2023 ASP Annual Meeting

Innovation Through Interaction

Rockville, MD
July 22 – 26, 2023

American Society of Pharmacognosy

Annual Meeting

Innovation Through Interaction

Rockville, MD
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By the 2023 ASP Organizing Committee

The 2023 Organizing Committee of The American Society of Pharmacognosy (ASP) is delighted to welcome you to Rockville, Maryland. The North Bethesda Marriott Hotel and Conference Center is steps away from the Metro which can quickly connect you with the three nearby airports as well as all that Washington D.C. has to offer. We hope you will agree that the program for this year's meeting is full of exciting topics and speakers. Additionally, there are a series of pre-meeting workshops that provide an opportunity to learn about botanical drug development regulations from the FDA, innovative assay technologies, fungal taxonomy, botanical safety, and public speaking for scientists. As always, there will be multiple opportunities to connect with old friends and make new ones during one of the organized social activities. Across the street from the hotel is the Pike and Rose district which is full of shopping and dining options. Of course, if you are coming to the meeting, you must take advantage of all the free attractions in the District. Whether it's a walk along the National Mall to see all the monuments or visiting one of the 13 Smithsonian Museums, you don't want to miss it. Regardless if this is your first visit to D.C. or your tenth, there is always something new to see and experience.

We want to hear from you #ASP2023

2023 ASP Annual Meeting

Organizing/Scientific Committee

Craig Hopp, Chair - (NCCIH)
Nandakumara Sarma Chair **-** (U S Pharmacopeia/USP)

Carole Bewley (NIDDK, NIH)
Lin Du (NCI at Frederick)
Ikhlas Khan (NCNPR, University of Mississippi)
Emily Mevers (Virginia Tech)
Barry O'Keefe (National Cancer Institute)
Timothy Ramadhar (Howard University)
Barbara C. Sorkin (Office of Dietary Supplements, NIH)

Meeting Planner/Registration

Laura Stoll (The American Society of Pharmacognosy)

American Society of Pharmacognosy

CODE OF CONDUCT

The American Society of Pharmacognosy (ASP) believes that the pursuit of scientific excellence is strengthened by the unique perspectives contributed by scientists from diverse backgrounds. The society strives for an inclusive environment that makes all our members feel included, welcomed and represented. We expect our members to interact with each other in a positive, professional manner, and to conduct themselves with kindness and courtesy. Members participating in discussions at our meetings should remain open-minded to different points of view and opinions and be professional and respectful when expressing dissent.

The ASP will not tolerate threatening, intimidating, or harassing behavior from any individual associated with the society or its events. For the purpose of this policy, harassment means unwelcome behavior directed at another person's sex, race, color, national origin, religion, sexual orientation, gender identity, disability, age, or other status protected under applicable law. For example, harassment can include comments or jokes that focus on gender differences or sexual topics, unwelcome advances or requests for dates or sexual activities, or the use of language or images that demean or degrade others.

Violations to this code of conduct may be reported to Laura Stoll, business manager for the American Society of Pharmacognosy (asphcog@ gmail.com) or Lesley-Ann Giddings (lgiddings@smith.edu) or Christine Salomon (csalomon@umn.edu) Co-Chairs of the ASP Diversity, Equity and Inclusion Committee. By registering for this conference, you have agreed to abide by the code of conduct. The ASP reserves the right to revoke the conference badge of any individual who violates the ASP code of conduct.

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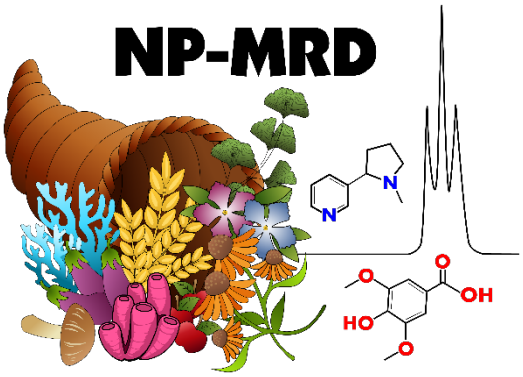

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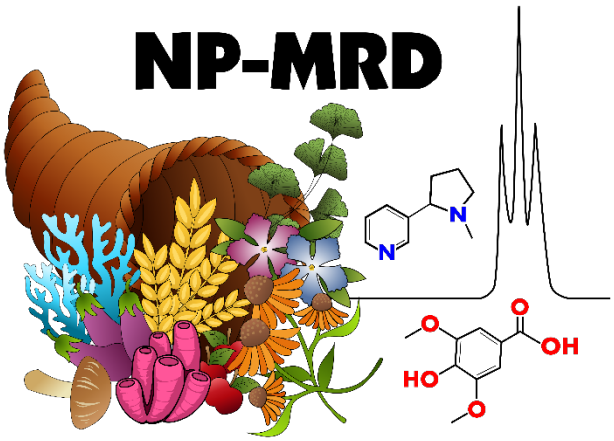

ASP Award Winners

Norman R. Farnsworth Research Achievement Award
Sheo B. Singh, Stevens Institute of Technology (2023)

Varro E. Tyler Prize
Djaja Djendoel Soejarto, University of Illinois at Chicago (2022)
Robert Verpoorte, Leiden University (2023)

Matt Suffness Young Investigator Award
Laura Sanchez, University of California, Santa Cruz (2022)
Jason Christopher Kwan, University of Wisconsin-Madison (2023)
Sandra Loesgen, University of Florida (2023)

Undergraduate Research Grant
Rodrigo Carrillo - Centro de Investigación CIentífica de Yucatán A. C.
Marvin Rositzki - University of Hawai'i at Hilo

Research Starter Grant
Benjamin Naman - San Diego Botanic Garden

D. John Faulkner Travel Award
Yuanyuan Ji - University of Maryland, Baltimore
Jeffrey Rudolf – University of Florida

David Carew Student Travel Award
Jose Alberto Gutiérrez- González - Universidad Nacional Autonoma de Mexico

Jerry McLaughlin Student Travel Award
Marcio Barczyszyn Weiss – Sao Paulo University
Vitor Lourenzon - University of Illinois at Chicago

Lynn Brady StudentTravel Award
Riley Blue - University of California, Santa Cruz
Timothy Bushman - University of Alabama
José D.D Cediel- Becerra – University of Florida
Cole Gannett – Virginia Tech
Kabre Heck – Auburn University
Thanh-Hau Huynh – Seoul National University
Eunah Jeong - Sookmyung Women's University
Lois Kwane Kyei – Virigina Tech
Diana Łomowska- Keehner - University of Florida
Huong Thi Pham - Sookmyung Women's University
Herma Pierre - University of North Carolina Greensboro
Liana Zaroubi – Simon Fraser University

Robert Krueger Travel Award
T'ea P Cameron - University of North Carolina at Greensboro
Maricarmen Corona - Centro de Investigación Científica de Yucatán, A.C.
Erin Marshall – University of Florida
Samuel Tanoeyadi – Oregon State University

Waqar Bhatti Student Travel Award
Logan Breiner – Virginia Tech

2023 Arthur E. Schwarting Award
Triculamin: an unusual lasso peptide with potent anti-mycobacterial activity. Frederikke D. Andersen, Katja D. Pedersen, Dennis Wilkens Juhl, Tobias Mygind, Paul Chopin, Esben B. Svenningsen, Thomas B. Poulsen, Marie Braad Lund, Andreas Schramm, Charlotte H. Gotfredsen, and **Thomas Tørring**, J. Nat. Prod. 2022, 85, 1514–1521.

2023 Jack L. Beal Award
Untargeted Identification of Alkyne Containing Natural Products using Ruthenium Catalyzed Azide Alkyne Cycloaddition Reactions Coupled to LC-MS/MS. Daniel Back, Brenda T Shaffer, Joyce E Loper, **Benjamin Philmus**. J. Nat. Prod. 2022, 85, 105-114.

American Society of Pharmacognosy

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Program Schedule

Saturday July 22, 2023

8:00 AM – 8:00 PM	**Registration** – *Ballroom Foyer*
9:00 AM – 4:00 PM	**Executive Committee Meeting** (Invitation Only) – *Lindon Oak Room*
9:00 AM – 12:00 PM	**AMWS1 – Botanical Drugs: A Regulatory Approach on how FDA reviews a Botanical Application -** *Forest Glen Room* Presenters: **Rajiv Agarwal** (DNDP1/ONDP/OPQ/CDER/FDA), **Donna Christner** (DNDAPI, ONDP/OPQ/CDER/FDA), **Margaret M. Kober** (DRORDPURM/ ORO/OND/CDER/FDA), **Mark Hirsch** (DUOG/ORPURM/OND/CDER/ FDA), **Charles Wu** (ONDP IO, ONDP/ OPQ/CDER/FDA)
9:00 AM – 11:00 PM	**AMWS2 - Biochemical and Biophysical Assay Design for Natural Product Discovery Campaigns -** *White Flint Ampitheatre* Presenters: **Shilpa Shenoy Kurian** (National Cancer Institute, National Institutes of Health) and **Brice A. P. Wilson** (National Cancer Institute, National Institutes of Health)

1:30 PM – 4:30 PM **PMWS1 – Current Topics in Botanical Safety -** *Forest Glen Room*
Presenters - **Michelle Embry** (HESI), **Josh Kellogg** (Penn State**), Robin Marles**, **Barbara C. Sorkin** (NIH), **Charles Wu** (USFDA)

1:30 PM – 4:30 PM **PMWS2 – Fungal Identification Using Molecular Tool**
White Flint Ampitheatre
Presenter: **Huzefa Raja** (University of North Carolina at Greensboro)

1:30 PM – 4:30 PM **PMWS3 – Public Speaking for Scientists** - *Glen Echo*
Nicholas Oberlies (University of North Carolina at Greensboro)

6:00 PM – 9:00 PM **President's Opening Reception** – *Salon E-H (Ticketed Event)*

Sunday July 23, 2023

7:30 AM – 3:30 PM **Registration** – *Ballroom Foyer*

Welcoming Remarks and Announcements

8:00 AM – 8:30 AM **Craig Hopp** (NCCIH), **Nandakumara Sarma** (USP) and **Amy Wright** (ASP President)

Symposium I – Where Chemistry Meets Biology – *Salon A-D*

Chairs: **Carole Bewley** (NIDDK, NIH) and **Barry O'Keefe** (National Cancer Institute)

8:30 AM – 9:15 AM PL-01 - **Jay Keasling** (University of California Berkeley)
Production of Supply-Limited Natural Product Therapeutics Using Engineered Yeast

9:15 AM – 10:00 AM PL-02 - **Bonnie Bassler** (Princeton University)
Quorum Sensing Across Domains: From Viruses to Bacteria to Eukaryotes

10:00 AM – 10:30 AM **Break** – Ballroom Foyer

Symposium II – NP that modifies Macromolecular Interactions: PPI, RNA-Prot, DNA-Prot etc. – *Salon A-D*

Chairs: **Carole Bewley** (NIDDK, NIH) and **Barry O'Keefe** (National Cancer Institute)

10:30 AM – 11:15 AM	PL-03 - **Anna Mapp** (University of Michigan) *Writing the Rules for Targeting Dynamic Transcriptional Coactivators with Natural Products*
11:15 AM - 11:45 AM	I-01 - **Oleg Tsodikov** (University of Kentucky) *Structural Insight into Antagonism of Oncogenic Transcription Factors by Mithramycin Analogues*
11:45 AM – 12:15 PM	I-02 - **Roberto DeGuzman** (University of Kansas) *Bacterial Nanoinjectors as Target for New Antimicrobials*

12:15 PM – 1:30 PM	**Journal of Natural Products Board Meeting** (Invitation Only) *Forest Glen Room*

12:15 PM – 2:30 PM	**Presenters for Poster Session I Set up Posters** - *Salon E-H*

Session S-PM1 – Old Molecules/New Purposes – *Salon A-C*

Chair: **Wendy Strangman** (University of North Carolina Wilmington)

1:15 PM – 1:45 PM	I-03 - **Patrick Grohar** (Children's Hospital of Philadelphia, University of Pennsylvania) *Development of Mithramycin and Analogs as Targeted Therapy for Pediatric Solid Tumors*
1:45 PM – 2:05 PM	C-01 - **Cole Gannett** (Virginia Tech) *Derivatives of the Antimicrobial Natural Product Blasticidin S Enhance its Antibiotic Activity*
2:05 PM – 2:25 PM	C-02 - **Mark Hamann** (Medical University of South Carolina) *The Unique Potential of the Platanosides as a New Class of Antibiotics with an Unreported MoA targeting a Protein Involved in Cell-Wall Biosynthesis as well as Providing Significant Ecological Implications Regulating Microbiome of the Tree Platanus Occidentalis*

2:25 PM – 2:45 PM C-03 - **Benjamin Blackburn** (Virigina Tech)
Repurposing of Medicinal Plant Metabolites, and Plant Metabolite-Inspired Compounds to Further Optimize Renewable Energy Technologies

2:45 PM – 3:05 PM C-04 - **Savannah Pierce** (University of the Pacific)
Rivularia spp. Cyanobacterial Fractions Selectively Regulate the Ubiquitin-Proteasome System

3:05 PM – 3:25 PM C-05 - **William Gerwick** (Scripps Inst Oceanography/UCSD)
Development of an Artificial Intelligence-Based Tool for Predicting Cancer Cell Cytotoxicity of Natural Products

Session S-PM2 – Microbiome/Probiotics Interactions – *Salon D*

Chair: **Barbara C. Sorkin** (NIH Office of Dietary Supplements)

1:15 PM – 1:45 PM I-04 - **Yasmin Belkaid** (NIAID)
Microbiome Control of Host Immunity

1:45 PM – 2:05 PM C-06 - **Lois Kwane Kyei** (Virginia Tech)
Bioassay-Guided Isolation of Pseudochelin A from Marine Egg Mass Microbiome

2:05 PM – 2:25 PM C-07 - **Jakub Piwowarski** (Medical University of Warsaw)
Revision of Current Concepts of Tannins Impact on Gastrointestinal Tract Homeostasis in Piglets

2:25 PM – 2:45 PM C-08 - **Yeong-Bae Yun** (National Institute of Forest Science)
Growth Characteristics of 10-Year-Old Wild-Simulated Ginseng (Panax ginseng C.A. Meyer) and Rhizosphere Soil Properties at Cultivation Sites

2:45 PM – 3:05 PM C-09 - **George Neuhaus** (Oregon State University)
Specialized Metabolites of the Herptile Gut Fungus, Basidiobolus

3:05 PM – 3:25 PM C-10 - **Margaret Hill** (University of Rhode Island)
Pseudoalteromonas rubra Package Prodiginine Antibiotics as Cargo in Membrane Vesicles

3:30 PM – 5:30 PM **Poster Session I** – *Salon E-H*
Poster #'s: P-001 – P-127
Chair: TBD

All posters need to be picked up by Monday 9:00 AM

ASP is not responsible for lost or damaged posters.

6:00 PM – 9:00 PM **Younger Members Event** (Ticketed Event)
Pinstripes
11920 Grand Park Ave, North Bethesda, MD 20852

Monday July 24, 2023

8:00 AM – 3:30PM Registration – *Ballroom Foyer*

Symposium III – Bridging the Gap: Collaborations with Predominately Undergraduate Institutions and HBCU's - *Salon A-D*

Chair: Timothy Ramadhar (Howard University)

8:30 AM – 9:15 AM PL-04 - **Jiangnan Peng** (Morgan State University)
SFC and NMR Methods for the Authentication of Botanical Raw Materials

9:15 AM – 10:00 AM PL-05 - **Katherine Maloney** (Point Loma Nazarene University)
Cryptic Species Membership as the Primary Driver of Secondary Metabolism in Sarcophyton glaucum: Leveraging Interdisciplinary Collaborations to Get Research Done at a PUI

10:00 AM – 10:30 AM Break – Ballroom Foyer

Symposium IV – Public/Private Partnerships

Chair: Craig Hopp (NCCIH)

10:30 AM – 11:15 AM PL-06 - **John Bencich** and **Rick Stewart** (Achieve Life Sciences)
Kicking the Habit: Development of Cytisinicline for Smoking Cessation

11:15 AM – 11:45 AM I-05 - **Stephanie Fertig** (NIH)
SEEDing Biomedical Innovation: Support for Small Businesses at NIH

11:45 PM – 12:15 PM I-06 - **Sharad Verma** (NCI)
Programs for Helping Extramural Investigators Develop Small Molecules for the Clinic: NCI Developmental Therapeutics Program

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12:15 PM – 1:15 PM **Fellows Meeting** (Invitation Only) – *Forest Glen Room*

12:15 PM – 2:30 PM **Presenters for Poster Session II - Set up Posters**
Salon E-H

Session M-PM1 – Unique Environments – *Salon A-C*

Chair: **Lesley-Ann Giddings** (Smith College)

1:15 PM – 1:45 PM I-07 - **Andrew Lowell** (Virginia Tech)
Enhanced Enzyme Stability: Extremophile Biosynthesis of Thermorubin

1:45 PM – 2:05 PM C-11 - **Joseph P. (J. P.) Gerdt** (Indiana University–Bloomington)
Chemical Signaling Regulates Aggregation and Chemotaxis in a Predator of a Human Pathogen

2:05 PM – 2:25 PM C-12 - **Joshua P. Torres** (University of Copenhagen, Denmark)
Biosynthesis of Conopeptides in Cone Snail Venoms

2:25 PM – 2:45 PM C-13 - **Sandra Dawn Bennett** (University of North Carolina Wilmington)
Microbial Diversity and Bioactivity of Longnose Gar Eggs and Ectoparasites

2:45 PM – 3:05 PM C-14 - **Xinhui Yu** (Oregon State University)
Influence of Geographic Variation on Chemistry and Bioactivities of Pacific Northwest Endemic Cyanolichens

3:05 PM – 3:25 PM C-15 - **Mira Liu** (University of California Berkeley)
Surface-Active Antibiotics Provide an Adaptive Advantage for Bacteria from Burned Soils

Session M-PM2 – Fungal Biosynthesis and Chemodiversity-LIN – *Salon D*

Chair: **Lin Du** (NIH, National Cancer Institute)

1:15 PM – 1:45 PM I-08 - **Yi Tang** (UCLA)
Discovery of New Structures, Enzymes and Functions from Fungal Biosynthetic Pathways

1:45 PM – 2:05 PM C-16 - **Dan Xue** (University of South Carolina)
Harnessing Bioactivity-led Metabolomics for Natural Products Discovery Targeting A Rare and Severe Cancer

2:05 PM – 2:25 PM C-17 - **Robert Stankey** (Terra Bioforge)
Directed BGC Cloning Combined with Bacterial and Fungal Expression Tools Uncover Novel Biosynthetic Mechanisms and Validate Metabologenomics

2:25 PM – 2:45 PM C-18 - **Kyo Bin Kang** (Sookmyung Women's University)
Mass Spectrometry-based Untargeted Metabolomics for Investigation on Chemical Interaction and Biotransformation by Fungi

2:45 PM – 3:05 PM C-19 - **Erin McCauley** (California State University DH)
Development of a Fungal Natural Products CURE for Upper-Division Chemistry & Biochemistry Laboratory Classes

3:05 PM – 3:25 PM C-20 - **Charles Wu** (Intact Genomics, Inc)
Fungal Artificial Chromosome Pipeline Enables the Discovery of Novel Compounds from Unique Biosynthetic Gene Clusters

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3:30 PM – 5:30 PM **Poster Session II – *Salon E-H***

Poster #'s: P-128 – P-255

Chair: TBD

All posters need to be picked up by Tuesday 9:00 AM

ASP is not responsible for lost or damaged posters

6:00 PM Buses Depart for Smokey Glen Farm

6:30 PM – 9:30 PM **An Evening at Smokey Glen Farm *(Ticketed Event)***

16407 Riffle Ford Rd, Gaithersburg, MD 20878

Tuesday July 25, 2023

7:45 AM – 1:00 PM Registration – *Ballroom Foyer*

Symposium V – Who's your Partner: Symbiosis and NP – *Salon A-D*

Chair: **Emily Mevers** (Virginia Tech)

8:00 AM – 8:45 AM PL-07 - **Mohamed Donia** (Princeton University)
Small-Molecule-Mediated Interactions in Microbe-Host Systems

8:45 AM– 9:30 AM PL-08 - **Monica Pupo** (University of São Paulo)
Chemical Ecology and Natural Products Discovery in Insect Microbiomes

9:30 AM – 10:00 AM Break – Ballroom Foyer

Symposium VI – Cannabis and Hemp/Delta8-THC, Terpenoids & Synthetic Impurities – *Salon A-D*

Chair: **Nandakumara Sarma** (USP)

10:00 AM – 10:30 AM PL-09 - **Lori Dodson** (Maryland Medical Cannabis Commission)
The Challenges in Cannabis Innovation from a Regulator's Perspective

10:30 AM – 11:00 AM I-09 - **Zhu Zhou** (York College CUNY)
Bridging the Knowledge Gap: Understanding Cannabinoids-Drug Interactions in Older Adults

11:00 AM – 11:15 AM C-21 - **Zach Stryker** (University of South Carolina)
Semi-Synthetic Phytocannabinoid Dimers as Potential Therapeutics for Neurological Disorders

11:15 AM – 11:30 AM C-22 - **Rahul Pawar** (Food And Drug Administration)
Quantification of Cannabinoids in Hemp-Derived Ingestible Products using improved LC-UV and LC-MS/MS Methods

11:30 AM – 11:45 AM C-23 - **Nam-Cheol Kim** (United States Pharmacopeia/USP)
Quality Considerations for Cannabis and Cannabis-Derived Products for Clinical Research

Symposium VII – How to Build Innovation and Creativity in Your Team: Perspectives from USP and NIH – Salon A-D

Chair: **Eduardo Caro-Diaz** (University of Puerto Rico) and
Elizabeth Kaweesa (University of Illinois Chicago)

11:45 AM – 12:05 PM PL-10 – **Debra Joy Perez** (United States Pharmacopeia)
Equity for Excellence: Empowering Diverse Teams through Inclusive Hiring Practices

12:05 PM – 12:25 PM PL-11 - **Pamela Tamez** (NIH)
Unleashing Innovation at NIH: The Power of Diversity in Scientific Workforce and Collaboration

12:25 PM – 12:45 PM Q&A/Discussion

Wednesday July 26, 2022

7:15 AM – 1:45 PM Registration – *Salon Foyer*

Award Symposium I – *Salon A-D*

Chair: **Cindy Angerhofer**

7:30 AM – 8:15 AM A-01 - **Laura Sanchez** (University of California, Santa Cruz)
2022 Suffness Award
Imaging Mass Spectrometry as a Powerful Tool for NP Research

8:15 AM – 9:00 AM A-02 - **Jason Kwan** (University of Wisconsin-Madison)
2023 Suffness Award
Using Genome-Resolved Metagenomics to Illuminate the Evolutionary History of Uncultured Symbionts that Produce Bioactive Small Molecules

9:00 AM – 9:45 AM A-03 - **Sandra Loesgen** (University of Florida)
2023 Suffness Award
Behavior-Guided Isolation of Natural Products

9:45 AM – 10:45 AM A-04 - **Djaja Soejarto** (University of Illinois at Chicago)
Tyler Prize 2022
Collaborative Research to Discover New Drug Leads and to Build Research Capacity

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10:45 AM – 11:15 AM Break – *Ballroom Foyer*

Award Symposium II – *Salon A-D*

Chair: John Cardellina

11:15 AM – 12:15 PM A-05 - **Robert Verpoorte** (Leiden University)
Tyler Prize 2023
Pharmacognosy: Mother of all Pharmaceutical Disciplines!

12:15 PM – 1:15 PM A-06 - **Sheo Singh** (SBS Pharma Consulting LLC)
Farnsworth Award 2023
Discovery and Development of Natural Product Leads and Drugs: A Life Story

Session W-PM1 – Recent Advances in Traditional Herbals - Quality/Authenticity and Analysis (Industry Perspective)– *Salon A-C*

Chair: **Amit Chandra** (Amway)

2:15 PM – 2:45 PM — I-10 - **Holly Johnson** (American Herbal Products Association)
Cross Pollination Between Herbal Industry and Pharmacognosy for Science Directed Towards Health Benefits, Quality and Safety

2:45 PM – 3:00 PM — C-24 - **Leena Pradhan-Nabzdyk** (Canomiks)
Standardization of the Biological Effect of Botanicals Using Genomics, Bioinformatics, and AI

3:00 PM – 3:15 PM — C-25 - **Claudia S. Maier** (Oregon State University)
Integration of Metabolomics with Bioactivity for Identifying Active Compounds of Ashwagandha Using a Stress-Induced Drosophila Melanogaster Phenotype

3:15 PM – 3:30 PM — C-26 - **Warren Vidar** (University of North Carolina Greensboro)
Unlocking New Opportunities for Natural Product Discovery with Interaction Metabolomics

3:30 PM – 3:45 PM — C-27 - **Maria Monagas** (United States Pharmacopeia (USP))
USP Standards for European Elder Berry Dietary Ingredients: Solutions to Address Quality and Adulteration Issues

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Session W-PM2 – NP and Infectious Diseases – *Salon D*

Chair: **Joshua Kellogg** (Pennsylvania State University)

2:15 PM – 2:45 PM — I-11 - **Robet Quinn** (Michigan State University)
Small Molecule Virulence Factor Production by an Opportunistic Pathogen is Shaped by Environmental and Polymicrobial Interactions

2:45 PM – 3:00 PM — C-28 - **Elizabeth Kaweesa** (University of Illinois Chicago)
Verticillin Cytotoxic Activity in High Grade Serous Ovarian Cancer in Preclinical In Vitro and In Vivo Models

3:00 PM – 3:15 PM — C-29 - **Liana Zaroubi** (Simon Fraser University)
The Chemical and Biosynthetic Response of Paraburkholderia megapolitana under Antibiotic Stress

3:15 PM – 3:30 PM — C-30 - **Christine Salomon** (University of Minnesota)
Discovery and Development of Anti-Parasitic Terpenes from Subterranean Fungi for Treatment of Cryptosporidiosis

3:30 PM – 3:45 PM	C-31 - **Jeffrey D. Rudolf** (University of Florida) *Genome Mining for Bacterial Terpene Synthases Reveals Novel Eunicellane Diterpenoids*

3:45 PM – 5:45 PM	**ASP Business Meeting – *Salon D*** ASP Members Welcome (Associate Members may attend but Voting Privileges are for Full Members Only)

6:00 PM – 7:00 PM	**Closing Reception – *Ballroom Foyer***
7:00 PM – 10:30 PM	**Banquet and Closing Program – *Salon E-H*** **(Ticketed Event)**

Banquet Sponsored by: Amway

Thank you for your participation in the 2023 ASP Meeting!

See you in Poland at ICNPR 2024!

American Society of Pharmacognosy

Award Speakers

A-01 – Laura Sanchez

Imaging Mass Spectrometry as a Powerful Tool for NP Research

Laura M Sanchez, Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA 95064

In nature, small molecules are often produced by macro- and microorganisms in order to facilitate communication and drive biological processes to the benefit (or detriment) of the community as a whole. Chemical gradients and chemical cues via the production of small molecules are ubiquitous across biological systems and my lab has used imaging mass spectrometry (IMS) to study these cues and gradients in cheese rind-derived microbial co-cultures, biofilm forming Gram negative microbes, and host-microbe interactions. In order to study the chemistry in specific microenvironments, we adapt IMS platforms to visualize the molecule maps that small molecules occupy in microbial and mammalian cultures or host tissues. IMS has previously been used to create small molecule maps in fresh frozen tissue sections and spheroids. We have also begun to adapt the platform to incorporate trapped ion mobility spectrometry (tims) and MS/MS directly from the IMS samples themselves. I will discuss how we've applied our MS based tools to study cyclic-di-GMP and biofilms in *Vibrio cholerae*, colonization of the light organ of *Euprymna scolopes* by *Vibrio fischeri*, and other systems that are hot off the bench.

A-02 – Jason Kwan

Using Genome-Resolved Metagenomics to Illuminate the Evolutionary History of Uncultured Symbionts that Produce Bioactive Small Molecules

Jason C. Kwan, Division of Pharmaceutical Chemistry, University of Wisconsin-Madison, Madison, WI 53705, USA

Multicellular eukaryotic organisms readily evolve symbioses with bacteria, taking advantage of their ability to synthesize primary and secondary metabolites. In return, hosts provide a stable and hospitable place to live. Symbionts in these relationships feel less selective pressure to maintain biosynthesis of metabolites the host provides, and so those functions are lost in a process of genome reduction. The longest established symbioses produce molecules that have been ecologically (and potentially therapeutically) import for millions of years, but genome reduction reduces the likelihood of laboratory culture. However, uncultured symbionts can now be studied through direct environmental sequencing (metagenomics). A genome-resolved approach has allowed my group to investigate various marine and terrestrial symbiotic partnerships, in tunicates, bryozoans, sponges and insects, to uncover the evolutionary history of both symbionts and their biosynthetic gene clusters (BGCs). I will present work on symbionts where BGCs appear to have been recently acquired by horizontal gene transfer (HGT), as well as others where the BGC is likely to be ancient. Instances of HGT as well as BGC duplication are only revealed by careful examination of metagenomes, and are likely underreported. I will also show different evolutionary trajectories of defensive symbionts in terms of genome reduction and a continuum from strict vertical to horizontal transmission between hosts. Finally, I will outline how the discovery of recent HGTs to uncultured bacteria may hold promise in finding related BGCs in free-living bacteria, potentially alleviating supply problems with small molecules from uncultured sources.

A-03 – Sandra Loesgen

Behavior-Guided Isolation of Natural Products

Sandra Loesgen[1], Annika Jagels[1], Jason Slot[2], Erin M. Marshall[1], James A. Strother[1], [1]University of Florida, the Whitney Laboratory, St. Augustine, FL 32080, USA, [2]Center for Applied Plant Sciences, The Ohio State University, Columbus OH, United States

Insect-associated microorganisms represent some of the oldest symbiotic relationships found on earth. Complex microbial communities evolved with their hosts and influenced insect traits, behavior, and reproduction. Similarly, chemical signals and mediators in form of small metabolites or and large biomolecules have been evolutionally selected in the holobiont to aid in interactions ranging from mutualistic symbiosis to all the way pathogenic warfare. The basidiomycete *Athelia* sp. also known as the 'cuckoo fungus', mimics termite eggs. Here we present novel alkaloids isolated from the sclerotia of the termite-associated fungus, with selective, aminergic activity. The discovery, isolation, structure elucidation, and *in vitro* and *in vivo* activity of the atheliapyrrolidines is disclosed. Our recently developed zebrafish anti-nociception assay enabled the 'behavior-guided' detection and isolation of several natural products from microbial extracts with potent analgesic activity.

A-04 – Djajja Soejarto

Collaborative Research to Discover New Drug Leads and to Build Research Capacity

Djaja D. Soejarto, Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA and Science and Education, Field Museum, Chicago, IL 60605, USA

I started my academic career in 1969 in Medellin, Colombia. In 1979 I started my academic career at the University of Illinois at Chicago and carried out collaborative research in various projects in the discovery of new drug leads from tropical plants, with emphasis on tropical America and tropical Asia, as potential candidates for pharmaceutical development. As a plant taxonomist, my share of the effort focused on explorations and the sourcing and taxonomic identification of the raw plant materials acquired. In the discovery research effort, the highlights of my collaborative endeavor include, among others, the discovery of new high-potency sweeteners, anticancer, anti-HIV, anti-TB, and antimalarial agents isolated from different species of seed plants collected in Paraguay, Thailand, Vietnam, and Sarawak (Malaysia). In the capacity-building effort, I trained students in Colombia, the USA, and Vietnam, while strengthening the host's research capacity through the founding of the Herbarium of the University of Antioquia, Medellin, Colombia (now the second largest Herbarium institution in the country) and through the upgrading of the Herbarium institution of Cuc Phuong National Park, in Vietnam, and the Institute of Traditional Medicine in Laos. I have also indirectly contributed to the development of Calanolide A as an HIV drug candidate, in the solidification of the UIC Intellectual Property policy in the discovery of new drug leads from plants, in the establishment of the Sarawak Biodiversity Center in Sarawak, and in the establishment and the functioning of a network of 27 Medicinal Plant Preserves in Laos.

A-05 – Robet Verpoorte

Pharmacognosy: Mother of all Pharmaceutical Disciplines!

R. Verpoorte, Natural products Laboratory, IBL, Leiden University, PO Box 9505, 2300RA Leiden, the Netherlands

In 1970, I started my MSc research internship in pharmacognosy in Sweden. At that time I had no clear idea what this pharmaceutical discipline meant. But in the first years, I was part of many discussions on the definition of pharmacognosy. I will not repeat these, but if you ask me for a description, I would answer you with the title of this abstract. That is a strength and a weakness at the same time. The weakness is that developing novel medicines is nowadays multidisciplinary. For example chemical synthesis and structure – activity relationship are used to improve the activities of already known compounds from medicinal plants. However, novelty is limited in this approach. A strength is giving credit to our ancestors and collaborating with native people to study their knowledge. It would strengthen pharmacognosy by leading us to novel activities and compounds. It would benefit native people with royalties and, not least, in evidence-based use of traditional medicines. Fortunately, we see that traditional medicine are a major research item in countries with well-documented traditional medicines. Based on the unique expertise in pharmacognosy and natural products research, the field has expanded enormously in food research, aiming at identifying health-affecting compounds. The problem of sourcing medicinal plants has resulted in new branches of research in pharmacognosy: plant cell biotechnology and synthetic biology. The Nobel Prize 2015 is a recognition of the importance of natural products research! We need this support to survive in science, as (self-)motivation is a driving force. If I ask you, what are you most proud of in natural products research, i.e. examples that you use to explain your work to family and friends? What would be your answer? I want to thank all people that worked in our lab in the past 50+ years. It is due to them that I am standing here to give some examples. Thank you so much all Leiden pharmacognosy alumni, colleagues, friends and family! I am very grateful to the ASP for the great honor of awarding me the Varro E. Tyler Prize.

A-06 – Sheo Singh

Discovery and Development of Natural Product Leads and Drugs: Highlight of 40-Year Career

Sheo B Singh, Department of Chemistry and Chemical Biology, Stevens Institute of Technology, Hoboken, New Jersey 07030

My fascination of natural products and its role in human health led me to pursue study of natural products for graduate studies leading to highly fulfilling 40-year career, culminating in discovery of over 400 biologically active natural products. These discoveries arose from screening efforts employing a wide variety of modalities and technologies including whole animal, whole cell, target based cellular, enzyme and receptor-based assays, targeting cancer, viral infections (HIV and flu), parasitic infections, bacterial and fungal infections, cardiovascular, and diabetes. These studies included bioassay-guided purification, structure elucidation, structural modification, medicinal chemistry, total synthesis, biosynthesis, in-depth biological evaluations, target identifications and target validations. These studies led to discovery and development of several clinical candidates and approved anticancer drugs. The presentation will highlight discovery and development of selected natural product leads and clinical candidates along with a few early screening leads that critically advanced target understanding enabling identification of pharmacophore and new leads. The presentation will mostly focus on the discoveries and developments of combretastatins, dolastatin-10, platensimycin, kibdelomycin, nodulisporic acids and a few others.

PLENARY SPEAKERS

PL-01 –Jay Keasling

Production of Supply-Limited Natural Product Therapeutics Using Engineered Yeast

Jay Keasling, Department of Chemical & Biomolecular Engineering University of California, Berkeley, Berkeley, CA 94720

Plants produce some of the most potent human therapeutics and have been used for millennia to treat illnesses. Two examples are vinblastine, the chemotherapeutic, and QS-21, an adjuvant used in several vaccines. Both molecules are large, highly decorated terpenes. Vinblastine is extracted from Catharanthus roseus and requires 1 ton of dried leaves to obtain 1 g. In a similar vein, QS-21 is extracted from the tree bark of Quillaja saponaria, and its isolation is complicated as the plant extract contains a multitude of different structurally related Quillaja saponins, rendering the purification process highly laborious and low yielding. To alleviate supply issues, we have engineered Saccharomyces cerevisiae to produce these molecules. For production of vinblastine, we have demonstrated de novo microbial biosynthesis of vindoline and catharanthine using a highly engineered yeast, and in vitro chemical coupling to vinblastine. We introduced 30 enzymatic steps beyond the yeast native metabolites geranyl pyrophosphate and tryptophan to catharanthine and vindoline. In total, 56 genetic edits were performed, including expression of 34 heterologous genes from plants as well as deletions, knock-downs, and overexpression of ten yeast genes to improve precursor supplies towards de novo production of catharanthine and vindoline, from which semi-synthesis to vinblastine occurs. As the vinblastine pathway is one of the longest monoterpene indole alkaloid biosynthetic pathways, this study positions yeast as a scalable platform to produce more than 3,000 natural MIAs and virtually infinite new-to-nature analogues. For QS-21 biosynthesis, we upregulated the yeast native mevalonate pathway to provide a high carbon flux towards 2,3-oxidosqualene, which is then cyclized by a heterologous β-amyrin synthase and site-selectively oxidized by plant cytochrome P450s to yield quillaic acid, the aglycone of QS-21. We further introduced plant nucleotide sugar synthetic pathways to make seven non-native UDP-sugars, which are used to add sugars onto the C3 hydroxy and C28 carboxy functional groups of quillaic acid via the co-expression of QS-21 pathway glycosyltransferases. Furthermore, an engineered type I PKS LovF, two type III polyketide synthases (PKSs), as well as two standalone ketoreductases (KRs) were expressed in yeast to form the dimeric acyl unit that constitutes the last step prior to the terminal arabinofuranose addition to yield QS-21. Owing to the promiscuity of several enzymes, structural analogues of QS-21 have further been generated and characterized using the biosynthetic platform, allowing for future establishment of a structure-bioactivity relationship as well as the rational design of novel potent vaccine adjuvants

PL-02 – Bonnie Bassler

Quorum Sensing Across Domains: From Viruses to Bacteria to Eukaryotes

Bonnie L. Bassler. Princeton University and Howard Hughes Medical Institute. Department of Molecular Biology. Princeton, NJ, 08544, USA

Bacteria communicate with one another via the production and detection of secreted signal molecules called autoinducers. This cell-to-cell communication process, called "Quorum Sensing", allows bacteria to synchronize behavior on a population-wide scale. We showed that behaviors controlled by quorum sensing are ones that are unproductive when undertaken by an individual bacterium acting alone but become effective when undertaken in unison by the group. For example, quorum sensing controls virulence factor production and biofilm formation in pathogenic bacteria. We found that eukaryotes that harbor quorum-sensing bacteria participate in these chemical conversations by providing the substrates bacteria need to make autoinducers. We also discovered that quorum-sensing autoinducer information can be hijacked by viruses that infect and kill bacteria. Thus, interactions across the eukaryotic, bacterial, and viral domains all rely on quorum sensing. Presumably, each entity in these combined beneficial and parasitic partnerships is garnering the information encoded in quorum-sensing autoinducers to optimize its survival and reproduction. Using what we have learned, we have built quorum-sensing disruption strategies for development into new anti-microbials. We have also engineered viruses to respond to user-defined inputs, rather than the bacterial autoinducers, to make phage therapies that kill particular bacterial pathogens on demand.

PL-03 – Anna Mapp

Writing the Rules for Targeting Dynamic Transcriptional Coactivators with Natural Products

Anna K Mapp, Life Sciences Institute and Department of Chemistry, University of Michigan, Ann Arbor MI 48104

Transcriptional coactivators and their partner transcription factors have been labeled as intrinsically disordered, fuzzy, and generally undruggable. Given this intrinsic disorder, selectively targeting the complexes using structural mimics has often been difficult. We propose that the identification of conserved mechanisms of engagement between coactivators and their cognate activators should provide general principles for the development of synthetic molecules that mimic the mechanism of transcription factor binding. In one example, biophysical characterization of the structurally divergent coactivator Med25 reveals that it forms short-lived and dynamic complexes with three different transcriptional activators and that conformational shifts are mediated by a flexible substructure of two dynamical helices and flanking loops. Analogous substructures are found across eukaryotic coactivators and in other dynamic cellular machines. Further, targeting the flexible structures with synthetic molecules

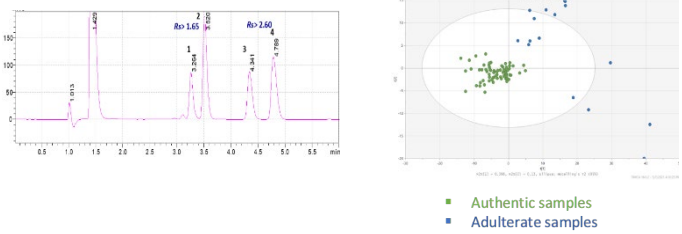
allows tunable modulation of various Med25-activator complexes. Thus, the two conclusions of the work are actionable for the discovery of small-molecule and natural product modulators of this functionally important protein class.

PL-04 – Jiangnan Peng

SFC and NMR Methods for Authentication of Botanical Raw Materials

Jiangnan Peng, Shagufta Perveen, Xiaoan Chen, Hongtao Yu*. Department of Chemistry, Morgan State University, Baltimore, MD21251*

The authentication and quality control of botanical raw materials (BRM) is challenging due to the complexity in chemical components and the unknown in bioactive components. Currently, analysis of marker compounds coupling with chemical profiling is used for the authentication of BRM. In this presentation, we will demonstrate the application of chiral supercritical fluid chromatography (SFC) and NMR chemical profiling in the authentication of tea tree oil (TTO). Some chemical components in TTO exist in two enantiomeric forms with characteristic enantiomeric ratios, which can be used for the authentication of the TTO. We developed a chiral SFC method to analyze the enantiomer ratios of RS-terpinen-4-ol and RS-α-terpineol for the authentication of TTO. This method shows the advantages of high resolutions (Rs > 1.5) and very short running time (5 minutes). NMR is a great analytical tool for chemical profiling for BRMs. A 1H MNR method coupling with multivariate analysis was developed to rapidly determine the authenticity of TTO samples. Figure 1. Left: SFC Chromatogram of TTO; Right: PLS-DA score plot of authentic samples and exclude the adulterate samples.



PL-05 – Katherine Maloney

Cryptic Species Membership as the Primary Driver of Secondary Metabolism in *Sarcophyton glaucum*: Leveraging Interdisciplinary Collaborations to Get Research Done at a PUI

Katherine N. Maloney, Department of Chemistry, Point Loma Nazarene University, San Diego, CA 92106

Establishing an externally-funded research program in natural products at a Primarily Undergraduate Institution (PUI) is a challenge made much easier through collaborations. In this talk, I'll share an example from my research program that started with my biology colleague's observation that the soft coral *Sarcophyton glaucum* was not a single species but a complex of at least seven genetically distinct clades, representing cryptic species. This abundant soft coral had been the subject of decades of study by natural products chemists, who observed a frustratingly variable pattern of production of biologically active natural products. We hypothesized that the seemingly arbitrary secondary metabolism of *S. glaucum* might actually not be random at all, but the natural result of researchers unwittingly collecting different (and perhaps mixtures of) cryptic species. That hypothesis formed the basis for a grant proposal, and a collecting trip in Palau. Ten years, an institutional move, one applied math collaborator, twelve undergraduates, a sabbatical, and a community mass spectrometry data platform later, we were able to show a strong correlation between cryptic species membership and chemical profiles, and to identify the chemical drivers of this difference as the cembranoid diterpenes sarcophine and isosarcophytoxides.

PL-06 – John Bencich and Rick Stewart

Kicking the Habit: Development of Cytisinicline for Smoking Cessation

John Bencich and Rick Stewart, Achieve Life Sciences

There are over 28 million smokers in the United States, and almost half of them try to quit the habit every year, often unsuccessfully. Cytisine is an alkaloid, similar in structure to nicotine, that is obtained from the seeds of *Cytisus laburnum* (L.). This compound has a long history of use in eastern Europe as a smoking cessation aid. It has been commercially available since the 1980s. More recently, Achieve Life Sciences, partially in collaboration with NIH, has been supporting research in the U.S. to demonstrate the safety and efficacy of this compound. As part of that effort, extensive preclinical toxicity studies were performed. This led to IND approval by FDA which allowed for multiple clinical trials to test its efficacy in supporting participants efforts to quit smoking and/or vaping. Data will be presented showing the history of this drug and the efforts from Achieve Life Sciences and their federal partners to move this toward approval in the U.S. as a smoking cessation aid.

PL-07 – Mohamed Donia

Small-Molecule-Mediated Interactions in Microbe-Host Systems

Mohamed S. Donia[1]. [1]Department of Molecular Biology, Princeton University, Princeton, NJ, 08554

In complex biological systems, small molecules often mediate important microbe-microbe and microbe-host interactions. Identifying these molecules and their producers, characterizing their biological activities, and explaining their relevance in a native context are challenging endeavors. Here, I will describe our ongoing efforts to develop computational and experimental tools for discovering small molecules and their producers from diverse microbe-host systems, and for revealing their role in the biology, ecology, and evolution of these systems.

PL-08 – Monica Pupo

Chemical Ecology and Natural Products Discovery in Insect Microbiomes

Mônica T. Pupo[1]. [1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14040-903, Brazil

Specialized metabolites (natural products) play important roles in mediating symbiotic interactions between microorganisms and their eukaryotic hosts. Insects represent one of the most diverse group of animals, and establish distinct types of symbiotic associations with microorganisms. Using a chemical ecology approach, our research group has described roles of microbial specialized metabolites in defensive and nutritional symbioses in microbiomes of different neotropical insects, such as fungus growing ants, myrmecophytes, stingless bees and membracides. Some compounds also have potential pharmaceutical applications as antimicrobials, which are likely related to their ecological roles. During this presentation I will discuss our approach and some results in this exciting field.

