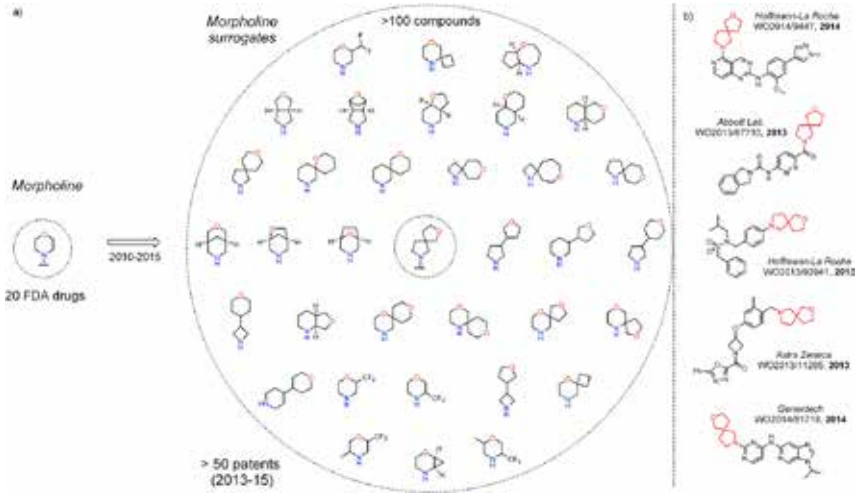

Figure 1. a) Novel *morpholine surrogates*; b) and their application in drug discovery.

Indeed, after publishing the synthetic details,[4] and making the compounds commercially available, they found a huge practical application in drug discovery (Figure 1b).[5] We do believe, that these structures will inevitably lead to discovery of novel drugs very soon.

Details of the compounds design, synthesis and application in drug discovery will be discussed.

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4) We published >40 manuscripts on these topics. Please, see: www.enamine.net / Research / Publications http://www.enamine.net/index.php?option=com_content&task=view&id=180
5) These morpholine surrogates are already covered in >50 patents (2013-15) by diverse pharmacompanies (Figure 1b).

SYNTHESIS AND APPLICATION OF UNNATURAL PROLINE ANALOGUES: ADVANCED BUILDING BLOCKS FOR MEDICINAL CHEMISTRY

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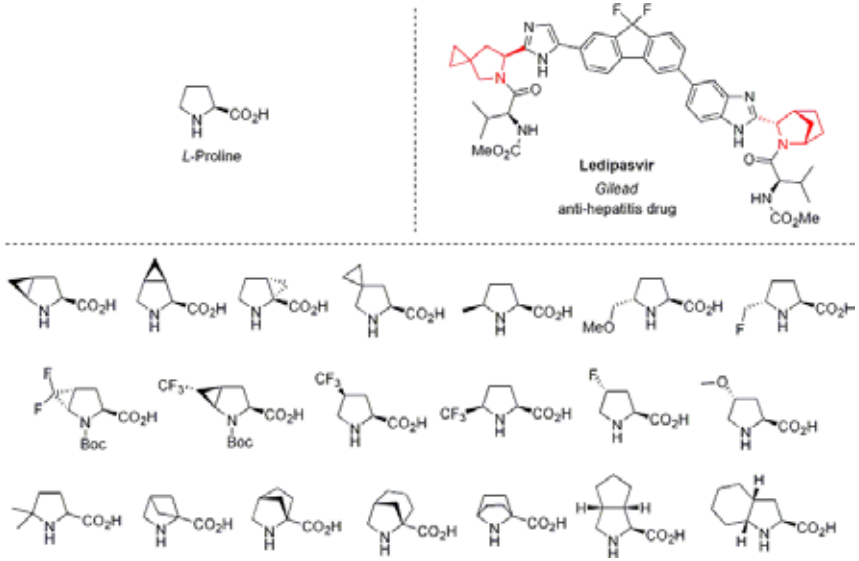
L-Proline is a natural amino acid playing an important role in drug discovery as a cheap chiral bifunctional building block. In this context, over the past decade unnatural analogues of Proline also became extremely popular. For example, in 2010 Gilead launched *Ledipasvir* – a drug bearing the residues of two unnatural analogues of *L*-Proline.



In this work, we have rationally designed, synthesized and applied a library of novel/previously scarcely available analogues of Proline in medicinal chemistry. Details of the synthesis and application of the obtained compounds will be discussed.[1-9]

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ENGINEERING OF P450 3A4 FOR THE HYDROXYLATION OF C-H BONDS IN THE SYNTHESIS OF PHARMACEUTICALS

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Department of Chemistry, McGill University, Canada

Despite decades of research, hydroxylation at unactivated carbons is still a great challenge for organic chemists. Traditional methodologies often require the use of toxic metals couples with high temperatures, or a series of protection/deprotection steps that negatively impact on the amount of generated waste. Over the last years the use of cytochromes P450 enzymes (P450s) as biocatalysts for the hydroxylation of C-H bonds has gained momentum. P450s are not only capable of oxidizing a broad spectrum of substrates, they also perform best in aqueous environment, under mild conditions of pressure and temperature. Here, we describe our approach to engineer P450 3A4, a human enzyme involved in the metabolism of > 50 % of marketed drugs. By using rational mutagenesis, we were able to demonstrate that it possible to control the regio- and stereoselectivity of hydroxylation, and that the methodology can be applied the synthesis of compounds of pharmaceutical interest including steroids and theobromine derivatives. A variety of techniques including chiral-HPLC, LC-MS, computational predictions, PCR-directed mutagenesis and synthetic methodologies will be discussed.

SOLID PHASE-ASSISTED SYNTHESIS OF PLINABULIN-OCTAARGININE CONJUGATE AS A DISULFIDE-TYPE PRODRUG

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Plinabulin, a microtubule depolymerization agent (**1**, IC_{50} = 15 nM, HT-29 cell), is a potent intravenous vascular disrupting agent and its clinical trial as antitumor drug is undergoing (Phase III).[1)] Recently, to improve its very low water solubility (< 0.1 mg/mL), we developed water-soluble prodrugs by liquid-phase reaction with hydrophilic amino acid derivatives, using a Huisgen reaction.[2)]

In the present study, to increase tumor selectivity of **1**, we conducted the synthesis of an alternative prodrug **2** possessing tumor-targeting octaarginine structure.[3)] However, the conjugation of these two units failed due to their distinct solubility with reaction solvents. To solve this problem, we developed a new solid-phase method to conjugate two units through disulfide bond by using the 3-nitro-2-pyridinesulfenyl chloride (Npys-Cl) resin (**3**).[4)] This resin **3** captures the compound with a sulfide group in the first step, resulting in the formation of an active disulfide on the resin, then release the newly produced disulfide into solution by addition of the thiol compound in the second step. Therefore, distinct solvent systems, which are suitable to solubilize respective reaction substrates, can be adopted in each step. For the synthesis of conjugate **2**, a prepared plinabulin sulfide derivative was first loaded on the resin **3** in CH_3CN or CH_2Cl_2. After loading (92%, measured from starting material disappearance), the resin was sequentially washed with CH_3CN and water, respectively, and reacted with a Cys-containing octaarginine peptide under various aqueous conditions to obtain the desired conjugate **2** (Plinabulin-SS-Arg_8) by disulfide exchange reaction. The pH sensitive second reaction was efficiently carried using solvent system of 40% CH_3CN/acetate buffer (pH5.0), and conjugate **2** was obtained in a 77% yield. Therefore, it is suggested that two-step solid phase disulfide conjugation method is useful to conjugate two units with different physicochemical properties. Conjugate **2** as a prodrug showed a 10-fold lower cytotoxicity than **1** against HT-29 cells. In vivo tumor targeting ability of **2** will be evaluated in near future.

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STUDIES TOWARDS A TOTAL SYNTHESIS OF ELEUTHEROBIN AND ANALOGUES

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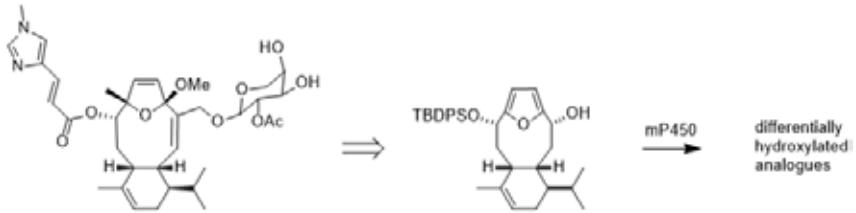
Eleutherobin (**1**) is a marine natural product that has been determined to possess promising anticancer activity arising through microtubule stabilisation, a mode of action similar to that of Taxol.[1] Its total synthesis has been reported in the past by two groups,[2,3] but the quantities obtained have not permitted a full in vivo evaluation of its therapeutic potential.

This project aims to develop a synthetic route that allows quick access to the minimally oxidised core of the natural product, which will then be subjected to late-stage oxidation methodologies for the generation of analogues. To this end a panel of mutant P450 oxygenases, developed by the Wong group to achieve site-specific oxidation of complex organic molecules,[4] will be employed to provide differentially hydroxylated analogues and at the same time gain information that would make this methodology more widely applicable.

A number of synthetic approaches to the eleutherobin core based on an intramolecular Diels-Alder reaction to construct the cyclohexene ring will be presented, culminating on a formal synthesis of the natural product that provides access to the desired core structure in the form of compound **2**. Preliminary results from mP450 oxidation studies will also be presented, along with initial results from cancer cell toxicity studies of intermediates.



1 **2**

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THE TOOLS FOR LEAD-ORIENTED SYNTHESIS: CASE STUDIES FROM KYIV

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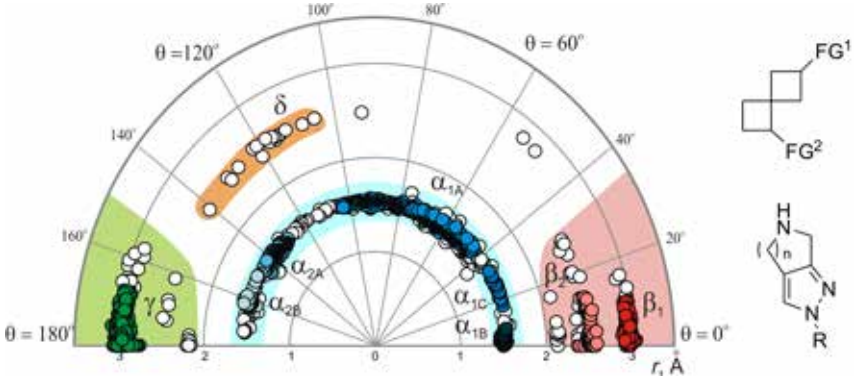
Since 2012, when the concept of lead-oriented synthesis (LOS) was introduced, it remains significant challenge for synthetic organic chemistry and related disciplines. While ADME physico-chemical parameters involved in the LOS idea can be easily predicted for novel molecules, the problem of analysis and visualization of three-dimensional nature of the molecular scaffolds (which is another feature usually associated with LOS) is still not fully solved. On the other hand, diversity-oriented synthesis (DOS) has provided many successful stories for chemical biology, whereas its use for the needs of LOS has been limited. In this presentation, we describe some case studies which address these topics:

- A simple geometric model was used to construct exit vector plots (EVP) for analysis and visualization of 3D chemical space covered by bifunctional scaffolds. Their use was demonstrated for disubstituted cycloalkanes [1] and bicyclic diamines [2]; in particular, correlation of the parameters with biological activity was found.
- Several examples of using “DOS for LOS” approach are described, namely, for the synthesis of pyrazole and spiro[3.3]heptane derivatives [3–5].



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NOVEL HIV-1 NNRTI AGENTS: OPTIMIZATION OF DIARYLANILINES WITH HIGH POTENCY AGAINST WILD-TYPE AND RIPLIVIRINE-RESISTANT E138K MUTANT VIRUS

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Following our prior studies about novel HIV-NNRTIs,[1-5] three series (**6**, **13**, and **14**) of new diarylaniline (DAAN) analogues were designed, synthesized, and evaluated for anti-HIV potency, especially against a rilpivirine (**1b**) resistant strain carrying the most prevalent E138K mutant. Promising new compounds were further assessed for physicochemical and drug-like properties, including aqueous solubility, log P value, and metabolic stability, as well as predicted lipophilic parameters of LE, LLE, and LELP indices. Among the synthesized new compounds, **6a**, **14c**, and **14d** exhibited high potency against wild-type (EC_{50} 5-6 nM) and the **1b**-resistahnt E138K (EC_{50} 10-12 nM) viral strains with low resistance fold changes (FCs) of 1.9–2.2 as well as good balance between anti-HIV-1 activity and desirable drug-like properties (such as aqueous solubility >25 μg/mL, log P 2.8-3.2). Further computational modeling studies also revealed insights into critical structural requirements for greater efficacy against the E138K drug resistant mutant.

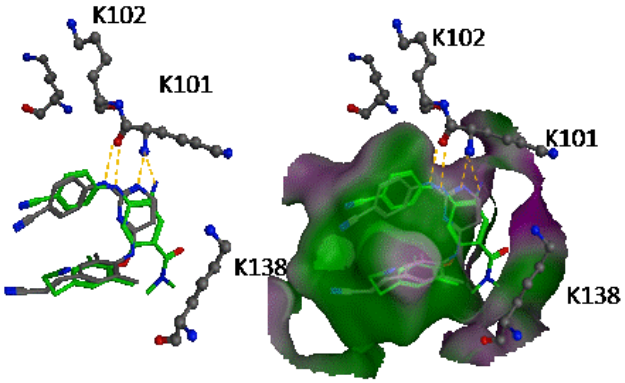

Figure 1. Predicted binding modes of **6a** (green stick) and ligand **1b** (rilpivirine, gray stick) with the E138K mutant viral RT crystal structure (2HNY).

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NOTES

POSTERS

Modulation of Protein-Protein Interactions - Novel Opportunities for Drug Discovery

EXPLOITING THE SELECTIVE NOXA PEPTIDE TO REGULATE THE PROTEIN-PROTEIN INERACTIONS OF THE PRO-SURVIVAL PROTEIN MCL-1 WITH SMALL MOLECULES

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The ability to evade apoptosis is a hall mark of cancer. Apoptosis is a highly conserved and controlled process, with the Bcl-2 family of proteins playing an important role as key regulators.[1] The family consists of both pro-survial (including Bcl-2, Bcl-x_L and Mcl-1) and pro-death proteins (including BAX and BAK) which are carefully balanced with regulating proteins (BID, NOXA, BIM and BAD) within the cell to control its fate.[2] High levels of the anti-apoptotic proteins are often observed in cancer and not only contribute to the development of the tumour but also confer resistance to current therapies including chemotherapy and radiation treatment.[3]

The anti-apoptotic Bcl-2 family of proteins are well-validated anti-cancer targets. The most successful small molecule inhibitors to date, ABT-737 and its orally available analogue Navitoclax, inhibit Bcl-2 and Bcl-x_L with sub-nanomolar affinity,[4] but like most small molecule Bcl-2 inhibitors, they do not inhibit myeloid cell leukemia-1 (Mcl-1) and lacks efficacy in tumours with high levels of Mcl-1.[5] Elevated levels of Mcl-1 are one of the most commonly observed abnormalities in human cancer,[6] and overexpression is linked to resistance observed against paclitaxel and vincristine.[7] Recent studies have shown that knockdown of Mcl-1 in pancreatic cancer cells treated with ABT-737 triggers apoptosis, indicating Mcl-1 is an important and significant therapeutic target in this type of cancer.[8]

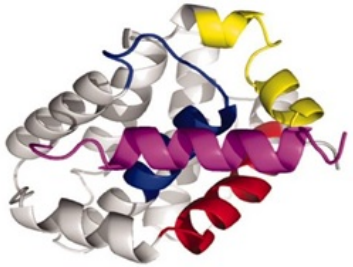

Figure 1. Ribbon diagram showing the mNoxaB BH3 (Bcl-2-homology region 3) peptide in purple and the BH1, BH2, and BH3 regions of hMcl-1 in blue, yellow, and red, respectively. (Reproduced from Czabotar PE, Lee EF et al.[9])

This led us to consider using the Noxa peptide to develop Mcl-1 selective inhibitors. Unlike some pro-apoptotic members of the Bcl-2 family which bind to several anti-apoptotic proteins, Noxa demonstrates high selectivity and affinity towards Mcl-1. For example a NoxaB-(75-93)-C75A peptide derived from the Noxa protein has been reported as a selective and high affinity binder (Figure 1).[9]

The work described focuses on the exploitation of the selectivity of the mNoxa peptide to exemplify a new strategy for drug discovery, using the peptide as a framework to transfer the efficacy and selectivity to small-molecule modulator leads.

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DISPIRO-INDOLINONES AS NOVEL SMALL MOLECULAR INHIBITORS OF P53-MDM2 INTERACTION

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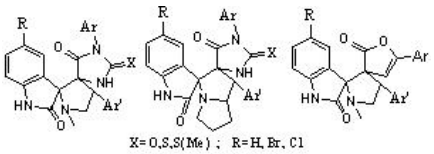
It is known that the cell protein p53 damages the genetic apparatus and it can stop the cell cycle and DNA replication, thereby it launches apoptosis. In regulation of the stability (and activity) of p53 protein a major role plays a protein MDM2 (in human HDM2). This protein binds with p53 and inhibits its action, so it is the MDM2 oncogene [1]. Most of the p53 inhibitors with a promising antitumor activity are complex compounds with conformationally constrained structures [2]. For this case, the synthesis and biological testing of targeting drugs with a conformationally rigid structure for the prostate cancer therapy is an important problem of modern organic chemistry.

Previously in our group [3] the «hit»-compound with the promising anticancer activity 2.1μM was found. For this compound the separation of two diastereomers was made and it was shown, that only one isomer had selectivity and cytotoxic effect for p53.

In this work, we describe the synthesis of dispiro-compounds shown below on the base of 2-thiohydantoins and their S-alkylated derivatives, as well as hydantoins and 1,3-oxazolones as 1,3-cycloaddition substrates:



The biological activity of synthesized compounds was determined in a standard MTT cytotoxicity assay for the cell lines HCT $p53^{(+,+)}$ and HCT $p53^{(-,-)}$, LNCap and PC3 and visualizing the distribution of compound in the cell show that compounds accumulate in cell nucleus.

This work was supported by Russian Foundation for Basic Research, project № 16-33-60166.

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PEX14-PEX5 INTERACTION INHIBITORS PROVIDE A NEW WAY TO TREAT TRYPANOSOMA INFECTIONS

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The parasitic protozoa of the *Trypanosoma* genus infect humans and many domestic mammals causing severe mortality and significant economic losses. The human diseases related to *Trypanosoma* are Human African trypanosomiasis (HAT, Sleeping sickness) caused by *Trypanosoma brucei* ssp. and Chagas disease caused by *Trypanosoma cruzi*. The existing therapies for both diseases exhibit serious side effects, require long treatment schedules and often fail to eliminate parasitemia [1,2].

Trypanosomatids couple glycolytic and peroxisomal function in a single organelle, called the glycosome. As glycosomes completely lack genetic information, all lumen active enzymes are translocated post-translationally. The PEX14-PEX5 protein-protein interaction has a pivotal role for protein translocation into glycosomes by docking of the cytosolic shuttling receptor PEX5 with the membrane-associated protein PEX14[3]. Therefore, inhibiting this interaction has been postulated as a potential way of disrupting glycosome function in *Trypanosoma* [4,5], leading to an accumulation of glycosomal enzymes in the cytosol, which in turn causes runaway phosphorylation of hexoses, ATP depletion and fatal metabolic catastrophe.

We report the first small molecule inhibitors that kill trypanosomes by disrupting the PEX14-PEX5 protein-protein interface. In our structure-based design we mimick the binding mode of the aromatic residues of PEX5 WxxxF/Y to the respective PEX14 pockets. Combining *in silico* screening, NMR, X-ray crystallography and medicinal chemistry we developed small molecular PEX14-PEX5 interaction inhibitors with trypanocidal activity comparable or better than currently approved therapeutics.

While protein-protein interactions are regarded as very difficult targets for drug development, our data indicate that glycosome import is indeed an “Achilles’ heel” of *Trypanosoma* that can be targeted with small, drug-like molecules. Our X-ray crystallographic structural data obtained at very high resolutions will assist future structure-based drug discovery efforts for this molecular target.

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ANTI-MALARIAL AGENTS TARGETING APICAL MEMBRANE ANTIGEN 1

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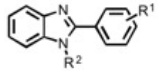
Malaria caused by *Plasmodium falciparum* (*Pf*) remains a global public health problem, causing an estimated 214 million cases and 438,000 deaths in 2015 alone. It is estimated that half of the global human population is at risk of malarial infection, in particular pregnant women and young children. The emergence of *Pf* resistance to first-line antimalarial therapies within South-East Asia has driven the search for new classes of antimalarial agents. Following a fragment screen[1], a range of fragment scaffolds was identified that interacted with the highly conserved hydrophobic cleft of *Pf* apical membrane antigen 1 (AMA1). AMA1 plays an essential role in the invasion of human red blood cells via the formation of a moving junction. Using a Fragment Based Drug Design (FBDD) approach, we developed small molecule inhibitors of the AMA1 hydrophobic cleft that have broad strain specificity.

Herein we report the synthesis and evaluation of the benzimidazole scaffold as a basis for the development of high affinity, strain-independent inhibitors of AMA1.



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A PROTEIN-TARGETING STRATEGY USED TO DEVELOP A SELECTIVE INHIBITOR OF THE E17K POINT MUTATION IN THE PH DOMAIN OF AKT1

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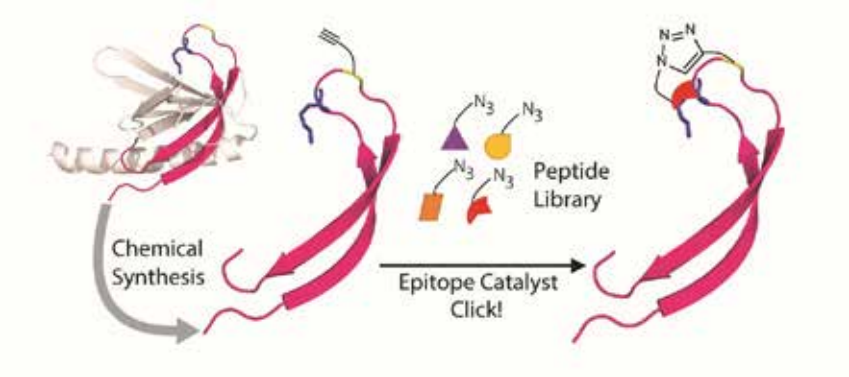
Ligands that can bind selectively to proteins with single amino-acid point mutations offer the potential to detect or treat an abnormal protein in the presence of the wild type (WT). However, it is difficult to develop a selective ligand if the point mutation is not associated with an addressable location, such as a binding pocket. Here we report an all-chemical synthetic epitope-targeting strategy that we used to discover a 5-mer peptide with selectivity for the E17K-transforming point mutation in the pleckstrin homology domain of the Akt1 oncoprotein. A fragment of Akt1 that contained the E17K mutation and an I19[propargylglycine] substitution was synthesized to form an addressable synthetic epitope. Azide-presenting peptides that clicked covalently onto this alkyne-presenting epitope were selected from a library using *in situ* screening. One peptide exhibits a 10:1*in vitro* selectivity for the oncoprotein relative to the WT, with a similar selectivity in cells. This 5-mer peptide was expanded into a larger ligand that selectively blocks the E17K Akt1 interaction with its PIP3 (phosphatidylinositol (3,4,5)-trisphosphate) substrate.



DRUGGING THE FBW7 E3 LIGASE: TOWARDS NEW CHEMINAL PROBES

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Despite a clear opportunity for the development of a new class of therapeutic agents, the scientific community has been slow to invest significant resources and efforts on targeting components of the Ubiquitin-Proteasome System (UPS) and this field is still in its infancy. This has been due to the historical lack of foundational tools and approaches and the significant complexity inherent in this biological system. The extensive protein complexes and multiple steps involved in the ubiquitination process have made targeting E3 ligases technically challenging from a drug discovery perspective. In addition, the general biology and chemistry of the E3 ligases are not as well understood, and these enzymes do not have an easily targetable active site.

One of the most commonly deregulated UPS protein in human cancers is the ubiquitin ligase component Fbw7,[1] which targets a range of substrates for degradation, including some key human oncoproteins including cyclin-E, MYC, Notch and Junk. Several therapeutic strategies to manipulate this protein and/or the multisubunit complex that it forms have been proposed;[2] however, so far, no potent small molecule directly targeting the Fbw7 complex to probe these strategies has been reported.

Using Mdmix,[3] a new computational platform developed in our group, we have identified *hot-spots* in the surfaces and interfaces of Fbw7. Virtual screening of the previously found regions has been performed using a pharmacophore filter created by Mdmix. These new inhibitors have been characterised primarily by Differential Scanning Fluorimetry (DSF). In parallel, a fragment-based screening campaign is being performed. A new collection has been put together from a range of vendors, taking into account the desirable range of physical and chemical properties of fragments and ensuring maximal coverage of chemical space. DSF is the frontline primary screen. Additionally, we are using Surface Plasmon Resonance (SPR) to screen for fragment binding directly against the protein immobilised on the chip. Orthogonal screening capabilities will allow cross-validation of hits, thereby maximising the chances of success in finding *bona fide* hits.

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Identification of Novel Non-Natural Supramolecular Ligands as Stabilizers of 14-3-3ζ Protein-Protein Interactions

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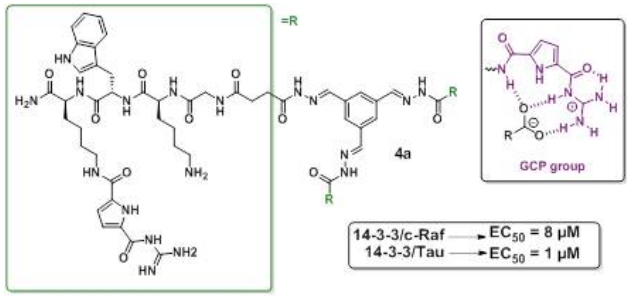
14-3-3 adaptor proteins play a central role in signal transduction. The recent resolution of crystal structures of different ligands in complex with these adaptor proteins opens up a formidable opportunity of modulating their physiological functions, and thereby, those implicated in pathological processes.[1] Herein is reported the identification of the first synthetic supramolecular stabilizers of the interaction between the adapter protein 14-3-3ζ and two of its effectors: c-Raf and Tau, which are involved in proliferative signal transduction and neurodegenerative diseases, respectively.[2],[3] These new ligands are decorated with the non-proteinogenic amino acid GCP (guanidinocarbonylpyrrole), an arginine mimetic designed by our group, which allows the stabilization of these interactions in the micromolar range.



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DREAM-MODULATORS A NOVEL SOURCE OF DRUG CANDIDATES FOR NEURODEGENERATIVE DISEASES

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DREAM (Downstream Regulatory Element Antagonist Modulator), also known as calsenilin or KChIP-3 (potassium channel interacting protein-3), is a multifunctional calcium binding protein, widely expressed in brain and with diverse subcellular localizations.[1] Recent studies have established a central role of DREAM in calcium homeostasis.[2] Considering that altered neuronal calcium homeostasis is a common feature of many neurodegenerative pathologies, DREAM modulation could open new avenues for the treatment of different neurodegenerative diseases.

Work from different laboratories has identified a growing list of interacting proteins that constitutes the DREAM interactome. These studies have highlighted a multifunctional role of DREAM, with specific actions in different cellular localizations. Among the different examples of DREAM protein-protein interactions, it is important to highlight those related to neurodegenerative diseases, such as the binding of presenilins,[3] the voltage-gated potassium channel Kv4,[1b] and the interaction of DREAM with ATF6 (Activating Transcription Factor 6), recently disclosed by us.[4] ATF6 is a protein involved in the unfolded protein response machinery (UPR).[5] Neuronal death related to accumulation of misfolded protein aggregates is central in the development of clinical symptoms of neurodegenerative disorders like Alzheimer and Huntington disease.[6] In this sense, considering that the formation of the DREAM/ATF6 complex blocks ATF6 sites-dependent transcription, an inhibition of protein DREAM would prevent the formation of this complex, releasing ATF6 and activating survival.[4]

In this communication we report the rational design and the synthesis of novel DREAM-binding molecules and their effects on the modulation of DREAM/protein interactions. This strategy involved structure-based design, synthesis and surface plasmon resonances studies. Moreover, we have determined the ligand binding site by directed-mutagenesis studies. Finally, *in cellulo* experiments have allowed the identification of novel and potent modulators of the DREAM/Kv4.3 and DREAM/ATF6 interactions.

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COMBINING CLICK CHEMISTRY & PEPTIDE SYNTHESIS TO GENERATE NOVEL INHIBITORS OF THE PRO-SURVIVAL PROTEIN MCL-1

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Apoptosis is a highly conserved and controlled process, with the Bcl-2 family of proteins playing an important role as key regulators. The family consists of both pro- and anti-apoptotic proteins and there is a careful balance within a cell which controls its fate. High levels of the anti-apoptotic proteins are often observed in cancer and not only contribute to the development of the tumour but also confer resistance to current therapies including chemotherapy and radiation treatment. In particular overexpression of myeloid cell leukemia-1 (Mcl-1) is one of the most common forms of genetic abnormalities in cancer with a variety of human cancers.

The anti-apoptotic Bcl-2 family of proteins are well-validated anti-cancer targets. ABT-.263 (Navitoclax) inhibits Bcl-2 and Bcl-XL with subnanomolar affinity and ABT-199 (Venetoclax) is currently in Phase III clinical trial as a selective Bcl-2 inhibitor. Although both compounds are in clinical trials, like most small molecule Bcl-2 inhibitors, they do not inhibit Mcl-1 and lacks efficacy in tumours with high levels of Mcl-1 deeming them ineffective as a single agent. Therefore compounds that specifically target Mcl-1 have the potential to overcome this resistance. Mcl-1 therefore represents an exciting and attractive target for the development of the next generation of cancer therapeutics. Here we report a novel approach to identifying small molecule inhibitors of Mcl-1, specifically exploiting the specificity and affinity of the pro-death BH3-only Noxa peptide to guide our discovery process.

DEVELOPMENT OF NON-BET BROMODOMAIN CHEMICAL PROBES

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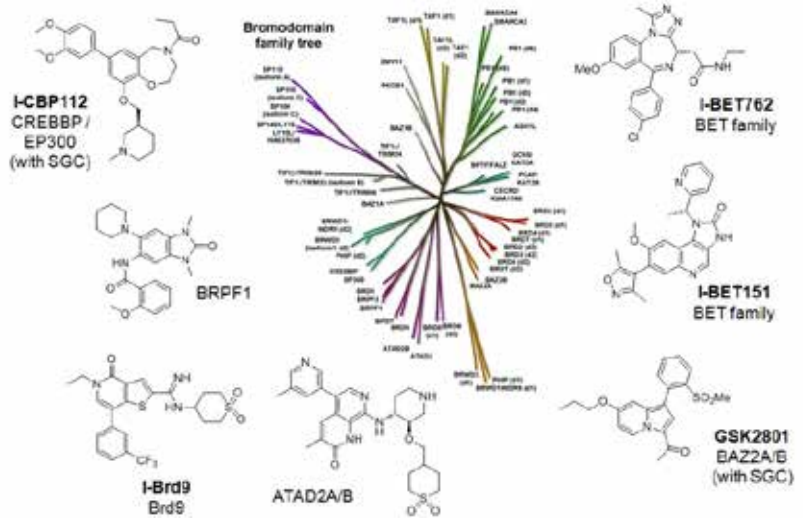
Bromodomains have emerged as an exciting target class for drug discovery over the past decade. Research has primarily focused on the bromodomain and extra terminal (BET) family of bromodomains, which has led to the development of multiple small molecule inhibitors and an increasing number of clinical assets. Central to this flurry of research in the BET field has been the ready availability of high quality small molecule chemical probes, in particular I-BET762 and (+)-JQ1. However, the BET family represents only eight reader domains of the bromodomain phylogenetic tree and the therapeutic potential of the remaining 53 family members is comparatively less explored. The development of non-BET bromodomain chemical probes will allow the community to gain a better understanding of their biology and potentially, help to identify and validate new targets for drug discovery. To this end, research within GlaxoSmithKline has led to the identification of high quality chemical probes for non-BET bromodomains. This presentation will communicate our work in developing small molecule bromodomain chemical probes, in particular inhibitors of BRPF1,[1] ATAD2A/B[2,3] and BRD9.[4]



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SYNTHESIS OF POTENTIAL ANTITUMOR DRUGS BASED ON DISPIRO-OXINDOLE DERIVATIVES OF 2-OXO, 2-THIOXO AND 2-SELENOXO-TETRAHYDRO-4H-IMIDAZOL-4-ONES

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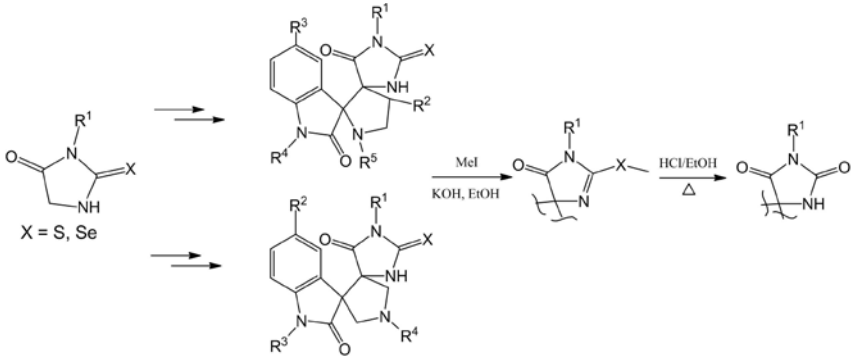
Synthesis of non-peptide small-molecular inhibitors for blocking p53-MDM2 interaction is an actual problem and new strategy in anti-cancer drug design. Protein p53 is a tumor suppressor and plays key role in controlling of cell cycle and apoptosis. MDM2 is endogenous oncoprotein and cellular inhibitor of p53. Thus, small-molecular inhibitors binds to MDM2 whereby released p53 activating process of destruction of the tumor cells. Inhibitors of MDM2 containing spiro-oxindole core are relatively new class of biological active compounds which had ability for effective blocking of the interaction between p53 and MDM2 [1].

In our laboratory we have developed synthetic approaches for preparation of spiro-oxindoles from commercially available reagents using 1,3-dipolar cycloaddition reactions [2]. As starting materials isatins, N-substituted amino acid and 5-arylmethylene-2-hydantoins, 5-arylmethylene-2-thiohydantoins or 5-arylmethylene-2-selenohydantoins derivatives were used. As the result, the compounds containing two spiro-fused cyclic fragments of different nature were obtained:



Spirooxoindole fragment imitates the structure of Trp23 in p53 and fills a hydrophobic pocket in MDM2. It is the common fact, that this structural element is the most important for creating connections, which can block the interaction between the proteins p53-MDM2, what is studied in this paper. Currently, over ninety compounds were tested on the cell lines HCT116 $p53^{(+/+)}$ and HCT116 $p53^{(-/-)}$, as well as LNCap and PC3, and the leader was detected. Then in vivo test at model p388 mouse lymphoma was performed and decrease of tumor growth was observed.

The work was supported by the RFBR, grant number 16-33-60166, RSF grant number 14-34-00017

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DESIGN AND SYNTHESIS OF THE LIBRARY OF sp3-ENRICHED α-HELIX-MIMETICS

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Although PPIs are among the most screened target class in high-throughput screening (HTS), success rate of finding hit compounds in many HTS campaigns using small molecule compounds remains generally very low. Several molecular-descriptors-based approaches have been developed to define PPI-related chemical space and therefore to reduce HTS attrition rate. However, such approaches are not indisputable, because, in fact, they equate non-drug-like and a PPI-related chemical spaces. This suggests that special design for PPI-focused libraries should be applied.

Since α-helices play a key role in many PPIs as the most common protein recognition element, we have designed a library of α-helix mimetics as potential modulators of PPIs. To ensure drug-likeness of library members, only sp^3-enriched scaffolds have been used for the library population. For scaffold selection we considered their ability to provide library members that are able to interact with a model 7-Ala α-helix. Details of mimicry modeling as well as examples of selected scaffolds and library features will be discussed.

LIGAND-BASED VIRTUAL SCREENING INTERFACE BETWEEN PYMOL AND LISICA AND ITS APPLICATION FOR THE DISCOVERY OF TLR7 AGONISTS

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By combining the flexible molecular visualization abilities of PyMOL [1] with the fast maximum-clique based algorithm [2] for small-molecule similarity comparison LiSiCA [3], we have developed a novel PyMOL plugin for efficient and user-friendly two- or three-dimensional ligand based virtual screening (freely available at http://pymolwiki.org/index.php/Lisica) (see Figure 1). This plugin was applied to the discovery of novel compounds that would exhibit agonistic activity on the Toll-like receptor 7 (TLR7). TLR7 is a transmembrane protein localized on the endosomal membranes, which plays a crucial role in the recognition of single-stranded viral RNA and in the subsequent activation of innate immunity [4]. The usage of a ligand-based virtual screening method was chosen because to date no crystal structure of the TLR7 receptor is available. Moreover, the TLR7 receptor is of special interest as recent computational studies suggest that its activity is based on preliminary monomer homodimerisation; therefore TLR7 agonists such as imiquimod are believed to be modulators of protein-protein interactions [5]. Using imiquimod structure as the reference compound, we employed the LiSiCA plugin to screen the Drugs Now ZINC database (containing approximately 10 million compounds) to obtain and visually compare topologically similar molecules to imiquimod. The highest scoring compounds were purchased from different vendors and biologically tested for the EC_{50} value on the HEK-Blue™, hTLR7 (InvivoGen) cell line. The two most active compounds found were shown to have an EC_{50} value of 7.4 and 8.3 μM (compared to the EC_{50} = 8.7 μM for imiquimod) while also possessing a different scaffold than the reference molecule; therefore we also demonstrated LiSiCA's ability for scaffold hopping.

Figure **1**. LiSiCA - PyMOL interface. 'Inputs' tab shown.

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PROTACTING LEADS: DESIGN OF A PROTEIN SURFACE-TARGETING FRAGMENT LIBRARY

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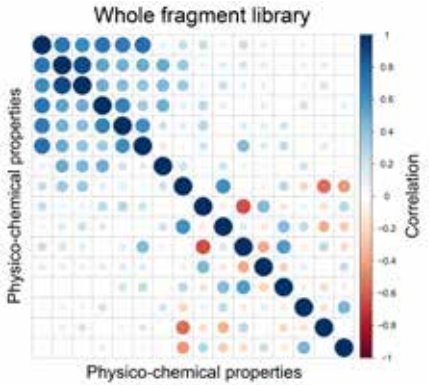
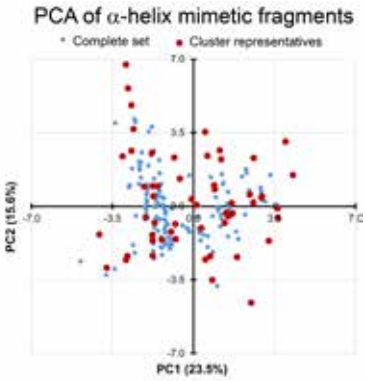
Fragments have been extensively used in early stages of lead discovery to probe a variety of protein functional sites, including enzymatic centres, protein–protein interaction (PPI) interfaces, and allosteric cavities [1]. A recently developed chemical biology tool, termed PROTAC (proteolysis targeting chimera), exhibits its biological activity regardless of the functionality of the anchoring site: It hijacks the catalytic activity of specific Cullin RING E3 ligases (CRLs) to trigger selective proteolysis of target proteins [2].
To take full advantage of this critical difference and thereby broaden the current application scope of PROTACs, we present here the first step towards developing drug discovery tools to purposely probe any patch of a protein surface. We have engineered a large (> 2.5 million) fragment library comprising relevant protein mimetic chemotypes, including biomimetics, peptidomimetics, and amino acid bioisosteres, among others, as well as biaryls and other structural motifs common in PPI modulators. Subsequently, we have applied physico-chemical property-based Principal Component Analysis (PCA) to each compound class to extract subsets of representative compounds with maximised diversity.





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MODULATION OF SYNAPTOGENESIS BY INHIBITION OF PROTEIN-PROTEIN INTERACTION. DISCOVERY OF NEW DRUGS FOR FRAGILE X TREATMENT

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Synaptogenesis or synapse formation is an important process in neurodevelopment. A balance between excitatory and inhibitory synapses must exist for proper function of neural circuits. When this balance is disturbed by some specific gene mutations, disorders such as autism, epilepsy, fragile X syndrome or schizophrenia appear. Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability within the autism spectrum disorders. FXS is caused by mutations in the gene that encodes the *Fragile X Mental Retardation Protein* (FMRP). FMRP regulates the translation of mRNAs involved in synaptic architecture, function and plasticity. Currently, there is no effective treatment for FXS which is an orphan disease from the therapeutic point of view [1].

The complex between the neuronal calcium sensor 1 (NCS-1) and the guanil exchange factor Ric8a regulates synapse number and probability of neurotransmitter release per synapse. Thus, thus NCS-1/Ric8a can be considered as a therapeutic target for the treatment of diseases in which these synaptic features are altered [2]. With the aid of structural and computational approaches we have recently discovered small molecules with phenothiazine scaffold that function on this therapeutic target as protein-protein interaction inhibitors. These compounds are able to cross the blood-brain barrier and have shown beneficial effects in modulating synapse number under pathological conditions such as FXS [3]. Therefore, the protein-protein interaction inhibitors discovered here could be a new pharmacological tool and/or innovative drug candidates to act on Fragile X Syndrome among other pathologies of the central nervous system.

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FRAGMENT BASED DRUG DISCOVERY APPLIED TO A PROTEIN-PROTEIN INTERACTION TARGET: FROM MILLIMOLAR FRAGMENTS TO NANOMOLAR DUAL ANTAGONIST OF XIAP AND CIAP1

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XIAP and cIAP1 are members of the inhibitor of apoptosis protein (IAP) family and 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. Using our fragment-based screening approach, Pyramid^{TM}, we identified non-peptidic fragments binding with millimolar affinities (ligand efficiency (LE) << 0.3) to both cellular inhibitor of apoptosis protein 1(cIAP1) and X-linked inhibitor of apoptosis protein (XIAP). Structure-based hit optimisation supported by a fast-turnaround of X-ray crystal structures allowed us to significantly increase the binding affinity and the LE of the starting hits. This led to the discovery of AT-IAP, a non-peptidomimetic, sub-10 nanomolar, orally bioavailable balanced dual cIAP1/XIAP antagonist, which is chemically distinct from previously reported, alanine-based, peptidomimetic antagonists.

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IDENTIFYING INHIBITORS OF THE TANKYRASE : SUBSTRATE PROTEIN PROTEIN INTERACTION

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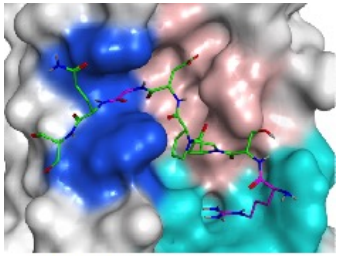
Tankyrase, a member of the poly(ADP-ribose) polymerase (PARP) family, catalyses the addition of ADP-ribose units to a diverse range of substrate proteins, a number of which have been implicated in cancer. For example, Tankyrase promotes telomere extension by binding and modifying telomere repeat binding factor 1 (TRF1), and supports Wnt signalling by targeting axis inhibition proteins (AXIN1 and AXIN2). Moreover, loss of Tankyrase gives rise to synthetic lethality of BRCA1/2-deficient cells. Therefore, inhibiting Tankyrase could expose cancer cell vulnerabilities and open up novel avenues for cancer therapy. Several research programmes centered on targeting Tankyrase catalytic function are under way. My project focuses on targeting Tankyrase through its substrate recognition domains – the ankyrin repeat clusters (ARCs).

Tankyrase ARCs recognise a peptide motif, with consensus RXX(P/G)XGXX.[1,2] I aim to develop tool compounds to disrupt the Tankyrase: substrate protein-protein interaction (PPI), targeting Tankyrase outside its catalytic PARP domain. Substrate binding antagonists could be used to address the challenges of drug specificity in targeting PARPs and may be an alternative therapeutic approach to inhibiting Tankyrase function. Much of our knowledge of substrate recruitment by Tankyrase comes from studying the model substrate 3BP2, a signalling adapter protein. I aim to modify the Tankyrase-binding motif (TBM) of 3BP2 into a more drug-like molecule via a peptide mimetic approach, targeting the three main interface hotspots; the glycine sandwich, central patch and arginine cradle.



The hotspots of the Tankyrase:3BP2 protein protein interface are highlighted above, with the conserved Arg and Gly residues coloured pink. PDB code: 3TWR

Initially, I explored the feasibility of replacing an arginine residue at the interface. A virtual screen was undertaken using GOLD docking software to model potential arginine replacements at position one of the TBM, and prioritise compounds for synthesis. Solid phase peptide synthesis was used to produce five arginine mimic peptides selected from the in silico screen. Tankyrase ARC constructs were expressed with a His-GST tag and purified using affinity and size exclusion chromatography. I developed a competitive fluorescence polarisation (FP) assay to measure the binding of synthesised peptides to TNKS ARC4.

I found that the guanidine group is optimal for binding to Tankyrase ARCs, however substitution of the guanidine moiety with imidazole groups showed potential for replacement of the arginine at the PPI interface with heterocycles containing a delocalised basic charge. Further alternatives for the arginine residue are being investigated. Replacements for the di-proline motif and glycine residue at the other interface hotspots will also be explored, using both peptide mimetic and fragment based approaches.

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DISCOVERY OF MOUSE-DERIVED HUMAN MYOSTATIN-INHIBITORY PEPTIDES AND ITS N-TERMINAL ACYLATION

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Endogenous mature myostatin negatively regulates skeletal muscle mass. Hence, myostatin is an attractive therapeutic target for muscle atrophic disorders including muscular dystrophy, cancer cachexia, sarcopenia and disuse muscle atrophy [1, 2]. The myostatin precursor protein is intracellularly cleaved in two parts, i.e., N-terminal prodomain and mature myostatin, by a furin-like protease. Two N-terminal prodomains can assemble with the homodimeric mature myostatin to form an inactive complex, which is then secreted and stored in the extracellular matrix. In these days, we successfully identified minimum myostatin inhibitory peptides **1** and **2** (24 and 23 amino acids, respectively) derived from an α-helical region of mouse myostatin prodomain sequence [3]. These peptides show the α-helical structure under the condition of 20 mM sodium phosphate buffer (pH 7.4) containing 10 % trifluoroethanol, and directly interact to myostatin with K_D values of 30-36 nM. Interestingly, it was noteworthy that a human myostatin prodomain sequence corresponding to peptide **1** derived from a mouse sequence did not show a significant inhibitory effect on human myostatin signaling. In addition, peptide **1** significantly increased tibialis anterior muscle mass in Duchenne muscular dystrophy model mdx mice. Then, we synthesized a series of N-acylated peptide derivatives (22aa) focused on the structure of N-terminal Trp residue, and successfully found that 2-naphthyloxyacetyl peptide **3** showed the significant inhibitory activity against human myostain, which was three times (IC_{50} value: 1.19 μM) more potent than the parent peptide **1** (IC_{50} value: 3.53 μM) [4]. Therefore, these peptides would be promising platforms for mid-size peptide-based medicinal chemistry towards the treatment of muscle atrophic disorders.

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TIME-RESOLVED DETECTION OF PROTEIN-PROTEIN INTERACTION AND MODIFICATION BY PRESSURE

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Protein-protein interactions are essence of life. To understand the molecular mechanisms of the protein-protein interaction, not only the static structure but also dynamical nature has to be clarified. Although many spectroscopic techniques have been developed so far to trace the reactions in time-domain, there are still a lot of dynamics that cannot be detected by the conventional methods. In order to overcome the limitations, we have developed a time-resolved technique based on the pulsed laser induced transient grating (TG) method. A photosensor protein is activated by a pulsed laser light, and subsequent dynamical changes such as conformational change, the fluctuation change, and the intermolecular interaction change are detected in time-domain by monitoring the diffusion coefficient, thermodynamical changes and some other properties. Here, I would like to focus our attention on the protein-protein interaction of a blue light sensor protein, PixD, and on a discovery that shows the pressure can control the interaction very sensitively.

PixD proteins are ones of photosensors containing the BLUF domain, and identified in cyanobacteria. They include Slr1694 of the mesophilic Synechocystis sp. PCC6803 (SyPixD) and Tll0078 of the thermophilic Thermosynechococcus elongatus BP-1 (TePixD). SyPixD regulates phototaxis of cyanobacterium. Crystallographic analyses showed that these homologous PixD proteins have a unique oligomeric structure: a decamer comprised of two stacked pentameric rings. Because oligomer formation of PixD proteins is also observed in solution, PixD may function as an oligomer in cyanobacterial cells. Indeed, the importance of decamer formation for signal transduction in SyPixD has been demonstrated. By using the TG technique, we discovered that the light controls the inter-protein interaction; e.g., changing the oligomeric structure. For example, the decamer of TePixD dissociates into the pentamer. Furthermore, we found characteristic dependences of the reaction on the concentration, on the intensity of the excitation light, and on the pressure. This result shows that the multiphoton excitation of this protein is important for the reaction.

For understanding the driving force of the reaction to detect the light, we tried to elucidate the transient enhancement of volume fluctuations during a chemical reaction in a time-resolved manner. The TG signal intensities representing the volume change depended significantly on the pressure. This result implies that the compressibility, which reflects the volume fluctuation, changes during the reaction and is dependent on the pressure. To clarify the relationship between volume fluctuation and reaction, the volume fluctuation of multiply excited TePixD was investigated. We showed that the enhanced volume fluctuation is an important factor for triggering the reaction of TePixD. These advanced techniques are applicable to many other protein systems.

DESIGN OF PPI AND EPIGENETIC TARGETED LIBRARIES

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A PPI Targeted library was designed using various chemoinformatics methods and protein docking procedure that were proven to be effective instruments in a number of studies described in literature.

Machine-learning method Library. Decision tree (DT) method is based on a cross-validation protocol. Using RDF 070m and Ui descriptors from PyChem software this protocol provides the balance between enrichment, sensitivity and specificity.

Docking Library. General information about protein-protein interaction and structural data were gathered from different X-rays of PDZs complexes with peptides and particular publications. Docking procedure was carried with Unity search engine, implementing H-bond donor/acceptor and volume constraints to achieve more reliable conformations and analysis scores.

Ligand-based and structure-based epigenetic libraries were also designed. They include both known and novel structures of compounds, which showed good results in *in silico* screening. The library includes 4 classes of epigenetic modifiers: DNA methyltransferases, Histone-Arginine Methyltransferases, Histone deacetylases, Histone cateyltransferases and a single family of Sirtuins. Besides the standard 2D similarity method, docking simulation approach was used, which gave compounds with good scores and novel structures, rather different from the known inhibitors.

NOTES

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POSTERS

Current Advances and Future Opportunities for the Treatment of Neurodegenerative Disorders

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL ABAD INHIBITORS FOR TREATMENT OF ALZHEIMER'S DISEASE

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Amyloid-beta peptide (Aβ), thought to be the main causative factor for the development of Alzheimer's disease, has been shown to interact with the mitochondrial amyloid-binding alcohol dehydrogenase (ABAD) [1]. *In vitro* experiments have shown this interaction to be cytotoxic and that enzyme activity is necessary for hallmarks of this cytotoxicity to be observed [2]. Thus, the direct inhibition of the ABAD may be of therapeutic merit in treating Alzheimer's disease (AD).

We have designed, synthesised and evaluated two novel series of benzothiazolyl urea analogues as direct ABAD inhibitors identifying three lead compounds (Fig. 1) that are markedly more potent than the previously described benzothiazolyl derived inhibitors *in vitro* [3]. The two most promising lead compounds fulfil the drug like properties required for CNS penetration, are reversible in nature with non-competitive or mixed non-competitive mechanisms of action and are nontoxic *in vitro*.

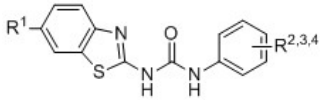

Figure 1: General structure of prepared ABAD inhibitors.

These compounds may form the basis for the development of more potent analogues which may be of therapeutic merit in treating Alzheimer's disease.

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PYRIDOPYRAZINE-1,6-DIONE AS A NEW SCAFFOLD FOR THE DESIGN OF GAMMA SECRETASE MODULATORS WITH IMPROVED CNS DRUG-LIKE PROPERTIES

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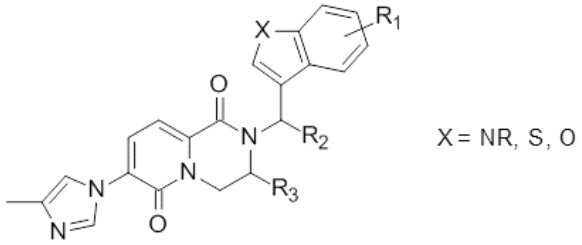
A defining characteristic of Alzheimer's disease (AD), the most common form of dementia, is the deposition in the brain of extracellular amyloid plaques which are mainly composed of amyloid beta peptides or Aβ peptides. It is the sequential cleavage of APP, the amyloid precursor protein, first by β-secretase or BACE, followed by γ-secretase (GS), that will generate Aβ peptides of different lengths, among which Aβ42 is the most prone to aggregate, the most neurotoxic. GS modulation has been proposed as a potential disease modifying anti-Alzheimer's approach. In contrast to γ-secretase inhibitors (GSIs), γ-secretase modulators (GSMs) cause a product shift from the longer amyloid isoforms to shorter, more soluble and less amyloidogenic isoforms, without inhibiting NOTCH proteolytic processing. Potent GSMs have been described in the literature and we have reported in a recent past our own GSMs from different chemical classes. Typically these compounds are suffering from poor drug-like physicochemical properties such as high lipophilicity, high molecular weight, low solubility and high aromaticity. In our continuous effort to design new scaffolds in a different chemical space we have identified a novel series of potent pyridopyrazine-1,6-dione derived GSMs with improved properties such as a lower lipophilicity, higher solubility, higher sp^3 character, resulting in compounds with higher free fraction. In this paper we will report the design and the synthesis of these new GSMs and discuss some aspects of the Structure Activity Relationships, as well as their in vivo pharmacological profile conducted in mouse and beagle dog.



DEVELOPMENT OF NEW MULTITARGET CANNABINOIDS: A PLAUSIBLE TREATMENT TO ALZHEIMER'S DISEASE.

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Dementia is one of the main causes of disease burden for developed regions. According to the WHO, these diseases will become the world's second leading cause of death by the middle of the century. This will have a dramatic impact in medical care, as well as important social and economic implications, unless more effective preventive treatments are available. Alzheimer's disease (AD) is the most common cause of dementia, accounting for probably 60-70 % of all dementias worldwide, followed by vascular dementia, mixed dementia, and Lewy body dementia.

Since 2003, when memantine was approved by FDA, it has not been approved any other drug to AD. In fact all the drugs that are being studied in clinical trials in recent years have failed. Therefore, it is a priority to increase resources to research the treatment of these diseases and to explore novel alternatives for the development of new drugs. For this purpose, the called multitarget-directed ligand (MTDL) approach has been the chosen strategy in our group.[1]

In this work, new 1-indazolyl ketones derivatives with a multitarget profile, as cannabinoid agonists and BACE-1 and/or BuChE inhibitors have been developed by means of a medical chemistry program using an iterative procces that comprises computational design, synthesis, *in vitro* studies and further studies by means of the mechanisms of survival/death in lymphoblasts of patients with Alzheimer's disease.

As results of this process two compounds with an interesting biological profile have been studied in an animal AD model (Tg APP mice). Following chronic oral administration (1 mg/kg/day in the drinking water) of the compounds to wild type and Tg APP mice, an AD model, spatial learning was assessed in the water maze. One of the drugs restored cognitive abilities of Tg APP mice.[2]

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DEVELOPMENT OF SELECTIVE S1P1 RECEPTOR AGONISTS FOR TREATMENT OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an inflammatory autoimmune disorder of the CNS. Fingolimod, developed as a S1P1 agonist by Novartis, is the first oral drug approved for relapsing forms of MS. Following phosphorylation *in vivo* , an active form of fingolimod, acts as a sphingosine 1-phosphate (S1P) receptor modulator, binding with high affinity to S1P receptors (S1P1,3,4,5). Although it shows high efficacy for treatment of MS, its low selectivity, in particular, high binding affinity to $S1PR_3$ causes unfavorable side effects such as bradycardia. Our therapeutic strategy through S1P receptors is to lower the circulating lymphocytes more efficiently by internalization of $S1P_1$ on lymphocyte and to enhance remyelination by modulation of $S1P_5$ on oligodendrocytes.

In this study, we have designed a new series of $S1P_1$ receptor agonists on the basis of the structure of Ono-4641. The preliminary *in vitro* evaluation of the synthesized compounds using calcium mobilization assay showed that their agonistic effects against $S1P_1$ are comparable to those of other reported agonist ligands. Indeed, KKSM07016 represented 90% and 85% activation values at the concentration of 10 μM and 1 μM respectively against $S1P_1$ receptor. EC_{50} value of KKSM07016 was 0.314±0.029 nM, which is comparable to that of BAF312 (0.103 ± 0.009 nM), a second generation MS drug developed by Norvatis.

Currently, we continue to perform lead optimization process for development of selective $S1P_1$ agonists with higher affinity.

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STUDIES OF PYRIDO[2,3-b]PYRAZINES AS CHOLINESTERASES INHIBITORS

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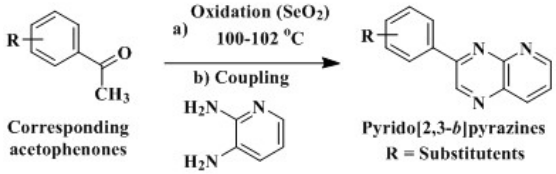
Alzheimer's disease (AD) is characterized by progress loss in memory, language skills, changes in moods, and many other cognitive functions. According to so called cholinergic hypothesis cholinesterases, include acetylcholinesterase (AChE), and butyrylcholinesterase (BChE), are considered as major pathogenesis of this brain disorder. Cholinesterases play an active role in cholinergic deficit in AD. Therefore, inhibition of these enzymes by employing small molecules carries significant importance in medicinal chemistry. So far, a range of molecules have reported as cholinesterases inhibitors. The available drugs for AD therapy such as galantamine, donepezil and rivastigmine etc. only reverse the disease symptoms transiently in early stages. Thus, development of new drug molecules has still substantial interests. In the present study, we have synthesized as series of novel pyrido[2,3-*b*]pyrazines and screened them against cholinesterease, both AChE and BChE. Biological screening shows varied activities of synthetic pyrido[2,3-*b*]pyrazines. The active compounds could potential serves as leads for further stages in drug development.



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DISCOVERY OF NOVEL BENZOQUINONES ABLE TO POTENTLY SUPPRESS LEUKOTRIENE BIOSYNTHESIS IN CELLULO AND BLOCK INFLAMMATION IN VIVO

Rosanna Filosa, Maria Maria Scuotto, Anja M. Schaible, Verena Krauth, Chiara Schiraldi, Daniela Schuster, Oliver Werz

5-Lipoxygenase (5-LO) is a potential target for pharmacological intervention with various inflammatory and allergic diseases. Leukotrienes (LT), formed from AA by catalysis of 5-lipoxygenase, are fast reacting pro-inflammatory mediators of the immune system; besides their physiological roles, they primarily mediate inflammatory and allergic reactions and are involved in the onset of inflammatory diseases such as asthma, allergic rhinitis, systemic lupus erythematosus (SLE), rheumatoid arthritis and also cardiovascular disorder. Recently published study showed that 5-LO expression appears to be upregulated in patients with neurodegenerative disease like AD. Continuing our studies on small molecules able to block 5-lox activity,[1-7] here we present the synthesis and biological evaluation of different libraries, belonging to quinones scaffold in which we systematically modified derivatives, yielding obvious structure-activity relationships and more potent analogues. Detailed pharmacological charaterization reveals quinone derivatives as higly potent 5-LO-selective lead compounds, without affecting 12/15-LOs, cytosolic phospholipase A_2, or cyclooxygenases. A highly selective and potent 5-LO inhibitor in intact human leukocytes was discovered with pronounced effectiveness in different models of inflammation. The lead compound 4,5-dimethoxy-3-dodecyl-1,2-benzoquinone (RF-22c) is a highly selective and highly potent 5-LO inhibitor in intact human leukocytes (IC50 =29 nM) with pronounced effectiveness in three different animal models of inflammation in vivo. RF-22c[8] exhibits >500-fold selectivity over related LOs or COX enzymes and lacks unspecific redox or iron-chelating properties, we exclude a redox-based mechanism for 5-LO inhibition but instead suggest specific binding of RF-22c to 5-LO, supported by molecular docking studies.

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DUAL GSK3B INHIBITION AND Nrf2 INDUCTION: A NEW FAMILY OF MULTITARGET COMPOUNDS TO FIGHT ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) bears the label of the most prevalent neurodegenerative disorder, with a number of patients that increases year by year. Taking into account the multifactorial nature of AD, a multitarget approach is increasingly considered as a suitable strategy to address its treatment. Due to their essential role in the progression of AD, neurofibrillary tangles (NFTs) formation,[1] oxidative stress[2] and neuroinflammation[3] have emerged as key targets for the development of new active compounds. Interestingly, these pathological hallmarks are closely related to the over-activity and over-expression of the kinase GSK3β[4] and to the downregulation of the antioxidant and anti-inflammatory *via* Nrf2-EpRE,[5] which have been observed in AD patients.

Under these premises, we have accomplished the design, synthesis and pharmacological evaluation of a new family of multitarget 2,4-dihydropyrano[2,3-c]pyrazoles, based on two simultaneous activities: inhibition of the kinase GSK3β and induction of the transcription factor Nrf2. The obtained compounds are able to inhibit GSK3β and to induce Nrf2 at the micromolar level, showing a successful implementation of both biological activities. Docking studies have allowed us to shed some light on the interesting structure-activity relationships displayed by the compounds. Furthermore, the combination of both biological activities in a single molecule have led into a remarkable anti-inflammatory effect, as well as good neuroprotective properties against tau hyperphosphorylation and oxidative stress *in vitro*. Finally, any of the obtained compounds have exhibited either neurotoxicity or hepatotoxicity, which confer them an important safety improvement in comparison to known electrophilic Nrf2 inducers.

In conclusion, the obtained compounds represent the first example of dual GSK3β inhibitors and Nrf2 inducers, two biological activities that endow them with interesting neuroprotective and anti-inflammatory properties, and with a remarkable interest in the long-standing research of AD's treatment.

Aknowledgements

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N-ALKYL-INDAZOLE-5-CARBOXAMIDES: A NEW SERIES OF BRAIN PENETRANT AND REVERSIBLE MAO-B INHIBITORS WITH SUBNANOMOLAR POTENCY

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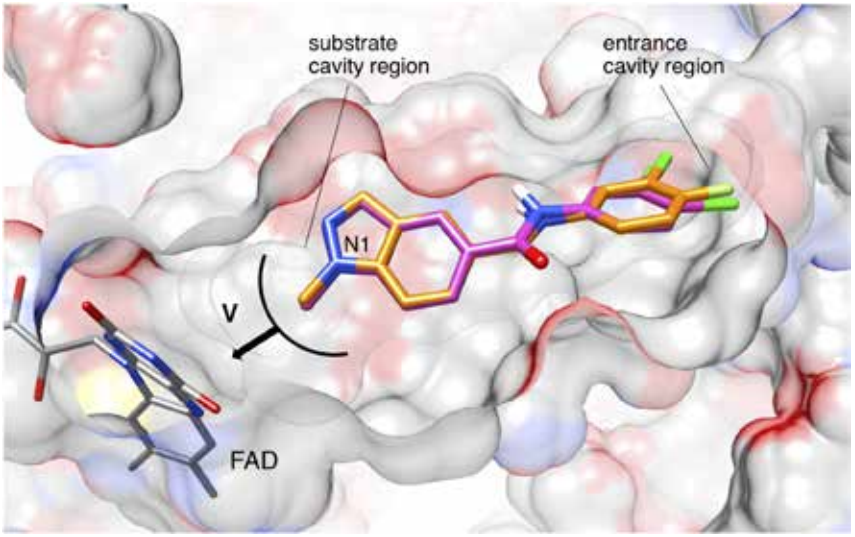
We recently discovered indazole-5-carboxamides (designated class I), indole-5-carboxamides (class II), and (indazol-5-yl)methanimines (class III) derivatives as highly selective, sub-nM inhibitors of MAO-B acting through a different mechanism of action than the standard treatment with levodopa [1,2]. The most potent derivatives were compounds that belong to the best-balanced MAO-B inhibitors reported to date.

The new series of N-alkylated indazole-5-carboxamides are not only highly potent, but also selective and brain penetrant. Compound NTZ-1441 can be highlighted because of its remarkable in vitro MAO-B inhibitory activity and selectivity - combined with a well-balanced physicochemical profile and BBB penetration ability. Compounds of class I series are highly useful as pharmacological tools for in vitro and in vivo studies, and may be suitable for the development of radioligands, including diagnostics for positron emission tomography (PET).

To rationalize the SAR detected and investigate further exploration steps, we analysed the binding mode of selected N1- and N2-alkylated indazole-5-carboxamide derivatives within the binding pocket of the human MAO-B enzyme using a novel Free Energy approximation concept ("HYDE"), originally published by Bayer, Hamburg University, and ourselves [3], now available in the software SeeSAR [4]; basic concepts behind the estimations and visualizations will be reported in this contribution. Moreover, selected compounds of class I series were profiled in a high-throughput ADME panel to access the most important physicochemical parameters and BBB permeability.



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4) www.biosolveit.de/SeeSAR

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF AMIDINE CONTAINING GABAA RECEPTOR AGONISTS

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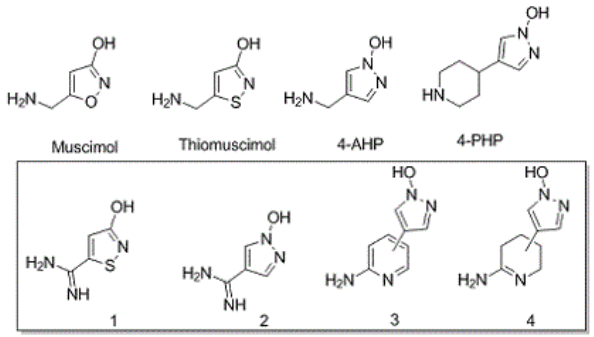
Synaptic inhibition in the brain is largely a result of γ-aminobutyric acid (GABA) signaling, where the fast inhibitory actions of GABA are mediated by activation of $GABA_A$ receptors ($GABA_ARs$). The $GABA_ARs$ belong to the Cys-loop receptor family of ligand-gated ion channels and play essential roles in numerous physiological and pathological processes. Consequently, the $GABA_ARs$ mediate the effect of a large number of clinical administered drugs and are putative drug targets in a wide range of neurodegenerative and psychiatric disorders, for example depression, schizophrenia, autism, anxiety and panic disorders.

Besides the potential as drug candidates, the development of compounds capable of activating the $GABA_ARs$ has contributed to the knowledge of the function and localization of the $GABA_ARs$ as well as the architecture of the orthosteric binding site. Conformational restrictions and bioisosteric replacement in the molecule of GABA have afforded a range of agonists with different pharmacological profiles. Classical agonists include muscimol, a naturally occurring compound in the mushroom *Amanita muscaria*, and thiomuscimol, the synthetic sulphur analogue of muscimol.

Bioisosteric replacement of the carboxylic group in GABA has been widely explored and include the 3-hydroxyisoxazole ring in muscimol, the 3-hydroxyisothiazole ring of thiomuscimol, and the 1-hydroxypyrazole ring of 4-AHP[1]. In contrast, the amino group in GABA has received much less attention despite the fact that transamination has been reported as a limitation for *in-vivo* studies of e.g. muscimol[2]. However, we have recently reported on a series of 2-aminotetrahydropyridine analogues of GABA and identified the amidine moiety as a valid bioisostere for the amino group[3].

Inspired by the above mentioned results we have explored amidine moity as an amino group bioisostere for the $GABA_AR$ agonists: 4-AHP, thiomuscimol, muscimol, and the low efficacy partial agonist 4-PHP in terms of pharmacological profile and metabolic stability (compounds 1-4). We here report on the synthesis and pharmacological characterization at the $GABA_AR$ of a series of amidine GABA analogues as novel $GABA_AR$ agonists.



The binding affinities of the target compounds at native $GABA_ARs$ were measured by displacement of [^{3}H]muscimol in rat membrane preparations. Functional characterization was carried out at the human $\alpha_1\beta_2\gamma_{2S}$ and ρ_1 $GABA_ARs$ using the FLIPR^{TM} Membrane Potential Blue Assay. The compounds were shown to be moderate to highly potent $GABA_AR$ agonists, some with low-nanomolar affinity and equipotency as agonist to the amino-containing analogues. Furthermore, the most potent compounds were examined as substrates for the GABA-metabolizing enzyme GABA transaminase.

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MAO INHIBITORY EFFECT OF 2-IMINO-3-PHENYLTHIAZOLIDIN-4-ONES AND 3-PHENYL-5-METHYLOXAZOLIDIN-2,4-DIONE

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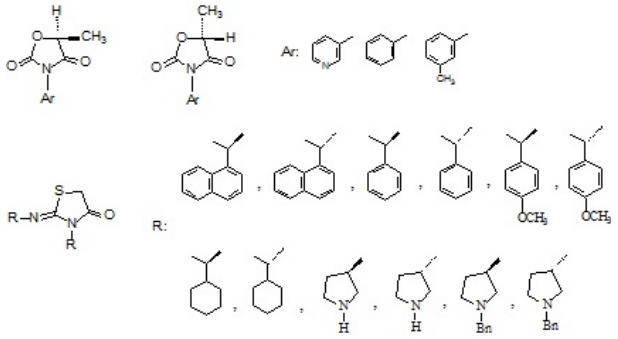
MAO-A and MAO-B are attractive targets for therapeutic intervention. MAO-A inhibitors are prescribed for the treatment of mental depression and anxiety while MAO-B inhibitors are used with L-DOPA and/or DA agonists in the symptomatic treatment of Parkinson's disease (PD). Knowing that many heterocycle nitrogen-containing derivatives, including thiadiazole, oxadiazole, thiazole, etc., behave as potential MAO inhibitors, and a common structural feature of substrates and inhibitors is an amino or imino group we have aimed to design novel potent MAO inhibitors regarding 3-phenyl-2-oxazolidinones and 3-phenylthiazolidin-4-ones. The newly synthesized chiral R,R- and S,S-2-imino-3-phenylthiazolidin-4-ones and 3-phenyl-5-methyloxazolidin-2,4-dione compounds were evaluated for their MAO inhibitory activity by fluorimetric Elisa assay method. It can be observed that oxazolidin-2,4-diones show obvious activity against MAO activity and inhibit MAO-A selectively and reversibly in a competitive manner while 2-imino-3-phenylthiazolidin-4-ones derivatives have no activity at 100 μM concentration.