PL-09- Lori Dodson

The Challenges in Cannabis Innovation from a Regulator's Perspective

Lori Dodson, MS, MT(ASCP). Office of Laboratory Services, Maryland Cannabis Administration, Linthicum, MD, 21061

While cannabis remains illegal at the federal level, adult use and medical markets are becoming increasingly common across the United States. As of May 2023, medical cannabis is legal across thirty-eight states, three territories, and the District of Columbia. Additionally, there are twenty-two states and the District of Columbia that have legal adult use markets. Revenue projections from an April 2023 MJBizDaily report show cannabis revenue topping $30 billion in 2022 and projected to hit $56.9 billion in 2028. With such a lucrative and dynamic market comes remarkable innovation, but that innovation inevitably introduces significant challenges regarding testing standardization and product safety. With a vast array of cannabis products readily available to consumers across the country, it is critical that standardized product testing models become available and consistently used as a tool to ensure product safety.

PL-10 – Debra Joy Perez

Equity for Excellence: Empowering Diverse Teams through Inclusive Hiring Practices

Debra Joy Perez, United States Pharmacopeia

Inclusive and equitable hiring practices lead to more diverse and high-performing teams with better outcomes, more creativity and more innovation than homogenous teams. At United States Pharmacopeia we believe Equity equals Excellence. The USP integrated DEIB strategy to advance excellence includes specific high-impact changes in the hiring life-cycle. These changes included specific improvements in the recruitment, screening, selection, search committee preparation and candidate interview experience that are replicable and sustainable. Come learn how to authentically operationalize DEIB for long-lasting change.

PL-11 – Pamela Tamez

Unleashing Innovation at NIH: The Power of Diversity in Scientific Workforce and Collaboration

Pamela Tamez, NIH

Diversity of thought and approach results in more productive collaborations and improves the quality of the science. Using evidence-based approaches, the Chief Officer for Scientific Workforce Diversity is NIH's thought leader in the science of scientific workforce diversity. We will discuss how NIH fosters creative and innovative teams through funding, data, and tools.

INVITED SPEAKERS

I-01 – Oleg Tsodikov

Structural Insight into Antagonism of Oncogenic Transcription Factors by Mithramycin Analogues

Oleg V. Tsodikov, University of Kentucky, Department of Pharmaceutical Sciences

Mithramycin (MTM) is a DNA binding antibiotic natural product that is known for its anti-cancer properties. MTM is a potent (nM) antagonist of oncogenic gene translocation products EWS-FLI1 in Ewing sarcoma, a bone and soft tissue cancer diagnosed predominantly in children, and TMPRSS2-ERG in aggressive prostate cancers. In both cases, MTM acts by perturbing the transcription function of these abnormal fusions through binding DNA. We obtained a series of crystal structures of MTM and its analogues alone, in complexes with DNA and with high-order transcription factor-DNA assemblies involving FLI1 and ERG. This structural information, supported by DNA binding studies, suggests DNA context-dependent direct and allosteric mechanisms of disruption of the oncogenic activity of the fusion proteins by MTM analogues. This work is driving development of selective MTM analogues as novel drugs against Ewing sarcoma.

I-02 Roberto De Guzman

Bacterial Nanoinjectors as Target for New Antimicrobials

Roberto N. De Guzman, Department of Molecular Biosciences, University of Kansas, Lawrence, KS

A potential target for developing new antimicrobials is the protein nanoinjector of Gram-negative pathogens. Many bacterial pathogens assemble a nanoscale protein injector to deliver virulence proteins into their hosts to cause infectious diseases. This nanoscale injector, or injectisome, is part of the bacterial virulence mechanism known as the type III secretion system (T3SS). The T3SS is essential for pathogenesis and is present only among pathogens. Currently, all pathogens that require the T3SS for infectivity have developed resistance to current antibiotics. The injectisome consists of a base, a needle, a tip, and a translocon. My lab studies the protein-protein interactions involved in the assembly of the needle, tip and translocon proteins of the injectisome. The injectisome is exposed on the bacterial surface, making it as an attractive target for developing new antimicrobials. Our goal is to understand the injectisome in atomic detail and use that knowledge in finding small molecules that could disrupt the assembly of the injectisome. Small molecules that can disrupt the assembly of the injectisome could be the next generation of antimicrobials.

I-03 - Patrick Grohar

Development of Mithramycin and Analogs as Targeted Therapy for Pediatric Solid Tumors

**Rebecca Kaufman[1], *Guillermo Flores[2, 3], *Elissa Boguslawski[1], Seneca Kinn-Gurzo[1], Maggie Chasse[2], Ian Bedows[2], Marie Adams[2], Matt Stout[1], Susan M. Goosen[2], Jenna Gedminas[1], Patrick J. Grohar[1]. [1]Children's Hospital of Philadelphia, 3501 Civic Center Blvd, Philadelphia, PA.[2]Van Andel Research Institute. [3]Michigan State University, College of Human Medicine*

It has been known for 25 years that Ewing sarcoma (ES) is absolutely dependent on the EWS-FLI1 transcription factor for cell survival. This mutation occurs in a genetic background characterized by few other recurrent somatic mutations. However, EWS-FLI1 is a transcription factor and widely regarded as a challenging if not "undruggable" target. A few years ago, we identified the natural product mithramycin (MMA) as an inhibitor of EWS-FLI1 in a HTS campaign in collaboration with the National Cancer Institute. Unfortunately, drug toxicity limited the accumulation of the compound in patients to concentrations insufficient to inhibit EWS-FLI1. Therefore, we have worked to identify mechanisms limiting the activity of the compound to optimize the schedule of administration and identify other related pediatric tumors that may benefit from this drug. In addition, we have characterized a second-generation inhibitor with an improved toxicity profile called, AIT-102 (AI Therapeutics). Here, we use molecular and in vivo xenograft modeling coupled with the combination of Chromatin Immunoprecipitation and Global Run-On sequencing (GROseq) to definitively characterize MMA and AIT-102 as EWS-FLI1 inhibitors. We show the mechanism of suppression and use the information to optimize the schedule of administration of AIT-102 to drive stunning xenograft regressions in Ewing sarcoma and rhabdoid tumor xenografts. These studies provide the basis for the clinical translation of AIT-102 as a targeted agent for these pediatric solid tumors.

I-04 – Yasmine Belkaid

Microbiome Control of Host Immunity

I-05 – Stephanie Fertig

SEEDing Biomedical Innovation: Support for Small Businesses at NIH

Stephanie Fertig, HHS Small Business Program Lead, Small business Education and Entrepreneurial Development (SEED), Office of Extramural Research, National Institutes of Health

The Health and Human Services (HHS) Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs, collectively known as America's Seed Fund, awards over $1.3 billion of non-dilutive funding every year to support early-stage small business research and development. The National Institutes of Health (NIH) Small business Education and Entrepreneurial Development (SEED) office supports this funding by providing multiple programs and resources to accelerate the conversion of scientific discoveries into healthcare solutions. This session will provide an overview of small business funding, the NIH's application and review process, and

the programs and resources available to small business innovators to further their product development efforts.

I-06 – Sharad Verma

Programs for Helping Extramural Investigators Develop Small Molecules for the Clinic: NCI Developmental Therapeutics Program

Sharad K. Verma, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 9609 Medical Center Drive, Rockville, Maryland 20850

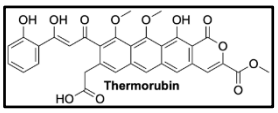
The NCI Developmental Therapeutics Program (DTP) provides services and resources to the extramural community to facilitate the discovery and development of new cancer therapeutic agents. DTP's discovery and development services cover the critical path of steps necessary to usher R&D programs from discovery through preclinical development and IND-enabling studies to support first-in-human trials. In recent years, DTP has expanded its capability to provide resources to the extramural community of drug discovery and development researchers who are working towards advancing their therapeutic candidates from 'bench to bedside'. For investigators who are developing small molecules and natural products, resources of particular interest include the DTP consultation service, the Stepping-Stones program which provides gap-filling development services, and the Natural Products library. Researchers can also access DTP's services through the NCI Experimental Therapeutic Program (NExT). Each of these resources will be described including details for how to leverage these assets to advance your research program.

I-07 – Andrew Lowell

Enhanced Enzyme Stability: Extremophile Biosynthesis of Thermorubin

Andrew N. Lowell,[1,2] Jennifer P. McCord,[1] Max Rivers,[1] Zachary A. Kohanov.[1] [1]Department of Chemistry and [2]Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Tech (Virginia Polytechnic Institute and State University), Blacksburg, VA 24061

Thriving in extreme environments requires organisms to evolve enzymes with high stability, a property valuable to chemists seeking robust biocatalysts. As its name suggests, thermorubin, a red pigment isolated from the thermophile *Laceyella sacchari* (formerly *Thermoactinomyces antibioticus*), possesses potent antimicrobial activities. Despite its known efficacy in eliminating MRSA in mouse models, the poor solubility of thermorubin stopped further development. However, facing the ascendent threat posed by antimicrobial resistant pathogens prompts us to re-engineer thermorubin and other potent forgotten antimicrobial natural products. Towards learning how thermorubin is naturally produced and then leveraging its biosynthetic enzymes as potential catalysts, we sequenced *L. sacchari* identifying a single gene cluster encoding the requisite type II polyketide synthase cassette and methyl transferases. Heterologous expression of this cluster in *E. coli* verified its output, furnishing thermorubin. Using the first enzyme in the pathway, a salicylate synthase, we created thermorubin's unusual starter unit *in vitro*. This enzyme has favorable thermal properties, retaining significant activity up to 50 °C. Annotating the cluster enabled us to propose a biosynthetic pathway containing other unprecedented steps, such as non-terminal pyrone formation. We are investigating these gene products to establish their properties and potential as biocatalysts for non-native substrates.



I-08 – Yi Tang

Discovery of New Structures, Enzymes and Functions from Fungal Biosynthetic Pathways

Yi Tang, Department of Chemistry and Biochemistry, Department of Chemical and Biochemical Engineering, University of California, Los Angeles, USA

Nature performs challenging synthetic transformations using powerful enzymes. These enzymes are frequently found in the biosynthetic pathways of natural products, many of which have served as inspirations for generations of synthetic chemists over the last fifty years. With recent advances in our abilities to manipulate the biosynthetic pathways, many powerful enzymes in novel natural product biosynthetic pathways have been revealed and characterized. In this talk, I will present a selection of recent work in the identification, characterization and engineering of structurally interesting new natural products and functionally diverse enzymes. I will present the use of different genome mining approaches to identify novel natural product scaffolds and biological activities, including these classified as unknown (BGCs)-unknowns (structures). Our work demonstrates there is significant potential in discovering new natural products from fungi.

I-09 – Zhu Zhou

Bridging the Knowledge Gap: Understanding Cannabinoids-Drug Interactions in Older Adults

Zhu Zhou, York College, City University of New York, New York, NY, USA

As older adults often suffer age-related comorbidities, an ever-growing segment of this special population has turned to a variety of cannabis (Cannabis sativa) products (e.g., marijuana, hemp, and hash oil, either smoked or ingested) to alleviate various ailments, including pain, depression, and insomnia. In the US, cannabis use among this population has doubled from 2015 to 2018. However, our understanding of how the aging process affects the safety of cannabis remains limited. This lack of knowledge is particularly concerning given that older adults are more susceptible to drug side effects and more likely to be on polypharmacy regimens, as the proportion of older adults with polypharmacy has tripled from 1994 to 2014. The two most widely studied cannabinoids in cannabis are the non-psychoactive cannabidiol (CBD) and the psychoactive Δ-9-tetrahydrocannabinol (THC). Previous studies have shown that both CBD and THC are metabolized by several cytochrome P450 (CYP) enzymes, which could lead to drug interactions if co-administered with medications that inhibit these enzymes. The combination of increased cannabis use among older adults who take multiple medications and the broad range of CBD and THC dosages highlights the urgency of understanding how age affects the pharmacokinetics of these cannabinoids. This presentation aims to provide a brief overview of current knowledge on the pharmacokinetics-based interactions between cannabinoids and drugs, the pharmacokinetic changes associated with aging, and potential approaches to addressing knowledge gaps in this area.

I-10 – Holly Johnson

Cross Pollination Between Herbal Industry and Pharmacognosy for Science Directed Towards Health Benefits, Quality and Safety

I-11 – Robert Quinn

Small Molecule Virulence Factor Production by an Opportunistic Pathogen is Shaped by Environmental and Polymicrobial Interactions

Cely Gonzalez[1], Christian Martin H.[1] and Robert A. Quinn[1].
Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects the airways, especially in people with the genetic lung disease cystic fibrosis (CF). The pathogen is notorious for its production of small molecule virulence factors, including phenazines, rhamnolipids, quinolones and siderophores. *P. aeruginosa* excretes some of these compounds in millimolar concentrations into its surrounding environment, with strong effects on host and other microbial cells. Here we show that changes in oxygen and nutrient concentrations in a polymicrobial infection model have significant effects on *P. aeruginosa* virulence factor production. We performed these experiments in a clinically relevant context by mimicking the effects of a novel therapeutic for CF called *Trikafta*, which greatly reduces mucus and amino acid production in CF airways. Reducing these primary nutrient sources and incubating a polymicrobial community in either aerobic or anaerobic conditions had significant effects on both the microbial community structure and *P. aeruginosa* small molecule virulence factor production. These metabolites were greatly reduced in aerobic conditions with low amino acid content of the media, even though the pathogen was still present at similar relative abundances to high amino acid content media. These experiments show that effects of therapeutics, such as Trikafta, that alter nutrient conditions of airway mucus, can reduce the virulence of *P. aeruginosa*. These findings have significant implications for the future of CF disease, because they indicate that *P. aeruginosa* has reduced virulence in people taking this highly effective new therapy.

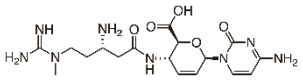
CONTRIBUTED SPEAKERS

C-01 – Cole Gannett

Derivatives of Antimicrobial Natural Product Blasticidin S Enhance the Antibiotic Activity

Cole Gannett,a Paige Banks,a Christina Chuong,b Emily Mevers,a James Weger-Lucarelli,b Andrew N. Lowella, a Department of Chemistry, b Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA 24061, United States*

Natural product cytotoxins are a potentially rich source of new antibiotics. Many of these compounds were largely ignored based on the assumption that their broad toxicity could not be restricted to bacteria. One particularly interesting compound is blasticidin S, a peptidyl nucleoside antibiotic that inhibits translation termination and has very broad toxicity. In this work, we describe semisynthetic modification of blasticidin S that successfully begins to refine its broad-spectrum activity toward bacteria. Production of a series of ester derivatives of this highly polar, zwitterionic compound was accomplished using a single semisynthetic step. The derivatives include alkyl and aromatic esters and were designed to probe the functional group tolerance at the carboxylic acid. These new analogs were screened against gram-positive and gram-negative bacteria, select fungal pathogens, and for cytotoxicity against a human cell line. Briefly, our derivatives had a marked increase in antibacterial activity and thus the selectivity for pathogenic bacteria versus human cells for the new analogs increased relative to blasticidin S. Work is ongoing to generate second generation derivatives and the preliminary data suggests some of our newer analogs have even more significant reduction in the human toxicity while similarly increasing the antibacterial activity.



C-02 – Mark Hammann

The Unique Potential of the Platanosides as a New Class of Antibiotics with an Unreported MoA targeting a Protein Involved in Cell-Wall Biosynthesis as well as Providing Significant Ecological Implications Regulating Microbiome of the Tree Platanus Occidentalis

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The platanosides are part of the very common flavone glycosides found in many plants. The only unique structural feature is the esterification of two of the OH groups on the carbohydrate unit with a p-coumaryl group. This functionality is critical for the antibiotic activity which includes significant activity both in vitro and in vivo against methicillin, vancomycin, erythromycin, gentamycin and ciprofloxacin resistant isolates. A proteomics analysis has identified a possible target protein with previously unreported function that appears to be key for cell well biosynthesis. Thus, this old molecule offers tremendous potential in the control of drug resistant G+/- infections caused by the ESCAPE pathogens. In plantations of American Sycamore (Platanus occidentalis), higher concentrations of this antibiotic Provides protection from the phytopathogenic bacterium called Xylella fastidiosa (Xf) which causes leaf scorch and cell death in plants. Further investigation of the plants microbiome and an assessment of the regulatory role of the platanosides on the plant microbiome we stumbled across a member of the tree's microbiome that generates rapamycin under highly specific culture conditions using rich media. This putative new strain of Bacillus amyloliquefaciens (Ba) was evaluated using MS imaging and contributes significantly to the natural products chemistry of the tree producing a diversity of secondary metabolites with antifungal activity. Both the Ba and rapamycin production appear to be highly regulated by the platanosides however, rapamycin can be detected and isolated from plant tissues. Some of the key roles of rapamycin to the host plant based on the literature and our own data suggest that it may promote autophagy of the leaf tissues in autumn and promotes and sustains vascularization of the plant. As in many other groups of organisms rapamycin has been shown to provide life-extending properties in plants; thus the success of the family Platanaceae may indeed be due in part to rapamycin expression at certain periods of time by the microbiome. Based on the fossil record, the family Platanaceae dates back over 50 million years and individuals in this family can live to be over 500 years old suggesting this relationship contributes to the success of this family of trees. The results of this ongoing study suggests that the platanosides may provide a very promising new class of antibiotics and plays a critical role in protecting the tree from phytopathogenic bacteria and regulating the plants microbiome.

C-03 – Benjamin Blackburn

Repurposing of Medicinal Plant Metabolites, and Plant Metabolite-Inspired Compounds to Further Optimize Renewable Energy Technologies

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Quinone-containing natural products have been recognized for their antimicrobial and anticancer properties.[1] Specifically, 1,4-naphthoquinone-based metabolites from plants and bacteria show activity towards treating mitochondrial diseases, and can experimentally carry out extracellular electron transfer (EET).[2,3] EET is vital to the functionality of Microbial Fuel Cells (MFCs), an emerging renewable energy source, which utilize the growth of Metal Reducing (MR) bacteria to generate an electric current. In this study, a small library of 1,4-naphthoquinone-based compounds was obtained through natural product isolation, commercial purchase, and semisynthesis. The library has been tested in biological assays that quantitatively measure the rate these compounds are able to pass electrons from MR bacteria used in MFCs (*Lactiplantibacillus plantarum* and *Shewanella*

oneidensis), to a Terminal Electron Acceptor. The assays show intriguing results relating to the compound's chemical properties such as; size, reduction potential, pKa, and LogP. How the compounds interact in the active site of the MR bacteria is also addressed in relation to EET assay results. The most active

C-04 – Savannah Pierce

Rivularia spp. Cyanobacterial Fractions Selectively Regulate the Ubiquitin-Proteasome System

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In eukaryotes, the ubiquitin-proteasome system (UPS) degrades proteins, and dysregulation of UPS components has been implicated in neurodegenerative diseases (Angelman's and Alzheimer's) and many types of cancer. This system works by selectively ubiquitinating proteins via a three-step enzyme cascade that involves the ubiquitin-activating enzyme (E1), -conjugating enzyme (E2), and -protein ligase (E3). UHRF1 is linked to E3 ubiquitin ligase activity. UHRF1 is overexpressed in high proliferating cells and tissues including breast carcinomas. During a 2019 collection trip to Puerto Rico, several cyanobacteria samples belonging to the genus *Rivularia* were collected from geographically diverse locations. The cyanobacteria were extracted, fractionated, and screened in an *in vitro* fluorescent ubiquitination assay. Increased ubiquitination activity was observed across the 70-50% EtOAc:MeOH fractions, indicating that *Rivularia* spp. produces a small molecule that can regulate the UPS. After testing against UHRF1, an E3 ligase, there was increased substrate and auto-ubiquitination activities indicating that the active fractions were activating the E2/E3 step. *Rivularia* spp. are known for producing loggerpeptins, a class of Ahp-containing cyclic depsipeptides, with potency towards elastase inhibition. Our collection of isolated loggerpeptins could allow a structure-activity-relationship study of small molecule regulation of the UPS. Interestingly, cyanobacteria are prokaryotes and do not contain ubiquitin, yet this exquisite suite of small molecules have a selective interaction with the eukaryotic UPS. Additional biochemical studies are underway to further characterize these observed activities. The activity and characterization of the compounds responsible for this specific activity will be presented.

C-05 – William Gerwick

Development of an Artificial Intelligence-Based Tool for Predicting Cancer Cell Cytotoxicity of Natural Products

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Predicting patterns of cancer cell cytotoxicity using deep learning would allow improved focus and dedication of resources to compounds having a greater likelihood of having promising activity. Machine learning techniques make use of patterns in data to learn to predict relationships, and many of these techniques are used as drug discovery tools with the intent to determine relationships between compound structure and biological function. Particularly in anticancer drug discovery, several models have been used to make binary decisions about biological activity for a narrow scope of drug targets. We take advantage of the strengths of neural networks in pattern recognition to develop a feed-forward neural network that simultaneously classified the antiproliferative activity of compounds against 59 cancer cell lines that are part of the NCI-60 in vitro panel of cancer cells. This novel tool predicts the activity to be one of six categories, not only indicating if activity is present, but also the degree of activity. By incorporating significant dropout during training, we ensure the model is robust and able to generalize to compounds not present in the training set. This makes our tool well suited to predicting antiproliferative activity for any number of compounds against these 59 targets. Using the TimTec Natural Products Library as an independent test set, we showed that our classifier can reach 57% accuracy in a six-way classification, and using a "within-one level of predicted cytotoxicity", it reached 91.5% accuracy for predictions to cell lines, and 86.8% accuracy for predictions to tumor panels.

C-06 – Lois Kyei

Bioassay-Guided Isolation of Pseudochelin a from Marine Egg Mass Microbiome

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Biofilms are a phenotype expressed during quorum sensing and have been described as surface-adhered communities of sessile bacteria that are enclosed in a matrix of extracellular polymeric substance (EPS). Biofilms commonly develop on medical devices in immunocompromised patients, which leads to persistent infections that are difficult to treat. In the biofilm state, bacteria are protected against both antibiotics and the host's immune system. Currently, efforts to target biofilms are focused on developing non-toxic compounds that do not threaten the viability of target pathogens but inhibit the formation of biofilms or disrupt already-formed biofilms. The goal of my research project is to an ecology-based approach to study the potential of marine egg mass microbiomes to produce specialized metabolites that can disrupt biofilms. Screening our library of >700 semi-crude bacterial fractions from moon snail egg masses for biofilm inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus* yielded 22 fractions inhibiting *P. aeruginosa* biofilm (~3% hit rate) and 38 fractions that inhibited biofilm formation in *S. aureus* (~5% hit rate). Bioassay-guided isolations on one particularly interesting fraction led to the identification of pseudochelin A as a biofilm inhibitor. Pseudochelin A is a known siderophore, but its biofilm-inhibiting activity has not been reported. Metabolomic analysis on our library reveals that this class of molecule is widespread among the fractions exhibiting antibiofilm activity in *S. aureus* but not in any

of the fractions that disrupted *P. aeruginosa* biofilms. Identification of the possible mechanism of action of pseudochelin A and dose-dependency assays are an on-going effort.

C-07 – Jakub Piwowarski

Revision of Current Concepts of Tannins Impact on Gastrointestinal Tract Homeostasis in Piglets

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Tannins are considered as anti-nutritional factors in piglets' nutrition. However, certain tannin-containing plants are well known for their anti-diarrheal properties, which have been utilized since ancient times in human and veterinary medicine but had been superseded by antibiotics, since discovery of penicillin in 1928 and introduction of antimicrobials in farm animal production since the 1950s. The conducted studies have shown that selected condensed and hydrolysable tannins-rich plant formulations are able to inhibit the enteropathogenic *E. coli* growth and adhesion to intestinal epithelial cells, and stimulate intestinal barrier formation through enhancement of TJ proteins expression. The tested tannin sources did not negatively affect diversity and metabolism of intestinal microbiota of post-weaning piglets *ex vivo*. Certain changes in microbial taxa were induced, which correlated with the formation of postbiotic metabolites- urolithins, which proven anti-inflammatory properties can contribute to the gut health of piglets during the weaning period. Financially supported by Polish National Science Centre grant OPUS LAP UMO- 2020/39/I/NZ7/02547.

C-08 – Yeong-Bae Yun

Growth Characteristics of 10-Year-Old Wild-Simulated Ginseng (*Panax ginseng* C.A. Meyer) and Rhizosphere Soil Properties at Cultivation Sites

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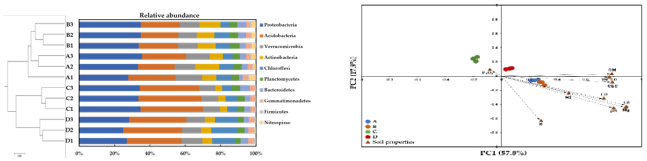
Wild-simulated ginseng (WSG) must be cultivated in mountainous forests without artificial facilities or chemicals. The aim of this study was to investigate correlations of soil bacterial community with soil chemical properties and growth characteristics of 10-year-old WSG. The most dominant bacteria in the bacterial community were *Proteobacteria* and *Acidobacteria* at all WSG cultivation sites. In principal coordinate analysis, soil bacterial community was affected by Ca, Mg, soil pH, TN, CEC, and OM. The most effective edaphic factor determined in this study was soil pH, which was recorded to be acidic at all studied cultivation sites. Results of this study could be used as important basic data for selecting suitable cultivation sites for WSG.



C-09 – George Neuhaus

Specialized Metabolites of the Herptile Gut Fungus, *Basidiobolus*

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Draft genomes of the herptile gut fungus, *Basidiobolus*, show evidence of horizontal gene transfer (HGT) of nonribosomal peptide synthetases (NRPS) from co-occurring gut bacteria. It is suspected that the peptidic metabolites encoded play a role in modulation of the herptile gut microbiome. Here, untargeted LCMS²-based metabolomics, using computational tools (GNPS, Sirius5, and CANOPUS) for structural class prediction, revealed that 10 unique *Basidiobolus* isolates produce cyclic peptides belonging to the same molecular family in laboratory culture. An analysis of the antimicrobial activity of extracts from a gecko isolate of *Basidiobolus*, using the Bioactive Molecular Networks Project protocol, highlighted 16 mass features that correlated with the observed antimicrobial activity against a panel of human pathogens. Analysis of tandem mass spectra assigned these mass features as peptides and depsipeptides. These experiments support the hypothesis that cyclic peptides from *Basidiobolus* can influence microbial growth and lay the groundwork for further study of their role in the herptile gut microbiome.

C-10 –Margaret Hill

Pseudoalteromonas rubra Package Prodiginine Antibiotics as Cargo in Membrane Vesicles

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Astrangia poculata, the northern star coral and official state coral of Rhode Island, is a model organism for the study of microbial-coral interactions due to the ease with which it can be cultivated under laboratory conditions. Coral larvae are thought to settle and metamorphose on substrates, such as crustose coralline algae (CCA), based on their microbial community composition and their associated chemical cues. Strains of Pseudoalteromonas rubra isolated from CCA have been shown to induce larval settlement of tropical corals. We hypothesize that P. rubra induces the settlement of A. poculata larvae by producing specialized metabolites packaged within membrane vesicles (MVs). In this study, we investigated MVs produced by P. rubra strains KB1 and CH007, which were isolated from CCA growing in A. poculata culture tanks at Roger Williams University. MVs were isolated by ultracentrifugation, lyophilized, and extracted with organic solvents. Analysis by UHPLC tandem mass spectrometry revealed that P. rubra MVs contain prodiginines, including prodigiosin, 2-methyl-3-hexyl prodiginine, and cycloprodigiosin, among others. Prodiginines are a class of red-pigmented specialized metabolites with diverse biological activities, including antimicrobial and algicidal activity, toxigenicity, and immunosuppressive properties. P. rubra MVs further demonstrated antimicrobial activities against Vibrio parahaemolyticus PSU5579, a problematic marine invertebrate pathogen. These results suggest a role for MVs from P. rubra as a delivery mechanism for mediating microbial and coral interactions.

C-11 – Joseph P. Gerdt

Chemical Signaling Regulates Aggregation and Chemotaxis in a Predator of a Human Pathogen

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Capsaspora owczarzaki is a single-celled eukaryote that can kill the parasites that cause schistosomiasis. This microbe was first isolated from the *Biomphalaria* snails that transmit schistosomes, and it has been shown to swarm and kill schistosomes *in vitro*. However, the chemistry and biochemistry that underlie its interactions with snails and schistosomes remain unstudied. We aim to elucidate which chemical factors *Capsaspora* senses from its snail hosts and its schistosome prey. Through bioassay-guided fraction of host serum, we have discovered that *Capsaspora* responds to specific host lipid molecules by aggregating—a phenotype that may help it to evade immune responses or feed more efficiently. Furthermore, by combining cell migration assays with analytical chemistry, we have discovered that *Capsaspora* responds to nutrients with a chemotactic response, which may enable it to efficiently hunt its prey. Now, we are employing the tools of proteomics, genetics, and chemical probes to characterize the signaling pathways that *Capsaspora* uses to regulate its chemically induced aggregation and migration phenotypes. These findings will reveal the chemical and biochemical foundations of this underexplored tripartite symbiosis. Our findings may reveal avenues to utilize *Capsaspora* as a biocontrol agent to reduce schistosome populations near human communities. Moreover, since *Capsaspora* is one of the closest unicellular relatives of animals, our work may inform the efforts of evolutionary biologists to reconstruct signaling pathways that existed in recent ancestors of multicellular animals.

C-12 – Joshua Torres

Biosynthesis of Conopeptides in Cone Snail Venoms

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The animal world has always been observed to be remit of biosynthetic pathways. Although several animal-derived natural products have inspired some of the life-saving drugs that humans use today, they pale in comparison to the diversity of biosynthetic pathways found in bacteria, viruses, fungi and plants. Recently, a few complex small molecules have been either genetically or biochemically linked to genes encoded by animals including worms, sponges, mollusks and birds. Among the pharmacologically useful natural products from animals are the conotoxins. The structural confirmations that underlie the basis of the conotoxins' exquisite receptor binding properties are due to the amino acid sequence and the diversity of posttranslational modifications (PTMs) that they contain. In this work, we used transcriptomic and genomic data of several cone snail species to propose the biosynthetic pathway of this very diverse family of ribosomally synthesized and post-translationally modified peptides (RiPPs). We also identified several elusive PTM enzymes (brominase, glycosyltransferase, and sulfotransferase) and utilized them in developing chemoenzymatic reactions to synthesize, and chemically modify several bioactive conotoxins. Our work, together with previous studies of natural product pathways from animals reveal the surprisingly rich secondary metabolism encoded by animal genomes akin to microbial systems.

C-13 – Sandra Bennett

Microbial Diversity and Bioactivity of Longnose Gar Eggs and Ectoparasites

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Drug-resistant infections are one of the most dire medical problems of the 21st century, causing an estimated 1.2 million deaths globally each year. Natural products derived treatments are commonly used

to counteract this threat with approximately 68% of all FDA-approved drugs in the last three decades originating from this source. However, very few new classes of drugs were approved during that time. Parasite and host microbiomes make up a unique ecological niche potentially providing access to novel secondary metabolites to help address this problem. Longnose gar fish carry ectoparasites and eggs known to be toxic. In our current research, bacterial communities from the parasites and gar eggs were examined using culture-independent microbiome analyses and were cultured on several media types. We hypothesize that the microbes involved in the protection of longnose gar eggs and parasitic isopods will produce diverse chemical compounds leading to new natural products. Differences were noted between culture-independent analyses and cultured microbes such as Bacteroidota making up 21% of the community composition of the isopod without being present in culture. Pure cultures of the bacterial isolates were grown to a larger scale for chemical extraction of secondary metabolites. Bioactivity was assessed with cultures against a panel of pathogens most likely to cause human infections, and by collaborators using extracts against multidrug resistant pathogens as well as against drug-resistant human cancer cell lines. High numbers of biosynthetic gene clusters were noted in a number of samples as well as activity against multi-drug resistant _*Staphylococcus aureus*_.

C-14 – Xinhui Yu

Influence of Geographic Variation on Chemistry and Bioactivities of Pacific Northwest Endemi Cyanolichens

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Lichens are a diverse and abundant group in the Pacific Northwest, consisting of symbiotic associations between photosynthetic partners (algae and/or cyanobacteria) and fungi. This study focused on the chemical diversity and effects of environmental changes on the production of specialized (secondary) metabolites in *Nostoc* sp. containing cyanolichens. Pre-fractionated complex mixtures were tested against a panel of BSL2 drug resistant human pathogens, and the results revealed that coastal individuals from Oregon produced more bioactive secondary metabolites compared to their inland counterparts. To discover more bioactive compounds with less wet lab work, untargeted LCMS/MS metabolomics using in silico predictions tools (Sirius5 and CANOPUS) were used to analyze chemical features. Several natural product databases, including GNPS, NPAtlas and newly developed secondary metabolite database Lichendex were used for quick dereplications. The predicted chemical classification results suggested that the cyanolichen *Lobaria anthraspis* from the coastal collections produced more terpenoids compounds and decreased the production of polyketides and alkaloids compared with their inland counterpart. In conclusion, these findings suggest that the environment plays a crucial role in the selectivity and production of bioactive lichen secondary metabolites.

C-15 – Mira Liu

Surface-Active Antibiotics Provide an Adaptive Advantage for Bacteria from Burned Soils

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Microbes face numerous challenges in burned soils, such as resource scarcity, poor nutrient solubility, and increased hydrophobicity. We know that fire-adapted microbes lie at the forefront of ecosystem recovery after a fire, and yet, the specific strategies that these microbes use to thrive in burned soils remain largely a mystery. Through bioactivity screening of bacterial isolates from burned soils, we discovered that several *Paraburkholderia spp.* isolates produce a set of unusual rhamnolipid surfactants with a natural methyl ester modification at the carboxy terminus. Identification of the *Paraburkholderia caledonica* str. F3 rhamnolipid biosynthesis genes led to discovery of the novel gene *rhlM*, which encodes for a putative integral membrane methyltransferase responsible for the unique methylation. We demonstrate that the methyl ester functionality imparts enhanced surfactant properties onto these rhamnolipid methyl esters (RLMEs). RLMEs exhibit antimicrobial activity against post-fire microbial isolates, including pyrophilous *Pyronema* fungi and *Amycolatopsis* bacteria. In addition, RLMEs facilitate bacterial motility on an agar surface. *In vitro* assays further demonstrate that RLMEs also improve aqueous solubilization of certain PAH compounds that are components of char, including naphthalene, phenanthrene, and benzo[a]pyrene. Collectively, these results point toward the significance of RL-MEs in *Paraburkholderia* isolates' dispersal and growth in a burned soil environment. Our findings shed light on some specialized metabolite-based strategies that bacteria employ in order to grow, survive, and outcompete other post-fire soil community members.

C-16 – Dan Xue

Harnessing Bioactivity-led Metabolomics for Natural Products Discovery Targeting a Rare and Severe Cancer

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Taking advantage of our unique, clinically derived cell lines of the rare cancer, pseudomyxoma peritonei (PMP), which urgently needs effective chemotherapy, we developed a bioactivity-led metabolomics procedure that integrated high-throughput cultivation and screening with metabolomics to rapidly discover microbial natural products with both novel structures and potent biological activities against PMP. To prioritize novel compounds from bioactive fractions for subsequent isolation, bioactive molecular networking was utilized. Microcrystal electron diffraction (MicroED) was incorporated to further prioritize chemical novelty and facilitate structure elucidation, even when the compounds were present as a mixture in bioactive fractions. Using this integrated procedure, we showcase the discovery of three marine fungus-derived compounds that inhibited multiple PMP cell lines with the potency comparable to mitomycin C, a clinically used drug for PMP treatment. Aspercyclicins A–C possess a unique 6/5/5/6/5/6 polycyclic scaffold that incorporates continuous fused, bridged, and spiro rings, which has not been previously described. Aspercyclicins represent a new class of potential leads against PMP and suggest that minor yet potent metabolites could be discovered in a targeted manner using an integrated bioactivity-led metabolomics.

C-17 – Robb Stankey

Directed BGC Cloning Combined with Bacterial and Fungal Expression Tools Uncover Novel Biosynthetic Mechanisms and Validate Metabologenomics

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Over 130+ biosynthetic gene clusters (BGCs) ranging from 12 to 150 kb from over 110 diverse bacterial and fungal strains were successfully captured and cloned using CRISPR enzymes to precisely excise pathways of interest. To improve heterologous expression, we developed a BGC expression vector (pDualP) for *Actinomycetota* and a companion vector for *Bacillus* that uniquely includes two inducible promoter elements that flank the cloning site. BGCs cloned and conjugally transferred to heterologous hosts include ACT, RED, nystatin, erythromycin, vancomycin, difficidin, bacillusin A, and many dozens of novel clusters. We rapidly de-orphaned the stravidin BGC from *Streptomyces* sp. NRRL S-98 using this inducible approach. Second, we observed a substantial enhancement of the antimicrobial activity of heterologously-expressed, soil-derived metagenomic BGCs through induction with pDualP and demonstrate the ability to reconstitute complete BGCs from metagenomic library fragments. Finally, we applied these directed cloning tools to known and novel fungal BGCs for successful heterologous expression in *Aspergillus*, including imizoquin and pestalamide. These results indicate that sequenced BGCs can be cloned intact from complex (meta)genomes, and that direct cloning into a dual-inducible expression vector can greatly accelerate downstream small molecule expression and characterization.

C-18 – Kyo Bin Kang

Mass Spectrometry-based Untargeted Metabolomics for Investigation on Chemical Interaction and Biotransformation by Fungi

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Specialized metabolites, also known as natural products, are the major way of interactions in the world of microorganisms. As the concept of chemical ecology has risen, the detailed roles and mechanisms of specialized metabolites are becoming interests of researchers. However, the chemical complexity of specialized metabolome makes it difficult to be investigated. Recent advances in mass spectrometry enabled us to observe thousands of molecules in samples in a short time, but the chemical diversity of natural products makes another bottleneck in untargeted metabolomics projects on specialized metabolites: the structural identification of molecules from the mass spectra. Fortunately, advances in data interpretation followed the advances of spectrometers; MS/MS spectral molecular networking and *in silico* annotation approaches have been useful solutions especially to natural product chemists. Here I will introduce two recent cases of successful application of mass spectrometry-based metabolomics for observation on fungal metabolism in our lab. In the first case, we applied metabolomics analysis to observe intra- and inter-kingdom interaction of *Penicillium* spp. isolated from marine environments. We could annotate multiple molecular families up-regulated or down-regulated by cocultivations. Multiple interesting hypothesis could be made based on the result, but here, our hypothesis on siderophore-mediated inhibition of competitor's specialized metabolism will be highlighted. As a second case, I will introduce our recent discovery of a previously unknown UDP-glycosyltransferase, designated UGT66A1, which is an enzyme catalyzing *O*-xylosylation on many different structural scaffolds having phenolic -OH groups. This discovery was driven by a metabolomics-based phenotyping on biotransformation of phytochemicals by wood decaying fungi.

C-19 – Erin McCauley

Development of a Fungal Natural Products CURE for Upper-Division Chemistry & Biochemistry Laboratory Classes

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Studies have shown that students are more engaged when their research activities address questions that are of current interest to

the scientific community, as opposed to traditional laboratory programs. Additionally, independent research experiences providing hands-on training for students, combined with faculty-student mentorship, can have extremely positive impacts on those students' careers. However, the number of students who can benefit from these research opportunities is limited by the number of research faculty in the department. Therefore, at California State University Dominguez Hills (CSUDH) at Minority-Serving and Hispanic-Serving Institution, we have developed a Mini Natural Products Research Project that can fit into a one semester Course Based Undergraduate Research Experience (CURE) and is available to all senior students. The objective of the student's research projects are to identify natural products from marine derived fungi, then screen them for anti-cancer and antibiotic activity. Over the course of the semester the students progress from a basic to an intermediate or advanced level in the following skill sets: 1) aseptic culturing techniques for microbial and mammalian cell lines; 2) molecule extraction and purification using high performance liquid chromatography; 3) molecule structure identification using mass spectrometry and NMR; 4) critical analysis of real-world experimental data; and 5) reporting of results through oral and written scientific communication. The overall goal of this CURE is to give every student at CSUDH a research opportunity that ensures they are workforce ready at the time of graduation.

C-20 – Chengcang Wu

Fungal Artificial Chromosome Pipeline Enables the Discovery of Novel Compounds from Unique Biosynthetic Gene Clusters

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Penicillium fuscum (2A) and P. camembertii/clavigerum (2B), two extremophilic fungus, have been extensively studied by the Stierle Research Laboratory as both axenic and co-cultures for natural products (NPs) discovery. The Stierle lab has isolated dozens of NP compounds including a novel antibiotic compound Berkeleylactone A, but the true biosynthetic potential of the 2 fungi is unknown. We have also successfully developed and patented Fungal Artificial Chromosome (FAC) technology for efficient discovery of biosynthetic gene clusters (BGCs) and their NP compounds from any filamentous fungi. Here we report the BGC and its FAC clone of the novel antibiotic: berkeleylactone A and related compounds from the P. camembertii/clavigerum (2B), although the novel antibiotics were discovered through co-culture of the 2A and 2B fungi. Furthermore, by integrating the fungal antiSMASH, MIBiG, and BiGSCAPE softwares with our fungal-FAC bioinformatics pipeline, we have also identified 15 unique BGCs from 2A and 12 unique BGCs from 2B fungi, of the 130 predicted BGCs of the TWO fungi. The progress of FAC-based heterologous expression of these BGCs and their chemical data will be presented.

C-21 – Zachary Stryker

Semi-Synthetic Phytocannabinoid Dimers as Potential Therapeutics for Neurological Disorders

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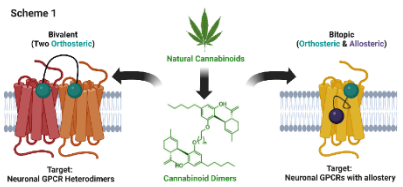
Natural products derived from *cannabis* have long been used for medicinal purposes. Beyond the direct engagement of known biological targets, the therapeutic benefits of *cannabis* may be attributed to an "entourage effect," where a combination of cannabinoids and other natural products in the consumed plant material induce a synergistic pharmacological outcome through interactions at diverse targets. Furthermore, rare cannabinoids such as dimeric Δ^9-THC (cannabisol) and dimeric CBD (cannabitwinol) may have unique effects and pharmaceutical potential. To explore the untapped capabilities of natural cannabinoid dimers and synergistic pharmacophore combinations, we have synthesized the first known series of semi-synthetic phytocannabinoid dimers. These dimers consist of two phytocannabinoid pharmacophores connected by PEG or alkyl linkers of varying lengths, which can be used to probe the optimal distance between putative binding regions. The development of these novel, semi-synthetic derivatives may result in useful pharmacological ligands, as well as tool compounds to further study the functional effects of GPCR dimerization and/or bitopic interactions at these targets [See **Scheme 1**].



C-22 – Rahul Pawar

Quantification of Cannabinoids in Hemp-Derived Ingestible Products Using Improved LC-UV and LC-MS/MS Methods

Rahul S. Pawar, Geoffrey A. Dubrow, Stephen Cahill, Christian Talavera . Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U. S. Food and Drug Administration, College Park, MD, 20740, USA

Since the passage of the Agriculture Improvement Act of 2018 (Farm Bill), the United States has seen a rapid increase in interest and availability of cannabidiol (CBD) products and other products derived from hemp. Sampling and testing of marketed hemp-derived products is an important component of FDA's work because little is known about the amounts of CBD and other cannabinoids in these products. Due to the diversity in products, varying matrix interferences, and the broad range of cannabinoid concentrations, accurate quantification of cannabinoid content is difficult. To overcome these challenges, methods pairing QuEChERS with UHPLC-UV and UHPLC-MS/MS to quantify 17

cannabinoids were developed. This provided a unified approach for sample preparation and measurement of analytes from diverse matrices. The method performance and the advantages of each method in overcoming challenges in analyzing various hemp-derived products matrices will be discussed. These methods provide a valuable tool for researchers and testing laboratories analyzing the hemp-derived products.

C-23 – Nam-Cheol Kim

Quality Considerations for Cannabis and Cannabis-Derived Products for Clinical Research

Nam-Cheol Kim, Dietary Supplements & herbal Medicines, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD, USA

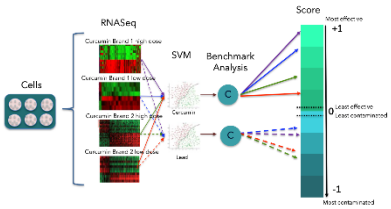
Active and growing interest in the use of cannabis inflorescence for medical purposes requires quality attributes to support sound and reproducible basic and clinical research. As cannabis is a heterogeneous matrix that can contain a complex secondary metabolome with an uneven distribution of constituents, ensuring quality means appropriate sampling procedures, a suite of tests, analytical procedures, and acceptance criteria to define the identity, content of constituents (e.g., cannabinoids), and limits on contaminants. Three main chemotypes based on the secondary metabolite profiles have been identified as useful for labeling for the following cannabinoid constituents: 1) tetrahydrocannabinol (THC)-dominant chemotype, 2) intermediate chemotype with both THC and CBD, and 3) cannabidiol (CBD)-dominant chemotype. Cannabis plants in each of these chemotypes could be further sub-categorized based on the content of other cannabinoids and/or terpene profiles. Morphologic and chromatographic tests are provided for the identification and quantitative determination of critical constituents. Limits for contaminants including pesticide residues, microbial contaminants, mycotoxins, and elemental contaminants are presented based on toxicological considerations and aligned with the existing USP procedures for general tests and assays. The principles could be used as the basis of public quality specifications for cannabis inflorescence and to facilitate scientific research on cannabis safety and potential therapeutic applications.