Acknowledgement: This study was supported by Scientific and Technoloical Research Council of Turkey (Project Number 114Z172).

LEAD OPTIMIZATION OF GABAA α5 RECEPTOR NEGATIVE ALLOSTERIC MODULATORS

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From HTS of 120,000 compounds using both binding and functional assays against the $GABA_A$ α5 receptor, we identified several chemical series of $GABA_A$ α5 receptor negative allosteric modulators (NAMs) as initial hit compounds. Optimization of the hit compounds led us to identify a potent and selective $GABA_A$ α5 receptor NAM. This compound significantly enhanced LTP (Long-term potentiation) in rat hippocampus slice and improved cognition in several animal models without anxiogenic or proconvulsant side effects. Currently further optimization is ongoing.

IDENTIFICATION OF NOVEL CB2 RECEPTOR AGONISTS WITH IN VITRO AND IN VIVO NEUROPROTECTIVE PROPERTIES

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G-protein-coupled cannabinoid receptors, CB_1 and CB_2, have emerged as promising therapeutic targets with a high potential for the treatment of neurological disorders. Classical cannabinoids showed potent activity *in vivo*, but they lacked of CB_1/CB_2 selectivity and most of them are psychoactive thus limiting their medical development. Consequently, novel strategies for exploiting cannabinoids as medicines need to be developed to overcome these side-effects. In this context, we proposed the development of novel CB_2 selective ligands based on the chromenopyrazoles scaffold previously described by us.

Structural modifications of the chromenopyrazole core led to the synthesis of novel derivatives allowing fine-tuning of cannabinoid receptor activity. The affinity of these compounds for CB_1 and CB_2 receptors was evaluated measuring their ability to displace the radioligand [^{3}H]CP55,940. Functional activity of the compounds with better CB_2 affinity and selectivity profiles was tested by cAMP accumulation experiments and GTPγS binding assays. In addition, docking studies using the active CB_1* and CB_2* receptor models provided structural information related to ligand-receptor interactions and validating the experimental structure-activity relationships.

Among this series of compounds, we have identified a very potent, efficacious and selective CB_2 agonist. Interestingly, this lead compound has shown neuroprotective properties in an *in vitro* neuroinflammatory model realized in M213 neurons. The neuroprotective capacity of this novel derivative was further confirmed in two *in vivo* models of Huntington´s disease and Multiple Sclerosis. Therefore, herein we have discovered a promising neuroprotective agent useful for those neurodegenerative pathologies in which the activation of CB_2 has a therapeutic value.

Acknowledgements: Supported by Spanish Grant SAF2015-68580-C2, SAF2012-40075-C02-02 and CANNAB-CM S2011/BMD-2308.

PAM-2, A POSITIVE ALLOSTERIC MODULATOR OF α7 NICOTINIC ACETYLCHOLINE RECEPTORS, ENHANCES MEMORY AND MODULATES ERK1/2 PHOSPHORYLATION IN MICE

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The positive allosteric modulators (PAMs) of human α7 nicotinic receptors (nAChRs) are promising therapeutic agents that improve cognitive deficit, especially important for the treatment of the neurodegenerative disorders such as Alzheimer's and Parkinson's diseases as well as schizophrenia.

Our findings, focused on 3-furan-2-yl-N-p-tolyl-acrylamide (i.e., PAM-2), the a7 PAM [1], indicate that the PAM-2: (a) improves memory acquisition/consolidation processes after acute treatment as well as memory consolidation after chronic treatment by using the passive avoidance (PA) test in male mice [2]. This activity was blocked by methyllycaconitine (MLA) (an α7-selective antagonist), confirming the role of a7 nAChRs in the PAM-2 promnesic activity [2]; (b) recovers the memory impairment in animals treated with the muscarinic antagonist scopolamine [2]. (c) Moreover, a synergistic (acute) effect between inactive doses of PAM-2 and DMXBA (a selective a7-agonist) was observed [2]. Furthermore, the intracellular signaling pathways involved in the promnesic activity elicited by PAM-2 were studied [2]. In particular, we found that (d) PAM-2 did not affect the a7 nAChR expression after acute and chronic treatment, whereas increased the extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation in the hippocampus and prefrontal cortex in mice after 21 consecutive days of treatment [2]. In conclusion, our findings clearly demonstrate that PAM-2 may constitute a promising therapeutic candidate for the treatment of cognitive impairments in neurological diseases such as Alzheimer's disease and schizophrenia where the cholinergic tone is altered.

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NOVEL 11β-HSD1 INHIBITORS FOR AGE-RELATED COGNITIVE DISORDERS AND ALZHEIMER'S DISEASE

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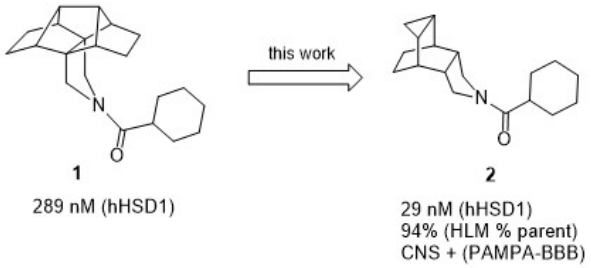
Growing evidence suggests that excessive glucocorticoid activity may contribute to age-associated memory impairment and Alzheimer's disease (AD).[1] It is known that glucocorticoids locally generated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) play a major role in age-related cognitive impairments, and that 11β-HSD1 deficiency prevents spatial memory impairments and cognitive decline with aging.[2]

Several groups and investigations have reaffirmed these findings with different *in vivo* experiments in rodent models. Acute and short-term treatments with 11β-HSD1 inhibitors have showed memory consolidation and improvements in cognitive function in aged mice and AD models.[3] Overall, these data suggest that 11β-HSD1 inhibitors provide a novel approach through a non-cholinergic mechanism to deal with these cognitive disorders.

Given that the enzyme active site includes a hydrophobic pocket to accommodate bulky lipophilic scaffolds, our group is currently focused on the design and synthesis of new 11β-HSD1 inhibitors taking advantage of our expertise in polycyclic compounds.

Our starting point was the hit compound **1**, which exhibited a submicromolar potency against 11β-HSD1. Based on this result, a medicinal chemistry program focused on the polycyclic scaffold optimization delivered more potent inhibitors that were characterized in terms of metabolic stability and brain penetration.[4] Our best candidate **2** was administered to twelve-moth SAMP8 mice during four weeks in their drinking water. Treated mice performed significantly better than control ones in the behavioral tests demonstrating an improvement in the cognitive decline of already aged mice. These findings also correlate with the results of the biochemical and molecular experiments carried out. Overall, these data contribute to confirming 11β-HSD1 as a plausible target for cognitive disorders including AD.



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COMBINING CINAMMATE AND ENONE SCAFFOLDS WITHIN NEW BIOLOGICAL ACTIVE HYBRIDS

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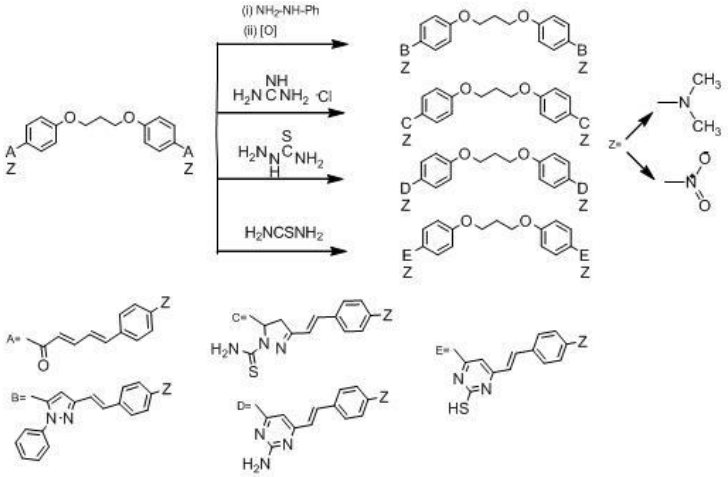
Chalcones are biogenetic precursors of flavonoids in higher plants displaying a wide variety of pharmacological properties. They are well known intermediates for the synthesis of various heterocyclic compounds. Cinammate derivatives also include anti-inflammatory, antioxidant, cytotoxicity towards cancer cell lines, antimitotic, antimutagenic, antibacterial, antiviral and antimalarial activities. A survey of literature in the recent past reveals that thiazine, pyrazoline and pyrimidine derivatives posses the same range of biological activities. These observations led us to synthesize [1,2,3] new bis-cinammate chalcones and their corresponding bis-pyrazoline, bis-pyrazole and bis-pyrimidines and to examine their bioactivities.

Chemistry : [1,2,3]



The compounds were tested *in vitro* for their ability to: a) inhibit *in vitro AchE*, b) inhibit lipid peroxidation of linoleic acid, c) inhibit *in vitro* soybean lipoxygenase, d) interact with luminole. The results were characterized based on the structural characteristics and physicochemical properties of the molecules.

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RATIONAL DESIGN TOWARDS THE FIRST CLASS OF POSITIVE ALLOSTERIC MODULATORS OF KAINATE RECEPTORS

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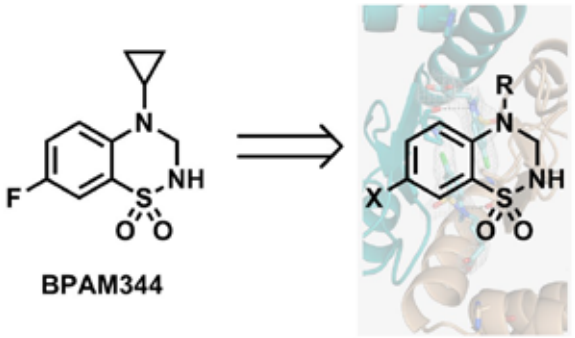
Ionotropic glutamate receptors (iGluRs) play a key role in the majority of fast central nervous system excitatory synaptic transmission, and are divided into three sub-groups: NMDA, AMPA and kainate receptors.[1] Of these subgroups the kainate receptors (KARs) remain the least understood, primarily as overlap with AMPA receptor antagonist sensitivity means suitable selective ligands are lacking.[2] Despite this, KARs are known for their ability to both excite and depress neuronal stimulation, with dysfunction leading to epilepsy, pain and psychological disorders such as bipolar and major recurrent depression.[3] Furthermore, their role in brain plasticity has identified KARs as plausible neuroprotectional targets in the fight against neurodegenerative diseases such as Alzheimer's and multiple sclerosis.[4] Thus, discovery of selective KAR modulators have broad therapeutic potential and appeal.



This research describes the design and synthesis of a new family of potential KAR modulators based on co-AMPAR/KAR positive allosteric modulator **BPAM344**, bearing a benzothiadiazine dioxide core.[5] Co-crystallisation of **BPAM344** with KAR subunit GluK1 elucidated the allosteric binding pocket of interest, and comparisons with AMPAR identified amino acid residues particular to KARs which could be targeted in order to increase compound specificity.[6] As such, a new generation of potential KAR modulators were designed and synthesised, bearing different acidic/basic moieties of varying lengths at the N-4 position. Biological evaluation of these compounds is currently underway.

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Nrf2 AND FREE RADICALS AS COMPLEMENTARY TARGETS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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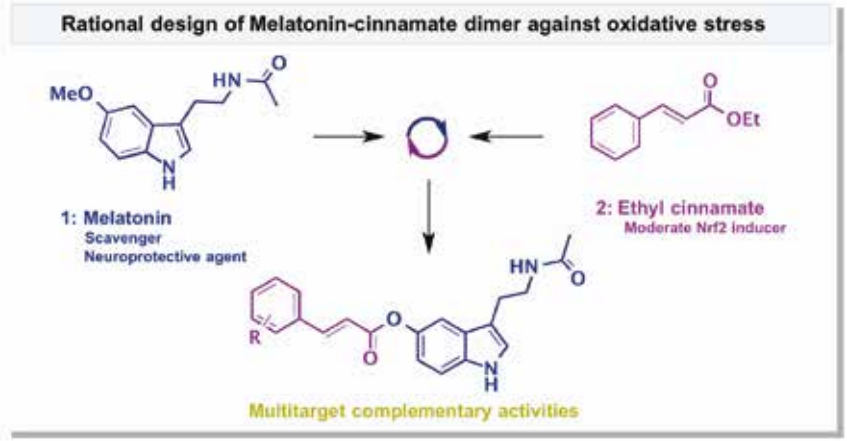
Neurodegenerative diseases (NDDs) share many pathological pathways, such as aberrant protein aggregation, mitochondrial dysfunction, oxidative stress and neuroinflammation. Although the multifactorial nature of NDDs hinders the development of efficient treatments, there is a great interest in the development of compounds directed to reduce oxidative stress and mitochondrial dysfunction, as the latter alterations play a crucial role in the evolution of those pathologies[1].

The transcription factor Nrf2 plays an important role in the defense against both, oxidative stress and neuroinflammation. Besides, the Nrf2 pathway is deregulated in NDDs [2]. Based on these observations, we propose the design, synthesis, and pharmacological evaluation of new compounds, hybrids of melatonin and cinnamic acid, as potential treatments for NDDs[3].



The biological evaluation of newly developed compounds showed an interesting pharmacological profile that includes Nrf2 induction capacity, potent scavenger ability and anti-inflammatory capacity. Besides, these compounds have demonstrated an interesting neuroprotective profile against different *in vitro* models of oxidative stress, and a broad security profile. Altogether, these results indicate these compounds should be further evaluated in *in vivo* models of NDDs.

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DEVELOPMENT OF NOVEL CANNABINOID TYPE 2 RECEPTOR TRACERS FOR PET IMAGING IN NEUROINFLAMMATION AND NEURODEGENRATIVE DISEASES

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Background. The cannabinoid receptor type 2 (CB2) is part of the endocannabinoid system and has gained growing attention in recent years due to its important role in neuroinflammatory/neurodegenerative diseases [1, 2]. Positron Emission Tomography (PET) allows quantification of neuroreceptors and their occupancy by drugs in preclinical and clinical studies. CB2 has very low concentration in brain tissue under basal conditions, however, it is up-regulated in cerebellum, cortex and brainstem in pathological conditions such as neuroinflammation and neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), Parkinson's and Alzheimer`s disease [1,2]. Therefore, CB2 receptor has attracted a lot of attention in recent years and is regarded as a very promising target for both non-invasive imaging and possibly therapy with a high clinical impact. Based on the literatures, we identified one 4-oxoquinoline (designated as KD2) as the most promising lead structure for the design of a potential imaging tracer for the CB2.

Methods. A series of novel CB2 ligands were designed and synthesized based on a 4-oxoquinoline lead structure designated as KD2, which was identified from the literatue [3]. Structure-Activity Relatioship (SAR) was studied using in vitro competitive binding assays with membranes containing human CB2 and CB1, respectively, using [^{3}H]-CP-55940 as the radioligand. The most promising compounds (designated as RS016 and RS126) were radiolabeled with C-11 and F-18 isotope, respectively. In vitro studies including stability, lipophilicity and autoradiography were performed. Further evaluation was performed in vivo in rats and mice using PET under baseline and blocking conditions.

Results. Up to eleven new KD2 derivatives were designed and synthesized in our laboratory. SAR studies led to the identification of N-(1-adamantyl)-1-(2-ethoxyethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (RS-016) and N-(1-adamantyl)-1-(2-fluoroethoxy)ethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (RS-126) as superior and more promising analogs of KD2. RS-016 and RS-126 exhibited excellent Ki values of 0.7 nM and 1.2 nM respectively, towards hCB2 and 10'000-fold selectivity over hCB1. Both [^{11}C]RS-016 and [^{18}F]RS-126 showed specific binding in autoradiographic studies on healthy rat spleen slices. PET imaging studies in rats showed high accumulation in the spleen, an organ with a high physiological expression of CB2. Of this high accumulation in vivo in spleen, ~ 78% was attributed to specific binding to CB2.

In a pilot study on human post mortem ALS spinal cord tissue sections, specific binding to CB2 was also demonstrated, which underlines CB2 as a potential target for imaging in ALS patients.

Conclusion. Based on the in vitro and in vivo results, [^{11}C]RS-016 and [^{18}F]RS-126 can be considered as valuable tools for the in vivo imaging of neuroinflammation and neurodegenerative diseases and have great potential to become successful CB2 PET tracers in the clinic.

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DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF RILUZOLE-BASED UREAS AS POTENTIAL ABAD MODULATORS FOR ALZHEIMER'S DISEASE TREATMENT

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Amyloid-beta peptide (Aβ) has been recognized to interact with numerous proteins, which may lead to pathological changes in cell metabolism of Alzheimer's disease (AD) patients. One such known metabolic enzyme is mitochondrial amyloid-binding alcohol dehydrogenase (ABAD), also known as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10). Altered enzyme function caused by Aβ-ABAD interaction, was shown to cause mitochondrial distress and consequent cytotoxic effect, therefore providing a feasible target in AD drug development [1]. Based on previous frentizole derivatives studies, we report a novel series of riluzole based benzothiazolyl ureas along with identification of two potent ABAD inhibitors, which exceeded the potency of previously reported benzothiazolyl compounds. One compound exhibited comparable cytotoxicity with the frentizole standard, however one fold higher cytotoxicity than the parent riluzole standard. The calculated and experimental physical chemical properties of the most potent compounds showed their promising features for blood-brain barrier penetration [2].

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EFFICACY OF A SMALL MOLECULE TARGETING GSK-3 IN A SPORADIC AMYOTROPHIC LATERAL SCLEROSIS MURINE MODEL

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron degenerative disease without any effective treatment up to date. Lack of knowledge of its pathophysiological molecular etiology together with limited animal models for the disease has hampered drug discovery development.
Recently, animals treated with β-N-methylamino-L-alanine (L-BMAA), a neurotoxic amino acid related to the appearing of ALS, have been proposed to be an appropriate sporadic ALS model to study the neurodegenerative pathological mechanisms of the disease as well as an important pharmacological tool for drug discovery [1].
In the present work, the neuroprotective role of VP2.51, a small heterocyclic molecule able to inhibit selectively glycogen synthase kinase 3 (GSK-3), is tested in this sporadic ALS murine model based on L-BMAA toxicity. VP2.51 intraperitoneal daily administration for two weeks, starting the first day after L-BMAA treatment, leads to total recovery of neurological symptoms and prevents the activation of autophagic processes in rats treated with this neurotoxic agent.
These results show that our toxin-based sporadic ALS murine model can be used to test the efficacy of new drugs and confirm the therapeutic potential of GSK-3 inhibitors [2], and specially VP2.51, for the disease-modifying future treatment of ALS.

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MOLECULAR DYNAMICS SIMULATION OF NMDA RECEPTORS. DESIGN OF ALLOSTERIC MODULATORS

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The glutamatergic system is of vital importance for cognition, learning and memory consolidation. Malfunctioning of this system leads to serious neurological disorders and is associated with neurodegenerative diseases. The molecular dynamics simulation and QSAR studies of ligand-receptor interactions were performed for the subtypes of one of the most important types of glutamate receptors, NMDA receptor. They are ligand-gated ionotropic receptors consisting of four subunits forming the ion channel, ligand-binding domains, and amino-terminal domains. The NMDA receptor antagonists, reversible ion channel blockers, and negative allosteric modulators can serve as neuroprotective compounds.

The molecular dynamics simulations and the analysis of motions of amino-terminal domains of the NMDA receptor comprised by the GluN1/GluN2B subunits as well as the modelled GluN1/GluN2X (X=A,C,D) receptors in complex with negative modulators have revealed the reasons for their high selectivity to the GluN1/GluN2B subtype. The role of negative modulators consists in the stabilization of the contact surface between the amino-terminal domains GluN1 and GluN2X because the complementarity of these surfaces without the bound ligand is quite poor. The docking results indicate very different binding modes for the two classes of known negative modulators of NMDA receptor –ifenprodil analogues and the aminoquinoline and styrylamidine derivatives. For the second type of ligands, an important contribution to the binding is made by the Y109 (GluN1) residue. The QSAR models are consistent with the revealed binding modes. It should also be mentioned that the binding of negative modulators such as ifenprodil leads to the disruption of zinc binding to the amino-terminal domains.

Based on the models obtained from the molecular dynamics simulations, the virtual screening of the ZINC database was performed that produced a number of potential negative modulators and their potency was confirmed by the *in vitro* studies using [^{3}H]ifenprodil.

COMBINED ANTIOXIDANT AND NOOTROPIC PHARMACOPHORES AS AGENTS AGAINST NEURODEGENERATIVE DISORDERS.

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Alzheimer's Disease (AD) is a chronic neurodegenerative condition that affects an increasing number of the population as life expectancy rises. Inflammatory reactions, surrounding cerebral microvasclature, are often observed. Inflammation products are found in the CNS of AD patients. 5-Lipoxygenase is overexpressed in AD and is responsible for neuronal vulnerability. Moreover, there is a significant role of oxidative stress in the evolution of AD. Glutathione levels are decreased in affected brain regions from AD patients.

GABA is the main inhibitory neurotransmitter of the central nervous system. In AD, an association has been found between behavioural problems and deficits of GABA in brain tissue. Decreased GABA levels have been detected in brain regions of patients with AD, suggesting that abnormalities of the GABAergic system may contribute to the pathogenesis of AD.

Finally, cyclised GABA derivatives such as piracetam and aniracetam, besides their anxiolytic activity, can interfere with AMPA receptor, demonstrating nootropic-neuroprotective activity.

Thus, the above pathological changes in the demented brain could be used as a starting point for rational design of multifunctional molecules for the medicinal treatment of cognition disorders.[1]

In this research, some antioxidant acids (e.g. 3,5-di-tert-butyl-4-hydroxybenzoic acid) were amidated with proline or 3-hydroxy-proline, expected to offer nootropic properties, since structurally related amides or esters of proline have nootropic action. Proline carboxylic group has been converted to an amide group by reaction with GABA. Additionally, the antioxidant acid was amidated with 2-pyrrolidinone, expected to offer neuroprotective activity.

The synthesised compounds were found to have in vitro anti-oxidant activity,[2] to exert anti-inflammatory activity, assessed as paw edema reduction and to inhibit lipoxygenase activity.[3] In an attempt to obtain an indication on the ability of the synthesised compounds to enter the brain, some introductory calculations concerning blood-brain barrier penetration were performed.

In conclusion, our results indicate that the design of agents able to be directed to different, selected biological targets, through different pharmacophores properly integrated in a single molecule, may be proven useful for the treatment of complex neurodegenerative conditions.

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COMPUTATIONAL PREDICTION OF BLOOD BRAIN BARRIER PERMEABILITY OF NOVEL PYRROLIDINONE DERIVATIVES WITH POSSIBLE NOOTROPIC AND ANTIOXIDANT ACTION

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The purpose of this investigation is to study the parameters and physico-chemical characteristics of novel compounds, designed for Blood Brain Barrier (BBB) permeation, in order to reach the Central Nervous System (CNS) where they are expected to develop their pharmacological activity. BBB, a specialized physiological structure, demonstrates a diverse role, both structural and functional, and has been a subject of great interest for the design and development of drugs with CNS action.

An in silico analysis for calculating measurements of physico-chemical properties of novel pyrrolidinone amides with antioxidant acids is presented, since these compounds are designed to demonstrate nootropic properties and antioxidant effect against oxidative stress caused by increased reactive oxygen species found in the demented brain. Besides the well known Lipinski's "rule of five" for predicting drug absorption, some more simple rules have been reported for prediction of CNS activity, based on the number of nitrogen and oxygen atoms (N+O) in a molecule or a combination of lipophilicity and (N+O). In addition to lipophilicity, the molecular polar surface area is a dominating factor for oral absorption and brain penetration of compounds transported by passive diffusion. Based on the above evidence concerning lipophilicity and polar surface area, some preliminary results are obtained. Furthermore, a prediction of BBB permeability is calculated using logBB values which express log(Cbrain/Cblood). According to our results, structural modifications of the above mentioned compounds which were designed as nootropic drugs are suggested, in order to ensure effective BBB permeation. An advantage of using this predictive method is that it can provide estimation of the probability of BBB permeability based on physico-chemical parameters prior to their synthesis. We found that most of the designed structures can easily penetrate BBB.

TARGETING TDP-43 PHOSPHORYLATION BY CASEIN KINASE-1δ INHIBITORS: AN INNOVATIVE TREATMENT OF FRONTOTEMPORAL DEMENTIA

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Frontotemporal lobar degeneration (FTLD) is the primary cause of early onset dementia after Alzheimer's disease (AD) and it is characterized by decline in brain cells which control behaviour, thinking and communication [1].One of the most common causes of frontotemporal lobar degeneration is mutations in the progranulin gene (*GRN*) which produces inclusions of the protein called TDP-43 (FTLD-TDP). Post-traslational modifications of TDP-43 are caused among others by abnormal activation of protein kinase CK-1δ responsible of TDP-43 phosphorylation, leading to the formation of cytoplasmic TDP-43 aggregates, which, in turn, may trigger neurodegeneration [2].

With the aim to target neuronal pathologies mediated by TDP-43 dysfunction, we have previously described a new family of small heterocyclic molecules able to inhibit CK-1δ with IC_{50} values in the nanomolar range and a great selectivity over more than 450 different kinases [3]. Moreover, these compounds were predicted as blood brain barrier permeable by PAMPA methodology.In the present work, we have used lymphoblast from FTLD-TDP patients carriers of a loss-of function mutation in *GRN* gene (c.709-1G>A) to evaluate the potential effects of two brain penetrant CK-1δ inhibitors on TPD-43 pathology. We have shown that our CK-1δ inhibitors are able to decrease TDP-43 phosphorylation and regulate its subcellular localization, increasing TPD-43 nuclear localization. Moreover, functional effects of nuclear TDP-43 as repressor of certain genes could be rescue by CK-1δ inhibitors treatment.

Our results show that lymphoblast from FTLD-TDP patients are a good technological platform to discover and study new drugs targeting TDP-43 diseases, and more importantly for translational research, that CK-1δ inhibitors, and specially compounds IGS-2.7 and IGS-3.27 [4], can be considered promising candidates for novel treatments for FTLD associated to *GRN* mutations and others pathologies in which TDP-43 is involved.

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DESIGN OF A STRUCTURE-BASED VIRTUAL SCREENING PROTOCOL, AS A TOOL IN THE DESIGN AND DISCOVERY OF INNOVATIVE SELECTIVE PDE5 INHIBITORS, WITH POTENTIAL ANTI-ALZHEIMER ACTIVITY

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The selection of phosphodiesterases (PDEs) as useful targets for the design of new agents actives in Alzheimer Disease (AD), is receiving in the last decade increasing attention (1-2). In AD patients, some PDE isoenzymes (PDE2, PDE5, and PDE9) are overexpressed in the memory and learning brain areas (3); some PDE inhibitors are proposed as learning and memory enhancers.

In this research, we have designed a new Structure-Based Virtual Screening (SBVS) approach, which, through the application of direct and reverse docking strategies, allows us to quantify the drug-target interactions of previously described selective inhibitors. The data obtained allow us to link objectively the selectivity against different PDE isoenzymes (PDE2, 3, 4, 5 and 9) with the structural characteristics and interaction properties and allow us, also, to predict, in an early phase of the drug design cycle, the selectivity of the new designed inhibitors. The total binding energy, the partial energy against the five regions that constitute the PDE active site, and the interactions with the selected residues located in the active site, were used to score the compounds. This energy scoring is capable of filtering the analysed compounds as selective, non-selective or no-inhibitor against the virtual screening targets.

The selected PDE models were obtained from the Protein database: 4HTX (PDE2) 1SOJ (PDE3), 3G4L (PDE4), 1XP0 (PDE5) and 3K3E (PDE9). The reference inhibitors were taken of PubMed, Zinc, DrugBank and BindingDB repositories. A series of pirimido- and piridazinoindole derivatives, previously reported by our team as PDE inhibitors (4-8), were used to validate the SBVS protocol.

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17-SPIRO-DEHYDROEPIANDROSTERONE DERIVATIVES AS SMALL MOLECULE MIMETICS OF NEUROTROPHINS

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Neurotrophins are a family of closely related secreted proteins that have been shown to control a number of aspects of survival, development and function of neurons such as axonal growth, dendritic branching and synaptic plasticity, in both the central and peripheral nervous systems. The members of neurotrophin families are nerve growth factor (NGF), brain-derived growth factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). There is a strong link between Alzheimer Disease (AD) and changes in neurotrophins levels. Degeneration is due, at least in part, to changes in expression of neurotrophins and/or their receptors. Preclinical studies point to the therapeutic potential of neurotrophic factors in preventing or slowing the progression of neurodegenerative conditions. Thus, neurotrophins have been proposed as therapeutic agents for neurodegenerative diseases, such as AD, PD, MS, to corneal neurotrophic ulcers. However, the poor pharmacokinetic properties of neurotrophins, mainly because of their sensitivity to proteolysis, restricted penetration of the blood–brain barrier and limited ability to diffuse in tissues, render their use as drugs prohibitive.

A potential approach for addressing neurotrophin limitations is the development of synthetic, small molecule, neurotrophin mimetics with favourable profiles of stability, tissue penetration and targeted biological actions.

We have recently synthesized 17-spiro-epoxy- dehydroepiandrosterone (DHEA) derivatives with anti-apoptotic and neuroprotective activity, selectively mediated through the neurotrophin receptors.[1-3] These compounds -in contrast to the parent molecule (DHEA)- are not metabolised to estrogens and androgens, and exhibit high affinity for the NGF receptor, TrkA.

Saturation transfer difference (STD) NMR spectroscopy revealed the interaction of the most active derivative (*R*)-3*β*,21-dihydroxy-17*R*,20-epoxy-5-pregnene (BNN27) with recombinant TrkA receptor, while, MD simulations at the 2TrkA:2NGF complex revealed that BNN27, developed stable interactions bridging TrkA and the NGF heterodimer.

In the context of our continuous efforts in obtaining small molecule mimetics of neurotrophins, and to probe the stereoelectronic requirements towards this aim, we designed and synthesized new DHEA derivatives bearing 5- or 6-membered 17-spiro substituents incorporating oxygen or nitrogen heteroatoms.

The new compounds were evaluated for their affinity for neurotrophin receptors, while, their anti-apoptotic activity was evaluated using the neural-crest-derived PC12 cell model. The obtained Structure-Activity Relationships will be discussed.

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NOVEL NMDA RECEPTOR ANTAGONISTS ADDRESSING THE GLUN2B SUBUNIT

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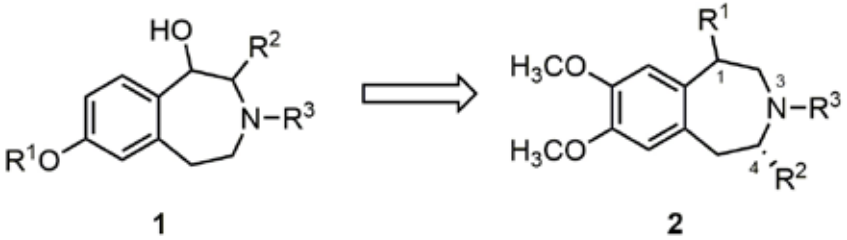
In the last decades neurodegenerative diseases like Alzheimer's disease and Parkinson's disease have attracted major attention due to increased incidence in overaging global population. Thus there is a great interest in developing selective therapeutic drugs for treatment. A promising approach is to address the NMDA receptor. Literature suggests that overactivation of the NMDA receptor leads to excitotoxicity which is associated with neurodegenerative diseases.[1,2]

An interesting binding site of the NMDA receptor is the ifenprodil binding site which is located at the GluN2B subunit. Ifenprodil served as promising lead compound for the design and development of novel GluN2B antagonists. Although ifenprodil shows high affinity (K_i = 10 nM, IC_{50} = 13.3 nM) its selectivity is rather low.[3,4]

Based on the ifenprodil structure our group developed antagonists **1** with a 3-benzazepine scaffold. Several compounds of type **1** possess high GluN2B affinity and antagonistic activity. The phenyl butyl derivative **1a** (R^1 = CH_3, R^2 = H, R^3 = $(CH_2)_4Ph$) with a K_i value of 5.4 nM and an IC_{50} value of 360 nM belongs to the most active and selective GluN2B antagonists.[5]

Based on these results, a chiral pool synthesis of diastereo- and enantiomerically pure 3-benzazepines **2** starting from (*S*)-DOPA was envisaged. The additional substituents in position 1 and 4 should allow the fine tuning of the GluN2B affinity. Moreover, the introduction of [^{18}F] to generate a tracer for positron emission tomography (PET) is possible. Evaluation of binding affinity is currently in progress.



1 **2**

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EFFECT OF SYNTHETIC CHALCONES ON THE ACETYLCHOLINESTERASE ACTIVITY

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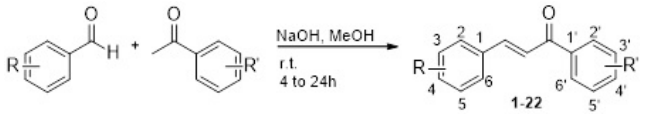
Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects about 44.3 million people worldwide [1]. The drugs that provide relief of symptoms are inhibitors of the acetylcholinesterase enzyme (AChE). It is also known that in patients with AD, the cellular damage caused by reactive oxygen species is higher than normal. Our aim was to synthesize chalcones derivatives and perform *in vitro* assay to develop multifunctional molecules with antioxidant and anticholinesterasic activities. In this work, we also conducted studies of molecular docking of the synthesized compounds.

Methodology

We synthesized twenty-two chalcones with electron-withdrawing and electron-donating substituents by the Claisen-Schmidt condensation [2] (Scheme 1).

Scheme 1



1: R = R' = H;**2:** R= 4-chloro, R' = H; **3:** R= 4-nitro, R' = H; **4:** R= 3,4-methylenedioxy, R' = H;**5:** R= 4-bromo, R' = H; **6:** R= 4-methyltio, R' = H; **7:** R= 4-chloro, R' = 3',4'-dimethoxy; **8:** R= 4-nitro, R' = 3',4'-dimethoxy; **9:** R= 4-bromo, R' = 3',4'-dimethoxy; **10:** R= 3,4- methylenedioxy, R' = 2',4'-dimethoxy; **11:** R= 4-methyltio, R' = 2',4'-dimethoxy; **12:** R= 4-methyltio, R' = 3',4'-dimethoxy; **13:** R= 2-fluor, R' = 3',4'-dimethoxy; **14:** R= 4-nitro, R' = 2',4',6'-triisopropyl; **15:** R= 2-fluor, R' = 2',4',6'-triisopropyl; **16:** R= 3,4- methylenedioxy, R' = 2',4',6'-triisopropyl;**17:** R= 3,4- methylenedioxy, R' = 2',4'-difluor; **18:** R= 3,4- methylenedioxy, R' = 2',5'-dichloro; **19:** R= 2,4-dimethoxy, R' = 3',4'-dimethoxy; **20:** R= 2,3-dichloro, R' = 3',4'-dimethoxy; **21:** R= 2-chloro-3-quinolinyl, R' = 2',4'-difluor; **22:** R= 3-quinolinyl, R' = 2',4'-dimethoxy.

AChE inhibition effects were evaluated using Ellman method [3]. The antioxidant activity were tested using the DPPH method [4] and docking simulation were performed in AutoDock Vina [5].

Results

Compounds were obtained in 53-98% overall yields. Compounds **14**, **15**, **16**, and **17** have not been described in the literature. Compound **1** presents IC_{50} value of 0.09 μM and shows promising activity. In respect of antioxidant activity, **15** has demonstrated antioxidant activity of 100%. Molecular docking studies of compound **1** and AChE (PDB: 1EVE) reveals a good interaction at the active site of AChE (-9.5 kcal/mol).

Conclusion

Chalcone **1** showed the best result in the *in vitro* AChE inhibition assay (IC_{50} = 0.09 μM). Molecular docking studies showed that compound **1** exhibits good interaction with AChE active site confirming the *in vitro* test. As antioxidant, chalcone **15** demonstrated the best result. We conclude that compound **1** is promising, requiring improvements in the structure to improve the antioxidant activity and further analysis.

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DEVELOPMENT OF INHIBITORS OF AMYLOID BETA PEPTIDE FIBRILLATION

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, and the most common cause of dementia which currently affects more than 44 million people worldwide (1). The amyloid cascade hypothesis states that the primary event in AD pathogenesis is the accumulation and fibrillation of amyloid beta peptide (Aβ) within the brain (2). Polyphenols, such as (–)-epigallocatechin gallate (EGCG), are potential disease-modifying drugs for AD treatment because of their ability to inhibit Aβ fibrillation (3,4). However, polyphenols are generally not druglike molecules and they have poor systemic and brain availability (5,6). This presentation highlights the development of novel inhibitors of $A\beta_{42}$ fibrillation, based on polyphenols, but with improved druglikeness. The ability of the compounds to modulate $A\beta_{42}$ fibrillation was evaluated using a variety of orthogonal techniques including fluorescence assays, transmission electron microscopy, and dot blotting. Inhibition of $A\beta_{42}$-induced neurotoxicity was assessed with SH-SY5Y cells, and blood-brain barrier permeability was estimated using a MDR1-MDCK II cell monolayer assay.

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DISCOVERY OF A SERIES OF BETA-SECRETASE INHIBITORS WITH THE ASSISTANCE OF STD-NMR

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Abeta played a central role in the Alzheimer's disease (AD) pathogenesis and progress, and beta-secretase (BACE) is a rate limiting enzyme in the biosynthesis of Abeta. In this sense, BACE-1 inhibition has been widely explored as a potential AD therapeutic drug target in the last decade. Most of the BACE inhibitors known to date are active-site directed competitive inhibitors, whereas a small portion of inhibitors are non-competitive or with no definite mode of action.

Our efforts have identified two weak BACE inhibitors from a library of natural products at first. Based on their non-competitive behaviour revealed by saturated transfer difference (STD)-NMR technique, we decide to link these two segment appropriately in order to find more potent BACE inhibitors. Encouragingly, a series of molecules were designed, some of which were found with enhanced inhibitory activity in both enzyme assay and the neuronal cells.

THE IMPACT OF HYDROXY GROUPS IN CONFORMATIONALLY RESTRICTED GLUN2B SELECTIVE NMDA RECEPTOR LIGANDS ON THEIR ANTAGONISTIC ACTIVITY – MOLECULAR MODELLING, SYNTHESIS AND BIOLOGICAL EVALUATION

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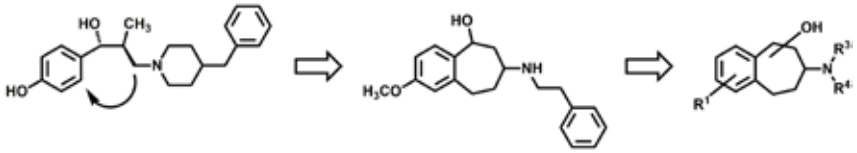
The hyperactivation of the NMDA receptor is connected to the development of several diseases like Alzheimer´s and Parkinsons´s disease, depression, Huntington´s disease, cerebral ischaemia and pain. GluN2B subunit containing NMDA receptors have shown to be involved in the damaging of neurons (excitotoxicity). Therefore this subunit is an interesting target for the development of new neuroprotective substances. GluN2B selective antagonists reduce the excitotoxicity and preserve the physiological function of the NMDA receptor at the same time [1].

Ifenprodil, a highly potent GluN2B receptor antagonist, provides the lead structure for novel antagonists [2]. To overcome the low selectivity of ifenprodil conformationally restricted antagonists were synthesised and tested in our group. Based on the promising results the conformational restriction approach was applied on a distinct and also highly potent GluN2B antagonist, Ro 25-6981 [3].



Ro 25-6981 1 2

The amino alcohol **1** represents one of the most promising compounds of the resulting benzo[7]annulen-7-amines with a binding affinity of 16 nM and an inhibitory activity of 12 nM [4]. These affinity and antagonistic activity data encouraged further investigations of this group of compounds. Molecular docking studies show interactions between the binding pocket and the hydroxy group attached to the seven-membered ring [5]. Therefore compounds with structure **2** were designed to increase affinity, selectivity and antagonistic activity. The antagonistic activity of these compounds is investigated in a functional assay resulting in novel structure activity relationships.

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TARGETING NEUROINFLAMMATION THROUGH POTENT NON-COVALENT INHIBITORS OF CASPASE-1

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Recent studies have shown the pivotal role of innate immune activation in the pathogenesis of major neurodegenerative diseases. It was discovered that microglia and other cell types components of the brain innate immune system, may be activated also by misfolded proteins or aberrant endogenous molecular patterns that are accumulated in the brain of patients affected by several neurodegenerative disorders. These stimuli are responsible for chronic neuroinflammation by triggering the inflammasome's formation and subsequent caspase-1 activation. Proinflammatory cytokines are processed by active caspase-1 into their mature form leading to cytokines production and, ultimately, chronic inflammation. Furthermore, the extensive generation of proinflammatory cytokines (IL-1β, IL-18) mediated from activated caspase-1, leads to pyroptosis that plays an important role in several neurological diseases. Moreover, recent findings indicate caspase-1 as a modulator for the activation of caspase-6 mediated axonal degeneration in AD. There is evidence that caspase-1 inhibition might effectively interfere with the onset and progression of neurological disorders so, caspase-1 inhibitors are very promising candidates for the treatment of neurodegenerative diseases.

We have designed and synthesized new potent and selective non-peptidic, non-covalent, cell permeable caspase-1 inhibitors, which have shown a very high activity in suppressing the formation of IL-1β in LPS induced inflammation and to cross the BBB in PET experiments.

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NOTES

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POSTERS

Big Data in Medicinal Chemistry

IN SILICO PREDICTION OF CYP3A4 INHIBITION BASED ON 2D AND 3D MOLECULAR DESCRIPTORS

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Drug-drug interactions (DDI) are important considerations during the process of developing new drugs. Some drugs are able to change the concentration of a second drug in the body by inhibiting and/or inducing enzymes involved in drug metabolism. For example, the activity of the important drug metabolising enzyme cytochrome P450 3A4 (CYP3A4) can be seriously impaired by inhibitors (by reversible or irreversible mechanisms) such as ketoconazole; this increases the concentration of co-administered drugs, eg. midazolam or nifedipine, that are largely eliminated by means of CYP3A4-mediated metabolism. The increase in concentration of the co-administered drug raises the possibility of it causing adverse side-effects. The effect of inhibitors on CYP3A4 activity can be measured experimentally and the results expressed in terms of an IC_{50} or K_i value, but results can be variable. Because effects of novel compounds on the pharmacokinetics of registered drugs can reduce the likelihood of their successful registration or restrict their post-registration use, determination of the likelihood of DDI is frequently performed early in drug discovery in order to obtain a timely estimate of inherent risk. *In silico* determination of inhibition of CYP3A4 and other drug metabolising enzymes therefore offers the potential of reducing initial costs. *In silico* prediction of IC_{50} values can be combined with *in silico* estimates of exposure to provide early screening of expected *in vivo* DDI for new compounds. Here, we focus on the prediction of IC_{50} for CYP3A4 inhibitors.

Prediction of IC_{50} values for CYP3A4 inhibitors has been explored on a diverse data set from Binding DB, spanning the IC_{50} range 10 nM - 10 µM. Using various pattern recognition approaches to build models for IC_{50} predictions, a number of different descriptor sets were tested to find the optimal set with the highest information content. These descriptor sets included RDKit structural descriptors, CDK structural descriptors and fingerprints, MOE 2D and 3D MM and QM molecular descriptors and CYP3A4-inhibitor docking scores via GOLD. From these initial studies, we have found that the models built using GOLD docking scores or MOE 3D QM descriptors yielded improved predictions compared to those using 2D or other 3D molecular parameters. We discuss these results, considering data set and the role of protein flexibility in these predictions. We also outline the potential for further application to modelling of DDI.

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF POTENTIAL DUAL BINDING SITE ACETYLCHOLINESTERASE INHIBITORS

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Alzheimer's disease (AD) is the most common form of dementia and this multifactorial neurodegenerative disease is characterised by a progressive and irreversible process of loss of cognitive function and memory. Some key neuropathological features have already been identified, such as the presence of amyloid plaque (aggregation and deposition of amyloid-β peptide) and neurofibrillary tangles (coiled wire Tau protein).[1]

The current approach for mild to moderate stages of AD aims the increased cholinergic transmission by inhibiting the enzyme acetylcholinesterase (AChE). Apart from that, AChE is also involved at the early phase of the disease by inducing amyloid-β aggregation through its peripheral active site. In this sense, the design of dual binding site inhibitors (inhibition of amyloid-β aggregation and AChE by targeting both the peripheral and catalytic active sites) has emerged as a promising strategy.[2]

The present work has addressed this approach to synthesise dual binding site inhibitors via "click chemistry" and peptidomimetic, which are relevant concepts in the discovery of bioactive molecules.[3,4] The target compounds have been designed based on the classical AChE inhibitor donepezil (Aricept ®) by replacing the 5,6-dimethoxy-1-indanone moiety to azido amino acids in order to afford structures bearing the 1,4-disubstituted 1,2,3-triazole ring (Figure 1).

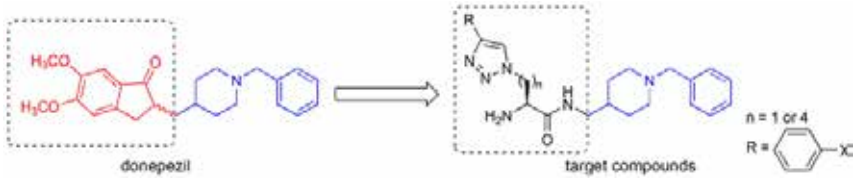

Figure 1. Target compounds based on the classical AChE inhibitor donepezil.

The amide coupling of Fmoc-azido amino acids (2 steps from Fmoc-Lys(Boc)-OH or Fmoc-Asn-OH) and the intermediate 1-benzyl-4-(aminomethyl)piperidine (2 steps from 4-piperidinecarboxamide) allowed the satisfactory synthesis of the azido-building blocks. Hence, the synthesis of the target compounds was successfully achieved (65-85% yields) via Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) with commercial terminal alkynes, followed by Fmoc deprotection for further biological assessment.[5,6]

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EXTRACTING AND EXPLOITING MEDICINAL CHEMISTRY ADMET KNOWLEDGE AUTOMATICALLY FROM PUBLIC AND LARGE PHARMA DATA.

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The remorseless increase in the cost of drug discovery requires medicinal chemists to generate compounds with properties acceptable for in vivo testing as efficiently as possible. An approach to this problem is to extract and record medicinal chemistry knowledge from measured data. The vast size of medicinal chemistry space, the global research efforts in compound design and intrinsically complex nature of drug sized molecules make the manual capture of such knowledge increasingly challenging. An automated approach based on advanced match molecular pair analysis combining two algorithms and capturing the local chemical environment will be presented. Case studies showing how such knowledge has been used to solve problems will be shared.

IDENTIFICATION OF PRIVILEGED SCAFFOLDS AND RECURRING FOLDING PATTERNS IN PROTEINS

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Binding site analysis is a widely established approach for the identification of similar ligand binding, which is typically based on the comparison of potential protein-ligand interaction patterns. Another level of conservation among different proteins is the similar spatial arrangement of secondary structure elements at the ligand binding site independent of the overall folding that can bind similar scaffolds (see Figure 1).[1] The knowledge about these similar ligand-sensing cores would allow for the rational identification of new inhibitors or the prediction of polypharmacology.[2]

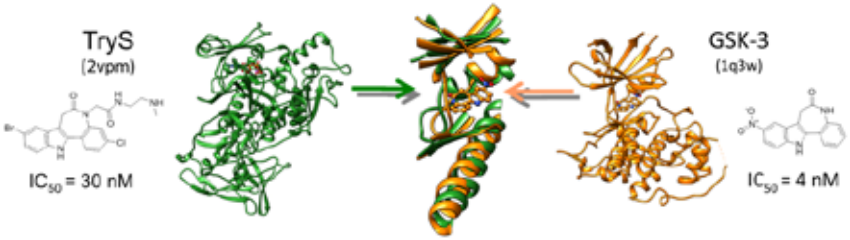

Figure 1: Glycogen synthase kinase 3 and trypanothione synthetase show a similar ligand-sensing core and known inhibitors that share a similar paullone scaffold.

A systematic analysis of this approach is still elusive due to the lack of appropriate computational tools. Therefore, we focused on the development of different tools for knowledge discovery and a day-by-day use in a rational molecular design workflow for the identification of new inhibitors and potential lead structures. A newly developed ligand-based tool was utilized to perform a chemogenomic analysis of Drugbank[3] and identified scaffolds that bind to unrelated proteins thus revealing conserved structural elements. Using this tool, a new ligand-sensing core was identified and applied to find new inhibitors for one of the proteins (BRD4) with an IC_{50} in the nanomolar range. Here, a subset of a high-troughput screening library was tested on BRD4 that contains all molecules showing a structural similarity to known ligands of the other proteins belonging to this ligand sensing core. A high-rate of ~11% in the primary screen clearly indicates a similarity in ligand binding between BRD4 and one of the proteins with impact on the identification of potential site-effects or predicting polypharmacology.

An automated method to determine ligand-sensing cores of otherwise unrelated proteins was also developed. The current implementation allows for a very fast comparision of one binding pocket to all known protein structures. An all-against-all comparison of all known protein binding pockets was performed within 28 days on a recent workstation. The final aim is a webserver including a database with all known ligand sensing cores that will hopefully be available soon for general use. This method is also to be used to analyze all kinases and find new trypanothione synthetase inhibitors for the fight against trypanosomatid diseases.

The underlying approach and newly developed computational tools will be discussed in detail and promising results will be presented to demonstrate the usefulness.

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STUDIES OF RELATIONSHIP BETWEEN BLOOD-BRAIN BARRIER PERMEABILITY AND CHEMICAL STRUCTURES OF DRUGS WITH APPLICATION OF DEEP NEUTRAL NETWORKS

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The ability of drugs to permeate the blood-brain barrier (BBB) is a key for its use in the treatment of central nervous system (CNS) disorders. Therefore the aim of the work was to develop a Deep Convolutional Neural Network (CNN) to predict permeability of active pharmaceutical ingredients (APIs) through the blood-brain barrier (BBB). Algorithm implementing the model was intended to work based on analysis of molecular descriptors (i.a. 3D electron densities) of APIs.

The database for the relationship were chemical structures of APIs labeled with binary positive/negative values depending on weather the APIs penetrate through BBB or not [1]. The geometries of molecules were subjected to conformation analysis following by optimization with DFT B3LYP 6-31G method. The algorithm for CNN was implemented using python language with Theano 0.9dev and Lasagne 0.2dev modules. Basing on optimal geometries, the 3D molecular descriptors were calculated and used as inputs for CNN. The applied model was trained and outer validated on test set of penetrating and nonpenetrating agents.

The study resulted in developing a mathematical model based on the CNN that allowed definition of a relationship between the 3D molecular descriptors of a large set of APIs and their permeability of BBB. Performance of model was evaluated with common validation measures for QSPR models: mean-squared error, cross-validation (Q^2) and least squares fit (R^2). The validation parameters were compared with those obtained with well-established approaches namely multiple logistic regression, artificial neural networks and support vector machines.

The pilot studies demonstrated that it is possible to create an algorithm defining a relationship between the chemical structures of APIs expressed as molecular descriptors (i.a. 3D electron densities) and BBB permeability. This approach is particularly valuable in predicting the BBB permeability of new APIs for CNS treatment.

Financial Support: This research was supported by The Ministry of Science and Higher Education in Poland

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SYNTHESIS OF A NOVEL POTENTIAL MULTITARGET CHOLINESTERASE INHIBITOR

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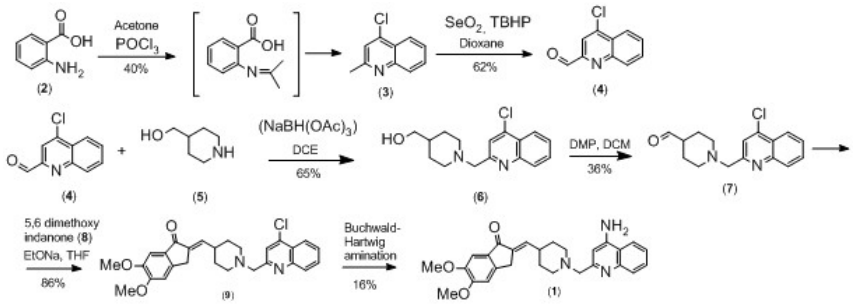
Nowadays the main strategy to treat AD is to inhibit the Acetylcholinesterase (AChE) in order to increase the neurotransmissor acetylcholine levels at synapses. Besides the impairment of cholinergic neurotransmission, the brain of the Alzheimer's disease patients shows β-amyloid plaques and neurofibrillary tangles. The binding pocket of AChE consists of two ligand binding sites: the catalytic anionic (CAS) and the peripheral anionic sites (PAS). Recent studies have demonstrated that the PAS might accelerate the aggregation and deposition of the neurotoxic beta-amyloid peptide[1]. It is known that a potential AChE inhibitor should bind in both sites of AChE, as a dual binding site which could disrupt the interactions between the enzyme and the β-amyloid peptide [2]. In this work is shown the synthesis of a novel AChE inhibitor **1**, designed as a tacrine-donepezil hybrid (**Scheme 1).**The synthetic strategy was based on classical methods, such as the cyclization reaction between the anthranilic acid (**2**) and acetone to afford the quinoline chloride derivative **3** (40% yield)[3], which methyl group was oxidized with SeO_2 to obtain the 4-chloro-quinolinyl-2-carbaldehyde (**4**) (62%)[4]. In a second moment, a reductive amination was performed among the aldehyde derivative **4** and the amine compound **5** in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) (65%)[5], resulting in the alcohol **6.** A mild oxidation with Dess-Martin reagent (DMP) of alcohol **6** afforded the aldehyde **7** (36%)[6]. Besides that, a type aldol condensation between the aldehyde **7** and 5,6- dimethoxy-indanone (**8**) enolate afforded the compound **9** (86%) [7]. Finally, The chlorine atom of **9** was substituted by amine group through the cross-coupling Buchwald-Hartwig amination, and the product **1** was obtained with 16% of yields[8] (**Scheme 1**).



The tacrine-donepezil hybrid **1** was tested in human AChE in modified Ellman's assay and showed an inhibition of 14±0.009 nM, which is more active than tacrine (230±0.069 nM) and at the same range as donepezil (5.7±0.0005 nM). Docking studies have shown that tacrine-donepezil hybrid **1**, has affinity for both sites, with potential capacity to disrupt the β-amyloid peptide aggregation induced by AChE. A novel 2-[(1-(4-aminequinolin-2-yl)-methyl)-piperidin-4-yl]-methylen]-5,6-dimethoxyindan-1-one (**1**) compound was synthesized, and showed a nanomolar range inhibitory activity towards human AChE. The tioflavin T test, to evaluate the β-amyloid peptide aggregation induced by AChE will be performed.

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DISCOVERY OF THE MGLU5 NEGATIVE ALLOSTERIC MODULATOR BASIMGLURANT (RO4917523)

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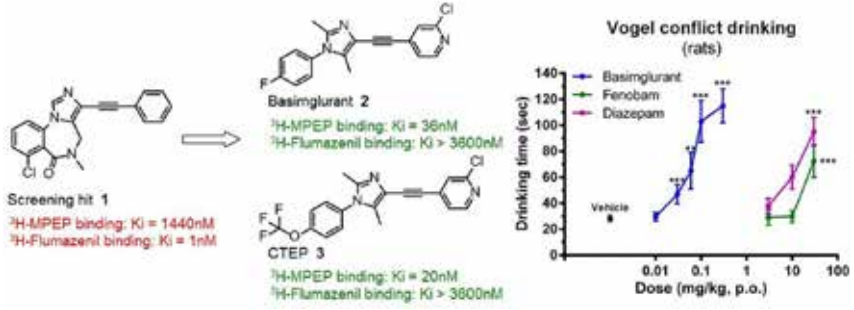
mGlu5 negative allosteric modulators (NAMs) have emerged as a novel approach for treating psychiatric indications including depression, Fragile X syndrome, anxiety, obsessive-compulsive disorders and levodopa induced dyskinesia in Parkinson's disease. Several mGlu5 NAMs are or have been in clinical development. An optimization process starting from a weakly active screening hit 1 led to the development candidate, basimglurant (2), a potent and selective mGlu5 NAM with favorable drug-like properties and CTEP (3), a first long acting mGlu5 NAM tool compound. Basimglurant is active in a broad range of anxiety tests reaching the same efficacy but at a 10- to 100-fold lower dose compared to diazepam and is characterized by favorable DMPK properties in rat and monkey as well as an excellent preclinical safety profile. Basimglurant shows a robust antidepressant-like profile, acting through a clearly distinct molecular mechanism compared to existing antidepressants.



RAPID TECHNIQUE FOR NEW SCAFFOLD GENERATION II: WHAT IS THE BEST SOURCE OF INSPIRATION?

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Scaffold hopping remains a central task in medicinal chemistry for generating and protecting intellectual property. We have previously presented a technique for rapidly generating reasonable yet novel scaffold replacements using molecular fields. We showed that our method, embodied in the scaffold hopping software Spark, competes favourably with other published methods including those derived from quantum mechanics.

In this poster we explore how different data sources affect the results of a scaffold hopping experiment. Common data sources include small molecule crystal structures, screening compounds, available reagents and literature reports of bioactive compounds. These data sources will be explored in relation to a set of Spark experiments (e.g., core replacement, R-group exploration, fragment growing) to determine how they affect the quality of the results.

NOTES

EFMC
International Symposium
on Medicinal Chemistry
Manchester, UK Aug. 28 – Sept.1, 2016

POSTERS

New Horizons in GPCR-targeted Medicinal Chemistry

FISHING FOR AN "OFF-TARGET": DECONVOLUTING THE MOLECULAR MECHANISM OF GPCR-INDUCED MACROPHAGE CHEMOTAXIS

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The cannabinoid receptors 1 and 2 (CB_1 and CB_2), are key components of the endocannabinoid system, and have a significant role in modulating pain perception, the immune system, metabolism and mood. CB_1 is mostly localized in the central nervous system (CNS) and modulation of this GPCR is thought be responsible for the psychotropic effects of cannabinoids. By contrast, CB_2 can be mostly found on cells of the immune system, and has been proposed as a therapeutic target for pain and inflammatory conditions such as atherosclerosis [1].

In a previous study to discover selective CB_2 agonists [2], whilst studying their effect on macrophage chemotaxis, we demonstrated that chemotaxis induced by selective CB_2 agonists can be uncoupled from CB_2 activation and be considered as an off-target effect at a non-CB_1/CB_2 $G_{i/o}$-coupled receptor [3].

Following that, we performed a ligand-based virtual screening of in-house library, using four positive for macrophage chemotaxis CB_2 agonists as templates. Further hit validation, revealed that several 2-thioxo-2,3-dihydroquinazolin-4(1H)-one derivatives share significant structural and electronic similarity to the reference compounds, as well as, hold potential as mediators of macrophage chemotaxis. As a result, a structure-activity relationship study was undertaken in order to uncover the sites in this scaffold, where a benzophenone photo-affinity label may be installed.

Gaining access to such a probe is of great importance as it will lead to the deconvolution of the CB_2 role during inflammation, and/or possibly lead to the identification of new players in macrophage chemotaxis and inflammation.

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DEVELOPMENT OF FLUORESCENT LIGANDS AS TOOLS FOR STUDYING CANNABINOID TYPE 2 RECEPTOR

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Cannabinoid type 2 receptor (CB2R), a G protein-coupled receptor (GPCR) found primarily in the peripheral tissue, is implicated in the pathologies of various diseases including osteoporosis, inflammatory bowel disease, atherosclerosis and cancer[1]. However, there is a significant lack of understanding of the specific role of CB2R in disease, as well as the variation of receptor expression levels between cell types and healthy and diseased tissue. Such knowledge is imperative for understanding disease pathology and for drug development. There are a variety of existing methods available for studying CB2R such as fluorescent immunohistochemistry, radioligands and reporter gene assays. However each of these have limitations such as poor receptor specificity and low reproducibility. Fluorescent ligands are excellent tools to study receptor structure and function in live cells and as such have been successfully designed for other GPCRs. Fluorescent ligands can be used to investigate dynamic receptor processes such as receptor activation and trafficking and can be employed in a variety of fluorescence based imaging techniques[2]. In this study a library of indole derivatives has been synthesised with the aim of developing a selective, high affinity fluorescent ligand for CB2R. The indole scaffold and attached substituents were selected on the basis of existing structure activity relationships (SAR) and a linker and fluorophore were coupled at a position thought to be tolerant of steric bulk. The synthesis was carried out over nine steps, generating seven final fluorescent compounds. These fluorescent compounds along with key synthetic intermediates underwent pharmacological evaluation. Radioligand binding assays were carried out to determine affinity and CBR subtype selectivity. Functional assays using HEK 293 cells were carried out on promising compounds to determine activity. The synthesis, pharmacological results and newly derived SAR will be presented.

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A THIENO[2,3-d]PYRIMIDIN-4-AMINE SCAFFOLD IS A NOVEL NEGATIVE ALLOSTERIC MODULATOR OF THE DOPAMINE D2 RECEPTOR

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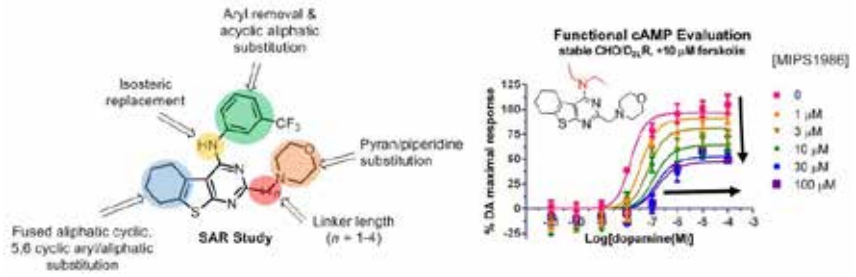
G protein-coupled receptor (GPCR) drug discovery is characterised by a high attrition rate, especially in the field of neuropsychopharmacology. This reflects the difficulty in treating complex diseases, such as schizophrenia. Current antipsychotics are predominantly efficacious at treating the positive symptoms of the disease through their action as antagonists at the dopamine D_2 receptor (D_2R) but this approach is limited by severe side-effects. To address this unmet medical need novel approaches and novel targets are required. Although the dopamine receptors are important drug targets, allosteric modulation of these receptors has not been exploited therapeutically. Allosteric modulators may have a number of advantages over approaches that target the orthosteric site of GPCRs, such as improved subtype-selectivity and thus reduced off-target side-effects, as well as maintenance of spatio-temporal patterns associated with endogenous neurohumoral signalling.[1] Recently, virtual ligand screening identified a negative allosteric modulator of the D_2-like dopamine receptors (**compound 7**)[2]. This ligand comprises a thienopyrimidine scaffold which does not feature in known dopaminergic ligands. We report the synthesis, and pharmacological profiling of a focused chemical library of **compound 7** analogues, with emphasis on the validation of allosteric virtual ligand screening and identification of key structural features guided by computational and synthetic methodologies. Our preliminary biochemical and functional characterisation at the D_2R has validated an allosteric mode of action for many structural analogues including evidence of non-competitive inhibition of the binding of the antagonist [^{3}H]raclopride. Additionally, these ligands were shown to non-competitively inhibit the action of dopamine *in vitro* with increasing concentrations of modulator causing a decrease in the maximal response observed in an assay measuring intracellular cAMP levels. These data are consistent with our identifcation of a novel thienopyrimidine scaffold as negative allosteric modulators of agonist efficacy at the D_2R.



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REGULATION OF CELL PROLIFERATION BY GPR55/B2 ADRENERGIC RECEPTORS USING (R,R)-4′-METHOXY-1-NAPHTHYLFENOTEROL IN RAT C6 GLIOMA CELL LINE

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(*R,R′*)-4′-Methoxy-1-naphthylfenoterol (MNF) is a potent inhibitor of the proliferation of rat C6 glioma cells with an IC_{50} of 0.45 nM and initial *in vivo* data indicate that the compound may be potentially useful in the treatment of malignant gliomas. MNF is an agonist of the β_2-adrenergic (β_2AR) receptor and an antagonist of the GPR55 receptor. Previous studies have demonstrated that MNF inhibits proliferation in 1321N1 astrocytoma and UACC-947 melanoma cells through the β_2AR and inhibits the growth of HepG2 hetapocallular carcinoma and PANC-1 pancreatic cancer cells *via* GPR55.

In this study, the mechanism of the observed MNF effect in C6 cells was investigated using [^{3}H]-thymidine incorporation, cell motility assay, western blotting, and internalization of the fluorescent GPR55 ligand, Tocrifluor 1117 (T1117).

MNF inhibited the ERK and AKT signaling pathways with half-maximal activity in low nanomolar concentration range. The effects of MNF were mimicked by Fenoterol and Isoproterenol, β_2AR agonists. ICI 118,551, β_2AR antagonist, blunted the effects of MNF towards ERK and AKT highlighting the role of β_2AR in MNFs activity. Moreover, cell pretreatment with AM251, GPR55 agonists, dose-dependently blocked the anti-mitogenic responses of MNF. Both AM251 and the atypical cannabinoid O-1602 increased cell motility and ERK phosphorylation, which was blocked by MNF. Finally, there was marked inhibition in the cellular accumulation of T1117 in MNF-treated C6 glioma cells.

These results indicate that MNF inhibits proliferation of C6 cells *via* activation of β_2-adrenergic receptor and abolishes oncogenic signaling elicited by GPR55 agonists. This dual activity of MNF is a promising feature in the context of glioma management, but may also be beneficial in other types of malignancies.

STRUCTURE-GUIDED DEVELOPMENT AND MOLECULAR MODELLING OF D2R/NTS1R HETERODIMER-SELECTIVE LIGANDS

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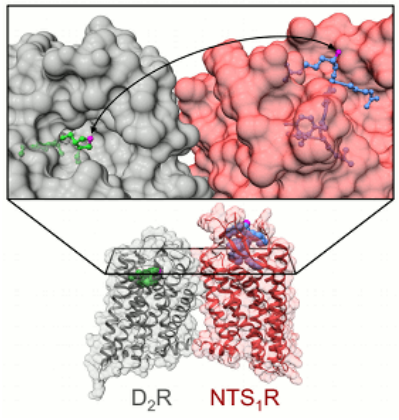
In the chronic disease of schizophrenia the dopamine (DA) system plays a very important role. With the discovery of many different types of dopamine D_2 heterodimer complexes the understanding of schizophrenia changed. It is presumed that the dopamine D_2 receptor (D_2R), interacting with many other G protein-coupled receptors (GPCRs), ion-channel receptors and DA transporters, acts as a switching point and a disturbance in several heterodimer complexes may promote the development of schizophrenia via a modified DA signaling [1]. One of these heterodimer complexes is formed by D_2R and the neurotensin receptor 1 (NTS_1R), in which neurotensin seems to produce antipsychotic effects. However, further studies are required to fully characterize this interaction.

A powerful tool to address and study dimers formed by GPCRs are bivalent ligands bridging the two neighbored orthosteric binding sites, of which the design can be quite challenging. However, high resolution crystal structures of GPCRs revealing a dimer orientation opened new opportunities to design bivalent ligands in a more rational way.

We made use of the crystal structure of the β_1-adrenergic receptor [2] (β_1AR) as a dimer and built a D_2R/NTS_1R heterodimer model. The D_2R protomer was a homology model and as NTS_1R protomer we selected a crystal structure of NTS_1R. The structure of β_1AR revealed a dimer interface involving transmembrane helix 1 (TM1), TM2 and helix 8. The generated dimer model could be used to select linker attachment points for the D_2R and NTS_1R pharmacophores as well as to determine suitable linker lengths. Molecular dynamics simulations with the bivalent ligands, performed to validate the ligand design, showed stable receptor-ligand complexes suggesting the development and experimental investigation of such ligands. In fact, the bivalent ligands showed receptor-bridging properties and high selectivity for the D_2R/NTS_1R heterodimer.



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NEW APPROACHES TO TREATMENTS FOR SCHIZOPHRENIA: TARGETING ?2-ADRENOCEPTORS

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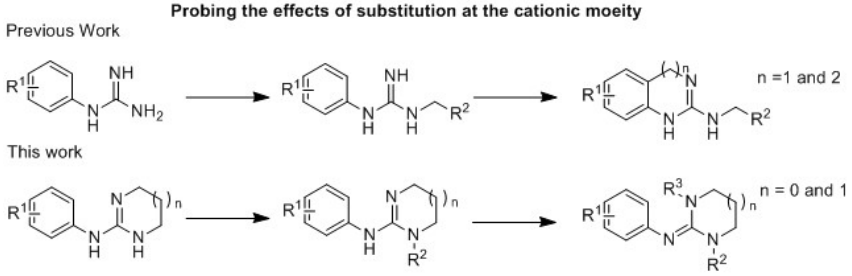
According to the World Health Organization (WHO), schizophrenia is a severe and debilitating mental disorder which affects more than 21 million people worldwide. Schizophrenia is more common, and manifests earlier in life, among males, with 12 million men *v.s.* 9 million women currently diagnosed.[1,2] This highly chronic condition manifests as a collection of different symptoms (positive, negative and cognitive) which interfere with patients ability to lead a normal life and earn a living, contributing to extreme emotional distress.[3]

Despite the broad range of pharmacological treatments currently available, an estimated third of schizophrenia sufferers are diagnosed as treatment-resistant, which refers to sub-optimal response to multiple antipsychotics in terms of positive symptoms.[4] The final option for these patients is the prototypical antipsychotic clozapine. However, this drug, which shows a wide range of affinities and activities at numerous CNS receptors, and consistently displays unparalleled efficacy, can lead to potentially life threatening side effects.[5] Moreover, negative symptoms and cognitive deficits are not treated by the currently available antipsychotics and this represents an unmet clinical need.[6]

The adrenergic system has been consistently proven to play an important role in neuropsychiatric disorders such as schizophrenia and major depressive disorder. Previous studies conducted within our group identified a number aromatic guanidine and 2-aminoimidazoline (cationic moeity) containing derivatives which display high competitive binding affinities (pKi 6.5 - 8.80) with varied functional activity at the α_2-adrenoceptors (α_2-AR) in human prefrontal cortex tissue.[8,9] The design, synthesis and pharmacological evaluation of these compounds has led to the development of numerous structure activity relationships (SARs) which are being utilized in the design and development of selective high binding competitive antagonists at the α_{2C}-AR.[10]

This work aims to identify key structural characteristics/motifs which impart subtype selectivity whilst maintaining high affinity and antagonistic activity, starting with the cationic moeity. Both previously synthesized and proposed compounds are undergoing selectivity screening using transgenic CHO cells stably expressing the human α_2-AR subtypes, α_{2A} and α_{2C}, at the pharmacology laboratory of Prof. Luis Callado at the University of the Basque Country, Spain.