C-24 – Leena Pradhan-Nabzdyk

Standardization of Biological Effect of Herbals Using Genomics, Bioinformatics, and AI

Leena Pradhan-Nabzdyk, PhD[1] and Manoj Bhasin, PhD[1,2]. [1]Canomiks, Inc, Rochester, MN, [2]Emory University, Atlanta, GA

Using cutting-edge omics science and machine learning, Canomiks, addresses the lack of standardization of biological effect of herbal ingredients by developing genomics-based benchmarks. The product is a multiplex gene expression benchmark assay and an artificial intelligence (AI) based score predictor software. The software can score the herbal ingredient's effectiveness or contamination *in vitro* in human cell culture, relative to the benchmarked gene expression of the analytical (USP grade) ingredient and/or the contaminants. The benchmark is developed using Next Generation Sequencing and innovative multiplexing technology to multiplex different samples together, to reduce cost and time. For developing the score predictor software, we are using the AI method called Support Vector Machine which is commonly used for developing life sciences AI machines. Following is an example of curcumin score predictor with lead as a contaminant. Based on the development of curcumin benchmark, other traditional herbals will be added to the platform. Acknowledgement: This work is funded by the National Science Foundation, award# 2136163.



Example of validation of benchmark assay and score prediction for curcumin using commercial brand curcumins and lead as a contaminant. The predictor will be able to discriminate between curc effectiveness, and lead contamination of different doses of commercially available curcumin.

C-25 – Claudia Maier

Integration of Metabolomics with Bioactivity for Identifying Active Compounds of Ashwagandha Using a Stress-Induced Drosophila Melanogaster Phenotype

Luke Marney[1,7], Jaewoo Choi[2,7], Md Nure Alam[1,7], Dani M Long[5,7], Alexander Law[5,7], Cody Neff [6,7], Kadine Cabey[6,7], Helen Holvoet[4], Burkhard Poeck[4] Roland Strauss[4], Jan F. Stevens[2,3,7], Amala Soumyanath[6,7], Doris Kretzschmar[4,7], Claudia S. Maier[1,2,7]. [1]Department of Chemistry, [2]Linus Pauling Institute, [3]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR, 97331, USA; [4]Institute for Developmental Biology and Neurobiology, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany; [5]Oregon Institute of Occupational Health Sciences, [6]Department of Neurology; [7]BENFRA Botanical Dietary Supplements Research Center, Oregon Health and Science University, Portland, OR 97239, USA

The botanical *Withania somnifera* (Ashwagandha) has a long history of traditional use in promoting health and wellbeing. The BENFRA BDSRC investigates WS for its potential to increase resilience against age-related changes, such as cognitive decline, sleep disturbances and depression. WS extracts and fractions were analyzed by LC-MS/MS metabolomics to characterize their chemical composition. Using wild type *Drosophila melanogaster* (DM), extracts and fractions were tested in behavioral tests for stress-induced depression using the stop-for-sweets and gap-

climbing tests. We then linked the observations made in the DM tests with the LC-MS/MS data and fraction information using GNPS networks. Surprisingly, withanolide-enriched fractions did not provide resilience against stress-induced depression in DM whereas the alkaloid-enriched fractions did. This work is supported by NIH grants U19 AT010829 and S10RR027878.

C-26 – Warren Vidar

Unlocking New Opportunities for Natural Product Discovery with Interaction Metabolomics

Warren S. Vidar[1], Tim U. H. Baumeister[2], Lindsay K. Caesar[3], Joshua J. Kellogg[4], Daniel A. Todd[1],Roger G. Linington[2], Olav M. Kvalheim[5], and Nadja B. Cech[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, [2]Department of Chemistry, Simon Fraser University, Burnaby V5A 156, BC, Canada, [3]Department of Chemistry and Biochemistry, James Madison University, Harrisonburg, VA 22807, [4]Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA 16802, [5]Department of Chemistry, University of Bergen, Bergen, 5020, Norway

Interaction metabolomics is a new approach that considers interactions within natural product mixtures, overcoming the limitations of classical metabolomics. Compound interaction terms (CITs) are used in this approach, which are calculated by multiplying the intensities of feature pairs in a metabolomics data matrix. To validate the approach, an antimicrobial and a synergist compound were added to inactive matrices and their LC-MS metabolomics profiles and antimicrobial activity were measured. Interaction metabolomics correctly identified the compounds responsible for the synergistic activity missed by classical metabolomics. This study also confirmed the synergistic activity of berberine and several capsaicinoids in extracts of goldenseal and habanero pepper. Interaction metabolomics could be useful for identifying synergists in natural products research and in other fields that study complex mixtures.

C-27 – Maria Monagas

USP Standards for European Elder Berry Dietary Ingredients: Solutions to Address Quality and Adulteration Issues

Joshua Bhattacharya[1], Jesse Applin[1], Allison Reitz[1], Natalia Kouznetsova[1], Nadejda Soukhova[1], Tiên Do[2], Eike Reich[2], Maria Monagas[3, 1]United States Pharmacopeia Convention (USP). Analytical Development Laboratory (ADL). 12601 Twinbrook Parkway. Rockville, MD 20852. USA. [2]CAMAG. Sonnenmattstrasse 11, 4132 Muttenz. Switzerland. [3]United States Pharmacopeia Convention (USP). Dietary Supplements and Herbal Medicines. 12601 Twinbrook Parkway. Rockville, MD 20852. USA

The demand for dietary ingredients derived from European Elder berry (*Sambucus nigra* L.), traditionally used to alleviate cold or flu symptoms remedy, has drastically increased due to the COVID-19 pandemic which has led to increased reports of adulteration. In 2021, in response to stakeholder inquiries, USP hosted the *Elderberry Standard Development Open Forum* to address the adulteration crisis and the need for new quality standards. Since then, USP has been actively working in the modernization of the currently official monograph for *European Elder berry Dry Extract*, upcoming in PF 48(3) (May/June 2023), and in the creation of new standards for other ingredients, including: aqueous dry extracts, liquid extracts, juice concentrates and dry juices. The use of confounding materials rich in anthocyanins such as black rice extract and purple carrot juice, undeclared synthetic dyes and species misbranding,are among the most common types of adulteration in elder berry products. In addition to adulteration related to identity and purity, manufacturing-related issues resulting in anthocyanin thermal degradation during the processing of juice concentrates as well as excessive use of carriers during the juice spray drying process can also lead to quality issues in these types of ingredients affecting both identity and composition. This presentation summarizes the workconducted by USP in collaboration with CAMAG for the development of new and modernized monographs, including: characterization by HPLC-DAD-MS at different wavelengths (535 nm and 365nm), HPTLC and HPLC-DAD identity tests based on anthocyanins and other flavonoids, HPTLC based detection of mixtures with adulterants, and HPLC-DAD quantification test for anthocyanins validated for each type of ingredient. Examples of the application of these tests using data collected in our labs to address issues related to ingredient specifications, species differentiation, detection of adulteration, and other quality matters in European Elder berry ingredients will be presented.

C-28 – Elizabeth Kaweesa

Verticillin Cytotoxic Activity in High Grade Serous Ovarian Cancer in Preclinical *In Vitro* and *In Vivo* Models

Elizabeth N. Kaweesa[1], Jaqueline M. Bazioli[1], Herma C. Pierre[2], Daniel D. Lantvit[1], Samuel K. Kulp[3], Kasey L. Hill[3], Mitchell A. Phelps[3], Christopher C. Coss[3], James R. Fuchs[4], Cedric J. Pearce[5], Nicholas H. Oberlies[2], Joanna E. Burdette[1]. [1]Department of Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL 60607, [2]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27412,[3]Division of Pharmaceutics and Pharmacology and [4]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210, USA. [5]Mycosynthetix, Inc., Hillsborough, NC 27278

Verticillins are epipolythiodioxopiperazine (ETP) alkaloids isolated from a fungus with nanomolar anti-tumor activity in high grade serous ovarian cancer (HGSOC). HGSOC is the fifth leading cause of death in women and new drug entities to help tackle

chemoresistance. Verticillin D was recently found in a new fungal strain and compared to verticillin A. Both compounds exhibited nanomolar cytotoxic activity against OVCAR4 and OVCAR8 HGSOC cell lines, OVCAR8 3D spheroids, and induced apoptosis. In addition, verticillin A and verticillin D reduced tumor burden *in vivo* using OVCAR8 xenografts in the peritoneal space as a model. Tolerability studies to optimize verticillin A formulation for *in vivo* delivery was performed and compared to a semi-synthetic succinate version. Formulation of verticillins achieved optimal drug delivery and found no serum biomarkers for liver or kidney toxicity. Thus, formulation studies are effective at improving tolerability and demonstrating efficacy for verticillins.

C-29 – Liana Zaroubi

The Chemical and Biosynthetic Response of *Paraburkholderia megapolitana* under Antibiotic Stress

Zaroubi, L.;[1] Paulo, B. S.;[2] Eustaquio, A. S.;[2] Linington, R. G.[1], [1] Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada. [2] Department of Pharmaceutical Sciences, and Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, IL, USA

A mass spectrometry-based parallel stable isotope labelling platform, named IsoAnalyst, has been recently developed as a universal characterization method for connecting natural products (NPs) to their cognate biosynthetic gene clusters (BGCs) rapidly and efficiently. This tool enables the design of experiments to further our understanding of the biosynthetic and chemical capacity of micro-organisms through the discovery of novel chemistry and NPs. Herein, we developed a generalizable method for NP induction in *Paraburkholderia megapolitana* using antibiotics at sublethal concentrations, and metabolically profiled this response over time using IsoAnalyst. Results from this study demonstrate large-scale changes in the metabolic profile of *Paraburkholderia megapolitana* under antibiotic stress which would have been difficult to observe using alternative methods such as untargeted metabolomics using MS/MS fragmentation. Our results highlight unique compounds that are induced under antibiotic stress including one class of putatively novel polyketides and non-ribosomal peptides hybrids that are robustly produced in the presence of the RNA polymerase disruptor rifaximin. Future work involves isolating, characterizing, and linking these NPs to their BGCs, to understand the full metabolic and biosynthetic response of these strains to antibiotic exposure.

C-30 – Christine Salomon

Discovery and Development of Anti-Parasitic Terpenes from Subterranean Fungi for Treatment of Cryptosporidiosis

Christine Salomon[1], Roberta O'Connor[2], Yudi Rusman[1], Jessica Williams[1], Harrison VanKoten[1], Selome Banini[1], Mary Piaskowski[2], Fernanda Fumuso[2], Alexis Cotto-Rosario[2], Chidiebere Onoh[2]. [1] Center for Drug Design, University of Minnesota, Minnesota, USA 55455, [2]Department of Veterinary and Biomedical Sciences, University of Minnesota, Minnesota, USA 55108

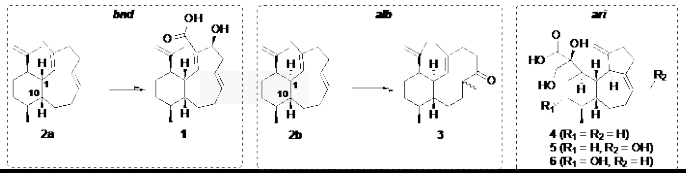
Cryptosporidiosis is an intestinal disease caused by apicomplexan parasites in the genus *Cryptosporidium*. *C. parvum* is distributed globally and one of the leading causes of water borne diarrhea in humans, and is especially detrimental to children under the age of 5 and patients with compromised immune systems such as those with HIV. As part of a drug discovery project to identify new anti-parasitic drug leads, libraries of extracts from diverse bacteria and fungi isolated from subterranean environments were screened using an in vitro infection inhibition assay. We identified a suite of structurally related norditerpene lactone metabolites from the fungus *Oidiodendron truncatum* with potent activity against both *C. parvum* and the related parasite *Toxoplasma gondii*. Three of the active compounds have sub-micromolar anti-parasitic activity, and vary in their off target cytotoxicity, pharmacokinetic stability, and permeability. We will discuss efforts to assess structure activity relationships, develop semi-synthetic analogs and the challenges and opportunities of translating in vitro activity into an in vivo mouse model system.

C-31 – Jeffrey Rudolf

Genome Mining for Bacterial Terpene Synthases Reveals Novel Eunicellane Diterpenoids

Zining Li,[1] Baofu Xu,[1] Zengyuan Wang,[2] Qian Yang,[2] Sandra Loesgen,[1,3] Liao-Bin Dong,[2] Jeffrey D. Rudolf[1]. [1]Department of Chemistry, University of Florida, Gainesville, FL, USA. [2]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing, China. [3]Whitney Laboratory for Marine Biosciences, University of Florida, St. Augustine, FL, USA

Terpenoids are the largest and most structurally diverse family of natural products. Of the 80,000 known terpenoids, less than 2% are of bacterial origin. Here, we describe the genome mining of three different families of eunicellane diterpenoids in bacteria. We discovered benditerpenoic acid (**1**), a *cis*-eunicellane, from *Streptomyces* sp. (CL12-4), its biosynthetic gene cluster, and the terpene synthase (TS) responsible for constructing benditerpe-2,6,15-triene (**2a**). We characterized AlbS, a TS that forms a diastereomer of **2a** (**2b**) and leveraged that finding into the identification of the *trans*-eunicellane diterpenoid **3**. Finally, we found a **1**-like gene cluster that produces highly oxidized eunicellane-derived diterpenoids, **4–6**, via a P450-catalyzed terpene cyclization.



Poster Presentations – Session I

P-001 – Victoria Anderson

Library Enabled Annotation for Botanical Natural Products (LEAF BotNP): Increasing the Representation of Botanical Spectra in Opensource Databases

Victoria M. Anderson[1], Warren S. Vidar[1], Daniel A. Todd[1], Nadja B. Cech[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412

The inclusion of spectral data in open-source databases increases the number and quality of annotations possible from mass spectrometry (MS) datasets. Additionally, the availability of targeted spectral libraries can prevent the nonsensical annotations that can occur when the spectra are searched across general libraries, many of which contain compounds that would never appear in the relevant sample. The Library Enabled Annotation for Botanical Natural Products **(**LEAF BotNP) initiative aims to collect high-quality positive and negative ionization mode MS-MS data for botanical natural products, and to deposit these in a specialized library on the opensource platform GNPS. The library will be available to any scientist seeking to annotate botanical MS data, and contributors around the world will be able to contribute their own spectral data. So far, data have been collected for 171 pure standards of botanical secondary metabolites. Existing MS spectral libraries typically include either the $[M+H]^+$ or $[M-H]^-$. Other adducts, especially dimers, are well represented in the LEAF library. In addition to aiding in annotation of botanical natural product data, this library can be used to investigate trends in ionization and fragmentation among a group of natural products.

P-002 – Riley Blue

Probing the Biological Function of Boron Via ^{11}B NMR and LCMS Analysis of Small Molecules

Riley M. Blue, Jocelyn M. Macho, Hsiau-Wei Lee, and John B. MacMillan. Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA 95064

Boron is a highly abundant element and is essential for plants and animals. Despite the essentiality and widespread availability of boron, its functional role in biological systems is poorly understood. Research into boron-containing compounds has been limited by the lack of analytical tools available to study them. Our lab recently optimized a ^{11}B NMR method to allow for the study of small amounts of boron in crude material. This method allows us to not only look for novel boron-containing natural products, but also probe the role of boron in biological systems. In this presentation, I will share two aspects of my research related to boron and small molecules.1) The use of ^{11}B NMR to isolate and characterize novel boronated natural products from our microbial fraction library and screen them for biological activity, and 2) the use of untargeted LCMS-based metabolomics and ^{11}B NMR to observe differential regulation of small molecules in wildtype (Col-0) and high boron requiring mutant (*bor1-1)* Arabidopsis in response to different levels of boron supplementation to probe the role of boron in plant function. This work will help to increase the understanding of the role of boron in biological systems.

P-003 – Timothy Bushman

Bacterial Espresso? Accelerated Solvent Extraction of Bacterial Cell Pellet as a Method for the Improved Extraction Recovery of Selected Metabolites

Timothy J Bushman, Hannah Berry, Maddie Dissinger, Jett Lane, Melanie Higgins, Lukasz Ciesla

Bacterial secondary metabolites have been a fruitful source of numerous biologically active compounds (Zhang et al. 2015). However, drug discovery from bacteria can be time- consuming and resource intensive. Organic solvents used in bacterial metabolite extraction are also costly to dispose of and can pose a hazard to human and environmental health (Barragán- Martínez et al. 2012, Pena-Pereira et al. 2015). New technologies like next-generation sequencing, molecular networking, and ultra-performance-liquid-chromatography have enabled a renaissance of bacterial natural product discovery (Traxler et al. 2015). Why then, have the techniques used to extract bacterial compounds remained relatively unchanged for the past 50 years (Katz and Baltz 2016)? Therefore, an extraction technique which substantially increases the yield of specialized metabolites per unit of biomass, as well as being more efficient than traditional methods would be an improvement in most experimental designs. Here we introduce the utilization of accelerated solvent extraction or ASE in the extraction of two distinct specialized metabolites, undecylprodigiosin and ectoine, from the model organism *Streptomyces coelicolor* A3(2). The ASE method was optimized using five key variables, sample preparation, solvent choice, temperature, static time, and number of cycles. Preliminary results of ethyl acetate extraction indicate a significant advantage to ASE when compared to lyophilization and subsequent sonication of the biomass in undecylprodigiosin recovery when ethyl acetate is the solvent.

P-004 – Mary Dawn Celiz

Sample Preparation of Microcystins in Dietary Supplements for LC-MS/MS Analysis

Mary Dawn Celiz and Jonathan Deeds, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, Maryland 20740, USA

Microcystins are cyclic heptapeptides produced by several cyanobacterial species (also known as blue green algae) such as *Microcystis aeruginosa*. The presence of microcystins, which are

hepatotoxins, in dietary supplements poses a potential health risk to consumers if present in unsafe quantities. Food and dietary supplements containing the cyanobacteria *Aphanizomenon flos-aquae* (AFA) as an ingredient have been found to be contaminated with microcystins when not adequately controlled for. Although AFA does not produce microcystins, AFA grown in the wild can be contaminated with other naturally co-occurring microcystin-producing cyanobacteria. The focus of this study is to optimize sample preparation for LC-MS/MS analysis of AFA-containing dietary supplements in capsule, tablet, and powdered forms by minimizing matrix effects while maintaining acceptable recovery of microcystins. Dispersive solid phase extraction, dilution, and use of nitrogen-labelled internal standards are investigated. Apparent recovery of ≥ 80% are achieved. The optimized method introduces a fast and easy way of determining microcystin contamination of AFA-containing dietary supplements.

P-005 – Sanem Hosbas Coskun

Quantification of Triterpene Glycosides of Black Cohosh Standard Reference Materials (SRMs)

Sanem Hosbas Coskun[1], Catherine A. Rimmer[2], Adam J. Kuszak[1, 1] Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA. [2]National Institute of Standards and Technology, Gaithersburg, MD, USA

Black cohosh (*Actea racemosa* L.) roots and rhizomes are among the most popular dietary supplement ingredients sold in the United States, traditionally considered for use in rheumatism and more recently to mitigate symptoms related to menopause. To support the analytical characterization of marker compounds and strengthen the chemical knowledgebase to authenticate these plant materials, the National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH ODS), is producing four black cohosh certified reference materials. Extraction studies were completed to identify the optimum gain for the triterpene glycosides. A selected ion monitoring (SIM) method using liquid chromatography-mass spectrometry (LC-MS) in negative mode was used for quantification studies. Additionally, cimifugin was selected to be a marker compound to identify the adulteration with other *Actea* species.

P-006 – Amy Keller

Acute (–)-Epicatechin Treatment Improves Thermoneutral-Diminished Vasoreactivity

Melissa M Henckel[1,2], Leslie A Knaub[1,2], Greg B Pott[1,2], Lori A Walker[3] and Jane E-B Reusch[1,2], Amy C Keller[1,2]. [1]Division of Endocrinology, Metabolism & Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, [2]Rocky Mountain Regional VA Medical Center, Aurora, CO 80045, [3]Division of Cardiology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

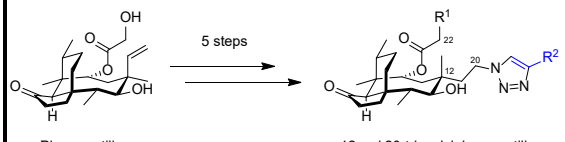
Cardiovascular disease (CVD), common in those suffering from diabetes, is a major cause of global mortality. Our previous work shows that (–)-epicatechin, a botanical compound known for its vasodilatory capacity, repairs thermoneutral-damaged vessel reactivity along with the stimulation of insulin secretion. Based on these results, we hypothesized that acute (–)-epicatechin may act globally by modulating the PI3K/Akt pathway, connecting insulin secretory activity with vasodilation. In a preliminary experiment, we housed Wistar rats at thermoneutral (30°C, TN) conditions for a total of 6 weeks, then moved half of the animals to room temperature (24°C, RT) for two weeks. Animals were treated with either 1 mg/kg body weight (–)-epicatechin or vehicle daily for 3 days. Aortic vasoreactivity and Akt protein expression were measured. Moving animals to RT housing failed to restore TN-caused diminished vasodilation. However, (–)-epicatechin significantly improved vasodilation response ($p<0.5$) in all animals (RT=15.7±5.9% vs. 57.5±16.9% and TN= 14.1±6.4% vs. 25.2±13.3%, control vs. treated, respectively, $p<0.05$ for both). There were no differences in Akt expression between any of the groups. These data illustrate (–)-epicatechin's acute improvement of vasoreactivity, perhaps by mechanisms other than PI3K/Akt pathway. Our ongoing work addresses (–)-epicatechin's modulation of cellular pancreatic and vascular physiology.

P-007 – Logan Breiner

1,2,3-Triazole Derivatives of the Antibiotic Natural Product Pleuromutilin

Logan M. Breiner[1], Roman P. Slowinski[1,2], Anthony J. Briganti[2], Anne M. Brown[2], and Andrew N. Lowell[1]. [1]Department of Chemistry, Virginia Tech, Blacksburg, Virginia, (US). [2]Department of Biochemistry, Virginia Tech, Blacksburg, Virginia, 24061, USA

Pleuromutilin-based semisynthetic drugs are used as critical human and veterinary antibiotics. Their unique mechanism of action has low development of resistance and their lack of use as livestock growth enhancers results in a low prevalence of resistance. Our previous work involving pleuromutilin-functionalized triazoles showed that C22 substituted compounds maintained activity while C20 substitution abolished it. By using computation and structure-based drug design to build on these findings, we show that epimerization of the C12 position provides potent lead compounds with C20 triazoles. Additionally, electrophilic intermediates identified en route to our final compound demonstrated enhanced activity. Currently, a series of electrophilic pleuromutilin analogs has been generated and is undergoing testing. These derivatives will reveal ribosome binding details and possibly a new mechanism of pharmacophore action as the source of increased activity.



Pleuromutilin 12-*epi*-20-triazolylpleuromutilins

P-008 – Susan Ensel

A New 5-Alkyl Resorcinol Isolated from *Gevuina papuana* with Antibacterial Activity

Susan Ensel,[1,2] Lucero Martinez-Fructuoso,[3] Rachel C. Mallory,[1] S. J. Ryan Arends,[4] Vitor Freire,[3] Jason R. Evans,[3] Brian D. Peyser,[3] Rhone K. Akee,[1] Christopher C. Thornburg,[1] Rohitesh Kumar,[1] Gina M. Morgan,[4] Leonard R. Duncan,[4] Barry R. O'Keefe,[3,5] and Tanja Grkovic[3,5, 1]Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702–1201. [2]Department of Chemistry and Physics, Hood College, Frederick, Maryland 21701–8599. [3]Natural Products Branch, Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702–1201. [4]JMI Laboratories, North Liberty, IA 52317. [5]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702–1201

Recently, the National Cancer Institute Program for Natural Product Discovery, in collaboration with the National Institute of Allergy and Infectious Diseases, presented the results of a high throughput screen of 326,000 natural product fractions against a panel of microbial and fungal pathogens. Fractions derived from the organic extract of the plant *Gevuina papuana* (Proteaceae) were identified as having antibacterial activity in this screen. Bioassay-guided isolation led to the identification of two known 5-alkyl resorcinol natural products that showed selective activity against *Staphylococcus aureus* subsp. *aureus* ATCC 29213, as well as a new alkyne-containing analogue. The activity of the compounds, together with the spectroscopic and synthetic data used to determine structure, will be presented.

P-009 – Christian Espinoza-Barrios

Metabolomic Investigation of Soil Fungi in the Search for Gram-Positive & Gram-Negative Antimicrobial Activity

Christian A. Espinoza-Barrios[1], Colby M. Laws[1], Victoria M. Anderson[1], T'ea P. Cameron[1], Tamam El-Elimat[1], Huzefa A. Raja[1], Nadja B. Cech[1], Nicolas H. Oberlies[1, 1]Department of Chemistry & Biochemistry, The University of North Caroline at Greensboro, Greensboro, NC, 27407, USA

A growing concern in clinical settings is the emergence of antibiotic-resistant bacterial infections, among the most concerning being Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii.* Despite a greater understanding of the selective forces that antibiotics have on bacterial strains, continued resistance drives the demand for novel sources of antibiotics. Natural products derived from fungi have been proven to be reliable sources of antibiotics since the discovery of penicillin in 1928. For this study, six soil fungal isolates and their chromatographic fractions were tested in vitro for antimicrobial activity. Three of these samples demonstrated antimicrobial activity against MRSA, while three were active against *A. baumannii*. Untargeted LC-MS metabolomics datasets from these samples were used to predict mass spectral features associated with activity, and comparison of these features against a fungal dereplication library indicated that activity was associated with the known compounds Destruxin B, Homodestruxin B, and Antibiotic PF 1052, and with several unknown compounds for which structural elucidation is ongoing.

P-010 – Oli Horyn

Investigating the Molecular Mechanism of Action of Sesquiterpene Lactone Laurenobiolide

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With constantly evolving bacteria threatening the efficacy of antibiotics, the search for novel antimicrobials is imperative. Natural products are used medicinally and have provided lead compounds for drug development. Laurenobiolide is a sesquiterpene lactone isolated from the North American tulip tree *Liriodendron tulipifera* with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Using disc diffusion assays, we validated its activity on a methicillin-sensitive strain of *S. aureus*, assessed its activity against some other bacterial species, and isolated laurenobiolide-resistant *S. aureus* mutants. To investigate what genetic changes might lead to resistance, we re-sequenced the genomes of the laurenobiolide-resistant mutants and wild-type cells. This allowed us to identify mutations present in the laurenobiolide-resistant mutants that are not present in the original laurenobiolide-sensitive cells. In all three resistant isolates, we found two mutations resulting in changes to coding sequences, which will be presented. Given these mutations result in changes to proteins which may be essential for cellular function, we hypothesize one of these two mutations may cause laurenobiolide-resistance. Together, we validated that laurenobiolide exhibits antimicrobial activity on *S. aureus*, assessed laurenobiolide for antimicrobial activity against other bacteria, and identified two potentially resistance-causing mutations. Our work suggests that laurenobiolide may be developed as a novel antimicrobial and our work is expected to provide insight into how laurenobiolide exerts its effects at the molecular level.

P-011 – Susan Egbert

Unlocking The Undiscovered Potential Of Lichen-Fungi Metabolites

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Lichen-fungi are symbiotic organisms composed of a primary mycobiont and a photobiont that can survive in harsh ecological conditions. Lichen often grow at a slow rate of one centimeter in diameter per year which limits the study of their natural product metabolome. This rate of growth creates a larger incentive for protecting their environmental niche due to the large relative investment in each colony. This ecological imperative encourages our investigation into their role in the environment, their production of secondary metabolites. The presented project is unique in that it will use genomics, advanced metabolomic techniques, and high throughput biological assays to understand not only why lichen make their metabolites but also how we can apply them as potential therapeutics. This presentation will describe our recent efforts to establish a comprehensive catalogue of lichen natural products and link their chemical structure to observed bioactivity.

P-012 – Jason Evans

NCI Program for Natural Product Discovery: Bioinformatics-Guided Approaches to Natural Product-Based Drug Discovery

Jason Evans[1,3], Tanja Grkovic[2], and Barry R. O'Keefe[1,4]. [1]Natural Products Branch, Developmental Therapeutics Program, National Cancer Institute; [2]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research (FNLCR); [3]Data Management Services Inc., FNLCR

Beginning in the 1980's, the National Cancer Institute (NCI) created the Natural Product Extract Repository. Now encompassing over 230,000 extracts from more than 40,000 species, the repository represents one of the world's largest publicly available collections of plant, marine invertebrate and microbial samples available for natural product-based research. The NCI also undertook the identification of active anti-cancer natural products using a collection of human tumor cells known as the NCI-60. Measuring growth inhibition and cytotoxicity, the assay generates a 60 x 5-point dose-response profile. Analysis and annotation of such a large amount of information can be challenging. Here we present a self- organizing map (SOM)-based analysis of the NCI-60 data on crude natural product extracts which enables the visualization of similar patterns of response in "biological space" to aid in the prioritization of chemistry efforts. The assay-driven approach was validated through the use of LCMS- and NMR-based metabolomics, as well as bioassay-guided isolation of active molecules. Examples of the use of the NCI- 60 SOM, will be presented demonstrating taxonomic, chemotypic and phenotypic relationships between extracts and enabling more efficient project prioritization.

P-013 – Camila M. Crnkovic

Developing an Algorithm for Cyanobacterial Biosynthetic Gene Cluster Prioritization

João Pedro B. Domingues[1], Laura P. Ióca[2], Márcio B. Weiss[1], Alessandra S. Eustáquio[3], Camila M. Crnkovic[1]., [1]School of Pharmaceutical Sciences, University of São Paulo, Brazil; [2]Department of Molecular Biology, Princeton University, Princeton, NJ; [3]College of Pharmacy, University of Illinois at Chicago, Chicago, IL

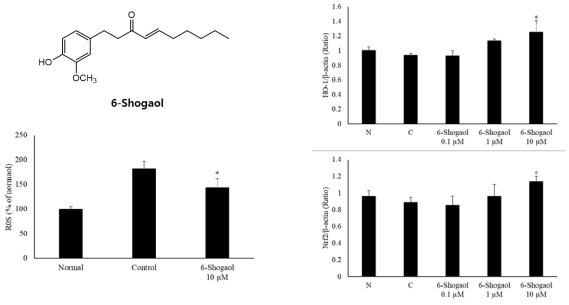
Cyanobacteria are a diverse group of microorganisms that produce secondary metabolites with potential pharmaceutical and biotechnological applications. In particular, Brazilian cyanobacteria still remain largely underexplored. Due to the increasing accessibility to genome data and the development of bioinformatics tools, genome mining strategies play a compelling role in natural product research to uncover biosynthetic gene clusters (BGCs) responsible for the production of novel molecules. To accelerate discovery, we developed an automated BGC prioritization algorithm focused on polyketide synthase (PKS), nonribosomal peptide synthetase (NRPS), and hybrid polyketide synthase-nonribosomal peptide synthetase (NRPS-PKS). Our pipeline is able to read .gbk antiSMASH output and to score BGCs within these metabolite classes. The algorithm generates a score based on the evaluation of the architecture and composition of the modules within the predicted BGCs, along with the presence of genes encoding for tailoring enzymes, transport and regulation. It is written in shell script and R. The rationale was validated using the genome of *Moorea producens* JHB, a previously studied cyanobacterium known to harbor the hybrid NRPS-PKSs that produce jamaicamides and hectochlorin. Then, we explored 55 Brazilian cyanobacterial genomes, which resulted in 23 prioritized BGCs. Future studies will include the chemical investigation of the prioritized BGCs, and the improvement of our algorithm in order to make it available to the natural product community.

P-014 – Sang Yoon Choi

Anti-Oxidative Effect of 6-Shogaol in Muscle Cells

Sang Yoon Choi[1], and Jinyoung Hur[1], [1]Korea Food Research Institute, Wanju 55365, Republic of Korea

The ginger is a zingiberaceae perennial plant of the subtropical or tropical regions, and its rhizome is edible. Gingerol and shogaol is the major essential oil contained in ginger, but shogaol has been reported to have higher radical scavenging and anti-inflammatory effect than gingerol. In this study, the protective effect of 6-shogaol on L6 muscle cells against oxidative damage was measured. As the results, 6-shogaol significantly inhibited hydrogen peroxide induced cell death in L6 skeletal muscle cells. The 6-shogaol inhibited the production of intracellular ROS and increased mRNA and protein expression of HO-1 and Nrf2. These results suggested that 6-shagol effectively inhibits oxidative damage of skeletal muscle cell.



P-015 – Geneive Henry

DNA Interaction and Cytotoxicity Studies of Synthetic Furanochromene-Quinoline Hybrid Derivatives

Blake Shellenberger[1], Olivia Basile[1], Joel Cassel[2], Ian Tietjen[2], Geneive E. Henry[1]. [1]Department of Chemistry, Susquehanna University, Selinsgrove, PA 17870, USA, [2]The Wistar Institute, Philadelphia, Pennsylvania, USA

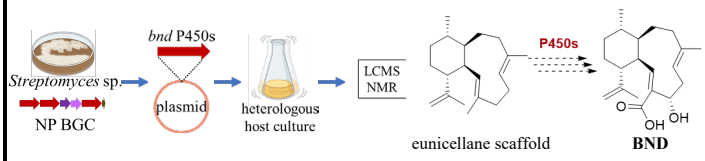
The furanochromene and quinoline scaffolds are present in many natural products and are considered as privileged structures. They are associated with a broad range of biological activities including anticancer, antibacterial and anti-inflammatory properties. The design of hybrid molecules incorporating two or more bioactive scaffolds is an effective strategy in drug design. In this study, a series of seven hybrid furanochromene-quinoline derivatives containing a hydrazone linker were synthesized by condensing a furanochromene hydrazide with 2-, 3-, 4-, 5-, 6-, and 8-quinoline carbaldehydes. In order to determine the anticancer potential of the derivatives, their interaction with DNA was determined through molecular docking studies and gel electrophoresis studies. All of the derivatives showed strong DNA docking affinity with preference for binding in the minor groove of DNA. In addition, the derivatives cleaved plasmid DNA to open-circular and linear forms in the presence of copper(II) ions. Copper ion concentration in cancer cells is higher than normal cells. Therefore, these data suggest that the derivatives may be effective in killing cancer cells. To further explore the anticancer potential of the furanochromene-quinoline derivatives, their cytotoxicity towards mammalian Vero cells was evaluated.

P-016 – Diana Łomowska-Keehner

Investigating *Streptomyces* Eunicellane Biosynthesis Through Heterologous Expression

Diana Łomowska-Keehner, Jeffrey D. Rudolf, Department of Chemistry, University of Florida, Gainesville, FL 32611

Eunicellane diterpenoid natural products (NPs) possess cytotoxic, antitumor, and anti-inflammatory properties. These NPs are oxidized by putative cytochrome P450 enzymes (P450s), likely imparting their bioactivities. Mostly found in corals, low yield and limited biosynthetic data preclude their pharmaceutical development. The discovery of bacterial eunicellanes offers an opportunity to study their biosynthesis due to the organization of biosynthetic gene clusters (BGCs). *Streptomyces* sp. CL12-4 produces benditerpenoic acid (BND), a eunicellane active against antibiotic-resistant Gram-positive pathogens. In this work, heterologous expression was used to isolate intermediates, establish the function of each P450 in the *bnd* BGC, and elucidate the full BND biosynthetic pathway. The results of heterologous expression experiments using different *bnd* P450 combinations will be presented.

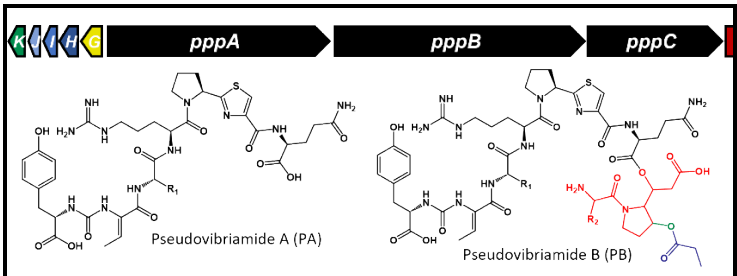


P-017 – Vitor Lourenzon

Biosynthesis and Biological Functions of Pseudovibriamides Isolated from the Marine Sponge-Associated Bacterium *Pseudovibrio Brasiliensis*

Vitor Lourenzon[1], Yitao Dai[1], Laura P. Ioca[1,2], Roberto G. S. Berlinck[2], and Alessandra S. Eustaquio.[1,1] Department of Pharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL. [2] Instituto de Quimica de São Carlos, Universidade de São Paulo, São Carlos, Brazil

Pseudovibriamides A- C (PA-PC) are non-ribosomal peptides (NRPS) and NRPS-Polyketide hybrid (NRPS-PKS) isolated from the marine sponge-associated strain *Pseudovibrio brasiliensis* Ab134. A library of knock-out mutants was generated by reverse genetics techniques to elucidate the biosynthesis of the pseudovibriamides. The compounds contribute to the swarming abilities and biofilm formation of *P. brasiliensis* and display an antibiotic antidote activity capable of protecting *Bacillus cereus* against the broad- spectrum antibiotic blasticidin S. However, PC was never isolated from the Ab134 culture, and PA and PB were isolated in low yields, limiting us from better exploring the biological activities of pseudovibriamide. Therefore, the heterologous expression of the *ppp* BGC is being designed to improve access to the compounds. The biosynthesis of the compounds, the biological activities, and the progress with the heterologous expression will be presented.



P-018 – Zacharie Maw

Discovery, Upregulation, and Synthesis of Cryptic Albubactin A-F using Rhamnolipid's as a Chemical Elicitor on Solid Fermentation of Marine *Streptomyces albus* RKJM-0023

Zacharie A. Maw[1], Christopher Cartmell[2], Alyssa L. Grunwald[3], Bradley Haltli[1,3], and Russell Kerr[1,3,4].[1]Department of Biomedical Science, Atlantic Veterinary College, Charlottetown, PEI, Canada, [2]Department of Pharmacology, College of Medicine, University of Arizona, Tuscon, AZ, USA, [3]Nautilus Biosciences Croda, Charlottetown, PEI, Canada, [4]Department of Chemistry, University of Prince Edward Island, Charlottetown, PEI, Canada

The use of chemical elicitors in *Streptomyces* is a proven method to upregulate or induce the biosynthesis of cryptic and silent natural products. However, there is limited research on the use of surface-active molecules as chemical elicitors with marine *Streptomyces*. Using the bacterial surfactant rhamnolipid, produced by *Pseudomonas aeruginosa*, the obligate marine *Streptomyces albus* RKJM-0023 significantly increased the accumulation of novel acyl-tripeptides albubactin A-F. Isolated in groups of three, albubactin A-C and D-F, the structure elucidation proved challenging with NMR, HRESIMS/MS, and Marfey's alone requiring a total synthesis of albubactin A to confirm the proposed structures. Albubactin's contain a never seen in nature sequence of three amino acid core with a C-terminus ethyl ester cap and an N-terminus featuring linear, iso-, or anteiso-acyl chains (C5–C13) dominated by the C9–C11 members. This research demonstrates the potential utility of exploring surface-active molecule interactions with *Streptomyces* as a novel approach to accessing cryptic natural products. It also shows that despite *Streptomyces albus* being a well studied strain, isolates from marine environments hold a high potential of producing novel chemistry.

P-019 – Carla Menegatti

Isotope-Labeled Monoterpene Reveals Insights into the Biosynthesis of Millipedes Secretions

Carla Menegatti,[1] Paige Banks[1], Emily Mevers[1].[1]Department of Chemistry, Virginia Tech, Blacksburg, VA*

Millipedes are a diverse class (Diplopoda) of arthropods that are distributed worldwide and are known to produce and store small molecules in repugnatorial glands. These specialized metabolites fall into three chemical classes, i.e., alkaloids, oxidized aromatics and hydrogen cyanide, and are predicted to have a role in chemical defense. Interesting these chemical classes clade well with the millipede phylogeny, where related clades of millipedes produce the same class of molecules. Millipede alkaloids are both the most chemically intriguing and least studied of all the chemical defense secretions. We recently reported a suite of new terpenoid-alkaloids, the ischnocybines, from the millipede *Ischnocybe plicata* (Pacific Northwestern United States). The ischnocybines add to the growing list of known alkaloids produced by phylogenically related millipedes. All the millipede alkaloids appear to be biosynthesized from geranyl pyrrolidine but are diversified during cyclisation and post-modifications. Probing this hypothesis, we synthesized an isotope-labeled geranyl pyrrolidine from geranyl chloride and d8-pyrrolidine. Crude protein extraction from preserved millipedes followed by incubation with the isotope-labelled geranyl pyrrolidine will provide insights into the accuracy of our hypothesis. Our long-term goal is to identify the millipedes genes that lead to the alkaloids and to eventually use this information to understand how this family of metabolites is diversified.

P-020 – Michael Pasquale

A Widely Distributed Biosynthetic Cassette is Responsible for Diverse Plant Side Chain Cross-Linked Cyclopeptides

Michael A. Pasquale, Williamjosh Brown, Bridgitte G. Ampolini, Stella T. Lima, Ethan B. Underwood, Tyler N. Graf, Cody E. Earp, Imani C. Khedi, Jonathan R. Chekan, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro

Burpitides are a newly defined class of cyclopeptides found in plants. This class of compounds is characterized by the key enzyme that is responsible for their biosynthesis called a BURP peptide cyclase. BURPs are responsible for the cyclization of the peptides through their side chains, often a tyrosine forming an ether bridge to an nearby amino acids β-carbon. Using a transcriptome-mining approach, a sub-class of burpitides called cyclopeptide alkaloids were linked to their **Ri**bosomally synthesized and **P**ost-translationally modified **P**eptide (RiPP) precursor peptides. This powerful approach allowed for the discovery of a widely distributed BURP peptide cyclase responsible for their biosynthesis. To validate these hypotheses, new compounds that perfectly matched the bioinformatic predictions were isolated from *Coffea arabica*, which we named arabipeptins. To build upon these results, 400 transcriptomes from diverse eudicots were assembled and analyzed for the capacity to

produce new cyclic peptides. This approach has allowed us to discover novel burpitides from *Gardenia jasminoides* which we n named gardenipeptins. These results reveal the wide distribution of burpitides across eudicots and the success of a bioinformatic guided approach to rapidly discover new members.

P-021 – Eric Schmidt

Biosynthesis of Natural Products in Animals

Zhenjian Lin and Eric W. Schmidt, Department of Medicinal Chemistry, University of Utah

Recently, many of the natural products previously attributed to the symbiotic microbiome have been found encoded in the animal genome. These are biosynthesized by widespread and unanticipated enzymes such as the animal fatty acid synthase-like polyketide synthases (AFLPs). We will focus on new discoveries concerning the biological distribution, natural products chemistry, and enzymology of AFLPs and other groups of recently discovered animal enzymes.

P-022 – Ayoola Smith

Engineering of New Biosynthetic Enzymes for Ribosomally Synthesized and Post-Translationally Modified Peptides (Ripps)

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Biosynthetic enzymes involved in the production of ribosomally synthesized and post-translationally modified peptides (RiPPs) in prokaryotic organisms often have two critical parts: 1) the RiPP precursor peptide recognition element (RRE) and 2) the catalytic domain. The former binds to the conserved leader portion of the bipartite precursor peptide(s) while the latter carries out modification(s) on the core peptide. This allows the enzymes to be specific yet promiscuously act on precursor peptides with diverse core sequences. Capitalizing on this unique biosynthetic logic, we sought to engineer new RiPP enzymes consisting of an RRE tethered to a well-studied protein post-translational modification enzyme, an *N*-glycosyltransferase. By nature, these protein post-translational modification enzymes are often promiscuous and act on peptidic substrates. Thus, they are well-suited as leads for fusion enzymes. We hypothesize that tethering the RRE to the promiscuous enzyme will increase the local concentration of the guided substrates (carrying a leader peptide) and improve catalysis due to the RRE-leader peptide interaction. We, therefore, sought to compare the catalytic efficiency and selectivity of the fusion enzymes to that of the native enzymes. Interestingly, preliminary data suggests that the fusion enzymes had better catalytic activity and selectivity for guided substrates than native enzymes. The success of this work will make it possible to create a toolbox of RiPP enzymes that can be used in combination with natural RiPP enzymes in known pathways to carry out custom modifications on peptide substrates. These enzymes can also be deployed for the improvement of the bioactivity, bioavailability, or stability of peptidic drugs.

P-023 – John Sorensen

Unlocking the Hidden Biosynthetic Potential of Lichen Fungi

John L. Sorensen, Department of Chemistry, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

There has been a recent resurgence of interest in the secondary metabolites produced by lichen fungi, driven by advances in genome sequencing and analytical chemistry technology such as LC-MS/MS. The lichen symbiont, resulting from cooperation primarily between a mycobiont (fungal) and photobiont (algal) partner has more recently been shown to house a more complex consortium of microorganisms.[1] Secondary metabolites are likely to play a critical role in the ecology of lichen fungi, and it is becoming clear that the chemical profiles are more complex than has been previously identified. We have recently used LC-MS/MS to identify the presence of usnic acid in strains of *Cladonia rangiferina* that have been traditionally described as lacking the ability to produce this ubiquitous secondary metabolite[2]. Genome sequencing of lichen fungi, by our group[3] and others[4], have revealed that there are a considerable number of 'cryptic' biosynthetic gene clusters that appear to code for novel chemical structures. We are now exploring these unknown genes through the use of heterologous expression in hosts such as *Aspergillus oryzae* and *E. coli*. We will describe our recent results with a lichen homologue (*cu-terA*) of the *terA* gene responsible for the biosynthesis of the polyketide terrein in *Aspergillus terreus*[5]. We will also describe our recent work on an O-methyltransferase that acts to further modify the *terA* polyketide product.

P-024 – Sarah Barr

Evaluation of Digestive Transformation of *Withania somnifera* (Ashwagandha) Plant Extracts and Bioactive Compounds via UPLC-MS

Sarah A. Barr[1], R. Thomas Williamson[1], and Wendy K. Strangman[1]. [1]Department of Chemistry, University of North Carolina Wilmington, Wilmington, NC 28403, USA

Ashwagandha (*Withania somnifera*) (WS) is primarily orally consumed and studied for its anti-inflammatory, anti-aging, and antioxidant properties. The primary bioactive agents in ashwagandha are a family of steroidal lactones known as withanolides. Well known withanolides, withaferin A (WFA), withanolide A (WDA), and withanoside IV (W4), have been isolated and studied for anti-cancer and neuroprotective effects. Preclinical in vitro studies normally use raw extract in bioassays exploring liver metabolism and plasma protein binding. This

practice fails to incorporate any changes to the metabolic profile from gastrointestinal digestion. This study investigates chemical transformations occuring after WS is orally consumed, and passes the stomach and small intestine, prior to entering the liver. In vitro identification of digestive metabolites is critical to downstream in vivo research. Both leaf and root ethanolic extracts along with WFA, WDA, and W4 standards, were analyzed in a simulated gastrointestinal digestion (SGID) model under both fasting and fed conditions. Results indicated that there there was more transformation in the leaf extract than the root extract. The epoxide placement on the withanolide skeleton made a large difference in compound stability in the gastrointestinal environment. A sizable portion of withaferin A was hydrolyzed, while withanolide A stayed largely intact. Withanoside IV was completely deglycosylated into its aglycone form sominone. The results from these experiments can provide valuable information regarding potentially bioactive digestive metabolites for future in vivo and clinical studies.

P-025 – Yuanyuan Ji

Do Other Hypericum Species are Promising as St. John's Wort (Hypericum perforatum)?

Yuanyuan Ji[1], Ruifei Zhang[2], Edward J. Kennelly[3], and Chunlin Long[4]. [1]School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA, [2]Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China, [3]Department of Biological Sciences, Lehman College, City University of New York, Bronx, NY 10468, USA, 4College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China

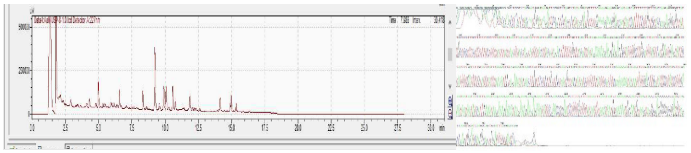
Plants in the genus Hypericum (Hypericaceae), include over 500 species, distribute in warm temperate areas worldwide. They have a broad-band medicinal use as antidepressant, antimicrobial, antitumor, and antiviral agents. More than 1000 secondary metabolites have been reported, including phloroglucinol derivates, xanthones, chromones, naphthodianthrone, and terpenoids. However only H. perforatum is official used as medicine to treat clinical depression and ranked at 21st out of the top-selling herbal supplements with its total sales of $23 million. We hypothesize that through detailed molecular network of marker compounds for evolutionarily related group of Hypericum species with bioactivity correlations will reveal promising species and drug lead compounds, which can be widely used in clinical like H. preforatum. By cut-edge mass spectrometry analytical instruments including GC-MS, LC-QTof-MS and LC-TQ-MS combing molecular networking, we examine intraspecific and interspecific chemical differences among eight species from two evolutionarily related botanical section, sect. Hypericum and sect. Ascyreia. H. attenuatum, H. erectum, H. faberi, H. hookerianum, H. bellum and H. pseudohenryi were verified are promising like H. perforatum through cytotoxicity, anti-inflammatory and antiplasmodia assessments. Cluster of promising leading compound also identified in bioactive species by molecule networking, most of them are novel phloroglucinol derivates.

P-026 – Yanru Li

Chemical Profiling and DNA Bar-coding Methods for the Investigation of the Authenticity of Ashwagandha

Shagufta Perveen[1], Yanru Li.[1].Seethapathy Saroja[1], Xiaoyan Chen[1],Hongtao Yu[1],Jiangnan Peng[1]:[1]Department of Chemistry, Morgan State University, MD21251

The root of Withania somnifera is used as an herbal medicine, ashwagandha. It is also called India ginseng, one of the most popular herbal medicines in India. Since the steady increase in the demand of ashwagandha in India and worldwide, the low-price aerial parts of the plants, which also contain withanolides, were seen on market as adulterants. In this study, we collected 88 ashwagandha samples from the grower, pharmaceutical company, and market and examined their authenticity using a holistic chemical profiling and DNA barcoding method. Chemical profiling was done with HPTLC and HPLC method. The extraction methods were compared. The pressurized heat method showed the highest extract efficiency, which is higher than the USP reflex method. The U.S. Pharmacopoeia HPTLC conditions are followed. The HPLC condition was optimized based on the USP method,



which showed better separation with short running time.

P-027 – Preston Manwill

There's Pepper in My Cinnamon: A Case of Contamination While Identifying Potential Herb-Drug Interactions

Preston K. Manwill[1], Rakshit S. Tanna[2], Colby H. Borges[1], Tyler N. Graf[1], Nicholas H. Oberlies[1], Mary F. Paine[2] and Nadja B. Cech[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27402. [2]Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, WA 99202

Consumers are turning increasingly to plant-based medicines (i.e., botanical dietary supplements) to combat chronic diseases and improve their quality of life. Due to the complexity of such products, there is the potential for accidental contamination or intentional adulteration of authentic botanical ingredients with other materials. In some instances, undisclosed ingredients are added to enhance the purported biological activity of the supplement. Inclusion of undisclosed ingredients in botanical supplements may increase the risk for herb-drug interactions and compromise the efficacy or safety of prescribed medications. In the present study, we analyzed a series of fractions prepared from a commercially available cinnamon (*Cinnamomum verum*)

supplement using biochemometrics, a multivariate statistical approach that combines chemical (e.g., untargeted ultra-high-performance liquid chromatography-high-resolution mass spectrometry) with biological (e.g., enzyme inhibitory activity) data. The goal was to identify potential cytochrome P450 (CYP) inhibitors. In addition to several known constituents of cinnamon, we identified piperine, the main constituent of black pepper (*Piper nigrum*) and a known inhibitor of select CYP enzymes. The piperine content measured in this product was too low (5.7 μg/g powder) to constitute a clinically relevant risk for pharmacokinetic herb-drug interactions. The study was expanded to identify piperine in 82 commercially available *C. verum* products, and 70% of them contained piperine at detectable levels, ranging from < 0.79 to 22 μg/g powder. The presence of other constituents of *P. nigrum* in the cinnamon products suggested that the source of this contamination was *P. nigrum*, rather than addition of purified piperine.

P-028 – Mirtha Navarro

USP Botanical Dietary Supplements and Herbal Medicines Pan American Expert Panel: First Anniversary Progress Report

USP Pan Am Expert Panel[1], Maria Monagas[2], Robin Marles[1] and Mirtha Navarro[1,3]. [1]United States Pharmacopeia (USP) Botanical Dietary Supplements and Herbal Medicines (BDSHM) Pan-American Expert Panel, USP BDSHM Expert Committee, Twinbrook Parkway, Rockville, MD 20852 [2]USP Dietary Supplements and Herbal Medicines, Twinbrook Parkway, Rockville, MD 20852 [3]University of Costa Rica (UCR) BIODESS Laboratory, Department of Chemistry, San Jose, Costa Rica

Considering the essential role that quality standards play and USP's commitment to global public health, the *Botanical Dietary Supplements and Herbal Medicines Pan American Expert Panel (BDSHM Pan-Am EP)* was launched last April 2022. The present poster presentation describes the charge and work of this Expert Panel in developing USP quality standards (e.g., monographs) and candidate reference standard materials for botanical ingredients and products used in traditional herbal medicines and marketed in the Pan-American region. USP monographs include specifications and tests for identity, composition, strength, purity, and limits for contaminants, which are required for consistent quality. The *BDSHM Pan-Am EP*, composed of experts from academia and industry as well as government liaisons of twelve countries from the Pan-American region, including Canada, USA, Mexico, Guatemala, Costa Rica, Panama, Ecuador, Colombia, Brazil, Peru, Argentina and Chile, has convened monthly during 2023. Among several activities, the *BDSHM Pan-Am EP* has worked on the proposal of a list of over one hundred plants and prioritization variables for the selection of topics for USP monograph development. To accomplish this, the EP has worked in gathering referenced data on agronomic, botanical, phytochemical, pharmacognosy and commercial aspects for such plants to complete the USP prioritization process. In addition, the members have provided insights from national health authorities and control agencies on the mechanisms to monitor the quality and safety of botanical products across the region. This input will facilitate preparing a comprehensive overview of herbal medicines national policies, regulations and best practices in the Pan-American region as a contribution to USP's endeavor toward Botanical Dietary Supplements and Herbal Medicines quality and public health assurance.

P-029 – Ruth Muchiri

Chemical Standardization of Milk Thistle (*Silybum Marianum*) Using Uhplc- Ms/Ms: Standard Addition Vs Calibration Curve Quantitation

Muchiri R and van Breemen RB Linus Pauling Institute, Oregon State University, Corvallis, Oregon

Milk thistle extracts known as silymarin are widely used as dietary supplements due to anti- inflammatory, anti-cancer and hepatoprotective effects. The main components of lipophilic extracts of milk thistle seeds and fruit are the abundant flavonoids and flavonolignans including silybin A, silybin B, isosilybin A, isosilybin B, silydianin, silychristin, taxifolin, and the less abundant 2,3-dehydrosilybins. Clinical studies using milk thistle have failed to provide consistent results, which probably result from use of inadequately standardized test materials. Inadequate standardization of test materials leads to inconsistent results due to variable and irreproducible levels of silymarin constituents, each of which might have different pharmacological activities. The aim of this study was to develop a validated method for the standardization of milk thistle silymarin. This method is applicable for measurement of silymarin constituents in test materials which will enable accurate correlation between pharmacological concentrations of individual compounds and the observed *in vivo* effects. Validation using the method of standard addition was employed to account for lack of blank matrix. Additionally, matrix effects were investigated by analyzing silymarin standards dissolved only in the UHPLC mobile phase. The quantities of isosilybin A, isosilybin B, silychristin, silydianin, taxifolin, silybin B, and 2,3-dehydrosilybin B in the milk thistle extract were comparable using both standardization methods. The experimental details and results from this study will be presented.

P-030 – Wilmer Perera

High-Performance Thin-Layer Chromatography PRO, a Powerful Analytical Tool for the Analysis of Botanicals

Wilmer H. Perera, CAMAG Scientific, Inc. 515 Cornelius Harnett Drive, Wilmington, NC 28401

High-Performance Thin-Layer Chromatography (HPTLC) is one of the most used techniques in the USA for the analysis of botanicals

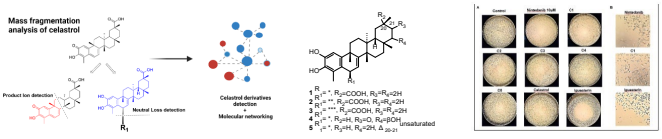
in the dietary supplements industry. With the new fully automated HPTLC PRO system, multiple samples can be analyzed in sequence, overcoming the environmental effects produced by the previous open system. HPTLC PRO also adds a more rigorous control of the gas phase and is a throughput technique for the quality control of plant materials among other applications. This presentation aims to discuss the evolution of HPTLC by revisiting important concepts developed through the last years, such as standardization, comprehensive fingerprint and also reviewing more recent ideas like the universal system suitability test, complementary developing solvents for untargeted analysis. The use of general developing solvents for targeted analysis and examples on how HPTLC can be used as an important and quick tool in drug discovery will also be covered.

P-031 – Jorge Ponce-Zea

LC-MS/MS Guided Isolation of Quinone Methide Triterpenes from Tripterygium Regelii

Jorge Ponce-Zea[1], Eun Jin Park[1], and Won Keun Oh[1]. [1]Korea Bioactive Natural Material Bank, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea

The current study used LC-MS/MS, mass fragmentation analysis, and molecular networking to isolate naturally occurring celastrol derivatives from *Tripterygium regelii* stems. Our research led to the isolation of five new quinone methide triterpene derivatives. Extensive spectroscopic analyses, chemical derivatization, and quantum chemical calculations were used to determine the structures of these derivatives. In addition, 45 compounds with the same scaffold were detected in LC-MS/MS analysis and were tentatively identified based on a building block rationale. Compounds **1**-**3** were discovered to significantly inhibit TGF-β-induced epithelial-mesenchymal transition migration in human A459 lung cancer cells.



P-032 – Zarna Raichura

Evaluation of Botanical Extracts for Cytochrome P450 Mediated Drug Interactions

Zarna Raichura[1], Kabre Heck[1], Jaewoo Choi[2,3], Mikah Brandes[5], Cody Neff[5], Claudia Maier[2,3,4], Amala Soumyanath[4,5], Robert Arnold[1], and Angela I Calderón[1]. [1]Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, AL 36849, USA, [2]Department of Chemistry and [3]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, [4]Botanicals Enhancing Neurological and Functional Resilience in Aging (BENFRA) Botanical Dietary Supplements Research Center and [5]Department of Neurology, Oregon Health and Science University, Portland, OR 97239

There has been remarkable growth in consumption of botanical dietary supplements (BDS), making it important to understand the safety profile of BDS with respect to the pharmacokinetic properties for any potential of botanical-drug interactions. One such botanical interactions which has gained significant attention involves inhibition of cytochrome P450 (CYP450) enzymes by co-administered drugs. Our study involved examining two widely used botanicals, ashwagandha and açaí for any potential inhibition of CYP450. For preliminary screening of ashwagandha extracts, aqueous leaf extract was the most active extract which resulted in enzymes CYP1A2, 2A6, 2B6, 2D6, 2C8, 2C9, 3A5, and 2E1 showing ≤ 50% enzyme activity. While CYP2D6, 2C9, and 2E1 showed ≤ 50% enzyme activity when treated with aqueous root, 70% ethanol leaf and root extracts at a human-relevant concentration of 1.89 µg/ml and 1.18 µg/ml of withaferin A and withanolide A. Whereas for açaí, only CYP2C9 showed ≤ 50% enzyme activity when treated with acidic methanol extract of açaí formulation at a human-relevant concentration of 2.321 ng/ml of cyanidine-3-glucoside. The results reflect that both botanical extracts showed potential of CYP450 inhibition, suggesting that compounds in BDS can prolong the half-lives of medications leading to extended action or toxicity. Next step involves, determination of IC_{50} values for those extracts and its respective enzyme who showed ≤50% activity.

P-033 – Rocío Rivera Rodríguez

Effects of Diterpenes from *Salvia Rosmarinus* on the Nrf2 Receptor Pathway in Intestinal Models

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The prevalence and incidence of intestinal diseases including Inflammatory Bowel Disease and colorectal cancer are increasing worldwide. This phenomenon is widely associated with a Western diet that promotes oxidative and inflammatory damage. Approved anti-inflammatory drugs have multiple adverse effects, especially the ones targeting chronic inflammation. Thus, approaches with less adverse effects including phytochemicals are a promising opportunity for IBD. *Salvia rosmarinus*, commonly known as rosemary, is approved as a food preservative in the European Union and has shown anti-inflammatory and antioxidant activity in various disease models. Diterpenes are present in oil-soluble rosemary extracts, however its anti-inflammatory potential in intestinal models remains understudied. The most abundant diterpene in rosemary is carnosic acid (CA) which accounts for 1.5-2.5% of the plant and has superior pharmacokinetics in mice. Yet, CA is not the only diterpene in rosemary, there are other diterpenes of interest like carnosol, the oxidized product of CA, rosmanol, and 12-O-methylcarnosic acid, a methylated product of CA Utilizing a luciferase reporter assay, other pure diterpenes and three hydrophobic fractions upregulated the nuclear factor erythroid 2-related factor 2 (Nrf2), key inflammation modulator in

the intestines. To assess the effects of rosemary diterpenes in the Nrf2 anti-inflammatory pathway, western blot, quantitative PCR, as well as an antioxidant assay were performed.

P-034 – Laxmi Sen Thakuri

Subcritical Water Extract of *Gracilaria chorda* Regulates Fat and Glucose Metabolism in Zebrafish Larvae Models

Laxmi Sen Thakuri[1,2], Ye Jin Jang[1,2], Hyung Jung Kim[3], Jin Woo Park[1,3] and Dong Young Rhyu[1,2]. [1]Department of Nutraceutical Resources, Mokpo National University, [2]Department of Biomedicine, Health & Life Convergence Sciences, BK21 FOUR, Mokpo National University, [3]Department of Pharmacy, Mokpo National University, Jeonnam 58554, Korea*

Insulin resistance (IR), known as impaired insulin sensitivity, is a hallmark of obesity and type 2 diabetes whose pharmacological medications have shown adverse effects. Thus, there is a dire need for alternative treatment. With an increasing therapeutic use of functional foods, particularly seaweed, a potential source of anti-obesity agents, this study aims to investigate the anti-obesity and antidiabetic activity of subcritical water extract of *Gracilaria chorda* at 210 °C (GCSW210) in high glucose (HG) and high fat (HFD)-induced IR zebrafish larvae models and to isolate the bioactive compound from GCSW210. In HG-induced larvae, GCSW210 increased glucose uptake and regulated the mRNA expression related to insulin production (insa). In HFD-induced larvae, it significantly recovered the glucose level, body weight and lipid accumulations as well as regulate fat metabolism and cytokine productions (PPARγ, C/EBPα, FAS, TNFα, IL6). Also, GCSW210 significantly increased glucose uptake and regulated glucose metabolism (irs1, akt, ampk) in both models. 5-Hdroxymethylfufural (5-HMF) and 2,5-bis(hydroxymethyl)furan were confirmed to be active substances isolated from GCSW210 through LC-PDA and LC-MS. These findings demonstrated that GCSW210 abbreviates lipid accumulation, pro-inflammatory cytokine productions, and improve glucose metabolism, which suggest use of GCSW210 as a potential treatment for IR and related metabolic disorders.

P-035 – Heather Winter

Antimicrobial Botanical Mechanisms of Action: A Hypericum Calycinum Case Study

Heather L. Winter[1], Colby M. Laws[1], Madeline N. Tillman[1], Nadja B. Cech[1]. [1] Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412

The goal of the studies described herein was to employ mass spectrometry metabolomics to reveal correlations between mechanism of action and chemical composition of botanical extracts. Antimicrobial activity of *Hypericum calycinum* L. (Hypericaceae) (creeping St. John's Wort) extracts was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA strain USA300 LAC AH1263). Six antibiotics were selected as positive controls based upon their clinical use in the treatment of MRSA infections. These antibiotics act via three different mechanisms of action: tetracycline and tigecycline (30S ribosomal subunit inhibition); moxifloxacin and levofloxacin (DNA gyrase inhibition); teicoplanin and vancomycin (cell wall disruption). The extract, its fractions, and the antibiotics all demonstrated antimicrobial activity against MRSA. Partial Least Squares (PLS) analysis using this antimicrobial data and the LC-MS metabolomics feature lists revealed clustering of the known antibiotics based on mechanism of action. The *H. calycinum* extract clustered with the 30S ribosomal subunit inhibitors, but purified hyperforin (a component of the extract) and one of the extract fractions clustered with the cell wall disruptors. These findings suggest that *H. calycinum* may exert activity by multiple mechanisms of action, a hypothesis that could be validated with further studies.

P-036 – Nathan Ezzone

Isolation of Cytotoxic Phytochemicals from *Aralia Vietnamensis*

Nathan Ezzone[1], Peter J. Blanco Carcache[1,2], Tran Ngoc Ninh,[3] Ermias Mekuria Addo[1], Eric D. Salinas-Arellano[1], Djaja D. Soejarto,[4,5] A. Douglas Kinghorn[1]. [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States, [2]The Ohio State University Comprehensive Cancer Center, Columbus, Ohio 43210, United States, [3]Vietnam Academy of Science and Technology, Hanoi, Vietnam, [4]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, [5]Science and Education, Field Museum, Chicago, IL 60605

The genus *Aralia* (Araliaceae) is well known for possessing a diversity of biologically relevant phytochemicals with potential antitumor effects, particularly triterpenoid saponins. The predominant phytochemicals reported in high content in this genus are terpenes including dammarane-type terpenoids, diterpenoids, triterpenoids, and sterols. Other known bioactive constituents are organic acids and their esters, flavonoids, polyacetylenes, and phenylpropanoids, all of which contribute to their historical medicinal use in the treatment of multiple diseases including hepatitis, rheumatoid arthritis, bruises, lumps and carbuncles. Previously, feature-based molecular networking has allowed for the dereplication of our extract to focus our isolation efforts toward unknown bioactive compounds. Thus, presented here are the current efforts to isolate constituents from the chloroform extract of *Aralia vietnamensis* using a bioassay-guided fractionation approach using reversed-phase column chromatography as well as high performance column chromatography (HPLC). Structure elucidation of the isolated compounds is completed by using 1D- and 2D- nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) techniques.

P-037 – Nathan Ezzone

Identification and Isolation of Potential Anticancer Agents from a *Penicillium* sp.

Nathan Ezzone[1], Ines I. Castro Dionicio[1], Eric D. Salinas-Arellano[1], A. Douglas Kinghorn[1], Esperanza J. Carcache de Blanco[1]. [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States

Penicillium species are a well-known and common fungi, occurring in a diversity of habitats worldwide. In a screen of various natural product extracts obtained from the NCI Natural Product Repository, we identified an extract of *Penicillium* sp. (F225059) as a potent cytotoxic agent against the HPAC pancreatic adenocarcinoma epithelial cell line (60.45% ± 1.06 inhibition at 10 mg/mL, IC_{50} = 1.72 mg/mL ± 0.009) and the MDA-T32 papillary thyroid carcinoma cell line (>50% inhibition at 20 mg/mL) in the Sulforhodamine B colorimetric assay. Meroterpenoids, polyketides, anthraquinones, oxygenated azaphilones, among others exemplify the anticancer compounds produced by *Penicillium* species suggesting their capability of producing structurally diverse and novel chemical structures that could be applied to cancer treatment. Therefore, our efforts focus on the identification of potential cytotoxic secondary metabolites from the active *Penicillium* species by metabolic study of the crude extracts. Multiple growth conditions were employed differing only in their carbohydrate source to explore the influence that different starch sources have on the abundance and chemical diversity of the secondary metabolites produced. LC-ESI-MS/MS was completed using the Thermo Q-Exactive Orbitrap with Vanquish-H UHPLC followed by feature-based molecular networking analysis hyphenated with *in silico* tools such as Global Natural Product Social Molecular Networking (GNPS) database and MZmine 3 for compound identification.

P-038 – Wen-Chi Wei

The Blockade of Mitogen-Activated Protein Kinase 14 Activation by Marine Natural Product Crassolide Triggers ICD in Tumor Cells and Stimulates Anti-Tumor Immunity

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Immunogenic cell death (ICD) refers to a type of cell death that stimulates immune responses. It is characterized by the surface exposure of damage-associated molecular patterns (DAMPs), which can facilitate the uptake of antigens by dendritic cells (DCs) and stimulate DC activation, resulting in T cell immunity. The activation of immune responses through ICD has been proposed as a promising approach for cancer immunotherapy. In this study, we investigated the effects of crassolide, a cembranolide isolated from the Formosan soft coral Lobophytum michaelae, on the induction of ICD, the expression of immune checkpoint molecules and cell adhesion molecules, as well as tumor growth in a murine 4T1 mammary carcinoma model. The results showed that crassolide significantly increased ICD and slightly decreased the expression level of CD24 on the surface of murine mammary carcinoma cells. An orthotopic tumor engraftment of 4T1 carcinoma cells indicated that crassolide-treated tumor cell lysates stimulate anti-tumor immunity against tumor growth. Crassolide was also found to be a blocker of mitogen activated protein kinase 14 activation. This study highlights the immunotherapeutic effects of crassolide on the activation of anticancer immune responses and suggests the potential clinical use of crassolide as a novel treatment for breast cancer.

P-039 – Chia-Hung Yen

Indigofera Suffruticosa and Its Major Compound Tryptanthrin Induce G2/M Arrest Through ATR/CHK1 Pathway in Jurkat Cells

Hong-Loan Tran[1], Kuei-Hung Lai[2], Hsun-Shuo Chang[3], Yi-Siao Chen[4], Hui-Chun Wang[1], Shuen-Shin Yang[3], Hsueh-Wei Chang[5], Chin-Mu Hsu[6], Hui-Hua Hsiao[6], and Chia-Hung Yen[1]. [1]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; [2]PhD Program in Clinical Drug Development of Herbal Medicine, College of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan; [3]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; [4]Ph.D. Program in Environmental and Occupational Medicine, College of Medicine, Kaohsiung Medical Uni-versity and National Health Research Institutes, Kaohsiung 80708, Taiwan; [5]Department of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; [6]Division of Hematology and Oncology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung 80708, Taiwan*

Indigofera suffruticosa Mill. is a folk medicine for treating leukemia, however the molecular mechanism and the active component remain unclear. Here we showed that ISAE triggered G2/M arrest in Jurkat cells in dose- and time-dependent manners. ISAE increased the inhibitory phosphorylation of CDK1 at Y15 by activating ATR/CHK1/Wee1/CDC25C pathway. Caffeine, an ATR inhibitor, reversed the effect of ISAE in Jurkat cells. Moreover, increased phospho-H2A.X stained cells indicated the involvement of DNA damage in the anti-leukemic effect of ISAE. Finally, qualitative analysis using UPLC-tandem mass spectroscopy and molecular networking revealed that tryptanthrin is the major metabolite in ISAE. At equivalent concentrations to ISAE, tryptanthrin induced G2/M arrest of Jurkat cells. Our findings provide evidence for the traditional use of *I. suffruitcosa* in leukemia treatment.

P-040 – Mohammed Ahmed

Isolation of Metallophore-Producing Bacteria from a Heavy Metal-Toxified Site

Mohammed M. A. Ahmed[1, 2], Cameron M. Hammers[1], Paul D. Boudreau[1]. [1]Department of BioMolecular Sciences, University of Mississippi, University, MS, USA, 38677, [2]Department of Pharmacognosy, Al- Azhar University, Cairo, Egypt, 11371

Heavy metal pollution is a major environmental concern. Due to the health risks, even at very low heavy metal concentrations, there is a need for remediation tools for these hazardous elements. Traditionally, the cost of this remediation is high, making use of biological strategies, such as bacterial bioremediation, may be a cost-effective alternative. Research groups around the world have engaged in efforts to isolate metal-tolerant bacteria (MTB) from Nature. These bacterial taxa have evolved numerous pathways to resist metal toxicity, including metal efflux pumps, redox-active metabolites or proteins, and small molecule metal chelators (metallophores). This project aims to isolate MTB from metal-toxified sites capable of producing metallophores to be used in different bioremediation scenarios. Our workflow begins with soil samples collected from metal-toxified mining sites in Montana. These samples are then plated on metal-supplemented media, from which colonies were picked onto chrome azurol S (CAS) plates. Copper and cerium were added as a primary selection step, to test the metallophores production, the CAS dye reveals excretion of metal-binding compounds. Of 51 colonies picked, 17 showed a positive result with the CAS assay. 16S rRNA sequencing of those hits suggested 8 distinct species of *Rhodanobacter*, *Dyella*, *Bradyrhizobium*, *Luteibacter*, *Cupriavidus*, *Arthrobacter*, and two separate *Paraburkholderia*. To study the biosynthetic capacity of the isolates, we nanopore sequenced these strains, antiSMASH analysis of these data revealed diverse biosynthetic gene clusters. Molecular networking using GNPS of the culture supernatants from these strains also showed distinct clusters of unknown metabolites that may be novel metallophore compounds.

P-041 – Margaret Banks

Ecologically Important Alkaloids from the Millipede *Andrognathus corticarius*

Paige Banks, Carla Menegatti and Emily Mevers. Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA

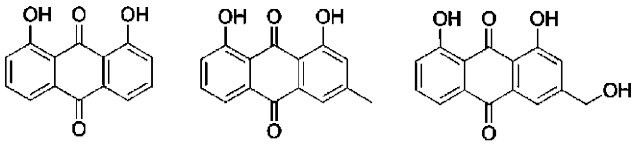
Millipedes can produce a diverse array of small molecules, used for defense, that are stored in specialized glands known as ozopores. Many of the small molecules, including alkaloids, produced by millipedes appear to cause disorientation in natural predators. *Andrognathus corticarius*, a social millipede found across the southeastern United States, has never been chemically investigated by GCMS analysis revealed that *A. corticarius* can produce many new alkaloids. Extraction and chemical evaluation allowed us to identify 21 new alkaloids that are chemically distinct from other known alkaloids, such as buzonamine, deoxybuzonamine and ischnocybines. Full 2D NMR datasets (^{1}H, ^{13}C, COSY, HSQC, H2BC, and HMBC) were acquired on three of the isolated metabolites and their planar structures elucidated. Tandem MS analysis suggest that most of these new metabolites contain the same bicyclic core but vary in the appended short chain fatty acids. *A. corticarius* alkaloids are strikingly different from all other millipede alkaloids in the sheer number of structural analogs found within individual millipedes. Hypothesizing that this chemical diversity has an ecological impact, we collected *A. corticarius* from different geographical regions across the Appalachia region and analyzed the alkaloid content of individual millipedes using LCMS in order to identify trends in alkaloid abundance across geographical locations. In addition, these new millipede alkaloids provide insights into a potential biosynthetic scheme that would lead to each of the different carbon backbones. Isotope labelling studies are being carried out to confirm this hypothesis and to identify key biosynthetic intermediates.

P-042 – Stephen Deyrup

Chemical Defenses of Adult Viburnum Leaf Beetles (*Pyrrhalta viburni*)

Isaiah I. Korostil, Alec P. Brundle, Jessica L. Pepe, Alexandra N. Robeson, Grace E. Zuchowski, and Stephen T. Deyrup, Department of Chemistry and Biochemistry, Siena College, Loudonville, NY 12211

Beetles are the most diverse group of macroorganisms on Earth. Many are known to produce or sequester chemicals to protect themselves from predators. The viburnum leaf beetle, *Pyhrralta viburni*, is native to Eurasia, but has become an invasive exotic pest in North America. The larval and egg stages of this beetle have been previously studied and shown to contain three anthraquinone compounds, chrysazin, chrysophanol, and dithranol, however, chemistry of the adult beetles has not been examined previously. Herein, we show that adult beetles also contain three anthraquinone compounds, chrysazin (**1**), chrysophanol (**2**), and, surprisingly, aloe-emodin (**3**) by using 1D and 2D NMR spectroscopic techniques supported by UPLC-MS data.



1 **2** **3**

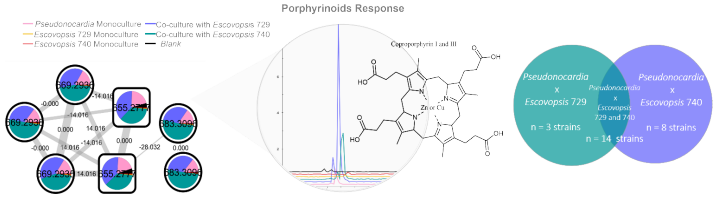
P-043 – Carlismari Grundmann

Porphyrinoids Production as a Response to Pathogen Infection in a Multipartite Symbiosis

Carlismari O. Grundmann[1], Ivan L. F. Migliorini[1], Weilan G. P. Melo[1], Pieter C. Dorrestein[2], Norberto P. Lopes[1] and Mônica T. Pupo[1]. [1]School of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo (FCFRP-USP). [2]Skaggs School of Pharmacy and Pharmaceutical Sciences - University of California San Diego (UCSD)

We have been developing a systematic study of the metabolic profiles from the interaction between symbiotic Pseudonocardia (36 strains) and the specialized parasite Escovopsis (2 strains),

isolated from attine ants. The analysis of the co-cultures (LC-MS/MS and Featured Based Molecular Networking) revealed the elicitation of bacterial metabolism in the presence of the pathogenic fungus, as evidenced by an increase in pophyrinoids production. This bacterial response appears to occur more robustly in co- cultures involving Pseudonocardia than Streptomyces strains from the same environment. The results also suggest that this bacterial elicitation is specific to the presence of the pathogenic fungi Escovopsis, as a form of competition for metals such as zinc and copper. Furthermore, complexes of these metals with the porphyrinoids impair the dispersion of the fungal pathogen, possibly assigning an ecological relevance to the ant colony microbiome.



P-044 – Jungmoo Huh

Identification and Biological Activity of a Lasso peptide from a *Pseudonocardia* sp. Associated with the *Trachymyrmex Septentrionalis* Fungus-Growing Ant

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Lasso peptides are an intriguing class of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products produced by microorganisms. This class of peptides has a unique lasso shaped topology consisting of a macrolactam ring with seven to nine amino acid residues. Various biological activities of lasso peptides have been reported, including antibacterial and antiviral activity as well as the ability to enhance the activity of other antifungal compounds. During the course of our research, we examined the clonality of *Pseudonocardia* strains obtained from a single colony of the *Trachymyrmex septentionalis* fungus-growing ants. Analysis of the biosynthetic gene clusters (BGCs) of several strains revealed their similarities with the exception of differences in a lasso peptide and a thiopeptide BGC in several strains. Additionally, strains with differing BGCs also expressed differing levels of bioactivity against the fungal pathogen, *Trichoderma* sp. Comparative metabolomics was used to identify a new lasso peptide from *Pseudonocardia* sp. JKS2562. Large-scale cultures were extracted to allow for isolation and identification of this lasso peptide, confirming its planar structure using 1D- and 2D-NMR and LC-MS/MS analyses. Experiments to characterize the absolute configuration of the isolated compound are ongoing.

P-045 – Mario Augustinovic

Chromatographic Method Development from Bioassays? Do it in a Flash!

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The chromatographic separation of complex mixtures of natural products requires meticulous adjustment of several instrument/system parameters, which to a non-expert presents a barrier to effective method development. As a result, it can be challenging to create efficient upstream chromatography methods that target a biologically active compound. This poster describes an automated process of calculating focused gradients to rapidly purify active compounds from extracts based on scouting gradients and bioassay data. Based on an initial scouting run, the Focused Gradient Generation within the Teledyne ISCO Peaktrak software generates a method that isolates only a selected analyte of interest, and considers parameters such as column delay volume, instrument dwell volume, mode and volume of injection, and effective retention time of a target analyte. An additional run is then performed to validate the method and purify the analyte of interest. This work demonstrates the utility of a focused gradient platform that can optimize purification of NPs based on bioactivity earlier on in the chromatographic process. The platform allows for a reduction in purification steps and thus in overall time needed to obtain a bioactive compound of interest. Importantly this function makes chromatographic method development more accessible to novice users.

P-046 – Yong Beom Cho

Molecular Networking-Guided Isolation of Diarylheptanoids from the Rhizomes of *Alpinia officinarum*

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The rhizomes of *Alpinia officinarum* are reported to be rich sources of a variety of diarylheptanoids, which exhibit various biological activities, such as anti-inflammatory, antioxidant, antibacterial,

and anticancer activities. In the course of research program for the isolation of bioactive constituents from the medicinal plant, the rhizomes of A. officinarum were extracted with MeOH, and sequentially partitioned with n-hexane, CH_2Cl_2, EtOAc, and n-BuOH. UPLC-MS/MS data of the four solvent-soluble fractions were analyzed by molecular networking at the GNPS web platform, and the cluster of unusual diarylheptanoids was revealed by the investigation of molecular network. These cluster was selected for the isolation, and as a result, a novel dimeric diarylheptanoid **1**, and two known terpene adducted diarylheptanoids **2** and **3** were isolated along with nine known monomeric diarylheptanoids **4-12** from the n-hexane fraction of the rhizomes of A. officinarum by various chromatographic methods. The chemical structures of isolated compounds were elucidated by interpretation of 1D-NMR, 2D-NMR and HR-ESI-MS data.

P-047 – William Crandall

Customizable Large-Scale HPLC Fraction Collection Using Low-Cost 3D Printing

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High-pressure liquid chromatography (HPLC) has been used for decades for the isolation of various molecules. In particular, small molecule isolation from natural sources (natural products) has been an area in which HPLC has been used extensively. Often, yields of novel compounds are extremely low from the source material. The acquisition of sufficient quantities for structural characterization relies on an adequate amount of starting material and iterative rounds of isolation. This reliance on preparative HPLC presents the issue of suitable equipment to perform large-scale isolation. Current machines are not adequate for specific use cases or come with a large cost barrier making them inaccessible to many researchers. Here we present a low-cost, efficient approach for building a large-scale HPLC fraction collector to isolate natural products. Using a hobbyist-grade three-dimensional (3D) printer (Creality Ender 3 Pro) and aluminum extrusions, the fraction collector can be built at a low cost. The motors and additional units to control them can then be salvaged from the 3D printer to be used to complete the build. An additional graphical user interface (GUI) allows for simple programming of the collection methods. This platform will increase the reproducibility of fraction collection methods, with the scale to run iteratively, while removing the cost barrier and allowing for a high degree of customizability.

P-048 – Hyeon Jeong Jeong

A Combination Extract of *Codonopsis pilosula* and *Trametes versicolor* Enhances Autophagy Activation Effects on Human Skin Cells

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Autophagy is a conserved self-digestion process associated with a wide variety of physiological roles, including cellular homeostasis, development, and differentiation. Canonical autophagy includes the formation of autophagosomes that enclose unnecessary or dysfunctional intracellular components and subsequently fuse with the lysosome, resulting in lysosomal-mediated break-down and digestions. Autophagy activation is also tightly linked in improving skin integrity and skin aging at the levels of the cells and tissue, therefore, autophagic control has become an emerging target in the development of anti-aging materials. In the present study, we examined the combination extracts of *Codonopsis pilosula* together with *Trametes versicolor* on the autophagy activation effect by the detection of LC3 that is a standard marker of autophagosomes. The extracts were treated on the two different human skin cell lines, and monodansylcadaverine and LC3 immunofluorescence staining have been conducted. We found that the combination extract of *C. pilosula* and *T. versicolor* significantly stimulated the expression level of LC3 and ameliorated cytotoxicity on the human skin cell lines relative to the single extract of *C. pilosula* or *T. versicolor*. The complex constituents of the combination extract of *C. pilosula* or *T. versicolor* have been analyzed by HPLC-MS, and active compounds have been identified.

P-049 – Shugeng Cao

Chemodiversity, Biosynthesis, and Bioactivity of Hawaiian Fungal Metabolites

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Investigation of fungi collected from unique environments in Hawaii has led to the discovery of new natural products with diverse structures. Besides polyketides, terpenoids, shikimic acid derived compounds, and non-ribosomal peptides, some secondary metabolites are hybrids that are biosynthesized from two or more enzymes. These Hawaiian fungal metabolites showed different biological properties, including antibacterial, anti-inflammatory, anti-proliferative, antithrombotic activities, and interestingly Sirtuin activation.

P-050 – Ines Castro-Dionicio

LC-MS/MS-based Dereplication of Cytotoxic Compounds in Two Extracts from the NCI Natural Products Repository

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The NCI Natural Products Repository contains an extensive collection of plant extracts that hold great potential for discovering novel anticancer drug candidates. To optimize the early stages of natural products research, annotation tools applied to LC-MS data are essential to increase efficiency and accelerate the discovery process. In this study, we examined a total of 352 plant extracts and 176 microorganism extracts obtained from the NCI Natural Products Repository (NPR) for their cytotoxic potential against bladder (HT-1376) and leukemia (BDCM) cancer cell lines using the SRB assay. Significant percentage of inhibition was observed for the extracts from *Parthenium incanum* Kunth (Asteraceae) (<50% for HT-1376, 66.33±2.0 for BDCM at 10 μg/mL) and *Thymophylla acerosa* (DC.) Strother (Asteraceae) (>50% for BDCM at 20 μg/mL). Both the active extract and following column chromatography active fractions have been analyzed using a UHPLC-ESI-Q Exactive system. The ESI-MS/MS data is being analyzed using various annotation tools (e.g., GNPS, Sirius, Compound Discoverer). Active fractions with the potential of containing novel cytotoxic compounds have been prioritized for structural characterization.

P-051 – Isabel Chauvin

Genetics-Guided Dereplication of Cryptophycin Analogues and Bioactive Peptides in Cyanobacteria

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Cyanobacteria are gram-negative photosynthetic autotrophs shown to be prolific producers of a variety of therapeutically relevant secondary metabolites and complex chemical scaffolds. Some genera of cyanobacteria such as *Nostoc* are capable of nitrogen fixation and produce diverse compound classes including non-ribosomal peptides (NRPS) and hybrid NRPS-polyketides (PKS). A genetics-guided approach to dereplication using DNA extraction, sequencing, assembly, and submission to antiSMASH identified the biosynthetic gene cluster of cryptophycin as well as other NRPS-PKS within *Nostoc* sp. UIC10890's genome. Cryptophycin analogues and other nitrogen-containing peptides were then found through ^{15}N stable isotope labeling and comparative metabolomics using tandem mass spectrometry (MS/MS). The structure of cryptophycin was confirmed through nuclear magnetic resonance (NMR) spectroscopy. The dereplication of cryptophycin and other peptides will be presented. The production of cryptophycin has previously been published in only two other *Nostoc* sp. strains. This work demonstrates how the use of a genetics-guided approach to dereplication alongside a traditional bioassay-guided approach expedites dereplication and potential discovery of novel compounds.

P-052 – Tamam El-Elimat

A 8,8'-Binaphthopyranone and Xylarenones with Cytotoxic Activity from A Freshwater Fungus (G708)

Tamam El-Elimat[1,2], T'ea Cameron[1], Perihan Gürbüz[1], Huzefa A. Raja[1], Manead Khin[2], Joanna E. Burdette[2] and Nicholas H Oberlies[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, [2]Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan, [3]Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Bioactivity-guided fractionation of an organic extract of a fungus isolated from submerged wood collected from fresh water (strain G708) led to the isolation of two known sesquiterpenes [xylarenones C (1) and D (2)] and one new polyketide [mycopyranone B (3)]. The structures were elucidated using a combination of spectroscopic and spectrometric techniques. The structure of 1 was further confirmed using X-ray crystallography, while the absolute configuration was established via a modified Mosher's ester method. The isolated compounds were tested against the OVCAR3 (ovarian) and MDA-MB-435 (melanoma) cancer cell lines. Compound 3 was the most potent, with IC_{50} values of 5.4 and 2.1 μM, respectively.

P-053 – Tamam El-Elimat

Dihydroquinoline-2-ones, Diketopiperazines, Cyclic Tetra and Pentapeptides, and a Polyketide from an *Aspergillus* sp. (MSX25804): Isolation and Antimalarial Activities

Tamam El-Elimat[1,2], Kristof B. Cank[1], Huzefa A. Raja[1], Steven P. Maher[3], Adriana A. Marin[3], Anthony A. Ruberto[3], Sarah Lee Mai[3], Blaise A. Darveaux[4], Dennis E. Kyle[3], Cedric J. Pearce[4], Nicholas H. Oberlies[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, [2]Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan, [3]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA 30602, [4]Mycosynthetix, Inc., Hillsborough, NC 27278

Antimalarial-guided fractionation of an organic extract of an *Aspergillus* sp. (strain MSX25804), led to the isolation of seven dihydroquinoline-2-one alkaloids (1-7), six diketopiperazine alkaloids (8-13), two cyclic tetrapeptide (14-15), a cyclic pentapeptide (16), and a polyketide (17). Compounds 7, 13, and 15 were new. The structures were elucidated using a combination of spectroscopic and spectrometric techniques. Compounds 1-17 were tested for antimalarial activity *in vitro*, where aflaquinolone A (1)

displayed a pEC_{50} value of 4.91 against malaria-causing *Plasmodium falciparum*. When evaluated for cytotoxicity, it was found to be inactive (IC_{50} >10.0 μM).