Probing the effects of substitution at the cationic moeity



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EXPLOITING SECONDARY BINDING POCKETS IN AMINERGIC GPCRs

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Fragment based drug discovery (FBDD) employs growing and linking strategies for optimization. Structural information on G-protein coupled receptors (GPCRs) made FBDD available on this class of targets, however, most reported programs applied a growing strategy starting from orthosteric fragment binders. We developed a sequential docking methodology to support the identification of primary (orthosteric) and secondary site binders and linking of these fragment hits. Predicting the binding mode of multiple fragments bound to a single target we assessed the sampling and scoring accuracy for the first and second site binders in self- and cross-docking situations. The prospective validation of this approach was performed on dopamine receptors using the human dopamine D3 receptor crystal structure and a human dopamine D2 receptor homology model. Two focused fragment libraries were docked in the primary and secondary binding sites, and best fragment combinations were enumerated. Similar top scoring fragments were found for the primary site, while secondary site fragments were predicted to convey selectivity. A set of linked compounds created from the best scored primary and secondary site binders were synthesized from which we identified a number of D3 favoring compounds including one with 200-fold D3 selectivity. The structural assessment of the subtype selectivity of the compounds allowed us to identify further compounds with high affinity and improved selectivity.

IMIDAZOTHIAZINONES SPATIAL REQUIREMENTS AND ACTIVITY AS GPR18 ANTAGONISTS

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Seven transmembrane, G-protein coupled receptors (GPCRs) are the largest family of receptors localized to the cell surface and the most common target 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. Receptor transcripts were found at high levels in testis, small intestine and cells associated with the immune system. As first demonstrated by McHugh [1], GPR18 may control the activity of microglial cell function. Since the cannabinoid agonist Δ^9-THC is an agonist at GPR18, it was suggested that GPR18 could be considered a third cannabinoid receptor subtype, however sequence homologies are low [2].

In our studies bicyclic imidazole-4-one derivatives were discovered as the first synthetic scaffolds active as GPR18 antagonists [3]. Further efforts allowed us to obtain new arylideneimidazothiazinones displaying antagonistic properties at GPR18. Long chain substitution, simulating natural lipids, were considered as a main chemical modification in a novel series of compounds. Selectivity toward GPR18 receptor vs. cannabinoid CB_1 and CB_2 receptors and another orphan cannabinoid-like receptor - GPR55 - was examined. Structure-activity relationships physicochemical properties (BBB-penetration) were analyzed and spatial requirements were deduced to create a GPR18 pharmacophore model using the MOE suite [4]. In vivo tests were performed.: In rats trained to discriminate THC (3 mg/kg, ip) from vehicle in a two-lever water-reinforced fixed ratio 10 task, neither GPR18 agonists in doses 1-10 mg/kg (ip) nor GPR18 antagonists CB-5 and CB-27 (1-10 mg/kg, ip) substituted for the training dose of THC showed any effect. Studies of combinations of GPR18 ligands and THC are currently underway.

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THE 7-SUBSTITUTED 1,2,3,4-TETRAHYDROQUINOLIN-2-ONE MOIETY OF ARIPIPRAZOLE IS A KEY DETERMINANT OF EFFICACY AT THE DOPAMINE D2 RECEPTOR

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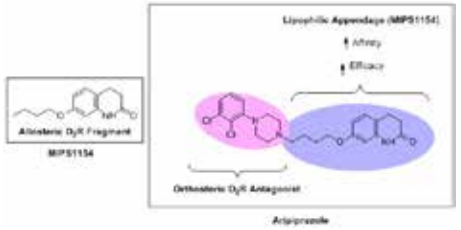
Schizophrenia is a complex mental disorder that currently affects approximately 1% of the population world-wide.[1] The majority of current treatments for the symptoms of schizophrenia are dopamine D_2 receptor (D_2R) antagonists. However, this approach can be associated with extrapyramidal side effects (EPS). The discovery of the third generation antipsychotic, aripiprazole, and its subsequent FDA approval for the treatment of schizophrenia, highlighted the utility of D_2R partial agonists as antipsychotics.[2] Furthermore, there is some evidence that aripiprazole may be a biased agonist.[3-5] Therefore it is of interest to understand the structural determinants of the efficacy of aripiprazole at the D_2R.

Using a truncated fragment-based structure-activity relationship (SAR) approach, we aimed to elucidate structural determinants that underlie aripiprazole's partial agonist efficacy. The evaluation of the truncated aripiprazole fragments led to the discovery of 7-butoxy-3,4-tetrahydroquinolin-2(1*H*)-one (**MIPS1154**), that acts as an allosteric modulator at the D_2R, to decrease the efficacy and affinity of dopamine as measured in a forskolin-induced cAMP assay. Surprisingly, however, merging 2,3-dichlorophenylpiperazine (**DCPP**) with **MIPS1154** to generate aripiprazole**,** caused an enhancement in affinity and efficacy as compared to **DCPP**. Similar enhancements of affinity and efficacy were also observed when **MIPS1154** was attached to a series of known orthosteric D_2R ligands. These results reveal that the lipophilic appendage is a key determinant the efficacy of aripiprazole and are consistent with the notion that aripiprazole may be able to engage the D_2R via an orthosteric/allosteric bitopic binding mechanism. These findings provide the basis for the design of novel D_2R partial agonists.



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DIMERIC NEUROPEPTIDE Y Y4 RECEPTOR AGONISTS: SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIPS AND RADIOLABELING

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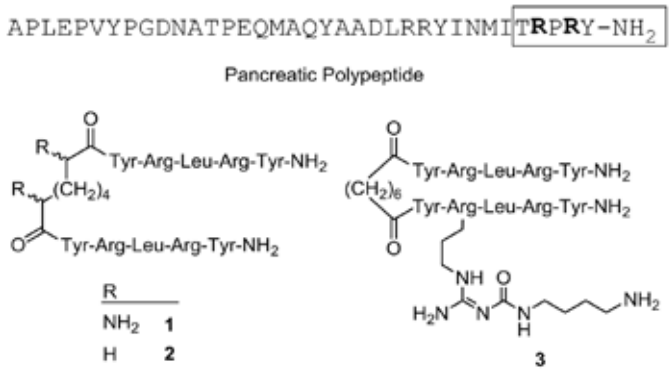
The diastereomeric mixture of **1** (BVD-74D) is a mimic of the C-terminal pentapeptide of the endogenous ligand pancreatic polypeptide and was described as a high affinity agonist at the human neuropeptide Y Y_4 receptor (Y_4 R) [1]. Here we report on the synthesis and pharmacological characterization of the pure diastereomers (2*R*,7*R*)- and (2*S*,7*S*)-**1** (*R* and *S* refer to the configuration of the stereocenters in the linker moiety) and a series of homodimeric analogs in which octanedioic acid was used as an achiral linker. To investigate the contribution of the individual amino acids in **2** to Y_4R binding, an alanine scan was performed. Backbone modifications were implemented by replacing one arginine or leucine in each of the two pentapeptide moieties with the respective aza-amino acid analog. Moreover, N^{ω}-(6-aminohexylaminocarbonyl)Arg was introduced as an arginine replacement enabling labeling at the primary amino group (**3**) [2].



(2*R*,7*R*)-**1** was superior to (2*S*,7*S*)-**1** in binding and functional cellular assays and equipotent with **3** on recombinant human Y_4 receptors. The two arginines in the pentapeptide sequences of **2** are crucial for Y_4R affinity. The replacement of the tyrosine adjacent to the linker was tolerated. [^{3}H]propionylation of one amino group in the linker of (2*R*,7*R*)-**1** or at the primary amino group in **3** resulted in high affinity Y_4R radioligands with subnanomolar K_d values.

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PHENOXYHEXYLAMINE DERIVATIVES AS HISTAMINE H3R RECPTOR LIGANDS

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Histamine H_3 receptors (H_3Rs) are one of the four known histamine receptors. They are mostly expressed in CNS and regulate the release of histamine itself and other neurotransmitters (*e.g.*, acetylcholine, noradrenaline, dopamine or serotonin). Blockade of these receptors could be useful in the treatment of various central nervous system (CNS), metabolic, pain and allergic disorders [1-3]. So far, many structurally diverse H_3R ligands have been synthesized and pharmacologically evaluated [4]. As a continuation of our previous works we synthesized a series of (methyl) (homo) piperidinyl-hexyloxyphenyl analogs of our lead structure DL77 (hH_3R K_i = 8.4 nM (CHO cells); K_i = 82 nM (HEK-293 cells); hH_4R K_i > 10 μM [5]). Compounds were screened at recombinant human H_3Rs stably expressed in HEK-239 cells, and exhibited moderate to good affinities (80 nM < K_i hH_3R < 400 nM). Moreover, several compounds were evaluated for anticonvulsant activity and neurotoxicity according to the standard protocols within Anticonvulsant Screening Program at the NIH/NINDS Bethesda (USA). For 4-methyl-1-(6-(p-tolyloxy)hexyl)piperidine good protection against MES induced seizures was observed but neurotoxicity was also detected.

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SYNTHESIS OF AZULENE-BASED MIMETICS OF OREXIN PEPTIDES

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G protein-coupled orexin receptors, OX_1R and OX_2R, and their activating peptide ligands, orexin-A and orexin-B, regulate sleep and arousal states. The orexin receptors could provide a clinical target for antagonism and agonism, to treat insomnia and narcolepsy, respectively.[1,2] Unlike the orexin receptor antagonists, whose discovery has been successful over a decade, most of the existing agonists are peptides, which are well known to be unsuitable as therapeutic molecules. The knowledge of the effective non-peptide orexin receptor agonists is sparse as only one such compound has been reported.[3] The lack of non-peptide orexin receptor agonists is not only a therapeutic pitfall but also hinder for the research of the orexin system. In order to reveal novel orexin receptor activating scaffolds, we have designed a combinatory database, which we subsequently docked to OX_2 R.[4] The compounds are based on the azulene scaffold with substituents in the 1-, 3- and 6-position. Currently, the top-scoring compounds are synthesized by utilizing our efficient synthetic methods for 1,3,6-trisubstituted azulenes.[5]

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DISCOVERY OF ORALLY ACTIVE KR-36996 AS A NOVEL UROTENSIN-II RECEPTOR ANTAGONIST

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Urotensin-II (U-II) is a cysteine-linked cyclic peptide that is expressed in a variety of tissues, including blood vessels, heart, liver, kidney, skeletal muscle, and lung.[1] U-II is known to be the most potent vasoconstrictor, displaying a 10 times greater potency than that of endothelin.[2] U-II is an endogenous ligand of the G protein-coupled receptor known as a GPR14 or the urotensin-II receptor (UT).[3] Activation of UT by binding of U-II promotes complex signal transduction pathways that control a wide range of physiological effects including vasoconstriction, vasodilatation, cell proliferation and hypertrophy.[4] Particularly interesting are UT's roles in cardiovascular functions that also include modulation of cardiac contractility, cardiomyocyte hypertrophy and fibrosis. These effects suggest that U-II and its receptors are involved in the pathogenesis of cardiovascular disease.[5] Furthermore, several studies have demonstrated that elevated plasma levels of U-II and increased levels of UT expression are associated with numerous cardiorenal and metabolic diseases, including hypertension, heart failure, atherosclerosis, diabetes,and renal failure. Therefore, UT has emerged as one of the most promising therapeutic targets for treating heart failure as well as a broad range of other cardiovascular maladies.[6] In the search for effective UT receptor antagonists,[7] we recently identified *N* -(1-(3-bromo-4-(piperidin-4-yloxy)benzyl)piperidin-4-yl)benzo[b]thiophene-3-carboxamide (KR-36996) as a novel and potent UT receptor antagonist.[8] The present studies demonstrated that KR36676 is a high and selective UT receptor antagonist (K_i = 4.44 ± 0.67 nM) and displays good pharmacokinetic profiles. Notably, the oral administration of KR-36996 (30 mg/kg, p.o.) after TAC for 2 weeks significantly decreased left ventricular weight by 40% and heart weight by 44.5% ($P < 0.05$). In summary, these results suggest that KR-36996 may be useful as an effective UT antagonist for pharmaceutical or clinical applications.

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USING SPLIT-LUCIFERASE COMPLEMENTATION FOR QUANTIFICATION OF Gαq –MEDIATED SIGNALLING

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Among the techniques established to investigate the function of $G\alpha_q$-coupled GPCRs are, for instance, Ca^{2+} assays, the determination of inositol phosphates from tritium-labeled *myo*-inositol and GTPase assays using ^{32}P- or ^{33}P-labelled GTP. Regardless of enabling a proximal (e. g. GTPase assay) or a more distal readout, the experimental set-up of these assays allow for the quantification of only one functional parameter.

Aiming at spatiotemporal analyses and the combined detection of alternate signal transduction pathways by optical methods, here we report on the application of a technique that uses split-luciferase complementation to quantify the activation of $G\alpha_q$ proteins in whole cells (cf. Fig. 1). A red light-emitting luciferase from the click beetle *Pyrophorus plagiophthalamus* (CBR) was dissected into two fragments, capable of combining to form a catalytically active enzyme [1, 2]. The C-terminal fragment of CBR was fused with the N- or the C-terminus of $G\alpha_q$ or introduced into three flexible loop regions of the protein. On the other hand, the N-terminal part of CBR was fused with either the C- or the N-terminus of phospholipase C-β3.

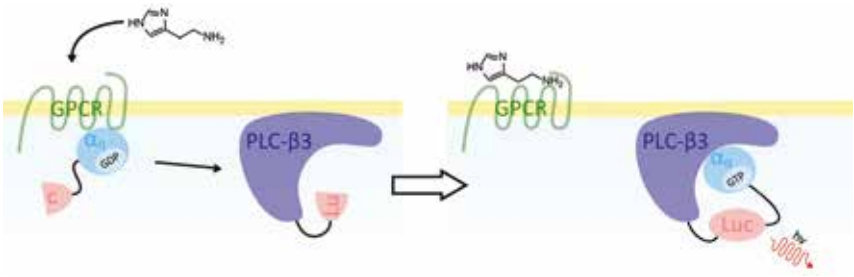

Fig. 1: Schematic illustration of the assay setup.

Two $G\alpha_q$ variants with CBR tags inserted in loop regions showed highest signal to background ratios when stimulated via prototypical $G\alpha_q$-coupling GPCRs. Furthermore, both variants allow for the construction of concentration-response curves of agonists and for the determination of pK_b values of antagonists in a 96-well format, as demonstrated for the human histamine H_1 and muscarinic M_3 receptor. The resulting pEC_{50}, pK_b and E_{max} values were in good agreement with published data.

The presented approach to the quantification of $G\alpha_q$ activation via split-luciferase complementation is a first step towards spatiotemporal investigations of $G\alpha_q$ signaling and harbors a potential with regard to multiparametric analyses of cellular signaling networks.

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A POSITIVE ALLOSTERIC MODULATOR OF SEROTONIN 5-HT2C RECEPTOR FOR OBESITY

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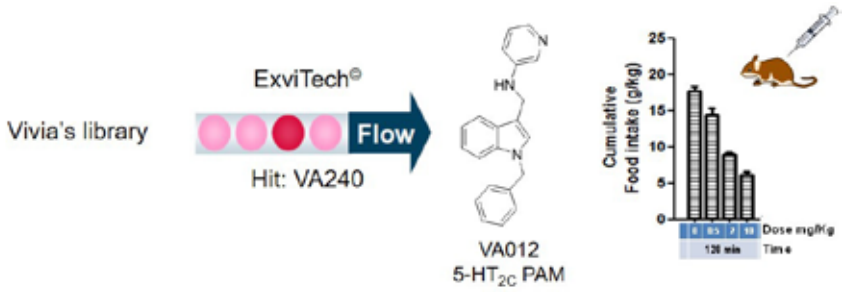
Stimulation of serotonin 5-HT_{2C} receptors (5-HT_{2C}R) is a promising intervention for the treatment of obesity.[1] However, one of the main obstacles of this approach is to obtain truly selective agonists, as related off-target effects on 5-HT_{2A} and 5-HT_{2B} subtypes are associated with unacceptable central nervous system and cardiac risks. The use of allosteric modulators (AMs) is an attractive alternative, as enhanced subtype selectivity profiles may be obtained, potentially leading to improved safety and pharmacology profiles.[2] [3] In this context, we initiated a research line aimed at the identification of 5-HT_{2C}R AMs as a new therapeutic approach for the development of safer drugs for the treatment of obesity.

In a screening of Vivia's library (ca. 1600 compounds) using a high sensitivity automated flow cytometry screening platform (ExviTech©), we have identified hit VA240 that was validated in IP1 assay as a moderate positive allosteric modulator (PAM) of the 5-HT_{2C}R (1.2 potentiation of 5-HT EC_{20}). Around this hit, we developed a medicinal chemistry program that led to VA012 that enhanced 5-HT potency (2-fold shift in EC_{50}) and efficacy (30% increase in E_{max}). Additionally, it does not bind the orthosteric binding site, no agonist or antagonist activity was observed, and no significant off-target activities were identified in a CEREP cellular functional GPCR profile. Despite its moderate pharmacokinetic properties, the compound enters the brain at reasonable levels (B/P = 3.8) and exhibited activity in feeding models in rats. VA012 inhibited food intake in a dose-dependent manner when administrated intraperitoneally (70% maximum inhibition at 10 mg/kg after 120 min). Also, a moderate effect in both food intake and body weight gain was observed in a semi-chronic study (7 days, 2 mg/kg).

These results support the interest of a 5-HT_{2C}R PAM as a promising therapeutic approach for the treatment of obesity.



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STRUCTURAL BASIS FOR IDENTIFICATION OF A D1 RECEPTOR PAM AS NOVEL DOPAMINE THERAPY FOR PARKINSON'S DISEASE

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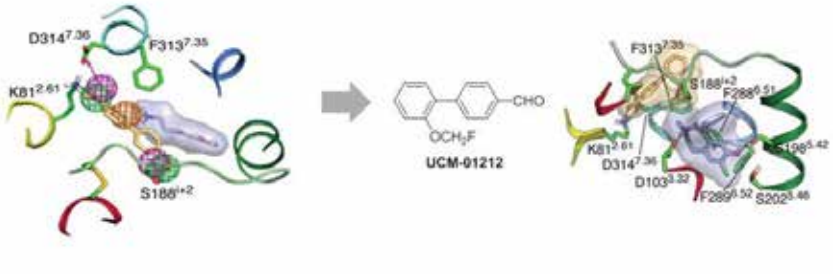
In recent years, novel approaches have emerged for drug development targeting GPCRs. Major advantages can be achieved with allosteric modulators (AM) that do not bind the orthosteric site for the endogenous ligand, but act in conjunction with it enhancing (PAM) or attenuating (NAM) its affinity, potency and/or efficacy [1] [2].

We initiated a research line aimed at the development of PAMs of the dopamine (DA) D_1 receptor (D_1R) as a new therapeutic strategy for the treatment of Parkinson's disease (PD). To start the project, we constructed a structure-based pharmacophore model to identify structural motifs for modulation of the receptor. Filtered compounds (~10.000) of the ZINC database were fitted to the pharmacophore model and ~200 putative PAMs were inspected for binding interactions with the D_1R model. 35 compounds were assessed for functional activity at human D_1R. Biphenyl derivative UCM-1212 has been characterized as a new D_1R PAM that increases the endogenous DA maximal effect in a dose-dependent manner (in 82% at 10 μM), is inactive in the absence of DA and exhibits subtype selectivity. UCM-1212 does not bind the orthosteric site, and its allosteric binding site has been studied through mutagenesis and docking experiments. The new compound influences motor coordination in a mouse model of PD [3], which represents proof of concept of the therapeutic interest of a D_1R PAM.



Acknowledgements: This work has been supported by grants from the Spanish Ministerio de Economía y Competitividad (MINECO, SAF2013-48271) and Comunidad de Madrid (SAL-2010/BMD2353).

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DESIGN OF NOVEL GPCR-TARGETED SCAFFOLDS: SYNTHETIC AND CHEMINFORMATIC EXPLORATION OF NOVEL CHEMISTRY SPACE

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Matching the synthetically accessible chemical space with biological target space is one of the core activities of medicinal chemistry. The content of today's compound collections is a reflection of the target families that have been addressed in the past, and chemical libraries are a reflection of the number and type of chemical reactions we can pursue in e.g. a 2 week chemistry/biology cycle time typically embedded in lead finding campaigns. Hence, there remains a substantial risk that currently pursued compound space might not match with the areas of biological target space the pharmaceutical industry will focus on in the near future.

Within our design and synthesis approaches we embark into a systematic exploration of spiro-cyclic systems in which a smaller ring (3 to 7 skeleton atoms) is associated with a medium-sized ring (7 to 12 skeleton atoms, figure 1). We will elaborate on the results of a systematic cheminformatics analysis of the charted bioactive compound space, followed by structure-based designs of novel bicyclic ring topologies. The focus will be laid on spiro-cyclic systems since a strong correlation of that topology with the target family of GPCRs was observed. Subsequent synthetic feasibility considerations are then fueling chemical validation of new bicyclic ring systems that qualify as scaffolds for 2D and 3D library expansion.

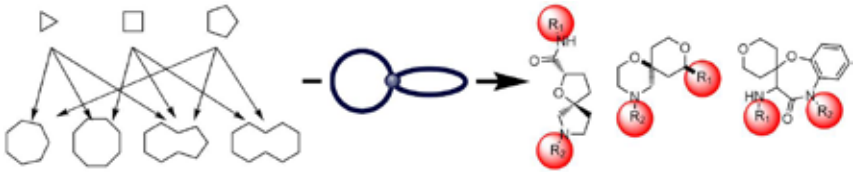

Figure 1: Schematic illustration of the design principles for spiro fused bicyclic topologies

In pursuit of this concept, we try to achieve an optimal balance between novelty on one hand, and proximity to bioactive compound space, i.e. resemblance of peptide secondary structure elements, and increased 3D skeletal complexity on the other hand. We consider this as a significant contribution to unlock the chemical accessible bicyclic ring system space that is often inaccessible in lead finding and lead optimization campaigns due to the underlying chemical complexity.

NMR-DRIVEN IDENTIFICATION OF OREXIN-1 SELECTIVE ANTAGONISTS

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Orexin-1 and Orexin-2 are Class A GPCRs primarily found in the hypothalamus and locus coeruleus. These receptors have been linked to a range of different physiological functions, including the control of feeding, energy metabolism, modulation of neuroendocrine function and regulation of the sleep–wake cycle. Importantly they are also associated with dopaminergic neurons of the ventral tegmental area (VTA) that are critical elements of the reward system.

Detailed, small molecule NMR studies employing the C4X technology[1] for a range of ligands was used to investigate the conformational preferences and sub-structural features responsible for these preferences. These data allowed the development of a predictive pharmacophore hypothesis that describes Orexin-1 antagonism. Furthermore, NMR guided conformational analyses as well as conformational design delivered multiple novel Orexin-1 antagonists building on the pharmacophore hypothesis. These series were subjected to iterative optimisation of their shape and drug like properties by routine input from NMR, resulting in the identification of highly potent and selective molecules. Moreover, exploration of the control features driving the conformational and pharmacophore profile guided problem solving to address shortcomings in ADME properties effectively.

Together, these approaches resulted in a unique combination of novel experimental data and design approaches driving medicinal chemistry decision making, and problem solving enabling the delivery of a highly efficient and effective project with a small resource overhead.

The presentation will describe the identification as well as conformational and property based optimisation of lead molecules resulting in the identification of orally active compounds with a range of kinetic and PK profiles.

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RADIOLABELED DIBENZODIAZEPINONE-TYPE MUSCARINIC RECEPTOR LIGANDS: MOLECULAR TOOLS FOR THE DETECTION OF ORTHOSTERIC, ALLOSTERIC OR DUALSTERIC BINDING?

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Muscarinic acetylcholine receptors (MRs) belong to the family A of G-protein-coupled receptors (GPCRs) and comprise five subtypes (M_1-M_5) which share a high structural similarity within the orthosteric binding pocket. Aiming at high-affinity and selective compounds, dualsteric/bitopic ligands (i.e. compounds that bind to the orthosteric and the allosteric binding site of an individual receptor) seem promising to solve the selectivity problem. However, the exploration of the role and extent of orthosteric and allosteric interactions involved in the binding of dualsteric ligands is highly challenging. To address this issue, we prepared a radiolabeled homodimeric and a monomeric MR antagonist ([^{3}H]UR-AP060 and [^{3}H]UNSW-MK259 respectively) based on the structure of DIBA, a dibenzodiazepinone derivative, which is closely related to the MR antagonist AF-DX-384 [1] . Both radioligands were obtained in high radiochemical purity (>97%). Saturation binding experiments at CHO-M_2 cells showed that both, the dimeric and the monomeric radioligands, bind to the M_2R orthosteric binding site with high affinity (K_d = 0.3 and 1.3 nM, respectively). The K_d values were in good agreement with binding data of the 'cold' versions of these compounds, UR-AP060 and UNSW-MK259 [2], determined by equilibrium competition binding with [^{3}H]N-methylscopolamine. Surprisingly, the dimeric ligand [^{3}H]UR-AP060 showed a drastic increase in (putative) non-specific binding.

Equilibrium competition binding experiments with [^{3}H]UR-AP060 and [^{3}H]UNSW-MK259 were performed at CHO-M_2R cells using various orthosteric and allosteric reference compounds. Furthermore, experiments performed with the monomeric radioligand [^{3}H]UNSW-MK259 at different concentrations in competition binding assay with the reported allosteric modulators W84 and gallamine, revealed a competitive interaction at the allosteric binding site. The results demonstrate that UNSW-MK259 is a dualsteric ligand at the M_2R.

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MOLECULAR INTERROGATION OF THE LOW-AFFINITY CONFORMATION OF THE β1-ADRENOCEPTOR

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The β_1-adrenoceptor exists in at least two distinct agonist conformations: a high affinity conformation (HAC) where responses are readily inhibited by antagonists and a low affinity conformation (LAC), of which the precise nature is unknown, where agonist responses are relatively resistant to antagonism. (1)

Conventional agonists (e.g. isoprenaline) stimulate responses via HAC, whereas others (e.g. pindolol, oxprenolol and alprenolol) can stimulate both conformations. CGP 12177, at low concentrations, is a high affinity antagonist of the HAC but, at higher concentrations, acts as an agonist at the LAC. There are several main pieces of evidence that sustains this two-conformation hypothesis:

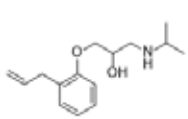
i) CGP 12177 agonist-induced responses are more resistant to antagonism than those stimulated by conventional agonists;

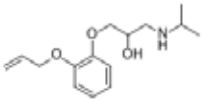
ii) the concentration of CGP 12177 required to stimulate an agonist response is considerably greater than the concentration required to bind to the conventional high affinity conformation;

iii) concentration response curves for alprenolol, pindolol and oxprenolol are biphasic, and the first high affinity component of this is readily inhibited by conventional antagonists whereas the second low affinity component is relatively resistant to antagonism. (2)

This phenomenon has been demonstrated in cells, tissues and animal but the clinical relevance, if any, remains unknown. (3)

In this communication, we report our investigation of this phenomenon by examining the ability of several analogues of alprenolol and oxprenolol to interact with the human β_1-adrenoceptor with a view to ultimately beginning to shed light on molecular descriptors contributing to LAC interaction and activation.


Alprenolol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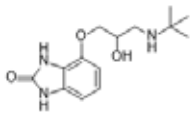
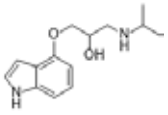

CGP 12177


Pindolol

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SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR MODELLING OF 1-OXA-4-THIASPIRO- AND 1,4-DITHIASPIRO[4.5]DECANE DERIVATIVES AS POTENT AND SELECTIVE 5-HT1A RECEPTOR AGONISTS

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Serotonin (5-hydroxytryptamine, 5-HT) is a relevant neurotransmitter both in the central nervous system and in periphery. It mediates several physio-pathological effects through at least 14 receptor subtypes. Among them, the 5-HT_{1A}R subtype has been extensively studied and still represents an attractive target for novel therapeutic uses. Agonists and partial agonists have been initially proven to be effective in anxiety, depression, and psychosis. More recently, they have shown pronounced neuroprotective properties indicating their potential benefit in the treatment of many neurodegenerative disorders, including Parkinson's disease and cerebral ischemia. Currently, it has been shown that 5-HT_{1A}R is involved at multiple levels in the regulation of nociception and 5-HT_{1A}R agonists may represent a new approach in pain relief therapy. Moreover it was found that 5-HT_{1A}R is implicated in oncogenesis and 5-HT_{1A}R antagonists demonstrated their efficacy in inhibiting the growth of different tumor (prostate, small cell lung). Thus, it is of paramount importance the discovery of more potent and selective 5-HT_{1A}R ligands. Recently, our research group reported 1-(1,4-dioxaspiro[4,5]dec-2-ylmethyl)-4-(2-methoxyphenyl)piperazine (**1**) as a potent 5-HT_{1A}R partial agonist (pD_2= 8.61). In an extension of this work a series of derivatives of **1,** obtained by combining different heterocyclic rings with a more flexible amine chain, was synthesized and tested. The results led to the identification of **15**, a novel 5-HT_{1A}R partial agonist with a 10-fold improved potency (pD_2= 9.58) and about 50 % of enhanced efficacy (E_{max}= 74%). MDCKII-MDR1 cells permeability assay predicted the *BBB* permeability of **15** that also showed a promising neuroprotective activity *in vitro*.

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AN INTEGRATED SCREENING PROCEDURE TO DISCOVER NOVEL OREXIN RECEPTOR LIGANDS

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Orexin system, G protein-coupled receptors OX_1R and OX_2R and activating peptide ligands orexin-A and -B, regulates states of sleep and arousal.[1] In man, the loss of orexins in CNS correlates with the occurrence of idiopathic narcolepsy, which provides a clinical target for orexin receptor activation therapy.[2] As peptides, the endogenous ligands are not suitable drug molecules raising a need for the development of non-peptide orexin receptor activators (agonists). However, discovery of such agonists to peptide binding receptors is challenging, and to date, literature reports only one non-peptide agonist for orexin system.[3] On the other hand, orexin receptor blockade promotes sleep, and several non-peptide orexin receptor blockers (antagonists) exist.[4]

We developed an integrated screening procedure to discover non-peptide orexin receptor ligands. The procedure consists of two phases: a pharmacophore-model-based virtual pre-screening and a cell-based in vitro screening (Ca^{2+} elevation). Our pharmacophore model combines previously reported orexin ligands and a docked orexin-A [5], and it is employed as a virtual screening filter. The filtered compounds undergo a subsequent functional screening, designed to detect their agonistic and antagonistic properties. After screening, we validate the possible hits using (1) a functional assay, in which the Ca^{2+} response to the studied compounds is abolished by a known orexin antagonist, and (2) an orthogonal method, such as a competition binding assay with radiolabeled orexin-A. The former method verifies the signal to be orexin receptor mediated, whereas the latter confirms the receptor binding.

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DESIGNED IPEROXO-BQCA (DERIVATIVE) HYBRID LIGANDS POSSESS SELECTIVELY ATTENUATED EFFICACY AT THE M3 MUSCARINIC ACETYLCHOLINE RECEPTOR

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In recent years several groups have reported hybrid ligands for the muscarinic acetylcholine receptors that are reported to engage the receptor 'bitopically' (i.e. both allo- and orthosterically), presumably in a binding orientation that is homologous to their constituent monomers. In an attempt to design a potent M_1 mAChR selective agonist, we have prepared hybrid ligands consisting of the potent agonist - iperoxo and a related analogue of the M_1 selective allosteric agonist/ positive allosteric modulator 1-(4-methoxybenzyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (BQCA) and compared their pharmacology with that of their constituent monomers at the M_1 - M_5 mAChRs, by pERK 1/ 2 and competitive radioligand binding assays. We found the hybrids to be less potent and efficacious than iperoxo, however generally more potent and efficacious than the corresponding *N*-linker iperoxo derivatives, suggesting that the hybrid alloster makes a receptor-ligand interaction which positively affects ligand potency and maximum inducible response, at several linker lengths. However compared with BQCA-d, the most potent, six, seven and eight polymethylene linker hybrids (hybrid-6, -7 and –8), displayed no selectivity for the M_1 over the M_2 - M_5 in either functional or binding assays. Instead the –C6 and –C7 hybrids displayed a moderate attenuation in efficacy at the M_3, relative to their orthoster, iperoxo. The –C8 hybrid displayed a much stronger attenuation in efficacy at the M_3 receptor resulting in moderate M_2/ M_4 selectivity at the level of efficacy. Though not obviously predictable from the monomer pharmacology, this property may be transferable to other partially selective orthosteric agonists, improving their subtype selectivity over the M_3 mAChR at the level of efficacy.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF A NEW 3-(1H-INDOL-3-YL)- PYRROLIDINE-2,5-DIONE DERIVATIVES WITH SEROTONIN RECEPTORS AND TRANSPORTER AFFINITY.

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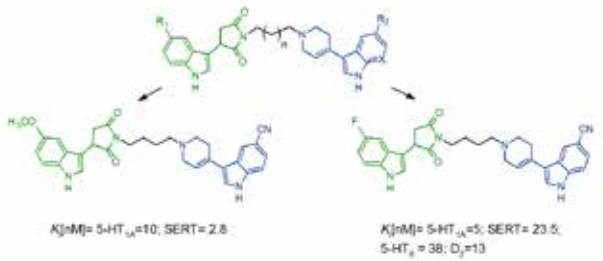
Major depressive disorder (MDD) is known to affect nowadays approximately 340 million people globally [1]. An important breakthrough in the pharmacotherapy of depressive disorders was the introduction of selective serotonin reuptake inhibitors (SSRIs). However, the efficiency rate of SSRIs was not satisfying[2]. Therefore, in 1993 there was proposed a hypothesis stating that the co-administration of 5-HT_{1A} receptor antagonists and SSRI-type medicines should enhance the effect of antidepressants. The validity of such an approach was confirmed by the registration of vilazodone and vortioxetine, the first dual binding SSRI (5-HT_{1A} receptor agonist/SSRI) medication[3]. This is the reason of our interest in this particular direction of investigating new medicines.

A series of 3-(1H-indol-3-yl)pyrrolidine-2,5-dione derivatives was synthesized and their biological activity was evaluated. The chemical structures of the newly prepared compounds were confirmed by ^{1}HNMR, ^{13}CNMR and ESI-HRMS spectra. All tested compounds proved to be potential 5-HT_{1A} receptor and SERT ligands. Among them, a few compounds showed significant affinity for both 5-HT_{1A}R and SERT. Selected compounds were evaluated for their affinity for D_{2L}, 5-HT_6, 5-HT_7 and 5-HT_{2A} receptors.

Figure 1. General structure of investigated compounds.



The test results obtained for exanimated series of *pyrrolidine*-2,5-*dione* derivatives indicate considerable potential of this group of compounds not only as the compounds of dual binding to 5-HT_{1A} receptor and SERT, but also as potential selective ligands with a high affinity to the above mentioned protein structures[4].

Acknowledgments: this study was supported by a grant 2013/09/B/NZ7/00748 from National Science Centre, Poland.

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NOTES

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POSTERS

Novel Approaches to the Treatment of Cancer

LIGAND-BASED IN SILICO SCREENING FOR THE DISCOVERY OF NOVEL INHIBITORS OF INDOLEAMINE 2,3-DIOXYGENASE (IDO1) AND TRYPTOPHAN 2,3-DIOXYGENASE (TDO2)

Simon Armitage, Thomas Pesnot, John Maclean, Sachin Mahale, Philip MacFaul, Sheenagh Aiken, Lucy Cartwright, Joshua Greally, Aleksandr Grisin, James Kelly, Kathryn Kilbride, Kyle Lyon, Elvis Maduli, William O'Neill, Robert Smith, Maria Tamara, Abhijith Thippeswamy, Andrew Tuffnell, Stuart Best, Matilda Bingham, Mary-Ann Campbell, Caroline Philips, Richard Armer

Redx Oncology, Duncan Building, Daulby Street, Liverpool, L69 3GA, UK.

Harnessing the immune system *via* immune checkpoint blockade (e.g. anti PD-1, anti PD-L1, anti CTLA4) has led to fast and long lived responses in cancer patients. Response rates however are low (typically 20%) and new treatments that enhance these rates are needed. Recent studies have shown that the administration of immune checkpoint blockers is associated with the overexpression of indoleamine 2,3-dioxygenase (IDO1).This, in turn, creates an immunoregulatory phenotype that counteracts immune checkpoint blockade and allows for cancer progression. The discovery of IDO1 inhibitors, and the potential to combine them with immune checkpoint blockers, therefore represents an attractive strategy to fight cancer.

Epacadostat and GDC-919 are the only IDO1 inhibitors in clinical development. Over the past two years both candidates have been involved in a multitude of phase I and II clinical trials (in combination with immune checkpoint blockers) for the treatment of solid tumours such as advanced NSCLC and melanoma. Although good efficacy was observed for both compounds in the clinic, high toxicologic risks were identified in preclinical and clinical stages. New, efficacious and safer IDO1 inhibitors are therefore highly sought after.

Redx Pharma is developing second generation IDO1 inhibitors with reduced toxicity risk. A ligand-based virtual screen identified 610 *in silico* hits, which were tested in IDO1 and TDO2 assays. TDO2 is a protein with similar biochemical activity to IDO1 that is essential to tryptophan homeostasis and is overexpressed in cancers such as glioblastoma. New chemotypes with IDO1-selective, TDO2-selective and dual IDO1/TDO2 inhibition profiles were confirmed as hits during this screening campaign. Chemical structures, pharmacological activity and *in vitro* DMPK profiles of selected hits will be presented. Further SAR optimisation of an IDO1-specific hit has led to the identification of highly novel molecules with IDO1 cellular activity comparable to that of clinical candidate GDC-919. The lead series displays a clean *in vitro* safety profile against hERG and CYP450 and is a promising start point for in-house lead optimisation.

(E)-INDOLE-3-ACRYLAMIDE DERIVATIVES AS POTENTIAL ANTI-HEPATOCELLULAR CARCINOMA AGENTS

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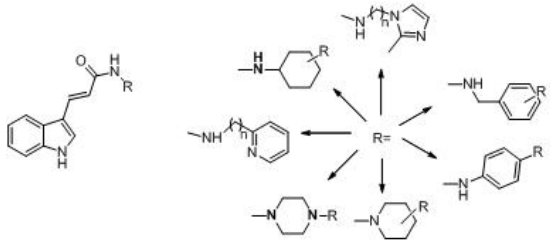
Hepatocellular carcinoma (HCC), one of the vascular solid tumors, accounts for 70%–85% of all malignant neoplasms of the liver burden worldwide. Although chemotherapy is the major treatment method for HCC patients, chemotherapeutic agents are known to have side effects or become ineffective through drug resistance mechanisms of tumor cells. Therefore, there is an urgent necessity to pay much attention to update and modify drug leads from the point of view of medicinal chemistry and drug design to fulfill more potent and effective therapies. By preliminary screening of indol-3-acrylamide type compounds synthesized by our group, 3-(1*H*-indol-3-yl)-*N*-(3,4,5-trimethoxyphenyl)acryl amide (SNB178) was identified as an inhibitor of cell proliferation of different human cancer cell lines acting as an inhibitor of tubulin polymerization and an inducer of apoptosis. In this study, we designed and synthesized a novel series of (*E*)-indole-3-acrylamide derivatives with the prime aim of developing agents with potential anti-proliferative activity towards HCC cells. Cytotoxic activities of indole-3-acrylamide derivatives were analyzed by SRB assay and by real-time cell analyzer on HCC cell lines. Cell cycle analysis through flow cytometry and cell staining methods were used to determine the mechanism by which these derivatives were showing their anticancer effect. Among 48 tested derivatives, 3 of them were chosen to be further studied on each cell line. Flow cytometric analysis of cultured cells treated with these molecules demonstrated that two of these compounds caused time dependent cell cycle arrest at the G2/M phase and also caused apoptotic cell death in Mahlavu and Snu-475 cell lines. Results were confirmed through western blot analysis where active molecules cause PARP cleavage in both cell lines indicating apoptosis and decrease in Cdc2 (CDK1) and CyclinB1 levels addressing cell cycle arrest in G2/M phase (This study was supported by TUBITAK research grant 113S973).



SYNTHESIS AND PRELIMINARY EVALUATION OF ANTICANCER EFFECTS OF INDOLE-3-CARBOXAMIDE DERIVATIVES IN HEPATOCELLULAR CARCINOMA CELL LINES

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Despite remarkable advances in the understanding of cancer biology and cancer genetics, cancer is continuing to be one of the largest causes of death claiming millions of lives each year in the world. Among other factors, inherent and acquired resistance to treatment and the dose-limiting toxicity caused by the narrow therapeutic window of many cancer drugs are major obstacles for effective cancer therapy. Since clinically useful drugs have problems with toxicity, drug resistance and bioavailability, there is an ongoing effort to find new compounds that might be safer or more effective. Several small synthetic molecules with an indole nucleus as core structure that act as anticancer agents have been identified within the past decade. (Indol-3-yl)glyoxamide, known as indibulin, has been described as a novel synthetic anticancer agent with significant antitumoral activity targeting the tubulin system. In view of the foregoing considerations, our work has been focused on the design and synthesis of a novel series of indole-3-carboxamide, indole-3-acetamide and indole-3-carboxamide derivatives. We aimed to define possible anticancer properties of newly synthesized indole derivatives their cytotoxic effect on HCC cell lines and to determine the molecular mechanism underlying this effect. Cytotoxic activities of these derivatives were analyzed by SRB assay and by real-time cell analyzer on HCC cell lines. Cell cycle analysis through flow cytometry and cell staining methods were used to determine the mechanism by which these derivatives were showing their anticancer effect. Two indole-3-carboxamide derivatives were found to have IC_{50} values below 10 μM, and chosen to be further studied on each cell line. Results suggest that these indole-3-carboxamide derivatives cause mitotic arrest. (This study was supported by TUBITAK research grant 113S973).

NOVEL PYRAZOLE CHALCONES: AS POTENT ANTICANCER AGENTS IN LIVER CANCER CELLS

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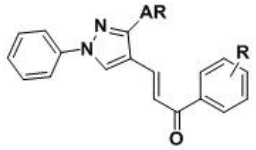
Cancer remains one of the leading causes of death worldwide and requires a pressing need for the development of novel and more effective treatments. Chemotherapy is employed as a crucial part of the multimodal treatment of cancer when surgery is not suitable. The narrow dosing window of current drugs with regard to their efficacy and safety and significant drug resistance resulting a failure of antitumor drugs to exert their effects in certain cancer types limit the use of contemporary cancer chemotherapy. Therefore, discovery of efficient cytotoxic agents with improved selectivity against cancer is still an attractive field. In this study, we aimed to define possible anticancer properties of newly synthesized pyrazole chalcone derivatives through evaluation of their cytotoxic effect on HCC cell lines and to determine the molecular mechanism underlying this effect. Cytotoxic activities of pyrazole derivatives were analyzed by SRB assay and by real-time cell analyzer on HCC cell lines. Cell cycle analysis through flow cytometry and cell staining methods were used to determine the mechanism by which these derivatives were showing their anticancer effect. Among 42 tested pyrazole derivatives, 14 of them were found to have IC_{50} values below 3 μM. Four of these molecules were chosen to be further studied on each cell line. Flow cytometric analysis of cultured cells treated with these molecules demonstrated that two of these compounds caused time dependent cell cycle arrest at the G2/M phase and also caused apoptotic cell death in both cell lines. Results were confirmed through western blot analysis where active molecules cause PARP cleavage in both cell lines indicating apoptosis and decrease in Cdc2 (CDK1) and CyclinB1 levels addressing cell cycle arrest in G2/M phase (This study was partially supported by Gazi University research grant 02/2012-41).



DESIGN OF PORPHYRIN-LOADED NANOBUBBLES FOR THERANOSTIC APPLICATIONS

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Nanoparticle formulations as theranostic agents for cancer therapy have gained a lot of attention for their ability to passively accumulate inside tumors through the enhance permeation and retention effect (1). A particular class of nanoparticles are nanobubbles (NBs), which are nanostructures constituted by perfluorocarbon gas stabilized by a lipid, surfactant and/or polymer shell (2,3). Because of their gas-core structure, NBs operate as an ultrasound (US) contrast agents without any further modification. Moreover, the robust and easily modifiable shell can be exploited to load drug, prodrug and/or vector in order to build the theranostic agent (4).

Recently, sonodynamic therapy has become a promising noninvasive approach for cancer therapy (5). The treatment exploits the ability of peculiar molecules (i.e. porphyrins) to be excited by US and to produce oxygen radical species during their decay process. Radical species, in turn, result in cell death. The advantage of using US is to induce the cytotoxic effect only in a definite region at a precise time, thereby decreasing side effects. In order to capitalize on the real-time visualization and on-demand delivery of ultrasound contrast agents, this project strives to combine porphyrins with the nanobubbles in order to obtain an US-activated theranostic agent. Here, porphyrins with different degrees of lipophilicity, molecular weight, shape and rigidity were synthesized, and were encapsulated in the NBs. Resulting porphyrin-NBs were characterized by ultrasound, microscopy imaging, dynamic light scattering (DLS), and extent of porphyrin loading.

Porphyrin-nanobubbles were found to be in the range of 200-300 nm in diameter, clearly visible under ultrasound (Contrast harmonic Imaging, frequency 8.0 MHz, mechanical index of 0.08), and have a loading of 25% with porphyrin A (more amphiphilic and more flexible) and 0.5% with porphyrin B (more rigid and more lipophylic). *In vitro* cell viability tests are currently ongoing. Overall, because of their nanoparticle footprint, ultrasound visibility combined with the switchable cytotoxicity of porphyrin, Porphyrin-NBs could make an exciting new class of theranostic agents.

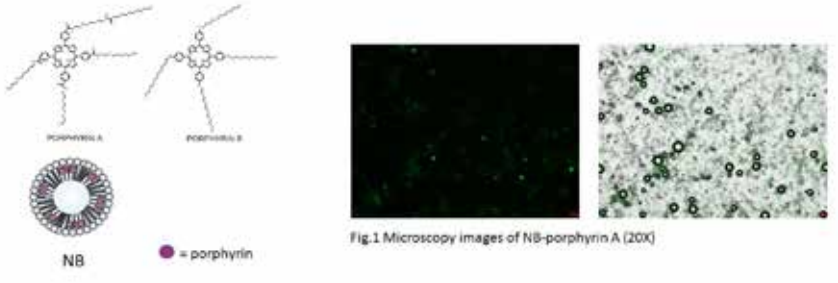

Fig.1 Microscopy images of NB-porphyrin A (20X)

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APPLICATION OF SCAFFOLD HOPPING TO GENERATE NOVEL HYDROXYAZOLE-BASED AKR1C3 INHIBITORS WITH IN VITRO ACTIVITY AGAINST PROSTATE CANCER CELLS

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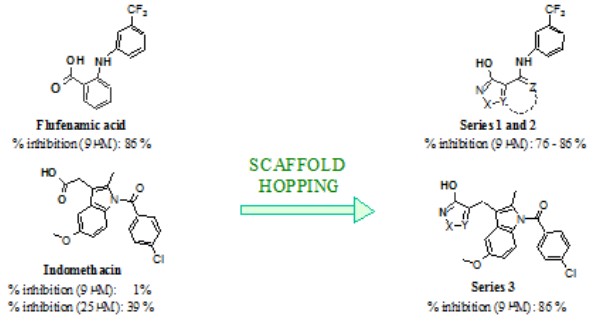
Prostate cancer (PCa) is the most commonly diagnosed cancer in men and the second leading cause of death in Western world. The resistance mechanisms occurring after the usual treatment with androgen deprivation therapy poses the urgent need of novel agents capable of targeting selectively the most critical features of resistance process.[1] Since the overexpression of the steroidogenic enzyme Aldo-keto reductase 1C3 (AKR1C3) in castration resistant prostate cancer (CRPC) cells is one of the most effective acquired drug resistance mechanism, development of highly potent and AKR1C3-selective targeting inhibitors is a viable strategy for the treatment of CRPC and metastatic diseases.[2] Flufenamic acid (FLU) and Indomethacin (INDO) have been shown to inhibit AKR1C3-dependent processes in human cell lines and murine xenografts.[2] However, the potential therapeutic usefulness of these drugs in the context of CRPC is limited because of undesired side effects associated with chronic COX inhibition.

Since 2006, the authors have directed their efforts towards the investigation of hydroxylated pentatomic heterocyclic systems in order to create sophisticate tools able to bio(iso)sterically mimic the carboxyl group, as well as other acidic moieties.[3, 4] This bioisosteric tool, combined with a more general scaffold hopping approach, was applied to design innovative AKR1C3 inhibitors: starting from FLU and INDO scaffolds, we identified three classes of structurally different AKR1C3 inhibitors, two series deriving from FLU and one deriving from INDO. The best candidates will be presented and their in silico design, synthesis, chemico-physical properties and biological evaluation will be fully discussed. Worth of note, some compounds were more active than their leads FLU and INDO on AKR1C3, and none of them maintained activity on COX enzymes, suggesting these structures can be developed as future lead compounds against castrate resistant prostate cancer.



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SYNTHESIS OF DOXORUBICIN GALACTOSIDE: A CHEMICAL GLYCODIVERSIFICATION STRATEGY

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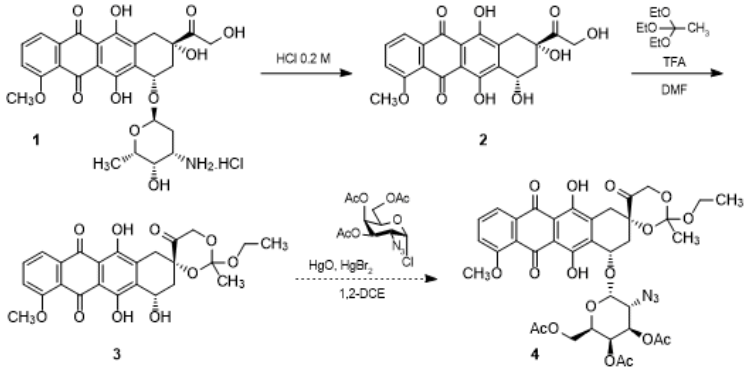
Doxorubicin is an antineoplastic drug from the anthracycline class, figuring amongst the current arsenal for cancer treatment as one of the most effective chemotherapeutics in clinical use. This glycoside drug is comprised by an unusual amino sugar known as daunosamine linked to a quinone/hydroquinone-type ring system, that acts as DNA-intercalating agent, oxidative stress inductor and topoisomerase II poison. Despite its broad efficacy, two major limitations are associated to doxorubicin, namely multidrug resistance and dose-related severe cardiotoxicity.

To overcome these drawbacks, new generation analogues have been synthesized, and the most successful structural variations were developed on the carbohydrate moiety, leading to epirubicin, pirarubicin and sabarubicin, for example, as well as azido-modified doxorubicin itself and some related derivatives. Such compounds have shown to be cytotoxic to tumor cells, even against drug-resistant lines, and some presented reduced cardiotoxicity[1]. This work exploits semi-synthesis from the natural product, aiming to prepare novel doxorubicin derivatives coupled to azido glycosides, in the search for compounds with enhanced antitumor activity and diminished toxicity. Glycones not previously combined with anthracyclines were given priority and a galactoside was selected, as part of a broader glycodiversification approach[2].

Accordingly, commercial doxorubicin hydrochloride (**1**) was subjected to acid hydrolysis, providing the respective aglycone (**2**) in 98% yield. To achieve practicable regioselectivity in the glycosylation step, doxorubicinone (**2**) was subjected to functional group protection. The ortho ester was chosen owing to its property of protecting 1,3-diols; thus the treatment of (**2**) with triethyl orthoacetate and TFA in DMF[3] afforded the 9,14-protected doxorubicinone (**3**) in 54% yield. Finally, glycosylation with 3,4,6-tri-*O* -acetyl-2-azido-2-deoxy-D-galactopyranosyl chloride under Koenigs-Knorr conditions (HgO, $HgBr_2$, DCM)[4] shall furnish the corresponding galactoside (**4**). After full deprotection, the compound will be assayed *in vitro* for cytotoxicity against tumoral cells and on cardiomyocytes culture, to reveal its therapeutic and toxic potential, respectively.



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NEW CLASSES OF MULTIDRUG RESISTANCE (MDR) MODULATORS

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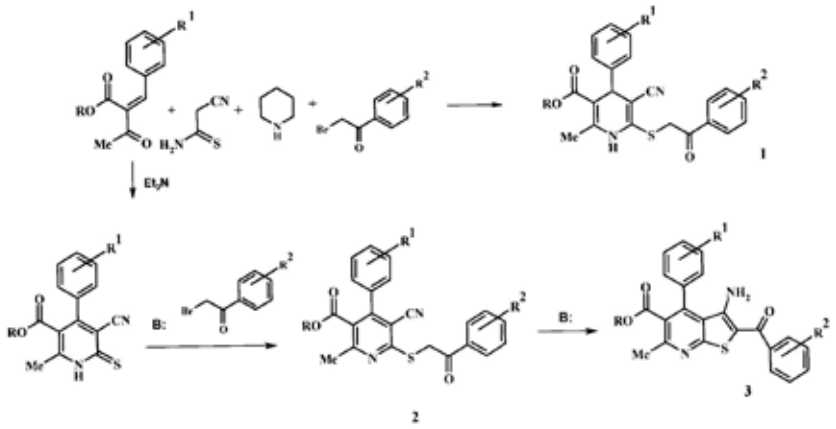
Multidrug resistance is the major obstacle in cancer chemotherapy. Tumor cells develop resistance against cytotoxic agents used in a previous treatment. A tumor may manifest also resistance to a cytotoxic agent to which it has not been previously exposed.

We discovered novel classes and groups of multidrug resistance modulators: thieno[2,3-b]pyridines [1] and relative compounds and synthesis intermediates – dihydropyridines[2], pyridines.



MDR is mainly related to the expression of ATP-binding cassette (ABC) transporters. P-glycoprotein(P-gp), multidrug resistance protein(MRP1) and the breast cancer resistance protein(BCRP1) transport a wide variety of structurally different substrates out of the tumor cells, thereby decreasing their intracellular concentrations.

So, comp.**1a** (R = Et, R^1 = 3,4,5-$(OMe)_3$, R^2 = 4-OMe) reveals enhanced activity (fluorescence activity ratio) on P-gp and MRP1 synthesis blocking; it has diminished antagonism on calcium channels (A7R5 cells; i.e., less side effects), lower cytotoxicity as verapamil.

Comp. **3a** (R = Et, R^1 = 3,4,5-$(OMe)_3$, R^2 = 4-OMe) has 23 times higher activity on P-gp synthesis blocking, 5,5 times higher activity on MRP1 synthesis blocking, 53 times higher activity on BCRP1 synthesis blocking as verapamil. At the same time it is 60 time less active Ca^{2+} channel blocker as standard verapamil.

Acknowledgement: This project is supported by Latvian State program Biomedicine and program InnovaBalt

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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF AOPCP DERIVATIVES: POTENT, METABOLICALLY STABLE AND SELECTIVE ECTO-5´-NUCLEOTIDASE (CD73) INHIBITORS

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Ecto-5′-nucleotidase (CD73) is a member of the *ecto*-nucleotidase family, which catalyzes the dephosphorylation of extracellular nucleotides. CD73 catalyzes the hydrolysis of nucleoside monophosphates, mainly of AMP, producing adenosine. Further *ecto*-nucleotidases include the nucleoside triphosphate diphosphohydrolases (NTPDases; subtypes 1, 2, 3 and 8), the nucleotide pyrophosphatases (NPP1-4) and the alkaline phosphatases (APs).[1] CD73 is often co-localized with adenosine receptors, and CD73 inhibitors reduce extracellular adenosine levels, which results in an indirect blockade of adenosine (P1) receptor activation. Therefore, they possess potential as novel drugs, e.g. for cancer immunotherapy or for the treatment of neurodegenerative diseases. α,β-Methylene-ADP (AOPCP, K_i= 197 nM, rat CD73), an analogue of ADP, is currently one of the most potent competitive inhibitors of CD73.[2] In the present study, AOPCP was used as a lead structure, and derivatives modified in various positions were prepared. We could dramatically increase potency of AOPCP by introducing large aromatic N^6-substitutens; among the best compounds were N^6 -(4-chlorobenzyl)- (PSB-12441, K_i 7.23 nM), and N^6-benzyl-purine riboside-5′-*O* -[(phosphonomethyl)phosphonic acid] (PSB-12379, K_i 9.03 nM).[3] Replacement of the 6-NH group in PSB-12379 by O (PSB-12431;K_i 9.20 nM) or S (PSB-12553; K_i 9.50 nM) yielded equally potent inhibitors. Selected compounds investigated at the human enzyme did not show species differences and displayed high selectivity and metabolic stability.[3] Moreover we obtained an X-ray crystal structure of PSB-12379 in complex with human CD73 at high resolution (2.05Å).The structure will allow the rational design of novel inhibitors with further improved potency. The synthesized compounds are the most potent and selective CD73 inhibitors known to date; they will become useful pharmacological tools to further elucidate the enzyme's (patho)physiological role and its potential as a drug target, e.g. for novel immunotherapeutic approaches for the treatment of cancer.

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DESIGN AND SYNTHESIS OF NEW NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS) ANALOGS WITH ANTI-CANCER ACTIVITY ON COLON RECTAL CANCER CELL LINES HCT-116, HCT-116 AND CACO-2.

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Cairo, Egypt

Nonsteroidal anti-inflammatory drugs (NSAIDs) lower the incidence of and mortality from colon cancer. In this study, the molecular structure of known NSAID was used as starting scaffold to design novel analogs and their effect on the proliferation of human colon cancer cells (HCT-116, HT-29 and CACO-2) were evaluated. Compared to the known NSAID and 5-FU inhibiting colon cancer cell proliferation, most of the derivatives displayed significantly increased activities. Especially compound 2 inhibited the growth of the three colon cell lines with IC_{50s} around 0.0127 ug/ml - 0.78 ug/ml. For further investigation cell cycle analysis was done for HT-29 finding that compound 2 makes cell cycle arrest at s phase. These data lend support for further studies on these new analogs as promising anti-colorectal agents.

THE DISCOVERY OF POTENT AND ORALLY AVAILABLE DOT1L INHIBITORS BY FRAGMENT GROWING, FRAGMENT LINKING AND FRAGMENTATION APPROACHES

Christoph Gaul (1), Frédéric Stauffer (1), Henrik Möbitz (1), Rainer Machauer (1), Philipp Holzer (1), Andrea Vaupel (1), Giorgio Caravatti (1), Clemens Scheufler (1), Cesar Fernandez (1), Martin Klumpp (1), Ulrich Hommel (1), Ralph Tiedt (1), Andreas Weiss (1), Kim Beyer (1), Keith Calkins (1), Michael Kiffe (1), Chao Chen (2), Hugh Zhu (2)

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Dot1L is the only known enzyme to methylate lysine 79 of histone 3 (H3K79), with the H3K79me2 mark being associated with active transcription. Under physiological conditions, Dot1L is critical for normal hematopoiesis, however, misdirected catalytic activity (methyltransferase) is believed to be causative for certain acute leukemias. Several oncogenic fusion proteins including MLL-ENL, MLL-AF4 and MLL-AF9 aberrantly recruit Dot1L to ectopic loci, leading to local hypermethylation of H3K79 and misexpression of genes (including HoxA9) which drive the leukemic phenotype. Inhibition of the methyltransferase activity of Dot1L in MLL-rearranged leukemias (mixed lineage leukemia, MLL) is predicted to reverse ectopic H3K79 methylation, leading to repression of leukemogenic genes (HoxA9, Meis1) and tumor growth inhibition. The recent quest for Dot1L inhibitors is spearheaded by Epizyme and culminated in the discovery of EPZ-5676, a SAM-competitive, nucleoside-containing Dot1L inhibitor, which is currently being evaluated in MLL patients in Phase 1b clinical trials. The agent is administered by uninterrupted, continuous intravenous (i.v.) infusion due to its physicochemical properties.

Herein, we will describe our Dot1L hit finding strategy, including biochemical, biophysical and virtual approaches, and our medicinal chemistry strategy, strongly influenced by structure-based design and property-based optimization. Among other concepts, a fragment growing and linking approach as well as a fragmentation method will be discussed, leading to the discovery of structurally completely novel (non-SAM like), orally bioavailable Dot1L inhibitors with excellent cellular activity.

A TRIPHENYLPHOSPHONIUM CONJUGATE OF A TYROSINE KINASE INHIBITOR LOCALIZES TO MITOCHONDRIA AND INHIBITS ACTIVATED MET IN NON-SMALL CELL LUNG CANCER CELLS.

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EGFR Tyrosine kinase inhibitors (TKIs) are drugs of choice for non-small cell lung cancer (NSCLC) patients that harbor activating EGFR mutations. 1 Unfortunately, the clinical effectiveness of these drugs is rapidly eroded by resistance due to secondary mutations at the kinase domain or constitutive activation of alternative signaling pathways that lie downstream of EGFR. 2,3 The HGF-MET signaling pathway is frequently cited as a bypass mechanism that is co-opted by NSCLC cells that are resistant to TKIs.4 We have found that MET overexpression in resistant NSCLC cells is associated with MET protein localization to the mitochondria. This has led us to hypothesize that targeting MET in these TKI resistant, MET amplified NSCLC cells would abolish the survival advantages that accrue to these cells and hence provide a means of overcoming resistance. To this end, a selective MET inhibitor, PHA665752, was chemically modified by conjugation to triphenylphosphine to yield a triphenylphosphonium analog, TM608. The triphenylphosphonium (TPP) cation is widely associated with mitochondrial targeting properties and has been conjugated to bioactive molecules (antioxidants, anticancer agents) for this purpose.5 By employing the fluorescent properties of PHA665752 and TM608, we showed that TM608, but not PHA665752, localized to the mitochondria of Hela cells. Investigations on NSCLC HCC827 cells which had normal levels of MET (HCC827A), and its isogenic derivative which is erlotinib resistant and MET-amplified (HCC827B),showed that mitochondrial MET was located in a similar intra-mitochondrial compartment as SDHA, an inner mitochondrial membrane protein. We found that TM608 suppressed MET phosphorylation at the catalytic residues Y1234/1235 in MET amplified HCC827B cells and purified mitochondria. PHA665752 suppressed MET phosphorylation of the same residues in whole HCC827 B cells but not in mitochondria. TM608 was also found to be more apoptogenic than PHA665752 in the HCC827B cells. These findings provide support for targeting mitochondrial MET with a TPP-TKI conjugate and the potential of this approach in addressing the problem of resistance to TKIs in NSCLC.

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PROTEASOME INHIBITION BY NEW DUAL WARHEAD CONTAINING PEPTIDO VINYL SULFONYL FLUORIDES

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The development of proteasome inhibitors has been an outstanding case showing that irreversible inhibitors may provide unique advantages by forming long-lived ties with their target[1]. Together with covalently reacting kinase inhibitors, proteasome inhibitors are part of the important arsenal of presently available crucial anti-cancer drugs.

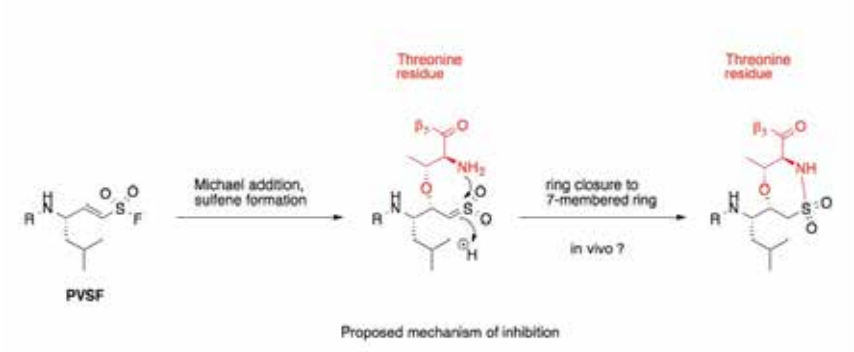
Recently we have developed powerful peptidomimetic proteasome inhibitors containing the sulfonyl fluoride warhead[2,3]. These peptido sulfonyl fluorides (**PSF**) displayed considerable selectivity of inhibition of the immunoproteasome[3].

Inspired by the "dual' warhead approach we now describe a new proteasome inhibitor concept in which a Michael electrophilic trap, is combined with a sulfonyl fluoride electrophile incorporated into a peptide sequence leading to a peptido vinyl sulfonyl fluoride (**PVSF**). Both electrophilic traps may then react with the nucleophilic amino and hydroxyl functional groups of the N-terminal threonine residue present in the active site of the proteasome leading to a 7-membered ring adduct.

The synthesis of peptido vinyl sulfonyl fluorides involved employing vinylogous amino sulfonates, which are accessible from amino acid derived aldehydes. To investigate the *in vitro* formation of the proposed 7-membered ring adduct, reactivity studies were carried out starting from simple amines and moving to more complex models for the amino alcohol part of the Threonine.

Furthermore, the proteasome inhibitory activity of two **PVSF** constructs was evaluated in a proteasome assay and compared to activities of **PSF** constructs.

The combined reactivity of this new dual warhead led to strong proteasome inhibition (IC_{50} 90 nM). Elucidation of the mechanism of inhibition within the proteasome awaits a crystallographic analysis.



Proposed mechanism of inhibition

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BENZIMIDAZOLONE SULPHONAMIDES - POTENT, SELECTIVE AND DRUG-LIKE INHIBITORS OF POLY(ADP RIBOSE) GLYCOHYDROLASE (PARG)

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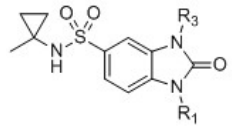
DNA repair proteins have attracted considerable interest during the last decade as potential targets for therapeutic intervention in cancer. Indeed, recent efforts in this area have culminated in the delivery of several novel drugs into clinical trials, as well as regulatory approval from the FDA, for the treatment of several cancers. Despite considerable progress in this area, drug discovery research pertaining to the DNA repair protein poly(ADP ribose)glycohydrolase (PARG), which plays a critical role in DNA single stand break repair, remains scarce. Although several compounds have been reported to inhibit PARG with varying degrees of activity in biochemical assays, these compounds generally exhibit weak or no activity in cells. There is therefore an urgent need for potent, cell-permeable inhibitors that could be used as chemical probes to elucidate the role of PARG in cancer and evaluate its potential as a therapeutic target.

As part of our innovative collaboration with AstraZeneca, we recently discovered a novel PARG-binding pharmacophore and employed this information to discover drug-like chemotypes; facilitating the development of several potent, selective and cell-permeable series of PARG inhibitors.



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In this poster, we provide a summary of our emerging results in this area, with particular emphasis on a novel series of benzimidazolone sulphonamides **1**. Notably, the best compounds in this series exhibit potent inhibitory activity towards PARG in biochemical and cellular assays with potencies of 40 and 60 nM, respectively. Moreover, these agents display pharmacological effects consistent with their anticipated mode of action and appropriate drug-like properties, as well as showing selectivity against PARP1 and the closely related glycohydrolase homologue ARH3. The medicinal chemistry optimisation of this scaffold has been described in detail, alongside our most recent biological results. Ultimately, this work has resulted in the development of potent, selective cell-permeable inhibitors of PARG, which may be used as tool compounds to elucidate the true pharmacology of PARG and its role in cancer and other diseases.

TARGETING THE WNT PATHWAY – DISCOVERY AND OPTIMIZATION OF A PORCUPINE INHIBITOR

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Porcupine (PORCN) has emerged as a molecular target of interest to academic groups and pharmaceutical companies in the search for biological and chemical entities useful for targeting Wnt-driven cancers. PORCN is a membrane bound O-acyltransferase (MBOAT) enzyme located in the endoplasmic reticulum (ER) of the cell. This MBOAT enzyme is of particular interest since it is essential for the palmitoleation of the Wnt ligands. PORCN adds a palmitoleoyl group to Serine 209 of the Wnt ligand before secretion. Upon secretion, the Wnt ligand activates the Wnt pathway to drive downstream gene expression. [1] The dysregulations of the various components of the Wnt/β-catenin signaling pathway is associated with a wide variety of diseases, in particular cancer described in various reviews. [2,3] The use of an orally bioavailable porcupine inhibitor that prevent Wnt secretion could potentially provide therapeutic effect to Wnt-driven cancers in the clinic.

To date, there had been several reported small molecule inhibitors of PORCN and the progress made in identifying human disease models had been described in a recent review. [4] Currently, there are two PORCN inhibitors in the clinic; LGK974 (ClinicalTrials.gov NCT01351103) and ETC-159 (ClinicalTrials.gov NCT02521844) [5] both in Phase 1 clinical trial.

Our high throughput phenotypic screen had identified several porcupine inhibitors with novel scaffolds. [6-8] ETC-159 bearing a xanthine moiety in the molecule came from our phenotypic screen. During the process of scaffold optimization, we had also been actively pursuing a xanthine replacement or bioisostere. With the help of the pharmacophore model developed in house [9], we have successfully morphed the xanthine scaffold to a potential preclinical candidate with a novel scaffold.