P-054 – Mary Ann Foglio

Cytoprotective Effect of *Fridericia Chica* (Bonpl.) L.G.Lohmann Extract Associated with Geranylgeraniol Enriched-Fraction on Epithelial Cells Treated with Bisphosphonate

Rogerio Jose Machado Junior[1], Julia C. Camilli[2], Rosanna Tarkany Basting[1], Patrıcia Maria Wiziacki Zago[2], Ilza Maria de Oliveira Sousa[1,2], Kaio Eduardo Buglio[2], Ana Lucia T.G Ruiz[2], Paulo Roberto Nogueira Carvalho[3], João Ernesto de Carvalho[2], Sara T. O. Saad[1] and Mary Ann Foglio[1,2], [1]Faculty of Medical Sciences, State University of Campinas, Sao Paulo, Brazil, [2]Faculty of Pharmaceutical Sciences, State University of Campinas, Sao Paulo, Brazil, [3] Institute of Food Technology, Campinas, Sao Paulo, Brazil

The chronic use of bisphosphonates is associated with the occurrence of medication-related osteonecrosis of the jaw (MRONJ). Previous data reported the positive effects of Geranylgeraniol on different cell types treated with Bisphosphonates. Foregoing work done by our research group demonstrated the wound healing capacity of Fridericia chica(Bonpl.) L.G.Lohmann standardized ethanol extract. Herein in vitro cytoprotective synergistic effect of the association of F.chica extract associated with an enriched geranylgeraniol fraction on keratinocytes exposed to zoledronic acid is reported. An association of F. chica at 1 and 5mg/mL with geranylgeraniol at 15mg/mL, increased cell viability by 73.5% and 71.1%, respectively. This treatment did not increase tumor cells viability, whereas the clonogenic potential assessment showed that, the association with F. chica(5mg/mL) reversed the effects of zoledronic acid on the cells. This study provides data for a potential treatment for MRONJ.

P-055 – Kirstie Tandberg Francis

Establishing a Marine Microbial Natural Product Drug Discovery Program at Mote Marine Lab

Kirstie Tandberg Francis, Mote Marine Laboratory, Sarasota, FL USA

Marine ecosystems are some of the most biologically diverse on planet Earth. This biodiversity translates to incredible chemical diversity. The intense competition for resources across marine ecosystems like coral reefs and deep-sea communities promotes the evolution of secondary metabolites, or marine natural products. Today, natural products and their derivatives make up 53% of all approved small-molecule drugs and 65% of approved small-molecule cancer drugs (Newman and Cragg 2020). Mote Marine Laboratory, a non-profit research institution founded in 1955, has a rich history of marine biomedical research. Discoveries from Mote scientists include antimicrobial activities in coral mucus-associated bacteria, antimicrobial activities in skate and ray epidermal mucus-associated bacteria, cytotoxic activity observed from bonnet head shark epigonal tissue against human cancer cell lines, and more. The new molecular microbiology program at Mote aims to expand the capacity of marine biomedical discovery by developing a marine microbial natural product drug discovery research program. This poster will describe the specific aims of the program: 1) maintain and expand a diverse microbial isolate library, 2) create a pre-fractionated library of microbial extracts, 3) screen for various biological activity, and 4) identify compounds through bioassay guided fractionation and structure elucidation. We plan to leverage Mote's history of marine biomedical success and unparalleled access to sampling and facilities to identify novel active compounds with unlimited potential in many therapeutic applications.

P-056 – Vitor Freire

National Cancer Institute Program for Natural Product Discovery: Combining FTIR and High Throughput Screening to Prioritize Chemistry Efforts on Bioactive Fractions

Vitor F. Freire[1], Masoumeh Dalilian[2], Shaurya Chanana[1], Lucero Martinez-Fructuoso[1], Rohitesh Kumar[3], Susan Ensel[3,4], Rhone K. Akee[3], Christopher C. Thornburg[3], Jason R. Evans[1], Barry R. O'Keefe[1,2], and Tanja Grkovic[1,2]. [1]Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD 21702, [2]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, [3]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. [4]Department of Chemistry and Physics, Hood College, Frederick, Maryland 21701

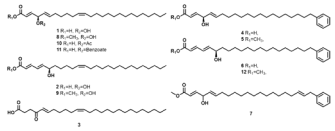
The National Cancer Institute Program for Natural Product Discovery (NPNPD) is an NCI initiative that aims to integrate natural products samples into high-throughput screening (HTS). The identification of chemotypes in mixtures following HTS is crucial for sample prioritization. To address this challenge, the development of strategies to use big datasets of NMR, LC-MS/MS and FTIR data are essential. For this goal, FTIR is an analytical technique with the advantage of being a non-destructive, solid-base procedure that requires only micrograms of sample and has well-established silicone-based 96- and 384- well-plates that enable automated sample preparation and rapid data acquisition. In this presentation, we explore strategies to use the FTIR data to perform sample prioritization, dereplication, and target isolation of natural products from the NPNPD prefractionated library.

P-057 – Ji-Yeon Hwang

Antiproliferative Fatty Acids with the Rare 1,4-Pentadien-3-ol Scaffold

Ji-Yeon Hwang[1], Masoumeh Dalilian[1,2], Lindsay Marron[1], Ekaterina I. Goncharova[1,2], Girma M. Woldemichael[1,2], Barry R. O'Keefe[1,3], Tanja Grkovic[1,3], and Lin Du[1,] [1]Molecular Targets Program, CCR, NCI, Frederick, MD; [2]Leidos Biomedical Res., FNLCR, Frederick, MD; [3]Natural Products Branch, DTP, DCTD, NCI, Frederick, MD

Deletion of the topoisomerase-3B (TOP3B) gene has been linked to various cancer types. The paired HCT116 cell lines with (TOP3B-WT) and without TOP3B (TOP3B-KO) were developed for discovering TOP3B-specific proliferation modulators. Seven new fatty acid derivatives (**1**–**7**), including four compounds (**1**, **4**, **5**, and **7**) with the rare 1,4-pentadien-3-ol scaffold, were isolated from the extracts of two *Lauraceae* species through bioassay-guided fractionation. The absolute configuration of the hydroxylated compounds (**1**, **2**, and **4**-**7**) was determined by acid methylation (**8**, **9**, and **12**) followed by the modified Mosher's method. Compound **1** showed potent antiproliferative activity against both the TOP3B-KO (IC_{50}KO, 0.58 μM) and TOP3B-WT (IC_{50}WT, 2.6 μM) cell lines with the selectivity index (SI) of 4.5 (IC_{50}WT/IC_{50}KO). Preliminary SAR studies via chemical modifications (i.e. methylation, esterification, and oxidation) indicated the intact 1,4-pentadien-3-ol scaffold was important for the antiproliferative activities.

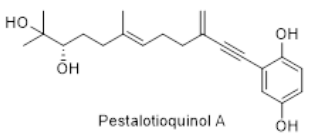


P-058 – Shinji Kamisuki

Pestalotioquinol A, A Novel Fungal Metabolite, Specifically Protects Cells Against Reactive Nitrogen Species and Ameliorates Inflammatory Bowel Disease

Yukine Tsurukawa1, Kazuki Kanno1, Yumi Katayama1, Naoyuki Aihara1, Hironobu Murakami1,2, Shiro Takeda1,2, Masaki Nagane1,2, Motoharu Sakaue1,2 and Shinji Kamisuki1,2. 1School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa 252-5201, Japan, 2 Center for Human and Animal Symbiosis Science, Azabu University, Sagamihara, Kanagawa 252-5201, Japan

We isolated a novel hydroquinone with a vinyl alkyne as a cytoprotective compound from the fungus, *Pestalotiopsis microspora* and named pestalotioquinol A (pesA). Herein, we revealed that pesA specifically protects cells against reactive nitrogen species such as peroxynitrite, but not against reactive oxygen species. The compound was also found to suppress lipopolysaccharide-induced production of interleukin-6, a proinflammatory cytokine, in RAW264.7 macrophages. Furthermore, we demonstrated that pesA ameliorated inflammatory bowel disease in mouse model.



P-059 – Sul Nur Karavus

Cytotoxic Daucane Esters from the Dichloromethane Extract of the Roots of Endemic *Ferula pisidica* Akalın & Miski (Apiaceae)

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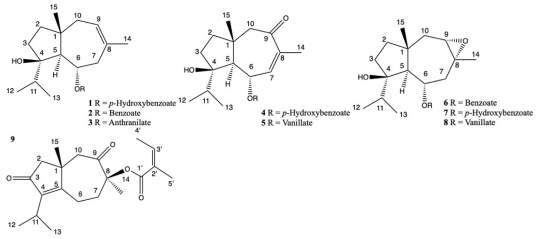
Ferula pisidica Akalın & Miski is a novel endemic species discovered in the vicinity of the Karaman province of Türkiye. As a part of ongoing studies to discover anticancer natural products, we isolated nine sesquiterpene esters, ferutinin (**1**), jaeschkeanadiol benzoate (**2**), elaeochytrin A (**3**), epoxyjaeschkeanadiol benzoate (**4**), epoxyjaeschkeanadiol *p*-hydroxybenzoate (**5**), epoxyjaeschkeanadiol vanillate (**6**), lancerodiol *p*-hydroxybenzoate (**7**), lancerodiol vanillate (**8**), webiol angelate (**9**) from the dichloromethane extract of the roots of *F. pisidica*. The structures of all isolated compounds were determined by spectroscopic methods including 1D and 2D NMR experiments, and HRMS. Extracts and isolated compounds were investigated *in vitro* for their cytotoxic activities against three human cancer cell lines (COLO 205, K-562, and MCF-7).



P-060 – Victoria (Tori) Klein

Pyrrole-Imidazole Alkaloids: Elucidating their Antibiotic Adjuvant Mode of Action

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Incidences of bacterial resistance have been recorded for every antibiotic in clinical use. Thus, it is critical to develop not only novel antibiotics, but also novel compounds to combat antibiotic resistance. Marine-derived, pyrrole-imidazole alkaloids can achieve both goals as they show broad-spectrum antibacterial activity and act as antibiotic adjuvants capable of restoring antibacterial activity of FDA-approved drugs in resistant pathogens. However, these alkaloids are underutilized because their antibacterial and adjuvant

mechanisms of action are unknown. To overcome this critical barrier, we have successfully isolated several known and novel pyrrole-imidazole alkaloids from extracts of three marine sponge *Agelas* species. Using these alkaloids, this study offers a robust combination of classic methods for analyzing bacterial growth and morphology with modern chemical genomics, fluorescence microscopy, and protein binding to gain a high-content, mechanistic understanding of these compounds and determine their cellular target(s) in a model organism, *E. coli*. Our chemical genomic screening demonstrates that a model compound, sceptrin, affects *E. coli* by damaging or modifying the bacterial cell wall biosynthesis. Supporting this hypothesis, we determined that sceptrin is bactericidal rather than bacteriostatic, which is common for antibiotics that affect cell wall biosynthesis as the antibiotic-induced structural changes to often lead to cellular pressure imbalances, triggering cell lysis and death. Additionally, transmitted light microscopy showed that sceptrin-treated *E. coli* form elongated, partially segmented cells, which is common to antibiotics that damage the bacterial cell wall or its biosynthesis. Florescence microscopy assays using a combination of membrane permeable and impermeable dyes demonstrated that sceptrin does not affect bacterial membrane permeability, a phenotype commonly coupled with cell wall inhibitors. Follow-up studies on sceptrin and the remaining alkaloids using cell wall-based fluorescence microscopy, high-content UPLC analysis of the cell wall precursors and components, and ITC-measured protein binding will be used to identify which cell wall biosynthesis protein is the target of this antibiotic class. This project will dramatically advance our ability to combat the antibiotic-resistance crisis by providing a pathway to generate a new class of urgently needed antibiotic adjuvants derived from these pyrrole-imidazole alkaloids.

P-061 – Maciej Korczak

Urolithin a Conjugates with Non-Steroidal Anti-Inflammatory Drugs: Synthesis, Impact on Phase II Metabolism and Caco-2 Monolayer Integrity

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Urolithin A (UA) is a bioactive postbiotic metabolite produced by gut bacteria following the consumption of ellagitannin-rich plants. Despite promising results of *in vitro* studies, the extensive phase II metabolism severely reduces its biopotency *in vivo*. To overcome this limitation, structural derivatives of UA were synthesized by conjugating it with ibuprofen, mefenamic acid, diclofenac, or acetylsalicylic acid. Subsequently, the bioavailability and impact of UA derivatives (UADs) on the glucuronidation rate were assessed using Caco-2 cell monolayer model. Although tested UADs did not penetrate cell monolayers, apical administration of all of them resulted in a significantly higher ratio of free UA to its metabolites on the basolateral side compared to treating Caco-2 cells with UA. Additionally, UADs improved intestinal barrier integrity *in vitro*, as demonstrated by increased transepithelial electrical resistance and elevated tight-junction proteins expression. Finally, next-generation sequencing revealed significant changes after the administration of UADs in Caco-2 cells. Project financially supported by Polish National Science Centre research grant Preludium Bis No. UMO-2019/35/O/NZ7/00619.

P-062 – Erin Marshall

New Drug Leads to Modulate Pain from Arctic Ocean Bacteria

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Chronic pain is a debilitating and widespread disease, affecting approximately 20% of adults. Current therapeutic approaches are effective but have severe side effects, including dependence, addiction, nausea, and constipation. Microbial secondary metabolites possess a wide range of biological activities and are a highly promising source for novel drug leads. Here we present a novel zebrafish assay for the identification of natural products with anti-nociceptive (pain relieving) activity and the results of our screen of metabolites from a collection of Arctic Ocean bacteria. Extracts from our collection of 85 Arctic Ocean bacteria were tested for *in vivo* pain-relieving activity using a zebrafish-based behavioral assay. This automated and medium-throughput assay allows 'behavior-guided' discovery of metabolites with analgesic activity that act on known or potentially new molecular targets. Next, *in vivo* zebrafish whole-brain calcium imaging experiments were used to pinpoint targeted neuron populations to elucidate mode of action. Assay hits are then tested for receptor specificity *in vitro* using cell lines expressing ion channels (hTRPA1) or GPCRs (opioid receptors) and submitted to the NIMH's Psychoactive Drug Screening Program (PDSP) for receptor binding and functional studies.

P-063 – Lucero Martínez-Fructuoso

A Screen for New Antimicrobial Natural Products from the NCI Program for Natural Product Discovery Prefractionated Extract Library

Lucero Martínez-Fructuoso[1], S. J. Ryan Arends[2], Vitor F. Freire[1], Jason R. Evans[1], Sean DeVries[2], Brian D. Peyser[1], Rhone K. Akee[3], Christopher C. Thornburg[3], Rohitesh Kumar[3], Susan Ensel[3,4], Gina M. Morgan[2], Grant D. McConachie[3], Nathan Veeder[2], Leonard R. Duncan[2], Tanja Grkovic[1,5], and Barry R. O'Keefe[1,5]. [1]Natural Products Branch, Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD, USA, and [2]JMI Laboratories, North Liberty, IA, USA, and [3]Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA, and

[4]Department of Chemistry and Physics, Hood College, Frederick, MD, USA, and [5]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD, USA

The continuing emergence of antibiotic resistant microbes highlights the need for the identification of new chemotypes with antimicrobial activity. The National Institute of Allergy and Infectious Diseases and the National Cancer Institute here report a large screen of 326,656 partially purified natural product fractions against a panel of four microbial pathogens, resulting in the identification of >3,000 fractions with antifungal and/or antibacterial activity. A small sample of these active fractions was further purified and the chemical structures responsible for the antimicrobial activity were elucidated. The proof-of-concept study identified many chemotypes, several of which have not previously been reported to have antimicrobial activity and discovered new natural products. The results show that there remain many unidentified antibiotic compounds from nature.

P-064 – Olumayokun Olajide

Natural Product Suppressors of SARS-CoV-2 Cytokine Storm: *In Vitro* Evidence

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It has now been established that severe infections with the SARS-CoV-2 induce activation of pulmonary immune cells to produce large amounts of lung-damaging pro-inflammatory cytokines and chemokines, resulting in pulmonary hyper-inflammation (cytokine storm). These events contribute significantly to acute respiratory distress syndrome (ARDS) and pneumonia in COVID-19, caused by SARS-CoV-2 infection. Dexamethasone and other anti-inflammatory steroids have been reported to provide improved clinical outcomes in patients with severe cases of COVID-19. However, their use is limited by their ability to suppress the overall immune response. Using an in vitro model of SARS-CoV-2 spike protein-induced hyper-inflammation and lung damage, investigations have been conducted on natural products with the potential to suppress SARS-CoV-2 cytokine storm. Results from these studies suggest that garcinoic acid (from *Garcinia kola*), andrographolide (from *Andrographis paniculata*), skimmianine (from *Zanthoxylum zanthoxyloides*), and thymoquinone (from *Nigella sativa*) demonstrated significance activity in reducing spike protein-induced increased release of pro-inflammatory cytokines TNFα, IL-6, and IL-1β, as well as chemokines IL-8, MCP-1 and MCP-2. The activities of these compounds were compared to that of dexamethasone. Further investigations revealed that the ability of these compounds to target inflammatory signalling pathways involving NF-kB, the MAPKs and NLRP3 inflammasome might be responsible for their activities *in vitro*. Natural products could provide viable alternatives to corticosteroids as pharmacological modalities in reducing cytokine storm in severe SARS-CoV-2 infection.

P-065 – Herma Pierre

Verticillin D: Media Studies, Streamlined Purification, and Absolute Configuration

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Verticillins are epipolythiodioxopiperazine alkaloids that have been explored for their pharmacological potential for decades. Many verticillins have been evaluated for their cytotoxic and anticancer properties; however, verticillin D has remained largely unexplored since its discovery in 1999. To assess its potential further, a larger supply was needed for semisynthetic analogue development and additional pharmacological assessment. To this end, we assessed the production of verticillin D in nine fungal strains, on two different solid media, and across varying fermentation times using our streamlined verticillin extraction and purification process. These experiments showed that the production of verticillin D was the highest in fungal strain MSX51257 and was substantially affected by solid media type and fermentation time. These experiments facilitated the supplying of verticillin D for absolute configuration determination via X-ray crystallography.

P-066 – Enrique Aguilar-Ramírez

Harnessing the Reactivity of Duclauxin to Obtain *H*ptp1b1-400 Inhibitors

Enrique Aguilar-Ramírez,[1] José Rivera-Chávez,*[1] Carlos Fajardo,[1] Valeria Reyes-López,[1] Carlos D. Quezada-Suaste,[1] Mario Carreón-Escalante,[1] Verenice Merlin-Lucas,[1] and Beatriz Quiroz-García.[1] [1]Departamento de Productos Naturales, Instituto de Química, UNAM

Duclauxin (**1**) and some analogues isolated from Talaromyces sp. IQ-313 were previously reported as putative allosteric modulators of the phosphatase activity of *h*PTP1B1-400. Based on these findings, an OSMAC experiment modifying biotic and abiotic parameters, on the culture of IQ-313 strain was applied to generate analogues of **1**. Moreover, a one-step semisynthetic approach guided by molecular docking towards *h*PTP1B1-400 was applied on this molecule, taking advantage of the isocumarin reactivity towards primary amines, and the likely elimination of MeOH. These methodologies generated 40 analogues divided in two series of duclauxamides (A and B), those incorporating a lactam

functionalization at C-1 (A serie) and the ones containing a lactam at C-1 and an extra unsaturation between C-7 and C-8 (B serie). *In vitro* evaluation coupled with Structure Activity Relationship (SAR) analysis for this set of molecules reveled some key aspects around the impact of the primary amine substituent and the introduced double bond on the modulatory activity towards *h*PTP1B1-400.

P-067 – Daniela Rebollar-Ramos

Antidiabetic Potential of a Trimeric Anthranilic Acid Peptide Isolated from *Malbranchea Flocciformis*

Daniela Rebollar-Ramos[1], Berenice Ovalle-Magallanes[1], Huzefa A. Raja[2], Mario Figueroa[1], Claudia Tovar-Palacio[3], Lilia G. Noriega[4], Rachel Mata[1]. [1]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, 04510, Mexico. [2]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, 27412, NC, USA. [3]Dirección de Nutrición, [4]Departamento de Fisiología de la Nutrición, Instituto Nacional Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, 14080, Mexico

Compound **3**, a trimeric anthranilic acid peptide, and another three metabolites were isolated from an organic extract from the culture medium of *Malbranchea flocciformis*. The chemical structure proposed previously for **3** was unequivocally assigned via synthesis and X-ray diffraction analysis. *In vitro* experiments included PTP-1B inhibition and insulin secretion in INS-1E cells. The results showed that **3** might act as an insulin sensitizer and a non-classical insulin secretagogue. These findings are in harmony with the *in vivo* assays using an oral glucose tolerance test and an acute oral hypoglycemic assay. Dereplication techniques aided by the Global Natural Products Social Molecular Network were used to analyze the chemical composition of the extract.

P-068 – Chin Piow Wong

Anti-Aging Activity of *Kaempferia parviflora* Extract (SIRTMAX®) and its Standardizing Constituent 3,5,7,3',4'-pentamethoxyflavone (PURESIRTMAX®)

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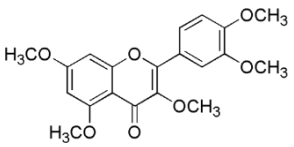
SIRTMAX® is an extract of Kaempferia parviflora rhizome Standardized to contain no less than 15% of total polymethoxyflavonoids. K. parviflora which is also known as black turmeric, is a well-known herbal medicine with various biological activities, including anti-aging. SIRTMAX® also possesses anti-aging activity, where SIRT1 activation and anti-glycation are revealed to be the contributing mechanisms. Among the standardizing polymethoxyflavonoids, PURESIRTMAX® (Fig) is the most potent in SIRT1 activation. The detailed mechanistic studies on SIRT1 activation using isothermal titration, NMR-spectroscopy, molecular dynamic, FRET, and known-down analyses will be reported in this presentation.



P-069 – Chase Clark

One Graph to Rule Them All: Socialgene Knowledge Graphs Facilitate Real Time Exploration of Chemical, Genetic, and Phylogenetic Space Across Hundreds of Thousands of Genomes

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Metagenomics enables us to peer into the life of creatures from nearly any environment. However, the transition from predicted DNA sequence to having a natural product in hand remains challenging. And, while we live in the era of "big data" and "machine learning", large scale analysis for natural product discovery still requires writing bespoke software, gathering disparate data (often requiring extensive reformatting and cleaning), and an incredible amount of time and skill to assemble. With SocialGene we have created a flexible, reproducible, open-source genome mining platform that can easily be extended with additional biological and chemical data. As proof of concept for large scale analysis we assembled a SocialGene graph containing >290,000 genomes from RefSeq, all biosynthetic gene clusters from MIBiG and all linkable chemical data from npatlas. This can be used for both non targeted analyses (e.g. How are terpene synthases distributed across phyla and environments?) and targeted searches (e.g. Find all organisms from sponges, with polyketide synthases near a halogenase and antibiotic resistance gene, not annotated by antismash, and available to purchase from ATCC.). Additionally, whole BGCs can be queried, allowing users to find previously-cultured strains that contain even distantly similar BGCs as those from metagenomic or other unique sources.

P-070 – Harman Gill

Bridging the Gap Between Genes and Natural Products: The First Functional Heterologous Expression a Tailoring Gene that Codes for a Decarboxylase from the Lichen *Cladonia uncialis*

Harman Gill and John L. Sorensen, Department of Chemistry, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

Despite the isolation of 1,000 known bioactive lichen mycobiont-derived secondary metabolites (SMs), conclusive links between the diversity of these SMs and biosynthetic genes remain cryptic. Some biosynthetic gene clusters (BGCs) have

been tentatively linked to a chemical structure by homology assignment to the genes. The Sorensen group reported the putative assignment of a number of BGCs from the lichen *Cladonia uncialis* that appear to code for lichen SMs. These BGCs are organized around a core gene such as a polyketide synthase (PKS) which in turn is flanked by various tailoring genes such as decarboxylases, methyltransferases, and monooxygenases among many other predicted functions. As part of our efforts to assign function to BGCs we have been examining specific tailoring genes. In particular we have focused on a gene that appears to code for a decarboxylase enzyme, in an attempt to determine the role in SM biosynthesis. A 964 bp gene was cloned from the genome of *C. uncialis* and inserted into the pQE30 expression vector which was used to transform *E. coli* (BL21(DE3) cells. The 35 kDa protein that was expressed after induction with IPTG was purified by use of a Ni^{+}-NTA resin. This presentation will report on a summary of our functional activity assays. We observed reversible carboxylation of resorcinol to 2,4-dihydroxybenzoic acid and of orcinol to orsellinic acid. This latter result may provide some insight into the role of this decarboxylase in SM biosynthesis in lichen fungi.

P-071 – Karla Piedl

Discovery of Lanthipeptide through Genome Mining

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Genome mining has allowed for the identification of new natural products in a faster, more targeted way than traditional activity-guided or mass spectrometry-guided isolation. As part of our on-going effort to identify new chemistry from bacteria associated with moon snail egg masses, we sequenced the genomes of 33 diverse strains. AntiSMASH analysis of these genomes led us to investigate the identity of an uncharacterized natural product biosynthetic gene cluster (BGC) in a *Lysinibacillus* sp. genome that was annotated as a Type III lanthipeptide. Lanthipeptides are a subclass of RiPPs (ribosomally synthesized and post-translationally modified peptides). RiPPs are typically excreted extracellularly and often exhibit selective antimicrobial properties. Employing molecular biology tools, a mutant strain was generated where the core lanthipeptide gene was knocked out. Chemical analysis of extracts from *Lysinibacillus* sp. WT versus *Lysinibacillus* sp. Δ*lanA* using LCMS for presence/absence of ions allowed for identification of the product of this particular BGC in the WT strain. Intriguingly, it appears that the leader sequence is not being cleaved from this 24-mer lanthipeptide. Work is ongoing to fully elucidate the two-dimensional structure and evaluate in biological assays.

P-072 – Conor Pulliam

Heterologous Expression Facilitates Discovery and Biological Evaluation of a New Group of Non-Ribosomal Peptides

Conor Pulliam, Dan Xue, Michael Madden, Ethan Older, Jie Li. Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA

During a recent screening project, we identified a compound putatively produced by a non-ribosomal peptide synthetase (NRPS) in a fraction exhibiting inhibitory activity against the SARS-CoV-2 Main Protease. We confirmed the link between the compound and the NRPS biosynthetic gene cluster (BGC) producing it by (1) deleting the BGC in the native strain, which led to no detection of the compound after LC-MS analysis, and (2) expression of the BGC in an optimized heterologous host, which enabled detection of the compound via LC-MS analysis. Heterologous expression of the NRPS BGC also allowed for increased compound yield, permitting more efficient isolation. The enhanced production also allows for investigation of the chemical structure of the compound and evaluation of its potential as a bioactive molecule, which will be discussed further.

P-073 – Daniel Zagal

Exploring Factors Affecting Microbiome Composition in Dietary Supplement Plants

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The fungal and bacterial communities constituting plant microbiomes are dynamic and influenced by various factors. Differences in microbiome composition among dietary supplement (DS) plants (DSP) can be a crucial factor explaining the frequently described metabolomic variability of cultivars and DS products. Metagenomic analysis of DSP bacterial and fungal microbiomes allows for genus and species level taxonomic annotation and provides insight into the factors affecting their composition. Field collections in Alaska, Colorado, and Pennsylvania produced 280 environmental DNA (eDNA) samples from two DSPs, *Rhodiola rosea* and *Actaea racemosa*, as well as their congeners, *R. integrifolia* and *A. rubra*. Endophytes and epiphytes were collected from leaves, stems and roots of collected specimens. Statistical analysis of alpha, beta, and gamma diversity shows differences in factors affecting composition of fungal and bacterial communities, where bacteria are selective for plant parts and fungi for plant species or locations. Moreover, endophytic and epiphytic microbiomes of individual DSP differ significantly in diversity, with the former being less diverse. The processing of over 40,000 distinct sequences, totaling four million sequences, illustrates that individual DSPs can have unique microbiomes that vary not only by species, but also by plant part and location. Increasing accessibility to sequencing technology makes it possible to characterize DSP microbiomes, which is a first step towards understanding host plant-microbe metabolomic relationships.

P-074 – Sarah Bonitatibus

Development of a Feature Prioritization Strategy for Deorphanizing the Human Gut Metabolome in an Inflammatory Bowel Disease Model

Sarah Bonitatibus[1], Matthew Henke[1]. [1]Department of Pharmaceutical Sciences, University of Illinois at Chicago

Although the prevalence of inflammatory bowel disease (or IBD) is increasing worldwide, much remains unknown about the etiology and pathogenesis of this incurable condition. With expanding evidence for the involvement of microbial communities as a possible environmental determinant of disease progression, many recent studies have sought to characterize the eubiotic and dysbiotic human microbiome. However, the large amounts and high complexity of the resulting multi-omics data from sources such as the Integrative Human Microbiome Project (HMP2) highlight an urgent need for robust new data processing and analysis workflows. In this study, a strategy is proposed and implemented for the identification of biologically significant features associated with the incidence and severity of human disease, using patient metagenomic and metabolomic data in combination with clinical metadata. Through comparisons of chromatographic methods, and the integration of differential abundance, dimensionality reduction, and correlation analyses implemented across classification boundaries (i.e., active versus inactive disease populations, as determined by clinical assessments), a shortened list of prioritized features was produced for future structural identification based on their greater suspected relevance to disease.

P-075 – Jeongho Lee

Veillonella Parvula, a Member of the Human Commensal Associated with Ulcerative Colitis Produces Inflammatory Peptidolipids

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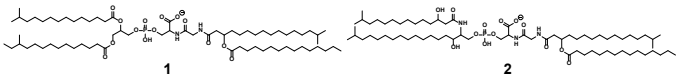
A cohort study in treatment-naive ulcerative colitis (UC) and microbiome study revealed association between UC and *Veillonella parvula*, an anaerobic, gram-negative member of the human commensal associated with diseases such as periodontitis and dental cavities. In this study, *Veillonella parvula* strain RJX1458 derived from UC patients was studied and an immunomodulatory assay was performed for bioassay-guided fractionation using murine bone marrow dendritic cells. Two peptidolipids, *N*-(3-*O*-acyl)acyl glycyl-serine phosphatidyl diacylglycerol (**1**) and *N*-(3-*O*-acyl)acyl glycyl-serine phosphatidyl dihydroceramide (**2**), were identified from the bioactive fractions of cell pellet extract.



1 **2**

P-076 – Michael Madden

Outer Membrane Vesicles Deliver Dual Immunomodulatory Sulfonolipids to Regulate Macrophage Immune Response

Michael Madden[1], Ethan Older[1], Dan Xue[1], Andrew Campbell[1], Dorathea Lee[1], Anna Goshow[1], Emily Quinn[1], Daping Fan[2], and Jie Li[1]. [1]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, USA, [2]Department of Cell Biology and Anatomy, School of Medicine, University of South Carolina, Columbia, South Carolina, 29209, USA

Outer membrane vesicles (OMVs) are spherical nanoparticles produced by gram-negative bacteria to deliver metabolites that mediate interactions with host cells and other organisms. Sulfonolipids (SoLs) are sphingolipid-like bacterial molecules that are also produced by gram-negative bacteria, including members of the human commensal gut microbiota. SoL-producing strains have been associated with protection again inflammatory diseases, so understanding the biological activity of SoLs and SoL bearing OMVs (SoL-OMVs) could help explain their effects on human health. This study shows the delivery of SoLs to host cells via OMVs by SoL-producing bacteria, reports the dual inflammatory activity of SoLs and SoL-OMVs *in vitro*, and discusses the transfer of SoL-OMVs from the gut microbiome into the bloodstream in mouse models. Together, these results demonstrate that SoL-OMVs produced by human gut microbial strains deliver immunoregulatory SoLs to host tissues and potentially other areas of the human body.

P-077 – Ethan Older

Human Gut Microbial Sulfonolipids are Linked to Inflammatory Bowel Diseases through Toll-Like Receptor 4 Signaling

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The trillions of microorganisms inhabiting the human gut are intricately linked to human health. While their abundances in the gut have been correlated with disease progression, understanding the functional metabolites these microbes produce is critical to deciphering their influence on our health.

Here, we use a new approach to directly connect the expression of human microbial sulfonolipid (SoL) biosynthetic genes with inflammatory bowel disease (IBD), revealing a negative correlation. We validate this connection using a mouse model of IBD, showing that SoL production is decreased while inflammatory markers are increased in diseased mice. In support of this connection, we apply bioactive molecular networking to show that SoLs consistently contribute to the immunoregulatory activity of SoL-producing human microbes. We further reveal that sulfobacins A and B, two representative SoLs, primarily mediate their dual immunomodulatory activity by interfering with Toll-like receptor 4 signaling, directly binding to myeloid differentiation factor 2 and competing with the natural ligand, lipopolysaccharide. Together, these results suggest that SoLs mediate a protective effect against inflammatory disease and showcase a widely applicable informatics-based approach to directly linking the biosynthesis of functional metabolites to human health.

P-078 – Hyun Bong Park

Gut Microbiota-Derived Tryptamine Dimer Is a Natural Agonist for GPR55

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Gut microbiota-derived small molecules play important roles in diverse human-microbiome crosstalk and specifically modulate local and systemic human inflammatory responses. Certain microbiota members are known to be tightly associated with the severity of inflammatory bowel disease (IBD), the diverse immunomodulatory small molecules encoded by the members are still largely unexplored. Here we anaerobically cultured IBD-relevant species *Ruminococcus gnavus* and isolated a previously unreported metabolite from the ethyl acetate culture extract. We characterized the chemical structure representing the urea core-linked tryptamine dimer by the interpretation of 1D and 2D NMR and high-resolution ESI-QTOF-MS spectral data, unambiguously confirmed by total synthesis. Next, we examined the metabolite against the non-olfactory G-protein coupled receptors (GPCRs) and found that the molecule significantly activates GPR55 that is an orphan GPCR member tightly linked to host inflammatory responses. The molecule also inhibits the activity of soluble Epoxide Hydrolase (sEH) with an IC_{50} (half-maximal inhibitory concentration) value of 420.5 nM.

P-079 – Roberto Berlinck

New Unusual Cytochalasans Produced by a Marine-Derived Fungal Strain, *Peroneutypa* sp.

Marcelo R. de Amorim[1,2], Sydney M. Schoellhorn[2], Camila de S. Barbosa[3], Giovana R. Mendes[3], Antonio G. Ferreira[4], Rafael V. C. Guido[3] Elizabeth Skellam[2], and Roberto G. S. Berlinck[1]. [1]Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, CEP 13560-970, São Carlos, SP, Brazil; [2]Department of Chemistry and BioDiscovery Institute, University of North Texas, 1155 Union Circle, Denton, TX 76203, USA; [3]Instituto de Física de São Carlos, Universidade de São Paulo, CEP 13563-120, São Carlos, SP, Brazil; [4]Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brazil

New unusual cytochalasans were isolated from cultures produced by the marine-derived fungus Peroneutypa sp. M16. Structures of the new metabolites were established based on spectroscopic data analysis and electronic circular dichroism calculations. Known cytochalasans isolated presented antiplasmodial activity against P. falciparum at low micromolar concentration. Full genome sequencing of Peroneutypa sp. M16 identified the biosynthetic gene cluster for the new cytochalasans and a biosynthetic route is proposed and under investigation.

P-080 – Sofia Kokkaliari

Discovery and Characterization of a New TACR2 Antagonist from a Marine Cyanobacterium from Guam

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Marine cyanobacteria are a rich source of secondary metabolites. In our continuous efforts to discover new bioactive metabolites, a cyanobacterium collected in Guam was investigated based on its cytotoxicity profile against HCT116 colorectal cancer cells. Following an NMR and mass spectrometry guided isolation, we isolated a new cyclic peptide-polyketide hybrid related to the apratoxins. The structure was determined by 1D and 2D NMR techniques, including *J*-based configuration analysis, and chiral analysis and derivatization of degradation products. We evaluated the compound for bioactivity through a series of studies, including RNA-sequencing and GPCR profiling, indicating that the compound is a tachykinin receptor 2 (TACR2) antagonist with selectivity over TACR1 and TACR3. This compound may serve as a probe for TACR2.

P-081 – Kabre Heck

Untargeted Metabolomics Analysis of *Euterpe oleracea* Mart. and *Euterpe precatoria* Leads to Potential New Species Marker

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Euterpe oleracea Mart., commonly known as açaí, is a palm fruit native to the Amazon region which has gained popularity due to its health benefits including antioxidant and anti-inflammatory activities. The bioactivity and potential health benefits of plant

material, as well as the results and rigor of laboratory experiments and clinical trials, are determined by their phytochemical makeup The objective of this work is to develop an efficient method for the untargeted characterization of various açaí extracts that can be potentially used for the chemical characterization of subsequent biological assessments. This workflow allowed the identification or tentative identification of 159 compounds of which 121 were described in açaí for the first time including fisetin, nevadensin, phlorizin, secoisolariciresinol, and tricin. Principle component analysis revealed that features obtained using positive mode ESI provided a better model than features obtained in negative mode. The most important feature was that of an unknown compound ("feature 1096") with *m/z* 549 $[M+2H]^{+2}$ and 1097 $[M+H]^{+}$. This compound has not previously been reported in açaí or any other plant, and structural elucidation underway using ESI-QTOF, MALDI-TOF, and HR-NMR is leading us to believe it is a flavanol-like trimer. Because this feature was important to the classification of açaí from multiple origins, it also led to the examination of organic and aqueous extracts of the *Euterpe precatoria* species where we also found a significant difference in abundance leading us to believe feature 1096 could be a chemical marker for authentication of açaí materials.

P-082 – Quanbo Xiong

Industrializing Molecular Networking Technology for Large Scale Natural Products Discovery

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Built on molecular networking technology, the Global Natural Product Social Molecular Networking (GNPS) has been utilized by numerous researchers all over the world to share, analyze, and connect MS/MS data since its establishment. However, GNPS is limited by scalability and intellectual property concerns at industry level due to its web-based open-access nature. Corteva Agriscience™, a multinational corporation that has a great history of success in natural products research and development, is leveraging advances in genomics, metabolomics, fermentation engineering, artificial intelligence, and analytics to reinvigorate industrial natural products discovery. In collaboration with Ometa Labs, a stand-alone molecular networking platform with core functions of GNPS was established at Corteva that has been serving as a foundational analytical engine to expedite natural products discovery. This presentation will share how Corteva Agriscience™ utilizes molecular networking to support discovery of the next generation of sustainable crop protection products.

P-083 – Yi Zhao

Cardiotoxicity of Diterpenoid Alkaloids from American *Aconitum*

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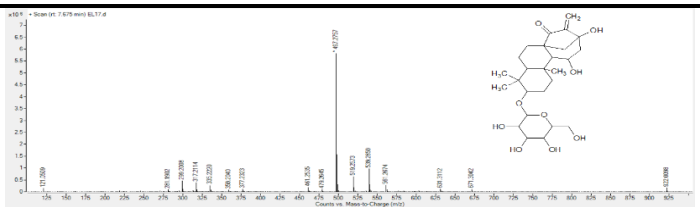
As one of the most well-known traditional herbal medicines in Asia, *Aconitum* species have been used to treat rheumatism, heart failure, inflammations, and pain due to their diterpenoid alkaloids. However, some of these alkaloids can induce acute cardiotoxicity because of their effect on voltage-dependent sodium channels in myocardium cells. We are interested in discovering bioactive diterpenoid alkaloids from *Aconitum* species that are not cardiotoxic. This research aims to study the diterpenoid alkaloids in American *Aconitum* species and assess the cardiotoxicity using a zebrafish model. Two American and five non-American species were either cultivated or collected in the field. Extracts of different parts, including roots, leaves, flowers, and stems, were analyzed using UPLC-qTOF-MS^{E}. Filtering for retention time and fragment intensity, 9180 chemical features were identified using Progenesis QI. PCA results show that the two American species cluster closely with the medicinally important Asian species, but separately from a toxic European species. The results suggest that American species are more chemically similar to the less toxic Asian medicinal species, and this could be due to their lower content of toxic diester diterpenoid alkaloids. The cardiotoxicity of the American *Aconitum* species is currently being assessed in the zebrafish model. Using OPLS-DA to compare the composition of the roots of American *Aconitum* species to Asian and European species, there are 31 chemical features found uniquely in American species, which will be explored for their analgesic potential.

P-084 – Idayat Akinwumi

Euphorbia Lateriflora: an Excellent Source of Diterpenes, Ellagic Acid, and Flavonoid Derivatives

Idayat A. Akinwumi, Shagufta Perveen, and Jiangnan Peng. Department of Chemistry, Morgan State University, Baltimore, Maryland 21251*

Euphorbia is the largest genus in the family Euphorbiaceae, comprising more than 2000 species. *Euphorbia lateriflora* is a shrub, native to Africa and used to treat urinary tract infections, blood disorders, venereal diseases, parasitic infections, and many other diseases. In a phytochemical investigation of the leaves of *E. lateriflora*, four diterpenoids, six ellagic acid derivatives, and three flavonoids were identified with the help of LCQTOF mass spectrometry. This is the first report on the identification of these secondary metabolites from *E. lateriflora*. The cytotoxic activities of the fractions were evaluated against different human cancer cell lines and potent activities were observed.

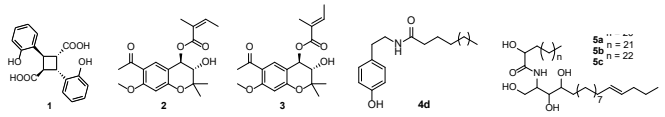


P-085 – José Alberto Gutiérrez-González

Compounds Isolated From An Organic Extract Of *Ageratina Grandifolia*

José Alberto Gutiérrez-González, Araceli Pérez-Vasquéz, José Luis Villaseñor and Rachel Mata. Universidad Nacional Autónoma de México, Ciudad de México, 04510, México

Extensive fractionation of an organic extract of the aerial parts of the traditionally used species *Ageratina grandifolia,* afforded 7 new compounds, including a truxillic acid derivative (**1**), two chromanes (**2** and **3**), one *N*-acylated tyramine derivative (**4d**) and three ceramides (5**a**-5**c**), along with 15 known compounds. The chemical structures of new compounds were elucidated using HR-MS, 1D and 2D NMR and computational calculations. The extract showed a mild inhibitory activity against the α-amylase enzyme.



P-086 – Madeline Hennessy

Unlocking the Therapeutic Potential of the Akuamma Alkaloids

Madeline Hennessy [1], Anna Gutridge [2], Meghna Gill[1], Yavnika Kashyap[1], Jim Wang[1], Richard van Rijn [2-5], and Andrew Riley [1], [1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago [2]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue [3]Purdue Institute for Drug Discovery, Purdue [4]Purdue Institute for Integrative Neuroscience, Purdue [5]Purdue Interdisciplinary Life Sciences Graduate Program, Purdue

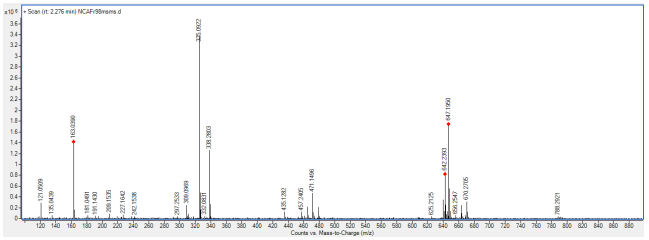
Clinically used opioids induce their analgesic effects by the activation of the mu-opioid receptor (μOR), however; their utility is limited due to their life-threatening side effects. To drive the discovery of improved analgesics, we are investigating a structurally unique class of natural product opioids known as the akuamma alkaloids. Isolated from akuamma seeds, these bioactive compounds are a class of monoterpene indole alkaloids with moderate and selective opioid receptor affinity. Herein, I sought to investigate how chemical modifications affect the alkaloids potency and selectivity of the opioid receptors. These structurally complex alkaloids are comprised of a methanoquinolizidine core and a furoindoline motif that generate six fused rings, five contiguous stereocenters, and a high sp3 fraction. The complexity of the alkaloids structure required the use of highly chemoselective transformations. Ultimately, the akuamma alkaloid analogues activity were evaluated at the opioid receptors. This research has provided insight into how these novel ligands can be utilized as chemical probes for investigating opioid receptor signaling pathways.

P-087 - Omosalewa Olaoluwa

LC-QTOF-MS Based Metabolomic Identification of New Cytotoxic Compounds from Medicinal Plant *Nelsonia Canescense*

Omosalewa Olaoluwa[1,2], Perveen Shagufta[1], and Peng Jiangnan[1]. [1]Department of Chemistry, Morgan State University, Baltimore, Maryland 21251, [2]Department of Chemistry, University of Ibadan, Nigeria*

Metabolomics is a useful tool for the identification of secondary metabolites in natural sources. *N. canescense* has been used to treat cancer, malaria, pain, cardiovascular, and inflammation. A phytochemical investigation of the aerial parts of *N. canescense* revealed two new chromone triglycosides, along with eleven known metabolites. These chromone glycosides are triglycoside derivatives containing one glucuronic acid showing unprecedented structural features. The structures of compounds were elucidated by LC-MS/MS mass spectrometric techniques. The isolated compounds were evaluated for their cytotoxic activity against two different human cancer cell lines, namely, A549 (lung) and MDA-MB-231 (breast), using MTT assay, and strong to moderate activities were observed against these cell lines.



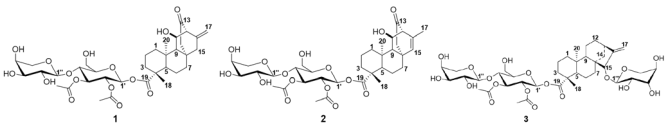
P-088 – Sofia Padilla-Mayne

Antidiabetic Potential of Compounds from the Aqueous Extract of Stevia Serrata

Sofia Padilla-Mayne, Berenice Ovalle-Magallanes, Robert Bye, Mario Figueroa, and Rachel Mata. Universidad Nacional Autónoma de México, Ciudad de México, 04510, México

A combination of open column chromatography and RP-HPLC of the aqueous extract of the roots of the antihyperglycemic species Stevia serrata yielded stevisalioside A (**1**) and two new diterpenoid glycosides (**2⊚3**). The new compounds were given the trivial names of stevisalioside B (**2**) and 19-O-2′,3′-diacetyl-b-D-glucopyranosyl-4′-O-a-L-arabinopyranosyl-15-O-a-L-pyranosyl-ent-kaurene (**3**),

respectively. The chemical structures of new compounds were elucidated using HRESIMS, 1D and 2D NMR techniques, and CD analyses. Stevia serrata is used for treating different ailments in Mexico. Therefore, a pharmacopeic UHPLC analytic method was developed and validated according to the International Conference on Harmonization (ICH) guidelines (ICH, 2015).



P-089 – Destini Thornton

Evaluation of Pharmacodynamic Interactions between Açaí Extracts and Anticancer Drugs

Destini Thornton[1], Rinbam Kromtit[1], Satyanarayana Pondugula[2], and Angela I Calderón[1]. [1]Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, AL 36849, USA, [2]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL 36849, USA

Açaí, *Euterpe oleracea* Mart., fruit has many bioactive compounds that contribute to anti-inflammatory and anti-proliferative effect in humans. To study the pharmacodynamic interactions between açaí botanical dietary supplements (BDS) and anticancer agents, açaí extracts will be tested in combination with the IC_{50}s of methotrexate and tamoxifen to determine any synergism or antagonism with combination therapy. Açaí fruit powder and two BDS capsule formulations were extracted using an aqueous, ethanolic, methanolic, and acidic methanolic solutions, respectively. An initial experiment of obtaining IC_{50} values for the anticancer agents was performed using a concentration of 10 pM to 100 μM. The IC_{50}s for MCF-7 against methotrexate and tamoxifen were 30.28 μM and 0.348 nm, and for MDA-MB-231 were 34.39 μM and 781 nM, respectively. The seven standardized açaí extracts, based on cyanidin-3-glucoside (C3G) content, were tested alone against the two cancer cell lines in a range of 10 pg/ml to 1000 ng/ml which includes the human equivalent dose of 2.321 ng/ml of C3G. All the extracts showed no cytotoxicity up to the testing concentration of 1000 ng/ml of C3G.The combinatorial assays of açaí botanical dietary supplements with anticancer agents on the cancer cell lines are underway. In brief, we have optimized the experimental design to measure, in vitro, pharmacodynamic interactions of açaí and the anticancer agents as well as completing the preliminary IC_{50} curves for the anticancer agents and açaí extracts.

P-090 – Márcio B. Weiss

Dereplication Using Metabolomics Tools for Prioritizing Cyanobacterial Samples for Natural Product Discovery

Márcio B. Weiss[1], Jaewon Yoon[1], João P. Brandão[1], Sandra R. C. Soares[3], Manoel O. M. Junior[1], Silvya S. Maria-Engler[1], José A. L. Lindoso[3], Roberto G. S. Berlinck[4], Ricardo M. Borges[2], Camila M. Crnkovic[1]. [1]School of Pharmaceutical Sciences, University of São Paulo, Brazil; [2]Center of Health Sciences, Federal University of Rio de Janeiro, Brazil; [3]Institute of Tropical Medicine of University of São Paulo, Brazil; [4]Institute of Chemistry of São Carlos, University of São Paulo, Brazil

Cyanobacteria is a diverse group of microorganisms that are found in almost every kind of ecosystem. These photoautotrophic prokaryotes are known as producers of an array of bioactive natural products. The underexplored diversity of Brazilian cyanobacteria offers great potential for prospecting biologically active molecules with unique structures. Nine strains of Brazilian freshwater cyanobacteria were cultured, extracted, and pre-fractionated. Extracts and fractions were analyzed by UPLC–HRMS/MS and tested against melanoma cancer cell lines and *Leishmania (L) amazonensis* promastigotes. *Nostoc* sp. fractions displayed antiproliferative activity against melanoma cells. *Komarekiella atlantica* extract and fractions showed activity against *L. amazonensis*. LC-MS data were processed with MZmine and a molecular networking was created with the GNPS platform. Additionally, Data Fusion-based Discovery (DAFdiscovery) was employed to determine features correlated with biological activity. Selected features were manually annotated using SIRIUS. The dereplication process suggested that both *Nostoc* sp. and *K. atlantica* produced potentially new natural products with biological activity against melanoma and leishmaniasis, respectively. This investigation combined the application of metabolomics tools for the rapid selection of bioactive features aiming for future isolation and structure elucidation.

P-091 – Neha Malhotra

Co-Cultivation Mediated Elicitation of Fungal Natural Products Against *Mycobacterium Tuberculosis*

Neha Malhotra, Helena Boshoff, Clifton Earl Barry 3rd, Tuberculosis Research Section, LCIM, NIAID, NIH, Bethesda, MD, USA*

Tuberculosis caused by *Mycobacterium tuberculosis* is a leading cause of global mortality with about eight million new cases and 1.6 million deaths ascribed to it annually. To combat the threat of drug- resistant strains of *Mtb*, newer drugs with novel mode of actions are needed. Microbial natural products (NPs) are gaining renewed attention as their complex structural scaffolds have evolved to specifically inhibit essential cellular targets. Herein, we utilized co-cultivation approach to identify cryptic biosynthetic gene clusters (BGCs) from fungal genomes eliciting the expression of genes that are silent or poorly transcribed in axenic cultures. Fungi were isolated from sphagnum peat bog collected from different regions of North-eastern USA because aspects of this ecological niche reflect the critical microenvironment of the human tuberculosis granuloma. In addition, sphagnum peat bogs are a natural habitat for slow growing mycobacteria that compete for limited nutrients with

other microbes. Bioactivity- guided assay against reporter strain mScarlet *Mtb* H37Rv led us to identify two fungal isolates that selectively produce growth inhibitory metabolites during co-cultivation with *Mtb*. Interestingly, counter- screening against ESKAPE pathogens and *M.smegmatis* did not cause any growth inhibition. Whole genome sequencing showed these isolates were a *Penicillium* and *Talaromyces* sp., denoted as F2 and Fun31 respectively in our study. Fungal mRNA sequencing from co-cultured isolates facilitated the identification of elicited Type I Polyketide Synthase BGCs that were silent in axenic cultures. These highly induced BGCs upon co-cultivation were bioinformatically linked to their products including patulin from F2 and unique emodin/chrysophanol derivatives from Fun31. These induced filtrates led to a highly responsive redox-stress homeostasis within *Mtb*. Our study illustrates a co-cultivation mediated elicitation of unique fungal NP resulting in a thiol-reactive oxidative stress mediated killing of *Mtb*. Subsequent chemical experiments will be helpful to confirm the molecular structure of the unknown metabolite as well as target identification would benefit us to validate the mode of *Mtb* killing. These results illustrate that two different fungi have convergently selected different molecules to target the same vulnerability of slow-growing mycobacterial species in nature and suggest that this target may be uniquely vulnerable in the context of human caseous lesions.

P-092 – Huong Pham

Bacillus Subtilis Inhibits the Biosynthesis of Verruculogens, the Antifungal Metabolites of *Penicillium Brasiliam*, Using Its Siderophore Bacillibactin

Huong T. Pham[1], Kyo Bin Kang[1]. [1]College of Pharmacy, Sookmyung Women's University, Seoul 04310, Korea

Siderophores are low-molecular-weight compounds produced by microbes to overcome metal starvation by binding to them. In this study, we suggest a previously unknown function of bacterial siderophore observed in the coculture between *Penicillium brasilianum* and *Bacillus subtilis*. To identify the chemical interaction, the LC-MS/MS analysis was performed to discover newly induced metabolites expecting initially them to be antibiotic metabolites. Surprisingly, contrary to our expectation, coculturing *P. brasilianum* with *B. subtilis* did not induce but decreased amounts of various specialized metabolites in the coculture plate. Targeted isolation on the compounds decreased by the coculture yielded two new verruculogen derivatives (**1**–**2**) as well as six known ones (**3**–**8**), viridicatumtoxin (**9**), and penicillic acid (**10**) that were identified by NMR spectroscopic analysis. Among the ten isolates, compounds **1**–**9** were reduced in the cocultivation. Further analysis of the LC-MS/MS data found that bacillibactin, a siderophore produced by *B. subtilis*, was significantly induced by the coculture. In the further assay, we screened the coculture *P. brasilianum* with *B. subtilis* ΔdhbF, which is a k/o mutant unable to produce bacillibactin, and evaluated the chemical interaction. Verruculogen production was not affected by *B. subtilis* ΔdhbF, suggesting that the iron deficiency caused by bacillibactin may have inhibited the biosynthesis of verruculogens. Our result suggests that bacterial siderophores can not only facilitate metal acquisition, but also play a role in inhibiting the toxic metabolite production of competitors.

P-093 – Luis Prieto-Costas

Exploring Natural products from Puerto Rico: Initial Studies of Extracts from Arracacia Xanthorrhiza and Chromobactrium Violaceum

Luis A. Prieto-Costas[1], Abel J. Baerga-Ortiz[1,2], Rafael Maldonado,[3] Alok Arun[4], Jacobo Morales-Méndez[4].[1]Center for Tropical Biodiversity, Molecular Sciences Research Center, San Juan, PR. [2] Department of Biochemistry, University of Puerto Rico, Medical Sciences Campus, San Juan, PR. [3]Department of Biology, university of Puerto Rico, Ponce Campus. [4]Institute of Sustainable Biotechnology, Inter American University of Puerto Rico

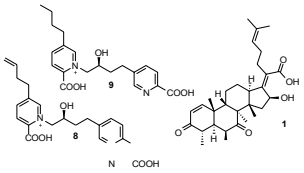
Puerto Rico, a Caribbean island, is recognized as an important region for biodiversity, housing numerous natural products derived from medicinal plants as well as microorganisms. The Puerto Rico Science, Technology and Research Trust is actively involved in conservation and bioprospecting initiatives on the island. One such effort is the Center for Tropical Biodiversity (CTB), which focuses on protecting the biodiversity resources of Puerto Rico and promoting the sharing of benefits from their commercial development. Herein, we present two research projects developed by Puerto Rican scientists with the support of CTB. The first project involves the determination of flavonoids from arracacia xanthorrhiza, also known as 'apio' plant, which has traditionally been used by the native population as a natural anti-inflammatory and antidiarrheal agent. In this study, we evaluated the antioxidant properties and total flavonoid content of the leaves in an effort to isolate potentially useful compounds. The results were obtained through antioxidant assays with DPPH and aluminum colorimetric assays, as well as HPLC studies. The second project involves the discovery of a new native amphibian species, *Eurotherodactylus juanariveroi*, which led to the identification of beneficial bacteria from its habitat. One such bacteria is *Chromobactrium violaceum*, the natural producer of antifungal violacein. This compound was isolated by FPLC/HPLC and studies are being conducted to confirm its potential use. We believe that these and other bacteria found in the habitat of *E. juanariveroi* play a crucial role in protecting the organism from common environmental pathogens. These research projects represent a fraction of the many conservation and bioprospecting efforts currently underway in Puerto Rico.

P-094 - KH Ahammad Uz Zaman

Uncommon Pyridiniums and Bacterial EF-G-GDP Inhibitory Fusaric Acid Analogs from Hawaii-Derived Fungi

KH Ahammad Uz Zaman[1], Xueliang Ge[2], Suparna Sanyal[2], Yousong Ding[3], and Shugeng Cao[1]. [1]Department of Pharmaceutical Sciences, DKI College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI 96720, USA; [2]Department of Cell and Molecular Biology, Uppsala University, Husargatan 3, Uppsala, SE-75124, Sweden; [3]Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA

A previously undescribed fusidic acid analog maunakeanolic acid C (**1**), and two unique fusaric acid derivatives, fusariumic acids A and B (**8** and **9**) were isolated from Hawaii-derived fungi, *Aspergillus terreus* and *Fusarium* sp., respectively, along with previously identified fusidic acid and fusaric acid derivatives (**2**–**7**). The fusidic acid analogs, maunakeanolic acids A⊚C (**1**⊚**2** and **4**), 6-deacetyl-1,2- dihydrohelvolic acid (**3**), helvolic acid (**5**), 1,2-dehydrohelvolic acid (**6**) and helvolinic acid (**7**), strongly bound to the elongation factor G (EF-G)-GDP complex of bacterial ribosome and inhibited both peptide translocation and ribosome disassembly, resulting in inhibition of protein synthesis. The structures of these compounds, elucidated by spectroscopic interpretation including HRESIMS and NMR, and ECD analysis, along with their mechanism of action will be presented in both oral and poster presentation.



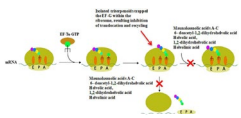


Fig. Schematic representations of MOA of isolated fusidic acid analogs during peptide synthesis

P-095 – Hye Jin Kim

Cinnamomum Verum-Derived O-Methoxycinnamaldehyde Is an Anti-Inflammatory Novel T-Lymphocyte NFAT Regulator with Therapeutic Potential in the Context of Depressive Disorder

Hye Jin Kim[1,2], Hyungjun Kim[1], Yujin Choi[1], Jun-Hwan Lee[1], Donghwan Kim[3], Sang Kook Lee[2,*], and Ki-Sun Park[1,*] [1] Division of Clinical Medicine, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea. [2] College of Pharmacy, Natural Products Research Institute, Seoul National University, Seoul 08826, Republic of Korea.[3]Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Homeostasis requires balanced co-operation between the nervous, immune, and endocrine systems. Inflammation is implicated in depressive disorder pathophysiology, including via cytokine-altered nervous system functions. On T-cell activation, inflammatory stimuli activates transcription factor NFAT to induce expression of itself and of depression marker cytokines TNFα and IL-2. Existing NFAT inhibitors such as cyclosporin A target nuclear translocation and exhibit unfavorable adverse effect profiles. Inhibition of alternate aspects of NFAT function is therefore desirable. Because *in vitro* and animal model studies support the anti-inflammatory and antidepressant effects of *Cinnamomum verum* (CV), the present study evaluated its impact on depression-like behavior in a murine model of inflammation-induced depression, including elucidating underlying molecular mechanisms. Pre-treatment with CV extract or constituent O-Methoxycinnamaldehyde (MCA) dose-dependently ameliorated both inflammation-induced depression-like behavior and increases in plasma TNFα and IL-2 levels. Non-cytotoxic levels of CV extract or MCA concentration-dependently ameliorated activation-induced increases in T-cell TNFα and IL-2 mRNA levels *in vitro* (accompanied by MCA-mediated decreases in TNFα and IL-2 protein secretion). Finally, CV extract or MCA ameliorated activation-induced increases in NFAT (but not NF-κB p65) mRNA and protein levels, but did not inhibit NFAT nuclear transcription. Instead, MCA p38 MAPK-independently decreased NFAT mRNA levels by promoting NFAT mRNA decay. Post-transcriptional regulation of T-cell NFAT is a novel anti-inflammatory mechanism of MCA. Overall, findings suggest that the CV extract-mediated decrease in inflammation and inflammation-induced depression-like behavior may be attributable to this MCA mechanism. Pending validation of this hypothesis and comprehensive safety and efficacy evaluation, MCA may represent an alternative or adjuvant to existing NFAT-targeting immunosuppressants for clinical prophylaxis or therapy in the context of inflammation-induced depressive disorder or other T-cell-associated inflammatory disorders.

P-096 – Manead Khin

Wheldone, a Fungal Secondary Metabolite Downregulates HNRNPD, a DNA Repair Protein

Manead Khin[1], Manuel Rangel-Grimaldo[2], Huzefa A. Raja[2], Ziwei Zhang[2], Julia Ekiert[2], Nicholas H. Oberlies[2], Yu Gao[1], Joanna E. Burdette[1]. [1]Department of Pharmaceutical Sciences, University of Illinois at Chicago, College of Pharmacy, Chicago, IL 60612. [2]Deparment of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402

Ovarian cancer is the most lethal gynecological malignancy, with high-grade serous ovarian cancer (HGSOC) being the most common and lethal histotype. Hence, there is an urgent need for novel therapeutics. Wheldone was isolated from a fungal co-culture and demonstrated cytotoxic in the low micromolar range against a panel of cancer cell lines. To investigate pathways altered in response to wheldone treatment, quantitative proteomics was performed, in which we identified that HNRNPD was the most downregulated protein by wheldone. HNRNPD is a heterogeneous nuclear ribonucleoprotein, which plays a role in DNA damage repair. We hypothesized that wheldone treatment would reduce HNRNPD thereby increasing DNA damage. Wheldone significantly upregulated γH2AX expression, which is a marker for the DNA damage. Since DNA damage can subsequently lead to apoptosis, we observed that wheldone led to significant PARP cleavage, an apoptosis marker, and increases in apoptotic cells. Additionally

wheldone led to a caspase-dependent apoptosis since caspase-3/7 enzyme activity was enhanced with wheldone treatment. Wheldone belongs to a novel chemical compound class for which the mechanism remains unknown, suggesting that wheldone may have an innovative mechanism of action. Future studies will focus on identifying whether wheldone sensitizes ovarian cancer cells to PARP inhibitors and platinum, which are both more effective in tumors with deficiencies in DNA repair.

P-097 – Md Nure Alam

Chemical Profiling of Greenhouse Grown *Centella Asiatica* Cultivars by High Resolution Mass Spectrometry

Md Nure Alam[1,6], Luke Marney[1,6], Liping Yang[1,6], Jaewoo Choi[3,6], Natasha Cerruti[4,6], Samuel Bassett[1], Kadine Cabey[5,6], Ramya Viswanathan[5,6], Sumana Rajagopal[5,6], Amala Soumyanath[5,6], Jan F. Stevens[2,3,6], and Claudia S. Maier[1,3,6].[1]Department of Chemistry, [2]Department of Pharmaceutical Sciences, [3]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA; [4]Oregon's Wild Harvest, Redmond OR 97756; [5]Department of Neurology, [6]BENFRA Botanical Dietary Supplements Research Center, Oregon Health and Science University, Portland OR 97239, USA

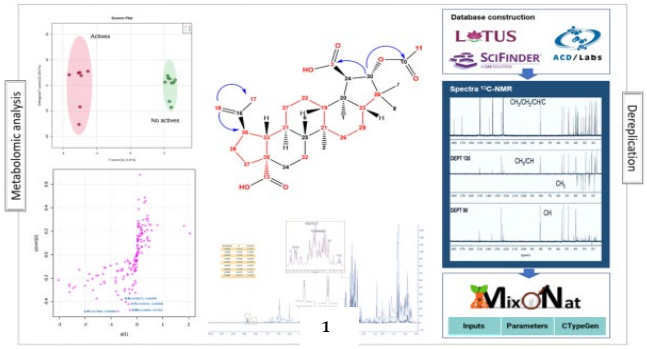
The tropical botanical *Centella asiatica* (CA) is under investigation in the BENFRA BDSRC due to its reputation for enhancing cognitive functions. To obtain standardized plant material with desirable levels of phytochemical marker compounds for future clinical trials, the cultivation of *Centella asiatica* in climate-controlled greenhouses in central Oregon was explored. Metabolite composition of green-house grown cultivars was investigated with respect to vegetative propagation competency, growth and harvest conditions and levels of compounds that were associated with bioactivity in previous studies, including mono- and di-caffeoylquinic acids, and triterpenoids. Four different cultivars BEN-CA-9 (Mountain valley), BEN-CA-10 (Hawaii), BEN-CA-11 (White cloud) and BEN-CA- 12 (9EZ) were each grown at three different propagation periods, during which leaves were collected at four different harvest times: week 8, 10, 12 & 14, respectively. Data reduction and multivariate methods were used to depict chemical variation among the cultivars and depict time-dependency of phytochemical levels. GNPS molecular network summarize chemical variations observed in the different cultivars. This study shows that cultivation of CA in climate-controlled greenhouses is a viable strategy to obtain plant materials for standardized formulations. This work is supported by NIH grants U19 AT010829 and S10RR027878.

P-098 – Maricarmen Corona-Vázquez

Application of Metabolomics and ^{13}C-NMR Dereplication Analyses in the Identification of Ceanotane Triterpenes with Antivirulence Activity from *Colubrina yucatanensis*

Maricarmen Corona-Vázquez[1], Gloria I. Hernández-Bolio[2], Naybi Muñoz-Cazares[1], María Claudia Peña-González[1], Séverine Dérbre[3], Luis Manuel Peña-Rodríguez[1]. [1]Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México; [2]Departamento de Física Aplicada, Centro de Investigaciones y Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, México; [3]University of Angers, SONAS Laboratory, Angers, France*

A chemometric analysis of the ^{1}H-NMR metabolic profiles of both the crude root extract and semipurified fractions of *Colubrina yucatanensis* (M.C. Johnst.) G.L. Nesom (Rhamnaceae) and the results of their inhibition of bacterial virulence factors in a *Pseudomonas aeruginosa* model, allowed the identification of several ^{1}H-NMR signals associated with the inhibitory activity. A combination of ^{13}C- NMR dereplication analysis and 2D-NMR experiments (HMBC and HSQC) of the bioactive fractions resulted in the identification of the bioactive metabolite as 3-*O*-acetyl-ceanotic acid (**1**), a ceanothane- type triterpene previously described in *C. greggii* var. *yucatanensis*. This is the first report of a ceanotane triterpene as a natural inhibitor of bacterial virulence factors, offering an alternative to antimicrobials.



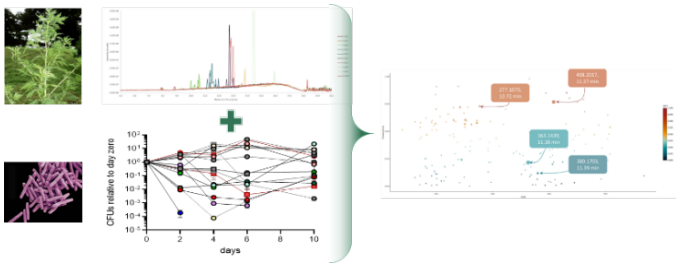
P-099 – Joshua Kellogg

Evaluation of Anti-tuberculosis Phytochemicals from *Artemisia* spp.

Joshua J. Kellogg[1], Teal Jordan[1], Maria Natalia Alonso[2], Melissa Towler[2], Pamela Weathers[2], and Scarlet Shell[2]. [1]Department of Veterinary & Biomedical Sciences, Pennsylvania State University, University Park, PA 16802, USA. [2]Department of Biology & Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609, USA

Tuberculosis (*Mycobacterium tuberculosis*, TB) kills 1.6 million people each year, and a major obstacle to lowering this number is the suboptimal nature of current TB therapies. *Artemisia* species are a rich source of phytochemicals, including artemisinin. Our preliminary studies indicate that *Artemisia* spp. extracts are antimycobacterial, yet the activity cannot be attributed to artemisinin alone. Using a biochemometric approach, we discovered compounds from *Artemisia afra* with potential anti-TB activity. Successive rounds of fractionation were profiled via untargeted LC-MS metabolomics and correlated with *in vitro* antimycobacterial assays using supervised machine learning methods. Tentative identification of bioactive metabolites highlighted phytochemicals from various classes possessing anti-

TB activity; next we will analyze combination effects to better understand their mechanism of action.



P-100 – Lily Peng

Edible Fruits as Potential Source for Anticancer Leads

Lily Peng[1,†], Srikar Bala[1,†], Ishika Jain[2,†], Perveen Shagufta[3]. [1]River Hill High School, Clarksville, MD 21029; [2]Glen Burnie High School, Glen Burnie, MD 21060; [3]Department of Chemistry, Morgan State University, Baltimore, MD 21251

As cancer remains in need of a cure, the search for treatments has sparked research of plants and their chemical components which offer new possibilities for cancer treatment. According to NCI, over half of all anti-cancer drugs were derived from natural sources. In the present study, we investigated the anti-cancer effects of seventeen fruits we collected, out of which three showed potent anti-cancer activities against human lung cancer cells A549. The chemical components of one of these potent fruits, Osage orange (*Maclura pomifera)* are characterized using LC-QTOF MS/MS methods. Our study demonstrated the presence of many interesting bioactive components, such as xanthones, isoflavonoids and flavonoids in the fruits. The fractions and compounds isolated from the fruit were further evaluated for cytotoxic activities, which showed strong activities against the cancer cell lines tested.

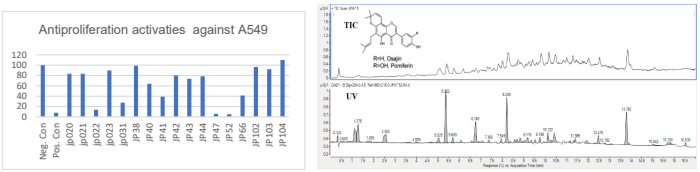


Figure 1. Left: Antiproliferative activities against A549 cells; Right: LC/MS profile of an active fraction

P-101 – Empress Williams

Exploration of Antimicrobial Compounds from the Medicinal Plant *Turnera Diffusa*

Empress V. Williams[1], Chantal V. Pelzer[1], Warren S. Vidar[1], Victoria M. Anderson[1], Fridah Rotich[1], and Nadja B. Cech[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27413, USA

Natural products span a great breadth of chemical space and as such play a valuable role in medicinal drug discovery. Novel antimicrobials are the need of the hour, to combat health-threatening issues posed especially by antibiotic-resistant pathogens. Natural products contain diverse secondary metabolites to fight these superbugs and offer a promising solution to microbial infections. *Turnera diffusa*, also known as damiana, a flowering shrub native to Mexico, South, and Central America, and the southern United States, has been utilized for centuries by indigenous peoples to treat a variety of ailments. In recent years, *T. diffusa* has been highlighted for its antimicrobial properties against Methicillin-resistant *Staphylococcus aureus* (MRSA), a Gram(+) skin bacterium that can lead to severe, life-threatening infections. This project aims to identify specific compounds from *T. diffusa* responsible for its antimicrobial activity. Ultra-high-performance liquid chromatography in tandem with high-resolution mass spectrometry (UHPLC-HRMS) data was coupled to biological activity data to guide the isolation of active compounds. Multivariate statistical analysis was used to predict compounds correlated to activity. Promising leads will be presented. Follow-up studies for this project are ongoing.

P-102 – Shmuel Carmeli

Hydanto-Anabaenopeptins from Lake Kinneret Brown *Microcystis* Bloom

Shira Weisthal Algor[1], Assaf Sukenik[2] and Shmuel Carmeli[1,1]Raymond and Beverly Sackler Faculty of Exact Sciences, School of Chemistry, Tel Aviv University, Tel Aviv, 69978, Israel. [2]The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic & Limnological Research Institute, P.O Box 447, Migdal, 49500, Israel

In the course of re-isolation of the known aeruginosins KT608A and KT608B for bioassays studies, we noticed the presence of some unknown anabaenopeptins in the extract of a brown *Microcystis* sp. cell mass collected during the 2016 spring bloom event in Lake Kinneret, Israel. The ^{1}H NMR spectrum of some of these compounds presented significant difference in the appearance of the ureido-bridge protons and their molecular masses didn't match any one of the 152 known anabaenopeptins. Analyses of the 1D and 2D NMR, HRMS and MS/MS spectra of the new compounds revealed their structures as the hydantoin derivatives of anabaenopeptins A, B, F and ^{1}[Dht]-anabaenopeptin A and oscillamide Y and a new anabaenopeptin, ^{1}[Dht]-anabaenopeptin A. The known anabaenopeptins A, B and F and oscillamide Y were present in the extract, as well. Some aeruginosins were isolated as well. We propose that these metabolites are the possible missing intermediates in the previously proposed partial biosynthesis route to the anabaenopeptins. The structure elucidation of the new compounds and the possible alternative biosynthetic routes to the anabaenopeptins will be presented.

P-103 – Hyo-Moon Cho

A Database-Guided Mass Defect and Molecular Networking Approach to Discover New Skeletal Structural Compounds From *Nocardia Brasiliensis*

Hyo-Moon Cho and Carole A. Bewley. Laboratory of Bioorganic Chemistry, 8 Center Drive, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

Recently, the common strategy for metabolite discovery has often used MS spectrometry. However, identifying components using MS and MS/MS data in complex extracts is tough and can lead to errors. To overcome such limitations, we applied mass defect calculations and molecular networking to identify promising new compounds with new skeletons. Six strains were selected from our own library consisting of desert-derived actinomycetes. Mass defect calculation inferred the class of unknown compounds by using the information of 118 extracted compounds isolated from *Nocardia* species collected from NPClassifier open-source database and the novelty of the compounds was given from the molecular networking. Here, three new eighteen-membered macrolides (**1**–**3**) were isolated from the ethyl acetate fraction and additionally four new linear peptides (**4**–**7**) and three known peptides (**8**–**10**) were isolated from the *n*-butanol fraction of *Nocardia brasiliensis*. Their structures were determined by NMR analysis and chemical derivatization.

P-104 – Winklet Gallimore

UWI at 75: Seventy Five Years of Natural Products Chemistry

Winklet A. Gallimore, The Department of Chemistry, The University of the West Indies, Mona Campus Roy B. Porter, The Department of Chemistry, The University of the West Indies, Mona Campus

Since its inception in 1948, the Department of Chemistry at the University of the West Indies, Mona Campus has enjoyed a rich tradition in the research area of natural products chemistry. The work in the early days was focused on the investigation of the toxin hypoglycin A in the edible fruit ackee. Currently, our research in the natural products arena includes work on terrestrial plants as well as marine species including brown algae, gorgonians, ascidians and sponges with a view to identifying bioactive compounds with potential as nutraceutical agents or to find applications in cosmetic or agricultural products. An overview of this work in the Department and its related natural products chemistry research efforts will be highlighted.

P-105 – Jaekyeong Kim

Targeted Isolation of Naphthol Oligomers From an Enodolichenic Fungus Daldinia Childiae Guided By LC-HRMS/MS-Based Molecular Networking

Jaekyeong Kim[1], Hyejin Ko1, Jae-Seoun Hur[2], Jin Woo Lee[3], Minsoo Noh[1] and Sang Hee Shim[1],. [1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, South Korea, [2]Korean Lichen Research Institute, Sunchon National University, Suncheon 57922, Republic of Korea,[3]College of Pharmacy, Duksung Women's University, Seoul 01369, South Korea*

Natural products of the endolichenic fungus are potential bioresources because they could produce novel bioactive compounds with distinct and diverse structural classes. Our chemical screening using in-house LC-UV-MS library led the chemical investigation of Daldinia childiae 047219. In addition, an LC-HRMS/MS-based molecular networking strategy was applied to investigate the potential naphthol oligomers of D. childiae, leading to the isolation of twenty polyketides including four new trimers and six new tetramers. The structures were determined by analyzing their 1D, 2D NMR, and HRESIMS data as well as ECD calculations. Among the compounds, the new naphthol trimer, 3,1′,3′,3″-ternaphthalene-5,5′,5″-trimethoxy-4,4′,4″-triol, and a known dimer, nodulisporin A, exhibited concentration-dependent adiponectin-synthesis-promoting activity (EC50 30.8 and 15.2 μM, respectively). Naphthol oligomers 3,1′,3′,3″-ternaphthalene-5,5′,5″-trimethoxy-4,4′,4″-triol and nodulisporin A represent novel pan-PPAR modulators and are potential pharmacophores for designing new therapeutic agents against hypoadiponectinemia-associated metabolic diseases.

P-106 – Haeun Kwon

Isoprenylated Cyclohexanol Derivatives from the Marine-Derived Fungus *Apiospora Piptatheri*

Haeun Kwon[1], Jaeyoung Kwon[2], Jae-Jin Kim[3], Dongho Lee[1]1Department of Plant Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea, [2]Natural Product Informatics Research center, Korea Institute of Science and Technology (KIST) Gangneung Institute, Gangneung 25451, Republic of Korea, [3]Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea*

Apiospora Sacc. (Apiosporaceae, Sordariomycetes, Ascomycota) is a cosmopolitan fungus found in various environments, including marine samples. The marine-derived *Apiospora* species has been reported to produce various bioactive compounds. Hence, LC-MS based metabolomic analysis was conducted to screen several marine-derived *Apiospora* spp.; *Apiospora piptatheri* was identified as a candidate with unique metabolites. Five new isoprenylated cyclohexanol derivatives, Apiosporiol A–E (**1**–**5**), were isolated from an EtOAc-extract of culture medium of marine-derived *Apiospora piptatheri*, isolated from the brown algae, *Sargassum fulvellum*. The isolation workflow was guided by a Molecular Networking-based dereplication strategy. The chemical structures of **1**–**5** were identified using MS and NMR spectroscopic techniques, and their absolute configurations were established by the chemical derivatization, quantum mechanical calculation, and a X-ray crystallography.

P-107 – Hee Ju Lee

Autophagic Regulation Activity of Benzylisoquinoline Alkaloids Isolated from the Stems of *Limacia Scandens*

Hee Ju Lee[1], *Eun Jin Park*[2], *Duc-Trong Nghiem*[3], *Ha-Thanh-Tung Pham*[3] *and Won Keun Oh*[2]. [1]*Natural Product Informatics Research Center, Korea Institute of Science and Technology, Gangneung 25451, Korea,* [2]*Korea Bioactive Natural Material Bank, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 00826, Korea,* [3]*Department of Botany, Hanoi University of Pharmacy, Hanoi 100000, Vietnam*

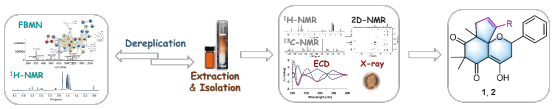
In search for novel compounds with autophagic regulation activity, the chemical composition of *Limacia scandens* was studied. Three new dimeric benzylisoquinoline alkaloids (**1**–**3**), one new 4-hydroxybenzoic acid-conjugated benzylisoquinoline alkaloid (**4**), and six known compounds (**5**–**10**) were isolated from the stems of *L. scandens*. All compounds (**1–10**) were screened for their ability to regulate autophagy in HEK293 cells stably expressing the GFP-LC3 plasmid. Among the isolated compounds, **1**, **2** and **4** showed autophagic regulation activity that blocked the process of combining autophagosomes and lysosomes. They also inhibit the protein degradation process from the autolysosome as inhibitors of autophagy. Novel benzylisoquinoline alkaloids from *L. scandens* showed potent potency for the inhibition of autophagic flux. This study provides potential candidates for developing natural autophagy inhibitors for disease prevention and treatment.

P-108 – Van-Hieu Mai

Cleistobutone A-D, Four Rare Phloroglucinols from *Cleistocalyx Operculatus* Buds as Potential Autophagy Regulation

*Van-Hieu Mai, Eun Jin Park, Byeol Ryu and Won Keun Oh, *Korea Bioactive Natural Material Bank, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea*

Chemical investigation *Cleistocalyx operculatus* buds by employing Feature-based Molecular Networking (FBMN) and ^{1}H-NMR as a dereplication tool, four unusual phloroglucinols (**1**-**4**) which featured by a rare decahydro-2*H*-cyclopenta[*i*]chromene skeleton, together with three new phloroglucinol meroterpenoids (**5**-**7**) were isolated. Structural analysis of all isolated compounds has lead to established a plausible biosynthetic pathway involving a late-stage integration of alkyl-CoA to the core structure 2,2,4-trimethylcinnamyl-β-triketone followed by a cascade of functionalization and cyclization lead to the formation of **1**-**4**. Compound **4** exhibits potential in activating autophagy in HEK293 cells expressing GFP-LC3 by reducing p62 expression and enhancing the excretion of autophagosomes in form of autolysosome.



P-109 – Kumudini Meepagala

A Plant Growth Stimulating Bacterium, and its Metabolites Isolated from *Hydrocotyle umbellata* (Dollarweed)

Kumudini M. Meepagala, Caleb M. Anderson and Stephen O. Duke. USDA-ARS, Natural Products Utilization Research Unit, P.O. Box 1848, University, MS 38677. National Center for Natural Products Research, University of Mississippi, University, MS 38677*

A bacterium growing on infected leaves of *Hydrocotyle umbellata,* commonly known as dollarweed, was isolated and identified as *Pantoea ananatis.* An ethyl acetate extract of tryptic soy broth (TSB) liquid culture filtrate of the bacterium was subjected to silica gel chromatography to isolate bioactive molecules. Indole was isolated as the major compound that gave a distinct, foul odor to the extract, together with phenethyl alcohol, phenol, tryptophol, *N*-acyl-homoserine lactone, 3-(methylthio)-1-propanol, cyclo(L-pro-L-tyr) and cyclo(dehydroAla-L-Leu). This is the first report of the isolation of cyclo(dehydroAla-L-Leu) from *Pantoea* species. Even though tryptophol is an intermediate in the indoleacetic acid (IAA) pathway, we were unable to detect or isolate IAA. We investigated the effect of *P. ananatis* inoculum on the growth of plants. Treatment of *Lemna paucicostata* Hegelm with 10^6 CFU (Colony Forming Units) of *P. ananatis* stimulated the growth of the plants. After 12 days of treatment, some control plants were browning, but treated plants were greener and no plants were browning. Growth stimulation occurred on *Cucumis sativus* (cucumber) and *Sorghum bicolor* (sorghum) plants when the rhizosphere was treated with the bacterium after germination at the same concentration.

P-110 – Dong-Chan Oh

Discovery and Biosynthesis of Cihunamides, Macrocyclic Antibacterial RiPPs with a Unique C-N Linkage Formed by CYP450 Catalysis

Joon Soo An[1], *Hyunbin Lee*[2], *Hyungyu Kim*[1], *Seungyeon Woo*[2], *Hyunsung Nam*[2], *Jayho Lee*[3], *Ji Yun Lee*[1], *Sang-Jip Nam*[4], *Sang Kook Lee*[1], *Ki-Bong Oh*[3], *Seokhee Kim*[2], *and Dong-Chan Oh*[1]. [1]*Natural Products Research Institute, College of Pharmacy,* [2]*Department of Chemistry, College of Natural Sciences, and* [3]*Department of Agriculture Biotechnology, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea, Department of Chemistry and Nanoscience, College of Natural Sciences, Ewha Womans University, Seoul 03760, Republic of Korea*

Cihunamides A-D (**1**-**4**), novel antibacterial RiPPs, were isolated from volcanic island-derived *Streptomyces* sp. The structures of **1**-**4** were elucidated as tetrapeptides composed of WNIW and cyclized by a unique C-N linkage between two Trp units. Genome mining of the producer strain revealed two biosynthetic genes encoding a cytochrome P450 enzyme and a precursor peptide. Heterologous co-expression of the core genes demonstrated the biosynthesis of

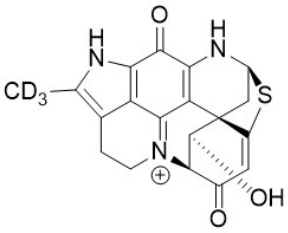
cihunamides via a P450-mediated oxidative Trp-Trp crosslinking. Cihunamides do not display non-canonical atropisomerism shown in tryptorubins, which are the founding members of the "atropitide" family. Therefore, we propose to use a new RiPP family name "bitryptides" for cihunamides, tryptorubins, and their congeners, wherein the Trp-Trp linkages define the structural class rather than non-canonical atropisomerism.

P-111 – Maria Orfanoudaki

Formation of Deutero-Methylated Artifacts of Pyrrole-Containing Natural Products

Maria Orfanoudaki,[1] Rhone K. Akee,[2,3] Lucero Martínez-Fructuoso,[2] Brian D. Peyser,[2] James A. Kelley,[4] Martin Schnermann,[4] Barry R. O'Keefe,[1,2] and Tanja Grkovic,[1,2] [1]Molecular Targets Program and [4]Chemical Biology Laboratory, Center for Cancer Research, NCI, Frederick, MD, USA, and [2]Natural Products Branch, Developmental Therapeutic Program, Division of Cancer Treatment and Diagnosis, NCI, Frederick, MD, USA, and [3]Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA

Pyrrole-containing natural products form a large group of structurally diverse natural products which occur in both terrestrial and marine organisms. In the present study the formation of deutero-methylated artifacts of pyrrole-containing natural products was investigated focusing on the class of pyrroloiminoquinones and specifically the discorhabdins. Three deuterated discorhabdins were isolated as artifacts of the isolation procedure caused by the presence of DMSO-d_6 during NMR sample preparation and handling. Five more semisynthetic derivatives were synthetized during the investigation of the mechanism of formation which was shown to be driven by deuteromethyl radicals in the presence of water, methanol, TFA, and traces of iron in the deuterated solvent. Generation of deuterated artifacts was also confirmed for other classes of pyrrole-containing metabolites, namely makaluvamines, tambjamines and dibromotryptamines which had also been dissolved in DMSO-d_6 during the structure elucidation process. The semisynthetic discorhabdins were assessed for their activity in the NCI-60 assay and 14-methyl discorhabdin L and 14-deuteromethyl discorhabdin L averaged micromolar potency against the cell lines tested.



P-112 – Harinantenaina Rakotondraibe

LC-MS and Proton NMR Spin Network Fingerprint Databases of Secondary Metabolites for Identification and Standardization of the Botanical Supplement *Centella asiatica*

Harinantenaina L. Rakotondraibe,[1,] Luke C. Marney,[2,3,4] Jaewoo Choi,[3,4] Liping Yang,[4,5] Nora Gray,[4,5] Jan F. Stevens,[2,4,5] Claudia S. Maier,[2,3,4] Amala Soumayanath[4,5]. [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA. [2]Department of Chemistry, Oregon State University, Corvallis, OR, USA. [3]Linus Pauling Institute, Oregon State University, Corvallis, OR, USA. [4]BENFRA Botanical Dietary Supplements Research Center, Oregon Health & Science University, Portland, OR, USA. [5]Department of Neurology, Oregon Health & Science University, Portland, OR, USA. [6]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR, USA*

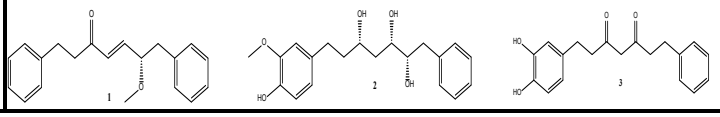
The botanical dietary supplement *Centella asiatica* (Apiaceae) is used in the United States for cognitive benefits and has been shown to not only activate Nrf2 in HepG2-ARE cells but also increase the expression of the antioxidant response gene Nrf2 *in vivo*. Due to the complexity of the metabolites present in these botanical supplement samples, their identification and standardization for clinical trial investigation remain challenges. Liquid chromatography coupled with mass spectrometry (LC-MS) is the most utilized method for detection, dereplication and characterization of metabolites because of the current refinement of ionization techniques and the expansion of the mass libraries of small molecules. Many of the constituents of *C. asiatica* however have not been fully determined and most isomers cannot be differentiated with current LC-MS methods. For this study, we have developed a comprehensive LC-MS coupled with Proton Spin Network Fingerprint database to quickly identify metabolites and standardize a sample of *C. asiatica*. This work was supported by the pilot project 1R03AT011872-01, funded by NIH/NCCIH, USA.

P-113 – Guijae Yoo

Chemical Constituents from the Rhizomes of *Alpinia Officinarum*

Guijae Yoo[1], Seung Hyun Kim[2], and Sang Yoon Choi[1]. [1]Korea Food Research Institute, Wanju 55365, Republic of Korea, [2]College of Pharmacy, Yonsei University, Incheon, South Korea

Alpinia officinarum (Zingiberaceae) is indigenous to Southeast China and Indochina, and has been used for the treatment of various diseases in traditional medicine. In this study, we isolated four new diarylheptanoids, (*S*,*E*)-6-methoxy-1,7-diphenylhept-4-en-3-one (1), (3*S*,5*S*,6*S*)-1-(4-hydroxy-3-methoxyphenyl)-7-phenylheptane-3,5,6-triol (2), 1-(3,4-dihydroxyphenyl)-7-phenylheptane-3,5-dione (3) and (4*Z*,6*E*)-1-(3,4-dihydroxyphenyl)-5-hydroxy-7-phenylhepta-4,6-dien-3-one (4) along with 48 known compounds, were isolated from the rhizomes of *A. officinarum*. Their structures were elucidated by extensive spectroscopic analysis including 1D, 2D NMR and HR-MS



P-114 – Korydwen Terrasson

New and Bioactive Compounds from the Branches of the Vietnam Plant *Beilschmiedia Yunnanensis*

Korydwen Terrasson[1,†], Ermias Mekuria Addo[1,†], Manead Khin,[2] Brenna Kirkpatrick,[2] Amanda Maldonado,[2] Tran Ngoc Ninh,[3] Harinantenaina L. Rakotondraibe,[1] Joanna E. Burdette,[2] Djaja D. Soejarto,[2,4] A. Douglas Kinghorn[1,] [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, 43210. [2]Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612. [3]Vietnam Academy of Science and Technology, Hanoi, Vietnam. [4]Science and Education, Field Museum, Chicago, IL 60605*

Beilschmiedia yunnanensis Hu (Lauraceae) is a tree endemic to southern China and Vietnam. A cytotoxic methanol extract obtained from its branches collected in Vietnam was subjected to bioassay-guided fractionation and isolation using various chromatographic techniques. Four new and eleven known compounds belonging to the phenylpropanoid, lignan, neolignan and flavonoid classes were isolated and characterized using NMR and mass spectrometry methods. Seven of the compounds were tested against the human OVCAR3 ovarian and the MDA-MB-435 melanoma cancer cells lines. Of those tested, the compounds 9′-*O*-(*E*)-feruloyl-5,5′-dimethoxylariciresinol and 9,9′-*O*-di-(*E*)-feruloyl secoisolariciresinol showed selective activity against the OVCAR3 cell line with IC_{50} values of 3.43 and 0.88 μM, respectively. The most active compound is currently being tested in an in vivo (hollow fiber) assay. The structure elucidation and the activity of these lignans are discussed in the poster presentation. This work was supported by program project P01 CA125066, funded by NCI, NIH, Bethesda, MD, USA.