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DEVELOPMENT OF NOVEL INHIBITORS AGAINST THE TRANSCRIPTION FACTOR HSF1 IN THE MULTIPLE MYELOMA

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Multiple myeloma (MM) is a malignant plasma cell disorder which is primarily localized in the bone marrow. Despite recent therapeutic progress, the disease remains largely incurable because the majority of the MM patients develops drug resistance.[1] Due to the continuous stress as a result of internal oncogenic processes as well as external factors (e.g. therapeutic agents) cancer cells are strongly dependent on heat-shock induced transcription factors (HSFs). HSF1 regulates heat shock protein (HSP) expression in mammalian cells under cellular stress conditions. Currently, HSF1 aroused interest as an anti-cancer target as it was found overexpressed in tumor cells leading to unusually increased tumor cell survival.[2-4] At the present time specific HSF1 inhibitors are lacking so that a series of α-acylaminocarboxamides was synthesized using Ugi four-component condensation[5] (Figure) and tested for the inhibitory activity using the strictly HSF1-dependent HSP70 regulation as screening assay in a MM cellular model. In order to identify potential targets the most potent inhibitor was biotinylated and used in an affinity capture experiment. Potential target proteins were affinity purified via streptavidin beads and identified by nanoLC-MS/MS analysis. As a result, a number of PI3K-induced kinases (PIIKs) including the DNA-dependent protein kinase (DNA-PK) were identified. Our findings are in line with previous reports showing dependence of HSF1 activity on DNA-PK or PI3K.[6, 7]

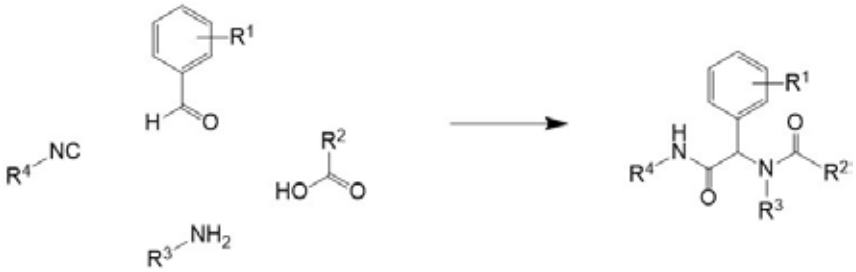

Figure: Schematic Ugi four-component condensation[5] obtaining α-acylaminocarboxamides as potential inhibitors of the HSF1 pathway in MM cells.

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DESIGN AND DISCOVERY OF 3-ARYL-5-SUBSTITUTED ISOQUINOLIN-1-ONES AS POTENT TANKYRASE INHIBITORS

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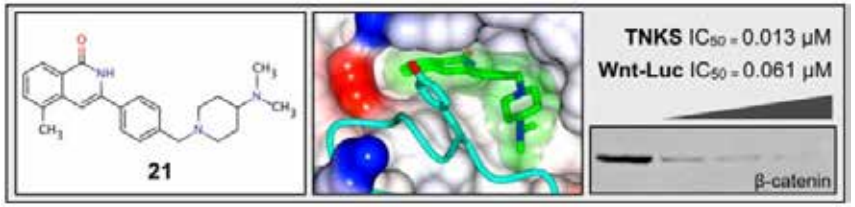
The tankyrase proteins (TNKS, TNKS2), members of the PARP superfamily of enzymes, are attractive anticancer drug targets, particularly as inhibition of their catalytic activity has been shown to antagonise oncogenic WNT signalling.[1] To identify chemical inhibitors of tankyrase we carried out an in silico small molecule screen using a set of 'PARP-binding' pharmacophores together with a generated (liganded) tankyrase homology model. This approach identified a structurally diverse set of ~1000 compounds for further study. Subsequent in vitro screening of recombinant tankyrase protein identified a subset of 59 confirmed inhibitors. Early optimisation followed by cell-based studies in WNT-dependent tumour cells, as well as cocrystallisation studies, identified a novel class of 3-aryl-5-substituted isoquinolin-1-ones, such as **21**[2], that exhibit potent inhibition of tankyrase activity as well as growth inhibition of colorectal cancer cells.



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NOVEL INHIBITORS OF GRP78: SCREENING A CHALLENGING TARGET USING THE CHEMETICS® SCREENING PLATFORM

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Nuevolution and Cancer Research Technology Discovery Laboratories (CRT-DL) have collaborated to successfully prosecute a screening campaign for inhibitors of Glucose-regulated protein 78 (GRP78, also known as BiP and HSAP5). GRP78 is an Endoplasmic Reticulum-localized chaperone required for proper folding of numerous plasma membrane-located and secreted proteins. GRP78 is a highly abundant protein and is further induced by conditions that lead to ER stress. Given that many cancers rely on potential GRP78 substrates for survival, or exist in a permanent state of ER stress, GRP78 is an attractive oncology drug target. However, it has proven difficult to drug with sufficient potency and selectivity by conventional methods. Using Nuevolution's Chemetics® screening platform we screened over 400 million DNA-encoded compounds, yielding a number of potent hit series. Confirmed actives were validated by biochemical and biophysical characterization and by subsequent X-ray crystal studies of key compounds. Multiple validated hit compounds are sub-µM in potency, exhibit selectivity for GRP78 over the closely related HSP72 protein and represent novel chemical space. Using X-ray co-crystallography 2 chemical series were found to induce a novel binding cavity discrete from the ATP binding site, suggesting a basis for the selective engagement of GRP78. Using structure-guided optimization we aim to develop potent cellular active GRP78 inhibitors for use in preclinical studies of e.g. multiple myelomas, ER+ breast cancers and gliomas.

NEW SEMI-SYNTHETIC DEHYDROABIETIC ACID DERIVATIVES TARGETING INFLAMMATION AND CANCER

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Natural products are excellent lead compounds in drug design and their chemical modification provides a sound strategy for finding new drugs with enhanced biological activities.[1] Abietane-type diterpenoids, such as abietic and dehydroabietic acids exist in the rosin of coniferous trees, widely available in the Finnish forests, and possess antimicrobial,[2] antiparasitic,[3] anticancer and anti-inflammatory activities, among others.[4]

According to estimates over 53 000 new cases of pancreatic cancer will be diagnosed in the US in 2016. The 5-year survival for this fatal disease remains at 7%.[5] The tumor microenvironment (TME) and the inflammatory process have major roles in the pathogenesis of cancer. Nitric oxide (NO) is one promoter of inflammation produced by inducible nitric oxide synthase (iNOS), and NO is overexpressed in the TME.[6]

As part of our ongoing research which focuses on the functionalization of natural products aiming to produce potent and selective compounds with targeted biological activities,[2,3,7] we have synthesized new dehydroabietic acid derivatives bearing anti-inflammatory and antitumor properties.

Our compounds inhibit the production of NO in mouse macrophages and inhibit growth in mouse and human pancreatic cancer cells lines. Several of the compounds are more potent than the parent dehydroabietic acid with IC_{50} values in the low micromolar range. Furthermore, our top five compounds induce monocyte differentiation in human leukemia cells as well as affect the expression of cell cycle proteins Cyclin D1 and p27 in pancreatic cancer cells, as measured by Western blotting.

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CARBON MONOXIDE-RELEASING MOLECULES AS NOVEL SYNERGISTIC AGENTS FOR ANTI-VEGF THERAPY OF TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is defined by the lack of expression of the oestrogen receptor, progesterone receptor and human epidermal growth factor receptor-2. Recently, CO was found to behave as an important endogenous signalling molecule and interestingly, to suppress VEGF receptor 2 and Akt phosphorylation. Given that anti-VEGF drug Avastin® exists as one of the few available targeted therapies for TNBC, we want to enhance its activity by combining it with new CO-releasing molecules (CORMs), whose design is based on commercially available ones. The new molecules will derive from the most potent available CORM, after corresponding testing of its activity.

Four commercially available CORMs are being screened for their effects on TNBC cell lines alone or in combination with Avastin®. IC_{50} range will be crucial for the selection of one leading compound, which will be subjected to structural modifications. A panel of two breast cancer cell lines serves as a model of TNBC, namely MDA-MB-231 and MDA-MB-436, alongside the non-cancerous human epithelial breast cells MCF-10A, as controls. The screening step is carried out through MTT assays and the use of the Seahorse Extracellular Flux Analyser. MTT assay is a colorimetric assay for detecting the cellular metabolic activity and viability of cells (cytotoxicity). Crystal violet assay is an alternative test used to measure the number of viable cells, based on their ability to absorb crystal violet dye. The Seahorse Extracelluar Flux Analyser helps to detect metabolic dysfunctions induced by CORMs, by calculating the extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR) of the cells, after corresponding treatment.

The results so far indicate that commercially available CORMs, without or in combination with low doses of Avastin®, have moderate cytotoxic activity on 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. However, ECAR levels of these cells are severely reduced, for example 100μM of CORM-A1 reduce the ECAR/pg protein level of MDA-MB-231 cells 5 times compared to control, proposing an interesting impairment of the cellular metabolism, due to the presence of CORMs.

Ongoing studies need to evaluate the ability of the combination of CORMs with Avastin® to alter the metabolic profile of TNBC cells, in order to select one leading compound for further investigation. The design of new derivatives will be based on the molecular characteristics of the chosen CORM and will add important desired characteristics, such as time – controlled release of CO with an appropriate mechanism. The collaboration with other universities will be decisive in elucidating the role of CO in TNBC and its potential benefit as a synergistic agent for existing anti-VEGF therapies.

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SYNTHESIS OF PYRROLIDINE RING-SUBSTITUTED ALKYLPHOSPHOCHOLINES AND THEIR CYTOTOXIC EFFECTS AGAINST HUMAN CANCER CELLS

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Alkylphosphocholines (APCs) are metabolically stable analogs of lysophosphatidylcholine in which an aliphatic side chain is bonded to phosphocholine via ether bonds. APCs are receiving much attention as a new class of potential antitumor agents since these compounds may act on cell membrane rather than on DNA. Miltefosine, a representative APC, has been approved for treatment of skin metastases from breast cancer. However, the clinical use of miltefosine is limited due to its poor pharmacokinetic property and moderate anticancer activity. Accordingly, several APCs have been synthesized to enhance anticancer activities or to reduce side effects of miltefosine. Recently, we examined whether the introduction of a cyclopentane ring as a spacer group between the polar head group and hydrophobic alky chain of the APC structure influenced on the anticancer activity. Some of the synthesized cyclopentane ring-substituted APCs showed more potent growth inhibitory effects than miltefosine against several human tumor cells (1,2). In the present study, a new series of pyrrolidine ring-substituted APCs was synthesized and evaluated for their growth inhibitory activities on selected human cancer cells: human lung cancer cell line (A549), human hepatocellular carcinoma (HepG2), human breast cancer (MCF-7), and epidermoid carcinoma (A-431). Most synthesized compounds inhibited more potently the growth of MCF-7 and A-431 cells than the growth of A549 and HepG2 cells. Some compounds exhibited greater activities than miltefosine on MCF-7 and A-431 cells. The structure of pyrrolidine ring-containing APCs and cell growth inhibitory activity relationships will be discussed.

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SYNTHESIS, DNA PHOTOCLEAVAGE AND ANTITUMORAL ACTIVITY OF TRIAZOLOQUINOLINIUM SALTS

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Recently, we have demonstrated that some triazolopyridine derivatives synthesized by our group are good DNA photocleavers and binders [1-2]. As a continuation of our research, triazoloquinolinium (TQ) disalts 1 and monosalts 2 were prepared (Figure 1). The DNA binding ability of this series of compounds has been investigated by means of UV-visible absorption, fluorescence titrations and viscosity measurements. Results have shown that all the compounds interact strongly at DNA grooves. All these TQ salts present photoinduced DNA cleavage activity (Figure 2a). Furthermore, they were tested for their anticancer activity against a panel consisting of 13 representative human cancer cell lines (glioblastoma, lymphoma, leukemia and pancreas, colorectal, breast and prostate adenocarcinoma). TQ monosalts resulted to be more active and selective than TQ disalts (Figure 2b).

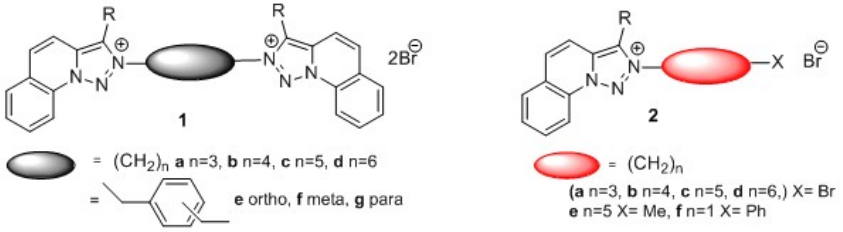

Figure **1.** Triazoloquinolinium disalts (**1**) and monosalts (**2**)

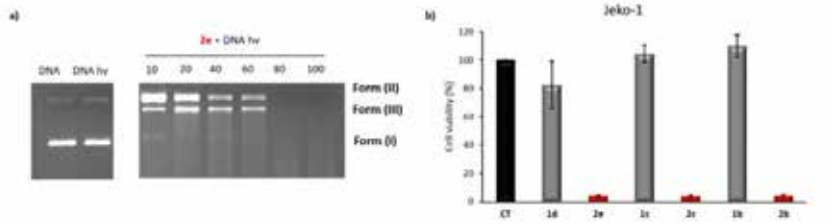

Figure 2. a) Photocleavage activity of **2e**. **b)** Cell viability of selected disalts and monosalts.

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AURONES AS POTENTIALS SERMs: SYNTHESIS, BIOLOGICAL ACTIVITY AND COMPUTACIONAL PREDICTION OF BINDING MODES

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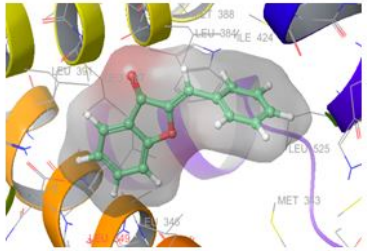
SERMs (Selective Estrogen Receptors Modulator) are chemical entities of low molecular weight that interact with estrogen receptors (ERa and ERb), they selectively stimulate or inhibit the estrogen receptors of different target tissues. [1] They have great pharmacological potential as they are prescribed in estrogen dependent breast cancer as well as in the treatment of menopause symtoms and in the treatment of osteosporosis in women.

Marketed SERMs as raloxifen and tamoxifen have achieved remarkable success in clinical therapies against, but its insufficient subtype selectivity causes adverse effects and drug resistance has appeared in the last decades. Therefore, discovery of new SERMs is of great importance for the clinical treatment of breast cancer. [2,3]

In this communication we will report the synthesis of a series of aurones through a biomimetic approach. [4,5] We will also report its estrogenic activity, docking studies and molecular dynamics simulations.



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ARMING ANTIBODIES WITH DNA CROSS-LINKING AGENTS DERIVED FROM THE DUOCARMYCINS

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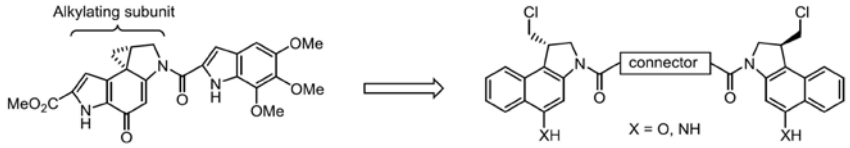
Antibody drug conjugates (ADCs) are composed of an antibody covalently linked to a therapeutic agent in such a way that the agent (or 'payload') is released selectively by antigen-presenting cells. Two ADCs armed with cytotoxic microtubule-binding agents have recently been approved for the treatment of HER2-positive breast cancer and CD30-positive lymphoma, prompting much work on the development of alternative ADCs as anticancer agents. An active area of exploration concerns the search for suitable payloads outside the class of microtubule-binding agents.

The duocarmycins are a small group of natural products that alkylate adenine in the minor groove of DNA. They possess several properties that make them attractive as ADC payloads, including high cytotoxic potency, activity against many multidrug-resistant cell lines, and activity against both cycling and non-cycling cells. Simplified and more synthetically accessible variants of the alkylating subunit have been reported which retain the cytotoxic potency of the natural products. This potency can be further enhanced by the preparation of dimeric analogues which cross-link DNA, providing, in some examples, remarkably toxic compounds with IC_{50}s in the fM range. This presentation will report on the synthesis and properties of homo- and heterodimers incorporating duocarmycin analogues, the preparation and antibody-conjugation of drug-linker constructs of the same, and the properties of these new ADC candidates.



(+)-Duocarmycin SA **New payloads for arming ADCs**

DEVELOPMENT OF NOVEL QUINAZOLINE-BASED DNMT3A SELECTIVE INHIBITORS

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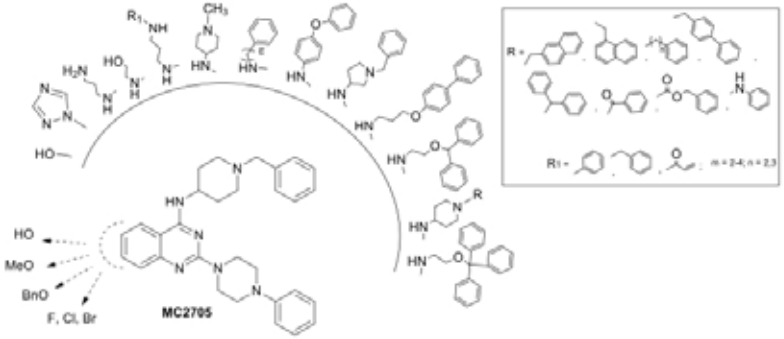
The DNA-methyltransferase 3A (DNMT3A) is a *S*-adenosyl-l-methionine (SAM) -dependent enzyme that in humans catalyzes *de novo* DNA methylation at the C5 position of the cytosine ring, mostly in the pericentromeric region of the DNA [1]. DNMT3A plays a crucial role in the epigenetic modulation of gene expression and represents a valuable target in cancer chemotherapy [2,3]. Through a process of hit-to-lead optimization, we have recently identified a lead structure (MC2705) as a selective non-nucleoside DNMT3A inhibitor (DNMT3Ai), with significant antiproliferative effects in different cancer cell lines [4]. With the aim to improve the potency and the anticancer effects of the prototype, and to confer it drug-like properties, we have performed a systematic medicinal chemistry optimization work on the structure of MC2705.



On the quinazoline ring of the lead, we have kept intact the 4-phenylpiperazin-1-yl substituent at the C2 quinazoline ring position, introducing several modifications on the C4 position: i) substitution of the *N*-benzyl moiety with other mono/bicyclic aromatic rings or with a methyl group; ii) oxidation/homologation of the methylene unit between the benzene and the piperidine rings; iii) replacement of the substituted 4-aminopiperidine moiety at the C4 quinazoline ring position with variously substituted aniline functions, or with a triazole or an hydroxyl function; iv) simplification of the 1-benzylpiperidin-4-amino group at the C4 quinazoline position with alkylamino functions variously substituted at omega position. We have then introduced methoxy, hydroxy, benzyloxy and halogen functions at the C6 and C7 quinazoline ring positions. Enzymatic and cellular tests carried on these new compounds, have shown that the inhibitory activity against DNMT3A reaches an IC_{50} value of 1.59 μM (about 6 times lower than MC2705) when the methoxy/benzyloxy groups are placed at C6/C7 positions of the quinazoline ring (MC3668).

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A SUSTAINABLE APPROACH TO RECYCLING CLASSIC ANTIMALARIALS TOWARDS NEW ANTIPROLIFERATIVE DRUGS

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Recycling classical drugs, by performing simple chemical modifications in order to improve their activity or repurposing them for other therapeutic targets, could be a sustainable manner to find new drugs.[1] Based on that, in the past few years, our group has been casting on classical antimalarials, such as chloroquine (**1**) and mepacrine (**2**), and discovered that a simple *N*-cinnamoylation on those scaffolds (compounds **3** and **4**, Figure 1) improved significantly their antimalarial activity.[2] When evaluated as antiproliferative drugs, compounds **3** and **4** showed activities in micromolar range against three different cancer cell lines (MKN-28, Caco-2 and MCF-7).[3] One of the most interesting and selective hits, from the family of compounds **4** (R = *p*F), was used to study the possible mechanism of action (MOA) behind the antimalarial and antiproliferative activity. The results that will be presented bring a new hope to search for antitumor leads with DNA binding, as possible MOA.

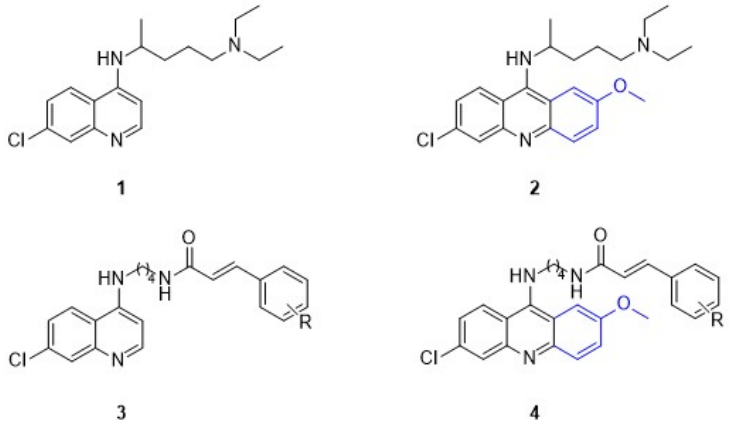

Figure 1: Chloroquine (**1**), mepacrine (**2**) and respective surrogates **3** and **4**.

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BENZIMIDAZOLE ANALOGUES AS KYNURENINE PRODUCTION INHIBITOR WITHOUT INDOLEAMINE 2,3-DIOXYGENASE INHIBITION

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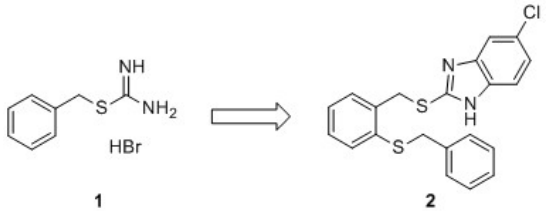
Kynurenine, which is a product of tryptophan catabolism through kynurenine pathway, and the metabolites play a variety of roles in the immune responses. It has been reported that kynurenine modulates T cells by suppressing their proliferation and inducing apoptosis, and activate regulatory T (Treg) cells. Recently, kynurenine was identified as an endogenous tumor-promoting ligand of the human aryl hydrocarbon receptor (AhR). Cancer cell-derived kynurenine promotes tumor-cell survival and motility, and suppresses the antitumor immune response through AhR in an autocrine/paracrine fashion. Therefore, the abrogation of kynurenine production in cancer cells is thought to be a promising approach to anticancer therapy.

Based on the importance of kunurenine in drug discovery, identification of modulator for kynurenine production is being investigated. In particular, indoleamine 2,3-dioxygenase (IDO) is remarkable target due to the regulatory enzyme for initial and rate-limiting step in kynurenine pathway. While several IDO inhibitors have been reported, we have also found *S*-benzylisothioureas **1** as IDO inhibitors.[1] However the isothiourea moiety should be avoided in drug development due to the potential toxicological property by possible nucleophilic substitution. Therefore, conversion of isothiourea to druglike structure is essential for drug development in promising anticancer therapy. Herein, we describe the design and synthesis of cyclized derivatives of isothiourea, as well as installation of substituents on the parent phenyl ring. The biological activities of these compounds on IDO inhibition and cellular kunurenine production in A431 cells are also reported. Especially, unexpected result for strong inhibition of cellular kunurenine production with barely IDO inhibition and the plausible mechanism of action (cpd **2**) will be also described.



1
IC_{50} = 61μM (IDO)
IC_{50} > 10μM (A431)

2
IC_{50} > 100μM (IDO)
IC_{50} = 0.46μM (A431)

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DISCOVERY AND OPTIMISATION OF THE FIRST SUB-MICROMOLAR, CELL PERMEABLE, SMALL MOLECULE INHIBITORS OF POLY(ADP-RIBOSE) GLYCOHYDROLASE (PARG)

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In recent years, many proteins involved in DNA repair have received considerable attention as potential cancer therapies, and several small molecule modulators have progressed to clinical evaluation or FDA regulatory approval in this field. However, the DNA repair protein poly(ADP-ribose) glycohydrolase (PARG), which plays a critical role in DNA single stand break repair, has so far eluded a successful drug discovery campaign. PARG is required for the hydrolysis of poly(ADP-ribose) chains, which are synthesized by poly(ADP-ribose) polymerase enzymes (PARPs), and serve to recruit repair proteins to the site of DNA damage. The dearth of potent, selective, cell permeable inhibitors of PARG has greatly limited research into the function and biological roles of this interesting target.

The poor druggability of PARG was evident from the low hit rate observed in a high-throughput screen (HTS) performed by AstraZeneca on a 1.4M compound screening library. Although HTS hit **1** displayed off-target toxicity at 72 h, X-ray crystallography revealed an unexpected binding mode to human PARG which was exploited by a programme of focused virtual screening and structure-based design to deliver several novel drug-like scaffolds. We disclose a series of quinazolinediones (**2**) as the first reported sub-micromolar cell permeable inhibitors of PARG. Non-cytotoxic, selective, drug-like chemical probes with low nanomolar cell activity will be presented, and SAR will be discussed with reference to their binding interactions to human PARG as observed by X-ray crystallography.

1 **2**

METAL-BASED PHYSIOLOGICALLY ACTIVE COMPOUNDS – CANDIDATES FOR ANTICANCER DRUGS

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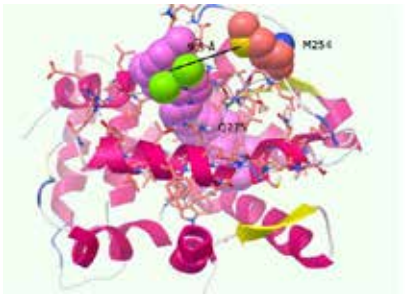
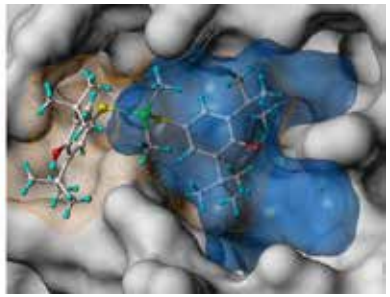
Nowadays the attention given to *medicinal inorganic chemistry,* which can be defined as the research field involving the use of metal-containing compounds for medicinal purposes, has steadily grown over the last years, due to their extensive application in pharmaceutical market.

A short review represents the main approaches to the design of protein target-oriented metal-based physiologically active compounds: the synthesis of known metal-based drugs analogues, the incorporation of organic pharmacophore groups into the ligand environment of metal, the use of organic drugs molecules as ligands in metal complexes. The protein target-focused syhthesis of novel metal compounds (M = Sn, Au, Ru), their physico-chemical properties and biological activity will be discussed. The biological activity has been studied in *in vitro, ex vivo, in vivo* experiments.





Acknowledgement

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TARGETING DNA AND DNA/TOPOISOMERASE(II) COMPLEX BY ANTIPROLIFERATIVE PYRAZOLO[1,2-A]BENZO[1,2,3,4]TETRAZINE-3-ONE DERIVATIVES, INSIGHTS ON THEIR MECHANISM OF ACTION

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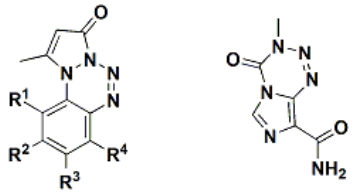
The development of new strategies aimed to discover new molecules able to act simultaneously upon multiple biotargets in fighting cancer, is an attractive approach to achieve synergistic effects for new therapeutically perspectives. Recently a new series of pyrazolo[1,2-a]benzo[1,2,3,4]tetrazine-3-one derivatives (PBTs), has been explored as potential anticancer candidates because of their promising antiproliferative activity, apoptosis induction in the low micromolar range, as well as cell cycle arrest promoters [1,2]. As an hopeful extension of these preliminary findings, we planned to investigate in depth on the mode of action the selected most active derivatives in an effort to get new insights on their anticancer potential.



PBTs

Temozolomide

At first, a DNA targeting is approached by means of flow linear dichroism (LD) experiments to evaluate the ability of a molecule to form an intercalative molecular complex. Additionally, we investigated the capacity of compounds to interfere with the catalytic cycle of topoisomerase II. Depending on the choice of functional groups, PBT scaffold exhibit diverse modes of action, including DNA complexation or inhibition of topoisomerase(II) activity. Preliminary *in silico* insights will also be shown on poster session.

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DESIGN AND DEVELOPMENT OF POTENT SERIES OF REVERSIBLE INHIBITORS OF LYSINE SPECIFIC DEMETHYLASE 1

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Background: LSD1 plays a key role in maintaining the balance between haematopoietic stem cell characteristics and differentiation to mature myeloid cells. In AML, LSD1 drives the oncogenic potential of leukaemic stem cells through the formation of immature blast cells by switching off this differentiation programming. Mechanism-based inhibitors of LSD1, developed from the monoamine oxidase (MAO) inhibitor tranylcypromine, have recently entered clinical trials. While the mechanism and inhibitory potential of these compounds are now well defined, the potential for effective reversible inhibitors of LSD1 as clinical agents is less clear.

Methods: Starting from existing literature and patent series, we employed rational medicinal chemistry and computational design using Cresset software to scaffold-hop into free IP space, while retaining activity against LSD1 in biochemical assays and by surface plasmon resonance (SPR). Selected compounds were tested in cellular assays and evaluated for their physico-chemical properties *in vitro* and *in vivo*.

Results: Several series of reversible LSD1 inhibitors have been designed and synthesised. These novel series demonstrate a clear pharmacophore for effective inhibition. The most active series displays K_D values of50values of

NOVEL EPIGENETIC HKMT INHIBITORS FOR EHMT1/2

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Despite the fact that a ll cells in an organism contain the same genetic code, the specific local and temporal expression of genes is regulated by posttranslational modifications on the DNA itself, and on the N-terminal tails of histone proteins that constitute the nucleosomes both, in normal cellular phenotypes but also in the development of human disease states.

Within the target landscape, the functional constituents of the epigenetic control machinery can be categorized into enzymes that covalently modify the DNA or, more predominantly, the N-termini of histone proteins by adding (epi-writers) or removing (epi-erasers) posttranslational marks to or from selected amino acid side chains. In addition to the enzymes, a broad range of receptor domains exists that recognize (epi-readers) the respective modification state of the affected side chain residues in a specific manner. The spectrum of posttranslational modifications ranges from conjugation of entire proteins over phosphate- or acetyl-groups to very small alterations such as adding or removing a single methyl-group to e.g. a lysine or arginine residue, thus controlling gene expression in a tight and accurate way.

HKMTs, histone-lysine(K)-methyl-transferases belong to the protein family of epi-writers and currently counts 96 family members. As HKMTs are still a relatively new target class, only a few inhibitors are currently known. These inhibitors either address the cofactor S-adenosyl-L-methionine (SAM) site or the substrate (histone) site. Since HKMT inhibitors are believed to become highly relevant e.g. as personalized cancer therapeutics, new strategies towards novel HKMT inhibitors are urgently needed.

Here we report on the concept of designing novel EHMT1/2-directed scaffolds that qualify as core structures addressing the histone binding site, and as such interfere in a protein-protein interaction with nanomolar biochemical activity.

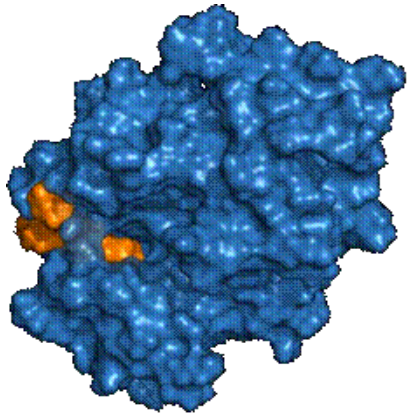

Figure 1: ME-06 docked in EHMT1 (IC_{50}=70nM)

DISCOVERY AND CHARACTERIZATION OF ANTIBODY-DRUG CONJUGATES WITH A NOVEL MODE OF ACTION

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Antibody Drug Conjugates (ADC) comprise of an antibody that targets a cell-surface antigen expressed by cancer cells, to which a cytotoxic small molecule is attached. Recently approved ADCs demonstrate the potential of this therapeutic class in the clinic. So far, mainly anti-mitotic (microtubule destabilizing agents) and DNA-damaging cytotoxic molecules have been used in the context of ADC. In this work, we describe ADCs based on a novel mode of action which utilizes inhibition of kinesin-5 as the cytotoxic payload principle. KIF11 (Kinesin-5, KSP) is a motor protein, a mitotic ATPase involved in spindle pole assembly, LMW inhibitors targeting KIF11 have previously been reported to be highly potent cytotoxic agents, since kinesin-5 is essential for mitosis and only present in dividing cells. We will describe the generation and characterization of novel ADCs, using KIF11 inhibitors, as the cytotoxic payload. In this context, ADCs with different linkers (cleavable vs non-cleavable) were compared in order to achieve cellular specificity, targeting tumor cells expressing the corresponding antigen. Further studies were also carried out to understand what is the active species in the cellular setting. The understanding of this provided a clearer roadmap for a rational inhibitor design, and a deeper understanding of the properties and characteristics that an inhibitor needs in order to be conjugated to the antibody, and to deliver an active ADC. Finally, we were also able to demonstrate in vivo efficacy in nude mice.

DESIGN, SYNTHESIS AND PHARMACOLOGICAL INVESTIGATION OF NEW DNA METHYLTRANSFERASE INHIBITORS

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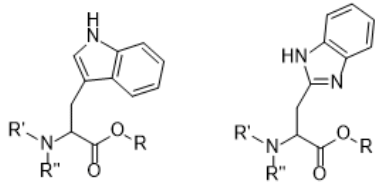
Epigenetic modifications without modifying the DNA sequence regulate gene expression in response to environmental factors. Epigenetic regulation is essential in physiological processes and is also involved in many diseases, including cancer.[1] In humans, DNA methylation is the most stable epigenetic mark and it is catalyzed by C5-DNA methyltransferases (DNMTs).[2] Two families of DNMT have been identified: the DNMT1, which is responsible for DNA methylation maintenance by methylating newly synthesized DNA strands; and the DNMT3A and DNMT3B, which are responsible for *de novo* DNA methylation. The expression of DNMT1, DNMT3B, or DNMT3A is increased in various tumors, making therefore DNMTs attractive therapeutic targets.[3,4] Several DNMT inhibitors (DNMTi) have been proposed to treat cancers. These compounds are endowed with nucleoside or non-nucleoside structure. Two nucleoside analogs have approved by the FDA and EMA against myelodysplastic syndrome, acute myeloid leukemia, and chronic myelomonocytic leukemia. Despite their high efficiency, their use is limited by the poor bioavailability and by undesired side effects due to their incorporation into DNA.[5] Thus non nucleoside DNMTi which do not need to be incorporated into DNA, might be safer and better drugable. Another feature of non nucleoside DNMTi is the possibility to identify inhibitors selective toward the DNMT isoforms expressed in the different tumors and physiological processes.

The findings led us to design and synthesize new series of DNMT inhibitors based on indole or benzimidazole templates.



In this communication, we report synthetic pathways and DNMT inhibition profile studies on the new inhibitor series.

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PRODRUGS SENSITIVE TO REACTIVE OXYGEN SPECIES FOR THE TREATMENT OF CHRONIC INFLAMMATORY DISEASES AND CANCER

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Insufficient drug selectivity causes severe side effects and host toxicity even in many of the first-line therapy treatments of diseases. Prodrugs are masked forms of pharmacologically active agents designed to undergo *in vivo* activation by specific stimuli. The use of prodrugs is generally proposed for the improvement of the ADME and "drug-like" properties of compounds. However, an additional feature is their application in targeted drug delivery, i.e. the released of the bioactive molecule by disease-specific stimuli.[1] Several pathologies, like cancer and chronic inflammatory diseases, are associated with increased levels of reactive oxygen species (ROS), due to the generation of inflammation.[2] This unique environment at the inflammatory tissue can therefore be used as a trigger stimulus. In this work we propose the use of prodrugs for the reduction of the undesired effects of drugs prescribed for inflammation related diseases. Making them inactive until they get activated predominantly or exclusively in inflammatory tissue was the strategy suggested (see Fig.1).

In order to achieve this goal, a series of promoieties sensitive to ROS were developed and synthesized. They were then coupled to different existing drugs and investigated. Promising results on the stability of the compounds in different physiological conditions, good ADME properties, activation at different ROS concentrations and comparable activity to the parent drug in cell-based assays were obtained. This indicates that the prodrug strategy is a promising tool for the improvement of current therapies for inflammatory diseases associated with serious side effects. This project presents a unique site-selective prodrug strategy based on ROS activation suitable for a wide range of diseases and different marketed drugs.

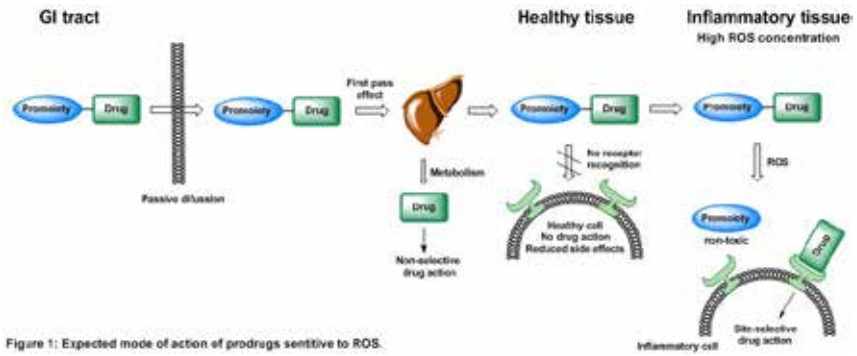

Figure 1: Expected mode of action of prodrugs sentitive to ROS.

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NOVEL ANTAGONISTS OF PREGNANE X RECEPTOR

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Pregnane X receptor (PXR) is a member of the NR1I nuclear receptor family and acts as a xenobiotic sensor and a paramount transcriptional regulator of drug metabolising enzymes and transporters. The overexpression of PXR in various cancer cells indicates the importance of PXR as a drug target for countering multidrug resistance (MDR) in anticancer treatments. The MDR of cancer cells contributes to the problematic 5% success rate of anticancer agents and the latest studies point to the PXR as one of the key players in the MDR of cancer cells.

We discovered novel PXR antagonists resulting from ligand-based approach and molecular modeling using marine sulphated steroids solomonsterols A and B as model compounds, which were isolated from a marine sponge, *Theonella swinhoei,* and were shown to act as PXR agonists in a Luciferase reporter assay on PYR transfected HepG2 cell line with potency similar to the effective PXR agonist rifaximin. The most active steroidomimetic bazedoxifene scaffold based antagonist exhibited IC50 value of 11 μM and in addition it down-regulated the PXR expression, exhibited an inhibition of PXR-induced CYP3A4 expression, which illustrates its potential to suppress PXR-regulated phase I drug metabolism (1). Further basedoxifene scaffold was replaced with the scaffold of diethylstilbestrol and the most active antagonist from this series exhibited IC50 value of 27 μM and also down regulated the CYP3A4 expression (2).

The suppression of PXR master target gene CYP3A4 highlights novel basedoxifene and diethylstilbestrole based compounds as PXR antagonists with the capacity to attenuate PXR-regulated phase I drug metabolism. Finally, new compounds represent a unique example of PXR antagonists that are shown to down-regulate the expression of PXR.

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NOVEL ASGP-RECEPTOR LIGANDS FOR TARGETED DELIVERY OF ANTICANCER DRUGS

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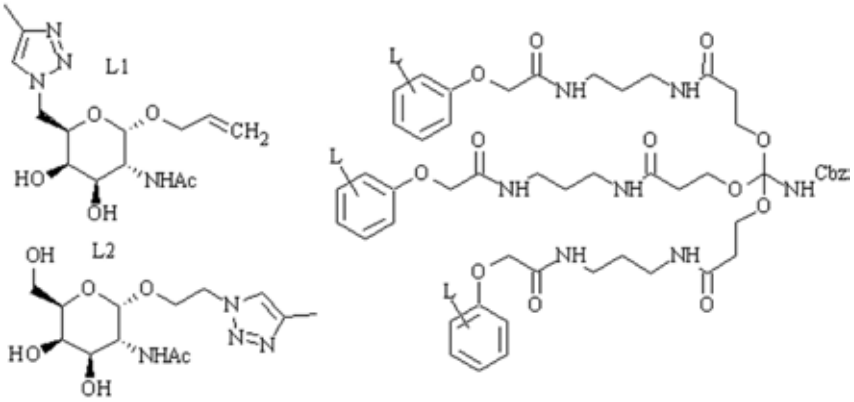
Hepatocellular carcinoma is the most common liver tumor, as a result of malignant transformation of hepatocytes. Every year more than 600 000 cases of this disease are diagnosed in the world[1]. One of the most promising ways to increase the effectiveness of chemotherapy is the targeted delivery of drugs in the liver cells using the hepatocyte ASGP-receptor which is galactose derivatives recognizing. This receptor is located only on liver cells surface and binds selectively with the hydroxyl groups in the 3rd and the 4th position of galactose. The outer part of the receptor consists of three subunits, each of which binds to galactose[2,3].



In this work we investigate the synthetic approaches to such vectors as shown above in Scheme, containing three N-acetyl-2-deoxy-2-aminogalactopyranose moiety having the high affinity for the receptor, for the delivery of anticancer drugs into hepatocytes.

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DESIGNING SMALL ORGANIC LIGANDS FOR THE ACTIVATION OF IMMUNE CYTOLYTIC REACTIONS AGAINST CANCER CELLS

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Immunotherapy is a new class of cancer treatment based on the innate powers of the immune system to fight cancer. Because of the immune system's unique properties, these therapies may hold greater potential than current treatment approaches to fight cancer more powerfully (1).

Intrinsically involved in the immunosurveillance of cancer, natural killer (NK) cells - a type of cytotoxic lymphocyte, critical to the innate immune system, that provides rapid responses to viral-infected and cancer cells - consist of a good target for these therapies.

Instead of acting via antigen-specific receptors, lysis of tumor cells by NK cells is mediated by alternative receptors, including NKG2D, NKp44, NKp46 and NKp30 (2).

In recent developments, B7H6, a surface protein present on a broad panel of tumor cells including lymphoma, melanoma, and carcinoma, was identified as a ligand for the NKp30 receptor. The structure of the NKp30-B7H6 complex has also been resolved (3), showing marked conformational changes that may be a key-factor for the NK-response activation role of B7H6.

Our current work aims at designing a family of small organic molecules (SOMs) capable of mimicking the effect of B7H6 on the NKp30 receptor. The main goal is to obtain an SOM capable of inducing an NK response, through binding to the NKp30 receptor, and structurally amenable to derivatization with tumor-targeting molecular units to produce a specific immune response against cancer cells.

A combination of computational docking and molecular dynamics tools was extensively used to scan several ligand libraries, yielding core-structures as possible ligands for the receptor. These were further optimized to generate lead structures for chemical synthesis. Data from mass spectrometry-based screening of the initial leads as NKp30 ligands will be presented.

Acknowledgements: This work has been carried out with financial support from Fundação para a Ciência e a Tecnologia (projects RECI/QEQ-MED/0330/2012, RECI/QEQ-QIN/0189/2012, and UID/QUI/00100/2013; and grants (SFRH/BD/110945/2015 to PFP and SFRH/BPD/108258/2015 to GCJ) and from Liga Portuguesa Contra o Cancro (LPCC/NRS-Terry Fox grant 2015).

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CHALCOGEN ENTITIES AND HETEROCYCLIC SCAFFOLDS: NEW HYBRIDS WITH ANTITUMORAL ACTIVITY

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In the last decade, the chalcogen selenium (Se) has generated a growing interest due to its efficacy and selectivity against cancer cells [1]. The biological activity of Se compounds is strongly dependent of its chemical form. Therefore, in this work we have decided to compare the activity between three Se-containing substituents that have shown promising results in previous studies: selenol, methylseleno and selenocyanate [2-4]. In addition, the corresponding sulfur (S) analogs were also synthesized in order to evaluate the effects of the isosteric replacement of S by Se in the anti-cancer properties.

The 27 novel derivatives were screened for their cytotoxic and antiproliferative activities against two human cancer cell lines: MCF7 (mammary adenocarcinoma) and PC-3 (prostatic adenocarcinoma). The most active compounds were also tested against a non-malignant human mammary epithelial cell line (184B5) in order to determine their selectivity. In general, the Se-containing derivatives were more active than their corresponding S analogs. However, we found that some S derivatives showed a greater activity or selectivity. Among the different substituents, seleno- and thio-cyanate seemed to be the most active groups in general.

On the basis of their potent activity and selectivity, the hybrids **7e** and **8f** were selected for further biological evaluation. Our results suggest that both compounds are able to induce caspase-dependent apoptosis and cell cycle arrest in G_2/M phase.

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SYMMETRICAL (DISELANEDIYLDIBENZENE-4,1-DIYLNIDE)BISCARBAMATES AS REDOX MODULATORS: A MOLECULAR MODELING APPROACH TO THEIR CYTOTOXIC AND REDOX ACTIVITIES.

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A molecular modeling study has been carried out on a previously reported series of (diselanediyldibenzene-4,1-diylnide)biscarbamate derivatives that show cytotoxic and antiproliferative *in vitro* activity against MCF-7 (breast adenocarcinoma) human cell line; radical scavenging properties were also confirmed when these compounds were tested for their ability to scavenge DPPH and ABTS radicals [1].

The data obtained through the approach used allowed us to classify the compounds into two different groups: (a) aliphatic carbamates for which the action mechanism could be related with a first nucleophilic attack (mediated by H_2O, for example) on the selenium atoms of the central scaffold, followed by the release of the alkyl N-(4-selanylphenyl) and N-(4-selenenophenyl)carbamate moieties. Then, a second nucleophilic attack on the carbamate moiety, to yield 4-aminobenzeneselenol and 4-selenenoaniline respectively, which can ultimately be responsible for the activity of the compounds; (b) aromatic carbamates, for which we propose a preferred nucleophilic attack on the carbamate moiety, yielding 4-[(4-aminophenyl)diselanyl]aniline, compound **F**, the common structural fragment for this series, for which we have previously demonstrated its cytotoxic profile. Then, selenium atoms of the central fragment may later undergo a new nucleophilic attack, to yield 4-selenenoaniline, **Y**, and 4-aminobenzeneselenol, **Z**. The phenolic moieties released in this process may also have a synergistic cytotoxic and redox activity.

[dplano_02.jpg]

The data that support this connection include the conformational behavior and the molecular topography of the derivatives which can influence the accessibility of the hydrolysis points, and some quantum descriptors values (bond order, atomic charges, total valences, ionization potential, electron affinity and LUMO 0 orbital location) that have been related to the biological activity of the compounds.

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EFFECTS OF BIOTIC AND/OR ENVIRONMENTAL FACTORS ON THE ANTITUMORAL EFFECTS OF LEAVES FROM DIFFERENT ACCESSIONS OF GRAPEVINE AGAINST CANCER CELL LINES

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Usually, most of the studies conducted on grapevine only consider the extracts obtained from the grapes in the evaluation of their potential effects on the human health. Furthermore, one big concern of the viticulture is the vast amount of grapevine pruning wastes (i.e. leaves and stems) that are underused and generate a great economic burden. In the search of a possible economic benefit for these pruning wastes, we have decided to evaluate the *in vitro* antitumor effects of the methanolic extracts obtained from the leaves against several cancer lines. Likewise, the grapevines were subjected to different biotic (mycorrhizal inoculation) and environmental (air temperature) factors to determine their effects on the cytotoxic activity.

A total of 16 foliar extracts were screened *in vitro* at five concentrations (from 250 to 15 µg/ml) using the MTT assay after 48 h of treatment on several cancer cell lines. The cytotoxic parameter values (LD_{50}, TGI and GI_{50}) were determined and reported for all of them. Most of the extracts showed a marked cell growth inhibition and, more interestingly, the modulation of the biotic and environmental factors had significant impact on these cytotoxic/cytostatic effects.

To our knowledge, this is the first report that demonstrates the modulating effect of biotic and environmental factors on the properties of grape leaves as a natural resource to provide human health benefits. These very promising results encourage us to further investigate the mechanism of action for these extracts. In conclusion, the preliminary data reported in this study could constitute an important finding for the viticulture.

NEW DELIVERY SYSTEMS BASED ON POLYMERIC SURFACTANTS FOR THE ENCAPSULATION OF SELENADIAZOLE DERIVATIVES AGAINST CANCER AND LEISHMANIA

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The pharmacology industry has been developing very fast in the past few decades. However 40% of newly discovered compounds are poorly soluble. The purpose of this work is to provide an innovative platform for the development of an efficient system for drug delivery using polymeric surfactants. Particularly, in this study, we have used five new selenadiazole derivatives substituted with a hydrocarbon chain of variable length (from 3 to 12 carbon atoms). This selenadiazole skeleton was chosen based on two aspects: its similarity with ebselen and the previously reported in vitro antitumoral effect for some related selenadizaole derivatives substituted on 5-position with different amides[1]. However, owing to the introduction of the hydrocarbon chain, the water solubility of these new active compounds is very poor.

Poloxamines, also known by their commercial name Tetronics®, are X-shaped amphiphilic block copolymers where each arm is formed by polyethylene oxide (PEO) and polypropylene oxide (PPO) blocks with a central ethylene diamine connector group. This allows the structure to self-assemble into spherical micelles with a lipophilic core and a hydrophilic outer shell, with responsiveness to pH changes due to the protonation of the central diamine. Depending on the architecture of these block-copolymers, their concentration and temperature, the micelles can aggregate into a long-range order array, inducing the formation of hydrogels[2]. The micellar core of the micelles offers a perfect environment for the encapsulation of the drug, allowing a controlled release through the body.

In this work, the structure of the micelles and gels formed by the combination of a direct and reverse poloxamine (with hydrophobic groups on the outside) have been characterized using small angle neutron scattering (SANS) and dynamic light scattering (DLS), as well as the optimal conditions for drug encapsulation determined by fluorescence and UV-vis spectroscopy and solubilization isotherms. Furthermore, in vitro studies were carried out to determine the radical scavenging activity and the cell growth inhibition against hormone-dependent cancer cell lines (mammary and prostate adenocarcinomas) for the new selenadiazole derivatives. Compounds with a lateral linear chain of three, four and six carbons (IV.B22, IV.B23 and IV.B26 respectively) showed a more potent radical scavenging in the DPPH assay in comparison with ebselen, a well-known antioxidant compound.

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SYNTHESIS, RADICAL SCAVENGING AND CYTOTOXIC ACTIVITIES OF NOVEL SELENADIAZOLE COMPOUNDS

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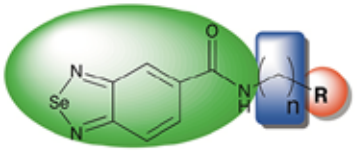
Selenium (Se) presents anticancer activity, this anticancer effect being highly dependent on the chemical form and on the selenium intake. In addition, some selenoproteins are involved in the modulation of the oxidative stress.

During the last decade, the incorporation of Se atom onto organic structures has achieved very promising agents against a plethora of diseases. One of these compounds is ebselen, a heterocyclic Se compound which acts as a mimetic of glutathione peroxidase. Continuing with our efforts seeking novel active Se compounds[1], a series of 28 new selenadiazole derivatives (**Figure 1**) were synthesized and evaluated *in vitro* to determine their cytotoxic and radical scavenging properties. The objective in the design of these compounds is the modulation of numerous structural features: **a)** the length of the linker with the amide group; **b)** different polarities for the R group (OH vs substituted phenyl rings); and **c)** several heterocycles with proven cytotoxic effects.



n = 0; 1; 2

R = OH; Ph; (4-CH_3NH_2)Ph; (4-$(CH_3)_2NH_2$)Ph; (4- $(CH_3)_3NH_2$)Ph; (4-$(CH_3)_4NH_2$)Ph; (4-CH_3)Ph; (4-Cl)Ph; (4-Br)Ph; (4- NO_2)Ph; (4-OCH_3)Ph; (4-SCH_3)Ph; (4-$SeCH_3$)Ph; (4-SeH)Ph; (4-SH)Ph; (4-SeCN)Ph; (4-triOCH_3)Ph; cyclohexyl; piperidine; 4-aminopyridine; piperazine; 4-morpholinoaniline; 2-aminothiazole; 1-(4-methoxibencyl)piperazine; 2-aminobenzothiazole;

All the compounds were isolated, characterized and screened using the MTT method in breast (MCF-7), prostate (PC-3) and colon (HT-29) cancer cell lines after 72 h of treatment at five different concentrations. Likewise, their radical scavenging activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Ebselen and ascorbic acid were used as standard compounds.

In conclusion, five compounds exhibit a dual activity as cytotoxic and radical scavenging agents and can be consider as very promising scaffolds to further develop.

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CHARACTERIZATION OF NOVEL METHYLSELENO DERIVATIVES

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Selenium (Se) is an essential micronutrient involved in many cellular mechanisms due to its incorporation into selenoproteins. However, at higher doses Se is cytotoxic and Se compounds have demonstrated to target many types of tumours. Their mechanism of action remains largely unknown, due to a strictly dose and chemical species dependency. Methylselenol is believed to be a key metabolite in selenium biochemistry and has shown promising features as an anticancer agent. For this reason, novel molecules containing a methylseleno moiety were synthesized. After testing them against a panel of tumour cell lines, the most active compounds were selected for further characterization. The metabolic processing of the compounds was analysed by assessing their reduction and interaction to the thioredoxin and glutaredoxin systems, known for their reduction of selenium metabolites. Potential ROS production and shifts in thiol status were also assessed in tumour cell lines after treatment with the compounds. 3D spheroid cultures, resembling the *in vivo* conditions much more than monolayer cultures, and more suited for cytotoxicity studies was thus used for drug delivery, viability and morphological studies. Our data clearly shows a great difference in effect between the novel compounds analysed.

EVALUATION OF NOVEL DISELENIDES AS CYTOTOXIC AGENTS AND APOPTOSIS INDUCERS IN VITRO

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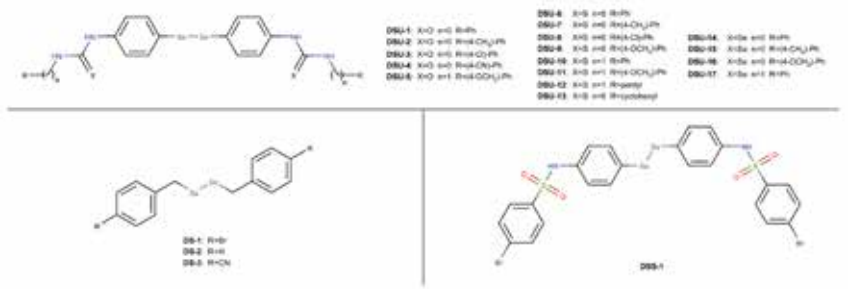
Incorporation of the Se atom into organic molecules is a promising rational design to achieve potent and selective cytotoxic compounds against several types of tumor cells. Therefore, the aim of this study was to evaluate the antitumor activity of 21 new diselenide compounds (**Figure 1**) against a panel of different tumor (CCRF-CEM, MCF-7, HT-29, HTB-54, K-562 and PC-3) and normal immortalized (184B5 and BEAS) cell lines.



Cytotoxicity was evaluated by the MTT method. Growth curves showed that most of the tested compounds were highly cytotoxic with GI_{50} values in the micro molar range. Here we report that DSU-4, the most cytotoxic among the tested compounds, was able to induce a time and concentration dependent cell death process in human lymphoblastic leukemia CCRF-CEM cells. However, no changes were detected in cell cycle phase distribution of the cell cultures. Since DSU-4 induced-cell death was suppressed by pre-treatment of the cells with the pan-caspase inhibitor z-VAD-fmk and it occurred with a decrease in *Bcl-2* levels, our results suggest that DSU-4 induces a caspase-dependent intrinsic apoptosis subroutine in CCRF-CEM cells.

DSU-7: A NOVEL SELENOCOMPOUND THAT INDUCES ENTOSIS IN MCF-7 CELLS

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Selenium (Se) is probably the most investigated trace element because of its dual action: whereas at low concentrations Se has antitumor properties, at high concentration it can be genotoxic and carcinogenic. Entosis is a non-apoptotic form of cell death, which is driven by homogeneous cell-in-cell invasion. Engulfment of the cell is paralleled by the activation of the Rho/ROCK/myosin II signaling pathway. Here we show that a novel selenocompound, DSU-7 can induce entosis in breast adenocarcinoma MCF-7 cell cultures in a concentration and time dependent manner. In fact, upon DSU-7 administration MCF-7 cells showed signs of G_2/M cell cycle arrest and DNA fragmentation as detected by TUNEL. Interestingly, DSU-7 induced-cell death was blocked by the Rho, ROCK and myosin II inhibitors CT04, H-1152, blebbistatin and Y-27632. However, neither the pan-caspase inhibitor z-VAD-fmk, nor the autophagy inhibitor wortmannin could suppress the effect of the drug. Besides, an increase in total protein levels of *ML-2* and no changes in *Beclin* and LC3B were detected.

SYNTHESIS AND BIOLOGICAL EVALUATION OF DIAROMATIC GUANIDINIUM-LIKE DERIVATIVES AS PROTEIN KINASE INHIBITORS FOR THE TREATMENT OF CANCER

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Kinases have emerged as one of the most intensively pursued targets in current medicinal chemistry research, especially for cancer, due to their critical roles in cellular signalling. To date, the US FDA has approved 28 small molecule kinase inhibitors, half of which were approved in the past 3 years.[1] The RAS–RAF–MEK–ERK pathway (ERK signalling) is an evolutionary conserved kinases' cascade that transmits signals from cell surface receptors to promote cell proliferation and survival. In physiological conditions, ERK signalling is tightly controlled by feedback loops at multiple levels, which are essential for maintaining regulated cell growth and homeostasis.[2] Components of the ERK signalling cascade are frequently mutated in cancer, with approximately 1/3 of human tumours expressing a constitutively activated mutant form of RAS2 and approximately 8% of tumours expressing an activated form of BRAF. These findings prompted the development of small-molecule inhibitors targeting components of ERK signalling to be used as cancer therapeutics.[2]

Previous studies in Rozas' group identified compound **1** (Figure 1) has a kinase inhibitor capable of inhibiting MAPK/Erk pathway through a type-III allosteric mechanism. As such, compound **1** provided a new chemical entity for further refinement of kinase selectivity and potency that may not face the limitations of ATP-competitive inhibition that challenge the translation of current generation PKIs towards the clinic.[3] Previous and on-going molecular-modelling studies point toward the idea that this compound forms hydrogen-bond/electrostatic interactions with one of the ATP-phosphates through the guanidinium moiety thus positioning the lipophilic (4-Cl-3-CF_3)-Ph group in the hydrophobic pocket of the enzyme.[4]

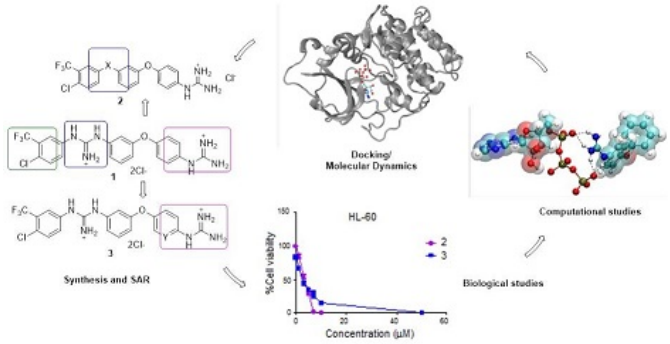

Figure 1: Optimization cycle of new guanidine-based kinases' inhibitors with anticancer activity.

New guanidinium aromatic derivatives (e.g. **2** and **3**) have now been synthesized to clearly identify the structural motifs that determine the allosteric inhibition of the MAPK/Erk pathway. The cytotoxic effect of these new derivatives has already been assessed, with a 3/4-fold improvement (IC_{50} = 3.08 μM for **2** and 1.53 μM for **3**) compared to **1** (IC_{50} = 9.72 μM). Moreover, their role as apoptotic inducers and inhibitors of different kinases will be evaluated by performing several biochemical assays.

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THE CLINICALLY USED IRON CHELATOR DEFERASIROX INHIBITS EPIGENETIC JUMONJIC DOMAIN-CONTAINING HISTONE DEMETHYLASES in vitro AND in vivo

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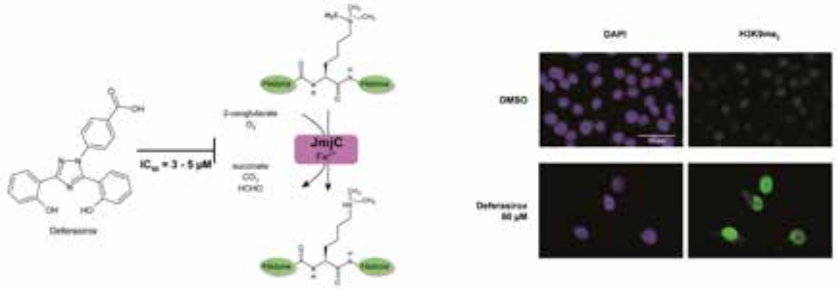
Epigenetic mechanisms determining the cellular phenotype are maintained by chemical modifications both to DNA as well as to the proteins around which it is wrapped, the histones. Iron(II)- and 2-oxoglutarate-dependent JumonjiC histone demethylases oxidatively remove methyl groups from lysine residues in the histone tails and are implicated in the reprogramming of cells and manifestation of numerous diseases, in particular cancers, making them viable drug targets.

We screened a collection of clinically used iron chelators in two orthogonal assays for their potential inhibition of these iron-dependent enzymes. While all exhibited micromolar inhibition *in vitro*, likely due to sequestration of iron from the assay buffer, for deferasirox, we could show that this compound is a bona fide active site-binding inhibitor of these enzymes. This was deduced from kinetic experiments showing competitive behavior with regard to the co-substrate 2-oxoglutarate as well as EPR spectroscopic investigations of the enzyme-bound metal center. A plausible binding model was established from molecular docking.



Deferasirox shows potent *in vitro* inhibition of JMJD2A, JMJD3, and JARID1A and *in vivo* antiproliferative activity on a range of esophageal cancer cells. On-target modulation of intracellular H3K9 trimethylation after deferasirox treatment could be demonstrated by immunofluorescence in KYSE-150 cells.

The fact that deferasirox is a licensed and safe drug already on the market for an unrelated disorder offers the exciting potential to develop this structure and derivatives further into potent anticancer medications based on their epigenetic regulatory potential.

(We thank the Deutsche Forschungsgemeinschaft for funding of research of inhibitors within CRC 992 "Medical Epigenetics" and the Studienstiftung des deutschen Volkes for a doctoral scholarship to M. R.)

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CYCLOMETALATED PLATINUM COMPLEXES OF ARYL GUANIDINES AS ANTICANCER AGENTS

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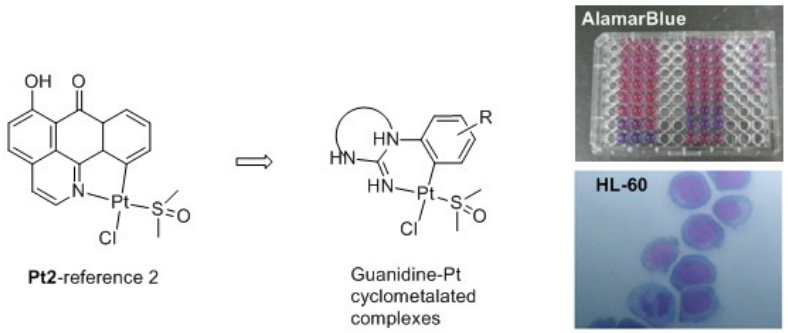
Platinum-based drugs such as cisplatin or oxaliplatin have been clinically used with much success against testicular, ovarian and head and neck cancers; however, their toxicity, limited efficacy in other tumours and potential for acquired and intrinsic resistance encourage further research in this area.[1] Recently, it has been reported that cyclometalated Pt derivatives (see **Pt2** in Figure 1) can exert their anticancer effect by binding to telomeric, c-myc and bcl-2 G-quadruplexes thus inducing senescence and apoptosis.[2] G-quadruplexes, which are four-stranded nucleic acid structures formed by guanine-rich sequences that fold into non-canonical secondary structures, have been found not only in telomeres but also in promoter regions of genes such as c-myc or bcl-2;[3] thus, stabilization of these particular G-quadruplexes may play a key regulatory role in the corresponding signalling pathways.[4]

For that reason, we aim to prepare cyclometalated Pt complexes of guanidine-based aromatic systems as novel anticancer therapeutics. In the past 10 years, we have developed a number of diaromatic, guanidine-based DNA minor-groove binders with very good affinity towards DNA. This affinity was assessed by means of different biophysical techniques such as DNA thermal denaturation, Circular and Linear Dichroism or UV titrations.[5] We have now prepared a small but novel library of cyclometalated guanidine-platinum derivatives (see Figure 1) as potential anticancer agents. Their preparation, full characterisation and crystal structures will be presented in addition to the results obtained in cell-viability assays on a human leukemia (HL-60) cell line.



Pt2-reference 2

Guanidine-Pt cyclometalated complexes

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EXTRACELULLAR PALLADIUM-MEDIATED DEALKYLATION OF BIOORTHOGONAL PRODRUGS

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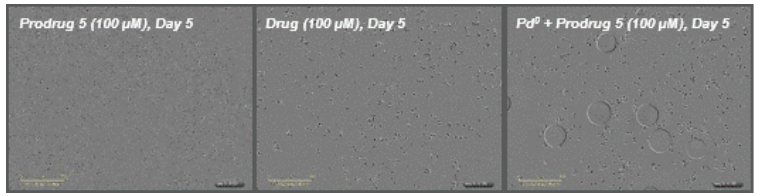
Chemotherapy is the treatment of cancer with antineoplastic drugs, which commonly operates by killing cells that divide rapidly, one of the principal features of cancer cells. However, the most effective cytotoxic drugs are limited by lack of selectivity since non-cancerous cells are also heavily affected[1]. To reinvigorate the medical use of approved drugs without a satisfactory safety profile, one of the main strategies followed by medicinal chemists is to transform chemotherapeutic agents into latent prodrugs[2]. While most popular prodrugs become active through a biochemical process, significant progress on the use of benign non-biological means to activate drug precursors is gaining ground. Palladium-activated prodrug therapy is an experimental therapeutic approach that relies on the unique chemical properties and biocompatibility of heterogeneous palladium catalysis to enable the spatially-controlled *in vivo* conversion of a biochemically-stable prodrug into its active form[3]. Using an alkylation strategy to mask functional groups essential for the cytotoxic mode of action of a clinically-used drug, different inactive Pd^0-sensitive derivatives were developed (**Prodrugs 1**-**5**). Particularly, cell viability studies on A549 cancer cells confirmed a ~100-fold reduction in cytotoxic activity for **Prodrug 5**. While Pd^0-resins and **Prodrug 5** displayed no cytotoxicity separately, combination of both exhibited equivalent antiproliferative properties to unmodified drug in A549 cancer cells, underlining the *in vitro* efficacy of this activation strategy and supporting further *in vivo* investigations.



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BMX-TARGETED SMALL MOLECULE DRUG CONJUGATES FOR PROSTATE CANCER THERAPY

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Standard of care strategies for the treatment of cancer still relies heavily on non-specific radiation and chemotherapy. These treatments are commonly associated with severe toxicities and in many cases only offer limited benefit for the patient. The targeted delivery of radionuclides, cytotoxic drugs and pro-inflammatory cytokines into malignant tissue, on the other hand, can markedly improve the therapeutic index and overall efficacy of such substances. Whilst monoclonal antibodies are the most widely used delivery vehicles to date, low molecular weight targeted agents are emerging as a promising alternative to antibody-based drug delivery.

Currently, extensive programs are underway in both academia and industry to optimize effector payloads, linkers and to discover new targets. The next quantum leap in the field of targeted cancer therapeutics will result from the miniaturization of targeting vehicles. Small organic ligands have negligible immunogenic risk and are easier to chemically modify enabling fine tuning of their ADME profile. As a result, it is possible to achieve a time dependent release of the cytotoxic payload at the target tissue maximizing its therapeutic efficacy while producing minimal damage to healthy cells. Most importantly, depth of tumour penetration and tumour-to-blood distribution ratio after injection should be significantly superior.

We will present our efforts towards the development of bone marrow tyrosine kinase in chromosome X (BMX) ligands for the targeted intracellular delivery of cytotoxic drugs into BMX-overexpressing tumours, namely prostate cancer. This project is expected to explore new avenues for drug delivery systems since the concepts here proposed go beyond prostate cancer therapy as they can be applicable to any ligand that interferes with up-regulated disease-related pathways.

SYNTHESIS AND ENANTIOMERIC SEPARATION OF A NOVEL SPIROKETAL DERIVATIVE: A POTENT HUMAN TELOMERASE INHIBITOR WITH HIGH IN VITRO ANTICANCER ACTIVITY

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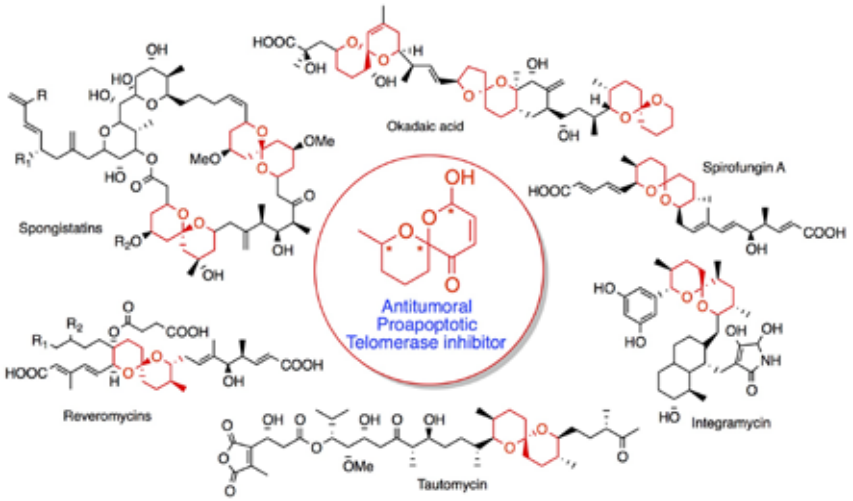
Numerous complex natural products containing spiroketals framework, with a wide range of important biological activities, have been isolated over the years from marine or terrestrial sources. Many groups investigated the possibility to reduce the complexity of spiroketal natural products while preserving their biological activity, showing that simple natural inspired spiroketals can be privileged scaffolds for new interesting lead compounds. We have synthesized a new structurally simplified spiroketal that has shown a potent antitumor activity against tumor cells of different nature and histotype. The simple spiroketalic structure of this compound has three stereocentres and its synthesis afforded a stereoisomeric mixture. We carried out the synthesis and the characterization of the stereoisomeric mixture, the stereoisomeric separation and the biological evaluation both of the stereoisomeric mixture and the enantiomerically pure spiroketals.



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CHEMICAL MODIFICATION OF THE ANTITUMOR ANTIBIOTIC OLIVOMYCIN A AND INVESTIGATION OF THE ACTIVITY AND MECHANISM OF ACTION OF NEW POTENT DERIVATIVES

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Although the interest to antitumor antibiotics of the aureolic acid group grew significantly after new perspective targets for these compounds have been discovered, chemical modifications of this chemical class are scarce. We developed new methods of modification of olivomycin A (**1**), the aureolic acid group antibiotic, including modification of the side chain, the aromatic ring of the aglycon and acyl groups of sugar branches (Fig. 1). The antiproliferative acitvity of newly synthesized semisynthetic derivatives was tested on a panel of tumor cell lines, and the most potent compounds were investigated for structure-activity relashionships and mechanism of action. N,N-Dimethylaminoethylamide of olivomycin SA (**2**) (N,N-dimethylaminoethylamide of 1'-de-(2,3-dihydroxy-n-butyroyl)-1'-carboxy olivomycin A) demonstrated the most promising properties and was selected for preclinical testing [1].

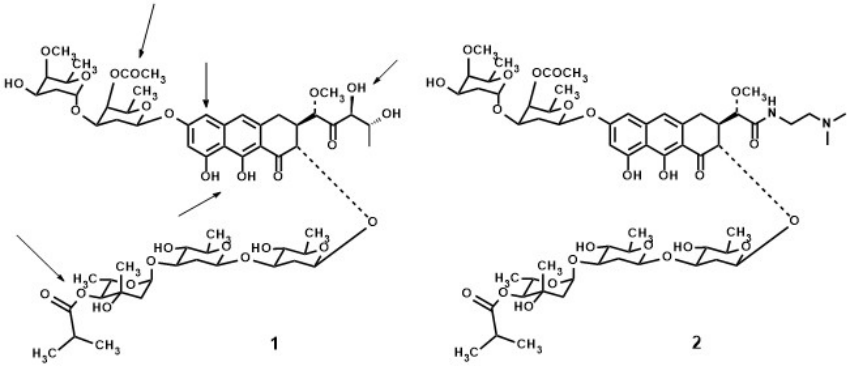

Fig. 1. Directions of chemical modification of olivomycin A (**1**) and the structure of the drug candidate N,N-dimethylaminoethylamide of olivomycin SA(**2**).

Studies of the mechanism of action of **2** in comparison with the starting olivomycin A (**1**) and *in vivo* experiments are under way.

This study was partly funded by theMinistry of Education and Science of Russia, contract №14.N08.12.0058.

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SYNTHESIS OF NOVEL BENZOTHIAZOLE-PIPERAZINE DERIVATIVES AND THEIR BIOLOGICAL EVALUATION AS ACETYLCHOLINESTERASE INHIBITORS AND CYTOTOXIC AGENTS

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Background - Chemotherapeutic agents that target cancerous cells selectively are still under investigation as drug resistance remains as a major problem [1]. In our previous study, benzothiazole-piperazine derivatives are shown to be cytotoxic against breast, hepatocellular, colon cancer cell lines [2]. In neurodegenerative disorders such as Alzheimer's disease (AD), AChEIs are prescribed primarily for the treatment of cognitive symptoms [3]. Recently compounds bearing benzothiazole and piperazine rings are reported for their potent inhibitory action on AChE [4].

Aims and Methods - In this study, we aimed to synthesize 11 novel N-(6-ethoxybenzothiazole-2-yl)-2-(4-substituted-piperazinyl)acetamide derivatives and investigate their cytotoxic, AChE/BuChE inhibitory activities. The compounds were tested for their cytotoxic activities against several cancer cell lines by sulforhodamine B assay and results were compared with 5-Fluorouracil. AChE/BuChE inhibitory activities of the compounds were determined by the modified Ellman method. In positive control experiments, donepezil was employed as the reference inhibitor. Synthesized compounds were identified with IR, ^{1}H-NMR, ^{13}C-NMR, LC-MS and UV spectra. Their purities were confirmed by elemental analysis. Docking of the inhibitor **2j** was carried out using the program Gold 5.1 to predict the interaction mode of these derivatives for AChE.

Results - Ellman study results show that all compunds are selective inhibitors on AChE rather than BuChE. Most active derivative against AChE is (1-methylpiperidine-4-yl)piperazine derivative **2j** (77.2%). In addition, **2j** has better selectivity over AChE in comparison with reference compound Donepezil (**2j**; AChE:BuChE, 77.2:18.6, **Donepezil**; AChE:BuChE, 94.8:70.5).

Conclusion - All compounds have cytotoxic activity against hepatocellular (HUH-7) and colorectal (HCT-116) cancer cell lines. In general, dihalo substituted benzylpiperazine derivatives (**2a**, **2e**) have the highest cytotoxic activities in all tested cell lines. In addition, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of synthesized compounds are investigated by *in vitro* Ellman's method. Compound **2j** leads to high and selective inhibition against AChE. Docking study was performed on compound **2j** to prove its high affinity to AChE where binding modes of **2j** and Donepezil on AChE were found to be similar.

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DEVELOPMENT OF NEW BROMOTHIAZOLE DERIVATIVES AND IN VITRO STUDIES ON THE INHIBITION OF COLON CANCER

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The cancer drug development process has become increasingly costly and dormant cancer cells in hypoxic and nutrient-deprived regions of solid tumors provide a major obstacle to treatment. Here, we describe the synthesis and characterization on new bromothiazole derivatives with core of nitazoxamide (Figure 1), an FDA-approved antiprotozoal drug [1].

Using a human adenocarcinoma-derived cell line (Caco-2 cell line), we then investigated the concentration-dependent antiproliferative (^{3}H-thymidine incorporation) and cytotoxic (extracellular lactate dehydrogenase activity) effect of these derivatives. At their highest concentration (1 mM), all compounds were able to reduce ^{3}H-thymidine incorporation by more than 80%, and all compounds presented a more marked antiproliferative effect than butyrate (5 mM). Also at this concentration, all compounds presented a cytotoxic effect.

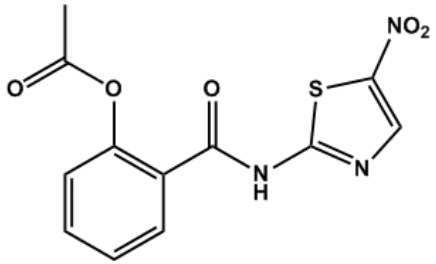

Figure 1. Structure of nitrothiazole benzamide drug, nitazoxamide.

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PHTHALIMIDE DERIVATIVES AS MODULATORS OF WNT PATHWAY

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The Wnt signaling pathway is a critical developmental pathway which operates through control of cellular functions such as proliferation and differentiation. Wnt proteins are secreted glycoproteins acting as growth factors regulating various cellular functions including proliferation, differentiation, death, migration and polarity. Aberrant Wnt signaling has been linked to the formation and metastasis of tumors. Our effort is to provide use of 6-substituted phthalimides for modulating Wnt activity and/or porcupine activity. The leading compound demonstrated subnanomolar inhibition of Wnt signaling in a paracrine cellular assay. It also showed excellent chemical, plasma and liver microsomal stabilities. Furthermore, compound exhibited good pharmacokinetic profiles with good oral bioavailability in rat. It also shows good efficacy in MMTV mouse Wnt model. Collectively, these results strongly support further optimization of this novel scaffold to develop better Wnt pathway inhibitors.

DISCOVERY OF 4,6-DISUBSTITUTED PYRIMIDINES AS POTENT INHIBITORS OF THE HEAT SHOCK FACTOR 1 (HSF-1) STRESS PATHWAY

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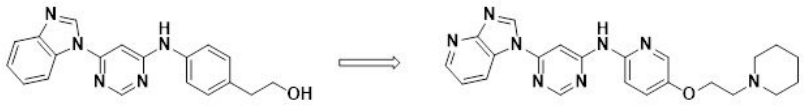
Heat shock factor 1 (HSF1) is a highly conserved transcription factor and is proposed to play an important role in oncogenesis and cancer progression, including providing a mechanism for cell survival under proteotoxic stress.[1] Therefore, inhibition of the HSF1 pathway could represent an exciting new opportunity in cancer treatment as an example of targeting non-oncogene addiction.[2]



CCT245287
HSF1 pathway assay IC_{50} = 2.0 µM

CCT250006
HSF1 pathway assay IC_{50} = 15 nM
CDK9 IC_{50} = 3 nM

Figure 1. Development of **CCT25006**

HSF1, as a ligandless transcription factor, is unlikely to be amenable to direct antagonism with small molecules, so we carried out a high-throughput unbiased cell-based phenotypic screen in U2OS human osteosarcoma cells to discover inhibitors of HSF1-mediated transcriptional activity. Two hit series were identified, representing distinct chemotypes, and were selected for further optimization. The first series, a 4,6-disubstituted pyrimidine, was confirmed and optimized using cellular SAR leading to a 130-fold improvement in HSF1-transcription inhibition, CCT25006 (IC_{50}=15 nM). These analogues were also shown to be potent inhibitors of CDK9, a kinase previously demonstrated to be important in general transcription regulation through the phosphorylation of RNAPII. Several CDK9 inhibitors currently under clinical investigation, such as Dinaciclib, were then screened and were also shown to be potent inhibitors of HSF1-mediated transcription. CCT25006 is a useful addition as a structurally distinct chemical tool to study the role of CDK9 and HSF1 transcription inhibition.[3] The optimization of the second series is currently ongoing.

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ANTI-TUMOR ACTIVITIES IN VITRO AND IN VIVO OF A NEW DRUG CANDIDATE 7-METHOXY-4-(2-METHYLQUINAZOLIN-4-YL)-3,4-DIHYDROQUINOXALIN-2(1H)-ONE (XLWX-18B) AND ITS RELATED DERIVATIVES

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In our prior studies on novel antitumor agents targeting the tubulin,[1-4] a potential new drug candidate, 7-methoxy-4-(2-methylquinazolin-4-yl)-3,4-dihydroquinoxalin-2(1H)-one (**1**, XLWX-18B, Figure 1), was discovered with high potency in vitro, a new chemo-type scaffold, simple synthesis, and desirable aqueous solubility and metabolic stability. In a continued study, compound **1** was further tested in a cellular panel of 60 human tumor cell lines by NIH-NCI and exhibited extremely high antiproliferative activity with low to sub-nanomolar GI_{50} values (at 10^{-10} M level). Meanwhile, its antitumor activity in vivo was evaluated in nude mouse BGC-823, H460, and S180 xenograft models, by intravenous (i.v.) injection at a dose of 1 mg/kg every 5 days. Compound **1** displayed significantly strong antitumor activity in vivo, suppressing corresponding tumor growth by 77%, 62%, and 73%, respectively, comparable to paclitaxel (at a 15 mg/kg dose) in the same assays. All mice treated with **1** were alive (8-10/group) and no obvious signs of toxicity were observed at the treatment dose and schedule; some of the mice even had increased body weight. Current data indicate that, based on its strong antitumor activity in vitro and in vivo on a well-tolerated dose schedule, compound **1** is a valuable potential new anticancer drug candidate for further development. Meanwhile, structural optimizations on **1** have provided new active derivatives series **2** and **3** as shown in Figure 1.

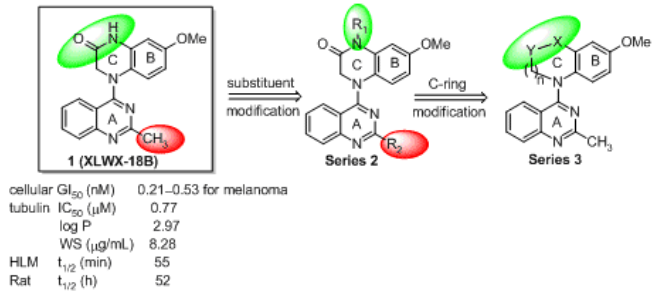

Figure 1. New drug candidate **1** and its derivatives

Acknowledgments. This investigation was supported by grants 81120108022 from NSFC awarded to L. Xie and US NIH grant CA177584-01 from NCI awarded to K. H. Lee.

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SELENOPHENE: A NEW CORE STRUCTURE FOR SUBTYPE-SELECTIVE ESTROGEN RECEPTOR LIGANDS

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Selenium is known as an important trace element involved in different physiological functions of the human body. Recently, research and clinical studies involving animal models gradually support the protective role of selenium against various types of cancer.[1]

Estrogen receptor (ER) is regarded as important pharmaceutical target for the treatment of breast cancer, and development of ER ligands has emerged as active study field in fight against breast cancer. Many of these ligands often having mixed agonist-antagonist and tissue-selective activities, some of these have been termed selective estrogen receptor modulators (SERMs).[2]

In the development of both SERMs and subtype-selective ligands, extensive investigation has been conducted to non-steroidal compounds having heterocyclic cores. As part of our ongoing interest in the development of ER ligands with different core structures.[3] Recently we developed a series of novel ER ligands based on a thiophene core, and noted, most of the 2,5- and 2,4-bis(hydroxyphenyl)-thiophenes were ERβ selective, whereas the bulkier 2,3,5-tris(hydroxyphenyl)-thiophenes were ERα selective.[3d] Intriguingly, some of the 2,5- and 2,4-diarylthiophenes show distinct superagonist activity in reporter gene assays, giving maximal activities 2-3 times stronger than that of estradiol.

For further investigation of the structure diversity of heterocycles for estrogen receptor, we reported herein the selenium-containing heterocycles for the first time as estrogen receptor ligands. Careful SAR analysis of their ER binding affinity output showed that most of selenophenes are ERβ-selective. In transcription assays, these selenophenes largely exhibit partial or full ERβ agonist activity, whereas these ligands display a wide range of ERα activity, including antagonist and agonist. Very interestingly, compared with the approved anti-breast cancer drug 4-hydroxytamoxifen, several compounds exhibited superior antitumor potency in breast cancer MCF-7 cell lines. On further examination, we found some compounds showed significant antiproliferative effects on ER (-) MDA-MB-231 cells. The most promising compound of this study has the highest binding affinity for ERβ and its antitumor potency in breast cancer MCF-7 cells is more potent than 4OHT; moreover, this compound has nontoxic to health VERO cells. These new ligands could act as scaffolds for the development of novel agents to improve therapeutics that target the estrogen receptor.

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NOTES

EFMC
International Symposium
on Medicinal Chemistry
Manchester, UK Aug. 28 – Sept.1, 2016

POSTERS

Emerging Topics

BUILDING A DIVERSE AND EXPERIMENTALLY CURATED FRAGMENT LIBRARY

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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**. 1H 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'**.

DEVELOPMENT OF BENZOTHIAZOLES AS DUAL 5-LIPOXYGENASE AND MICROSOMAL PROSTAGLANDIN E2 SYNTHASE-1 INHIBITORS

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Prostaglandins (PGs) and leukotrienes (LTs) are powerful bioactive lipid mediators that have a large number of biological actions in the human body [1, 2]. The common precursor of PGs and LTs is arachidonic acid (AA). The 5-lipoxygenase (5-LO) and the microsomal prostaglandin E_2 synthase-1 (mPGES-1) are both enzymes within the arachidonic acid cascade. 5-LO is the initial enzyme which catalyzes the conversion of AA to the corresponding LTs; whereas the mPGES-1 is responsible for the transformation of PGH_2 into PGE_2 which is one of the most prominent mediators of inflammation, pain and fever. A valuable pharmacological approach for anti-inflammatory therapy is the dual inhibition of 5-LO and mPGES-1. In contrast to the traditional NSAIDs the dual inhibition of PGs and LTs might be superior over single interference with PGs in terms of anti-inflammatory effectiveness as well as regarding reduced side effects [3]. In the post area of selective COX-2 inhibitors different approaches for dual inhibition of PGs and LTs have been pursued, like dual COX/LO, dual COX-2/LTA_4-Hydrolase or dual 5-LO/mPGES-1 inhibitors [4], [5], [6], [7]. Within the dual 5-LO/mPGES-1 inhibitors the pirinixic acid derivatives are the most advanced one. However pirinixic acid derivatives are well known compounds with many various biological activities especially PPARα and PPARγ activation [8]. Therefore, in this series we replaced the central scaffold of the pirinixic acid, the chlorinated pyrimidine core, by a benzothiazole, which was identified by a virtual screening approach [9].

Here we present the synthesis and in vitro pharmacological characterization of the benzothiazole derivatives and we were able to identify compounds, which are about equally potent to the most potent pirinixic acid derivatives.

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ZINC FINGERS IN DRUG DISCOVERY: IN SILICO IDENTIFICATION OF KEY INTERACTIONS BETWEEN DOF-ZFS AND COGNATE DNA

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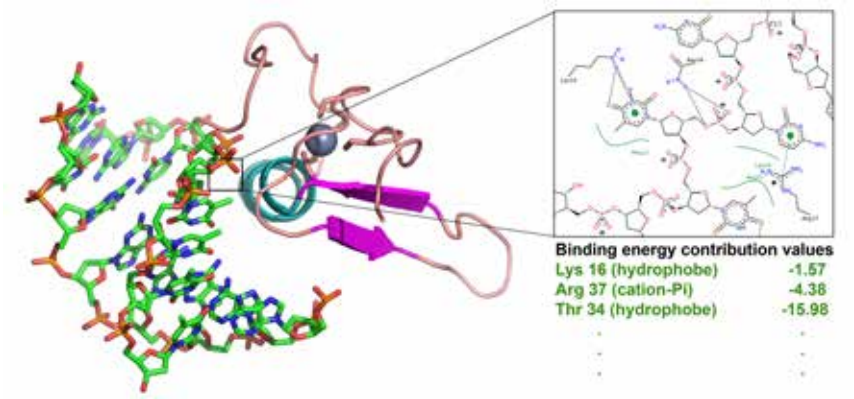
Zinc-finger proteins (ZFs) designed to recognize specific DNA sequences are powerful tools with many potential uses in drug discovery and therapeutics. DOF (DNA-binding with one finger) proteins, a family of DNA-binding transcription factors, are members of zinc fingers unique to plants. Until now, there is no report of experimentally solved structure for DOF proteins, making empirical investigation of DOF-DNA interaction more challenging. The current advances in comparative modelling and the availability of refined molecular mechanics force fields allow feasible prediction of interaction energies for macromolecular complexes.

Therefore, the approaches considered in this work were to model the 3D structures of DOF zinc fingers (ZFs) from Arabidopsis thaliana in complex with DNA molecule, (*i*) to calculate their binding energies, (*ii*) to identify key interactions established between ZFs and DNA, and (*iii*) to determine the impact of the different interactions on the binding energies (Figure 1).



The theoretical binding energies were correlated to the presence of the identified interactions and the contribution of each interaction type was quantified to provide structure-activity relationship (SAR) rules for the DOF-ZF–DNA interaction. The results of such SAR analyses can be used to predict the binding free energies of the hypothetical DOF-ZFs for the purpose of designing novel DNA binding ZFs with variety of therapeutical applications.

FRAGMENT-BASED DESIGN WITH VISUAL AFFINITY AND ADME GUIDANCE

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Fragment-based design, overall, is considered a success story - and it is being pursued by commercial and academic institutions. The key challenges to have emerged from publications in recent years have referred to synthetic access and relevance to intellectual property (IP), finding a "good" link to merge two or more fragments and to target and meet the "relevant properties" of the project. A "good" link is considered a fragment that connects initial fragments in such a way that their binding mode is not distorted; "relevant properties" are primarily "affinity", but these certainly also refer to Absorption, Distribution, Metabolism and Excretion (ADME) related properties such as solubility, or blood-brain-barrier penetration.

Extremely fast algorithms such as indexing techniques for the hit finding procedure [1], atom-based affinity visualizations [2] that are computed on-the-fly and that take solvent effects into account, and Optibrium's integrated ADME property modeling [3] integrated in the software SeeSAR[4], now provide technologies to support *in silico* fragment-based design in a true multi-parametric optimization context.

We will outline the procedures and demonstrate the practicability and relevance using several structure-based examples.

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EXPLORATION OF THE CHEMICAL SPACE AROUND C(5) POSITION OF THE BENZIMIDAZOLE NUCLEUS IN BRP-7 TOWARDS MORE POTENT INHIBITORS OF HUMAN 5-LIPOXYGENASE-ACTIVATING PROTEIN (FLAP)

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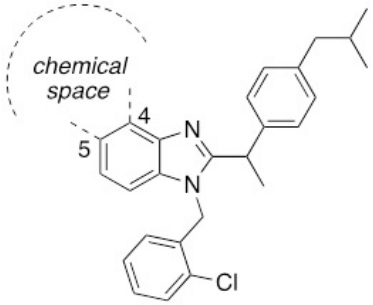
Leukotrienes (LTs) are pro-inflammatory mediators playing pivotal roles in progression inflammatory diseases including asthma, allergy, cancer and atherosclerosis. LTs are produced by 5-lipoxygenase (5-LO) pathway from arachidonic acid (AA) by the key enzyme 5-LO with concomitant involvement of 5-LO activating protein (FLAP). In the first step of LT biosynthesis, 5-LO catalyzes the production of the unstable epoxide LTA_4 from AA, which is further metabolized to LTB_4 or cysteinyl LTs (cys-LTs) such as LTC_4, D_4 and E_4. This first step also requires the involvement of the 5-LO-activating protein (FLAP), which acts as a regulatory protein by interaction with 5-LO for the transfer of AA to 5-LO for efficient metabolism. FLAP inhibitors such as MK-886, MK-0591, BAY-X1005 and AM803 (GSK2190915) showed promising clinical profiles in small or large clinical studies for several indications as a result of inhibition of LT formation. However, none of those compounds reached the market yet. Inspired by the therapeutic potential of FLAP inhibitors, we recently identified BRP-7 [1-(2-chlorobenzyl)-2-(1-(4-isobutylphenyl)ethyl)-1*H*-benzimidazole] as a LT synthesis inhibitor targeting FLAP in intact neutrophils, without direct effects on 5-LO. Here, we show that introducing substituents to explore the chemical space around C(5)-position of benzimidazole was quite beneficial for obtaining very potent BRP-7 analogues, which are able to potently inhibit LT biosynthesis in intact neutrophils. Therefore, our data reveal the potential of additional polar interactions at this position for development of more potent FLAP inhibitors (This study was supported by TUBITAK Research Grant 112S596).



BRP-7

CHEMICAL TUNING OF ANTHRANILAMIDES TOWARDS SELECTIVE PPARδ AGONISM

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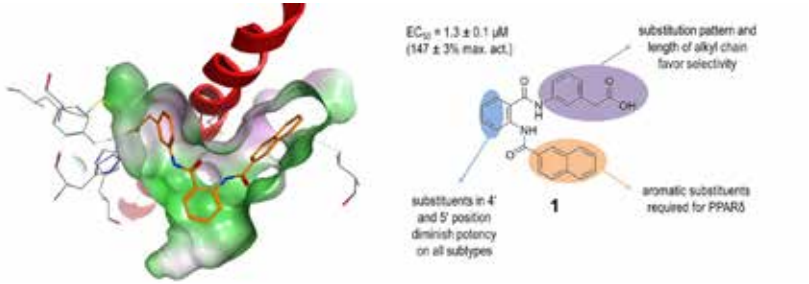

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor family that function as ligand-activated transcription factors.[1] PPAR activation by endogenous ligands - fatty acids and eicosanoids - leads to the expression of various genes involved in proliferation of liver peroxisomes, metabolic regulation of lipid and glucose homeostasis, as well as inflammation.[2–5]

In mammals, three subtypes have been identified which differ in expression and physiological function. Whereas PPARα and PPARγ agonists have been extensively studied because of hypolipidemic and antidiabetic properties, the physiological role of PPARδ (also referred to as PPARβ) remained unknown for a long time. By now, it has been figured out that PPARδ is ubiquitously expressed and plays a pivotal role in fatty acid oxidation in key metabolic tissues such as skeletal muscle.[6] Besides, PPARδ activation exhibits anti-inflammatory effects and hence gained interest as therapeutic target. However, in contrast to PPARα and PPARγ, no PPARδ ligand has been approved as drug so far. Although first clinical trials with PPARδ agonist GW501516 demonstrated promising results such as decreased plasma triglyceride levels, elevated HDL levels, and enhanced insulin sensitivity in obese patients,[7,8] GW501516 promoted the growth of intestinal adenomas.[9]

In initial studies, we have already shown that anthranilamides are promising candidates to overcome the need for selective PPARδ agonists.[10] In the process, compound **1** proved to be selective over PPARα and PPARγ, showing a low micromolar EC_{50} value on PPARδ. Starting from computational docking of **1** into the PPARδ ligand binding domain (LBD), we investigated the acidic head group, substitution of the aromatic moieties, as well as introduction of heteroaromatic systems to exploit the interaction between the ligand and the binding pocket. In this structure-activity relationship (SAR) study, we chemically optimized the potency of anthranilamide **1** in several cycles to come up with a selective, nanomolar PPARδ agonist, which was tested in a PPAR-Gal4 transactivation assay for each subtype. Further research including *in vivo* investigations will reveal whether this compound class is suited as novel strategy for treatment of metabolic syndrome.

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DESIGN AND SYNTHESIS OF OPIOID-FLUOROPHORE CONJUGATES FOR BIOIMAGING

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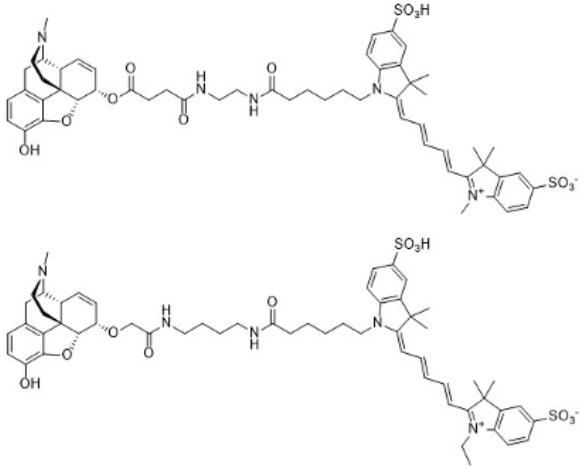
Bioimaging using fluorescent probes has become an important tool in the study of G protein-coupled receptors, allowing for real-time tracking of receptor translocation and recycling. Fluorescent probes have been previously used to characterize other receptor systems, such as the adrenergic and purinergic systems [1–3]. We are keen on applying this technology to the opioid receptor systems. Small molecule fluorescent probes for the opioid receptors have been previously described [4,5], and we wish to continue the development of these probes using clinically relevant ligands and alternative fluorescent systems.

We have synthesized fluorescent opiate agonists that may be used to study the opioid receptors. Morphine was used as the initial targeting ligand, and was synthetically modified to allow for conjugation to the desired fluorophore. Two linking methods were selected and full morphine-fluorophore conjugates synthesized, consisting of the targeting ligand morphine, an appropriately sized linker, and Cy5 as the fluorophore.

In addition to this, we have created small molecule targeted nanoparticles, also to be used as bioimaging agents. Nanoparticles have gained interest as an alternative to organic fluorophores, as single particles may be visualized, giving higher resolution data [6,7]. For this work, gold nanorods were selected. We have created protocols that allow for functionalization of these nanoparticles using a discrete monomeric biocompatible surface coating in addition to a morphine congener. Preliminary data suggests successful coating of these particles, and their maintained ability to activate the μ-opioid receptor.

This work will assist in elucidating the underlying mechanisms behind opioid tolerance and dependence, allowing for the design of the next generation of analgesics lacking these side effects.



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TO DESIGN AND DEVELOP CHEMISTRY TO MAKE NOVEL HIGH QUALITY FRAGMENTS

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Fragment-based drug discovery is an established powerful paradigm to deliver clinical candidates, using small molecules as start points that are structurally simple and typically bind with low affinity, but high ligand efficiency.[1,2] Once a fragment hit is identified by using biophysical techniques, it is optimized in order to improve binding affinity, while retaining drug-like physicochemical properties.[3]

The aim of this project is to exploit the concept of chemistry efficiency by developing a range of chemistries that can be carried out on a common intermediate. Moreover, attention is focus on generating bicyclic systems, made up of unsaturated heterocycles fused to saturated cycles (increase sp^3 features), since several studies have highlighted the importance of increasing the proportion of sp^3 atoms/saturated rings, to improve developability parameters including solubility, selectivity etc.[4]

For this purpose, we have synthesized high-quality fragments by employing two distinct strategies: reagent-based DOS (Diversity Oriented Synthesis) and substrate-based DOS (Figure 1).[5]

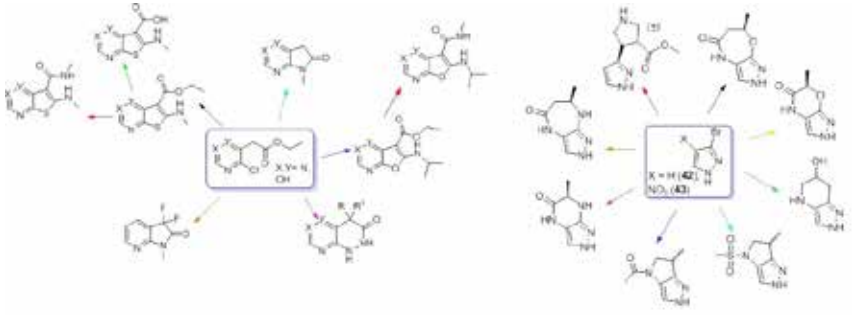

Figure 1. Reaction maps displaying how diverse scaffolds were generated.

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CONFORMATIONAL DESIGN – WHY SHAPES OF MOLECULES MATTER

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The principal focus in drug design is the optimisation of the pharmacophore while improving or maintaining drug-like properties. Synonymous with pharmacophore optimisation is the improvement of affinity and potency for the primary target in parallel with increasing selectivity over secondary and anti-targets. To achieve this, medicinal chemists apply well-documented workflows and principles that concentrate mostly on the systematic, rational and iterative modification of pharmacophoric groups. To maximise the affinity contribution of each pharmacophoric or molecular interaction group, two principal conditions have to be met. In the first place, well-matched properties of the pharmacophoric group with its interaction site are important. Secondly, and in many cases more significantly, the geometric disposition of the pharmacophoric groups with their complementary groups on the binding partner need to match precisely to meet the geometric and distance dependencies of electrostatic and hydrogen bond based molecular interactions in particular. Consequently, medicinal chemistry should give substantial attention to the rational control of the geometric disposition of pharmacophoric features (by conformational design).[1]

The ability to use conformational design in this regard is dependent on conformational analysis and measurement techniques, which experimentally comprises solution NMR and X-ray crystallography. These methods allow both the specific and general conformational preferences of individual groups to be measured, understood and applied to medicinal chemistry geometric design questions. Combined with theoretical considerations, these data are being used in the development of a framework for conformational design, with guidelines and principles that medicinal chemists can apply in the optimisation of the geometric disposition of pharmacophoric features during drug development.

Here we will present a framework, language and principles for conformational design and illustrate its application with examples that highlight the importance of the control of molecular shape in drug discovery.

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QUANTIFICATION OF FREE LIGAND CONFORMATIONAL PREFERENCES BY NMR AND THEIR RELATIONSHIP TO THE BIOACTIVE CONFORMATION

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Accurate, experimentally determined solution 3D-structures of free ligands provide unique opportunities and understanding for drug design, especially in enabling the purposeful manipulation of molecular shape by conformational design. Previous experimental methods to derive solution 3D-structures have had neither the accuracy, generality nor resolution needed for drug design and therefore the information contained in the free ligand's preferred conformational envelope has been unavailable to the medicinal chemist. Here, we describe a novel NMR methodology for accurately measuring the solution conformational preferences of free ligands that is based upon conventional small molecule NMR techniques and can be performed in physiologically-relevant solvents. The method fits experimental data against a dynamic model during refinement, with an order of magnitude more experimental data being correctly predicted than previous NMR approaches. Importantly, there is no reliance upon force-fields or molecular dynamics simulations.

The method will be exemplified by a discussion of the solution conformational envelopes of the aminoglycoside streptomycin[1], the ACE inhibitor lisinopril and the insomnia drug suvorexant (Belsomra®). Comparison with the target-bound (bioactive) conformation of each of these molecules gives important and fresh insight for the practicing medicinal chemist. Accurate free ligand solution conformations can, in many cases, provide a subsidiary route to the bioactive conformation when co-complex crystallographic data is unavailable and is moreover powerfully synergistic when co-complex crystal data is available.

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DESIGN, VIRTUAL SCREENING, AND SYNTHESIS OF TOLL-LIKE RECEPTOR 7 MODULATORS

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Toll-like receptors (TLRs) are pattern-recognition receptors involved in the host cell recognition and initiation of immune responses against microorganisms.[1] Among 12 functional TLRs identified in vertebrates, TLR7 is considered as an emerging therapeutic target for the treatment of viral infections and autoimmune diseases.[2] To search for potential novel ligands of TLR7, small-molecule TLR7 agonist imiquimod[3] and its analog were used as query compounds in three-dimensional similarity-based virtual screening using the Rapid Overlay of Chemical Structures (ROCS) software.[4] Six new compounds with three new chemical scaffolds were discovered as initial hit antagonists of TLR7, with IC_{50} values in the micromolar range, as determined by reporter assays.[4] Additionally, simple and straightforward synthetic pathway for synthesis of analogues with chromeno[3,4-*d*]imidazol-4(1*H*)-one scaffold was developed and optimised.[4] Sixteen novel chromeno[3,4-*d*]imidazol-4(1*H*)-one derivatives were synthesized and evaluated for TLR7 agonist and antagonist activities in a reporter assay based on colorimetric determination of alkaline phosphatase in the hTLR7-HEK239 cell-culture supernatants, which is released by activated cells. The IC_{50} values determined were in the low micromolar range, and the most potent agonist showed similar potency as imiquimod with an IC_{50} value of 1.8 μM.[4,5] Furthermore, we obtained useful information about the structure-activity relationships of chromeno[3,4-*d*]imidazol-4(1*H*)-one agonists, which represent an important starting point for further studies of small-molecule agents that target the Toll-like receptors.

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AMINOADAMANTANES BOUND TO INFLUENZA WT AND S31N M2TM - EVIDENCE FOR A WEAK BINDING - NO BLOCKING INHIBITION OF PROTON CONDUCTANCE OF S31N M2 PROTEIN

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Unrestrained 80-ns molecular dynamics (MD) simulations showed that amantadine analogues have a strong, specific orientation with the amine turned inward (C-end) towards the central cavity in the S31 M2 transmembrane (M2TM) pore in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayers but have variable orientation and a propensity to remain outward (N-end) pointing in N31 M2TM. The mobility of the drugs inside the N31 M2TM suggests weak binding and the experimental binding constants determined using Isothermal Titration Calorimetry (ITC) fully account for less stable complexes compared to S31 M2TM pore. The S31N-aminoadamantane ligand complex stability was tested through synthesis of new ligands with slight increased size leading to a bit higher affinity which was nevertheless lower than 5 μM. The ITC data suggest an entropy driven binding to N31 M2TM in agreement with the variable orientation compared to the enthalpy driven binding to S31 M2TM. Solid state NMR data revealed interaction of aminoadamantane drugs with both S31 and N31 M2TM but chemical shift changes are bigger and linewidths are narrower for S31 M2TM where binding is stronger and complex is more stable respectively in agreement with the ITC data. Electrophysiology experiments show that aminoadamantane variants are able to block only S31 M2 protein. Overall the data suggest a weaker binding of aminoadamantanes to the N31 M2TM compared to the S31 M2TM resulting to insufficient blockage of the proton currents of N31 M2 protein.

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POTENTIATOR DRUGS FOR BAD BUGS - HAMAMELITANNIN ANALOGUES POTENTIATE ANTIBIOTICS IN THE FIGHT AGAINST STAPHYLOCOCCUS AUREUS

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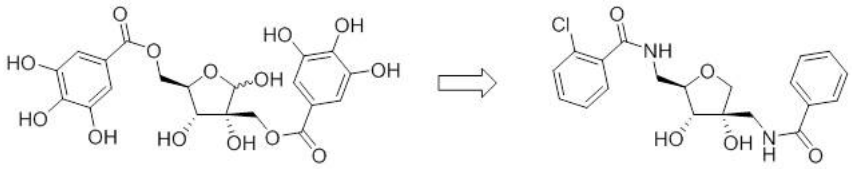
Antimicrobial resistance is a global public health challenge and the development of new antibiotics is scarce. The inherent property of conventional antibiotics to impose selective pressure on bacteria, together with their misuse and overuse, contributed to the development of multi-resistant pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of these 'superbugs'. In both healthcare and community settings, MRSA is a major cause of infections worldwide.[1] In addition to this, staphylococcal pathogens are a frequent cause of biofilm-associated infections.[2] Sessile cells within a biofilm are more resistant to several kinds of stress (e.g. antibiotics, immune system) compared to planktonic cells.

Hamamelitannin (HAM), a natural product isolated from the American witch hazel (*Hamamelis virginiana*) was recently identified as an antimicrobial potentiator.[3,4] It increases the susceptibility of *S. aureus* towards a wide range of antibiotics by affecting peptidoglycan thickness and eDNA release through the quorum sensing receptor TraP.[5]

However, the stability and activity of this natural molecule are not optimal. Therefore, we wanted to investigate the structure activity relationship of HAM in order to identify derivatives which are more active and metabolically more stable. Our work resulted in the identification of a metabolically stable compound (**1**) with potent *in vitro* activity and exceptional activity in a *Caenorhabditis elegans* infection model and a murine mastitis model, while lacking cytotoxicity against MRC-5 lung fibroblast cells.[6]



HAM
cLogP: -0.47
EC_{50}: 145.5 µM

1
cLogP: 2.26
EC_{50}: 0.3890 µM

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DESIGN OF SP3-ENRICHED FRAGMENT LIBRARY

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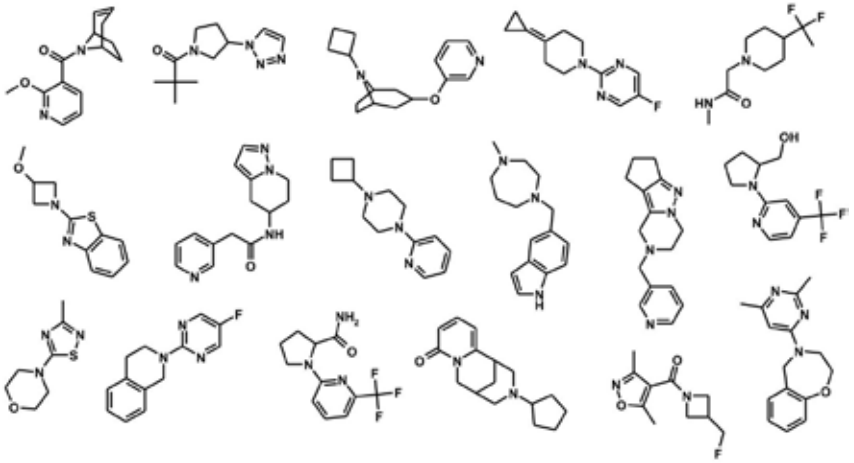
Fragment-based drug discovery (FBDD) is an important approach to design of potential lead compounds, which has started to deliver first approved drugs recently. Many companies have their own FBDD programs, and many vendors propose their fragment libraries on market. However, most of the commercial fragment libraries are obtained by filtering or extension of the already available collections, which leads to uneven coverage of the chemical space by these compounds. In this presentation, we propose the database of 8000 compounds which is specially designed for fragment-based drug discovery. The database is generated by virtual coupling of carefully selected set of building blocks, which define the following features of the resulting library:

- a cornerstone of the design is high quality of the fragments which is defined by rigorous control of Phys-Chem properties (Ro3 extension), as well as a number of structural filters applied (incl. PAINS filters);
- a key feature of the library is high fraction of sp^3 carbons in the compounds (average Fsp^3 is 0.58, whereas most commercial libraries have average Fsp^3 0.3–0.4);
- the diversity of the library is controlled by a number of methods (Tanimoto from chemical FPs; clustering by pharmacophoric FPs; scaffold analysis);
- novelty of the library is ensured by selection of reaction partners (at least one of the building blocks used for coupling has limited availability on market).

Currently, synthesis of the fragment library is in progress; 500 compounds have been already prepared.



SYNTHESIS OF NOVEL PYRAZOLE-DERIVED BISPHOSPHONATES AND THEIR BIOLOGICAL EVALUATION IN VITRO AND IN RATS

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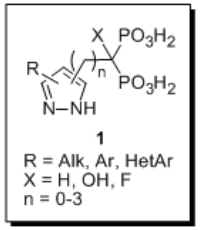
Bisphosphonates (BPs) are synthetic analogues of inorganic pyrophosphate. They are widely used for therapy of numerous metabolic disorders such as postmenopausal and glucocorticoid-induced osteoporosis, Paget's disease, bone metastasis in cancer patients, hypercalcemia, hereditary skeletal disorders in children, etc. BPs have their therapeutic effect due to their ability to inhibit osteoclast activity and decrease hydroxyapatite degradation. After getting into osteoclasts, BPs inhibit the synthesis of some key metabolic enzymes, promote structural changes in cytoskeleton, and decrease the concentration of protein markers for resorption of bone tissue and calcium in blood serum. Since introducing nitrogen atoms into the molecules of BPs increases their antiresorptive effect significantly, in this work, we have designed and synthesized a library of novel pyrazole-derived BPs, which were evaluated *in vitro*.

The first step of the library design included virtual generation of BPs of general formula **1**. These compounds were studied *in silico* as inhibitors of pharnesylpyrophosphate synthase by docking, using zolendronic acid as a reference structure. Five pyrazole-derived BPs (I-12, I-40, I-42, ISP-30, and ISP-50) were selected and synthesized for biological evaluation. *In vitro* studies showed that compounds I-12, I-40 and ISP-30 showed milimolar-range proapoptotic activity against RAW264.7 cells. Of these, bisphosphonate I-12 had the highest antiresorptive efficiency and the ability to regulate nutritional mineral under condition of alimentary osteoporosis in rats.



1
R = Alk, Ar, HetAr
X = H, OH, F
n = 0-3

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DISCOVERY OF SMALL MOLECULE UTROPHIN MODULATORS FOR THE THERAPY OF DUCHENNE MUSCULAR DYSTROPHY (DMD)

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Duchenne Muscular Dystrophy (DMD) is a devastating, X-linked 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. By transcriptionally reprogramming the temporal and spatial expression of the dystrophin-related protein utrophin, we aim to develop a pharmacological therapy applicable to all DMD patients by targeting the primary defect and restoring sarcolemmal stability. In partnership with Summit Therapeutics, the 2-aryl benzoxazole utrophin modulator ezutromid (formerly SMT C1100),[1] which demonstrates reduced dystrophic symptoms in the *mdx* mouse, has progressed to human clinical trials. As a potential First-In-Class molecule, ezutromid, following successful Phase 1b evaluation,[2] is about to start open label Phase 2 trials in DMD patients.

The successful clinical progression to date of ezutromid provides crucial proof-of-concept for the strategy which is being undertaken, and a comprehensive pipeline of future generation utrophin modulators is being developed. A series of Second Generation Utrophin Modulators which are structurally related to ezutromid, but with improved physicochemical and metabolism profiles have also been evaluated in the *mdx* mouse, and results were published recently.[3] In parallel, novel utrophin modulator chemotypes have been discovered using an alternative *in vitro* dystrophin null myoblast screening assay where the reporter gene has been directly knocked into a utrophin exon. Multiple new structural classes which significantly increase utrophin expression in both murine and human DMD myoblasts have been identified and are now being optimised. Importantly, initial evidence suggests that some of these small molecules modulate utrophin transcription through an alternative regulatory mechanism to ezutromid. These new compounds exhibit favourable solubility, stability, oral absorption and are well tolerated in the *mdx* mouse. Structure-activity studies are underway, with the objective to improve compound effectiveness and exposure. This poster will summarise progress made on the Utrophin Modulator drug pipeline.

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DISCOVERY OF POTENT AND ORALLY AVAILABLE FRUCTOSE 1,6-BISPHOSPHATASE INHIBITORS AS NOVEL ANTI-DIABETIC AGENTS

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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, a representative candidate BJB-2936 was evaluated extensively in terms of pharmacodynamic and pharmacokinetic properties. Long-term administration of BJB 2936 to diabetic animal models (KKA^{y} mice and db/db mice) resulted in significant glucose lowering and HbA1c reduction. Glucose lowering was linked to inhibition of gluconeogenesis and endogenous glucose production for BJB2936. In fact, the FBPase activity of liver in mice was inhibited by 39% and 96%, respectively, at oral doses of 50 mg/kg and 200 mg/kg. The pharmacokinetic parameters of BJB2936 in rats were also investigated. It has been demonstrated that BJB2936 was orally available and exhibited high plasma concentration.

In summary, a new class of structurally distinct FBPase inhibitors with low molecular weight was identified. The pronounced glucose lowering potency and the acceptable pharmacokinetic properties warrant its further development as a novel therapeutic approach for the treatment of type 2 diabetes mellitus.

Acknowledgement

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POSTERS

Innovation in Kinase Drug Discovery

DISCOVERY OF PQR620, A HIGHLY POTENT, SELECTIVE, BRAIN PENETRABLE AND ORALLY ACTIVE MTORC1/2 INHIBITOR

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Various tumors and central nervous system (CNS) disorders share aberrant activation of the mTOR pathway. Drugs targeting the mTOR pathway which plays a fundamental role in cell growth and proliferation, represent therefore a valuable path to address multiple therapeutic areas.

The development of specific mTOR inhibitors is challenging due of extensive conservation of the ATP-binding pocket of this particular PI3K-like family. Knowledge from empirical structural-activity relationships investigations on morpholino-triazinyl derivatives was combined with an understanding of the molecular interactions in the X-Ray co-crystal structure of PI103 bound to mTOR (PDB code 4JT6)[1] to improve selectivity and potency. A structure-activity systematic variation of the hinge region and affinity binding motifs including, biological validation assays and preclinical pharmacological assessments leading to the development of PQR620 will be presented.

Here, PQR620 displays excellent selectivity versus PI3K lipid kinases, protein kinases and unrelated targets.

In A2058 melanoma cells PQR620 demonstrated inhibition of protein kinase B (pS473) and ribosomal protein S6 (pSer235/236) phosphorylation with IC_{50} values of 0.2 µM and 0.1 µM, respectively. PQR620 showed anti-proliferative inhibition in a panel of 66 NTRC Oncolines™ cancer cell lines (GI_{50} : 140-4050 nM).

In mice and rats oral application of PQR620 exhibited a dose-proportional PK. Plasma to brain ratio was at least 1 and Cmax was reached after 30 minutes, indicating that PQR620 is brain penetrant. PQR620 potently inhibited mTOR signaling in vivo in tumors, brain and other tissues. Importantly, no effect on plasma insulin levels was observed. The anti-proliferative action of PQR620 translated into in vivo anti-tumor activity in various mouse models with PQR620 being well tolerated (100 mg/kg).

A 14 day GLP toxicological study in rats showed very good tolerability (MTD=30 mg/kg). Only minor toxicities such as dose-related changes in body weight were observed.

A robust 4 step synthetic route to PQR620 was established, which provides rapid access to quantities required for pre-clinical testing. In conclusion, PQR620 inhibits mTOR potently and selectively, and shows anti-tumor effects in vitro and in vivo. PQR620 is currently in pre-clinical development.

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SAR156497, AN EXQUISITELY SELECTIVE INHIBITOR OF AURORA KINASES

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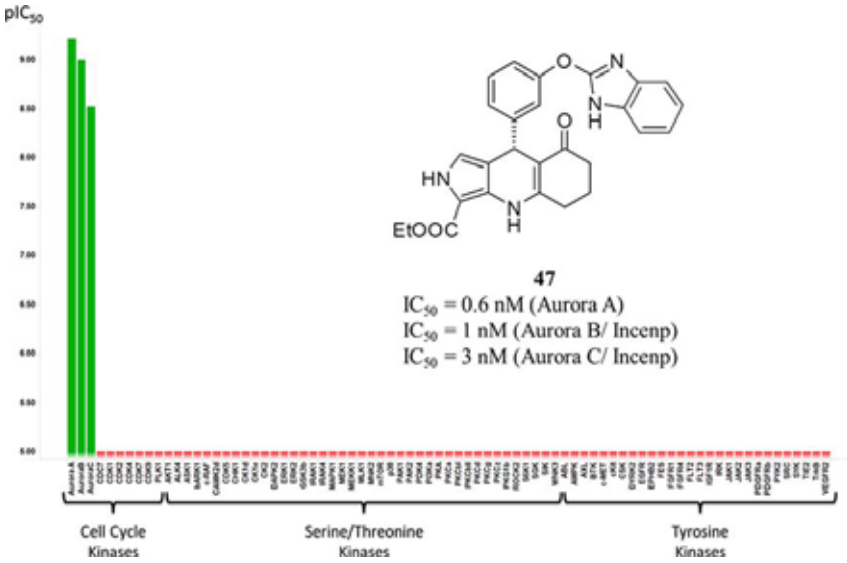

The Aurora family of serine/threonine kinases is essential for mitosis. Their crucial role in cell cycle regulation and aberrant expression in a broad range of malignancies have been demonstrated and have prompted intensive search for small molecule Aurora inhibitors. Indeed, over ten of them have reached the clinic as potential anticancer therapies. We will report the discovery and optimization of a novel series of tricyclic molecules that has led to SAR156497, an exquisitely selective Aurora A, -B and -C inhibitor with *in vitro* and *in vivo* efficacy. We will also provide insights into its mode of binding to its target proteins, which could explain its selectivity.

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N-METHYLPICOLINAMIDE TETHERED BENZOTHIAZOLE: A PROMISING SCAFFOLD FOR TARGETING DIVERSE CANCER RELEVANT PROTEIN KINASES

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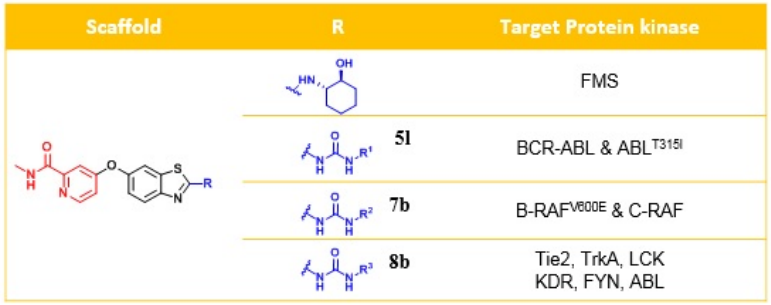
Inspired by the impressive anticancer activity of certain picolinamide and substituted benzothiazole (BTZ) derivatives, new BTZ members bearing *N*-methylpicolinamide motif have been designed and synthesized as potent kinase inhibitors. The first series of target compounds (**A**) was tailored to selectively target both the wild ABL-1 kinase and its gatekeeper mutant ABL^{T315I}. In this series, the ureidobenzothiazole **5l**, with terminal methylpiperazine fragment, proved to be the most potent derivative with two-digit nanomolar IC_{50} values against both native and mutant ABL kinases as well as selective anti-leukemic effect towards K562 cell line. Changing the aliphatic side chain in series (**A**) with different aromatic groups afforded a novel series of compounds (**B**) with broad spectrum anticancer activities. Kinase profiling of the most potent ureidobenzothiazole **7b** (with 3,5-bis-trifluoromethylphenyl group) revealed its selective inhibitory activity towards both B-RAF^{V600E} and C-RAF kinases. On the contrary to the limited kinase activity (ABL and RAF) of the aforementioned mentioned benzothiazoles, Compound **8b**, possessing the hydrophilic ethylpiperazinyl moiety and *m*-trifluoromethylphenyl group, displayed pronounced multikinase inhibitory activity over a number of oncogenic kinases with low nanomolar IC_{50} values. For example, it showed IC_{50} values of 0.8 nM, 3.8 nM and 6.7 nM against Tie2, TrkA and LCK kinases, respectively. Such multikinase activity could justify its broad spectrum anticancer activity over numerous cancer cells, particularly the leukemia K562 (GI_{50} = 51.4 nM) and colorectal carcinoma KM12 (GI_{50} = 19 nM) cell lines. Moreover, profiling of CYP450 inhibitory effects for **8b** demonstrated its low possibility to exhibit undesirable drug–drug interactions. In addition, in vivo PK profiling of **8b** disclosed its reasonable oral bioavailability. Taken together, it could be concluded that fine adjustment of the substituent's nature at C-2 of benzothiazole may modulate its kinase selectivity profile, which may represent a promising approach for targeted cancer therapy.



Keywords: *N*-methylpicolinamide, ABL kinase, B-RAF^{V600E}, C-RAF, Multikinase, Anticancer agents

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DISCOVERY OF MK-8449, A POTENT 2ND GENERATION SYK/ZAP70 INHIBITOR

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We previously described the discovery of MK-8457, our first generation SYK/ZAP70 inhibitor. In this presentation we will highlight the strategy we embarked on towards a 2nd generation clinical candidate. In particular we will highlight the following aspects of our optimization campaign which culminated in the discovery of MK-8449

- Maintained and improved the high kinase selectivity of >99% of all kinases tested having IC50's >100-fold the SYK IC_{50}
- Optimized compound activity in human whole blood
- Explored and enhanced the electrostatic interaction of our inhibitors with the SYK enzyme
- Maximized structural diversity relative to MK-8457
- Improved PK in preclinical species consistent with a predicted lower human dose and $t_{½}$ consistent with a *q.d.* dosing regimen

SYNTHESIS OF CHIRAL SUBSTITUTED PIPERAZINES AS LINKERS FOR MACROCYCLIC KINASE INHIBITORS

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The role of kinases has been widely studied in recent years and many Drug Discovery programs are about finding inhibitors of kinases, mainly in Oncology but also in other therapeutic areas.

Kinase inhibitors display often a heteroaromatic scaffold and one of the major and very common hurdles is finding selectivity against off target kinases.

The strategy we use to improve the selectivity issue is the formation of macrocycles of the heteroaromatic scaffold as a way to reduce the number of possible conformations.

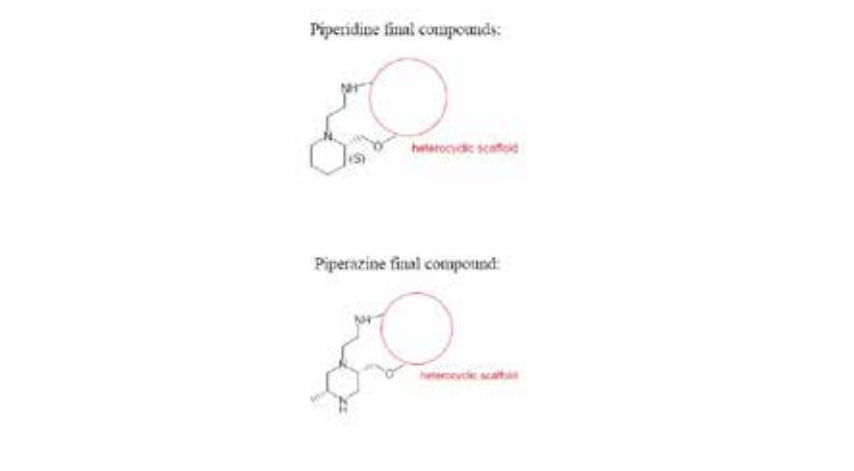
Here we present the synthesis of chiral piperidines and piperazine linkers for these macrocycles. The piperidine and piperazine moiety greatly improves water solubility and the correct stereochemistry proved to be necessary for activity.

Synthesis of piperazines:

This methodology allows access to chiral substituted piperazines with chemical selectivity on either nitrogens of the piperazine ring.

The classical diketopiperazine formation followed by reduction of the amides as a way of synthesising piperazines leads to two amines therefore doesn´t allow selectivity of one nitrogen of the piperazine over the other to grow the linker on.

Selectivity in this synthesis is achieved by first forming an amine then an amide and reaction of the amine before reduction of the amide.



NOVEL QUINAZOLINE BASED PI3K DELTA-SELECTIVE INHIBITORS

Klemens Hoegenauer (1), Nicolas Soldermann (1), Frederic Stauffer (1), Pascal Furet (1), Nadege Graveleau (1), Alexander B. Smith (1), Christina Hebach (1), Gregory J. Hollingworth (1), Ian Lewis (1), Sascha Gutmann (1), Gabriele Rummel (1), Mark Knapp (2), Romain M. Wolf (1), Joachim Blanz (1), Roland Feifel (1), Christoph Burkhart (1), Frederic Zecri (1)

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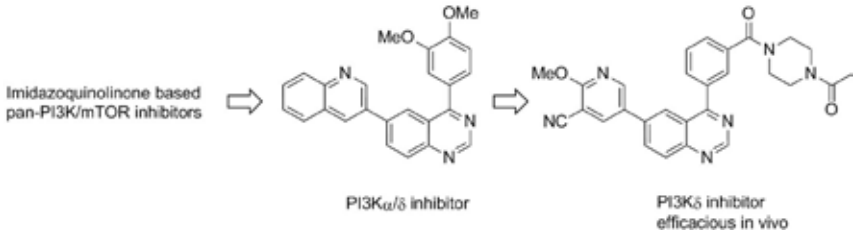
Inhibition of the lipid kinase PI3Kδ is a promising principle to treat B- and T-cell driven inflammatory diseases.[1] The strong biological rationale resulted in a medicinal chemistry program aimed at the discovery of PI3Kδ–selective inhibitors with suitable properties and efficacy to allow for development as an anti-inflammatory therapeutic. Using a scaffold deconstruction–reconstruction strategy starting from pan-PI3K/mTOR imidazoquinolinones based inhibitors,[2] we identified 4-aryl quinazolines that were optimized into potent PI3Kδ isoform selective analogues with good pharmacokinetic properties in rats and dogs. With one compound (right structure in figure), we demonstrate that biochemical PI3Kδ inhibition translates into modulation of isoform dependent immune cell response (human, rat and mouse). After p.o. administration of this compound to laboratory rats, proximal PD markers are inhibited in a concentration-dependent manner. Using a mechanistic plaque forming cell assay,[3] dose-dependent efficacy after oral dosing could be demonstrated.



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DEVELOPMENT OF A NEW CLASS OF ALK2 INHIBITOR FOR THE STUDY OF THE MOST AGGRESSIVE PAEDIATRIC BRAIN CANCER, DIPG

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ALK2 is a transmembrane serine/threonine kinase and member of the TGFβ superfamily of signalling proteins. Mutations in ALK2 are known to cause fibrodysplasia ossifficans progressiva (FOP); an extremely rare condition where patients are completely disabled by the growth of a second skeleton. This often occurs in response to injury, hence surgery accelerates the condition and no other treatments are available. More recently, many of the ALK2 mutants observed in FOP patients have been observed in approximately 25% of diffuse infiltrative pontine glioma (DIPG) cases. DIPG is the most aggressive paediatric brain cancer – representing only 10% of cases but responsible for 80% of deaths. As with FOP surgery is considered impossible due to the infiltrative nature of these cancers and their location in a vital region of the brain. Furthermore there is no beneficial treatment for DIPG (which has a median age of mortality of 9; less than 12 months from diagnosis).

The vast majority of reported ALK2 inhibitors share the same chemotype, and although some show promise in FOP models they are ineffective in DIPG models. Independent chemical tool compounds are needed to study the role of mutant ALK2, primarily in the context of DIPG.

We identified a 6-pyrazolo-quinazalinone fragment from another kinase project as a low micromolar inhibitor of ALK1, which shares 79% identity with ALK2. Screening of this and related quinazalinones strongly suggested the binding mode and confirmed that sub-micromolar inhibitors were possible for ALK2 with excellent ligand efficiencies (L.E. ~0.50). We prepared analogues with a range of central pocket pyrazole replacements, as well as a range of solvent channel groups – leading to inhibitors with low nM IC_{50}s– an increase in potency 390x the initial hit fragment.

Crystallography is ongoing with our collaborators at the SGC to aid the design of more potent inhibitors. Pleasingly, some early compounds are effective in killing DIPG cell lines with mutant ALK2 and have typical dose response curves in the low μM EC_{50} range – which is rarely recapitulated by existing compounds. Additional tests are ongoing.

PYRAZOLO[4,3-D]PYRIMIDINE INHIBITORS OF CYCLIN-DEPENDENT KINASES

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Cyclin-dependent kinases (CDK) are a group of enzymes involved in many cellular processes including regulation of the cell cycle and transcription. Deregulation of the cell cycle connected with CDK hyperactivity is a common feature of tumor cells and provides a rationale for the development of specific CDK inhibitors.

The purine heterocycle became one of the first investigated scaffolds of cyclin-dependent kinase (CDK) inhibitors leading to the early discovery of roscovitine. Roscovitine is a pan-selective CDK inhibitor with multiple effects on cancer cells and was among the first CDK inhibitors that entered clinical trials. Inspired by the success of roscovitine, we have prepared a pyrazolo[4,3-*d*]pyrimidine bioisostere of roscovitine [1] and recently introduced novel class of nanomolar CDK inhibitors, 3,5,7-trisubstituted pyrazolo[4,3-*d*]pyrimidines [2].

To date, several other CDK inhibitors built on heterocycles isosteric to purine have been described [3], but among them, only pyrazolo[1,5-a]pyrimidines, pyrazolo[1,5-a]-1,3,5-triazines, and our pyrazolo[4,3-d]pyrimidines exceed the activity of corresponding purines. Compounds based on the latter group, the pyrazolo[4,3-d]pyrimidines, display nanomolar anti-cancer activity and some derivatives suppress abnormal proliferation related to the pathogenesis of restenosis in vascular smooth muscle cells or tumor angiogenesis

The presence of a N^6-biaryl substituents were proven the most advantageous for the activities of purines compared to monoaryl substituted. Therefore, we newly prepared a library of 5-substituted 3-isopropyl-7-[4-(2-pyridyl)benzyl]amino-1(2)Hpyrazolo[4,3-*d*]pyrimidines, a selective inhibitors of cyclin-dependent kinases displaying nanomolar potency against CDKs and cancer cell lines [4].

Our work is focused on the biological and biochemical characterization of these novel derivatives, using enzymatic cellular assays, immunoblotting and flow cytometry performed with recombinant proteins or human cancer cell lines. Some of the compounds induce apoptosis, as proved by activation of the caspase cascade. In addition, all the compound increases cellular levels of the tumor suppressor protein p53 and some p53-regulated genes. The studied compounds significantly surpass purine bioisosteres in terms of their antiproliferative and anticancer properties and could yield a lead structure for development of a new anticancer therapeutics.

The work was supported by grant from GACR (14-19590S).

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PYRIDYLAMIDE BASED UREIDOBENZOTHIAZOLES: IDENTIFICATION OF NEW RAF KINASE INHIBITORS WITH IMPROVED ANTICANCER ACTIVITY

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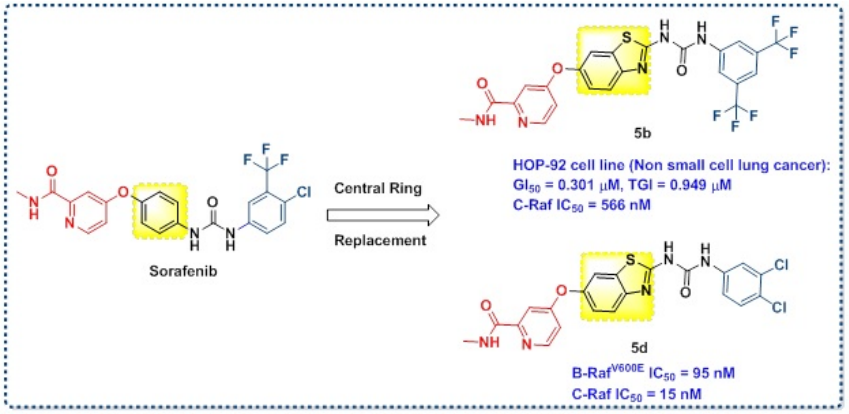
A new series of amido and ureidobenzothiazole derivatives linked with the substantial pyridylamide moiety *via* ether linkage at the 6-position of benzothiazole has been designed and synthesized as potent anticancer sorafenib congeners. A selected array of 12 derivatives was assessed for its antineoplastic activity over a panel of 60 human cancer cell lines at 10 uM at National Cancer Institute (NCI, USA). The amidobenzothiazole **4b** and ureidobenzothiazoles **5a**, **5b** and **5d** exhibited promising growth inhibitions and thus were further tested in 5-dose testing mode to determine their GI_{50} values. The cell based assay results disclosed that 3,5-bis-trifluoromethylphenyl (**5b**) urea member is the best member with superior potency and efficacy compared to sorafenib as well as broad spectrum activity including 57 human cancer cell lines. Kinase profiling of compound **5b** showed its kinase inhibitory activity against both B-Raf^{V600E} and C-Raf. Moreover, the most potent derivatives in cell assay were examined for their RAF inhibitory activities, and the results were justified by means of molecular docking. Profiling of CYP450 and hERG channel inhibitory effects for the active compounds indicated their low possibilities to exhibit undesirable drug–drug interactions and cardiac side effects.



Keywords: Pyridylamide, Benzothiazole, B-RAF^{V600E}, C-RAF kinase, Anticancer activity

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IMIDAZOPYRIDINE DERIVATIVES AS POTENT DUAL Bcr-Abl/Src TYPE II INHIBITORS.

Shinae Kim, Seungyeon Lee, Dayea Kim, So Young Kim, Dong Kyu Choi, Jung Beom Son, Eunhwa Ko, Sun-Hwa Lee, Hwan Geun Choi

DGMIF, New Drug Development Center 80, Cheombok-ro, Dong-gu, Daegu, Korea South

After the launch of imatinib in 2001, targeted therapies based on the kinase inhibitors represent most significant in the cancer research area. The development of kinase inhibitors proceeds both towards selective compounds and dual or multi-targeted inhibitors. The most of dual Abl/Src inhibitors are Type I inhibitors targeting the ATP-binding site in DFG-in conformation. Src family kinases regulates triple-negative/basal-like and metastatic human breast cancer MDA-MB-231 cells and Bcr-Abl is found in 90% patients with chronic myelogenous luekemia(CML). Dual Abl/Src inhibitors are very promising compounds against the treastment of leukemias and solid toumors.

Here we will present the synthesis of a library of novel imidazopyridine type II inbibotors and the structure-activity relationship of these compounds was discussed based on enzymatic and cellular activities. Our result showed that imidazopyridine derivatives potently inhibit not only TNBC cell line MDA-MB-231 but also Bcr_Abl^{T315I}. These imidazopyridines can lead to the development of novel targeted therapy for the breast cancer and chronic myelogenous leukemia (CML).

DISCOVERY OF A POTENT AND SELECTIVE JAK1 INHIBITOR FOR THE TREATMENT OF RHEUMATOID ARTHRITIS (RA)

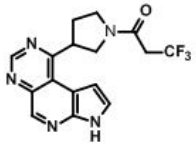
Bheemashankar Kulkarni, Anish Bandyopadhyay, Dinesh Barawkar, Robindro S, Tanushree Bende, Jignesh Doshi, Yogesh Waman, Partha Mukhopadhyay, Rajesh Bonagiri, Dilip Jadhav, Santosh Kumar, Prashant Dalvi, Rushikesh Jadhav, Umesh Singh, Sreekanth Rouduri, Satheesh Avaragolla, Hari Prasad, Manoj Pothuganti, Sujitha Balaji, Dhananjay Umrani, Ahmed Nadeem, Madhu Bala, Abhijeet Bhalerao, Sachin Bhamre, Ravi Bhamidipati, Satyanarayana Reddy, Ashwani Gaur, Narsimha Munagala, Siddhartha De, Avinash Dhanave, Azfar Quraishi, Joshi Vasa, Anita Chugh, Narayanan Hariharan, Kasim Mookhtiar

ADVINUS THERAPEUTICS LTD QUANTUM TOWERS PLOT NO 9 RAJIV GANDHI INFOTECH PARK HINJAWADI PHASE 1 PUNE MAHARASHTRA INDIA 411057

The Janus kinase (JAK) family consists of four cytoplasmic tyrosine kinases, JAK1, JAK2, JAK3 and TYK2 that are critical regulators of signaling by multiple cytokines. JAK1 and JAK3 are key players in inflammatory cytokine signaling and thus are attractive targets for chronic inflammatory diseases such as Rheumatoid Arthritis (RA). Tofacitinib, the first JAK inhibitor approved for RA, has shown robust efficacy in RA.

Selective inhibition of JAK1 may provide robust efficacy as it plays a major role in IL-6 cytokine signaling Selective JAK1 inhibitor GLPG0634 demonstrated efficacy in preclinical inflammatory disease model of RA[1] and also in phase-II clinical trial[2]. Thus different selectivity profile may offer better therapeutic window and opportunity to differentiate from other JAK inhibitors in clinic.

This poster will disclose discovery of a novel, potent JAK1 inhibitor having 200 fold selectivity over JAK3 in biochemical kinase assay. The compound showed >100 fold selectivity towards JAK1 (IL-6 induced STAT1 phosphorylation) and >40 fold selectivity towards JAK3/JAK1 (IL-2 induced STAT5 phosphorylation) over JAK2 (GM-CSF induced STAT5 phosphorylation) respectively. The Compound showed acceptable *in vitro* ADME, PK properties in rodents and dog that translated into robust efficacy in rat model of Adjuvant-induced arthritis (AIA) with excellent PK-PD correlation.