P-115 – Paulo Vieira

Exploring Fungal Co-Cultivation to Access Chemical Diversity

Vitor de Souza Mazucato[1], and Paulo Cezar Vieira[1]. [1]Department of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14040-903, Brazil

The co-cultivation of microorganisms has been known as one important approach to chemical diversity production. This approach involves the cultivation of two or more fungal species together, which can lead to the production of unique chemical compounds that may not be observed when each species is grown separately. We are studying seven fungi (*Fusarium guttiforme*, *F. proliferatum Pestalotiopsis diospyri*, *Phoma caricae-papayae*, *Colletotrichum horii*, *C. gloeosporioides* and *Phytophthora palmivora*) isolated from papaya and pineapple. These fungi have been cultivated in various co-cultivation combinations and in different media such as PDB and rice. We have found that the chemistry has changed quite a lot when moving from axenic to cocultured strains. Also, the cocultivation in rice has yielded a much higher amount of extract with a chemical profile different from the PDB medium. The changes in the chemical composition have been followed through the analysis of the extracts and fractions by ^{1}H NMR where clear differences can be detected. This presentation will discuss the recent results obtained using this approach to chemical diversification focusing on a series of compounds belonging to different chemical classes identified as peptides, terpenoids, and carboxylic acids among others.

P-116 – Kojo S. Acquah

Variation in Specialized Metabolite Production by Bacterial Symbionts of the Hawaiian Bobtail Squid upon Challenge via Co-Culture or Unique Elicitors

Kojo S. Acquah[1], Mariam A. Zedan[2], Shekar Sunderesh[2], Kathryn R. McBride[1] and Marcy J. Balunas[1,3]. [1]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA, [2]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA, [3]Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA*

The Hawaiian bobtail squid, *Euprymna scolopes*, has been extensively studied due to its symbiosis with the bioluminescent bacterium, *Vibrio fischeri*, which colonizes the squid light organ. Adjacent to the light organ of the female squid is the accessory nidamental gland (ANG), another specialized organ that harbors a consortium of bacteria that are subsequently passed to eggs to provide protection during embryogenesis. Although the two organs are adjacent, *V. fischeri* has not been detected in either the ANG or egg jelly coats (JC), suggesting that ANG and JC bacterial symbionts may produce metabolites that deter colonization by *V. fischeri*. In addition, while many ANG bacteria have exhibited antimicrobial activity, some have not shown activity under normal laboratory culture conditions likely due to the presence of silent biosynthetic gene clusters (BGCs). In this study, we employed two modalities to elicit metabolite production, including co-culture of ANG strains with *V. fischeri*, as well as utilization of the rare earth element (REE) cerium to induce BGC expression. Although there was modulation of metabolite production in the induced cultures across the strains, *Leisingera* sp. ANG-DT elicited substantial metabolite production especially when induced with cerium. Comparative metabolomics of the monoculture and induced cultures of *Leisingera* sp. ANG-DT showed metabolites exclusive to co-culture and/or cerium induced cultures, suggesting that some BGCs are induced by specific elicitation methods. Isolation, identification, and bioactivity of induced metabolites from the large-scale culture of *Leisingera* sp. ANG-DT will be presented.

P-117 – Hadi Pourhadi

Fluorination of Diepoxin-η: Characterization and Anticancer Activity Evaluation of Semisynthetic Derivatives of Spirobisnaphthalenes

Hadi Pourhadi[1], Tamam El-Elimat[1], Manuel R. Grimaldo[1], Huzefa A. Raja[1], Cody Earp[1], Tyler N. Graf[1], Warren S. Vidar[1], Manead Khin[2], Nadja B. Cech[1], Joanna E. Burdette[2], Cedric J. Pearce[3], and Nicholas H. Oberlies[1]. [1]Depatment of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, USA. [2]Department of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA. [3]Mycosynthetix, Inc., Hillsborough, NC, USA

Diepoxin-η belongs to the spirobisnaphthalene class of fungal secondary metabolites. Since the initial description of the founder of this structural class, MK 3018 in 1989, more than two hundred spirobisnaphthalenes have been identified from fungi or synthesized. However, among these, there are only four fluorinated analogues reported, and all of those are from total synthesis (i.e., related to palmarumycin B6). In recent years, the introduction of fluorine into secondary metabolites has earned significant consideration due to enhancing the pharmacokinetic and pharmacodynamic profiles by changing acid/base properties, electronegativity, lipophilicity, and metabolic stability. For instance, it can be estimated that globally more than 20% of drugs in the market have at least one fluorine atom in their structure. As such, we pursued the semisynthesis of fluorinated analogues of diepoxin-η. Our investigation of the biological activity of the products illustrates micromolar inhibition against human ovarian (OVCAR3) and melanoma (MDA-MB-435) cancer cell lines. In addition, we have combined a variety of research tools and techniques to isolate and characterize these semisynthetic analogues.

P-117B – Zachary Kohanov

Current Progress on the Total Synthesis of Thermorubin

Zachary A. Kohanov[1], Suzzudul I. Shuvvo[1], Andrew N. Lowell[1]. Department of Chemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA, 24061, USA

An increase in the rise of antibiotic resistance in pathogenic bacteria is a growing health concern. FDA approvals have been limited to natural product derivatives, because discovery of new classes with unique mechanisms of action has become increasingly rare. Previously discovered antibiotics with novel mechanisms of action and limited past exploration warrant examination as potential candidates as a lead molecule. Of significant note is thermorubin (**1**), a tetracyclic antibiotic containing an α-pyrone and a salicylate moiety with the majority of functionality aligned along one side of the molecule. Thermorubin's unique bacteriostatic mechanism of action that disrupts protein translation by preventing protein elongation and keeping the ribosomal unit bound together means it holds promise as a potential antibiotic, although it must be obtained in larger quantities for study. We have devised a retrosynthesis with construction of the tetracyclic core originating from the mono or bis-sulfoxide **2a** or **2b** through annulations with **3** and **4**. Intermediate **2** is achieved by cyclization of acetylacetone **5a** or its disulfide analogue **5b** with dimethyl-3-oxoglutarate **6**. In addition to synthesizing thermorubin, the addition of the salicylate portion as a last step will allow for addition of functionalized salicylate derivatives.

ASP Summer Research Fellowship Posters P-118 – P-125

P-118 – Selome Banini

Development of Methods to Produce Novel Semi-Synthetic Norditerpenes to Treat Cryptosporidiosis

Selome Banini[1], Yudi Rusman[1], Harrison VanKoten[1], Mary Piaskowski2, Fernanda Fumuso2, Chidiebere Onoh2, Roberta O'Connor[2], Christine Salomon[1]. [1]Center for Drug Design, University of Minnesota, Minneapolis, MN 55414, USA, [2]Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108, USA

Cryptosporidium is an intestinal parasite that causes cryptosporidiosis and is one of the most common causes of diarrheal disease across the globe. Cryptosporidiosis is problematic for immunocompromised patients, and lacks any reliable and effective therapeutic options. We have identified a collection of norditerpene lactones from a subterranean fungus known as Oidiodendron truncatum that were tested and identified to have potent bioactivity against Cryptosporidium. The goal of this project is to develop methods to produce semi-synthetic terpene derivatives with improved bioactivity, low cytotoxicity, and ideal pharmacokinetic characteristics. Communic acids are labdane diterpenes that can be isolated from a number of different conifer species, and have been used to synthesize diverse bioactive terpenes. We are developing methods to efficiently extract, purify, and identify communic acids from Juniperus communis berries. We plan to utilize the isolated communic acids as building blocks to develop methods to produce a structurally diverse library of norditerpene lactones. The synthesized compounds will be tested and compared to the natural products and the structure activity relationships will be determined. The goal of this work will be to determine the best lead structure for future animal studies to develop a treatment for cryptosporidiosis in immunocompromised patients.

P-119 – Aria Gonzalez

Characterizing the Structure Activity Relationship of Lipids that Induce Cellular Aggregation within *Capsaspora owczarzaki*

Aria Gonzalez[1], Lorin Brokaw[1], Ria Kidner[1], J.P. Gerdt[1]. [1]Department of Chemistry, Indiana University Bloomington, 800 East Kirkwood Avenue, Bloomington, IN 47401

Symbioses dictate the behavior of organisms on earth. An understudied symbiosis exists between a freshwater snail, *Biomphalaria glabrata*, and a protozoan, *Capsaspora owczarzaki*. Our lab has recently shown that lipids present in *Biomphalaria* hemolymph trigger cellular aggregation in *Capsaspora*. In order to better understand this phenomenon, we performed a structure-activity relationship study to compare the activity of various lipids. Lipids containing a zwitterionic head group tended to be more active than lipids containing a positively or negatively charged head group. Additionally, to see more robust aggregation, lipids need a tail containing at least one degree of unsaturation as well as more than sixteen carbons. The trends discovered in this SAR study will help us to further characterize this cellular behavior, which may shed light on this understudied symbiosis between *Capsapora* and its host snail, *Biomphalaria.*

P-120 – Melvin Osei Opoku

Discovery of Novel Terpenoids from Predicted Terpene Synthases Genes

Melvin, Alsup, Tyler A., Jeffrey D. Rudolf, Department of Chemistry,Chemical Biology Division, University of Florida

Natural products are chemical compounds produced by organisms and they possess pharmaceutical and commercial relevance; contributing to about 50% of commercial drug products[2]. Terpenoids are the largest and most structurally diverse family of natural products [2]. Surprisingly, the fascinating mechanisms of how terpene synthases (TSs) make terpenes are not fully understood. Approximately 50,000 terpenoid metabolites, encompassing nearly 400 different structural families, such as monoterpenes, sesquiterpenes, and diterpenes, have been found in both terrestrial and marine plants, liverworts, and fungi. However, only a small portion of these widespread metabolites have been identified in prokaryotes. Our research aims to identify novel terpene scaffolds by screening predicted bacterial TS genes in an engineered terpene precursor overproduction system.

P-121 – Jorge Hernandez Garcia

Identifying Novel Chemical Scaffolds from Marine Derived Fungi using Tandem Mass Spectrometry based Molecular Networking

Jorge Hernandez Garcia & Erin McCauley. Department of Chemistry & Biochemistry, California State University Dominguez Hills, Carson, CA 90474, USA

One of the major challenges in natural products drug discovery is the redundant purification and structure elucidation of previously identified compounds. The objective of this research was to use Tandem Mass (MS/MS) Spectrometry and the Global Natural Products Social (GNPS) Molecular Networking platform to identify novel secondary metabolites from marine-derived fungi. To achieve this over 100 taxonomically distinct marine-derived fungal strains were grown in five different media types. The resulting extracts were analysed using LC-MS/MS and evaluated using the Library Search and Molecular Networking of the GNPS platform. Putatively novel secondary metabolites were purified and structurally elucidated.

P-122 – Anna Pons

Biosynthesis of Pseudovibriamides and its Genetic Complementation of a Knock-Out Mutant

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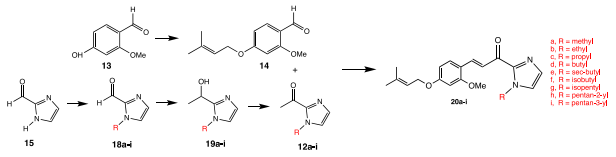
Pseudovibriamides A, B, and C (PA, PB, PC) are non-ribosomal peptides-polyketide hybrids isolated from *Pseudovibrio brasiliensis* Ab134, a bacterium associated with marine sponges (Ioca *et al*. 2021, PMID: 33961724). Evidence suggests that *Pseudovibrio* are beneficial to marine sponges (Romano 2018, PMID: 29453252). Pseudovibriamides affect *Pseudovibrio*'s motility and biofilm formation, behaviors that are important for host colonization. To elucidate the biosynthesis of the pseudovibriamides, a library of knockout mutants was generated. The gene *ppp*A encodes for a non- ribosomal peptide synthetase involved in the biosynthesis of PA, a heptapeptide. The ⊙⊙*ppp*A mutant does not produce PA, as expected, and motility and biofilm are reduced in this mutant. In this project, the ⊙⊙*ppp*A mutant will be complemented with a plasmid containing the *ppp*A gene aiming to restore the production of pseudovibriamides. This plasmid will be built through Gibson assembly and it will be transferred into *P. brasiliensis* Ab134 using conjugation from *E. coli* S17-1. Once mutants are obtained, metabolite analyses will be performed, along with motility and biofilm assays. Genetic complementation is important to confirm that the observed phenotypes are indeed due to loss in PA biosynthesis. It is also a prospective technique that can be used to engineer new compounds by replacing *pppA* with other genes.

P-123 – Roxana Gonzalez

Synthesis and Antiproliferative Evaluation of Licochalone A-Inspired Chalcones in Cancer Cell Models

Roxana Gonzalez, Esveidy Oceguera, Guanglin Chen, and Qiao-Hong Chen. Department of Chemistry and Biochemistry, California State University-Fresno, Fresno, CA 93740, USA

Licochalone A (LA), a naturally occurring chalcone isolated from licorice root, was revealed to have micromolar antiproliferative activity in various cancer cell lines including prostate and breast cancer cells. Inspired by our previous success in enhancing the antiproliferative potency of curcumin by replacing the substituted phenol with a 1-alkyl-1H-imizadol-2-yl moiety, the same strategy was employed to design a group of new LA-inspired chalcones for evaluation of their anti-proliferative activity in prostate and breast cancer cell models. Nine target chalcones were designed, and the synthetic approach to these target chalcones has been secured. One target compound and several intermediates towards the remaining target chalcones have been achieved, which have been characterized through ^{1}H-NMR, ^{13}C-NMR, IR, and HRMS. The antiproliferative activity of the synthetic chalcone has been evaluated using WST-1 cell proliferation. Intriguingly, the synthetic chalcone demonstrated a greater potency towards androgen receptor-positive cancer cells.



P-124 – Ma. Andrea Gatmaitan

Cockroach-Associated Bacteria and Their Antimicrobial Activity

Ma. Andrea Gatmaitan[1], José D.D Cediel-Becerra[2], Julia Charske[3], Mark Ou[2], and Marc G. Chevrette[2,4]. [1]Department of Biology, University of Florida, Gainesville FL, 32611, [2]Department of Microbiology and Cell Science, University of Florida, Gainesville FL, 32611, [3]Department of Food Science and Nutrition, University of Florida, Gainesville FL, 32611, [4]University of Florida Genetics Institute, Gainesville FL, 32610

With the issue of antimicrobial resistance on the rise, the quest for novel antimicrobials has gained utmost importance. Traditionally, most antibiotics have been sourced from soil-dwelling *Streptomyces* species. However, recent studies discovered insect-associated *Streptomyces* with promising bioactive molecules against antimicrobial-resistant pathogens, such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. Cockroaches, notorious for their resilience in extreme environments, may possess unique bacterial communities that contribute to their remarkable adaptability. In this study, we isolated bacteria from the American cockroach *Periplaneta americana* and the Australian cockroach *Periplaneta australasiae* to explore their potential as a source for natural products. With 16S rDNA gene sequencing, we characterized the bacterial diversity residing in their microbiome. Additionally, we evaluated the inhibitory effects of these bacterial isolates through bioactivity assays against clinically relevant target strains such as *Acinetobacter baylii* and *Pseudomonas aeruginosa*. Future directions include employing genome mining approaches in promising strains to identify biosynthetic gene clusters to uncover the natural products encoded by those species.

P-125 – Jacqueline Vargas

Elicitation and Secretion of Specialized Metabolites in Hairy Root Cultures of Annatto (*Bixa orellana*)

Jacqueline R. Vargas Ulloa[1,3], Gaurav Gajurel[1,2], and Fabricio Medina-Bolivar[1,3]. [1]Arkansas Biosciences Institute, [2]Molecular Biosciences Graduate Program, [3]Department of Biological Sciences, Arkansas State University, Jonesboro, AR 72401, USA

Plants are a major source of drug discovery since they produce a great diversity of specialized metabolites. Annatto (*Bixa orellana*) is a plant native to South America that has been used in traditional medicine for its antibacterial, anti-inflammatory, and antimalarial properties. These biological activities can be largely attributed to specific types of terpenoids which can be produced in response to stress. Hairy roots allow for the stable production of metabolites as they can grow indefinitely in liquid medium and are not affected by environmental factors. In this study, annatto hairy root cultures were stressed with chemical elicitors (methyl-β-cyclodextrin, methyl jasmonate, hydrogen peroxide, and magnesium chloride) to induce terpenoid production and establish a sustainable production platform for these compounds. After 192 hours of elicitor treatment, the compounds that were secreted into the culture medium were extracted using ethyl acetate and analyzed by high-performance liquid chromatography. Non-elicited hairy root cultures were used as a control. While there was no compound production in the control, the elicited cultures yielded six compounds with similar absorption spectra. Ongoing work focuses on purifying these compounds and elucidating their structure. Ultimately, this research will enable the identification of novel specialized metabolites with potential health applications.

P-126 – Lesley-Ann Giddings, Brian Murphy and Christine Salomon

Equity in Action: The ASP Summer Research Fellowship Program

Lesley-Ann Giddings[1], Brian Murphy[2], and Christine Salomon[3]. [1]Department of Chemistry, Smith College, Northampton, MA 01063, Department of Pharmaceutical Sciences, [2]University of Illinois at Chicago, Chicago, IL 60607, [3]Center for Drug Design, University of Minnesota, Minneapolis, MN 55405

One critical challenge facing STEM fields in the US, which is reflected within the ASP, is a lack of racial and ethnic representation among our membership, leadership, award recipients, and meeting speakers. To correct for the underrepresentation of Black, Indigenous and Latinx (BIL) students in our field, the ASP DEI and Executive Committees, ASP Foundation, and ASP Fellows collaborated to develop the Summer Research Fellowship (SRF) program. The program offers 1) a 2.5 month stipend for students to engage in research under a mentor in the natural product sciences; 2) 11 weekly online professional development workshops focused

on science communication as well as exposure to graduate programs and careers in natural products; and 3) a special online ASP webinar for students to present their research to an international audience. The first two cohorts (2021-2022) were highly successful, with seven students accepted into graduate or professional programs and several others receiving prestigious research fellowships and awards (remainder of the cohorts are still engaged in undergraduate studies). Importantly, most students in the first cohorts are continuing to pursue research in the field of natural products chemistry. Students in the third cohort (2023) have begun their research, participating in the weekly workshops and preparing for their presentations. All eight current SRF students are attending the 2023 ASP meeting and presenting posters, so please be sure to visit their posters and welcome them. The DEI committee is is seeking additional support through grants to continue the successful SRF program.

P-127 – ASP Younger Members Committee

Natural Product Careers and Opportunities

ASP Younger Members Committee

The ASP membership possesses a diverse range of highly sought-after skills. Nonetheless, navigating the digital era to identify the next career move can be an intimidating challenge, as a single competitive position may attract hundreds of applicants. The ASP annual meeting serves as an invaluable platform to facilitate face-to-face connections and discussions about employment opportunities with other members. This space is reserved for the assortment of academic, industrial, and government positions offered by companies and academics actively seeking natural product talent. Stop by and explore the current available positions, and discover the key individuals to engage with for networking for future career opportunities. ARE YOU LOOKING FOR STUDENTS, POSTDOC's, EMPOLYEES?? – bring your printed job ad and post it or email it to scarlson1@pacific.edu and we will post it for you!

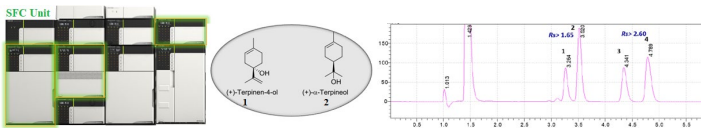
Poster Presentations Session II

P-128 – Shagufta Perveen

A Fast and High-resolution Supercritical Fluid Chromatography Method for the Chiral Separation of Tea Tree Oil Enantiomeric Components

Shagufta Perveen[1], Hongtao Yu[1,], Jiangnan Peng[1,*]. [1]Department of Chemistry, Morgan State University, MD21251*

The enantiomer ratios of tea tree oil (TTO) components are characteristic for the authentication of TTO. Currently, ISO and European Pharmacopoeia measure these ratios using a chiral GC method, which has a running time of 60 minutes without baseline separation of the enantiomers. Here, we report an eco-friendly and fast supercritical fluid chromatographic (SFC) method for the chiral resolution of the TTO key components, *RS,* (+/-) terpinen-4-ol and *RS* (+/-) α-terpineol. A polysaccharide-based Lux Amylose-1 chiral column was chosen to separate the enantiomers. Resolution values of > 1.6 and > 2.5 were reached within 5 minutes for *RS,* (+/-) terpinen-4-ol and *RS* (+/-) α-terpineol, respectively. This method is validated with good linearity, repeatability, and recovery.



P-129 – Qingxi Su

Identification of Sweet Modulators from a Botanical Extract by Combination of Sensory Guided-Fractionation and Untargeted Metabolomics Approach

Qingxi Su, Zhichun Shang, Li Chen, Yanpeng Hou, Niels Christensen, Jack Bikker, Jing Li, Jeanmarie Carr, Diana Klaser Cheng. International Flavors and Fragrances, Union Beach, NJ 07733

Sweetness modulation is a technology to address flavor perception challenges with sugar-reduced foods and beverages. Many non-caloric sweeteners in the market, such as steviol glycosides and mogrosides, have higher sweetness intensity than sucrose, but possess lingering and bitter aftertastes that consumers dislike or an absence of sugary mouthfeel that matches the intensity. Plants are a vast source of chemical diversity for discovering functional molecules that can modify the perception of flavor. In this study, we identified compounds from a botanical extract that can increase the perception of sweetness. These compounds, or extracts containing these compounds, can be applied as flavorings in foods and beverages to improve the overall taste quality especially for sugar free or low sugar applications. We performed sensory-guided fractionation, that led to isolation of seven sweetness modifying compounds. As a complimentary approach, we also performed LCMS-based untargeted metabolomics. LC/MS/MS profiles of the extracts were correlated with sensory panel data on perceived sweetness intensity using multivariate latent class analysis. The untargeted metabolomics approach led to the identification of three additional sweetness modifying compounds; one of which is a novel structure with a similar scaffold. Additionally, all the identified compounds are newly reported to display sweetness modifying effects. Thus, we demonstrated the combination of taste-guided fractionation and untargeted metabolomics is a powerful approach to discover compounds with taste modifying properties.

P-130 – Mei Wang

Quality Evaluation of Peppermint Oils and Commercial Products: An Integrated Approach Using Conventional and Chiral GC/MS Combined with Chemometrics *Shukria*

Mei Wang[1], Joseph Lee[2], Jianping Zhao[2], Shamba Chatterjee[2], Amar G. Chittiboyina[2], Ikhlas A. Khan[2,3].[1]Natural Products Utilization Research Unit, Agricultural Research Service, United States Department of Agriculture, University, MS 38677, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, [3]Divison of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677

Peppermint essential oil (EO) has a multitude of applications such as a fragrance in cosmetics, personal care, and industrial products; or as a flavoring ingredient in food and beverages. Despite its popularity and economic significance, peppermint EO is often adulterated in order to reduce production costs and to increase profits. Although ISO standards for peppermint EO exist, detecting sophisticated forms of adulteration remains challenging. In the current study, GC/MS, chiral GC/MS, and chemometric techniques were used to evaluate an extensive set (n = 58) of peppermint oils and commercial products purported to contain peppermint oil. Specifically, thirty-six terpenoids were examined in each sample and compared with the ISO standards. Seventy-five percent of the commercial products did not meet the ISO specifications. Chiral GC/MS was used to measure eight terpenoids, *viz*. α-pinene, β-pinene, limonene, isomenthone, menthone, menthol, pulegone, and menthyl acetate. The enantiomeric compositions of 28 commercial products were above or below the norm measured from authentic peppermint oils. Of the 28 samples, one met the ISO standards. Using authentic oils, a class prediction model based on partial least squares-discriminant analysis (PLS-DA) was constructed. The model can distinguish the most common types of peppermint oils (US, India, and US/India blend) sold in US market and was then used to analyze commercial samples. Overall, by combining conventional and chiral GC/MS along with chemometrics, the quality and authenticity of peppermint EO can be easily assessed.

P-131 – Jianping Zhao

Chemometrics-aided NMR for Differentiation and Characterization of Peppermint Essential Oil

Jianping Zhao [1], Mei Wang [2], Joseph Lee [1], Zulfiqar Ali [1], Ikhlas A. Khan[1]. [1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677; [2]Natural Products Utilization Research Unit, ARS. Department of Agriculture, University, Mississippi 38677, USA

The plants of the Mentha genus are generally aromatic and can be used to produce essential oils – referred to as "mint oils". Peppermint oil, a mint oil obtained from the fresh overground parts of *Mentha piperita*, has been widely used in foods, beverages, soaps, and cosmetics. It is also used for health purposes to address a number of conditions, such as common cold, sinus infections, headaches, and mental problems. The mint oil obtained from *Mentha arvensis*, called cornmint oil, is another commercially important essential oil with massive production but is less expensive than peppermint oil. It was reported that the peppermint oil market is vulnerable to adulteration as ingredients are not always consistent with label claims. There is a critical need to develop methodologies for the authentication and characterization of peppermint oil. In this study, the variation and distribution of chemical composition in peppermint oil samples (n = 124) were evaluated, and the outlier samples were detected and explored for possible adulteration based on NMR and chemometric analysis. As the result, significant variation in the chemical composition was observed for some of the investigated samples by comparing their NMR spectral fingerprints, and the adulterants, such as triethyl citrate and bis(2-ethylhexyl) adipate, were identified in some of the commercial samples obtained from the market.

P-132 – Hyo-Jung Kwon

Protective Effects of *Prunella Vulgaris* Extract (HFN2-007) on Testosterone-Induced Benign Prostatic Hyperplasia in Rats

Eun Bok Baek, Eun-Ju Hong, Jee Hyun Kang, Kyu-Pil Lee, Hyo-Jung Kwon. College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea

Benign prostatic hyperplasia (BPH) is a urogenital disorder that affects approximately 85% of males who are over 50 years of age. *Prunella Vulgaris (PV)*, a well-known traditional medicinal plant, is used for the cure of abscess, scrofula, hypertension and urinary diseases. The present study evaluated the therapeutic effects of PV extract (HFN2-007) in the BPH animal model. BPH was induced in rats via subcutaneous (sc) injections of testosterone propionate (TP). Rats were also administered daily oral gavage of HFN2-007 or vehicle. After four weeks of induction, all animals were euthanized humanely and their prostate glands were removed, weighed and processed for further analysis. HFN2-007 treatment significantly reduced the prostate weight, epithelial thickness, and proliferating cell nuclear antigen (PCNA) expression, with the levels of cleaved caspase-3 and Bcl-2-associated X (Bax) protein considerably increased compared to BPH group. HFN2-007 also decreased inflammatory cell infiltration and pro-inflammatory cytokine levels compared with BPH group. Furthermore, the expression of phosphor-nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) were reduced by HFN2-007 treatment. These results indicate that HFN2-007 suppresses the development of BPH associated with anti-proliferative, pro-apoptotic, and anti-inflammatory effects, suggesting it is a potential alternative therapeutic agent for BPH.

P-133 – Zachary Lane

Hydroxamic Acids and their Potential Applications as Glycolytic Inhibitors Against Enolase

Zachary Lane, Jolanta Krucinska, Lee Wright, and Dennis Wright. Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA

Over the last 85 years there has been a desire to generate new antibiotics. This is largely in part due to the resistance that microbes have begun to generate and has led to about 35,000 deaths annually in the United States. With the emergence of new resistances and increased spreading from these microbes, generation of new antibiotics from natural products offers alternatives that can be pursued. Hydroxamates, or hydroxamic acids, comprise of a carbonyl attached to a hydroxylamine and are common secondar metabolites that have been demonstrated to have many therapeutic applications. These compounds have been demonstrated to have enolase inhibition activity, an essential step in the glycolytic pathway for the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Like a lot of biological enzymes, enolase functionality is depended on a divalent interaction between magnesium ions. The hydroxyl and carbonyl functional groups of the hydroxamate have been demonstrated to be a potential key player for magnesium binding and enolase inhibition. In this study, we are analyzing three compounds, SF-2312, alahopcin and dealanylalahopcin. These are secondary metabolites produced by microbial species are suggestive of enolase inhibition and continued studies will help to determine the crucial functional groups necessary for magnesium interaction and enolase inhibition.

P-134 – Emily Mevers

Discovery of a Complex Terpene Produced by Fungus-Growing Ants Bacterial Symbiont

Paige Banks,[1] Munhyung Bae,[2] Eric Helfrich,[3] Jon Clardy,[4] Emily Mevers[1]. [1]Department of Chemistry, Virginia Tech, Blacksburg, VA, USA, [2]College of Pharmacy, Gachon University, Incheon, South Korea, [3]Institute of Molecular Bioscience, Goethe University Frankfurt, Frankfurt, Germany, [4]Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Natural products have played a critical role in drug discovery and innovation for many decades, and this is especially true for the treatment of infectious diseases. The success of natural products in the clinic is due to their evolutionary history, their structures and functions having evolved over millions of years of selective pressures to carry out an essential role for the producing organism. One particularly important role is the production of defensive metabolites by symbiotic microorganisms to protect their eukaryotic host. Recent investigations into a bacterial symbiont from fungus-growing ants has led to the discovery of a new complex metabolite that likely defends the host from fungal pathogens. The producing bacterial strain, *Pseudonocardia* sp., showed impressive antifungal activity in binary intruder assays. Bioassay-guided isolation and structure elucidation revealed that the active metabolite belongs to a novel class of highly modified C35 terpene that incorporates a high degree of oxidation, and new sugar moiety. This unprecedented structure raises additional unanswered biosynthetic questions and potential biomedical applications.

P-135 – Edwin Chavez Santana

Identification of Natural Products that Exhibit Antibiotic Activity Against *Pseudomonas Aeruginosa*

Edwin Chavez Santana & Erin McCauley. Department of Chemistry & Biochemistry, California State University Dominguez Hills, Carson, CA 90747, USA

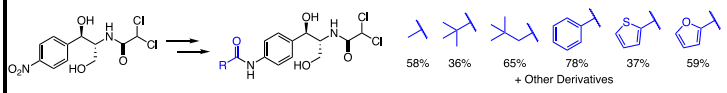
A major challenge facing the future of human health is the rise of antibiotic-resistant pathogenic bacteria. In 2017 the World Health Organization (WHO) published a list of 12 pathogens that will pose the greatest threat to human health due to antibiotic resistance. The objective of this research was to identify natural products from marine-derived fungi that have antibiotic activity towards an antibiotic-resistant strain of *Pseudomonas aeruginosa.* To achieve this over 100 taxonomically distinct marine-derived fungal strains were cultured in a minimum of five different media types, and their extracts were screened against *P. aeruginosa* using a minimum inhibitory concentration (MIC) assay. For any extract that exhibited the desired activity the metabolites present in the extract were purified and structurally elucidated.

P-136 – Suzzudul Islam Shuvo

A Semisynthetic Approach of Chloramphenicol Derivatization

Suzzudul Islam Shuvo, Logan M. Breiner, Andrew N. Lowell, Department of Chemistry, Virginia Polytechnic and State University, Blacksburg, VA 24061, USA*

Chloramphenicol, a peptidyl transferase inhibitor, binds to the 50S ribosome and hinders protein synthesis. Among the different functional groups this molecule possesses, the *p*-nitro group is uncommon and a known toxicophoric, which may be responsible for the off-target effects of this antibiotic. Replacing the *p*-nitro group has been a common strategy for chloramphenicol derivatization, but these derivatives have been done synthetically so far. To improve chloramphenicol's activity and lower toxicity, we are replacing the *p*-nitro group with an amide by semisynthetically modifying the natural product, creating novel derivatives. By creating a series of these derivatives, we can investigate their activity and toxicity. The lead molecule will be subjected to additional modification with a view to further improving the drug.



P-137 – José D.D Cediel-Becerra

PreQ0 Gene Clusters in *Streptomyces* Reveal an Unexplored Biosynthetic Reservoir

José D.D Cediel-Becerra[1], Valérie de Crécy-Lagard[1,2], and Marc G. Chevrette[1,2],[1] Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA, [2] University of Florida Genetics Institute, University of Florida, Gainesville, FL, USA

Genome mining has become an essential approach to unearthing the diversity of natural products in the microbial world, including antibiotics, anti-cancer compounds, and photoprotectants. Uncovering new natural products through mining non-canonical BGCs (*i.e.*, those not detected by algorithms such as antiSMASH), remains a challenge. 7-cyano-7-deazaguanine ($PreQ_0$) is a molecule involved in nucleoside modifications (in DNAs and tRNAs) and serves as the scaffold for the synthesis of pyrrolopyrimidine nucleosides, such as the antibiotic's toyocamycin and sangivamycin in *Streptomyces rimosus.* $PreQ_0$ itself has anti-cancer activity, suggesting that natural products incorporating $PreQ_0$ may have bioactive properties. As expected, the $PreQ_0$ loci elude identification by the antiSMASH algorithm, which currently lacks any $PreQ_0$ BGC identification rules. To address this challenge, we have developed a pipeline to accurately identify and delimit the boundaries for $PreQ_0$ BGCs using *S. venezuelae* ATCC 10712 as a focal strain for $PreQ_0$ BGC organization. Interestingly, according to the metrics tested, the top-scoring strains possessed the main proteins for $PreQ_0$ biosynthesis (*i.e.*, FolE, QueD, QueE, and QueC), and appear to be novel putatively BGCs as they have different genomic contexts than toyocamycin and nucleic acid modifying $PreQ_0$ loci. Some strains evidenced insertions/deletions (with scarce annotations) in the proposed pathway. The putative $PreQ_0$ BGC variations found here are being investigated by evolutionary approaches to prioritize strains for BGC structure elucidation based on their genomic signatures.

P-138 – Trevor Clark

Annotation Before Investment: Applying Similarity Network Fusion o Natural Products Discovery

Suzie K. Hight[1], Trevor N. Clark[2], Kenji L. Kurita[2], Elizabeth A. McMillan[1], Walter Bray[3], Anam F. Shaikh[2], Aswad Khadilkar[3], F. P. Jake Haeckl[2], Fausto Carnevale-Neto[2], Scott La[3], Akshar Lohith[3], Rachel M. Vaden[1], Jeon Lee[4], Shuguang Wei[1], Scott R. Lokey[3], Michael A. White[1], Roger G. Linington[2], and John B. MacMillan[3].[1]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, [2]Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada, [3]Department of Chemistry, University of California Santa Cruz, Santa Cruz, CA 95064, [4]Department of Bioinformatics, University of Texas Southwestern Medical Center, Dallas, TX 75390

Natural product isolation is a time-consuming process, often burdened by low yields, difficult isolation procedures, and complex structures to elucidate. Recently the HIFAN consortium developed Similarity Network Fusion (SNF), a platform that sophistically combines data from multiple bioassay platforms, to improve biological assessment prior to isolation efforts. Using SNF analysis combined with metabolomics data from 628 microbial extracts and 2027 pure standards from a Selleck library, six chemical targets from four different extracts/mechanism of action (MOA) clusters were prioritized for isolation. The first three extracts were found in clusters that also contained compounds from the Selleck library giving concrete MOA prediction, i.e the compound, trichostatin A, a known HDAC inhibitor, whose extract was found to cluster with other HDAC inhibitors. Examination of the cluster with the unique MOA profile, a cluster comprised solely of natural product extracts, led to the discovery of parkamycins A and B, two azoxy-containing biaryl compounds. The MOA of parkamycin A was explored by profiling gene transcripts for up and down regulation at 10 μM. Several genes and their associated metabolic pathways have now been linked to with parkamycin A and will be detailed.

P-139 – Hua Deng

Apply Machine Learning to Determine Cannabinoids Concentration in Cannabis

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Cannabis has gained legal and social acceptance in North America and globally, leading to an increasing demand for quick and on-site determination of major active components. This study presents a machine learning based analytical pipeline to predict the concentration of CBDA, total CBD, and total THC based on FTIR spectroscopy. A total of 122 samples were collected from Maryland farms, and their FTIR spectra were acquired. The concentration of CBD and THC varieties obtained from HPLC were used as standards (or target variables). The FTIR spectra underwent outlier detection, baseline correction, smoothing, and normalization, followed by LASSO regression and logistic regression with an 80:20 train-test ratio. The coefficients of determination (R^2) were 0.92 and 0.91 for CBDA and total CBD, respectively. Determined total THC concentration values were directly used to distinguish Industry Hemp from marijuana using 0.3% as the threshold, with an accuracy of 96%. Different machine learning models were also compared in terms of accuracy and applicability. This study provides an example of quantitative analysis of botanical samples using traditional spectroscopic data with assistance from machine learning models.

P-139B – Jiah Kim

Comparative LC-MS and GC-MS Analysis of Metabolites from Medicinal Herb Plants Stem and Root Part Extract

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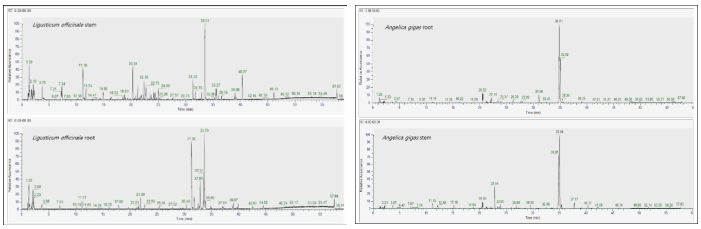
Ligusticum officinale and *Angelica gigas* are highly used in traditional medicine, and its root is valued for primary and secondary metabolite contents. However, evidence suggests that other plant parts such as leaves and stems are also potential sources of bioactive compounds. Therefore, this study aimed to provide insight into the differential accumulation of metabolites in stem and root parts collected from two species of medicinal herb plants. We conducted secondary metabolite profiling analyses of extracts of *L. officinale* and *A. gigas* using Liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS). Twenty secondary metabolites were profiled in *L. officinale* and *A. gigas*, respectively. The kinds of and contents of secondary metabolites were different by stem and root parts of each species. Also, primary metabolite contents contained in stems and roots were shown to the different depending on the plant parts. The accumulation pattern of primary and secondary metabolite in different tissues or organ is highly suggestive of utilizing leaf, and stem tissues along with roots for value addition of *L. officinale* and *A. gigas*.



P-140 – Arvie Grace Masibag

Membrane Vesicles as Microbial Delivery Systems for Mediating Competition in Pathogen-Probiont Interactions

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Membrane vesicles (MVs) are nanosized single- or double-membraned particles produced by bacteria through blebbing and, for some, explosive cell rupture. MVs may contain biologically active components, such as lipopolysaccharides (LPS), phospholipids, and outer membrane proteins (OMPs), as well as periplasmic components, allowing MVs to be involved in diverse cellular processes such as cell-to-cell communication, stress responses, antimicrobial resistance, horizontal gene transfer, and immune evasion. MVs can also package virulence factors and specialized metabolites, thereby contributing to host colonization, microbial competition, and infection-associated pathology. In this study, MVs were isolated and purified from the marine bacteria *Pseudoalteromonas piscicida* JC3, *Vibrio coralliilyticus* RE22, *Vibrio parahaemolyticus* PSU5579, and *Phaeobacter inhibens* S4. *Vibrio* strains RE22 and PSU5579 are notable for their ability to cause disease outbreaks in oyster and shrimp aquaculture systems, respectively. JC3 and S4 are putative probiotic strains that show promise as microbial additives to mitigate host infections. The MVs were subjected to an array of *in vitro* assays to assess their possible roles in microbial interactions. MVs were found to demonstrate various biological effects, including antibacterial activity, alteration of biofilm formation, and iron-binding properties, suggesting their role in microbial competition and nutrient acquisition. The results demonstrate that MVs produced by phylogenetically diverse marine bacteria exhibit ecological roles for mediating bacterial and environmental interactions.

P-141 – Dhammika Nanayakkara

Molluscicidal Activity of Selected Essential Oils against *Biomphalaria Havanensis* and *Planorbella Trivolvis*

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The trematode *Bolbophorus damnificus* causes losses in catfish aquaculture in the Southeastern United States. Two snail species, *Biomphalaria havanensis* and *Planorbella trivolvis* are known to transmit this infection to catfish. The most practical way to control this infection is to eliminate snails in catfish production ponds. Copper sulfate ($CuSO_4$) is currently used for this purpose. However, its usefulness is limited by its toxicity towards non target species. As part of a collaborative project to search for selective and environmentally benign snail controlling agents, we evaluated five commercially available essential oils for molluscicidal and ovicidal activities using copper sulfate as the positive control. Some of these oils showed potent molluscicidal and ovicidal activities. We also evaluated major constituents present in active oils for these activities.

P-142 – Victor Ribeiro

Antifungal and Cytotoxicity Activity of the Main Compounds From Bazilian Red, Green and Brown Propolis

Victor P Ribeiro[1], Prabin Tamang[2], Jairo K. Bastos[3] and Kumudini M Meepagala[2]. [1]ORISE fellow-Agricultural Research Service, U.S. Department of Agriculture, University of Mississippi, MS, 38677, USA. [2]Natural Products Utilization Research Unit, USDA-ARS – University of Mississippi. 3 School of Pharmaceutical sciences of Ribeirão Preto, Brazil

Due to a vast biodiversity, Brazil has many different types of propolis. The main types of Brazilian propolis are green, red and brown. Our objective was to isolate the major compounds of these tree main types of Brazilian propolis and evaluate their cytotoxic and antifungal activities. From green propolis, were isolated kaempeferol, drupanin, artepellin C and baccharin. Red propolis furnished vestitol, isoliquiritigenin, formonometin, neovestitol, methylvestitol, medicarpin, oblongifolin A and gutiferone E. From Brown propolis isopimaric acid, desydroabietic acid, communic acid and totarol were isolated. Cytotoxicity was determined in four tumor cell lines BT-549, SK-MEL, SK-OV-3, KB and two normal cell lines VERO and LLC-PK1 at the range of concentrations 100 – 0.78 μg/mL. Pronounced cytotoxicity activities were observed for medicarpin with IC_{50} of 1.8 μg/mL against SK-OV-3 and communic acid with 3.5 μg/mL against KB cell lines. All isolated compounds indicated no cytotoxicity against normal cell lines evaluated. The TLC bioautography assay was conducted to evaluate the antifungal activity of these fractions against the fungal plant pathogen *Colletotrichum fragariae*. Compounds isolated from green propolis drupanin and baccharin and compounds isolated from red propolis vestitol, medicarpin and neovestitol showed the major inhibitory zones against *C. fragariae.*

P-142B – Mee-Young Lee

Anti-inflammatory and Anti-allergic Effects of Cheonwangbosim-dan Wwater Extract: An *in vitro* and *in vivo* Study

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Cheonwangbosim-dan is a traditional herbal prescription that is widely used to improve or treat physical and mental illnesses in East Asian countries. The aim of the present study was to investigate the preventive and protective effects of a Cheonwangbosim-dan water extract (CBDW) against allergic inflammation using *in vitro* and *in vivo* models. BEAS-2B and MC/9 cells were treated with various concentrations of CBDW and stimulated with different inducers of inflammatory mediators. The production of various inflammatory mediators was subsequently evaluated. BALB/c mice were sensitized and challenged by repeated application of ovalbumin (OVA). CBDW was administered by oral gavage once daily for 10 consecutive days. We assessed the number of inflammatory cells and production of Th2 cytokines in bronchoalveolar lavage fluid (BALF), the plasma levels of total and OVA-specific immunoglobulin E (IgE), and histological changes in lung tissue. Our findings showed that CBDW significantly decreased the levels of various inflammatory mediators (eotaxin-1, eotaxin-3, RANTES, LTC_4, TNF-α, MMP-9, 5-LO, ICAM-1, and VCAM-1) *in vitro*, significantly reduced the accumulation of total inflammatory cells, the production of Th2 cytokines (IL-5 and IL-13), the levels of IgE (total and OVA-specific) *in vivo*, and remarkably inhibited histological changes (infiltration of inflammatory cells and goblet cell hyperplasia) *in vivo*. These results suggest that CBDW possesses anti-inflammatory and anti-allergic properties by lowering allergic inflammation.