Lead Compound

Kinase assay
JAK1 IC_{50}: 0.005 µM
JAK3 IC_{50}: 1.0 µM
Cell based assay
JAK1 IC_{50}: 0.37 µM
JAK3/JAK1 IC_{50}: 0.94 µM
JAK2 IC_{50}: 38.5 µM

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DISCOVERY OF CCT251921: A POTENT, SELECTIVE AND ORALLY BIOAVAILABLE SMALL MOLECULE MODULATOR OF THE MEDIATOR COMPLEX-ASSOCIATED KINASES CDK8 AND CDK19

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The Mediator complex-associated kinase CDK8 has been implicated in human disease,1 particularly in colorectal cancer where CDK8 has been reported as a putative oncogene.2 We previously reported the discovery of CCT251545, a potent, orally bioavailable small molecule inhibitor of Wnt signalling from a cell-based pathway screen.3 We identified protein kinase paralogs CDK8 and CDK19 as the primary targets of this chemical series.4 Protein X-ray crystallography studies of the chemical probe CCT251545 in complex with CDK8/cyclin C revealed an unusual protein binding conformation invoking a C-terminal loop insertion into the ATP binding site and enabled the design of improved CDK8/19-selective compounds. We optimized the metabolic stability and aqueous solubility of the chemical probe CCT251545 in order to facilitate further *in vivo* evaluation of CDK8/19 pharmacology and progression into preclinical *in vivo* studies. Here we describe the medicinal chemistry optimisation of CCT251545 to CCT251921, a potent, highly selective and orally bioavailable dual CDK8/19 ligand with excellent translation to cell-based activity and improved pharmacokinetic and pharmaceutical properties.5 Demonstration of *in vivo* activity following oral dosing in solid human tumor xenograft model will also be shown. Scaffold hopping and biochemical HTS versus CDK8 led to additional compound series with a Type I binding mode and resulted in structurally differentiated back-up candidate with equivalent pharmacological profile to CCT251921.6-8

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SYNTHESIS, MOLECULAR DOCKING AND BIOLOGICAL ACTIVITY OF DIHYDROPYRANBENZOQUINONES AS CK2 INHIBITORS

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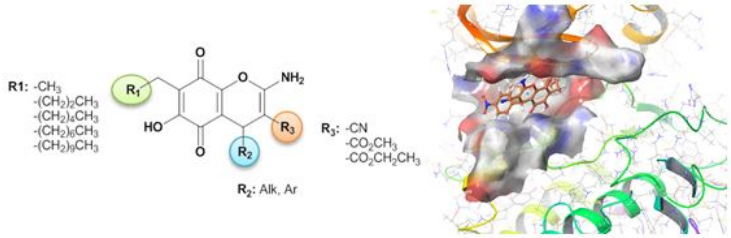
Protein Kinase CK2 is a newly validated therapeutic target which has emerged as an attractive drug discovery target in oncology. [1-2] This kinase, which has been closely related with many humans cancer, has been shown to play a key role in cell cycle control, cellular differentiation and proliferation. After screening of a library of quinonic compounds it was found that 2-amino-5,8-dioxo-4*H*-chromenes are promising inhibitors of CK2.

Herein, we report the synthesis of a set of these compounds following a direct and highly efficient approach based on a multicomponent reaction from different 2-hydroxy-[1,4]-benzoquinones [3], aldehydes and cyano derivatives. Inhibitory activities of CK2 and molecular docking studies are also included.



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TOWARDS THE GOAL OF CURING CML: DISCOVERY OF ABL001 A NOVEL ALLOSTERIC INHIBITOR OF BCR-ABL PREVENTING DISEASE RELAPSE BY DUAL TARGETING

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The discovery of ABL001, the first allosteric selective receptor tyrosine kinase inhibitor in Phase I clinical trial for the treatment of patients with chronic myelogenous leukemia (CML) and a subset of acute lymphoblastic leukemia (ALL) will be reported. Two aspects of this ground breaking discovery have significant impacts on cancer research. Firstly; ABL001 is a potent BCR-ABL inhibitor with a novel, allosteric mechanism of action. In contrast to inhibitors such as imatinib and nilotinib that bind to the ATP-site of the kinase domain ABL001 binds to a distinct allosteric site on the kinase domain. Secondly this presents a unique opportunity to treat patients with Ph+ leukemia using a combination of two potent, mechanistically distinct BCR-ABL inhibitors. Pre-clinical efficacy studies have illustrated the potential of this approach with complete regressions being achieved in animals receiving a ABL001/nilotinib combination with no evidence of disease relapse despite treatment being withdrawn. A similar combination approach in the clinic would be anticipated to provide patients with a deeper and more sustained reduction in tumor burden with a reduced risk of relapse. Achieving such a goal would be an important step towards the next paradigm shift providing a cure for patients with CML.

Low molecular weight compounds previously identified to bind to the myristoyl-pocket of BCR-ABL, failed to progress to clinical candidates. To develop superior starting points for medicinal chemistry we performed fragment-based screens and the resulting hits were optimized using in silico docking, crystallography and NMR studies. We discovered that myristoyl-pocket binders must induce a critical "bend" in the C-terminal helix for the kinase to form the auto-inhibited conformation. Small molecule medicinal chemistry starting points were discovered using a NMR conformation assay confirming that the molecules could bend the helix. Furthermore we will report the structure-based optimization to improve potency, selectivity and in vivo pharmacokinetics, leading to the discovery of ABL001. We will discuss how combinations of ABL001 with the ATP-competitive inhibitor nilotinib, prevents the emergence of drug resistance in vivo. Clinical testing of ABL001 in combination with catalytic-site inhibitors is underway to determine if the pre-clinical observations translate to the clinic and provide potentially curative treatment regimens for patients.

SYNTHESIS AND CHARACTERIZATION OF NEW DGKα INHIBITORS

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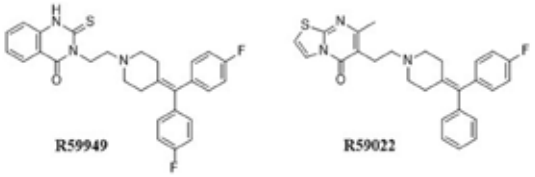
Mutations of the SAP gene (SH2D1A), which encodes for an immune-specific protein adaptor containing an SH2 domain, results in X linked lympho-proliferative disease (XLP), a syndrome associated to Epstein-Barr virus infection and characterized by defective restimulation-induced cell death (RICD) of T cells, Th2 cytokine release and NKT development and function [1].

We previously showed that upon TCR stimulation SAP acts by mediating the negative regulation of diacylglcyerol kinase alpha (DGKα), which, by converting diacylglycerol to phosphatidic acid, determines the spatiotemporal organization of diacylglycerol-mediated signaling and regulates PKCtheta- and RasGRP-mediated activation of NFAT, NF-kB transcription factors [2].

No specific DGKα inhibitor is actually available: the two generic DGK inhibitors available (R59949 [3] and R59022 [4]) are limited by strong binding to serum proteins (R59949) and toxicity for several cell lines (R59022). We intend to develop DGKα inhibitors specific for this isoform, to measure their pharmacokinetics.

This comunication focus on the rationally driven discovery of small organic molecules able to inhibit the α isoform of DGK.



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CDK8 INHIBITORS WITH LONG RESIDENCE TIME EMERGING FROM A RETRO-DESIGN APPROACH: BINDING KINETICS IS KEY

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Upregulation of CDK8 has recently been described for colon cancer, gastric cancer, and melanoma, rendering CDK8 as an attractive target for the development of selective and efficacious anti-cancer drugs.
Based on the recent findings that in contrast to almost all other CDK family members, CDK8 is amenable to a type II inhibition mode, we set out to design selective CDK8 inhibitors pursuing a privileged structure-based approach. The employed privileged structures are tailor-made for disrupting the hydrophobic R spine within the N-terminal lobe of a kinase, thereby leading to an induced-fit mechanism of derived inhibitors that will exhibit a pre-engineered binding kinetic signature. This “Retro-Design” approach allows keeping the molecular complexity of inhibitors at a minimum level since the seed scaffold is targeted towards the deep pocket of the conformationally rearranged binding.
Here we report on the discovery and optimization of a new class of CDK8 inhibitors. Frontrunner compounds exhibit excellent biochemical inhibition data and a high cellular efficacy in a variety of mechanism-of-action models as well as phenotypic models such as inhibition of anchorage-independent cell growth. The front-runner compounds show superior selectivity over a huge panel of kinases when compared to market approved drugs or to competitor CDK8 inhibitors. This selectivity is attributed to the distinct inhibition mechanism which is corroborated by detailed binding kinetic studies which reveal residence times in the range of several hours. Detailed structure-kinetic relationships will be discussed.

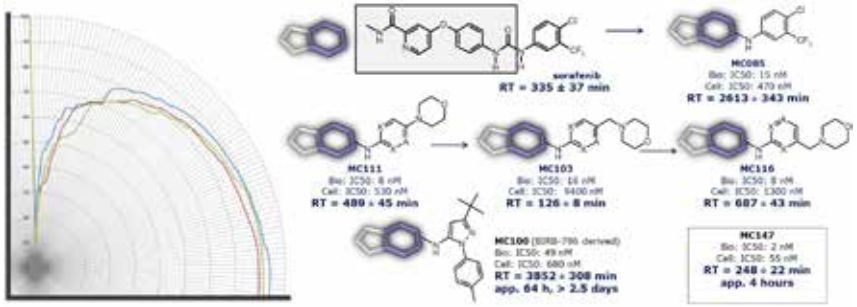

Figure1:
Left – radar plot highlighting the selectivity of front-runner compounds in a 400 membered kinase panel; right – detailed structure-kinetic relationships emerge.

DESIGN AND SYNTHESIS OF SELECTIVE PIM1 INHIBITORS BY UTILISING UNIQUE STRUCTURAL FEATURES IN THE HINGE

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PIM kinases are constitutively active serine/threonine kinases, and are reported to overexpress in certain types of cancer. Currently, pan-PIM inhibitor PIM447 is under clinical investigation as anti-leukemia agent. Among three family members, PIM1 is reported to overexpress in asthma, peanut-allergy and pulmonary arterial hypertension, indicating that PIM1 is a potential therapeutic target to treat chronic diseases.[1)] Uniquely, PIM kinases do not form a canonical hydrogen bond with ATP or kinase inhibitors because PIM kinases are the only kinases to have a proline at the hydrogen donor position in the hinge.[2)] In addition, there is an extra space in the hinge of PIM, as a result of insertion of amino acid residues after the proline.[3)] Previously, we reported a 7-azaindole derivative as potent but non-selective PIM1 inhibitor.[4)] We hypothesized that the 7-position nitrogen forms a canonical hydrogen bond with backbone amide in hinge region of off-target kinases to cause low kinase selectivity, and tried to sterically mask the 7-position nitrogen to improve the selectivity by utilising PIM's unique structural features mentioned above. We synthesized a series of 6-substituted-7-azaindole derivatives, and found 6-methyl or 6-ethyl substituted derivatives show clean kinase selectivity profile while retaining potency. Results of SAR and ADMET study will be discussed in this presentation.

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DISCOVERY OF NEW PROTEIN KINASE INHIBITORS WITH THE FURO [3,2-B]PYRIDINE CORE

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Development of new protein kinase inhibitors has been a very active field in the academic as well as in the industrial sector. Up to date, 30 compounds that are currently clinically used have been identified. The central hypothesis of our project was that the furo[3,2-*b*]pyridine motif could serve as a proper bioisostere of the pyrazolo[1,5-*a*]pyrimidine pharmacophore, which was successfully used in numerous series of potent and selective inhibitors of various protein kinases. Interestingly, only a few series of furo[3,2-*b*]pyridine-based protein kinase inhibitors were documented in the (patent) literature. In addition, furo[3,2-*b*]pyridines with NHR substituents at the 7 position, which are generally important for the interaction with the hinge regions of kinases, were not known at all. In order to prepare the initial set of furo[3,2-*b*]pyridines with particular substitutions patterns at positions 3, 5, 6 and 7, we optimized two known methods to assemble the furo[3,2-*b*]pyridine core and developed one new annulation methodology. While some direct analogs of known pyrazolo[1,5-*a*]pyrimidine inhibitors proved to be less potent, the series with proper substituents at positions 3 and 5 of the furo[3,2-*b*]pyridine scaffold contained some highly potent (IC_{50} < 50 nM) and selective inhibitors of CLK and HIPK kinases, which emerged only recently as possible therapeutic targets. Of note, the activities of the most potent compounds would be hardly predictable from the available crystal structures - they would suggest that the size of the ATP binding site's cavity would be insufficient to accommodate some "most active" substituents at position 3 of the core.

NOVEL ORALLY AVAILABLE JAK2 SELECTIVE INHIBITORS AS POTENTIAL TREATMENT OF MYELOPROLIFERATIVE DISORDERS

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The Janus Kinases (JAK1, JAK2, JAK3, TYK2) are non-receptor tyrosine kinases mainly involved in the signal transduction mediated by cytokines. They play a crucial role in hematopoiesis and immune response. While JAK1, JAK3 and TYK2 have an important role in the regulation and development of immune system, JAK2 is a critical mediator for cytokines essential for growth and differentiation of myeloid cells, such as erythropoietin (EPO), thrombopoietin (TPO), interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF).

Activating mutations of JAKs are found in association with malignant transformation. The best characterized gain-of-function mutation, JAK2-V617F in the pseudo-kinase domain of JAK2, is present in hematopoietic cells of patients with myeloproliferative disorders (MPD). The point mutation renders the kinase constitutively active and induces cytokine-independent proliferation of hematopoietic cell lines. In particular, the JAK2-V617F mutation is found in >95% of patients with polycythemia vera (PV), *circa* 50% of patients with essential thrombocythemia (ET), and *circa* 50% of myelofibrosis (MF) patients.

Although JAK inhibitors have been approved in oncological and autoimmune settings (e.g. the JAK1/JAK2 inhibitor ruxolitinib in MF and PV and the pan-JAK inhibitor tofacitinib in rheumatoid arthritis) and multiple agents are in clinical testing, JAK2 selective compounds might provide an advantage for long-term MPD therapy or in association with immunotherapy, given that inhibition of other JAK family members leads to immunosuppressive effects. Due to the high homology in the adenosine triphosphate (ATP) binding pocket among the JAK family kinases, the discovery of JAK2 inhibitors having JAK family selectivity is not trivial. Despite this difficulty, we engaged in a program aimed at identifying potent inhibitors of JAK2 and possibly gaining a better insight into JAK family selectivity.

Herein we report the optimization efforts that led to the identification of potent and selective JAK2 inhibitors. Screening of the NMS compound collection led to the identification of the pyrrole derivative NMS-P901, with promising activity against JAK2, but low selectivity in cells. A structure based and medicinal chemistry driven optimization allowed the identification of derivatives[1,2] active against JAK2, with selectivity over the other JAK family enzymes both in biochemical and cellular assay. Furthermore, a selection of potent and orally available JAK2 inhibitors showed interesting *in vivo* efficacy, with evidence for JAK2 pathway suppression demonstrated by *in vivo* pharmacodynamic effects.

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DISCOVERY OF A POTENT, HIGHLY SELECTIVE AND EFFICACIOUS RAF KINASE INHIBITOR TO TREAT KRAS MUTANT SOLID TUMORS - A HYPOTHESIS DRIVEN AND PROPERTY FOCUSED DISCOVERY STORY OF RAF709

Gisele Nishiguchi (1), Benjamin Taft (2), Alice Rico, Robert Aversa (1), Huw Tanner, Matt Burger (1), Hanneke Jansen (2), Polyakov Valery (2), Lifeng Wan (2), Sharadha Subramanian (2), Victoriano Tamez Jr (1), Paul Barsanti, Lina Setti, Aaron Smith, Beatrix Wagner (3), Mohammad Hekmat-Nejad (2), Mulugeta Mamo (2), Richard Zang (2), Linda Xiao (2), Shengtian Yang (2), Dazhi Tang (2), Lesley Griner (1), John Tellew (4), Jake Haling (4), Vesselina Cooke (1), Brent Appleton, Anne Van Abbema, Hanne Merritt, Sylvia Ma (2), Elaine Ginn (2), Kent Wong (2), Qin Yue (2), Colin Lorentzen (2), Vijay Sethuraman, Payman Amiri, Mallika Singh, Wenlin Shao, Yan Lou, Michael Dillon, Darrin Stuart (1), Savithri Ramurthy (1)

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More than 30% of human cancers harbor mutations in the Mitogen Activated Protein Kinase (MAPK) pathway, most prevalent of which are RAS or BRAF mutations. Effective therapies to treat RAS mutant cancers remain an unmet medical need. CRAF has been demonstrated to be the critical mediator of mutant KRAS-driven tumorigenesis, and to play an essential role in mediating pathway reactivation following MEK inhibitor treatment. Therefore, CRAF presents an attractive target for the therapeutic intervention of RASmut tumors. We initiated a drug discovery program to develop small molecule inhibitors of both B- and CRAF kinase activity in RASmut cancer models. Hits were identified through accelerated hit finding. Data mining enabled us not only to quickly understand the issues to be addressed but also helped us to set the project strategy and define the target compound profile. Hypotheses were postulated, tested utilizing X-ray crystal structures and medicinal chemistry principles. Herein, we will describe how emphasis on physical chemical properties and cellular potency led to the discovery of ATP-competitive, potent, selective inhibitor of RAF that is highly efficacious in KRAS mutant driven models.

LDC1267, A NOVEL TAM FAMILY KINASE INHIBITOR

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The receptor tyrosine kinases Tyro3, Axl, and Mer constitute the TAM kinase family. Their signaling is involved in cell survival, proliferation, migration and adhesion, vascular smooth muscle homeostasis, platelet function and erythropoiesis, and regulation of inflammatory cytokine release. Abnormal expression and activation of TAMs has been implicated in several malignancies and is correlated to poor prognosis. Here we report our discovery and optimization of a series of quinoline derivatives as potent and selective inhibitors of the TAM kinase family. During medicinal chemistry optimization the exploration of the back pocket-binding fragment of these type II kinase inhibitors yielded substituted pyrazoles as the most active ones. These elaborated new compounds demonstrate *in vitro* potency in the nM range against TAM kinases and high selectivity against other kinases in a selected panel. Many of these new inhibitors dramatically reduce the Axl phosphorylation levels in HEK293 cells in a dose-dependent manner. ADME profiling and *in vivo* mouse PK studies resulted in the identification of the lead candidate LDC1267. Furthermore, *in vivo* studies showed that LDC1267 reduces metastasis formation in a syngeneic B16F10 mouse model. Taken together, these newly identified inhibitors of the TAM kinase family represent an approach for cancer therapy. Further development is pursued by our partner Qurient Therapeutics.

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IDENTIFICATION OF POTENT, SELECTIVE AND ORALLY BIOAVAILABLE QUINAZOLINE BASED INHIBITORS OF IRAK4 FOR THE TREATMENT OF INFLAMMATION - A CAUTIONARY TALE.

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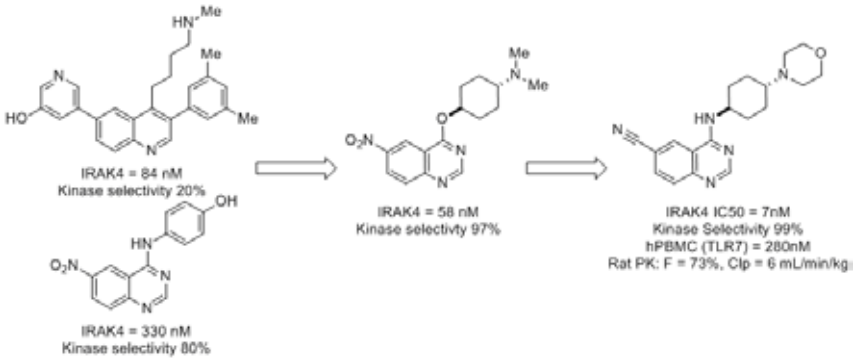

Interleukin-1 receptor associated kinase 4 (IRAK4) has been implicated in IL-1R and TLR based signaling. Therefore selective inhibition of the kinase activity of this protein represents an attractive target for the treatment of inflammatory diseases. Medicinal chemistry optimization of high throughput screening (HTS) hits with the help of structure based drug design led to the identification of orally-bioavailable quinazoline based IRAK4 inhibitors with excellent pharmacokinetic profile and kinase selectivity. These compounds show activity in vivo via the oral route in a TLR7 driven model of inflammation.

DISCOVERY OF INHIBITORS OF SRC AND mTOR USING A LIGAND-BASED CHEMOCENTRIC APPROACH IN BREAST CANCER MODELS

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Using an agile approach that combines ligand-based design of highly-focused compound libraries and phenotypic screening in an iterative manner, we have rapidly developed novel kinase inhibitors with optimal drug-like properties that display very potent antitumoral activity against breast cancer cells. The strategy consisted of using a highly promiscuous kinase inhibitor as a template to design high quality small molecule collections that would facilitate the search for enhanced physicochemical properties and, at the same time, the exploration of novel pharmacological features. To accelerate the advance from hits to leads to drug candidates, compounds were screened across a suite of 2D and 3D phenotypic assays quantifying cancer cell proliferation, invasion and survival in the search for derivatives with potent anticancer properties. Chemical design was biased towards breast cancer treatment (rather than to a particular target) by using human breast cancer cells as a discriminating cell model. Such pseudo target-agnostic strategy enabled the rapid identification of compounds that inhibited pathways involved in breast cancer survival and cell invasion and also disregarded compounds with low cell penetrability. Using this pragmatic approach, target deconvolution of identified hits and leads was largely simplified (= focused kinome screening), thereby assisting the mechanistic elucidation of the molecular targets and antitargets involved in the observed phenotype. This led to the discovery of novel ATP-competitive kinase inhibitors with unique properties, including the exquisitely selective mTOR inhibitor eCF309 [1] and the orally-available SRC inhibitor eCF506 [2], which is the first small molecule with subnanomolar IC_{50} for SRC that requires 3 orders of magnitude greater concentration to inhibit ABL. This is of relevance because the manifestation of cardiac events -especially in elderly patients- is a well-established adverse effect of ABL inhibition and many studies have shown that ABL plays a paradoxical anti-oncogenic role in breast cancer. Such off-target activities are absent in eCF506, which exhibits excellent water solubility and an optimal DMPK profile, halts SRC-associated neuromast collective cell migration in zebrafish embryos without inducing life-threatening heart defects and inhibits *in vivo* SRC phosphorylation in tumor xenografts [3].

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DESIGN AND SYNTHESIS OF NEW DIARYLAMIDES WITH PYRIMIDINYL PYRIDINE SCAFFOLD AND BIOLOGICAL EVALUATION OF THEIR ANTI-PROLIFERATIVE EFFECT ON CANCER CELL LINES

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A new series of diarylamides, having a pyrimidinyl pyridine scaffold, was designed and synthesized. The target compounds were synthesized in three steps. A selected group from the target compounds was tested over a panel of 60 cancer cell lines at a single dose concentration of 10 lM, and the most active compound, 5j, was further tested in a five-dose testing mode to determine its IC_{50} value over the 60 cell lines. In single-dose testing mode, compound 5j showed the highest growth inhibition against the NCI-60 cancer cell lines, while other tested compounds showed a weak to moderate inhibitory activity against a range of different cancer cell lines. In five-dose testing mode, compound 5j showed strong inhibitory activity in micro molar range against many cancer cell lines. Its major activity was against melanoma cancer cell lines. Therefore, compound 5j is a promising hit compound targeting this severe form of cancer.

TARGETING SPECIFIC INTERACTIONS TO IMPROVE EGFR-LIGAND BINDING

Paul Gane

Chemical Computing Group

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.

POSTERS

The Importance of Solute Carrier Transporters in Drug Discovery

DEVELOPMENT OF LIGANDS TARGETING THE BETAINE/GABA TRANSPORTER 1 (BGT1)

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GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). GABAergic signaling is mainly terminated by the GABA transporters (GATs) via neurotransmitter reuptake. Owing to their role in regulating extracellular GABA level, the GATs are proposed drug targets in a number of neurological disorders.

Four GAT subtypes have to date been identified: GAT1, GAT2, GAT3 and the betaine/GABA transporter 1 (BGT1). Due to its high abundance, the GAT1 subtype is the most explored transporter. However, the emerging pharmacological potential of non-GAT1 subtypes has increased the need for development of improved non-GAT1 selective compounds.

We have recently reported on a series of guanidine-containing β-alanine analogues and several derivatives of 2-amino-tetrahydropyridine/pyrimidinecarboxylic acid (**1**-**3**), examined for their ability to inhibit GATs (figure 1). This study led to the identification of several analogues with pronounced selectivity for BGT1 over the three other GAT subtypes [1].

The present study is based on the most potent and selective compound, the guanidine containing compound **3**, identified in the above-mentioned study. The target compounds have been obtained through structural modifications, including C- and N-alkylation with different R_1 and R_2 substituents and isolation of obtained stereoisomers. The synthesized compounds were pharmacological characterized at human GATs expressed in mammalian cell lines.

The synthesized compounds all retained selectivity towards the BGT1 subtype and showed IC_{50} values in the mid- to high micromolar range. Introduction of a methyl group in the 4-position (R_1) generated four enantiomers whose inhibitory activities indicate a preference for the orientation of the methyl- and carboxylic group. Substituting R_2 with smaller substituents resulted in an up to 60-fold decrease in activity, however the results also suggest that larger lipophilic substituents could lead to the regain of inhibitory effect and avoiding transport as a substrate.

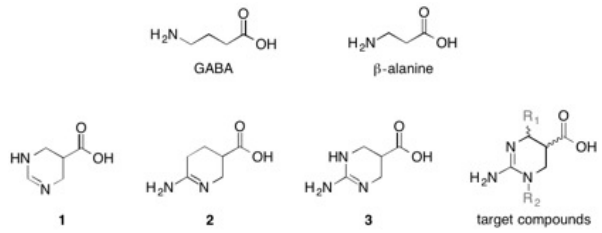

Figure 1. Structures of GABA, β-alanine, selective BGT1 compounds 1, 2 and 3 and the synthesized target compound

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SELECTIVITY PROFILING OF THE HUMAN MONOAMINE TRANSPORTERS: A CASE STUDY ON CATHINONE ANALOGS

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The human monoamine transporters including hSERT (human serotonin transporter), hDAT (human dopamine transporter) and hNET (human norepinephrine transporter) belong to the solute carrier 6 (SLC6) gene family (also referred to the neurotransmitter-sodium-symporter family (NSS) or Na^+ /Cl^- -dependent transporters). They play an important role in the central and peripheral nervous system by regulating the signaling among neurons. Numerous compound classes have been identified to interact with these transporters, and they are used either in a therapeutic setting or are abused as illicit drugs.

This project concentrates on the molecular basis and the chemical characteristics of ligand transporter interaction and selectivity at hSERT and hDAT. By exploring the chemical space of hSERT and hDAT interacting compounds via the Open PHACTS Discovery Platform by using KNIME, we analyzed the scaffolds appearing selective for either hSERT or hDAT. As use case for further studies we used the class of cathinones. They represent a subclass of the amphetamines and are a quite prominent group of abusive drugs with a rising trend of consumption. Throughout the data extraction process, we collected 56 compounds sharing this scaffold and being tested in the same biological assay type. The main structural variations include the substituent on the nitrogen atom, the substituent at the aromatic ring, as well as some modifications at the C_α-atom. Subsequent structure-activity relationship studies with hDAT pIC50 values and selectivity as dependent variables, as well as docking of selected compounds into protein homology models of hSERT and hDAT revealed first insights into the molecular basis of transporter selectivity by pointing out an influence of the substituent at the C_α-atom to the carbonyl group.

Acknowledgements:

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115191, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution. We further wish to acknowledge the Open PHACTS Foundation, the charitable organisation responsible for the Open PHACTS Discovery Platform, for providing access to the Platform as well as the Austrian Science Fund (FWF), grants F03502 and W1232.

MOLECULAR DOCKING OF NOVEL NON-COMPETITIVE hBGT1 INHIBITORS TO EXPLORE THEIR STRUCTURE-ACTIVITY RELATIONSHIP

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3) Medical University of Vienna, Center for Brain Research, Dept. of Biochemistry and Molecular Biology, Austria

The human betaine/γ-aminobutyric acid-transporter 1 (hBGT1) facilitates the reuptake of γ-aminobutyric acid (GABA) into neuronal and glial cells [1]. The inhibition of hBGT1 increases GABAergic transmission and has therefore emerged as a promising target for the treatment of epilepsy [2,3].

In this study, we explored the structure-activity relationship of new analogs of the first known non-competitive hBGT1 inhibitor N-(1-benzyl-4-piperidinyl)-2,4-dichlorobenzamide (BPDBA) [4] by computational methods. BPDBA is selective for BGT1 over the other three GATs. The recently released crystal structure of human serotonin transporter (hSERT) shares high sequence identity (44.7%) and similarity (66.4%) with hBGT1 and was used as a template for hBGT1 homology models. Noteworthy, the structure of hSERT was co-crystalized with a ligand revealing an allosteric binding site [5]. Therefore, active BPDBA analogs were docked into the postulated corresponding allosteric binding site of hBGT1 between transmembrane regions 1, 6, 10, 11, 12. Docking with flexible side chains was performed in the software package GOLD [6] and 100 poses per compound were generated. The compounds were analyzed using an in-house protocol for common scaffold clustering with an RMSD of less than 3Å [7]. Calculation of Protein-Ligand-Interaction-Fingerprints (PLIFs) and visual inspection was carried out with MOE [8]. 1000 poses of 10 active BPDBA analogs were assembled into 87 clusters.

The two most populated clusters, which contained poses of all docked compounds, were able to explain the structure-activity relationship of an experimentally tested BPDBA analog series. Accordingly, the 2,4-dichlorobenzamide ring of BPDBA fits into a lipophilic pocket that is stabilized by pi-pi interactions with Tyr453. Removing the Cl substituents in the series of BPDBA compounds leads to a loss of van der Waals (vdW) interaction and gradually decreases affinity. Introducing a third Cl substituent in the meta position increases affinity due to increased vdW interaction. However, introduction of a trifluoromethyl substituent in the ortho-position leads to a slight decrease in affinity due to steric hindrance. In addition, the removal of the benzyl ring of BPDBA leads to a drastic affinity drop due to the loss of VdW interaction. This docking study reveals the first insight into a new possible molecular binding mode of non-competitive hBGT1 inhibitors that might be driven by vdW interaction.

Acknowledgement

We gratefully acknowledge financial support provided by the Austrian Science Fund grant number W1232.

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IDENTIFICATION OF IMIDAZOLE-4-ACETIC ACID AS A NEW POTENT TAUT INFLUX INHIBITOR THROUGH CHARACTERIZATION OF TAUT-MEDIATED RETINAL DELIVERY

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Retinal diseases leading to impaired vision and ultimately blindness include disorders such as age-related macular degeneration and diabetic retinopathy, which are the leading causes of blindness in the developed countries. There is currently no effective cure or treatment to reduce the ischemic/hypoxic stress generated in these diseases. A proposed therapeutic approach consists in decreasing the retinal neuronal activity, which reduces the metabolic demands of the retina.[1] The ρ-containing $GABA_A$ receptors (ρ $GABA_A$Rs) are mainly responsible for the inhibitory control in the retina, which makes this type of receptors a particularly suitable target for regulating retinal neuronal activities. These receptors are predominantly localized in the axons of bipolar cells but they have also been identified in other regions of the central nervous system, which highlights selective ocular targeting as an important issue.

The taurine transporter (TAUT) is a Na^+-dependent carrier playing a key role in the transport of taurine and also γ-aminobutyric acid (GABA) at the blood-retinal barrier (BRB). It has been previously demonstrated that the taurine transport at the BRB is almost 30 times higher than across the blood-brain barrier.[2] Therefore, ρ $GABA_A$ R ligands, which also are substrates for TAUT, would preferably be delivered into the retina, thereby minimizing the off-target effects on ρ $GABA_A$Rs in the brain. This transporter has previously been shown to be expressed in ARPE-19 cells, a spontaneously arising human retinal pigment epithelial cell line.[3] Consequently, this cell line was explored as an *in vitro* model of TAUT at the BRB (Figure 1).

In order to investigate the structural requirements of $GABA_A$Rs ligands for interacting with TAUT at the BRB, different standards $GABA_A$R ligands were tested for their ability to inhibit the TAUT-mediated influx of taurine in ARPE-19 cells.

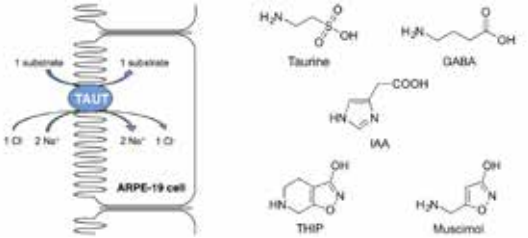

Figure 1. The taurine transporter (TAUT) in ARPE-19 cells and structures of two endogenous substrates, GABA and taurine, along with three GABA analogs (IAA, THIP, and muscimol).

Results showed that taurine influx was significantly inhibited by GABA and imidazole-4-acetic acid (IAA) in a concentration-dependent manner displaying similar IC_{50} values of 646 µM and 659 µM, respectively. Moreover, IAA demonstrated higher inhibitory properties than other GABA analogs such as 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) or muscimol (Figure 1). These studies may indicate a TAUT-mediated retinal delivery of IAA, which could therefore be used as a new lead structure for the development of potent ρ $GABA_A$R ligands that, for delivery purposes, are also TAUT substrates.

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NOTES

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POSTERS

Covalent Drugs Revisited

FROM CATALYTIC MECHANISMS TO THE DEVELOPMENT OF EMBM, A COMPUTATIONAL TOOL FOR THE DESIGN OF TS ANALOG INHIBITORS OF PROTEASES

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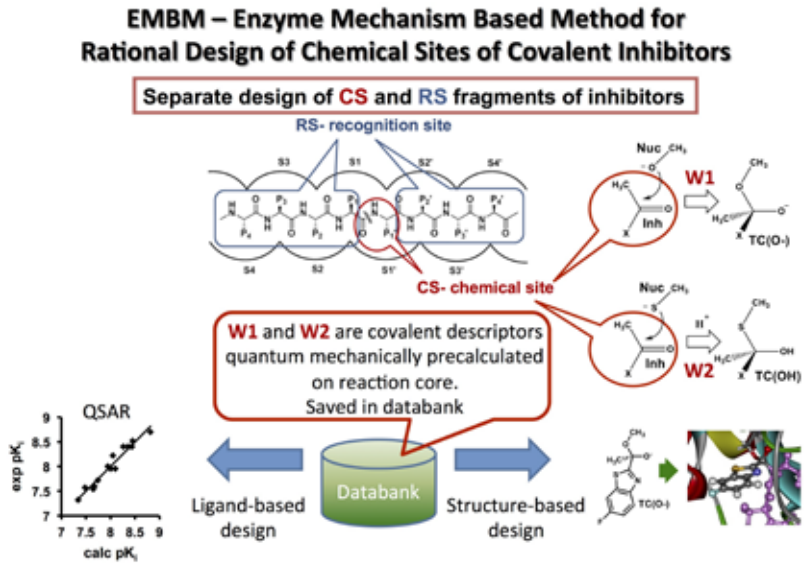
Mechanistic studies of catalysis and inhibition of serine and cysteine proteases afforded new and sometimes surprising insights, challenging conventional dogmas in enzymology. They provided a mechanistic basis for the understanding of the trend in binding affinity of "warheads" of reversible covalent transition-state analog (TS) inhibitors.

These studies led us to the development of EMBM (enzyme mechanism based method), a unique CADD methodology for the prediction of binding affinity and the design of TS inhibitors. EMBM is unique among other computational tools by accounting for both covalent and non-covalent interactions of TS inhibitors with their target enzymes. The method was implemented in both ligand-based and structure-based design protocols, demonstrating its prediction ability on series of TS inhibitors of seven serine- and cysteine hydrolases.

Thus, EMBM may provide a practical and efficient tool for the design of drugs that are based on TS analog inhibitors.



ACRYLAMIDES AND INFLAMMASOMES: ANOTHER STEP TOWARDS DIRECT NLRP3 INHIBITION

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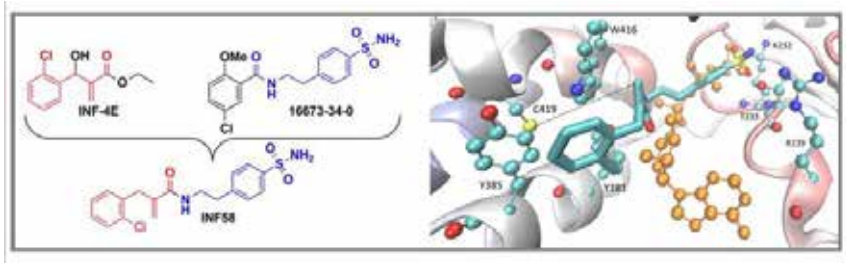
NLRP3 inflammasome plays a crucial role in activating caspase-1, processing the pro-inflammatory interleukin-1β (IL-1β), and triggering pyroptotic cell death cascade.[1] Gain of function mutations in NLRP3 determine its abnormal activation which is a key factor in the pathogenesis of autoinflammatory diseases known as cryopyrin-associated periodic syndromes (CAPS). NLRP3 inflammasome is also relevant in the progression of several diseases, such as atherosclerosis, type-2 diabetes, Alzheimer's disease, and gout.[2] Several studies suggested that covalent drugs may be the strategies to dampen NLRP3 activity.[3]
In this study, the synthesis of acrylamide compounds and their pharmaco-toxicological evaluation as potential inhibitors of NLRP3 network are described. Tuning down Michael acceptor reactivity led to compounds endowed with low toxicity profile, determined by cell viability and serum albumin binding assays. Best hits were also able to inhibit IL-1β release from different macrophage subtypes, including CAPS mutant macrophages. Mechanism of action of these acrylamides involves direct interaction with NLRP3, confirmed by NLRP3 ATPase activity inhibition on isolated protein. 2-(2-Chlorobenzyl)-*N*-(4-sulfamoylphenethyl)acrylamide (INF58) was able to concentration-dependently inhibit NLRP3 ATPase with an $IC_{50}$of 74 μM: in silico prediction of its binding mode in the catalytic pocket indicates that a putative interaction with Cys419 residue might account for this activity.



This study shows that these acrylamides could be good candidates to develop safe covalent inhibitors of NLRP3 inflammasome.[4]

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DEVELOPMENT OF NOVEL SELECTIVE AND POTENT INHIBITORS OF RHOMBOID INTRAMEMBRANE PROTEASES

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Intramembrane proteases control important biological processes by cleaving membrane proteins in their transmembrane helices. Rhomboid intramembrane proteases regulate intercellular signaling, mitochondrial dynamics, invasion of eukaryotic parasites and membrane protein quality control. They have emerging medical potential, but their specific inhibitors have been lacking. Rhomboids are serine proteases using a catalytic dyad of serine and histidine.

In our recent work we have elucidated the structural basis of their substrate specificity, and based on this we have identified substituted peptidyl ketoamides as novel covalent reversible inhibitors of rhomboid proteases. We show that optimization the sequence of the P5 to P1 residues of the peptidic part of the inhibitor enhances its potency and selectivity, and substitutions at the nitrogen of the ketoamide dramatically improve the potency of the inhibitor. Our co-crystal structure of the peptidyl ketoamides bound to E.coli GlpG rhomboid shows the binding mode of this class of compounds. The moiety modifying the ketoamide nitrogen (the 'tail') extends into the prime side of the active site cavity, while the ketoamide warhead covalently binds the catalytic serine and extensively hydrogen-bonds to the oxyanion stabilizing residues of GlpG.

COVALENT INHIBITORS OF LGTC: A TEMPLATE FOR THE DISCOVERY OF DRUG-LIKE INHIBITORS AGAINST BACTERIAL GLYCOSYLTRANSFERASES

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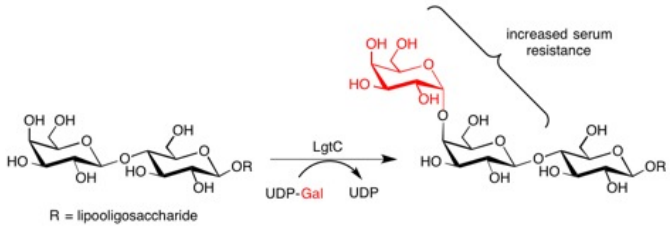
Bacterial glycosyltransferases (GTs) are key enzymes for the biosynthesis of complex glycans and glycoconjugates. Many of these enzymes have a direct role for bacterial virulence and viability [1]. Inhibitors of bacterial GTs are therefore sought after as tool compounds for anti-bacterial drug discovery and chemical microbiology. However, most existing GT inhibitors are substrate analogues with limited suitability for such applications, while non-substrate-like GT inhibitors are rare [2].



Here, we describe the discovery of a novel, non-substrate-like inhibitor chemotype for LgtC [3], a retaining α-1,4-galactosyltransferase involved in lipooligosaccharide biosynthesis in Gram negatives (Figure). In non-typeable *Haemophilus influenzae*, LgtC expression is required for the installation of a terminal digalactoside epitope, and has been associated with high-level serum resistance [4]. The new inhibitors have low to sub-micromolar inhibitory activity, comparable to the best substrate-based inhibitors. We provide enzymological and spectroscopic evidence that the new inhibitors, which are structurally unrelated to either the donor or acceptor of LgtC, react covalently with a non-catalytic cysteine residue in the LgtC acceptor binding site. We also outline applications of the new inhibitors as tool compounds for chemical microbiology, e.g. for the imaging of virulence factors.

We also present a detailed analysis of available sequence and structural data, which reveals that non-catalytic cysteines are a common motif in the active site of many bacterial GTs. This suggests that the new GT inhibitors can serve as a broadly applicable template for the rational development of drug-like inhibitors against a target class of bacterial enzymes, for which almost no such inhibitors exist to date.

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DRUGGING THE "UNDRUGGABLE" WITH MCR SCAFFOLD MANIFOLD: THE DESIGN AND SYNTHESIS OF COVALENT INHIBITORS AND MACROCYCLES

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High-throughput screening (HTS) is one of the most utilized methods of identification of chemical probes and drug leads in today's drug discovery of many pharma companies. However the limitations and pitfalls of HTS technologies are more obvious than ever. The discovery of novel scaffolds and exploitation of the still high-degree unknown chemical space for the unmet medical needs is urgent. Companies more than ever need highly customized libraries based on the needs of their in-house biophysical screening and co-crystal structure analysis.

TelesisPharma, based on its unique experience in Multi-Component Reactions (MCRs) chemistry engineers highly functionalized small molecule and macrocyclic scaffolds in 1 to 3 synthetic steps.

Amongst the scaffold manifold that we can easily access and demonstrate the power of MCRs, TelesisPharma reassesses an important class of drugs: Covalent inhibitors. We design and synthesize covalent target binders based on unique and yet underexplored scaffolds equipped with a variety of electrophilic warheads, to target mutant proteins, e.g. *KRAS (G12/13C), p53 (Y220C), IDH1 (R132C) or DNMT3a (R882C)*. Moreover, we present an efficient synthesis and virtual screening of macrocyclic libraries. Competent access to hundreds of macrocyclic scaffolds using MCRs will be presented.

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POSTERS

Novel Molecular Probes for in Vivo Chemistry

SYNTHESIS OF PYRROLOCYTOSINE-GUANIDINIOCARBONYL PYRROLE CATIONS AS A NEW TYPE OF MOLECULAR PROBES

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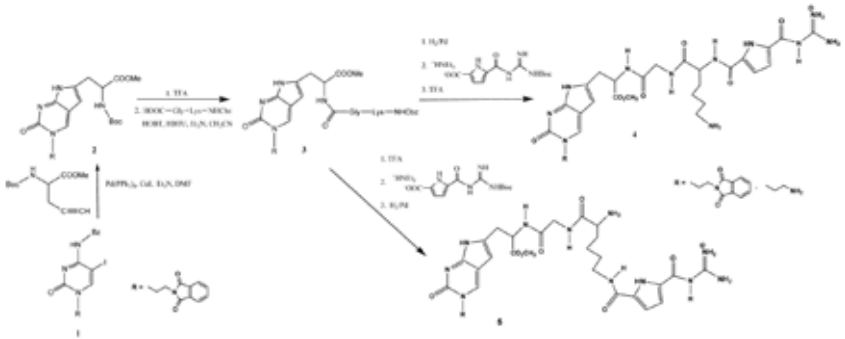
Division of Organic Chemistry and Biochemistry, Laboratory for Biomolecular Interactions and Spectroscopy, Ruder Boskovic Institute, Bijenicka 54, 10000 Zagreb, Croatia

Hybrid compounds which combine several different binding modes (intercalation, groove binding and electrostatic interactions) can distinguish various ds-DNA/RNAs based on very small differences in their secondary structures. We designed and synthesized new type of molecular probes combining two fragments which are well known to interact with nucleic acids: fluorescent pyrrolocytosine nucleobase[1] and guanidiniocarbonyl pyrrole cation known as a highly efficient DNA minor groove binder.[2] Novel fluorescent analogue of cytosine **2** was synthesized from N^4-benzoyl-5-iodocytosine derivative **1** by a one pot sequential Sonogashira cross-coupling and annulation with propargylglycine (Scheme 1). Amino acid monomer **2** was then coupled to the dipeptide Gly-Lys giving compound **3**, which is attached to guanidinocarbonyl pyrrole carboxylic acid, resulting in hybrid multipurpose probes **4** and **5**, and which may have potential as *in vivo* sensors.



Scheme 1. Synthesis of pyrrolocytosine-guanidiniocarbonyl pyrrole cations.

Synthetic details of new hybrid probes, their spectrophotometric properties and the interaction with different DNA/RNAs will be reported.

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PALLADIUM NANOPARTICLES FOR THE SPECIFIC MODIFICATION OF NATIVE PROTEINS

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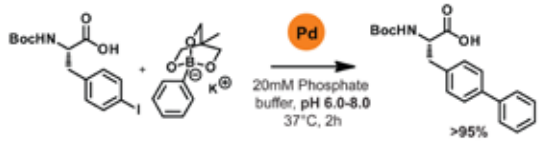
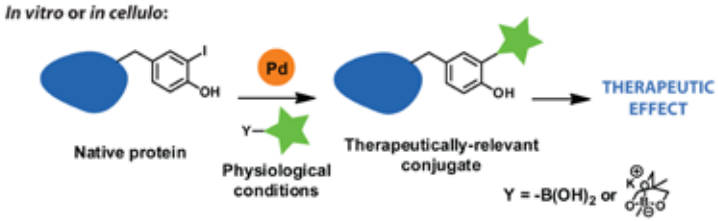
Chemical transformations that can be performed selectively under physiological conditions are highly desirable tools to manipulate proteins and generate conjugates with new or improved properties.[1] Owing to their broad functional group tolerance and biological compatibility, palladium-catalyzed *Suzuki-Miyaura* reactions emerge as attractive strategies for the formation of functional protein conjugates.[2] In addition, the low toxicity associated with palladium opens perspectives for specific protein modifications of therapeutic interests.[3]

Palladium nanoparticles stabilized by polymers demonstrated excellent catalytic activity for the modification of halogenated amino acids through *Suzuki-Miyaura* cross-coupling reactions in water. Interestingly, up to 98% conversion into the coupled amino acid could be achieved in 2 h at 37°C using stable, water-soluble cyclic triolborates as organometallic partners in the presence of only 1 mol% of palladium.[4]



These nanocatalysts demonstrated the ability to modify thyroglobulin, a naturally iodinated protein involved in the production of thyroid hormones, presenting the first example of cross-coupling reaction on a native protein. Given the stability, selectivity and remarkable properties of palladium nanoparticles in biological settings, these systems open exciting therapeutic perspectives involving the manipulation of naturally-occurring proteins in living systems.[5]



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CHARACTERIZATION OF NOVEL MONOMETHINE CYANINE DYES AS INTERCALATING AGENTS AND FLUORESCENT PROBES

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The novel asymmetric cyanine dyes were synthesized, their spectral characteristics and interaction with double stranded (ds)DNA have been investigated. Used as sensitizers for colour photography and recently as fluorescent labels for biomolecules and nucleic acids, cyanines are characterized by their high molar absorptivity and relatively high affinity for nucleic acids. Upon binding to double helical DNA, dyes exhibit large fluorescence emission intensity enhancements. Additionally, cyanine dyes are used as molecular probes in detection of specific DNA sequences important for genetic screening, clinical diagnostics and microchip analysis of gene expression, whereas fluorescence is the most common form of detection. In this work, we have analysed fluorimetric, spectrophotometric, thermal melting, circular dichroism measurements in order to elucidate the mode of binding and the specific affinity for different polynucleotide secondary structure motifs.

Our further research was focused on elucidating the biological activity of novel cyanines with potential antitumor activity. We have screened our own designed and synthesized asymmetric dicationic monomethine cyanine dyes (AK-A1, AK-A2, AK-A3, AK-A4 and AK-A7) for antiproliferative activity (MTT assay) and showed negligible activity on human carcinogenic lung (H460) and breast cancer (MCF-7) cells. AK-A7 dye showed weak antiproliferative activity (IC_{50} = 7,74 x 10^{-4} M) toward H460 cells. Considering that the spectroscopic measurements indicated intercalative mode of binding with the DNA, low antiproliferation activity was a surprising result. Having two positive charges, safe assumption was that the dyes do not pass the semi permeable membrane of mammalian cells. Therefore, dyes AK-A1 (abs/em ~483/516 nm) and AK-A4 (abs/em ~510/533 nm) have been further checked by confocal microscopy for subcellular localization and the results suggested their mitochondrial localization in human cells. Commercial dyes such as Mitotracker Deep Red and DiOC6(3) were used for colocalization experiments. Another colocalization strategy involved transient transfection of human cancerogenic cells with pMito-ECFP vector. After adding specific dyes (AK-A1 and AK-A4) and their incubation, colocalization of cyan fluorescent protein produced in mitochondria and dyes was successfully observed. The correlation of the fluorophores colocalization was calculated using Pearson correlation coefficient (PCC). Image J (Wayne Rasband, NIH, Bethesda, USA) and LAS X Leica Microsystems software packages have been used for overlaying of images and calculation of PCC. To conclude, tested cyanine dyes are excellent imaging agents with low cytotoxicity and no photobleaching effects observed.

NOTES

POSTERS

Recent Advances on Approaches to Treat Pain

NOVEL OXIDATIONS OF OPIOID DERIVATIVES

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Opioids are a group of well-known pain relieving medicines most commonly used to treat severe pain conditions, such as cancer-related pain, chronic low-back pain and chronic pain associated to diseases such as osteoarthritis.[1] Morphine is a classic example of opioid analgesics, relieving pain by agonizing mu opioid receptor.[2] The existing opioid receptor agonists have several disadvantages, like tolerance and hyperalgesia, especially in long-term clinical use.[1]

Oxidation products of opioids, such as the 7,8-epoxide derivatives of morphine, have been suggested to bear less liability to dependence, with activity comparable to the parent compounds both *in vitro* and *in vivo*.[3] However, direct oxidations of opioid substrates are challenging and thus scarcely reported in the literature. Oxidation protocols have mostly focused on optimizing the production of 14-hydroxyl derivatives from thebaine and oripavine, in route to the preparation of drugs such as naloxone and naltrexone.[4]

In continuation of our research with opioid derivatives[5], we are currently exploring oxidation reactions on several opioid derivatives in search for novel compounds with improved potency and fewer side effects.[6] Our synthetic efforts are being supported by molecular modelling methods to help to focus the synthesis on the most promising modifications. Docking simulations are conducted to the major three crystallized opioid receptors[2,7] (mu, kappa and delta) to prioritize the synthesis, and later on bioactivity testing of the synthesized compounds will be performed on these opioid receptors.

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BIFUNCTIONAL PEPTIDE BASED OPIOID AGONIST - NOCICEPTIN ANTAGONIST LIGANDS FOR THE DUAL TREATMENT OF ACUTE AND NEUROPATHIC PAIN

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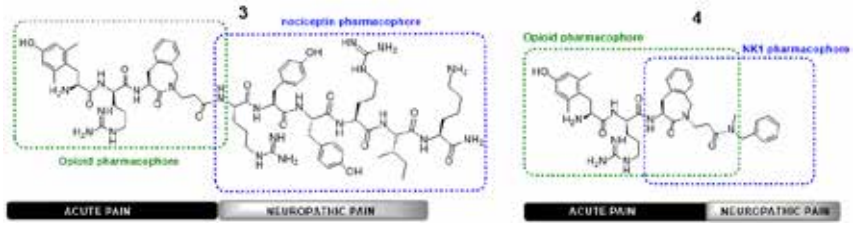
To eliminate or reduce opioid-related side effects, other receptors involved in pain can be targeted. Examples of this strategy include the use of neurokinin-1 antagonists, neurotensin agonists or cholecystokinin receptor antagonists,[1] in combination with opioids. Activation or blockage of distinct targets can be achieved by combination therapy (i.e. using drug cocktails) or by the use of designed multiple ligands (DMLs).[2] The latter are single chemical entities able to bind two or more well-chosen receptor types.

In our work, the opioid pharmacophore H-Dmt-D-Arg-Aba-β-Ala-NH_2 **1** was linked to peptide ligands for the nociceptin receptor.[3] Combination of **1** and NOP ligands (e.g., Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2**2**) led to binding affinities in the low nanomolar domain. *In vitro*, the hybrids behaved as agonists at the opioid receptors, while weak antagonism was determined at the nociceptin receptor. Intravenous administration of hybrid H-Dmt-D-Arg-Aba-β-Ala-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2**3** to mice resulted in potent and long lasting (> 3 hours) antinociception in the tail-flick test, indicating that this compound was able to permeate the BBB. This was further supported by a cell-based BBB model. All hybrids alleviated allodynia and hyperalgesia in neuropathic pain models. Especially with respect to hyperalgesia, they showed to be more effective than the opioid and NOP parent compounds. Hybrid **3** did not result in significant respiratory depression, in contrast to an equipotent analgesic dose of morphine. In comparison to earlier reported opioid agonist – neurokinin 1 receptor antagonists (e.g. H-Dmt-D-Arg-Aba-β-Ala-*N*Me-Bn **4**),[4] the best opioid – NOP hybrids have ED_{50} values about 1,000 times lower than those of the opioid agonist - NK1 antagonist hybrids such as **4** in the von Frey test and about 20 times lower in the cold plate test.[3,4] These opioid – NOP hybrids hence represent a promising avenue towards analgesics for the dual treatment of acute and neuropathic pain devoid of respiratory depression.



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DISCOVERY OF A G-PROTEIN BIASED MU OPIOID ANALGESIC WITH REDUCED SIDE EFFECTS

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Morphine and related opioids are well-known traditional analgesics and remain until today essential components in pain therapy. Their powerful analgesic effect is mainly referred to their ability to stimulate the μ opioid receptor (μOR). While the wide range of μOR agonists are relieving pain extremely efficacious, they all suffer from similar adverse effects like respiratory suppression, sedation, constipation, physical dependence and tolerance. The side effects often limit the dose for opioid analgesics resulting in an inadequate treatment of pain.

Recent studies with morphine in β-arrestin-2 knock out mice suggest that the analgesic effect results from signaling through the G-protein pathway, while many side effects may be an outcome of activated β-arrestins. Thus, specific μOR agonists with bias towards G-protein promoted signaling are required. [1]

The determination of the crystal structure of the μOR [2] offers an opportunity to seek μOR ligands with new chemotypes via structure-based approaches. In silico screening of over three million commercially available compounds against the orthosteric pocket and further structure-based optimization of initial docking hits led us to the identification of a novel μOR agonist scaffold. Starting from an originally determined hit as an isomeric mixture, we were able to provide an optically pure, subtype specific and G-protein biased μOR agonist (PZM21) by an appropriate synthetic strategy. Based on the crystal structure of the μOR in the active state [3], the binding mode of PZM21 could be determined using molecular dynamics and SAR studies involving diffusible and covalently binding analogs. PZM21 displays a unique efficacy profile in mice models. The compound generates substantial analgesia with only little depression of respiration compared to morphine. Results of mouse open field locomotion and conditioned place preference experiments are promising, indicating that PZM21 may have less reinforcing activity than classic opioids. Uniquely, PZM21 seems also to be able to distinguish between the affective component of pain and reflexive behaviors. Hence, this novel scaffold may serve both as a probe to investigate μOR signaling and as a therapeutic lead for safer opioid analgesics devoid of many of the dose-limiting side effects. [4]

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INJECTABLE PEPTIDE HYDROGELS FOR CONTROLLED-RELEASE OF OPIOIDS

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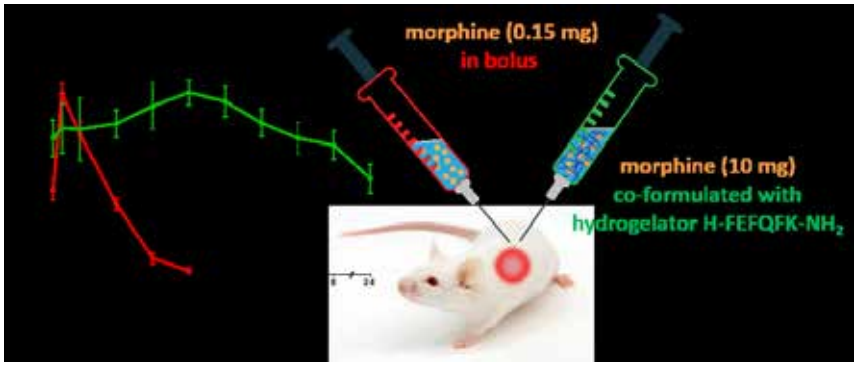
Currently, most drugs are directly administered into patients orally or systemically, without any specific formulation or via parenteral routes. Therefore, to get the desired therapeutic effect, high doses are required due to substantial biodegradation of the drug prior to interaction with the biological target. These high doses can however also result in the appearance of adverse effects. To overcome the need of repeated high dose administration, hydrogels have been reported as suitable controlled drug-delivery systems. More specifically, peptide hydrogels loaded with active ingredients can liquefy during injection (i.e. shear thinning behavior), followed by quick hydrogel reformation once injected. These systems present several advantages such as the protection of the drug against the enzymatic degradation by encapsulation in the hydrogel network, while maintaining the therapeutic plasma drug concentration over a long period via diffusion from the hydrogel or by degradation of the gel network. Consequently, lower dosage and frequency of administration are possible and result in an improvement of the drug efficacy while reducing the risk of side effects.

In this work, a new set of hydrogel-forming peptides was designed starting from the short, tunable and amphipathic hexapeptide hydrogelator H-Phe-Glu-Phe-Gln-Phe-Lys-OH. This peptide showed interesting results in terms of gelation and in vitro drug release profile.[1] All hydrogels were characterized at macroscopic and microscopic level by rheology, cryogenic transmission electron microscopy (TEM) analysis. In order to study their eventual therapeutic potential, the hydrogels have been used for entrapment and sustained release of opioid drugs. The *in vitro* drug release properties and hydrogel toxicity (cell viability experiments) were also determined. Based on the best physicochemical, mechanical, and non-cytotoxic properties, selected hydrogels were investigated for *in vivo* release of opioids. Opioid administration by subcutaneous injection and subsequent testing in the tail-flick assay (acute pain model), showed sustained antinociceptive effects over longer periods of times, as compared to drug injections in saline solutions.[2]



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TOWARDS THE VALIDATION OF THE LYSOPHOSPHATIDIC ACID RECEPTOR LPA1 THROUGH THE DEVELOPMENT OF SELECTIVE LIGANDS

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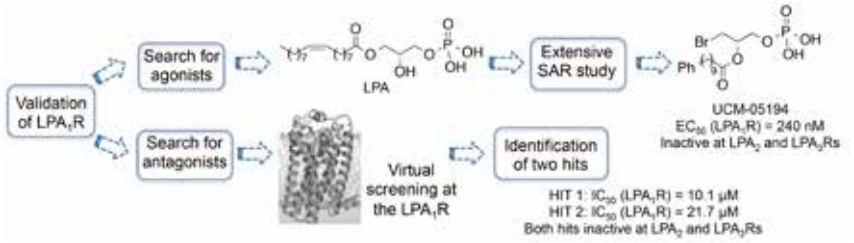
Lysophosphatidic acid (LPA) is a bioactive phospholipid involved in many physiological functions in the nervous, vascular, immune and reproductive systems, as well as in diseases such as cancer, fibrosis, obesity, and pain.[1,2] Some of its effects, especially in the nervous system, are due to the binding to the LPA_1 receptor (LPA_1 R). However, LPA has affinity by six receptors (LPA_1–LPA_6), so to establish the actual potential of LPA_1R as a therapeutic target high affinity and selective ligands (agonists and antagonists) are needed. Towards this objective we have started two medicinal chemistry programs, one focused on the development of new agonists and the other one aimed at identifying new antagonists. In the first case, we have used as starting point the structure of the endogenous ligand LPA to carry out an extensive structure-activity relationship (SAR) study where modifications of both the hydrophobic and acid moieties were carried out. This work led to the identification of a potent and selective LPA_1R agonist, compound UCM-05194, which is currently under investigation in an in vivo pain model.[3] With respect to the development of antagonists, we used the crystal structure of the LPA_1R recently disclosed[4] and the ZINC compound database to carry out a virtual screening (VS). After visual inspection and selection, the highest ranked ligand molecules from the VS (18 compounds) have been assayed for antagonism at the LPA_1R and among them we have identified two compounds with antagonist activity at the LPA_1R which are selective versus LPA_2 and LPA_3 Rs. We are currently exploring these initial hits as suitable scaffolds for the development of structurally new potent and selective LPA_1R antagonists.



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BENZOTHIAZOLE-BASED INHIBITORS OF N-TYPE AND T-TYPE CALCIUM CHANNELS

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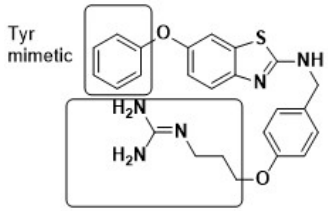
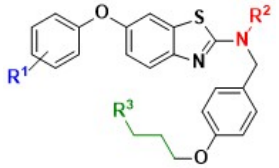
Over the last decade, there has been extensive research into the identification of drug leads to treat chronic pain. Of relevance to this work are the peptides ω-Conotoxins, isolated from the marine cone snail that belongs to the genus *Conus*. ω-Conotoxin GVIA binds essentially irreversibly and selectively to N-type calcium channel, a validated target for the treatment of neuropathic pain and is considered as a potential lead for further drug development. ω-Conotoxin GVIA has a number of key aminoacid residues that are crucial for its biological activity. Small molecule peptidomimetics of ω-conotoxin GVIA, that possess a benzothiazole core scaffold structure and appropriate side-chain mimics, have previously been reported.[1, 2]

In the hope of improving biological activity and selectivity for the N-type calcium channel, we have undertaken a structure-activity relationship study of the previously reported benzothiazole mimetic (**1**).[2]The effect of variation of the substituent on the tyrosine side chain mimetic and substitution of the secondary nitrogen were investigated. All compounds were tested for N-type and T-type calcium channel inhibition using the FLIPR SH-SY5Y neuroblastoma assay. Our synthetic approach to these compounds, their biological activity and pharmacokinetic properties, as well as investigations on other novel promising scaffolds will be discussed in this presentation.



(1)

EC_{50} = 5.8 µM (radioligand displacement assay)



This work

29 compounds synthesized; R^1= H/F/CF_3/OH/CN; R^2= H/CH_3; R^3 = NR^2_2 or N=C(NH_2)$_2$

Best: IC_{50} = 11 µM (N-type); 84 µM (T-type) (FLIPR SH-SY5Yneuroblastoma cell assay)

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POSTERS

High Throughput Screening Strategies to Obtain High Quality Leads

VIRTUAL PHARMACOKINETIC PREDICTION FOR COMPOUND SELECTION IN EARLY DRUG DISCOVERY

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In order to be successful, a drug must have appropriate pharmacokinetics (PK). By definition, it must be possible to achieve a sufficient concentration at the therapeutic target for a sufficient duration in order to achieve the desired therapeutic effect, whilst avoiding concentrations likely to trigger unwanted effects at off-target sites. The provision of PK-related information should proceed in step – in terms of cost, effort and value – with the provision of information regarding therapeutic and toxic effects. In this way, estimation of the therapeutic window, and the consequent likely success of the drug can be most reliably performed. Prediction of PK is becoming well supported in later drug discovery and development by methods such as physiologically based pharmacokinetic (PBPK) modelling for *in vitro-in vivo* and interspecies extrapolation. There is, however, less support for early prediction of PK that can be used alongside receptor screens for hit identification and early pharmacology/safety profiling.

We present a novel *in silico* approach developed to fill this gap, enabling the virtual estimation of human PK without the need for generation of any *in vitro* ADME data or for compound synthesis. The approach is thus complementary to early target binding/safety profiling. It can be used in conjunction with these screens to direct chemistry and to prioritise for synthesis and further investigation compounds that have the appropriate combination of efficacy, safety and PK properties for the proposed therapeutic indication.

The core of the approach is a PBPK model parameterised (with organ sizes, blood flows, intestinal transit times, etc.) for humans. The compound-specific parameters, related to absorption, distribution, metabolism and elimination, that determine the PK behaviour of any given compound are predicted by quantitative structure-property relationship (QSPR) models. These models require as input structural descriptors calculated from a 2D representation of the chemical structure. This 2D structure representation is, therefore, the sole requirement for enabling PK prediction. Currently, the QSAR models within the PBPK represent partition coefficients for 10 major organs, tissues or tissue groups, and the rates of intestinal absorption, hepatic metabolism and renal elimination. Changes to compound structure thus lead to changes in predicted *in vivo* PK via consequent changes in any, or all, of these properties. The PBPK model has been developed by optimisation against several hundred *in vivo* concentration-time profiles following intravenous or oral administration, followed by rigorous statistical model building using an advanced in-house statistical pattern recognition system.

Results for a test set of 62 drugs indicate that PK prediction by the model is robust, with 5 PK parameters (total clearance, half-life, mean residence time, and steady state and elimination phase volumes of distribution) predicted with an average mean-fold error of between 2- and 2.5-fold.

A STRATEGY FOR IN SILICO PREDICTION OF IN VIVO TOXICITY IN EARLY DRUG DISCOVERY

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Manifestions of *in vivo* adverse responses to a drug arise from the drug's intrinsic toxicity coupled with exposure to the drug of the molecular interaction sites through which its toxic effects are mediated. Besides the obvious dose-dependent exposure effect, whereby increasing the amount administered results in increasing the risk of an unwanted effect, exposure of interaction sites is dependent on the individual pharmacokinetic properties of a compound that determine its rate and extent of accumulation at a given location (organ, tissue, cell type, organelle, etc) *in vivo*. The ability to identify these drivers of *in vivo* toxicity via appropriate consideration of pharmacokinetics (PK), tissue accumulation, receptor binding and other relevant molecular interactions is potentially of significant benefit for compound selection. The capacity to predict these properties from compound structure would help in designing out potential toxic liabilities, whilst maintaining *in vivo* efficacy.

Here, we present results on the prediction of *in vivo* toxicity from compound structure using a combination of quantitative structure property relationship (QSPR) and physiologically based pharmacokinetic (PBPK) modelling. PBPK modelling is used to predict the time- and dose-dependent exposure of organs and tissues via the bloodstream. A novel feature of the PBPK model is that, as input, it requires only structural information in the form of structural descriptors calculated from a 2D representation of compound structure. QSPR modelling is used, both within the PBPK model (e.g. for predicting compound-dependent aspects of PK, such as the rate of gastrointestinal tract absorption and extent of partitioning into tissues), and for combining PK-related properties and intrinsic toxicity data for the final *in vivo* toxicity prediction.

We demonstrate initial results on the prediction of human *in vivo* drug tolerability from compound structure. In addition, we show that, using publicly available data for model development, *in silico* prediction of rat acute LD_{50} following oral administration is more reliable than using extant models generated from *in vitro* cytotoxicity data.

THE MEDICINAL AND BIOLOGICAL CHEMISTRY (MBC) LIBRARY: AN EFFICIENT SOURCE ON NEW HITS.

Victor Sebastian (1,3), Carlos Roca (1,3), Mahendra Awale (2,3), Carmen Gil (1), Ana Martinez (1), Jean-Louis Reymond (2), Nuria E. Campillo (1)

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Drug discovery is a time-consuming and expensive process, with an estimated time of at least 12-14 years before the release of a new drug into the market and an approximate cost of 1 billion dollars. Consequently, there is a need to optimize the methods for discovery and optimization of pharmaceutical candidates in order to reduce costs and duration of this step. The identification of new molecule hits remains as one of the biggest challenges in drug discovery today. To succeed in this process it is essential to properly design and characterize chemical libraries.

Our Medicinal and Biological Chemistry (MBC) laboratory has developed an in-house chemical library containing over 1000 compounds primarily focused on neurological and neurodegenerative diseases. This collection of chemicals, which is continuously growing, has been constructed through more than thirty years of experience of our research group in the design and discovery of new drugs for unmet diseases. Our chemical library, in combination with different *in silico* and *in vitro* methods, has been applied to various types of screening programs, resulting in the successful identification of a number of hits against different therapeutic targets, with some of these compounds being currently under clinical trials. [1-4]

Drug-like properties of MBC library has been analyzed using QikProp[5] application which allowed us to compare drug-like properties of candidates with commercially available drugs (Schrödinger software package[6]) and MQN-Mapplet, application that allowed us to visualize projections from 42 molecular quantum numbers (MQNs), defined as counts for simple structural features, creating a multidimensional grid called MQN space. [7-8] Using this last study we have performed a comparative analysis with other known marked chemical libraries.

The theoretical prediction has been assessed through correlation between experimental and predicted values, which bechmarked the reliability of QikProp and confirmed the great potential of MBC chemical library for lead discovery and optimization.

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HIGH-THROUGHPUT PROFILING OF ESTERASES INVOLVED IN HYDROXAMATE LEADS HYDROLYSIS

Paul Hermant, Catherine Piveteau, Alexandre Biela, Damien Bosc, Matthieu Roignant, Benoit Deprez, Rebecca Deprez-Poulain

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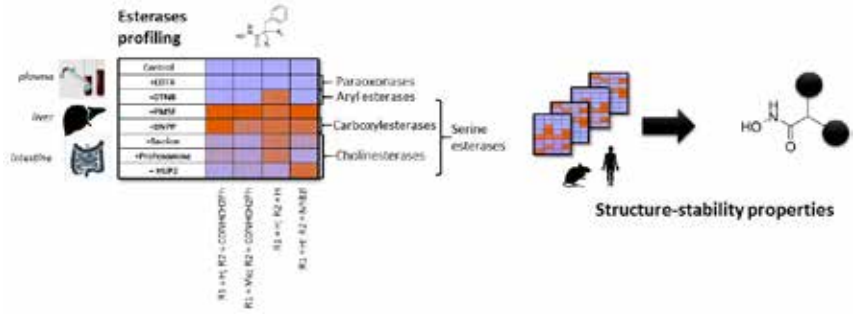
Hydroxamates are valuable leads in drug discovery and tools for chemical biology and target validation. Several hydroxamates have reached the market among which the new class of HDAC inhibitors (vorinostat, belinostat, panobinostat). Developpement of hydroxamate leads to target metalloenzymes requires to take as early as possible in consideration the pharmacokinetic properties. Indeed, some hydroxamates are prone to hydrolysis in plasma (rodent or human). This property can impair their use as in vivo probes or leads since the resulting carboxylic acid is generally less active and presents a different distribution pattern.

To help medicinal chemists to rationalize plasma stability, we have performed the first study of structure-plasma stability for hydroxamates and defined some structural rules to predict or correct this issue in a preclinical stage.

Several esterases are present in plasma and microsomes (both intestinal and hepatic). Importantly, the esterases distribution and proportion differ between rat and human plasma.

Carboxylesterases and cholinesterases are the most important enzymes involved in hydroxamic acids metabolism in plasma. Major species and localization differences (plasma, intestine, liver) have been observed and are commented. In order to have a better understanding of the degradation mechanism of hydroxamic acids that occur *in vivo,* the role of each esterase was investigated using a high throughput methodology.

Structure-stability relationships of different hydroxamic acids compounds in blood plasma, microsomes (intestinal or hepatic) all free of- or combined with-specific esterase inhibitors, allowed identifying which esterase was involved in their degradation. We provide a toolbox for developing molecules containing hydroxamic acid with an improved metabolic stability.



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NOVEL SMALL MOLECULE-BASED INSULIN-REGULATED AMINOPEPTIDASE (IRAP) INHIBITORS IDENTIFIED BY HIGH-THROUGHPUT SCREENING

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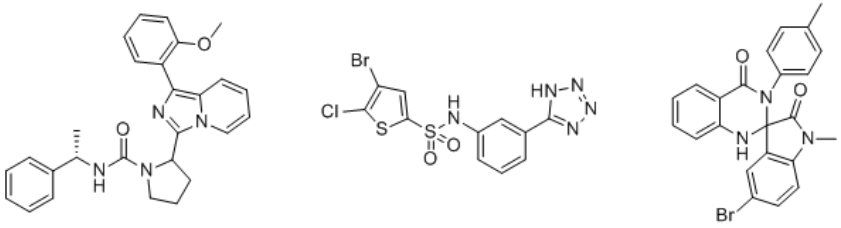
Insulin-regulated aminopeptidase (IRAP), a single-spanning transmembrane zinc-dependent metallopeptidase, is a potential target for cognitive enhancers. Administration of previously known inhibitors has resulted in facilitation of memory and enhanced performance in spatial memory tasks. These abilities have attracted considerable interest in recent years but the majority of known inhibitors are peptidic based with limited CNS penetrance. To identify nonpeptidic IRAP inhibitors, we adapted an established enzymatic assay based on membrane preparations from Chinese hamster ovary cells and a synthetic peptide-like substrate for high-throughput screening purposes.[1] The 384-well microplate-based absorbance assay was used to screen a diverse set of 10,500 compounds for their inhibitory capacity of IRAP. The assay performance was robust with Z′-values ranging from 0.81 to 0.91, and the screen resulted in 23 compounds that displayed greater than 60% inhibition at a compound concentration of 10 μM. After hit confirmation experiments, purity analysis, and promiscuity investigations, three structurally different compounds were considered particularly interesting as starting points for the development of small-molecule-based IRAP inhibitors. After resynthesis, all three compounds confirmed low μM activity and were shown to be rapidly reversible. Additional characterization included activity in a fluorescence-based orthogonal assay and in the presence of a nonionic detergent and a reducing agent, respectively. Importantly, the characterized compounds also showed inhibition of the human ortholog, prompting our further interest in these novel IRAP inhibitors.



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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL CYCLOPROPANE-CONTAINING SEMICARBAZONES DERIVATIVES AS POTENTIAL ANTICONVULSANT AND ANTITUMOR AGENTS

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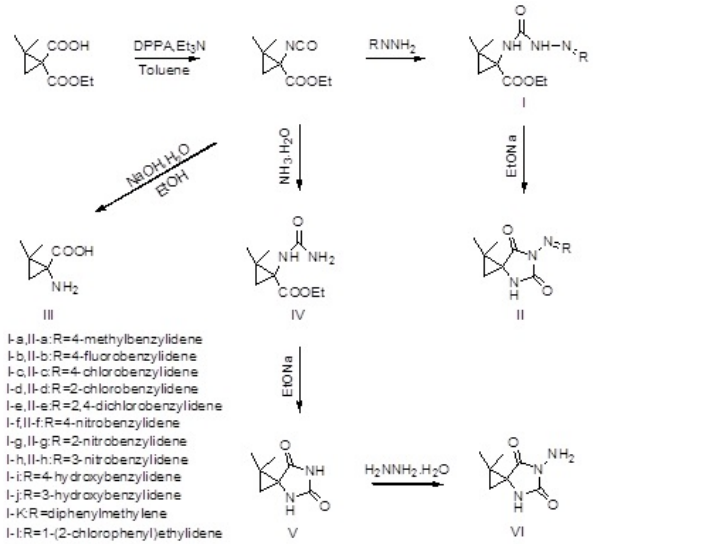
A novel series of cyclopropane-containing semicarbazones derivatives were synthesized and screenedas potential anticonvulsantandantitumor agents. According to combination principles, we found a way to mash up hydantoin core with semicarbazone. In other words, some atoms in those agents would not only be a part of the hydantoin core but also a member of the semicarbazone group and evaluated for their anticonvulsant activities using maximal electroshock shock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure models in mice. Nineteen novel target compounds I-a~l and II-a~h were synthesized. The physiological activity of the new compounds is not due to cyclopropane structure. The activity experimental study showed that II-a~d contained the lowest median effective dose (ED_{50}) of 100mg/kg in MES test, and II-b~d the lowest ED_{50} of 300 mg/kg in scPTZ test. In addition to compound III, VI and I-j, all compounds had severe neurotoxicity. In the mechanism of drug action and drug side effects, among these compounds IC_{50} value of compound II-d against Hela was 23.7μM; IC_{50} values of compound I-d against HepG2 was 26.7μM. they did not show activities against the other cell lines which had been tested. These promising data suggested that the new compounds can be a new class of anticonvulsantand antitumor agents with high effectiveness and low toxicity for the treatment of epilepsy.



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LEARNINGS FROM HIGH THROUGHPUT SCREENING IN ANTIFUNGAL RESEARCH

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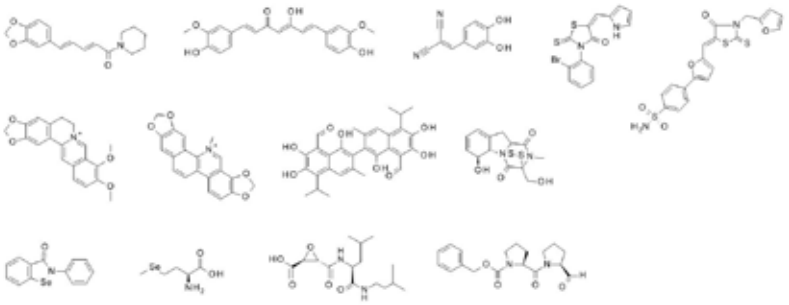
There is not a lot of literature covering the topic of PAINS and other frequent hitters in phenotypic screening.[1] In this poster, we would like to share the learnings of high throughput screening (HTS) of 41000 compounds on phenotypic antifungal assays. The molecules reported in the Figure 1 are examples of compounds showing some antifungal activities at 20 ppm but failed once progressed on higher tier tests. The problems remain very similar to what has been reported for the *in vitro* assays with Michael acceptors, rhodanines, natural products embedded with undesirable features and other reactive groups.



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NOVEL POTENTIAL PROTEASOMAL INHIBITORS BASED ON SALICYLAMIDES

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Inhibition of protein degradation is one of strategies for suppression of uncontrolled proliferation of cancer cells. Proteolytic degradation is in cells mainly ensured by proteasome and its inhibition by bortezomib showed benefit in clinical use for the treatment of cancer. To date, most proteasomal inhibitors can be divided into 5 major groups: peptide aldehydes, peptide boronates, peptide vinyl sulphonates, peptide epoxyketones and β-lactones. In addition, there are several groups of natural proteasome inhibitors and ther drug-like synthetic compounds carrying a non-peptide scaffold.

Preliminary screening of a compound library for proteasomal inhibitors generated positive hits from a library of 2-hydroxy-*N*-[1-(2-hydroxyphenylamino)-1-oxoalkan-2-yl]benzamides, hereafter referred to as diamides [1]. These diamides show structural similarity to the proteasome inhibitor and clinically used anticancer drug bortezomib. We have prepared new compounds (*O*-benzyl-5-chlorosalicyl-tripeptide aldehydes, *O* -benzyl-5-chlorosalicyl-dipeptide aldehydes and other derivatives) and tested them for their antiproteasomal activities in cells.

For monitoring anti-proteasomal activity of novel compounds, we used a cell-based assay involving U2OS cells expressing green fluorescence protein (GFP) fused to a short degron that is rapidly degraded by a proteasome. Inhibition of the proteasome then leads to the accumulation of GFP in treated cells. Inhibitor-treated cells were visualised by immunofluorescence microscopy or live-cell imaging system.

In addition, we also monitored the accumulation of polyubiquitinated proteins in treated cells by indirect immunochemical methods, using specific antibodies towards polyubiquitin that recognise polyubiquitin chains linked by Lys48, which predominantly targets proteins for proteasomal degradation. We also analysed levels of proteins with a high turnover that are degraded by a proteasome, e.g. MDM2 . Because bortezomib has been found to strongly induce protein levels of CDK inhibitors p27 and p21 independently of p53 we also evaluated levels of these proteins in treated cells using specific antibodies.

Bortezomib has been shown to promote apoptotic cell death in some cancer cell lines, therefore, we evaluated the mechanism of cell death for novel active compounds. Activation of apoptosis was verified biochemically by measuring the activity of caspases 3, 7 and 9 using the fluorescently-labelled substrates Ac-DEVD-AMC and Ac-LEHD-AMC, respectively. These results will be complemented by immunoblotting of active caspases 3, 7 and 9, an 89-kDa fragment of poly-ADP-ribose-polymerase (PARP-1).

The authors greatfully thank for the financial support to the Ministry of Education, Youth and Sport (not specified SG support project for Ph.D. students of the Faculty of Chemical Technology).

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MCR FOR NOVEL HETEROCYCLE SYNTHESIS

Pravin Patil, Alexander Dömling

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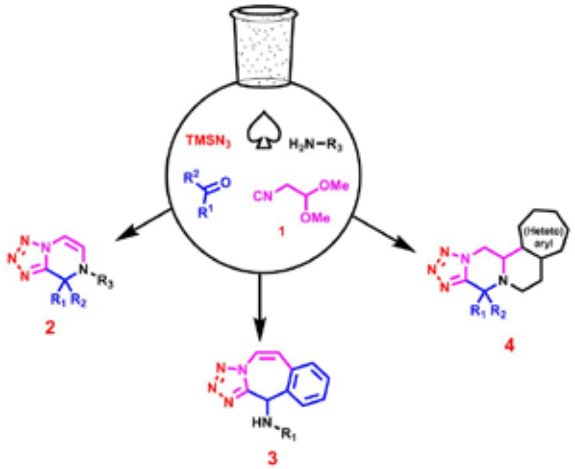
The European Lead Factory (ELF) is a collaborative public-private partnership aiming to deliver innovative drug discovery starting points. By 2017, 500.000 novel compounds should compose the ELF library.[1] Prof. Dömling laboratory is one of the research partner of ELF to develop new scaffolds for molecular libraries synthesis using multicomponent reaction (MCR) chemistry. MCR leads to target scaffolds in very few synthetic steps in minimum time to accomplish libraries of suitable size.

Here we report three different novel heterocyclic scaffolds easily accessible *via* isocyanide based MCR. These scaffolds have in common – a facile Ugi tetrazole reaction which is further expanded with the use of a bifunctional isocyanide.[2,3]



Depending on the target molecules various amines and aldehydes or ketones were employed for the reaction. The isocyanide derived from dimethyl acetal afforded **2**, **3** and **4** in good to excellent yield *via* post condensation.[2,3]

Funding: *The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution*

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PREPX FOR STREAMLINING CRYSTAL EXPLORATION IN DRUG DISCOVERY

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The European Lead Factory is a pan-european platform for lead discovery and optimization by High Throughput Screening, available to users across academia and industry. The main output from HTS and secondary validation is the "improved hit list" (IHL); but further optimization of these compounds, whether for potency, specificity or cellular activity, requires more information. Within ELF, our team provides support for one of the most powerful tools for guiding this process, namely direct observation of compound binding by X-ray crystallography. The primary challenge in ligand soaking or co-crystallization studies is availability of an appropriate crystal system: not only are published crystals notoriously difficult to reproduce at all, but in general access to multiple crystal forms and crystallization conditions are required to ensure success.

Our team is embedded in the Structural Genomics Consortium (SGC), which a decade ago established the crucial role of high-throughput approaches and parallel exploration of expression constructs for producing reliable protein crystals. To implement these learnings for the ELF challenge, we have further streamlined the experiments by developing a process, Parallel Rapid Expression, Purification and Crystallization (PREPX) that allows testing of large numbers of constructs with minimal manual effort, up to 48 per week. PREPX has enabled us to explore multiple solubilisation strategies simultaneously, and investigate a large number of surface mutations for their effect on crystallization. We show how this process allows obtaining new crystal forms for a number of different ELF targets, and their importance in crystal-based ligand binding studies, and present progress on applying PREPX to expression systems other than *E.coli*.

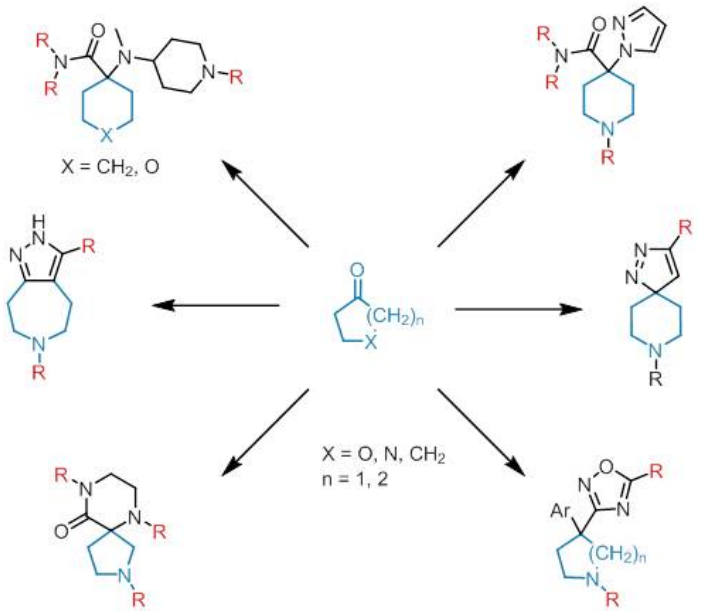
The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement nÂ° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies™ in-kind contribution.

CYCLIC KETONES AS BUILDING BLOCKS FOR DIVERSE LIBRARY SCAFFOLDS

Christopher Pearce, Jack Thomas, Ian Strutt, Gavin Milne, Guillame Parra, Lauren O'Neil, Daniel Hamza, Iain Miller, Brett Stevenson, Po Man Liu, Stefan Jones, Hayley Watson, Geraint Jones

Sygnature Discovery, BioCity, Pennyfoot Street, Nottingham, NG1 1GF

Cyclic ketones have proven to be a very useful and versatile starting point giving rise to a diverse set of sp^3-rich scaffolds in only 2 – 3 steps. This poster will present the synthesis and properties of a number of libraries that utilised simple cyclic ketones as common starting materials.



Chemistry includes a combination of nucleophilic aromatic substitution and oxadiazole formation to afford scaffolds containing 3 points of diversity. We also present a range of libraries utilising the Bargellini reaction that provides access to cores containing carboxylic acids next to a quaternary centre. Additionally, an interesting 3+2 cycloaddition followed by a sigmatropic ring expansion gives rise to spirocyclic and fused pyrazoles respectively.

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HETEROGENEOUS CATALYSIS: A KEY TOOL IN THE SYNTHESIS OF SP3-RICH SCAFFOLDS

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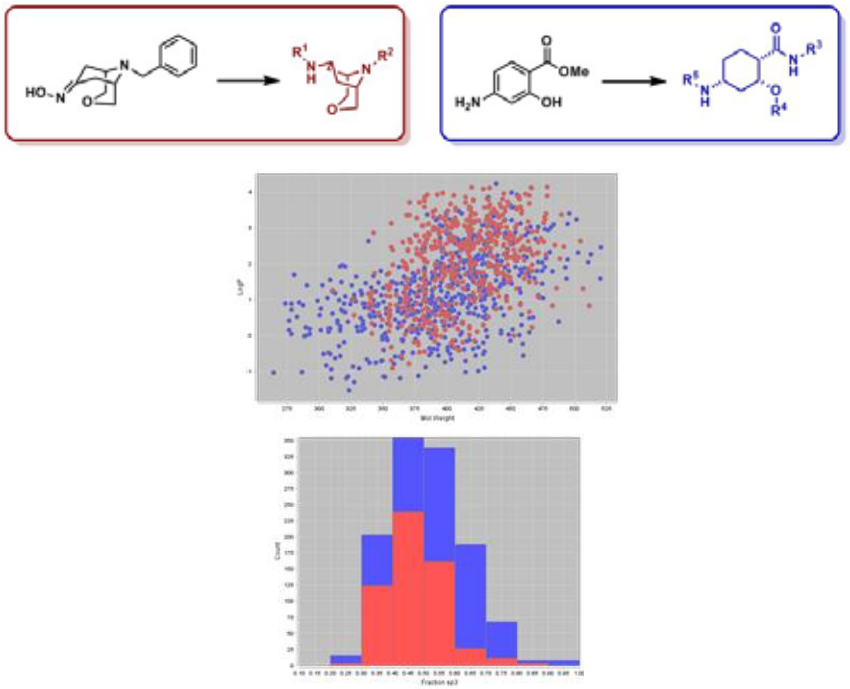
Heterogeneous catalysis is a key tool that has allowed access to multiple sp^3-rich scaffolds that have been further diversified to provide two distinct IMI European Lead Factory libraries. This poster will describe the chemoselective rhodium-catalysed reduction of an oxime to a primary amine in the presence of an *N*-benzyl-protected amine and the hydrogenation of a benzoate ester to give a highly functionalised all-*syn* cyclohexane product. Our subsequent elaboration of these scaffolds gave rise to libraries with highly desirable properties encompassing a wide range of chemical space.



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ENCODED SELF-ASSEMBLING CHEMICAL (ESAC) LIBRARIES: A POWERFUL TECHNOLOGY FOR LIGANDS DISCOVERY AND AFFINITY MATURATION

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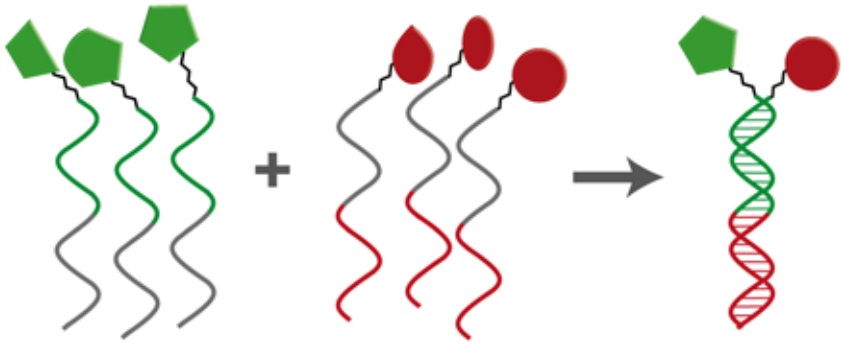
1) Philochem AG, 8112, Otelfingen, Switzerland
2) Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Science, ETH Zurich, 8093, Zurich, Switzerland

The isolation of small organic molecules capable of high-specific binding to biological targets is a central problem in chemistry, biology and pharmaceutical sciences. Small organic molecules with high binding affinity and specificity to disease-associated antigens can find application for selective pharmacodelivery of bioactive payloads at the site of disease. [1] In spite of advances in combinatorial chemistry and other chemical methodologies, the generation of high-affinity ligands to biological targets remains a formidable challenge. Encoded Self-Assembling Chemical (ESAC) libraries allow facile identification of small molecular-weight binders to macromolecular targets. [2] ESAC technology uses libraries of organic molecules linked to individual oligonucleotides that mediate the self-assembling of the library and provide a code associated with each organic molecule. By means of self-assembly, relative small sub-libraries (requiring A + B synthesis steps) can easily yield very large DNA-encoded libraries (A x B dual pharmacophores). Using this approach, we have successfully discovered new high-affinity binders to targets of pharmaceutical interest. [3]



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POSTERS

Late Breaking News

EUROPEAN LEAD FACTORY – GAME CHANGING FOR INNOVATIVE MEDICINE

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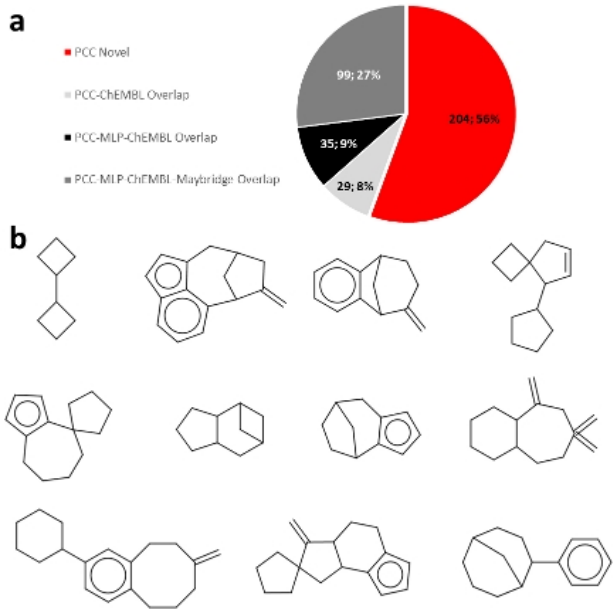
The so-called "patent cliff" has been a much discussed and feared event in the pharmaceutical industry. Numerous blockbuster drugs have come off patent in the recent years and more will do so until nowadays. In order to sustain Europe's position in the global health care business, new approaches in the pharmaceutical R&D process are being considered: crowd sourcing and open innovation.
The European Lead Factory (ELF) - a pan-European initiative for drug discovery organized by the Innovative Medicines Initiative (IMI) as a public private partnership – was set in 2013 to address this issue and to give a major boost to early drug discovery in Europe. It is composed of two main elements: the Joint European Compound Collection and the European Screening Center.
High throughput screening (HTS) represents a major cornerstone of drug discovery. The availability of an innovative, relevant and high quality compound collection to be screened often dictates the final fate of a drug discovery campaign. As the chemical space to be sampled in research programs is practically infinite and sparsely populated, significant efforts and resources need to be invested in the generation and maintenance of a competitive compound collection. The novelty, diversity, structural complexity, physicochemical characteristics and overall attractiveness of the first 100.000 Public Collection of ELF Compounds for HTS purposes will be presented in order to illustrate the innovative approach. The ELF public compounds collection (PCC) will be compared with molecules from i) a commercial, diverse screening collection; ii) Molecular Library Program (MLP) of the National Institutes of Health in the USA (NIH) and iii) ChEMBL collection.



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2) The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115489, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in-kind contribution.

ALCHEMICAL FREE ENERGY CALCULATIONS AND ISOTHERMAL TITRATION CALORIMETRY MEASUREMENTS OF AMINOADAMANTANES BOUND TO THE CLOSED STATE OF INFLUENZA A/M2TM

Harris Ioannidis (1), Antonios Drakopoulos (1), Christina Tzitzoglaki (1), Felix Kolarov (2), Kathrin Freudenberger (2), Paraskevi Gkeka (3), Günter Gauglitz (2), Zoe Cournia (3), Antonios Kolocouris (1), Nadine Homeyer (1), Christos Liolios (2), Holger Gohlke (3)

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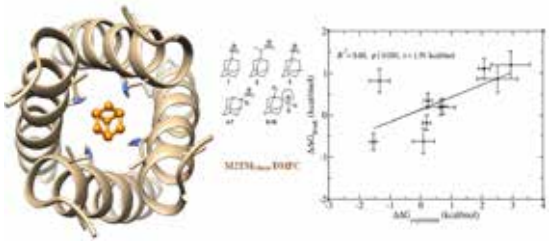
3) Biomedical Research Foundation, Academy of Athens, 11527 Athens, Greece



Adamantane derivatives, such as amantadine and rimantadine, have been reported to block the transmembrane domain (TM) of the M2 protein of influenza A virus (A/M2) but their clinical use has been discontinued due to evolved resistance in humans. Although experiments and simulations have provided adequate information about the binding interaction of amantadine or rimantadine to the M2 protein, methods for predicting binding affinities of whole series of M2 inhibitors have so far been scarcely applied. Such methods could assist in the development of novel potent inhibitors that overcome A/M2 resistance. Here we show that alchemical free energy calculations of ligand binding using the Bennett acceptance ratio (BAR) method are valuable for determining the relative binding potency of A/M2 inhibitors of the aminoadamantane type covering a binding affinity range of only ~ 2 kcal mol^{-1}. Their binding affinities measured by isothermal titration calorimetry (ITC) against the A/M2TM tetramer from the Udorn strain in its closed form at pH 8 were used as experimental probes. Two different A/M2TM peptide sequences were used for binding affinities measurements, Udorn (WT) and Weybridge bearing the two mutations, i.e. V28I and L38F. The relevant peptides were synthesized through Solid Phase Peptide Synthesis. Two series of alchemical free energy calculations were performed for Udorn complexes using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipids to mimic the membrane environment. A fair correlation was found for DPPC (R2=0.58) that was significantly improved using DMPC (R2=0.87), which resembles more closely the DPC lipids used in the ITC experiments. This demonstrates that binding free energy calculations by the BAR approach can be used to predict relative binding affinities of aminoadamantane derivatives towards M2TM with good accuracy. For Weybridge sequence the correlation was low possibly due to the lack of an experimental structure. These results led to the synthesis of a new analogue proved to be a nanomolar inhibitor against S31 WSN/33 virus.

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5) ACKNOWLEDGMENT. The research was supported from Chiesi Hellas.
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THE DISCOVERY OF NOVEL P2X7 ANTAGONISTS FOR THE TREATMENT OF DEPRESSION

The P2X7 receptor is a ligand-gated ion channel that is expressed in glial cells in the CNS and peripherally in monocytes. Activation of the P2X7 ion channel leads to activation of downstream signaling pathways and secretion of IL-1β, among other pro-inflammatory cytokines. Secretion of IL-1β from glial cells in the CNS is hypothesized to participate in the initiation of a neuroinflammatory cascade and this neuroinflammation likely contributes to a variety of neurological disorders. In order to characterize the role of activation of the P2X7 receptor in neuroinflammation Janssen Neuroscience became interested in the identification of novel P2X7 antagonists that penetrate the CNS. This presentation will focus on a discussion of efforts that led to the discovery of highly selective and potent P2X7 antagonists that are brain penetrant and demonstrate target engagement of the P2X7 receptor in rat brain as measured by ex-vivo autoradiography. The presentation will also discuss medicinal chemistry efforts that lead to the identification of a candidate for clinical development.

NOTES

EFMC
International Symposium
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Manchester, UK Aug. 28 – Sept.1, 2016

POSTERS

Showcase Brazil

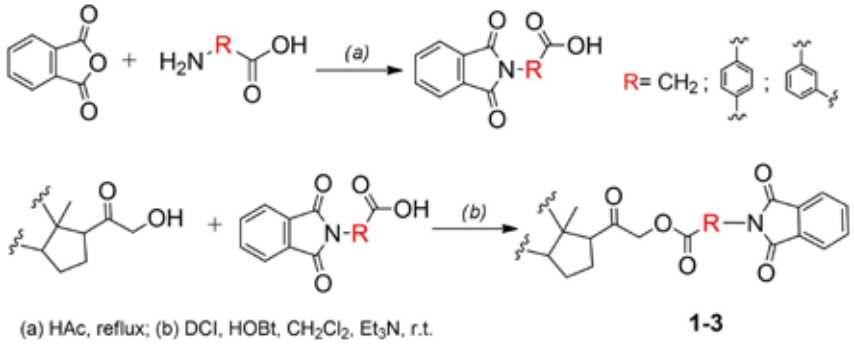
SYNTHESIS, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF NEW PREDNISOLONE DERIVATIVES

Marcella Gabrielle Mendes Machado, Cauê Benito Scarim, Rafael Consolin Chelucci, Jean Leandro dos Santos, Chung Man Chin

Faculdade de Ciências Farmacêuticas, UNESP – Univ Estadual Paulista, Rodovia Araraquara-Jaú Km 1, CEP 14800-903, Araraquara, SP, Brazil

Introduction. Therapy anti-tumor necrosis factor α (anti-TNF-α) has become an interesting approach in the treatment of inflammatory diseases, especially those patients who do not respond to conventional treatment [1]. Phthalimide derivatives, with modulating properties of cytokine TNF-α, have been reported in the literature with multiple potential therapeutic effects including anti-inflammatory, antiangiogenic and anticancer effects [2]. The glucocorticoids are used in the treatment of chronic inflammatory diseases, however, the various side effects of long-term glucocorticoid therapy have limited their use in clinical practice [3]. So, in this work, using molecular hybridization prednisolone was condensed with phthalimide derivatives using different spacers to obtain new compounds (**1-3**) in order to achieve a synergism of action useful for the treatment of inflammatory diseases.

Methodology. *1.Synthesis*. The compounds (**1-3**) were obtained in two steps: a) synthesis of phthalimide derivatives by condensation of appropriately functionalized amine to phthalic anhydride using acetic acid as solvent; b) nucleophilic substitution using coupling agents (DCI, HOBt) in dichloromethane at low temperature (Fig 1). *2. Antinociceptive activity*. Analgesic activity was determined *in vivo* with the acetic-acid-induced (0.6%, 0.1 mL/10 g) abdominal constriction test in mice. The compounds and prednisolone (used as the standard drug) were administered orally (100 µmol/kg) as a suspension in 5% arabic gum in saline (vehicle) 1 h before acetic acid solution administration (*i.p.*). The number of constrictions per animal was recorded for 20 min. Antinociceptive activity was expressed as percentage inhibition of the constrictions compared with those in the vehicle-treated control group. *3. Anti-inflammatory activity*. Anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema model. Edema was induced by subplantar injection of carrageenan (1%, 0.1 mL/paw) into the right-hind paws. The compounds were administered orally (100 µmol/kg) 1 hour before carrageenan injection. The control group did not receive any oral treatment. Paw thickness (mm) were measured just before the treatment and then hourly for 6 hours after carrageenan injection.



Results. *1. Synthesis*. The compounds were obtained after silica gel column chromatography purification with global yield variable between 41-52%. All compounds were characterized by NMR 1H, NRM 13C, infrared spectroscopy and mass spectrometry. *2. Analgesic activity.* All compounds demonstrated inhibition of abdominal constriction between 34-46% while prednisolone inhibited 30%. *3. Anti-inflammatory activity*. Derivatives **1** and **2** showed anti-inflammatory activity superior to the parent drug (prednisolone) after 60 min. Derivative **3** demonstrated weaker anti-inflammatory activity than the parent drug after 120 min.

Conclusions. The hybrid compounds were obtained with good yields and showed analgesic and anti-inflammatory activity superior to prednisolone. In vitro studies anti-TNF-α activity are in progress.

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POSTERS

Other

DESIGN AND SYNTHESIS OF BENZOTHIAZOLE SCHIFF BASES OF POTENTIAL ANTITUMOR ACTIVITY

Thuraya Al Harthy (1), Raid Abdel-Jalil (1), Wajdi Zoghaib (1), Maren Pflüger (2), Elisabeth Hofmann (2), Harald Hundsberger (2)

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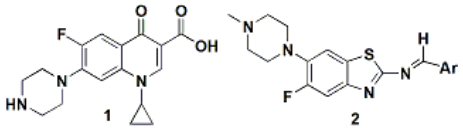
Benzothiazoles considered as privileged structure known to exist in many natural product and pharmaceuticals. Moreover, fluorine and piperazine are common appendages in medicinal chemistry due to their immense utilities in drug design and their unique bioactivities. The combination of both fluorine and piperazine moieties may enhances the biological activity.1 For instance, ciprofloxacin® (1) is a powerful antibiotic with a broad spectrum of biological activity and the optimum activity, which is attributed to the presence of fluorine and piperazine2 Figure 1.



1

2

As part of our ongoing research devoted toward the synthesis of potential bioactive heterocyclic systems, herein we report the synthesis and cytotoxicity of a novel series of benzothiazole Schiff bases, (4-sbstituted-benzylidene)-[5-fluoro-6-(4-methylpiperazin-1-yl)-benzothiazol-2-yl]-amines (2) Figure 1. The preliminary results show that at least one compound, (4-Fluorobenzylidene)-[5-fluoro-6-(4-methyl-piperazin-1-yl)-benzothiazol-2-yl]-amine, has specific anticancer cytotoxicity while leaving primary non-transformed cells unharmed which can be studied further.

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ANTIOXIDANT ACTIVITIES OF MANNICH BASES OF KOJIC ACID DERIVATIVES

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Among antioxidant compounds, kojic acid which is a fungal metabolite commonly produced by many species of *Aspergillus*, *Acetobacter*, and *Penicillium* has been highlighted because it presents antioxidant activity by chelating iron ions as well as the copper ion present in the active site of tyrosinase enzyme (1). So, it presents depigmentation activity preventing enzymatic browning, and in cosmetic preparations as a skin-lightening or bleaching agent. However, kojic acid is not stable at high temperatures and presents a labile oxidative property against light. Therefore, many kojic acid derivatives are synthesized to solve this problem.

Previously, some Mannich bases of kojic acid derivatives were synthesized in our laboratory and examined for their various biological activities including anticonvulsant, antibacterial, antifungal and antiviral activities with cytotoxicity (2-7). Hence, in the light of these findings, herein, some new Mannich bases of kojic acid were synthesized and their antioxidant activities were evaluated.

Antioxidant activity of the compounds and kojic acid is determined by the methods of 2,2-diphenyl-1-picrilhydrasil (DPPH), *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) radical scavenging activity, metal chelation effect, iron-(FRAP), phosphomolybdenum-(PRAP) reducing antioxidant power (8-12). An examination of the results of these methods revealed that Mannich bases (58.17-67.57 µg/ml) at a dose amount of 1000 µg/ml has a mild iron chelation effect higher than kojic acid (8.89±0.75 µg/ml) but lower than the reference compound ethylenediaminetetraacetic acid (EDTA, 97.66±0.12- 2000 µg/ml). Since iron ion and other transition metal ions catalyze oxidation in the body, it is important to examine metal chelation effect of an antioxidant. A comparison of antioxidant power of phosphomolybdenum structure and antioxidant effects demonstrates that Mannich bases (0.154-0.187 µg/ml), though lower than the reference compound flavonoid quercetin of vegetable origin (0.320±0.005 µg/ml), shows a higher antioxidant effect than kojic acid (0.103±0.006 µg/ml).

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DISCOVERY OF 4,5-DIARYLISOXAZOL-3-CARBOXYLIC ACID SKELETON AS A NOVEL CHEMOTYPE FOR INHIBITION OF 5-LIPOXYGENASE ACTIVATING PROTEIN (FLAP)

Burcu Caliskan (1), Ersan Celikoglu (1), Jana Gerstmeier (2), Susanna Voelker (2), Ulrike Garscha (2), Abdurrahman Olgac (1), Andrea Carotti (3), Antonio Macchiarulo (3), Oliver Werz (3), Erden Banoglu (1)

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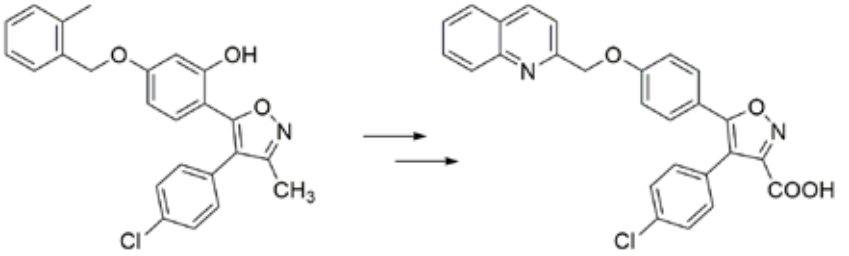
Pharmacological intervention with leukotriene (LT) biosynthesis is a clinically validated strategy for treatment of respiratory and cardiovascular diseases such as asthma and atherosclerosis. 5-Lipoxygenase (5-LO) catalyzes the first step of LT biosynthesis to produce the unstable epoxide LTA_4 from arachidonic acid (AA), which is further metabolized to LTB_4 or cysteinyl LTs (cys-LTs). This first step also requires the involvement of a helper protein, namely 5-LO-activating protein (FLAP), which acts as a regulatory protein by interaction with 5-LO for the transfer of AA to 5-LO for efficient synthesis of LTs. Although no FLAP inhibitor has yet reached the market, several FLAP inhibitors to date such as AM803 (GSK2190915), AZD6642 and BI665915 were reported to be in various stages of preclinical and clinical studies for treatment of asthma and COPD. In this presentation, we report a novel FLAP inhibitor based on the previously identified 4,5-diaryl-3-methylisoxazole derivative (**1**), which was moderately effective in a cell-based assay (IC_{50}=4.4 µM) and directly inhibited isolated 5-LO (IC_{50} =1.9 µM). Structural optimization of (**1**) resulted in 4-(4-chlorophenyl)-5-[4-(quinolin-2-ylmethoxy)phenyl]isoxazol-3-carboxylic acid (**2**), which exhibited remarkable inhibition of cellular LT biosynthesis targeting FLAP (IC_{50} of 8 nM and 10 nM in human monocytes and neutrophils, respectively) with negligible inhibition of 5-LO (IC_{50} = 8 µM). (**2**) also demonstrated good in vivo efficacy by inhibiting zymosan-induced peritonitis with ID_{50} of 7 mg/kg in mice. Together, our results exemplify the 4,5-diarylisoxazole-3-carboxylic acid scaffold as a new chemotype for further development of effective FLAP inhibitors (This study was supported by TUBITAK research grant 112S596).



1 (5-LO inh.)
IC_{50}PMNL=4.4 µM
IC_{50}5-LO=1.9 µM

2 (FLAP inh.)
IC_{50}PMNL=0.01 µM
IC_{50}mono=0.008 µM
IC_{50}5-LO=8.0 µM

PHOSPHOLIPID-BASED PRODRUGS FOR THE TREATMENT OF IBD: DRUG TARGETING STRATEGY

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Phospholipase A2 (PLA2) expression/activity is significantly elevated in inflamed intestinal tissue in inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis. PLA2 hydrolyses the *sn*-2 fatty acyl bond of phospholipids (PL) liberating a fatty-acid and a lysophospholipid. By replacing the *sn*-2 positioned fatty-acid with a drug, PLA2 may be exploited as a prodrug activating enzyme, liberating the free drug from the PL-complex. Therefore, orally delivered PL-based prodrugs will release the free drug at the inflamed sites, effectively targeting the regions of intestinal inflammation. We have utilized a modern computational approach to simulate the PLA2-mediated activation using the candidate drug, and to predict the most appropriate linker length. We have synthesized PL-diclofenac conjugates and shown in-vitro activation of these synthesized conjugates by isolated bee venom PLA2 and conditioned medium from inflamed Caco-2 cell line. We showed that depending on the linker length between the PL and diclofenac, PLA2 could be exploited as the activating enzyme *in-vitro*, liberating the free diclofenac from the PL complex. We have compared the computational calculations to our experimental data, and obtained excellent correlation between the *in-silico* predictions and the *in-vitro* experiments. The proposed research may significantly improve drug therapy in IBD patients, enabling higher efficacy and lower toxicity profiles.

COMPUTATIONALLY DRIVEN DRUG DISCOVERY IN STRUCTURE-ENABLED PROGRAMS: FREE ENERGY CALCULATIONS

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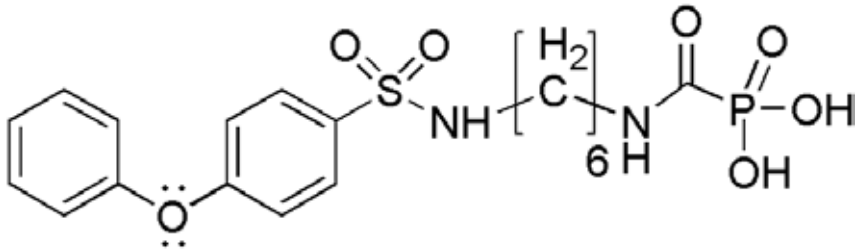
Over the past decade, free energy calculation methods have matured to the point where they can have a significant positive impact on drug discovery programs. These methods provide insight into the energetics of waters in the active site hich greatly facilitates drug design and the ability to predict relative binding affinities allowing for optimal selection of compounds for synthesis. This presentation will focus on the workflows utilized by Schrödinger's drug discovery group with articular emphasis on the free energy calculation methods implemented in FEP+. Both retrospective and prospective examples will be included that illustrate the use of FEP+ in the rapid and efficient optimization of potency, selectivity and solubility; including the use of these approaches in Nimbus drug discovery programs.

CARBAMOYLPHOSPHONATE ENZYME INHIBITORS AS ANTICANCER DRUGS

Eli Breuer

Hebrew University of Jerusalem
Israel

The tumor microenvironment contains certain extracellular zinc enzymes that safeguard the cancer and support its proliferation and dissemination. These water soluble enzymes are (a) matrix metalloproteinase-2 (MMP-2), that degrades basement membrane and opens the way to the dissemination of metastases; (b) carbonic anhydrases CAIX & XII that regulate tumor micro-environment pH to support tumor survival; and finally (c) autotaxin, (ATX) that converts lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA) which supports cellular proliferation, tumor growth and metastasis. We developed water soluble carbamoylphosphonic acid (**CPO**) inhibitors to maximize their chances to encounter **and inhibit the enzymes in the extracellular space**, away from cancer or healthy cells.



Our compounds inhibit all three types of enzymes mentioned. Inhibition of MMP-2 prevents the dissemination of metastases.[1] Inhibition of CAIX or CAXII raises the pH of the extracellular fluid disabling the tumor cells,[2] or the same CPO on ATX stops the generation of LPA and tumor proliferation.[3,4] Successful inhibition of these enzymes by CPOs disables cancer and results in a new kind of extracellular, nontoxic antimetastatic & anticancer therapy.

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TOWARDS THE NOVEL SEQUESTRATING AGENTS FOR USE IN BIOLOGICAL SYSTEM

Abeer BUKHARI, Peter QUAYLE

School of Chemistry. University of Manchester

Sidrophores are a small iron sequestrating/chelating compounds which coordinates with an iron metal (Fe^{3+})to form a chelate or complex. sidrophore is used for the sequestration of iron in living tissues because the free ferric ion is toxic in living systems. Sidrophores can find in nature such as enterocheline[1] or synthetic such as vibriobactin A[2].

Enterochelin is a naturally siderophores, which are found in bacteria and fungi. The work presented in this poster the synthesis of macrocyclic template which is mimic enterochelin based upon a 2,3-dihydroxtbenzenesulfonamide (Enterocheline analogue), which is prepared by reacting hydrochloric lactone with sulfonyl chloride, to develop properties.

We wish to use of this approach in the design of sensors for application in the early diagnosis of neurodegenerative diseases.

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SYNTHESIS AND EVALUATION OF A PDE1 PET LIGAND

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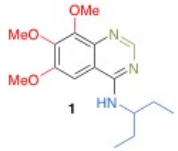
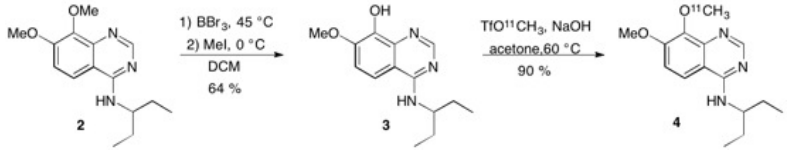
3) Department of Discovery Chemistry/DMPK, H. Lundbeck A/S, Ottiliavej 9, 2500 Valby, Denmark.

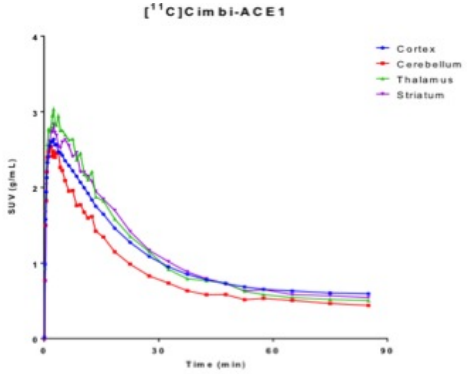
Phosphodiesterase (PDE) catalyzes the breakage of cAMP/cGMP to AMP/GMP and are therefor important regulators in intracellular messaging. Inhibition of one of the eleven isoforms, PDE1, has shown positive effect on cognition, thus being a potential drug target for Alzheimer´s and Huntington´s disease.[1] An effort was undertaken to develop a potent PDE1-inhibitor by a Structure Activity Relationship (SAR) study of an known PDE1 inhibitor[2] (**1**) by altering 1) the three methoxy groups, 2) the quinazoline moiety or 3) the C-4 amine.



Compounds synthesized were all evaluated based on the potency and selectivity towards PDE1 and the inhibitor with the highest potency and selectity (**2**) was demethylated followed by ^{11}C-labelling.



Injection into Danish landscape pig and Positron Emission Tomography (PET) monitoring shows passage of **4** across the blood brain barrier (BBB) but is rapidly removed from the brain and is rapidly metabolized.



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IMMOBILIZATION OF METAL ORGANIC FRAMEWORK MATERIALS INSIDE CAPILLARY FOR ELECTROCHROMATOGRAPHIC SEPARATION OF PHARMACEUTICALS

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Metal organic framework materials (MOFs) are a group of new materials. Scientists are paid attention on their applications in different fields. We are interested in the study of MOFs application in separation science. In our group, we have successfully developed several immobilization methods of MOFs such as HKUST-1, MOF-5 for the preparation of capillary electrochromatographic and solid-phase microextraction columns. I like to present recent achievements in this topic in my group. For example, we have developed a new method for the growth of HKUST-1 on the inner wall of capillary by using liquid-phase epitaxy process at room temperature. The fabricated HKUST-1@capillary can be successfully used for the separation of substituted benzene including methylbenzene, ethylbenzene, styrene, chlorobenzene, bromobenzene, o-dichlorobenzene, benzene series, phenolic acids, and benzoic acids derivatives. High column efficiency of 1.5 x 10^5 N/m for methylbenzene was achieved. The formation of HKUST-1 grown in the capillary was confirmed and characterized by scanning electron microscopy images, Fourier transform infrared spectra and X-Ray diffraction. The column showed long lifetime and excellent stability. The relative standard deviations for intra-day and inter-day repeatability of the HKUST-1@capillary were lower than 7%.

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SMART CHEMICAL LIBRARIES FROM PRESTWICK CHEMICAL: POWERFUL TOOLS FOR HIGH-QUALITY HIT DISCOVERY

Jean-Marie Contreras (1), Laurent Schaeffer (1), Christophe Morice (1), Laurence Jung (1), Jean-Marc Simon (1), Marie-Louise Jung (1), Bruno Didier (2)

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Despite all current efforts dispensed in drug discovery, safety-related problems continue to be one of the major causes for drug attrition in preclinical and clinical development. In order to circumvent this concern, toxicity issues should be considered as early as possible and smart chemical libraries be privileged for screening. Therefore this poster will present the features of three smart chemical libraries – the Prestwick Chemical Library ® (PCL), the Prestwick CNS Drug Library and the Prestwick Drug-Fragment Library –. Designed to ensure high-quality hits and therefore reduce cost of the initial screening, they can be considered as powerful tools for hit discovery. The PCL is a product arising from medicinal chemistry expertise, comprising 1280 off-patent drugs, thus presenting a large degree of chemical and pharmacological diversity within a relatively small number of compounds. The Prestwick CNS Drug Library is a unique collection of 320 structurally diverse approved and marketed drugs carefully selected for their known pharmacological effects on the central *nervous system.* The Prestwick Drug-Fragment Library is a collection of 2228 fragments arising from the smart fragmentation of the PCL approved drugs. The fragmentation process was performed manually, based on our expertise in medicinal chemistry.

Over the 16 past years, “hit-likeness” and “hit-workability” of our smart chemical libraries have been substantially reported by users.

MONITORING LIGAND-ASSOCIATED STRUCTURAL CHANGES OF A FLEXIBLE ENZYME WITH A GENETICALLY ENCODED NON-NATURAL FLUORESCENT AMINO ACID

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Flexible drugs targets are often incompatible with standard structure-based drug design and discovery methods. This is because they are capable of adopting many conformations in solution, which can vary widely in structure and can be difficult to predict. This study examines the flexible enzyme glutamate racemase (GR), a bacterial cell wall enzyme responsible for the turnover of L- to D-glutamate, an essential component of bacterial peptidoglycan, that has been identified as a promising antibiotic drug target. Previously, GR has been shown to take on distinct conformations upon binding unique competitive inhibitor scaffolds, which has complicated structure-based drug design and discovery efforts. In this study, a mutant GR possessing a genetically encoded non-natural fluorescent amino acid, L-(7-hydroxycoumarin-4-yl) ethylglycine (7HC), located at Tyr53, an allosteric region known to undergo dynamic changes due to ligand binding, and therefore named $GR^{Y53/7HC}$, was engineered to provide insight into ligand-associated structural changes. Binding of one competitive inhibitor type to $GR^{Y53/7HC}$ produced fluorescence quenching while binding of another produced fluorescence enhancement. A parallel computational study – including essential dynamics, ensemble docking, and MD simulations – was performed to examine the cause of the experimentally observed differential fluorescence pattern. Differences in solvent exposure of the 7HC moiety occurring upon ligand binding accounted for the experimentally determined results. Further experimental data revealed significant structural differences between the two $GR^{Y53/7HC}$-ligand complexes supporting these computational claims. $GR^{Y53/7HC}$ serves as a model approach for predicting ligand-associated structural changes common amongst flexible enzymes.

ZANAMIVIR CONJUGATES: BETTER ANTI-INFLUENZA DRUGS?

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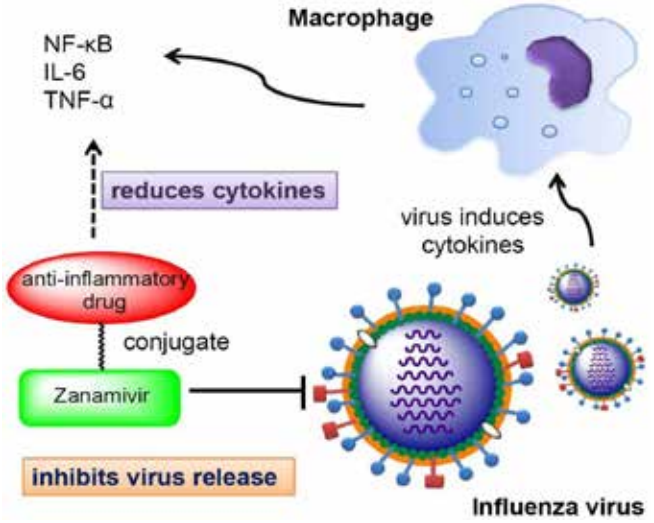
2) The Genomics Research Center, Academia Sinica, Taipei, 115, Taiwan.

Influenza is a respiratory infection that causes severe health problems. For treatment of seasonal flu and possible pandemic infections, it is needed to develop new anti-influenza drugs that have good bioavailability against a broad spectrum of influenza viruses including the resistant strains. Our anti-influenza drug discovery program has focused on the inhibition of the activity of the neuraminidase on the surface of influenza virus. Zanamivir is a potent neuraminidase inhibitor that has rare drug resistance, but has low bioavailability. In the influenza virus infected patients, the uncontrolled virus-induced cytokines can cause high mortality. We shall demonstrate in this presentation that the bifunctional therapeutic drug comprising zanamivir conjugated to an anti-inflammatory agent is beneficial for simultaneous inhibition of influenza virus neuraminidase and suppression of pro-inflammatory cytokines. The zanamivir conjugates are synthesized and their enhanced anti-influenza activities are confirmed by enzymatic and cell-based assays. The mice experiments further show that these anti-influenza conjugate drugs improve the survival rates against H1N1 or H5N1 viral infection. The synergistic protection effect of zanamivir conjugate is better than the combination treatment of zanamivir and anti-inflammatory drug.



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POTENTIAL ANTI-HSV-1 XANTHINE FUSED HETEROCYCLIC DERIVATIVES FOR CANCER TREATMENT

Anber Abdelrahim (1,2), Alaa Hayallah (2,3), Samia Abdel-Moty (2), Abdel-Alim Abdel-Alim (2), Claire Simons (1)

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2) Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt.
3) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Deraya University, Minya, Egypt.

Background: Herpes simplex virus-1 (HSV-1) can cause cold sore eruptions, genital skin lesions, corneal infections, or encephalitis with the latter accompanied usually with marked neurological damage and death.[1] Moreover, HSV-1 has been extensively explored in gene therapy, since HSV-1 thymidine kinase (HSV-1 TK) is the most well characterised suicide gene used in combination with ganciclovir (GCV) for cancer therapy and in other diseases without inducing considerable systemic toxicity.[2] The main problems associated with HSV-1 TK/GCV system therapy are, due to the toxic side effects result from the high doses or the systemic administration of GCV to achieve tumour death, or GCV limited penetration in the brain and the CNS compartment.[2]

Methods: The present work includes design, synthesis and biological evaluation of new tricyclic xanthine derivatives as potential anti-HSV-1 agents and as analogs of penciclovir (T.PCV) that could be used further in suicide gene therapy for cancer. Docking simulations were performed using the crystal structure of HSV-1 TK complexed with GCV followed by organic synthesis to prepare the generated new series of compounds.

Results: The new compounds interact with the key amino acids of TK active site, Figure (1). Structure elucidation of the synthesized compounds was confirmed by NMR, HR-MS and/or microanalysis and will be evaluated for anti-HSV-1 activity at Rega Institute for Medical Research, KU, Leuven, Belgium.

Conclusion: The relatively increased lipophilicity of the newly synthesized compounds is targeted to enhance the potency and bioavailability of the parent drug and also for the better uptake of the drug into the CNS. Taking into account lack of genotoxicity of PCV over GCV, chemical modification for PCV may render it a safe and alternative drug in suicide gene therapy.

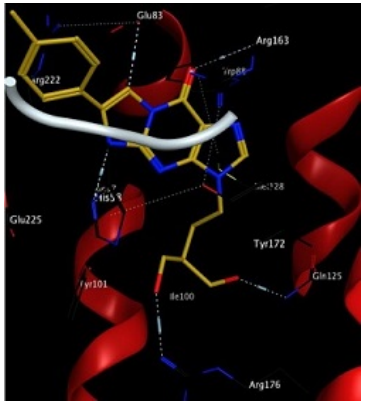

Figure 1: 3D docking pose for a new TPCV compound in TK active site

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CAN WE BEAT THE KETAMINE TIGER?

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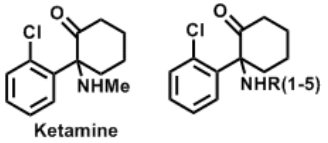
While ketamine is a useful non-opioid anaesthetic and analgesic, the combination of a long half-life and severe hallucinogenic effects are drawbacks. We prepared a series of aliphatic esters of ketamine, considering that these would be pharmacologically active but would be rapidly hydrolysed to much more hydrophilic and inactive acids. We explored the properties of the esters as shorter-acting analogues in an infused rat model, measuring the time after infusion to recover from both the anaesthetic (righting reflex) and the analgesic (response to painful stimulus) effects. No significant relationship was seen between the chain length and the potency of these esters as sedatives, but the Me, Et and i-Pr esters were the more dose potent (up to 2-fold less than ketamine), whereas n-Pr esters were less potent (from 2-6-fold less than ketamine). For the Me, Et and i-Pr esters, recovery from anaesthesia was 10-15-fold faster than from ketamine itself, and for the n-Pr esters, it was 20-25-fold faster than from ketamine. A new dimethylamino ketamine derivative (homoketamine) had ketamine-like sedative effects but was slightly less potent than ketamine, and ester analogues of homoketamine had very weak sedative effects. We will present the synthesis of these esters and their structure-activity relationships.

Examples:



R1 = $(CH_2)_4CO_2$)Me (Racemate)
R2 = $(CH_2)_4CO_2$Me (S enantiomer)
R3 = $(CH_2)_2CO_2$Et
R4 = $(CH_2)_3CO_2{}^{i}$Pr
R5 = $(CH_2)_2CO_2{}^{i}$Pr

Agent potency and speed of offset in rats

	R1	R2	R3	R4	R5	Ketamine
Dose to LORR(mg/kg)	33.6(7.1)	35.9(2.7)	48.0(23.0)	41.6(8.6)	33.1(5.7)	21.1(0.9)
Dose to PWS=1(mg/kg)	44.1(9.5)	43.6(2.5)	55.0(25.1)	66.4(16.8)	36.7(5.7)	26.4(1.3)
SI	10.9(0.4)	21.1(2.3)	19.4(6.6)	16.5(1.7)	16.4(4.3)	4.3(0.18)
NI	6.6(0.3)	11.6(1.9)	10.3(4.6)	14.9(4.5)	4.5(0.4)	1.9(0.16)
Time to righting (sec)	98.7(16.4)	95.0(17.6)	177(51)	36.7(21.9)	82.7(18.8)	1075(74)

LORR : loss of righting reflex

PWS : pedal withdrawal score

SI: sedation index (weight adjusted mean dose per minute of righting reflex loss)

NI: nocioceptive index (weight adjusted mean dose per unit decrease in PWS score from baseline)

All data are means (SEM)

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL AMINO BIOISOSTERES FOR THE GABAa RECEPTOR

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Several neurotransmitters are known in the mammalian central nervous system (CNS) where γ-aminobutyric acid (GABA) exerts the main inhibitory function. GABA is known to activate ionotropic GABA$_A$ receptors (GABA$_A$Rs) and metabotropic GABA$_B$ receptors. GABA$_A$Rs are member of the Cys-loop receptor superfamily of ligand-gated ion channels and composed by five subunits assembled around the chloride ion conducting pore. Several subunits have been identified so far (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ, π, ρ_{1-3}) and they build at least 26 native and mainly heteromeric GABA$_A$R subtypes. GABA$_A$Rs composed of ρ_{1-3} subunits assemble as homo- or pseudohomomers and are also known as the GABA$_C$ receptors. Different subtypes have different pharmacodynamic properties and they are located in disparate brain regions. Consequently, they probably exert heterogeneous functions throughout the central nervous system, which have to be revealed in details.[1]

Taking this knowledge, it would be important to disclose the function of each GABA$_A$R subtype not only in physiological condition but also in certain pathologies, and investigate the possibility of using orthosteric ligands as therapeutic agents. However, there is still a lack of GABA$_A$R subtype selective compounds targeting the orthosteric binding site. To meet the need, a large number of analogues in the GABA$_A$R setting has been synthesized aiming for subtype selectivity. A bioisosteric approach has been used extensively and most efforts have been directed to the replacement of the acidic moiety of GABA.[2] On the other hand, few examples of bioisosteric replacement of the amino moiety of GABA are reported, which include imidazole-4-acetic acid (IAA, depicted in *Figure 1*) described as a GABA$_A$R partial agonist.[3]

In the present study, a bioisosteric replacement of the amino moiety of IAA using a variety of five membered non-aromatic heterocycles is presented. Dihydroimidazole and 2-amino analogues of dihydrothiazole, dihydrooxazole, and dihydroimidazole (*Figure 1*) were chosen and shown to translate into valid novel amino bioisosteres in the GABA$_A$R area. The synthesis, the pharmacological and physicochemical properties of these novel heterocycles are reported and discussed.

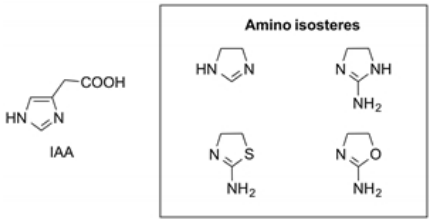

Figure 1. *Left: structure of lead compound imidazole-4-acetic acid (IAA). Right: structures of novel amino bioisosteres in the GABA$_A$-R setting.*

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TRIAZINE NITRILES AS HUMAN CATHEPSIN L INHIBITORS: INVESTIGATION OF II-STACKING INTERACTIONS IN A BIOLOGICAL MODEL

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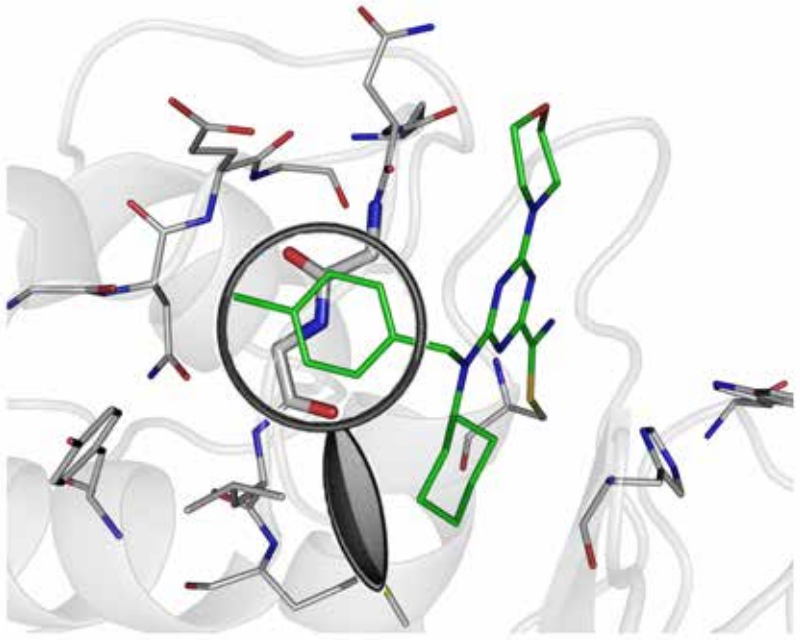
In 2013, we highlighted the importance of π-stacking of heteroarenes on peptide amide bonds in a computational study.[1] Herein we report a complete study of this interaction in a biological model. Human cathepsin L (hCatL) is a cysteine protease used as drug target against various cancers,[2] and was identified as a model system of choice. It possesses a flat dipeptide fragment formed by Gly67–Gly68 in the S3 pocket of hCatL. A series of over 50 triazine nitriles featuring pyridines, pyrimidines, pyridazines, indazoles, (iso)quinolines, (benzo)thiophenes, (benzo)furans, and (benzo)thiazoles as S3 pocket vectors, was synthesized, and their biological activity evaluated in a fluorimetric assay. With the help of crystal structures obtained during this program, the π-stacking of heterocycles in a biological model was investigated.



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CHARACTERIZATION AND MICROBIOLOGICAL ANALYSIS FOR QUALITY CONTROL OF AN ANTI-PSYCHOTIC DRUG SUBSTANCE, OLANZAPINE

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AIM:

Olanzapine, an atypical anti-psychotic drug with a thienobenzodiazepinyl structure is indicated for the treatment of schizophrenia. Our present work deals with the identification and characterization of the active pharmaceutical ingredient Olanzapine and its related substances by HPLC and the evaluation of its microbiological quality.

METHODS:

Olanzapine was identified through its organoleptic characteristics, its melting point and by an infrared absorption using Spectrum One **FTIR** spectrometer. The determination of its purity, identification and the dosage of related substances of olanzapine were carried out using a SHIMADZU® LC-2010 CHT HPLC, equipped with a UV detector at 260 nm and Phenomenex C8 column (150 mm X 4.6 mm X 5 µm) which is maintained at room temperature. The flow rate was about 1.5 ml per min for Assay and about 1 ml per minute for related substances. A mixture of acetonitrile and 6.9 g/l sodium monohydrate phosphate, pH= 2.5 (1: 1) was used as mobile phase for the assay and a gradient mobile phase elution consisting of (**A**): acetonitrile: purified water (20:80, V/V) and (**B**): acetonitrile: purified water (60:40, V/V) for related substances. Microbiological control was based on enumeration of total viable bacteria and on the search of specified germs « Escherichia Coli ».

RESULTS:

The identification of olanzapine active substance and the evaluation of its chemical quality showed conformity with Eur. Ph. 8.0 norms. (Results of the stydy carried out on june 2015)

The percentage content of Olanzapine calculated was about 99.93%. The analysis of this substance by HPLC showed one non specified impurity, its relative retention time was 0.81 and its percentage content was 0.03%.

Microbiological analysis showed that it is free of total viable aerobic bacteria, yeasts, molds and Escherichia coli.

CONCLUSION: Our drug substance is therefore consistent with the standards required by the pharmacopoeia, reflecting its good chemical and microbiological quality.

PURINE-MIMICKING 3'-ETHYNYLRIBOFURANOSE NUCLEOSIDES: NEW OPPORTUNITIES FOR AN OLD SUGAR MODIFICATION

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Nucleoside analogues can be considered as cornerstones in the treatment of cancer and viral infections, with research dating back more than 50 years. Despite this fact, agents with intriguing and improved efficacy, tolerability etc. have still been discovered over the past decade.[1] Recently, research in our laboratory was initiated to re-evaluate certain nucleoside scaffolds and to uncover untapped potential. This presentation will discuss a library subset comprising nucleosides that combine a fixed 3-ethynyl-D-ribofuranose moiety[2] with purine-mimicking pyrazoles[3] as nucleobase surrogates.

The central ribofuranose moiety was prepared according to literature procedures. Glycosylation via a one-pot Vorbrüggen reaction,[3] followed by deprotection and carboxamide formation gave the final products. Initial screening of a small subset of derivatives delivered a low micromolar hit against RSV virus, with a good selectivity index (>10). Follow-up series with a modified carboxamide moiety, additional C-4 & C-5 substitution and the parent ribofuranose analogue were prepared and profiled.

A small library of purine-mimicking *C*-3'-ethynylribofuranose nucleosides was successfully synthesized via Vorbrüggen glycosylation as the key step. Screening of the prepared analogues showed interesting activity against RSV-virus. Preliminary structure-activity exploration of this series points to a unique antiviral profile of our initial hit.

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SELF-IMMOLATIVE SPACERS: TOOLS FOR PRODRUG STRATEGIES

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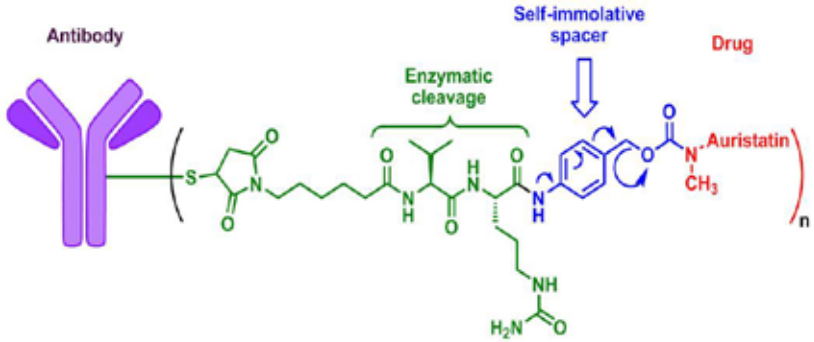
Self-immolative spacers are covalent assemblies that are tailored to correlate the cleavage of two chemical bonds (see scheme 1). This permits to build caged compounds which can be uncaged by successive rupture of two bonds; the cleavage of the first one triggering the cleavage of the other one.

Scheme 1: General process of self-immolative spacers

This property was originally exploited to overcome limitations of drug delivery by introducing a spacer core which bypasses steric hindrance and biocompatible activation. When it is necessary to introduce an additional group to link the targeting moiety to the active compound, self-immolative spacers can be considered as powerful tools. This is effectively the case in chemotherapy, as proved by the use of this kind of spacers in Adcetris® (see scheme 2).



Scheme 2: Adcetris®, approved for lymphoma treatment.

Since few years, our team has worked on physicochemical aspects of spacers to determine their ability to release active compounds[1-5]. This work presents recent advances and last results we got in the field of prodrugs containing self-immolative spacers.

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EFFICIENT SYNTHESIS OF ANTI-INFLAMMATORY LIPID MEDIATOR RESOLVIN E1 AND ITS ANALOGUES

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 Resolvin E1 (RvE1), a metabolite of eicosapentaenoic acid, is a lipid mediator with highly potent anti-inflammatory activity.[1] Although the RvE1s' polyunsaturated chain moiety (C6-C16) is conformationally constrained, its alkyl chain (C1-C5) containing carboxyl is flexible. Thus, we hypothesized that the relative positioning between these two moieties impacts its bioactivity and therefore designed CP-RvE1, in which the flexible C2-C4 alkyl chain is replaced with conformationally restricted stereoisomeric cyclopropanes (Figure 1). Results of structure-activity relationship in these compounds would give important information about the bioactive conformation. First, we have already established a new synthetic route for RvE1 efficiently applicable to the synthesis of the designed CP-RvE1 (Figure 2). According to this route, we are now synthesizing CP-RvE1s including various *cis* and *trans*cyclopropanes.

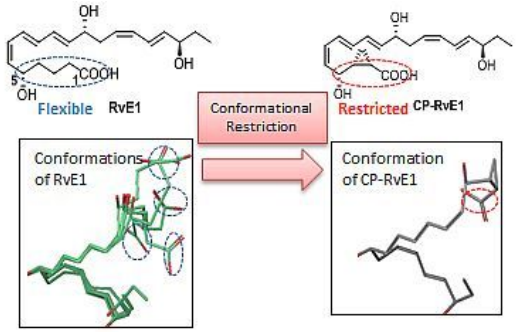

Figure 1. *Design of CP-RvE1*

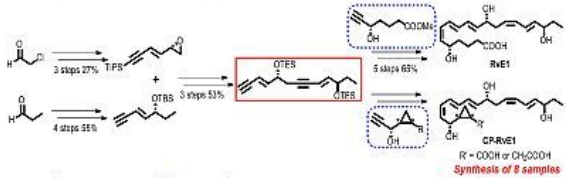

Figure 2. *Synthesis of RvE1 & CP-RvE1*

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IDENTIFICATION OF COMPONENTS WITH INHIBITORY ACTIVITIES ON 3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE FROM ASTER GLEHNI

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The leaves of *Aster glehni* Fr. Sckm. (Compositae), a Korean traditional herb, have been reported to exhibit antioxidant, anti-inflammatory and anti-adipogenic effects, and prevented increases in atherogenic index and body weight in high-fat diet-fed rats (1). 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 (2), the present study was aimed to search for HMGCR inhibitory components from *A. glehni*. The ethyl acetate soluble fraction of *A. glehni* (AGE) showed potent inhibitory effect on HMGCR (IC_{50} = 150.3 μg/mL) in comparison to other solvent fractions. To identify HMGCR inhibitory components, various chromatographic separations of the AGE led to isolation of five new caffeoylglucoside derivatives, together with 13 known compounds. All isolated substances were evaluated for their inhibitory activities on HMGCR. Among them, methyl 3,5-*O*-dicaffeoylquinate showed the most potent inhibitory activity with an IC_{50} value of 9.0 μM, while 3,5-*O*-dicaffeoylquinic acid exhibited little inhibitory activity. Pravastatin used as a positive control elicited the most potent inhibitory activity as expected with an IC_{50} value of 1.3 μM. In addition, for the purpose of improving the inhibitory activity of the AGE, components of the AGE were derivatized to methyl esters with acid resin in methanol. The methylated AGE residue (AGEM, IC_{50} = 98.1 μg/mL) was separated into five fractions on column chromatography on DIAION HP-20 by eluting with 30, 50, 70, 90 and 100% methanol. The most potent inhibitory activity was found in the fractions eluted with 70 and 90% methanol (IC_{50} ≈ 20 μg/mL). The results suggest that *A. glehni* has potential to be a new source of agents for controlling cholesterol biosynthesis.

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IN SILICO FRAGMENT-BASED HIT IDENTIFICATION FOR EFFICIENT DISCOVERY OF NEW COSMETIC INGREDIENTS

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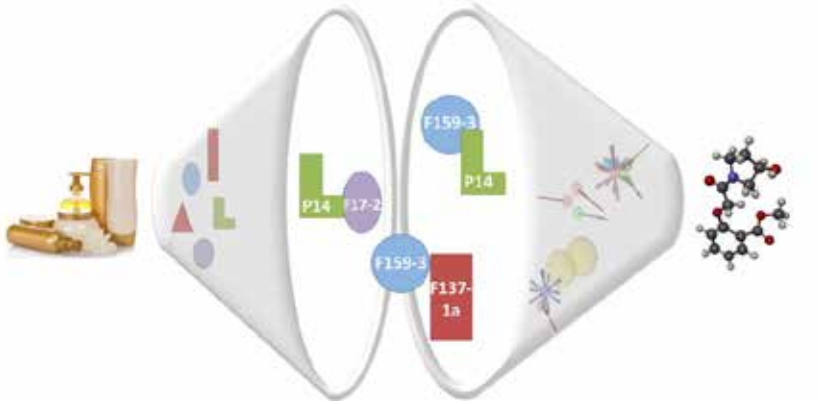
The purpose of this study was to quickly get access to an active, safe and easily accessible candidate molecule for development in topical cosmetic applications using innovative *in silico* methods.

Through a process of fragmentation, functionalization, and recombination of 274 market approved molecules for cosmetic usage, we customized an *in-house* virtual library of 92,000 molecules ideally suited for virtual screening.

With this library in hand, we used fragment cosmetophore*-based virtual screening to establish the proof of concept of our approach on a novel skin protein target. Hence, virtual screening of the *in-house* library followed by a short and oriented hit-to-lead optimization process led to the discovery of novel development candidates having intrinsically suitable specific skin beneficial properties for cosmetic applications.

This successful innovative strategy could be definitely extended to other applications and targets of interest.



* in the context of cosmetic research, the term cosmetophore is employed instead of pharmacophore.

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SELECTIVITY DETERMINING FEATURES IN N-MYRISTOYLTRANSFERASES – A POTENTIAL DRUG TARGET WITH A HIGHLY CONSERVED BINDING SITE

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One major goal of each drug design project is to obtain high affinity ligands for a certain target while maintaining selectivity over potential off-targets and thereby reducing side effects. That however proves to be challenging when facing a highly conserved binding site. In this project we are focusing on a pair of well investigated enzymes - the N-myristoyltransferases of *Leishmania major* (*Lm*NMT) and the human homolog *Hs* NMT1 which share an overall sequence identity of over 40% and identical first shell protein-ligand interactions. For *Lm*NMT many non-selective inhibitors have been found, but there are only few selective ones known[1-5]. In the case of selective inhibition the molecular basis for it is still unclear. In our work we aim to reveal the reasons for selective inhibition by molecular dynamic simulations (MDs), isothermal titration calorimetry (ITC), enzyme inhibition assay and X-ray crystallography analyzing the protein dynamics, second shell interactions and water network formation. Through simulations we were able to narrow down the selectivity determining amino acids to two independent regions – one close to the catalytic domain, the other more distinct altering the water network within the binding site. Currently, we are working on the proof of concept by site directed mutagenesis and X-ray crystallography.

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PHOTOSWITCHABLE 4-(PIPERIDIN-4-YL)-1-HYDROXYPYRAZOLE ANALOGUES FOR THE GABA TYPE A RECEPTORS

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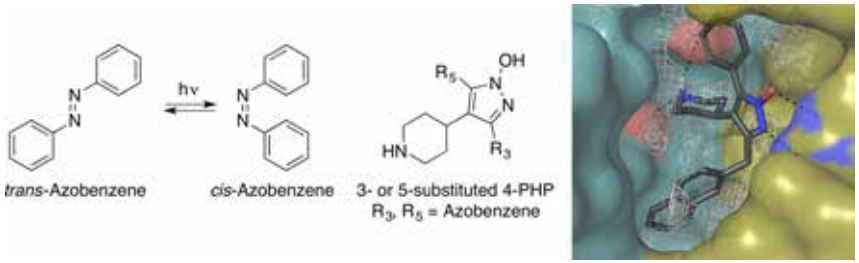
γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and exerts the majority of its effects through the $GABA_A$ receptors ($GABA_AR$). The $GABA_ARs$ are pentameric ligand-gated ion channels that mediate fast synaptic responses to GABA and belong to the Cys-loop receptor superfamily. $GABA_ARs$ are widely distributed in the CNS and proposed to be involved in several neurodegenerative and psychiatric disorders including anxiety, schizophrenia, and Alzheimer's[1].

Photopharmacology provides an opportunity to regulate biological processes by light. Light is non-toxic and non-invasive and can be delivered with high spatiotemporal precision. Furthermore, qualitative and quantitative regulation of light is possible by adjusting wavelength and intensity, respectively. Photoswitches are small molecules, which upon absorption of a photon undergo reversible conformational changes. The commonly used photoswitch azobenzene undergo *cis/trans* isomerization upon photon absorption resulting in a large change in geometry and polarity[2]. Coupling of a photoswitch to a ligand enables reversible control by light of not ordinarily light-sensitive receptors.

We have previously shown that 3- or 5-substituted 4-(piperidin-4-yl)-1-hydroxypyrazoles (4-PHP) bind to the orthosteric binding site of $GABA_ARs$ and the identified cavities are capable of accommodating relatively large substituents on 4-PHP[3,4]. These cavities could enable introduction of photoswitches such as azobenzene with retained affinity of the ligands at the $GABA_AR$.



Based on the abovementioned observations we introduced azobenzene in the 3- or 5-position of 4-PHP, which led to a series of photoswitchable analogues. The analogues behave similar to azobenzene with *cis/trans* isomerization upon irradiation with UV-Vis light and thermal relaxation over time.

Pharmacological characterization at $GABA_ARs$ was performed in a [^{3}H]muscimol binding assay using rat brain cortical membranes. Binding affinities of all dark adapted analogues were in the low micro- to low nanomolar range at native rat $GABA_ARs$.

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DEVELOPMENT OF AN ACCESS TO DNA-HETEROCYCLE CONJUGATES BY Au(I)-CATALYZED A3 MULTICOMPONENT REACTIONS

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The screening of large, pooled DNA-encoded small molecule libraries (DELs) is a validated technology for the target-based identification of bioactive compounds. DELs are generated by iterative organic synthesis and encoding steps. Prerequisite for library synthesis is therefore DNA-compatibility of synthesis methodology. Currently, reaction methodology meeting this requirement is very limited mainly due the incompatibility of the purine nucleotides with many catalysts such as transition metal ions, e.g. Au(I). We hypothesized that the use of oligopyrimidine-sequences in the first step of DEL synthesis might broaden the spectrum of applicable catalytic systems to initiate DEL synthesis. In our newly developed "**TIDEC"** (oligo**T**hymidine **I**nitiated **D**NA-**E**ncoded **C**hemistry) strategy, we employ the solid phase-bound 5'-aminolinker-modified hexathymidine sequence serving as an adapter oligonucleotide.[2] Here, we demonstrate the applicability of Au(I) catalysis to furnish TIDEC-conjugates of highly substituted pyrazolines[3] from readily accessible starting materials. The TIDEC-alkyne **1** was reacted with an aldehyde **2** and a hydrazide **3** using a catalytic system consisting of a Au(I)-complex and a Ag(I)-salt giving a rise to TIDEC-pyrazoline **4.** We are currently synthesizing DNA-encoded combinatorial libraries based on these heterocyclic structures.

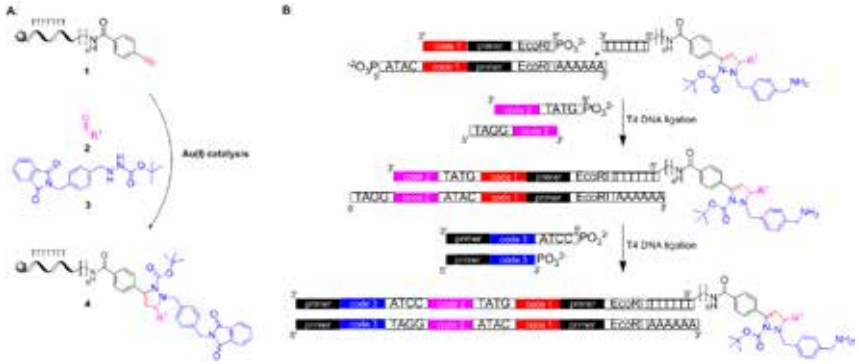

Fig. 1A: Au(I)-catalyzed A3 multicomponent reaction to synthesize hexa-T pyrazoline conjugates; **1B**: DNA-encoding of TIDEC-pyrazolines by T4 DNA ligation

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COMBINING ANTIOXIDANT, ANTIINFLAMMATORY & HYPOLIPIDEMIC ACTIVITY BY DESIGN: NEW MULTI-POTENT MORPHOLINE DERIVATIVES FOR ATHEROSCLEROSIS

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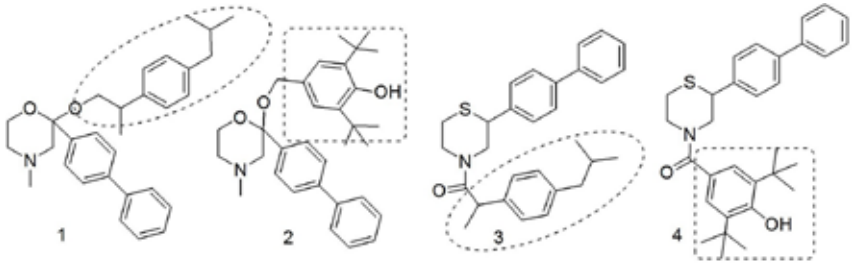
Multifunctional agents that address two or more targets of a multifactorial disease, can be expected to produce superior *in vivo* effects as compared to higher-affinity single-targeted compounds.[1,2,3] Based on the pathophysiological mechanisms involved in atherosclerosis (inflammation/hyperlipidemia/oxidative stress), we designed new aromatic thio/morpholine derivatives (1-4) by combining several different anti-inflammatory and antioxidant pharmacophores.



These novel compounds, combining antioxidant, antihyperlipidemic and anti-inflammatory activities by design, exhibited increased *in vitro* activities against oxidative stress (protection against lipid peroxidation) and inflammation (lipoxygenase inhibition). This activity profile was shown to be extended *in vivo* a) in a hyperlipidemic mouse model where cholesterol/triglyceride levels were reduced up to 70% and plasma total antioxidant capacity (TAC) increased 2-fold and b) in an inflammatory mouse model (carrageenan-induced mouse paw edema) where compounds exhibited more potent anti-inflammatory action than parent or reference molecules.

Based on these results, it appears that the incorporation of the specific antioxidant/anti-inflammatory pharmacophores, into the aromatic thio/morpholine lead structure, enhanced and broadened the pharmacological profile of the new compounds that may find successful therapeutic applications in atherosclerosis and metabolic syndrome disorders.

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DEVELOPMENT OF BISPECIFIC BICYCLIC PEPTIDES

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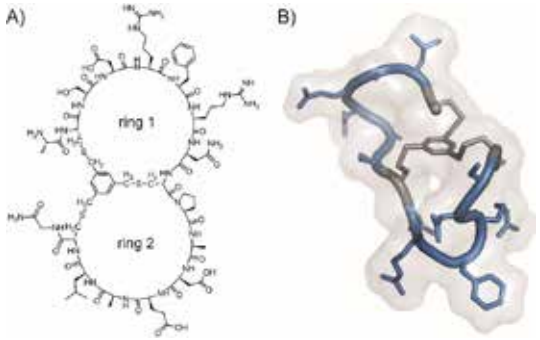
Bicyclic peptides (figure A) are constrained peptides that show higher binding affinity and selectivity for a target as compared to a linear or monocyclic peptide.[1] The constrained structure reduces the entropic penalty upon binding and therefore increases affinity, and the rigidity and chemical conjugation render the peptides more resistant to proteases. Several bicyclic peptides previously developed in our lab demonstrated a high stability in plasma, as well as in intestinal extract, greatly increasing their usefulness as potential drug candidates.[1]

In this study we develop bispecific bicyclic peptides using phage-display[2] to target two homologous serine proteases, the coagulation factor XIIa and plasma kallikrein. Both serine proteases are involved in the intrinsic coagulation cascade, which is activated by coagulation factor XII (FXII, Hageman factor). Activated FXII cleaves plasma prekallikrein (PPK) to generate active plasma kallikrein (PK), which generates FXIIa in a reciprocal fashion, and an additional inflammatory mediator. Both PK and FXIIa are important targets in coagulation and inflammatory disorders.[3] By blocking both targets simultaneously the reciprocal activation of the intrinsic coagulation cascade will be inhibited, which ideally can be done with the administration of only one drug instead of two.

In order to make a bispecific bicyclic peptide, our previously established libraries are evolved with phage-display where we alternate panning against each target, FXIIa and PK. Peptides have been found that show consensus sequences within the same molecule, comparable to the consensus of previously developed FXIIa and PK inhibitors. Their inhibition constant for both targets and anti-coagulation properties are currently being tested.



Example of bicyclic peptides. A) chemical structure. B) three dimensional model.

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SCREENING OF NATURE BANK AND DIVERSE SCAFFOLD LIBRARY FROM COMPOUNDS AUSTRALIA AGAINST AMPK

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Adenosine 5'-monophosphate-activated protein kinase (AMPK), a sensor of cellular energy status, plays a key role in whole-body energy homeostasis. Therefore, AMPK is an interesting target for the treatment of pathological conditions involving abnormal energy regulation like metabolic disorders including type-2-diabetes and obesity. AMPK is a promising target to fight cancer due to its influence on cell proliferation. Additionally, pharmacological targeting of AMPK could be beneficial for the treatment of Alzheimer´s disease and other neurological diseases.[1]

A high-throughput screening method was set up using the luciferase-based Kinase-Glo® assay kit from Promega on an Agilent BioCel™ automation system. The assay conditions were optimized so AMPK activators and inhibitors can be detected using the same conditions.

Selected compound libraries from Nature Bank[2], a unique source of natural products and natural product extracts, and Compounds Australia[3] were then screened against one isoform of AMPK in order to discover novel AMPK activators and inhibitors for further development in biological and medicinal chemistry projects.

So far, >700 compounds from Nature Bank and >5´300 compounds from a diverse scaffold library from Compounds Australia were screened. Five scaffolds including >40 molecules from the diverse scaffold library and >20 natural products were identified to modulate the activity of AMPK. The structure-activity relationship information that is already present in the scaffold hits as well as molecular modeling studies of the hit structures will be used for the development of more potent AMPK modulators.

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DESIGN AND SYNTHESIS OF 2,2'-DIINDOLYLMETHANES TO SELECTIVELY TARGET CERTAIN G-QUADRUPLEX DNA STRUCTURES

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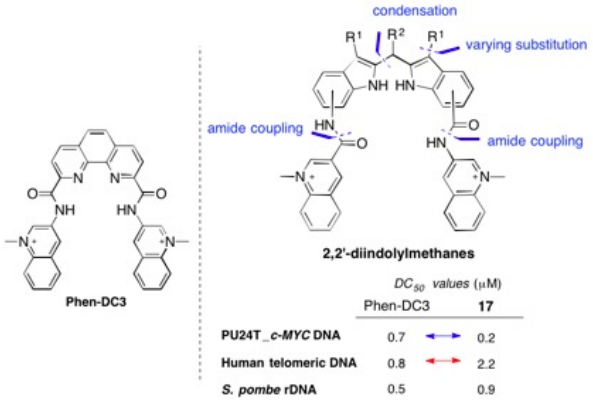
G-quadruplexes (G4) are secondary DNA structures that are evolutionary conserved and enriched at e.g. telomeres, promotor regions, and ribosomal DNA. G4 DNA structures are important in many biological systems for example in genetic regulation, control of the latency of viruses as well as the protection of the telomere ends from degrading. However, there are still big gaps in the knowledge of the biological interplay of these systems. G4-stabilizing molecules are thus of interest both as chemical research tools and because they hold great therapeutic potential for the treatment of cancer and infectious diseases. We have developed a set of new G4-stabilizing ligands, to be used as chemical tools.[1] Our main structure is inspired by one of the most frequently used G4-ligands, Phen-DC3.[2] Our strategy was to replace the central phenantroline part in Phen-DC3 with a 2,2'-diindolyl motif to increase the flexibility of the ligand. The ligands were first evaluated for their G4-binding properties through our *in vitro* assay based on Thioflavin T fluorescence.[3] The assay showed a dose dependent binding with all of the synthesized ligands. For three of the ligands, the binding was comparative to, or even better than, the binding of Phen-DC3. Interestingly, the ligands showed selectivity between the different G4 structures in this assay with up to 11-fold preference for one of the G4 structures compared to the others. The ligand binding has also been investigated using circular dichroism (CD), which showed a less intrusive binding with our ligands when compared to the binding of Phen-DC3. Additionally, the ligands ability to stabilize G4 structures was also investigated using CD, which confirmed their selectivity for certain G4 structures.



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FROM STEM CELL SCREENING TOWARDS SMALL MOLECULE TOOLS FOR TGF-BETA-SIGNALLING AND HEART REGENERATION

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The transforming growth factor β (TGFβ) pathway is a key player in various biological processes in the context of cell proliferation and differentiation. Targeting this signalling pathway with novel small molecular agents holds great promise in the field of regenerative medicine.[1,2] From a high-content screen in murine embryonic stem cells (mESCs), we identified a specific subclass of 1,4-dihydropyridines (DHPs) that inhibit TGFβ signalling and thereby act as stimulators of cardiomyogenesis.[3,4]

Since the TGFβ pathway is inhibited by the discovered DHPs in a unique fashion and the concrete cellular mechanisms and targets have yet to be resolved, there is a pressing need to develop chemical probes to decipher this new mode of action. Moreover, based on the evident role of TGFβ in cardiac remodelling and fibrosis, our second line of research is devoted to demonstrate proof-of-concept *in vivo*. To do this, the compounds require "early hit-to-lead" optimization.

Here, I will summarize a multidisciplinary chemical biology- and medicinal chemistry-based workflow based on a 'screening hit' to agents for several *in vitro* and *in vivo* applications. For this, various techniques were applied, including ligand-based (quantitative) structure-activity relationships (SARs), X-ray crystal structure analysis, signalling pathway selectivity and *in vitro*-pharmacokinetic profiling. This set-up allowed for the development of selective, potent and drug-like candidates.[5] Thus, we designed compounds that meet key requirements of "chemical probes" and can be utilized for the elucidation of the unknown mode of action. Furthermore, key obstacles that typically limit *in vivo* applicability, such as poor compound solubility and stability, could be addressed. Moreover, we demonstrated efficacy in an engineered heart tissue (EHT, from neonatal rat cardiomyocytes) as a 3D-tissue model of hypertrophy and fibrosis.

Taken together, medicinal chemistry-driven efforts led to a set of highly attractive small molecules that can both be used to investigate a novel mechanism of TGFβ inhibition and as *in vivo* pharmacology tools to study heart regeneration and remodelling after myocardial infarction.

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DESIGNING MORE SELECTIVE DRUGS BY PUTTING MODELLING INTO CHEMISTS HANDS WITH LiveDesign

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LiveDesign is Schrödinger's next generation web-based application for collaborative drug design. LiveDesign enables scientists to analyse new chemical ideas in the context of existing experimental data, computational predictions and other data.

In a recent collaboration between medicinal chemists at Syros Pharmaceuticals and modellers at Schrodinger, LiveDesign was used to give easy access to customised predictive models. We will show how this resulted in a significant improvement in experimentally determined target selectivity.

SEMIEMPIRICAL AND AB INITIO METHODS FOR MODELLING CYCLODEXTRIN - CEFUROXIME AXETIL COMPLEXES

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Cyclodextrins (CDs) are oligosaccharides of great importance for pharmaceutical industry. The possibility of active pharmaceutical ingredients (API) to form inclusion complexes thanks to lipophilic cavity inside of this polymer is useful property for designing pharmaceutical dosage forms. Cyclodextrins found their applications as modifiers of APIs in regards to solubility, taste, stability and release profile. Each of mentioned modifications requires inclusion of API inside lipophilic cavity of CDs in suitable stoichiometric ratio. Proper determination of stability of complexes with given API as well as domain of API which includes into cavity plays crucial role in early stage of designing of pharmaceutical dosage forms. Molecular modelling can be applied to such task in order to get insight into API – cyclodextrin interactions on molecular level.

In presented work, complexes of β-lactam antibiotic – cefuroxime axetil with α-, β-, γ-, hydroxypropyl-α-, methyl- β-, hydroxypropyl-β- and hydroxypropyl-γ- cyclodextrins were prepared by co-precipitation and characterised with FT-IR and DSC methods. Molecular modelling approach was applied in order to analyse possible modes of binding of cefuroxime axetil in CDs. Moreover, FT-IR spectra of obtained complexes conformations were calculated in order to acquire close correlation with experiment.

Modelling of CD complexes *ab initio* is demanding task due to extensive variety of possible modes of interaction and stoichiometry ratios of API and CD molecules. We proposed the method based on application of semiempirical PM7 (Parametrization method 7) and PM6 (Parametrization method 6) with DH+, DH2 and DH3+ corrections. PM6 and PM7 methods were applied for calculation of spectra for different conformations of API – CD complexes The corrections were applied to accurately model interactions between investigated API and CDs.. The most energetically favored complex was also modelled with computationally demanding method DFT (Density Functional Theory) with B3LYP (Becke, three-parameter, Lee-Yang-Parr) and compared against results obtained with semiempirical methods. Instead of analysis of the most stable conformation, mathematical optimization methods were applied in order to find combination of not necessarily energetically optimal conformations comprising samples of cyclodextrins in prepared samples.

The study demonstrated the ability of new semiempirical methods and corrections to experimentally model interactions. We conclude that simple modelling of one, most energetically favoured conformation of complex is not enough to catch variety of different binding modes existing in real sample of prepared complexes.

Acknowledgments

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NEW 18 KDA TRANSLOCATOR PROTEIN LIGAND GML-1 IS PROMISING AS NEW FAST ANXIOLYTIC

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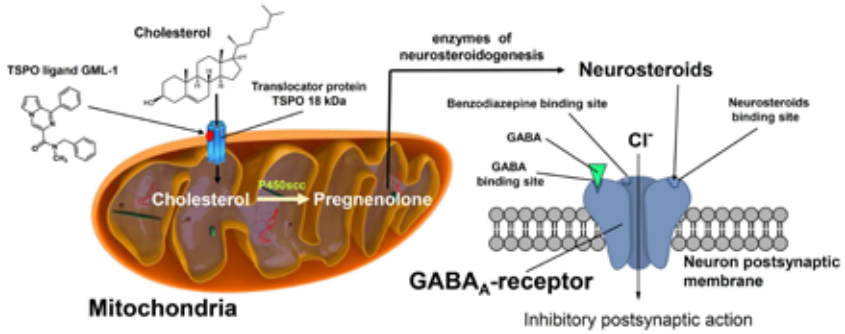
TSPO (18 kDa translocator protein) – is a new therapeutic target for neurological and psychiatric disorders [1]. This receptor activates cholesterol transport from the outer to the inner mitochondrial membrane, that is the rate-limiting step of neurosteroids biosynthesis. Neurosteroids are potent positive allosteric modulators of $GABA_A$ which plays an important role in the pathophysiology of anxiety disorders; they have their own binding sites on $GABA_A$ receptors, which are different from the benzodiazepine. Since TSPO plays an important role in regulating the synthesis of neurosteroids, the ligands of this receptor may be fast anxiolytics free from side-effects of benzodiazepines.



In this work we designed and synthesized a new type of TSPO ligands relating to 1-arylpyrrolo[1,2-a]pyrazine-3-carboxamides [2]. Using *in vitro* and *in vivo* screening methods compound GML-1 was selected as the most promising for development as new anxiolytic. This compound has high TSPO affinity ($IC_{50} = 5.4*10^{-8}$M) and no affinity against benzodiazepine site on $GABA_A$ receptor. GML-1 shows high anxiolytic activity in standard animal behavior tests comparable with that of diazepam, and it doesn't demonstrate negative side effects of benzodiazepines. The involvement of TSPO and neurosteroids in the mechanism of anxiolytic activity of GML-1 was proved using TSPO selective antagonist PK11195 and inhibitors of neurosteroids biosynthesis enzymes. GML-1 has low toxicity, $LD_{50} < 1000$ mg/kg. In contrast to the benzodiazepines, GML-1 demonstrates the nootropic and neuroprotective effects. The results obtained allow to consider the compound GML-1 as a new promising anxiolytic.

This work was supported by the Basic Research Program of the Presidium of the Russian Academy of Sciences (The project "Design, synthesis and pharmacological properties of the original ligands of the mitochondrial protein TSPO»).

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AMINOPYRIDINES: OPTIMIZING hERG PROFILES WITHOUT COMPROMISING PERMEABILITY

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Aminopyridine is a key motif of a lead chemotype in our Plasma Kallikrein (PKL) project, binding into the enzyme's S1 pocket to elicit a potent inhibition. Despite the excellent ADME profiles generally exhibited by this chemotype, however, the aminopyridine motif entails a persistent hERG affinity, which led the team to pursue various avenues to overcome the issue while keeping good permeability of compounds. Approaches discussed hereby include modulation of cLogP & pKa, replacement of basic groups with neutral groups, and application of global & local QSAR models.

X-RAY CRYSTAL STRUCTURES OF HITS FROM A FRAGMENT BASED DISCOVERY PROGRAM ON THE EPIGENETICS TARGET BRD3

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The bromodomain (BRD) containing proteins are a family of proteins responsible for reading epigenetic acetylated lysine marks on histones and are rapidly emerging as a target class amenable to pharmacological intervention with small molecules[1]. The Bromodomain and Extraterminal (BET) sub-family which includes BRD2, BRD3, BRD4, and BRDT are perhaps the most widely explored of the class. Initially identified as a result of phenotypic screening for modulation of ApoA1, subsequent target identification and compound optimisation has revealed multiple small molecule inhibitors of BET bromodomains, which have progressed to preclinical and clinical evaluation. BRD3 in particular is associated with a number of disease phenotypes. For example, depletion of BRD3 slows growth in cancer models including prostate cancer[2] and medulloblastoma[3] and BRD3 has been implicated in NUT midline carcinoma (NMT)[4].

While there are a number of available pan-BET inhibitors[5], the design of specific BRD3 inhibitors may lead to a beneficial clinical outcome with reduced off-target effects. However, bromodomains of the BET family have a high degree of structural similarity, especially in the acetylated lysine binding pocket, making the design of selective inhibitors problematic. BRD3 therefore represents an intriguing and challenging drug discovery target.

By carrying out fragment based screening using surface plasmon resonance (SPR) a number of hits to the first bromodomain in BRD3 (BRD3/BD1) were identified with K_D ranging from 10 μM to 250 μM. These were characterized further by determining their X-ray crystal structures in complex with the BRD3/BD1. While the resulting structures all showed the hits binding at the acetyl-lysine binding site analysis of the structures and further chemical elaboration of these fragments may lead to a BRD3 or BRD2/3 specific molecules that could be utilized as a tool compounds for elucidating the individual roles of the members of the BET-family.

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PURIFICATION OF DORSAL LECTIN FROM THE REEF STONEFISH, SYNANCEIA VERRUCOSA

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A large number of venomous and poisonous animals exist in aquatic environments worldwide. More than 200 of the approximately 22,000 species of fish in the ocean are considered to be venomous. Most of these venomous fish are non-migratory, slow moving, and mainly live in shallow waters in protected habitats. Venomous scorpaeniform fish include the lionfish and scorpionfish from the family Scorpaenidae, devil stinger and stonefish from the family Synanceidae, and waspfish from the family Tetrarogidae. These fish possess 11-17 dorsal, 2 pelvic, and 3 anal spines, with the venom secretory complex being located within the anterolateral grooves of these spines.

The reef stonefish, *Synanceia verrucosa* has 13 dosal and 3 anal spines, which contain venom glands that are covered by an integumentary sheath. *S. verrucosa* lives from shallow tropical marine waters in the Pacific and Indian Oceans from the Red Sea to the Great Barrier Reef. Envenomation occurs when people carelessly step on the fish, and are stung by the dorsal spines. Envenomation appears immediately as intense, sharp, and persisting local pain, and swelling around the sting. Symptoms dependent on the amount of venom injected. Systemic effects including fever, delirium and shock have been reported. However, only a limited number of studies have investigated the toxicity of *S. verrucosa*. Therefore, we herein examined the dorsal venom of the reef stonefish, *S.verrucosa* using column chromatography and separated a novel lectin that induced mitogenic activity.

Dorsal lectin was purified from the reef stonefish, *Synanceia verrucosa*, using a combination of affinity chromatography techniques. A single band was detected on a native PAGE gel with a relative molecular mass of 45 kDa. The agglutination of rabbit erythrocytes by the 45 kDa lectin was inhibited most effectively by methyl α-D-mannoside. The 45 kDa lectin stimulated mitogenesis in murine splenocytes. This is the first study to examine the dorsal lectin of *S. verrucosa* and one of very few studies on venom lectin from stonefish. These results suggest that the reef stonefish, *S.verrucosa* may be a novel resource for biologically active substances.

The author has no conflict of interest to disclose with respect to this presentation.

HIGHLY POTENT, SELECTIVE, AND ORALLY BIOAVAILABLE INHIBITOR OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 (11β-HSD1)

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Chronic high level of glucocorticoids can result in insulin resistance from impairment of insulin-dependent glucose uptake, increased hepatic gluconeogenesis, and reduced insulin secretion from the pancreas. Due to the physiological relationship between glucocorticoids and metabolic disease risk factors, 11β-HSD1 has been regarded to be a potential target for the treatment of metabolic syndrome as well as type II diabetes. 11β-HSD1 is a key enzyme that acts as an NADPH-dependent reductase and converts inactive cortisone into active cortisol, which is an actual circulating glucocorticoid in humans. 11β-HSD1 is mainly distributed in specific tissues, such as liver, adipose, and brain, and that regulates tissue-specific glucocorticoid levels. In our research, a series of pyrimidine-4-carboxamide based inhibitors of 11β-hydroxysteroid dehydrogenase type 1 was synthesized and evaluated to optimize the privious picolinamide lead compound. Combination of the replacement of a pyridine ring of lead compound with a pyrimidine ring and the introduction of an additional fluorine substituent at the 2-position of the phenyl ring resulted in the discovery of a potent, selective, and orally bioavailable inhibitor SKI2852, which demonstrated no CYP and PXR liabilities, excellent PK profiles across species, and highly potent and sustainable PD activity. Moreover, repeated oral administrations of SKI2852 significantly reduced the blood glucose and HbA1c levels and improved the lipid profiles in *ob*/*ob* mice, and these efficacies were synergistically enhanced by combination with metformin.

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Synthesis and optimization of picolinamide derivatives as a novel class of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors

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Chronic high level of glucocorticoids can result in insulin resistance from impairment of insulin-dependent glucose uptake, increased hepatic gluconeogenesis, and reduced insulin secretion from the pancreas. Due to the physiological relationship between glucocorticoids and metabolic disease risk factors, 11β-HSD1 has been regarded to be a potential target for the treatment of metabolic syndrome as well as type II diabetes. 11β-HSD1 is a key enzyme that acts as an NADPH-dependent reductase and converts inactive cortisone into active cortisol, which is an actual circulating glucocorticoid in humans. 11β-HSD1 is mainly distributed in specific tissues, such as liver, adipose, and brain, and that regulates tissue-specific glucocorticoid levels. Based on the docking results of our initial hit compound, *N*-cyclohexyl-6-(piperidin-1-yl)picolinamide from high throughput screening of in-house library, we performed SAR studies by the synthesis of a series of 6-substituted picolinamide derivatives and their inhibitory activities against 11β-hydroxysteroid dehydrogenase type 1. Several compounds were identified as novel and potent inhibitors of 11β-HSD1. As a result, High potency toward human 11β-HSD1 was achieved by the incorporation of a hydroxy-adamantyl group. In addition, the replacement of piperidine ring with 1-(4-substituted phenyl)piperazine led to a substantial improvement in metabolic stability. In conclusion, the best compound possessed desirable potency in both human 11β-HSD1 enzyme assay and the mouse 11β-HSD1 enzyme assay, and demonstrated oral efficacy in lowering liver and adipose 11β-HSD1 activities in a mouse *ex vivo* model. Finally, the best compound exhibited good *in vivo* efficacy in HF/STZ mice and reduced fasting blood glucose and insulin levels after oral dosing.

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NOVEL CINNAMIC ACID-PRIMAQUINE CONJUGATES OF SEMICARBAZIDE TYPE

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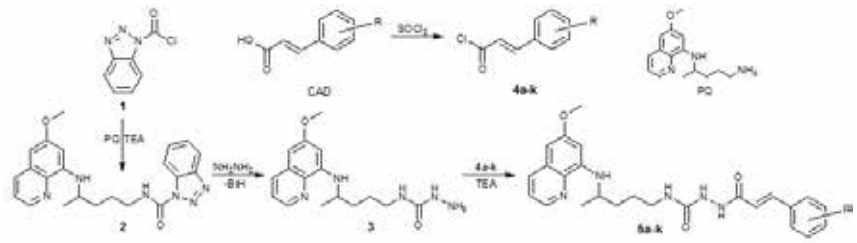
Molecular hybridization approach based on the combination of pharmacophoric moieties of different compounds was used to produce new hybrid molecules with cinnamic acid derivatives (CAD) and primaquine (PQ) motifs bound via spacer. CAD, a large group of compounds appearing naturally in plants, have a long history of human use. Impressive number of papers report on their biological activities (antitumor, antimicrobial, antioxidative, antidiabetic, hepatoprotective, hypolipemic, antimalarial) and low toxicity (1-3). On the other hand, primaquine is a well-known antimalarial drug with antiproliferative potential. In our recent work we have shown that primaquine derivatives with substituted terminal amino group possess significant cytostatic activity in low micromolar concentrations towards various cancer cell lines (4, 5) or high selectivity towards MCF-7 (breast cancer).



The starting compound benzotriazole carboxylic acid chloride **1** was used for the preparation of primaquine benzotriazolide **2**, which reacted with hydrazine hydrate and gave semicarbazide **3**. The title CAD-primaquine conjugates **5a-k** were prepared by acylation of product **3** with corresponding CAD chlorides **4a-k**. The following CAD were used: cinnamic acid, a-methylcinnamic acid, methoxy, dimethoxy, trimethoxy, methylenedioxy, chloro, fluoro, trifluoromethyl and bistrifluoromethyl cinnamic acid. Structures of newly prepared *N* -cinnamoyl-primaquine conjugates were confirmed by IR, ^{1}H and ^{13}C NMR and MS spectroscopy. Evaluation of their biological activity is in progress.

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EVALUATION OF [18F]IMA201 AS A NOVEL RADIOTRACER FOR AGGREGATED ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

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Objectives: Parkinson's Disease (PD) is one of a number of neurodegenerative conditions, which may be collectively grouped as "protein deposition diseases", associated with the conversion of native soluble proteins into deposited aggregates. Abnormal accumulation of the protein α-synuclein, in combination with a misfolding process, leads to the formation of insoluble aggregates, also referred to as Lewy bodies and Lewy neurites, which are a definitive marker for the pathological diagnosis of Parkinson's Disease.

The ability to monitor the progression of α-synuclein deposition *in vivo* will aid understanding of disease progression, including the onset of dementia in PD patients and in time may lead to improved diagnosis as well as support drug development initiatives. The aim of this study was to evaluate [^{18}F]IMA201 as a potential PET ligand for delineation of α-synuclein in patients with PD.

Methods: 4-fluoro-*N*-phenylbenzenesulfonamide has been found to provide protection in cellular models of α-synuclein-mediated dysfunction [1]. Its radiolabelled analogue, [^{18}F]IMA201, was prepared by copper II-mediated 18F-fluorination of the tetramethylpinacol phenylboronic ester precursor [2]. [^{18}F]IMA201 specificity for α-synuclein fibrils in PD brain tissue was evaluated using quantitative autoradiography. Human tissue was ethically sourced from the Parkinson's UK Brain Bank. Anatomically adjacent fresh frozen tissue sections (10 μm) from the grey matter area of the midbrain or cortical region of three individual PD patients were cut and fixed in 4% paraformaldehyde. Sections were incubated with increasing concentrations of [^{18}F]IMA201 (0.3, 1, 10 nM) and apposed to phosphor-imager plates. Specific binding was identified by homologous block (10 μM). The presence of α-synuclein was confirmed with immunohistochemistry in anatomically adjacent slides. Dynamic PET scans were also conducted under baseline conditions and following pre-treatment with unlabelled IMA201 (2 mg/kg *i.v.*) to determine *in vivo* regional brain uptake of [^{18}F]IMA201 in a healthy male rat.

Results: IMA201 was successfully labelled with ^{18}F with a radiochemical yield of 150-400 MBq and mean specific activity of 10 ± 11 GBq/μmol (n=3). [^{18}F]IMA201 demonstrated a heterogenous binding signal in brain tissue of three patients at three different concentrations (**0.3nM:** 6.2, 22 and 6.5 DLU/mm^2/Bq; **1nM:** 0, 120 and 40 DLU/mm^2/Bq; **10nM:** 390, 370 and 100 DLU/mm^2/Bq). Percentage of specific binding ranged from 0 - 87%, which was consistent with the heterogenous expression of α-synuclein between patients as determined by immunohistochemistry. [^{18}F]IMA201 demonstrated good brain penetration in a healthy rodent with reversible kinetics during the time of the scanning period.

The successful labelling, in vitro binding characteristics and good *in vivo* kinetic profile of [^{18}F]IMA201 suggest that this is a potential candidate for monitoring α-synuclein in the CNS. Radiosynthesis optimisation to allow clinical translation and further studies investigating the selectivity of [^{18}F]IMA201 for α-synuclein over other misfolded proteins in patient brains, including the evaluation of a SB signal in a rodent model of PD, are required to confirm the potential of [^{18}F]IMA201 in clinical applications and neurodegenerative drug development.

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GMP-COMPLIANT SYNTHESIS AND PRECLINICAL EVALUATION OF THE SELECTIVE ALPHA-V BETA-6 TRACER [18F]IMAFIB FOR IMAGING AND QUANTIFICATION OF FIBROSIS

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Imanova, Burlington Danes Building, Imperial College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK

Objectives: The expression levels of the epithelial-specific integrin $\alpha_v\beta_6$ are up-regulated following injury as well as carcinomas and fibrosis. The peptide NAVPNLRGDLQVLAQKVART (A20FMDV2), derived from the foot and mouth disease virus, has been identified as a potent selective binder of $\alpha_v\beta_6$. Initial studies in animal models have indicated that [^{18}F]FBA-A20FMDV2 ([^{18}F]IMAFIB) is a promising tool compound for noninvasive imaging of $\alpha_v\beta_6$ expression *in vivo*.[1] The objectives of the present work was to evaluate the specificity of the [^{18}F]IMAFIB uptake in rodent $\alpha_v\beta_6$ expressing tissues and to enable the tracer translation for clinical use.

Methods:[^{18}F]IMAFIB was labelled using a GMP compliant and fully automated process as previously described.[2] Briefly, [^{18}F]IMAFIB was labelled with fluorine-18 by conjugation of the resin bond precursor (A20FMDV2 peptide on rink amide resin) to the prosthetic group [^{18}F]-fluorobenzoic acid ([^{18}F]FBA), followed by acidic cleavage from the resin, purification by semi-preparative HPLC and reformulation in saline. Quality control methods for clinical batches of [^{18}F]IMAFIB were developed in accordance to EP guidelines. Plasma metabolite analysis was performed using the so-called 'Hilton method'.

A whole body distribution of [^{18}F]IMAFIB in healthy Sprague-Dawley rats was performed to estimate the radioactivity exposure of humans. Rodent tissue data were adjusted to reflect human values based on the different proportions of organ to total body mass in rat and human. Using these data, dosimetry calculations provided the individual organ doses and the whole body effective dose.

Rodent studies were performed under baseline conditions and following administration of homologous block of IMAFIB (2 mg/kg). PET-CT scans were carried out and metabolite-corrected arterial input functions were acquired. Quantitative compartmental analysis of the tracer regional kinetics was performed using a parent plasma input function. The regional volumes of distribution (V_T) were generated for all scans as outcome measure.

Results: Typically, 450 MBq of [^{18}F]IMAFIB were synthesised with a SA of 39 ±2 GBq/μmol and with high radiochemical purity (>97%). An estimated human effective dose of 0.035 mSv/MBq of injected activity was determined from the rodent preclinical dosimetry study. The regional time-activity curves under baseline conditions and homologous blocking conditions showed a decrease of activity uptake in both lung and liver following treatment. Metabolite analysis showed a rapid metabolism of [^{18}F]IMAFIB in rat plasma with approximately 5% of intact radiotracer still present at 30 min. The tracer kinetics were well described by a compartmental modelling. The V_T values for lung and liver were reduced (56%, 94%, respectively) following administration of the unlabelled IMAFIB demonstrating a displaceable binding component in these regions. A whole body distribution of [^{18}F]IMAFIB in rodents was acquired to estimate the radioactivity exposure to human volunteers. The highest uptake was observed in the stomach content and small intestine content, followed by the kidney, pancreas and liver.

Conclusion: [^{18}F]IMAFIB *in vivo* data showed a significant displaceable binding component in the rodent liver and lung. Clinical studies are ongoing and will be reported separately.

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HUMAN INDUCED PLURIPOTENT STEM CELL (hiPSC)-DERIVED CARDIOMYOCYTES: CHEMICALLY-DEFINED DERIVATION, GENETIC MANIPULATION AND APPLICATIONS IN DRUG DEVELOPMENT

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The Nobel Prize awarded discovery that somatic cells can be reprogrammed to an induced pluripotent stem cell-like state and the ability to generate cardiomyocytes (CMs) from these iPSCs using directed differentiation methods have provided a fertile ground for many applications. Such applications are not only of therapeutic nature (= regenerative medicine) but also include technologies for the drug discovery process (= safety pharmacology and toxicology) and disease modelling (= disease-in-a-dish) for basic and applied research in cardiac biology. Although current differentiation protocols enable the derivation of CMs from hiPSCs in high yields under chemically-defined conditions, there are still several obstacles for the above outlined applications. [1-3]

Here, we provide an overview on our hiPSC-based approaches to the discovery and characterization of new modulators of cardiac differentiation and regeneration. These efforts build on using a robust and efficient chemically-defined differentiation protocol that allows us to establish a platform of new high-content assays.[4] In this regard, we are exploring different methods for generating hiPS reporter cell lines harboring fluorescence reporter cassettes (e.g., bacterial artificial chromosome transgenesis) to determine CM yields, constitution and their proliferative capacity. One aim is, for example, the identification of pro-proliferative small molecules by 'forward chemical genetics'. This approach would not only allow the expansion of early, immature CMs for transplantation but also increase our knowledge of mechanistic cues that direct the postnatal loss of CM proliferation.

Moreover, we are using the assay platform to study mechanistic aspects of (new) small molecule Wnt/β-Catenin inhibitors as this pathway is a key player within cardiopoietic differentiation. These studies might be useful to enable cell context-specific Wnt inhibition (= safety for *in vivo* applications) and improving the quality of hiPSC-derived CMs.

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BIOCATALYTIC C-H ACTIVATION OF THE JAK INHIBITOR RUXOLITINIB

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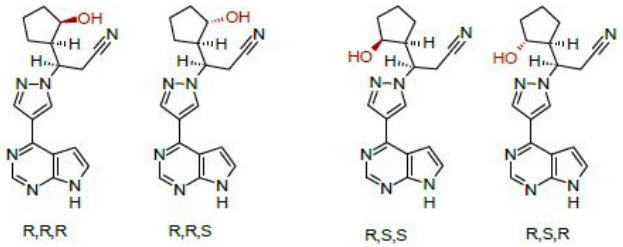
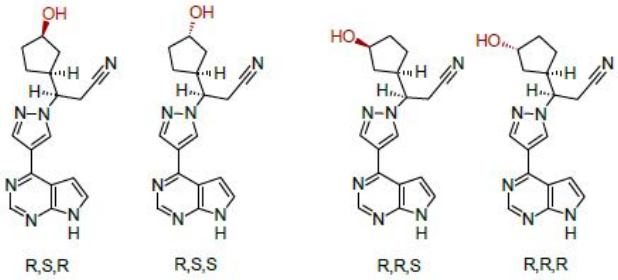
The first-in-class JAK inhibitor, ruxolitinib (trade names Jakafi®and Jakavi®), is primarily metabolized to a complex mixture of stereoisomeric cyclopentyl hydroxyl and keto metabolites. Application of Hypha's microbial-based biocatalytic C-H bond activation to ruxolitinib resulted in the production of an array of hydroxylated and further oxidized keto metabolites, many of which corresponded to circulating human metabolites. All possible oxidized isomers of the aliphatic cyclopentyl moiety were derived from a variety of microbial species which were readily scaled up, enabling efficient production of stereoisomer metabolite standards for structural characterization and bioanalytical monitoring.

Putative 2– and 3-cyclopentyl hydroxylated stereoisomeric metabolites of ruxolitnib produced via aliphatic methylene hydroxylation by a selected bacterial strain from Hypha's biotransformation panel. Corresponding cyclopentyl ketone derivatives of metabolites were also produced and identified.

2-Hydroxylation of Cyclopentane Moiety



3-Hydroxylation of Cyclopentane Moiety



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SYNTHESIS AND ANTI-INVECTIVE EVALUATION OF SUBSTITUTED 3-(PHENYLCARBAMOYL)PYRAZINE-2-CARBOXYLIC ACIDS AND THEIR ESTERS

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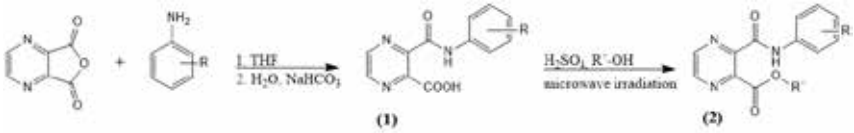
Pyrazinamide is a first-line antitubercular drug and has been used for over sixty years. Pyrazinamide has a significant role in shortening of tuberculosis treatment. As a small molecule pyrazinamide and metabolically derived pyrazinoic acid offer many ways how to affect mycobacteria: acidification of cytoplasm[1], inhibition of trans-translation (liberation of ribosomes trapped in faulty protein synthesis)[2], inhibition of Fatty Acid Synthase I (synthesis of mycolic acids)[3] and aspartate dexarboxylase (involved in energetic metabolism)[4].

Series of substituted 3-(phenylcarbamoyl)pyrazine-2-carboxylic acids **(1)** and their methyl and propyl esters **(2)** were prepared in this project.



R - H, 2,5-diCH$_3$, 4-CH$_2$CH$_3$, 2,4-diOCH$_3$, 2-OH, 4-NO$_2$, 4-N(CH$_3$)$_2$, 2,4-diF, 3,4-diCl, 4-Br, 3-CF$_3$, 4-CF$_3$, 2-CH$_3$, 5-F, 5-CH$_3$, 2-Cl, 2-OH, 5-NO$_2$, 2-OH, 5-Cl

R'- methyl, propyl

The starting compound pyrazine-2,3-dicarboxylic anhydride reacted with substituted aniline to obtain compound with amide and carboxylic moiety **(1)**. In the following step the carboxylic group was esterified by methanol or propanol **(2)**. Microwave irradiation was used to form ester.

Prepared compounds were characterized with analytical data and tested *in vitro* for their antimycobacterial (*M. tuberculosis* H37Rv, *M. avium*, *M. kansasii* and *M. smegmatis*), antibacterial and antifungal activity. Structure activity relationship will be discussed.

The study was supported by the Grant Agency of Charles University, project B–CH/1594214, SVV 260 291 and Zentiva company.

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SYNTHESIS OF NEW 4-ARYL-PYRIDO[1,2-C]PYRIMIDINE DERIVATIVES AS POTENTIAL ANTIDEPRESSANT AGENTS

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Research on the synthesis and biological evaluation of pyrido[1,2-c]pyrimidine derivatives has been carried out in the Department of Drug Technology and Pharmaceutical Biotechnology, Medical University of Warsaw, for the last decade. Earlier work in the mentioned subject described a series of derivatives of pyrido[1,2-c]pyrimidine with 3-(piperidin-4-yl)-1H-indole residue in the pharmacophore element. A series of compounds with a high affinity to both molecular targets – $5HT_{1A}$-R and SERT - as well as a suitable functional profile with 5-HT_{1A} receptor binding (pre- and postsynaptic agonism) was obtained [1-4]. The aim of the current research was to implement 3-(piperidin-3-yl)-1H-indole structure in place of 3-(piperidin-4-yl)-1H-indole residue in order to receive a more serotonine-like pharmacophore element.

The concept of combining SSRI activity with 5-HT_{1A} agonism was proposed and extensively studied in recent years as a promising strategy for potential new antidepressants development [5-7]. The validity of this approach was confirmed by the registration of vilazodone (Viibryd), which entered the American market in 2011 as the first dual acting antidepressant (SSRI/5-HT_{1A} agonist). Moreover, in clinical trials vilazodone proved to be well tolerated, with a low discontinuation level and no severe, life-threatening adverse effects [8]. In 2013, the Food and Drug Administration approved another SSRI/5-HT_{1A} agonist – vortioxetine (Brintellix) – for the treatment of major depressive disorder in adults.

Novel 4-aryl-pyrido[1,2-c]pyrimidine derivatives are obtained by way of a multi-step chemical synthesis and subjected to analytical studies, using the methods of 1H NMR and 13C NMR spectroscopy as well as HRMS. The pharmacological profile of the obtained compounds was assessed in radioligands binding assays (5-HT_{1A}, SERT). In vivo functional studies will be conducted in the Institute of Pharmacology, Polish Academy of Sciences and Department of Pharmacobiology, Jagiellonian University Medical College in Kraków; metabolic stability evaluation in the Department of Pharmaceutical Chemistry, Medical University of Gdańsk. The results of in vitro and in vivo studies will allow us to draw conclusions regarding structure-activity relationship in the tested group of compounds and to select compounds for further pre-clinical evaluation.

We acknowledge the financial support of the Polish National Science Center grant, OPUS 6, No. UMO-2013/11/B/NZ7/01638.

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FXR ANTAGONISTS BASED ON A LIBRARY OF OLEANOIC ACID 3-O-ESTERS

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Farnesyl X receptor (FXR) is an attractive pharmacological target especially for the treatment of various liver and metabolic diseases, e.g., non-alcoholic steatohepatitis, cholestasis, atheroscalerosis and diabetes. Based on the previous finding that a natural product oleanolic acid (OA) exhibiting antagonism on FXR, we initiated a structure optimization effort through the incorporation of a variety of esters to C-3 position of OA. To maximize the structural diversity of C-3 esters, we selected 285 representative carboxylic acids from a virtual library containing more than 30,000 carboxylic acid members in MDL® ACD database and screened those OA C-3 esters by autodock to generate four hits, which were synthesized and bioassayed to confirm their FXR antagonistic activity. This study not only led to the discovery of some OA C-3 ester based FXR antagonists with moderate potency, but also validated the model for this series of ligands in complex with FXR which will promote further investigation on more potent triterpene-based FXR interacting agents.

FRAGMENT-BASED HIT DISCOVERY FOR THE EPIGENETIC TARGET, BRD3

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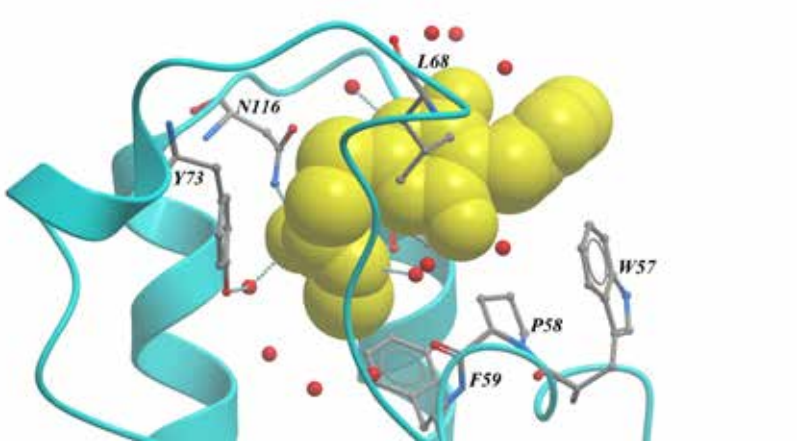
2) Peak Proteins, BioHub, Alderley Park, Alderley Edge, Cheshire, SK10 4TG, UK

Sygnature Discovery has designed and synthesised a proprietary fragment library with a high degree of novelty that covers a wide range of chemical space. Comprised of over 900 compounds, most of which are not commercially available (>60%), the library was designed using the 'rule of 3' as a guideline and with a strong emphasis on diversity.

To demonstrate its effectiveness in fragment-based drug discovery, the library was screened against an epigenetic target with little previous literature on drug discovery, Bromodomain-containing protein 3 (BRD3, also known as RING3L). BRD3, like other members of the Bromodomain and Extra-Terminal motif (BET) family, contains two tandem homologous bromodomains and an extra terminal motif. BRD3 functions by binding acetylated lysine residues on chromatin and transcriptional regulators, exemplified by the role it plays in the regulation of transcription by promoting the binding of GATA1. Due to this involvement in regulation, BET members often play a role in several types of cancer. BRD3 in particular is associated with a number of disease phenotypes. For example, depletion of BRD3 slows growth in cancer models including prostate cancer and medulloblastoma and BRD3 has been implicated in NUT midline carcinoma (NMT).

While there are a number of available pan-BET inhibitors, the design of specific BRD3 inhibitors may lead to a beneficial clinical outcome with reduced off-target effects. However, bromodomains of the BET family have a high degree of structural similarity, especially in the acetylated lysine binding pocket, making the design of selective inhibitors problematic. BRD3 therefore represents an intriguing and challenging drug discovery target.

Using Surface Plasmon Resonance (SPR), Sygnature's proprietary fragment library and protein provided by Peak Proteins, we screened for fragments that bound to domain 1 of BRD3 (BRD3D1). Of 908 fragments screened, nine were found to bind with K_D ranging from The binding of the fragments was then confirmed by X-ray crystallography, six of which returned structures with resolutions better than 1.7 Å. By using these fragments as starting points to develop tool ligands, we could further interrogate BRD3 involvement in disease phenotypes as well as a potential starting point for a new drug discovery program.



SYNTHESIS OF NOSCAPINE ANALOGUES AS POTENTIAL ANTI-CANCER AGENTS

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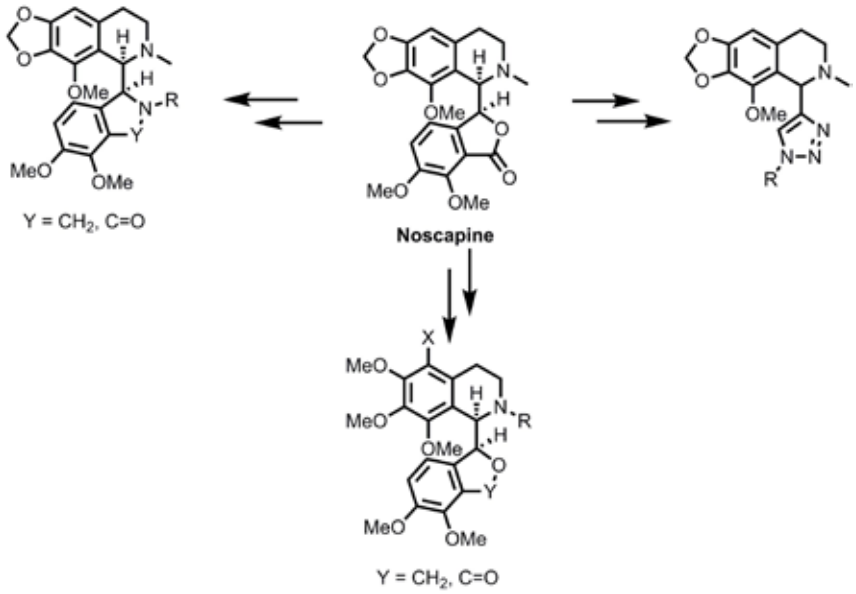
Noscapine, an opioid isolated from *Papaver somniferum*, has been used as an anti-tussive agent for over 50 years. Despite widespread use, it has shown low incidence of side-effects. More recently, noscapine and its derivatives have gained widespread interest as anti-cancer agents through interruption of microtubule polymerisation.[1,2] A wide range of semi-synthetic noscapine analogues have been synthesised so far, improving activity up to 40-fold in breast cancer. Most of these derivatives have involved the addition or modification of side-chains and functional groups, but little has been done in altering the core structure.

The goal of this project was to expand the library of noscapine analogues with modified ring systems. To this end, the noscapine 6,7-dimethoxyphthalide ring was replaced in a series of triazole derivatives, and modified towards isoindoline and isoindolinone moieties.

A series of noscapinoids was also synthesised in which the 1,3-dioxolane moiety of the native tetrahydroisoquinoline ring was cleaved and converted to give a range of methoxy-substituted analogues, including the 1,2,3-trimethoxyphenyl group, a motif that appears in a number of existing microtubule-binding anti-cancer drugs.[3,4] These analogues were further modified with side-chains that have proven to improve noscapine activity in prior work.

The activities of these compounds in breast, prostate and pancreatic cell lines are presented.



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ACTIVITY OF ETHIONAMIDE-MESOPOROUS SILICON NANOPARTICLES AGAINST MYCOBACTERIUM TUBERCULOSIS

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Ethionamide (ETH, Figure 1) is an important second-line antituberculosis drug used for the treatment of patients infected with multidrug-resistant mycobacteria [1]. Recently, we reported the loading of ETH into thermally carbonized-porous silicon (TCPSi) microparticles, and we studied the solubility, toxicity, permeability, and metabolic profiles of the drug-loaded TCPSi. The solubility and permeability of ETH was clearly enhanced after loaded into TCPSi particles at different pH-values and showed a fast metabolization process in the presence of the TCPSi particles [2].

After that, carboxylic acid functionalized thermally hydrocarbonized porous silicon nanoparticles (UnTHCPSi-NP) were synthesized with ETH and we evaluated the antimicrobial effect of this conjugate against *Mycobacterium tuberculosis* strain H37Rv, the reference strain commonly used in research laboratory settings. In this communication, it is reported the minimal inhibitory concentration (MIC_{50}) of the conjugate and ETH, as the lowest concentration of antibiotic that inhibits 50% of the growth of the microorganism.

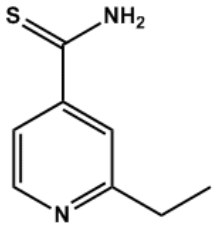

Figure 1. Structure of ethionamide (ETH).

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PROBING ALLOSTERIC REGULATION OF AN EXECUTIONER CASPASE

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The caspase family of cysteine-dependent, aspartate-specific endoproteases have attracted widespread attention as drug targets due to their fundamental roles in apoptosis and inflammation pathways. Caspases employ a catalytic cysteine-histidine dyad to judiciously hydrolyze peptide bonds, in order to carry out their essential cellular functions. Apoptotic caspases are further segregated into initiator and executioner caspases. Initiator caspases (-8, -9) activate the executioner caspases (-3, -6, -7) in order to carry out the final steps of apoptosis. Cell death resulting from aberrant caspase activation has been implicated in numerous cardiovascular and neurodegenerative diseases.

Numerous screening efforts have been directed towards the development of caspase inhibitors. To date, the overwhelming majority of these compounds target the active site through a form of mechanism-based inhibition. However, due to the aspartate preference of the executioner caspases, these molecules are inherently charged and lack drug-like properties. Targeting a known allosteric site at the dimer interface is proposed to yield molecules with superior drug properties. We also hypothesize that current HTS libraries do not contain the complementary chemical space for the allosteric site of the caspases, and that fragment screening will provide the necessary diversity to discover allosteric caspase inhibitors.

In order to maximize screening chemical diversity, we leveraged fragment-based drug discovery. A fragment library was screened against caspase-7 by differential scanning fluorimetry (DSF). Compounds identified from the screen were further tested by DSF, functional assays, and surface plasmon resonance. Two non-competitive inhibitors from the screening effort have been characterized by X-ray crystallography and were confirmed to bind at the aforementioned allosteric site. Further work is being done to understand the mechanism by which these compounds are allosterically inhibiting caspase-7.

TOWARDS THE ELUCIDATION OF THE MECHANISM OF ACTION OF SMALL MOLECULE UPREGULATORS OF UTROPHIN USING CHEMICAL PROTEOMICS

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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 approaches (e.g. exon skipping, read through of stop codons, gene therapy) are being developed none of them have yet shown efficacy in man and gained FDA approval. Our strategy to deliver an effective therapy for DMD is to develop an oral small molecule upregulator that replaces and compensates for the missing dystrophin with utrophin, its autosomal paralogue. This 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 small molecule utrophin modulator that reduces dystrophic symptoms in the *mdx* mouse,[1] is in a Phase 2 clinical trial.[3,4]

Ezutromid demonstrates proof of principle for the strategy, but we still need to rapidly parallel track 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 utrophin modulators we have conducted structure-activity relationship studies within one of the novel compound classes and as a result pull-down chemical probes have been prepared for chemoproteomic analyses. Finding optimal conditions for the pull-down assay is a challenging process because the cellular location of the potential target(s) and binding affinity of the probe to target(s) remains unknown. To overcome these problems and poor cell-permeability of biotin-tagged probes we have prepared improved dual tagged probes which incorporate both a photoaffinity group (diazirine) and an alkyne tag which retain comparable activity to their unlabelled counterparts. By applying Cu(I)-catalysed azide-alkyne “click chemistry” the biotin tag is introduced after cell lysis. Pull-down experiments and cellular thermal shift proteomics analysis[5] using the improved dual tagged probes are underway.

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NOVEL INDOLE-FLUTIMIDE HETEROCYCLES WITH ACTIVITY AGAINST INFLUENZA PA ENDONUCLEASE AND HEPATITIS C VIRUS

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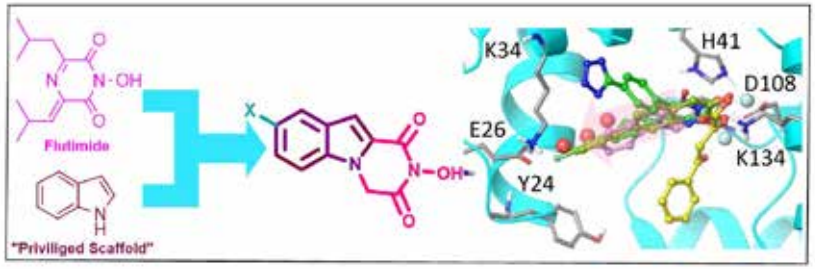
Influenza viruses cause considerable morbidity and mortality, whether in the context of annual epidemics, sporadic pandemics, or outbreaks of avian influenza virus. For hepatitis C virus (HCV), an estimated 170 million people are chronically infected worldwide. These individuals are at high risk of developing progressive liver injury or hepatocellular carcinoma. Since the efficacy of currently approved antiviral drugs is threatened by emerging viral resistance and the cost remains high, new antiviral drugs are still required.

By utilizing a structure-based approach, novel substituted indole-flutimide heterocyclic derivatives (1,2-annulated indolediketopiperazines) were rationally designed, synthesized and evaluated as influenza PA endonuclease and HCV NS5B polymerase inhibitors. The compounds were also tested for their antiviral effect against HCV and cytotoxicity.

All *N*-hydroxyimides were potent PA endonuclease inhibitors while displaying low cytotoxicity. The novel unsubstituted indole-flutimide heterocyclic derivative proved to be the most active analogue, while the most favorable indole substitution was fluorine at position 8. The chloro-derivative showed additional potent anti-HCV activity and exhibited remarkable selectivity (>19). In accordance with the SAR data, removal of the hydroxyl group from the imidic nitrogen caused a complete loss of activity against influenza PA as well as HCV.

Our findings suggest that the novel pyrazino[1,2-α]indole-1,3(2*H*,4*H*)-dione framework that we have developed, following mild and experimentally convenient protocols, offers a promising motif for further construction of new analogues with optimized antiviral properties through appropriate substitution on the diketopiperazine or indole ring nuclei.



DEVELOPMENT OF AN ALBUMIN-BINDING LIGAND FOR PROLONGING THE PLASMA HALF-LIFE OF PEPTIDE THERAPEUTICS

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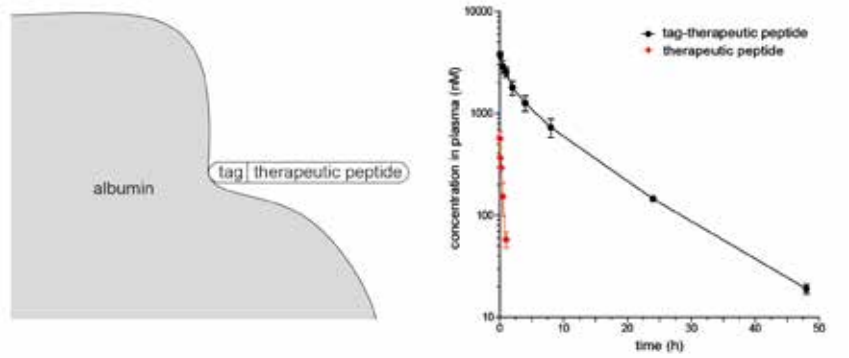
Peptide therapeutics applied intravenously are rapidly cleared from the blood circulation by renal filtration. The short half-life prevents their application to diseases that require drug exposure of several hours or days (1). An attractive strategy to hamper filtration of peptides in the kidneys is to tether them non-covalently to a long-lived serum protein such as human albumin (2). Several albumin-binding ligands based on peptides or small molecules were developed but they suffer from relatively low affinities for human albumin as well as a poor solubility in physiological buffers, reducing their potential application to peptide therapeutics.

To overcome these limitations, a chimeric peptide-small molecule albumin ligand with low nanomolar affinity for human, rat and rabbit albumin, a high solubility and a small size suitable for automated synthesis of complex conjugates was successfully developed. Peptides conjugated to the tag retained their bioactivity and displayed around a 30-fold increase in half-life in rats.



Schematic structure of the albumin-binding ligand (tag) (left panel) and pharmacokinetics of a therapeutic peptide and its conjugated format in rat plasma upon i.v. injection (right panel).

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SYNTHESIS OF 5-FLUOROPYRIMIDINE NITRILES AS POTENT HUMAN CATHEPSIN L (hCatL) INHIBITORS

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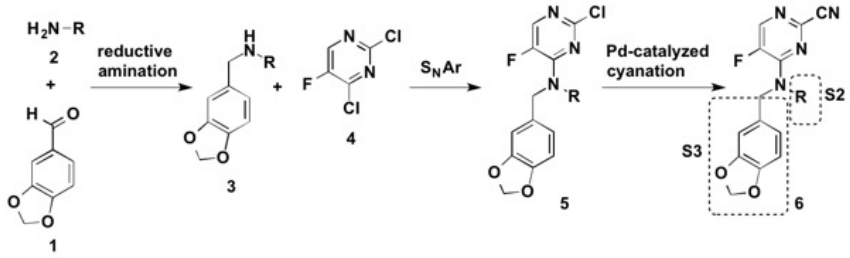
The human cysteine protease cathepsin L (hCatL) has been of interest in our group for several years.[1,2] It is an enzyme known to be upregulated in a variety of human cancers and has been considered as target for cancer treatment.[3] Similarly, we investigated the inhibition of the parasitic hCatL-like enzymes rhodesain and falcipain-2, which are promising targets for the treatment of African sleeping sickness and malaria, respectively.[4]

Structure-based drug design on rhodesain, falcipain-2 and hCatL led to the discovery of potent diaminotriazine nitrile-based inhibitors. The active site of hCatL consists of three pockets, and reversible covalent binding of the catalytic Cys to the activated nitrile provides the major stabilization of the formed protein–ligand complexes. Extensive structure-activity relationship studies revealed that substituents in the S1 pocket have negligible impact on binding affinity and can therefore serve as useful handle to tune selectivity and pharmacokinetic properties. On the other hand, ligand substituents pointing towards the S2 pocket are major contributors to binding affinity with a preference for hydrophobic substituents. The proper occupation of the S3 pocket by aryl vectors, which undergo stacking interactions with the peptide backbone at the entrance of the pocket, also has a large role in providing binding affinity.[5] However, high electrophilicity of the nitrile head group of triazine nitriles leads to high cytotoxicity caused by off-target effects.[6]

We therefore used molecular modeling to design a series of ligands with lower electrophilicity of the nitrile head group, which are based on 5-fluoropyrimidine nitriles bearing a 1,3-benzodioxolyl group as S3 substituent and various alkyl, aryl, and heteroaryl moieties as S2 substituents.

The final ligands were synthesized by a three-step protocol. Reductive amination of piperonal (**1**) with corresponding primary amines **2** furnished secondary amines **3**, which were attached to 2,4-dichloro-5-fluoropyrimidine (**4**) by nucleophilic substitution. Final nitriles **6** were obtained by palladium-catalyzed cyanation of chloropyrimidines **5**.



R: cycloalkyl, aryl, heteroaryl; all connected directly or via methylene, ethylene, or propane-1,3-diyl units

This work was supported by Roche Basel and a grant from the ETH research council.

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