P-143 – Reema Al-Qiam

Increased Conversion of Hypocrellins to Hypomycins Under Anaerobic Growth Conditions

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Hypocrellins and hypomycins are two main subgroups of fungal perylenequinones with unique photochemical and redox properties. These naturally occurring photosensitizers are known to readily generate singlet dioxygen ($^1O_2$) upon light-activation acting as phototoxins. It was previously proposed that hypocrellin-producing fungi may reduce these native photosensitizers as a self-protection mechanism. To investigate this redox-regulated protection mechanism, in our initial study, we examined their electrochemical and chemical redox behavior and demonstrated that hypomycins are derived from the hypocrellins via chemical reduction under anaerobic conditions. Our results indirectly supported the presence of a reductive pathway for self-protection against these natural phototoxins and explained the chemical diversity observed in the fungal metabolites. Herein, we present our analyses of the secondary metabolic profile of a *Shiraia*-like sp. (strain MSX60519) under aerobic and anaerobic growth conditions to further understand this redox regulation in fungus. Under anaerobic fermentation, up to 20-fold improvements in the production of hypomycins A, C, and E were observed while the production of *ent*-shiraiachrome A was slightly diminished. This finding supports our supposition that the reduced forms of hypocrellins under anaerobic growth are more prevalent and can undergo an intramolecular ring closing metathesis leading to increased production of hypomycins. These results indicate that the chemical profile of the fungal metabolites can be directly controlled by factors such as the availability of O_2.

P-144 – Tahir Ali

Identification of Putative Glycosyltransferases from Environmentally Isolated Bacteria Involved in Glycosphingolipids Biosynthesis

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As exemplified by the COVID-19 pandemic, vaccines are a powerful tool to reduce the incidence and fatality rates of infectious diseases. A typical vaccine is composed of an antigen and an adjuvant to enhance the immune response. Natural products, such as glycosphingolipids, offer potential as new adjuvants for vaccines. Bacterial glycosphingolipids (GSLs), including α-galactosylceramide and glucuronosylceramide, have been identified as important modulators of the immune response, but the enzymes (glycosyltransferases) responsible for their biosynthesis are not well characterized. We set out to identify and characterize the glycosyltransferase involved in the biosynthesis of GSLs from two strains of environmentally isolated bacteria belonging to the *Novosphingobium* genus. This study used nanopore sequencing to draft genomes of these strains and identified putative glycosyltransferases by interrogating genome annotations with the RAST tool. The putative glycosyltransferases with other available glycosyltransferases were assembled into a sequence similarity network (SSN) using the Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST) and visualized through Cytoscape. Interestingly, the network showed clustering of two groups of homologs linked to the known glucuronosyltransferase (GlcA-T) proteins. One hypothesis for this outcome is that one homolog is a GlcA-T while the other works on other sugar chemistry with similar or dissimilar acceptor substrate e.g., ceramide or diacylglycerol. However, some of the identified hits are proteins of smaller size, which could be attributed to sequencing errors. In future research, we aim to correct these errors. Additionally, we plan to examine the roles of these two proteins discovered in our strains through knockout experiments, comparing the metabolomic profiles of the knockout and wild

strains for changes in GSLs production using LC-MS/MS analysis to identify the products of these enzymes.

P-145 – Elise Ballash

Genome Mining Unveils Prenylated and Methylated Diketopiperazine Natural Products

Elise Ballash, Jessia Rahe, and Amy Lane, Ph.D. Department of Chemistry and Biochemistry, University of North Florida, Jacksonville, FL, 32224

Actinomycete bacteria excel at producing structurally and functionally diverse diketopiperazine (DKP) natural products that are important in drug discovery. The formation of DKP scaffolds can be catalyzed by cyclodipeptide synthases (CDPS), which form an amide bond between two aminoacyl tRNAs (aa-tRNAs). We aimed to characterize the natural products encoded by a cryptic actinomycete biosynthetic gene cluster that includes homologues of CDPSs, prenyltransferases, methyltranserases, and racemases. The cryptic gene cluster and individual genes from the cluster were heterologously expressed in a *Streptomyces* host. LC-MS and HPLC-UV profiling of metabolites produced by these hosts revealed that the CDPS catalyzes formation of cyclo(L-Trp-L-Trp). This scaffold is further tailored by the prenyltransferase, methyltranserase, and racemase into decorated DKPs, whose structures we elucidated by NMR spectroscopy. The structure elucidation of these DKPs opens door for use of this actinomycete DKP pathway to probe the biological assembly of these natural products, provide biosynthetic access to novel DKPs, and biochemically characterize individual tailoring enzymes.

P-146 – Keelie Butler

Genome Mining Reveals New RiPP Natural Product in Pathogenic Streptococci

Keelie S. Butler[1], Jonathan R. Chekan[1]. [1]University of North Carolina at Greensboro

Ribosomally synthesized and post translationally modified peptides (RiPPs) are a growing class of natural products. Through bioinformatic advancements, genome mining for these RiPP natural products is leading the way for the discovery of new analogs and modifications. In this study, we focus on using this genome mining strategy to search for putative new RiPP classes. Using this strategy, a well conserved RiPP gene cluster was found within several human commensal and pathogenic bacteria, including members of the *Streptococcus* genus, such as the important human pathogen *Streptococcus pneumoniae*. Successful heterologous expression and enzymatic activity assays revealed the formation of a product confirmed by LC-MS and a putative new enzymatic reaction. Further enzymology and substrate scope studies reveal the important residues in the peptide substrate essential for processing by the biosynthetic enzymes. Understanding the biosynthesis of this new natural product may lead to important insight into the pathogenesis of clinically relevant Streptococci.

P-147 – Ashley Clements

Bioactivity-Driven Metabologenomics Identifies Biosynthetic Pathway for Stemphones

Ashley Clements[1], Alexa Lee[1], Raveena Gupta[2], Navid Ayon[2], Cody Earp[3], Fatma Butun[2], David Dainko[2], Matt Robey[4], Jin Woo Bok[5], Nancy Keller[5], Huzefa Raja[3], Nicholas H. Oberlies[3], Neil Kelleher[2,4], Lindsay K. Caesar[1]. [1]Dept. of Chemistry and Biochemistry, James Madison University, [2]Dept. of Chemistry, Northwestern University (NU), [3]Dept. of Chemistry and Biochemistry, University of North Carolina at Greensboro, [4]Dept. of Molecular Biosciences, NU, [5]Dept. of Medical Microbiology and Immunology, University of Wisconsin-Madison (UW)

Fungi represent a diverse yet underexplored source of chemodiversity, and less than 5% of natural products from fungi have been identified. Natural products research increasingly applies -Omics technologies to guide discovery efforts and link natural products to their biosynthetic gene clusters (BGCs). Recently, we conducted a paired metabologenomics study on 110 fungi and found thousands of significant natural product-BGC correlations. To prioritize from these correlations, we combined our dataset with cytotoxicity data to predict active constituents, after which we linked prioritized molecules to their BGCs using metabologenomics. The predicted bioactive components were purified and characterized as new stemphone analogs and their bioactivity confirmed. We identified a BGC with both non-reducing and highly-reducing polyketide synthase components, as well as prenyltransferase and terpenoid cyclase components that is likely responsible for biosynthesizing these meroterpenoids. Confirmation of this pathway is underway. This approach illustrates the promise of bioactivity-directed multi-Omics analysis to prioritize discovery of bioactive molecules and their biosynthetic machinery.

P-148 – Sajan Green

Expansion of Natural Product Diversity Through a Novel Diketopiperazine Isomerase

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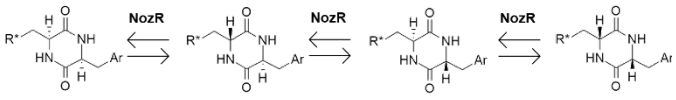
Cyclo-D-Trp-D-Trp (DD-cWW) forms the backbone of the nocardioazines, diketopiperazines (DKPs) with unique biological activity produced by a marine Nocardiopsis sp. actinomycete. Our previous work elucidated the biosynthetic pathway used to create these decorated DKPs. Along this pathway, we discovered NozR, the DKP isomerase responsible for the inversion of stereochemistry on the DKP backbone. To our knowledge, this marks the first reported enzyme to catalyze the inversion of configuration of any DKP substrate. The substrate specificity of NozR has been tested *in vitro* and shown a great degree of promiscuity and reversibility for any DKP

with an aromatic amino acid. The data illuminating this discovery and characterization of this novel isomerase will be presented.



R=any amino acid residue
*(if equivalent to Ar then only three isomers are observed)
Ar=any aromatic amino acid residue (Phe, Trp, Tyr)

P-149 – Manuel Rangel Grimaldo

Strain Heterogeneity of *Aspergillus Fischeri* with Respect to Gliotoxin Biosynthesis

Manuel Rangel Grimaldo[1], Huzefa A. Raja[1], Gustavo H. Goldman[4], Antonis Rokas[2,3] and Nicholas H. Oberlies[1]. [1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, USA. [2]Department of Biological Sciences and [3]Evolutionary Studies Initiative, Vanderbilt University, Nashville, TN, USA., [4]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil

Aspergillosis is a major cause of human morbidity and mortality, which can be caused by inhalation of asexual spores produced by *Aspergillus fumigatus,* resulting in over 200,000 life-threatening infections each year worldwide. The most severe form of this disease is invasive aspergillosis, which primarily affects individuals with compromised immune systems or preexisting lung conditions. Approximately 70% of these infections are caused by *A. fumigatus*, whereas the remaining 30% are caused by other species in the genus. Multiple virulence traits related to invasive aspergillosis include the ability to grow at 37 °C and under low-oxygen conditions and the production of a diverse set of secondary metabolites that influences the virulence profile, such as gliotoxin, a well-known metabolite that inhibits the host immune response. Although *A. fumigatus* is a major opportunistic fungal pathogen of humans, most of its close relatives are not known to be pathogenic, including *A. fischeri*. However, recent studies demonstrated that the genome of *A. fischeri* contains a biosynthetic gene cluster that is homologous to the *A. fumigatus* gliotoxin cluster and is able to biosynthesize gliotoxin under the same growth conditions. To gain further insight, we analyzed four strains of *A. fischeri* in four different culture media that were grown at 37 °C to simulate the infection conditions. This revealed that all the strains have similar metabolomic profiles, but there is strain heterogeneity with respect to the biosynthesis of gliotoxin.

P-150 – Daniel Icenhour

Investigating the Biosynthesis of Terpenes in Actinobacteria Using CRISPR-based Gene Editing

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Actinobacteria are known for their ability to produce novel natural products, but studying the biosynthesis of these compounds in the native host presents several difficulties. To overcome these challenges various CRISPR-Cas9 gene editing systems have been developed to target genes with high precision guided by a designed sgRNA. Taking advantage of the high GC content of Actinobacteria, we utilized the cBEST single-point mutation strategy to knock-out genes predicted to be involved in the biosynthesis of benditerpenoic acid and diazepinomicin. In each gene cluster, we have knocked out and identified genes vital for biosynthesis, allowing us to identify new compounds produced as a result of these knockouts. Further purification and structural elucidation of these compounds will help to reveal their biosynthetic function and gain an understanding of their impact involved in secondary metabolism.

P-151 – Sanath Kandy

Bioinformatic Discovery of a Fused Prenyltransferase-Cyclase Containing RiPP Gene Cluster

Sanath Kavuthian Kandy[1] and Jonathan R. Chekan[1]. [1]University of North Carolina at Greensboro

Natural products have long played a crucial role in being a primary source of bioactive molecules and drug leads. They have also been shown to be a source of new chemistry and enzymology. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a growing superclass of complex natural products characterized by diverse bioactivities produced by extensive post-translational modification on the linear peptide. The discovery of RiPP natural products has been expedited due to recent advancements in genome mining and bioinformatics, resulting in over 40 distinct classes being identified. Using a class-agnostic genome mining approach, we discovered a new RiPP modification catalyzed by a prenyltransferase+cyclase fusion enzyme (PTC). Prenyl groups are frequently present in various natural products, and the prenylated natural products have been shown to enhance the cell penetration capabilities of bioactive molecules, also improving the stability and pharmacokinetics. In vitro reconstitution of this biosynthetic enzyme and analysis by LC-MS/MS has confirmed site-specific prenylation of a single amino acid on the precursor peptide substrate. The preliminary results indicate that the prenylated product is being cyclized. Efforts to characterize the prenylated product fully structurally are currently ongoing and the successful structural characterization may lead to the identification of a new RiPP modification. The distribution of the fusion enzyme across diverse bacterial taxons and genomic contexts, as revealed by bioinformatic analysis, suggests that this modification may exist in a variety of RiPP natural products.

P-152 – Vanderlan Bolzani

Cytotoxicity Activity of New Circular Miminiproteins from *Pombalia Atropurpurea*

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Cyclotides from stems of *Pombalia atropurpurea*, Violaceae family, were extracted, defatted, lyophilized and resulted in the crude extract, which was submitted to SPE-C18 cartridges and eluted with the mixture of 20%, 40%, 60%, 80%, and 100% buffer B (90% CH_3CN, 0.1% CF_3COOH) in an aqueous solution of 0.1% CF_3COOH. The 40%, 60%, and 80 % fractions showed rich concentration in cyclotides, and were evaluated against human colorectal carcinoma (HCT-116) and breast cancer cell (MCF7), and both have showed high and moderated cytotoxicity, respectively. From the 40% fraction, four cyclotides were isolated and all of them showed high cytotoxicity against HCT116, but just three peptides showed high cytotoxicity activity against MCF7. From the 60% fraction, three cyclotides were isolated, but only one exhibited high cytotoxicity against HCT116 and MCF7 cells. Grant support FAPESP no 2013/07600-3, CAPES no 88887.373581/2019-00 and CNPq no 465637/2014-0 for research financial support.

P-153 – Zoie Bunch

Metabolomics Studies to Identify *Stephania Tetrandra* Alkaloids with In Vitro Antiviral Activity Against SARS-CoV-2

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Botanical natural products are often consumed for their purported efficacy against COVID-19. In this study, we have identified several constituents from the medicinal plant *Stephania tetrandra* with in vitro efficacy against SARS-CoV-2. A *S. tetrandra* methanolic extract was partitioned to obtain an alkaloid-rich partition (ST01Alk), which was further fractionated using flash chromatography. The resulting fractions were then tested for anti-viral efficacy against human 293T epithelial cells over-expressing ACE-2 and TMPRSS2 protease (293TAT). We identified two fractions with potent anti-viral activity and minimal cytotoxicity. Untargeted LC-MS metabolomics data were acquired for all fractions and evaluated using partial least squares and selectivity ratio analysis using the antiviral data as the dependent variable. The analysis revealed that three alkaloids; cepharanthine, tetrandrine, and fangchinoline, were correlated with anti-viral activity. Follow up analysis confirmed the activity of the purified alkaloids. Additionally, several unknown features in the dataset were also observed to correlate with anti-viral efficacy, putatively assigned to minor *S. tetrandra* alkaloids. Overall, our findings shed light on the potential of *S. tetrandra* alkaloids as anti-viral agents against SARS-CoV-2. Further studies are warranted to elucidate the precise mechanisms underlying their antiviral effects and to evaluate their safety and efficacy in clinical settings.

P-154 – Angela Calderón

Sandwich-Cultured Hepatocyte Model to Assess Drug Transporter Induction by Açaí Extracts

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Açaí, Euterpe oleracea Mart., is a highly popular botanical in the United States known for its antioxidant and anti-inflammatory properties. Its consumption in botanical supplements and food products is predicted to grow exponentially in the coming years. We have established that açaí extracts can produce an inductive effect on CYP enzymes, and we are now investigating its inductive effects on P-gp and OATP-B drug transporters. The materials obtained were certified organic açaí fruit powder and two dietary supplement capsules containing aqueous extracts of açaí fruit. The açaí powder is typical of what is seen in açaí food products and was extracted separately in acidic methanol (AC), 95% ethanol (ET), methanol (ME), and water (AQ). Two açaí capsules formulations were selected based on the 2019 Amazon Market Report and their commercial availability. These capsules were separately extracted in ME and AC. A basement membrane matrix (Geltrex) was added to the plated human hepatocytes after 4 hours of incubation, thereby producing a sandwich-culture hepatocyte model to assess the effects on the drug transporters. These sandwich-cultured primary human hepatocytes (SCHH) were treated with a human-relevant dose concentration of 2.321 ng/mL cyanidin 3-glucoside (C3G) of each standardized açaí extract. Initial analyses showed an induction potential of OATP drug transporters by the AQ and AC extracts of the açaí fruit powder. These effects will be further analyzed at a higher concentration (100 ng/mL C3G), comparable to açaí food concentrations, by RT-PCR for transcriptional induction and substrate probe assays for activity level confirmation. This study accentuates a rigorous experimental design assessing induction of drug transporters by interaction with açaí products.

P-155 – Serge Fobofou

Discovering Antiviral Agents from Plant Sources

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Infectious diseases caused by emergent or re-emergent pathogens pose serious health problems and viral diseases dominate the World Health Organization (WHO)'s list of ten threats to global health. The recent Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) and monkeypox/mpox outbreaks have unmasked the urgent need for antiviral drugs that can be speedily deployed to combat viral infections before a vaccine is developed. Indeed, only 10 antiviral therapeutic agents are currently approved out of more than 220 viruses known to cause diseases in humans. Thus, there is an urgent need for new and readily affordable drugs against viral infections and drug-resistant pathogens. Medicinal plants have been used against viral infections for centuries, but several of these plants still remain uninvestigated both pharmacologically and chemically. Our work investigating the anti-mpox, anti-HIV, and anti-COVID-19 activities of medicinal plant extracts will be presented as well as some biologically active compounds we isolated and characterized.

P-156 – Nora Gray

Centella Asiatica Administered in the Drinking Water Attenuates Age-Related Changes in Cognition, Anxiety and Sleep-Deprived Cognition More Robustly Than When Administered in the Diet

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The medicinal plant Centella asiatica has a long history of use as a rejuvenating herb to improve brain health. Here, we evaluated the effects of a water extract of Centella asiatica (CAW), administered either in their drinking water or diet for 4 weeks, in healthy eighteen month old male and female C57BL6 aged mice to investigate its effects on cognition, anxiety and depression and sleep-deprived cognitive function. During the final 2 weeks of treatment mice underwent behavioral testing. Results were compared to 18-month- and 3-month-old mice that did not receive any CAW. CAW given in the drinking water improved age-related deficits in learning, executive function, recognition memory and sleep-deprived cognition and attenuated the increased measures of anxiety observed in aged mice. CAW given in the diet did not elicit the same magnitude of effects. These results suggest that route of administration is important for the effects of CAW on resilience to age-related behavioral and cognitive changes. This work is supported by the BENFRA center NCCIH U19 AT010829.

P-157 – Sophie Fadime Aydoğan

Novel Triterpenoid Derivatives from Astragalus Strictispinus and Assessment of their Immunostimulatory Activity

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Astragalus L. (Fabaceae), one of the largest genera in Angiosperm, is represented with approximately 2500-3000 taxa. Astragali Radix represents old and well-known drugs in traditional medicine for the treatment of nephritis, diabetes, and uterine cancer as antiperspirant, diuretic, and tonic. The major phytochemicals of *Astragalus* taxa displaying beneficial properties include saponins, flavonoids, and polysaccharides. In particular, cyloartane-type triterpenoids and their glycosides such as cycloastragenol and astragaloside IV are the main bioactive constituents (Figure 2). Herein, we report the isolation and structural identification of 27 cycloartane type triterpenoids including twelve undescribed cyloartane derivatives (**1-12**) from the roots of *Astragalus striptisipinus* and assessment of their immunostimulatory and anti-inflammatory activities.

P-158 – Sophie Fadime Aydoğan

Phytochemical and Biological Studies on Teucrium Taxa from Anatolia

Fadime Aydoğan, Zulfiqar Ali, Shabana I. Khan, Ikhlas A. Khan, National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, 38677, Oxford, MS, USA

Teucrium L (Lamiaceae) is a polymorphic genus consisting of 300 species distributed in Europe, America, Asia. Aerial parts of the *Teucrium* L (Germander) have been used to prepare herbal teas and supplements as tonic, carminative, spasmolytic, diuretic, antiseptic, antirheumatic, antipyretic, anthelmintic, and weight loss. *Neo*-clerodane diterpenes have been reported as the major constituents in *Teucrium* spp. Moreover, clinical studies involving many cases reported an outcome of the use of aerial parts of *Teucrium* spp. causing toxic hepatitis. In particular medicinal plants are an important source of herb-drug interactions (HDI), and hepatotoxicity. The pregnane X receptor (PXR) is a nuclear receptor that plays a critical role in the metabolism of xenobiotics by increasing the expression of numerous genes, including CYP450s. Herein, this study investigated both the isolation and determination of different class phytochemicals from *Teucrium* species in Anatolia and the assessment of the PXR activation of seventeen neo-clerodane diterpenoids and three abietane diterpenoids.

P-159 – Chunlin Long

Dietary Flowers as Herbal Medicine in Yunnan

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Yunnan harbors the richest biological and cultural diversity in China. We reported that edible flowers from 303 species have been consumed by different linguistic groups in Yunnan. Edible flower consumption has formed a cultural phenomenon in the province, and about half the species of edible flowers in Yunnan are also used as herbal medicine. For example, "maisuo" in the Dai language is the flower of Gmelina arborea Roxb. in the family Lamiaceae. It has been used to treat various ailments by Hani, Dai and Yi ethnic groups, and its chemical composition includes acylated iridoid glycosides, acylated rhamnopyranoses and verbascoside. The cytoprotective activity of 11 selected compounds from Gmelina arborea against carbon tetrachloride-induced cytotoxicity on liver was determined. Six compounds showed hepatoprotective activity while 6-O-a-L-(200, 300-di-O-trans-p-hydroxycinnamoyl) rhamnopyranosylcatalpol exhibited the most potent cytoprotective effect with an EC50 value of 42.5 mM (SI = 19.3) compared with biphenyldimethylesterate (DDB, EC50 = 277.3 mM, SI = 9.8) and bicylo-ethanol (EC50 = 279.2 mM, SI = 12.2).

P-160 – Matthew Menkart

Inhibition of Cytochrome P450 Isoforms by Ethanolic Extracts of *Cinnamomum* spp. (Lauraceae)

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Herbal medicine is a key component of both traditional and Western medicines, but many herbs lack sufficient research on their ability to inhibit or induce cytochrome P450 enzymes (CYPs). Concomitant use of prescription or over-the-counter drugs with certain foods and medicinal herbs may lead to potentially dangerous drug-herb interactions. One such botanical, cinnamon (*Cinnamomum* spp.), was found to possess CYP inhibitory activity through prior high-throughput screening efforts. These studies aimed to determine species variation in CYP inhibition by *Cinnamomum* spp. and identify which CYP isoforms are most affected. Five isoforms, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A5, were tested against crude ethanolic extracts of four *Cinnamomum* species: *C. burmannii, C. verum, C. cassia,* and *C. camphora*. Extracts were prepared at concentrations ranging from 2 μg/mL to 64 μg/mL and inhibition was quantified using a half-maximal inhibitory concentration value (IC_{50}). The greatest inhibition was observed in CYP2C9, with *C. burmannii* (IC_{50}=7.166 μg/mL) and *C. verum* (IC_{50}=7.434 μg/mL) exhibiting more inhibition than *C. cassia* (IC_{50}=8.621 μg/mL) and *C. camphora* (IC_{50}=12.515 μg/mL). This trend was seen across other isoforms, while CYP2D6 showed negligible inhibition by the *Cinnamomum* spp. extracts. Subsequent liquid-liquid partitioning and fractionation via reverse-phase high-performance liquid chromatography and bioactivity testing of *C. burmannii* revealed the most active fraction (2017C-PF7) did not contain any cinnamic acid derivatives. These results indicate that cinnamon can inhibit multiple CYP isoforms, but the extent of this activity varies across species, with *C. burmannii* and *C. verum* being the most inhibitory.

P-161 – Andrés Oliveros-Díaz

Bioassay-Guided Fractionation of Larvicidal Colombian Flora Against *Aedes aegypti* and Its Possible Toxic Effect on Non-Target Organism

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Plants are a potential source of mosquito control molecules due to their ability to produce a wide diversity of natural products through their metabolism. In this work, the ethanol-soluble extracts of 5 4 plant species from the Colombian Caribbean region were evaluated as potential larvicides against *Aedes aegypti* mosquito. The seed extracts of *Tabernaemontana cymosa* Jacq. and *Mammea americana* L. were the most active against mosquito larvae; Bioassay-guided fractionation and structural identification through NMR gave as a result five indole alkaloid and twelve coumarins isolated from *T. cymosa* and *M. americana*, respectively. The indole alkaloid voacangine (TcK001) showed a high activity against *A. aegypti* susceptible (Rockefeller) and resistant (Puerto Rico) strain, with an LC_{50} of 5.13 and 8.64 ppm, respectively. Toxic effects of voacangine related alkaloids on non-target organisms were evaluated using molecular docking with 975 human proteins and *in vivo* tests on the model organism *Caenorhabditis elegans.* Docking calculations showed low affinities of voacangine with human proteins, these results are correlated with the lack of activity of the compound evidenced on the *Caenorhabditis elegans* model at all tested concentrations.

P-162 – Evelyn Assis de Andrade

UPLC-MS/MSe Untargeted Metabolomics Study for Differentiation of *Kalanchoe* spp. Aqueous Extracts

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Several species of the genus *Kalanchoe* are known as miracle-leaf, because of their traditional use in folk medicine, around the world. Various *Kalanchoe* species are broadly used for the treatment of infections, inflammations, wounds and injuries, digestive dysfunctions, and other diseases. Cardioactive glycosides (such as the bufadienolide class) and phenolic compounds (mainly flavonoid derivatives of quercetin and kaempferol) are the metabolites already described in the literature and correlated with these biological activities for *Kalanchoe* spp. However, precise authentication of these *Kalanchoe* species is a challenge due to morphological similarities. Hence, it is imperative to establish comprehensive methods for its differentiation. In this study an UPLC-MS/MSe untargeted metabolomics analysis was performed in order to differentiate specific metabolites of five *Kalanchoe* species. Leaves of *K. crenata*, *K. daigremontiana*, *K. pinnata*, *K. marmorata*, and *K.* x *houghtonii* were collected and extracted with water, following the ethnopharmacological usage, and metabolite profiling of the extracts was analyzed using a Waters I-Class UPLC coupled to a Waters G2-XS Quadrupole Time of Flight (Q-TOF) Mass Spectrometer. Replicate samples were classified using metabolomics software Progenesis QI. This is the first report of the metabolomic profile of these species. The metabolome data and the chemical markers highlighted in this study can be used as a reference to accurately differentiate the *Kalanchoe* species.

P-163 – Cuiying Ma

USP Monograph Methods Differentiate Different Cinnamons

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Cinnamons from barks of the Cinnamomum trees are used as a spice. The benefit of blood glucose control makes cinnamons to be popular dietary ingredients. The most important source of cinnamon is the true cinnamon, also called Ceylon cinnamon from the bark of *Cinnamomum verum*. The other popular cinnamons on market are cassia cinnamon from the bark of *Cinnamomum cassia* (Syn. *Cinnamomum aromaticum*), Indonesian cinnamon from the bark of *Cinnamomum burmanni*, and Saigon cinnamon which is similar to cassia cinnamon grown in Vietnam. The marketed *C. verum* products are often adulterated with other *Cinnamomum* species that are lower in price. For appropriate quality control and labeling of cinnamons, specifications and quality testing are required. USP monographs for *Cinnamomum verum* Bark and *Cinnamomum cassia* Bark provide quality control procedures by both HPLC and HPTLC with suitable reference standards to help ensure that the intended cinnamon with good quality is used. The HPLC method in USP cinnamon monographs can efficiently identify different cinnamon bark species and distinguish each cinnamon ingredient from others. The HPLC of *C. verum* bark contains a significant peak of eugenol which can differentiate it from *C. cassia* and *C. burmanni* barks. The HPLC and HPTLC of *C. cassia* bark do not show significant peak/band of cinnamtannin B1 which can differentiate it from *C. verum and C. burmanni* barks.

P-164 – Lobna Elsadek

Grassystatin G, a New Cathepsin D Inhibitor from a Marine Cyanobacterium: Discovery, Synthesis, and Biological Characterization

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Through mining marine cyanobacteria, a prolific source of structurally diverse secondary metabolites, we isolated grassystatin G (**1**), a new statine-containing linear peptide. The planar structure was determined by analysis of 1D, 2D NMR and MS/MS fragmentation data. We employed chiral HPLC analysis and Modified Marfey's method to assign the absolute configuration of constituent amino acids. As the statine moiety is a known pharmacophore with an inhibitory effect against aspartic proteases, we screened a panel of aspartic proteases (human and HIV) against grassystatin G. In contrast to cathepsin E, a notable inhibitory action against cathepsin D was found. Molecular docking provided insights into the structural features responsible for the selectivity towards cathepsin D. Cathepsin D is well documented to play a role in cancer proliferation and metastasis, particularly in the context of breast cancer. To overcome the lack of material for further biological studies and mechanistic characterization, we developed a 3+3 convergent synthesis and have accessed the peptide with an overall yield of 19% using standard peptide coupling. Using grassystatin G, we described the molecular mechanisms governed by cathepsin D inhibition in triple-negative breast cancer.

P-165 – Peter Blanco Carcache

Pilot Profiling of Old-World Spice *Myristica fragrans* Using Molecular Networking and Molecular Docking for Future Cancer Prevention Nanotechnology Application

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In a previous report (*Food Chemistry* 2016, *202*, 269-275), it was found that the ethyl acetate extract and fractions of *Myristica fragrans*, a traditional Old-World spice used for culinary and medicinal applications, afforded enhanced PARP-1 and NF-kB activity *in vitro*. These protein markers have been closely associated with cancer chemopreventive and anti-inflammatory activity. Among the compound classes involved in the bioactivity are lignans, such as licarin derivatives, thus demonstrating the potential of *M. fragrans* constituents for chemoprevention. Using UHPLC-ESI-Q Exactive system hyphenated ESI-MS/MS was used for chemoinformatic sample analysis via Global Natural Products Social (GNPS) Molecular Networking combined with *in silico* molecular docking and *in vitro* bioassays. Quinone reductase, NF-kB, and PARP-1 assays were selected to obtain new insight into further natural product development for prostate specific nanoparticle targeted delivery in the field of cancer chemoprevention. This work was supported by the Training Program for Cancer Prevention and Control Project 5T32CA229114-05, funded by NCI, NIH, Bethesda, MD, USA

P-166 – Dayani Sarath Parakumge

N-Acetyl Balanol: A Balanol Analog from the Fungus *Cosmospora* sp. with Potent Protein Kinase A Fusion Inhibitory Activity

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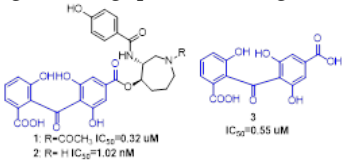
In fibrolamellar hepatocellular carcinoma the chimeric DNAJB1-PRKACA gene produces a fused protein kinase A (PKADJ) and induces tumor pathogenesis. High throughput screening of a fungal extract library received from the University Oklahoma using a sandwich ELISA assay found the extract of Cosmospora sp. to selectively inhibit PKADJ. Bioassay guided fractionation of this extract yielded N-acetyl balanol (**1**), the first naturally occurring balanol analog, together with balanol (**2**) and its biosynthetic precursor di-benzophenone. Among these three isolated compounds balanol potently inhibited PKADJ with IC_{50} 1.07 nM while N-acetyl balanol and its benzophenone precursor had IC_{50} 0.32 μM and 0.55 μM PKADJ inhibition respectively.



P-167 – Ana Ponce

Identification of Fungal Natural Products that Exhibit Cytotoxic Activity Towards a Brain Cancer (U87) Cell Line

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The National Institute of Health Surveillance, Epidemiology, and End Results (SEER) Program estimates there was a total of 24,810 new cases of brain and other nervous system cancers diagnosed in the US in 2023 and an estimated 18,990 deaths. The overall goal of this research was to identify secondary metabolites from marine-derived fungi that exhibit cytotoxic activity towards a brain cancer (U87) cell line. A total of 100 taxonomically distinct fungal strains were cultured in five different media types. The extracted natural products were screened against a brain cancer (U87) cell line using a sulforhodamine B (SRB) cytotoxicity assay. This research led to the identification of a family of structurally novel terpenes that exhibit cytotoxic activity towards the U87 cell line.

P-168 – Nolan Barrett

Probing Ecological Tradeoffs Between Antibiotic Detoxification and Allelopathy in Lake Water Bacteria Using Multi-'Omics Methods

Nolan H. Barrett[1], Princess Pinamang[1], Janet K. Hatt[2], and Konstantinos T. Konstantinidis[1,2]. [1]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA; [2]School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA, USA

Natural microbial communities present many challenges to studying ecological phenomena. One such challenge is determining the effects of horizontal gene transfer (HGT) on genes linked to survival during interspecies interactions in a complex community. However, advancing methodologies, including meta-'omics analyses and bioinformatics tools, prove valuable for probing interactions that are unresolvable to the naked eye. Genes of high value to population survival, such as those that code for antibiotic detoxification or competition by allelopathy, present a nuanced tradeoff to have only one of these traits as bacteria are unlikely to acquire, maintain, and express both traits equally. In this study, a well-characterized microbial community from the water of Lake Lanier, Georgia, USA was challenged in mesocosms with benzalkonium chlorides (BACs, a group of commercially used antimicrobials) to provide a selection pressure for detoxification. Live cells of *Pseudomonas nitroreducens*, which possess natural resistance to BACs, were added in varying abundances to provide a non-

native competitor for allelopathy to act against as well as a source of BACs detoxification genes to horizontally transfer. We tested the hypothesis that the acquisition of BACs detoxification genes through HGT will increase due to the selection pressure of the BACs compared to the selection pressure of allelopathic competition, resulting in the decreased prevalence/expression of allelopathy biosynthesis genes as a tradeoff. The resulting metagenomic and metatranscriptomic data were analyzed with in-house bioinformatics tools.

P-169 – Lesley-Ann Giddings

Isolation and Characterization of a Red-Pigmented *Massilia* from an Antarctic Microbial Mat

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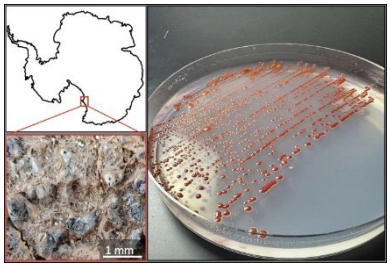
Microbial mats in the McMurdo Dry Valleys of Antarctica are exposed to a range of selective pressures, including extreme seasonal variation in temperature, water and nutrient availability, and UV radiation. Here, we describe the isolation and characterization of a Gram-negative, rod-shaped, motile, red-pigmented species of Massilia frigida, strain DJPM01, from a microbial mat within the Don Juan Pond (DJP) basin (inset figure). Genome analysis indicated genes associated with cold and salt tolerance as well as 17 putative biosynthetic gene clusters, many of which are involved in the biosynthesis of nonribosomal peptides, ribosomally synthesized and post-translationally modified peptides, and the red antimicrobial pigment prodigiosin. Genome analyses of sequenced Massilia indicated prodigiosin biosynthesis is unique to Antarctic strains. When cultivated on complex agar, multiple prodiginines were detected by LC/MS. UV-A radiation, an ecological stressor in the Antarctic, was found to significantly decrease the abundance of prodiginines produced by strain DJPM01. Genomic and phenotypic evidence indicate strain DJPM01 can respond to the ecological conditions of the DJP microbial mat, with prodiginines produced under a range of conditions, including extreme variation in UV radiation.



P-170 – Melany Puglisi

Understanding the Role of Chemical Mediation of the *Caulerpa* spp. Microbiome

Melany Puglisi,[1] Marina Ghazar,[1] Savannah Pierce,[2] Shane Roesemann,[3] Jason Kwan,[3] and Skylar Carlson[2].[1]Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628, [2]Department of Chemistry, University of the Pacific, Stockton, CA 95204, [2]University of Wisconsin at Madison, 777 Highland Ave, Madison, WI 53705

Understanding chemical mediation of the micro- and macroalgal microbiome is important in the discovery of natural products and understanding opportunistic disease outbreaks in the ocean. *Vibrio* spp., a major component of the microbiome associated with the invasive species *Caulerpa cylindracea*, cause seafood-associated gastroenteritis. The objective of this study is to explore the role of chemical mediation in the formation of the microbiome. Eight species of *Caulerpa* were collected from the Florida Keys. Swabs of the algal surface were collected in sterile tubes for isolation of surface associated bacteria (SAB) and to explore the microbiome. Previously, minimal growth inhibition (8.4%) and growth promotion (6.6 %) were observed when solvent partitions were screened against 38 SAB. However, 90% of the bacterial panel settled in response to the same partitions. To better explore the complexity of these interactions, the non-polar algal partitions were analyzed using MADByTE (Metabolomics And Dereplication By Two-Dimensional Experiments), an NMR based metabolomics technique. In addition, 16S amplicon data were analyzed using the mothur analysis pipeline. Prior to analysis, ambiguous bases were removed, chimeric reads were detected by VSEARCH, and non-bacterial reads were removed. Reads were clustered into OTUs and community differences were determined by PERMANOVA. The resultant community structures will be presented with the information from MADBYTE networks to provide insight regarding the complex settlement data and refine the SAB panel for bioassay-guided fractionation of novel settlement compounds.

P-171 – Samuel Tanoeyadi

Studies on the Ecological Functions of the Antidiabetic Drugs Acarbose

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As part of an ongoing effort to understand the ecological roles of bacterial secondary metabolites, we characterized the possible benefits of the antidiabetic drug acarbose for the producing bacteria. We found evidence that acarbose could be used to suppress the growth of other bacteria by inhibiting their GH enzymes, while the producing bacteria are equipped with acarbose-resistant GHs. In addition, we found that acarbose-resistant GHs, e.g., AcbE and GacZ1, have a larger active side pocket, which plays an important role in conferring resistance towards acarbose. Point mutation of the acarbose-resistant AcbE, resulted in a GH variant that has smaller active site pocket and is more sensitive to acarbose. The results support the hypothesis that acarbose functions as a competitive exclusion agent for the producing bacteria in their natural environment. In addition, we

found that the putative α-glucanotransferase AcbQ, whose gene is part of the acarbose BGC, has both transglycosylase (TG) and glycosyl hydrolase (GH) activities. The TG activity is ~ 8 times more efficient than that of the GH activity. It catalyzes transglycosylation reactions between acarbose and maltose (G2) or maltotriose (G3) as well as acarbose 7-phosphate and maltose or maltotriose as well. We propose that the main function of AcbQ is to produce longer chain acarbose derivatives, which have better inhibitory activity against amylases. Consequently, the TG activity of AcbQ supports the competitive model for acarbose and related compounds. However, the GH activity of AcbQ may support the carbophore model. Under starvation conditions and the lack of G2 and G3, AcbQ may hydrolyze acarbose-G1 and acarbose-G2 to produce glucose for energy production and anabolism.

P-172 – Gabriela Toninato De Paula

Natural Products from Actinobacteria Associated with the Stingless Bee *Tetragona Clavipes*

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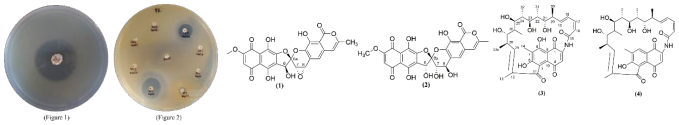
This project aims on the chemical and ecological analyses of actinobacteria associated with *T. clavipes* bees. Antagonism antimicrobial assays (Figure 1) and bioguided fractionation (Figure 2) allowed the isolation of compounds with activities against human pathogens and entomopathogens. Two compounds, griseorhodin A (**1**) and C (**2**), were isolated from *Streptomyces* sp. strain C6.2. Two compounds were also isolated from *Micromonospora* sp. strain C5.7, 16-demethyl-34a-dehydroxy rifamycin W (**3**) and its new analogue compound (**4**). Our results show the microbiomes stingless bees are a very promising niche for the isolation of new and biologically active compounds.



P-173 – In Jin Ha

A Novel Method for Preparation and Analysis of the 2-Phenoxychromones-Enriched Fraction of *Artemisia Capillaris* and Its Anti-Cancer Effect on Colorectal Cancer Cells

In Jin Ha [1, 2], Sanghee Han [2], Min Young Lee [1], Kwang Seok Ahn [3], Dongwoo Nam [1,3], and Seok-Geun Lee[1, 2, 4*]. [1]Korean Medicine Clinical Trial Center, Kyung Hee University Korean Medicine Hospital, Seoul 02454, Republic of Korea, [2]Graduate School, Kyung Hee University, Seoul 02447, [3]College of Korean Medicine, Kyung Hee University, Seoul 02447, [4] BioNanocomposite Research Center, Kyung Hee University, Seoul 02447*

A preparation method for a 2-phenoxychromones-enriched fraction of *Artemisia capillaris* (PFAC) was established using a HEMWat solvent system designed to extract selectively targeted compounds, and succeed to prepare and confirm the PFAC to contain highly concentrated amounts of capillarisin, 6-methoxycapillarsin, and 6-demethoxy-4'-methylcapillarisin. The components in the PFAC were identified using LC-QTOF MS/MS with molecular networking analysis and charcterized with various mass scan modes. Also, the potential anti-cancer effects of the PFAC were explored in human CRC cells by analyzing cell viability, cell cycle, and apoptosis. The analytical and biological data of the PFAC will be presented.

P-174 – Yurry Um

Global Metabolite Profiling of Wild-Simulated Ginseng and Ginseng Based on GC-MS and LC-MS Analyses

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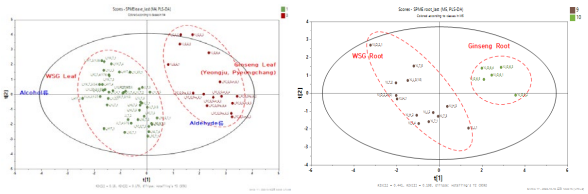
Ginseng is produced using artificial facilities. Wild-simulated ginseng (WSG) is grown naturally by planting seeds in the forest. In the present study, to globally characterize metabolites of WSG and ginseng, GC-MS/SPME, GC-MS, and LC-MS were used and a multivariate statistical analysis was performed. Partial least squared discriminant analysis (PLS-DA) data showed a clear separation in metabolite profiles among WSG and ginseng samples. Major volatile metabolites of WSG were caryophyllene, humulene, and α-panasinsen, while those of ginseng samples were styrene, δ-cadinene, and isospathulenol. Major non-volatile metabolites of WSG were tartaric acid, sucrose, and glucose, while those of ginseng samples were glucuronic acid, monomethyl phosphate, and phosphate.



P-175 – Yan-Hong Wang

Characterization of Key Metabolites from *Eleutherococcus senticosus* and Ci-wu-jia Tea by UHPLC-UV-QToF MS

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Eleutherococcus senticosus Maxim. (syn. Acanthopanax senticosus Harms) leaves have been developed as a functional beverage called ci-wu-jia tea in China and Siberian ginseng tea in the United States and Europe. Phytochemical studies revealed that organic acid derivatives, triterpene glycosides, and flavonoids were the major secondary metabolites in the leaves of E. senticosus. Several analytical methods have been reported for the quantitative or qualitative analysis of different classes of secondary metabolites in E. senticosus leaves, but no analytical method has been done for the chemical analysis of key metabolites in a single analytical method. This study developed a sensitive UHPLC method combining UV and MS/MS to characterize the triterpene glycosides, organic acid derivatives, and flavonoids from E. senticosus leaves. Fragmentation patterns of three sub-groups of triterpene glycosides in E. senticosus leaves were investigated. A compound screening library including 241 compounds reported in the literature was created and used to confirm the compounds in the samples. The developed UHPLC-UV-MS/MS analytical method combined with the UNIFI processing platform can simultaneously characterize key metabolites from E. senticosus. It provides a simple and sensitive way to perform quality control of E. senticosus and related ci-wu-jia tea products.

P-176 – Esperanza Carcache de Blanco

Bioassay-Guided Study of *Pyrenacantha Kaurabassana* from the NCI Natural Products Repository

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Cancer represents one of the most lethal diseases worldwide. Natural products (NPs) and their derivatives are one of the major sources of lead candidates for drug development. Besides, NPs are considered as a promising approach to cancer control. In the present work, 352 plant extracts and 176 microorganism extracts from the NCI Natural Products Repository (NPR) have been screened against human pancreatic (HPAC), and thyroid (MDA-T32) cancer cells. The methanolic extract of the plant *Pyrenacantha kaurabassana*, showed cytotoxic activity against HPAC and MDAT-32 cells, exhibiting an IC_{50} of 8.72 ± 0.03 μg/mL in MDAT-32 thyroid cancer cells. Thyroid cancer is among the rare cancer types as treatment options are limited and the most common option is left to surgery. Thus, it is important to find non-surgical options for this rare disease. The extract of this species was analyzed by LC-HRMS to and generate a Molecular Networking using GNPS for the discovery potential new drug leads and designate structurally similar compounds into clusters. This plant could be considered a potential source of bioactive compounds as new leads for the treatment of cancer.

P-177 – Esperanza Carcache de Blanco

Bioassay-Guided Study and Molecular Networking of *Cupressus arizonica* from the NCI - NPR

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Natural products (NPs) and their derivatives are one of the major sources of lead candidates for anticancer drug development. Nevertheless, the frequency of isolation of novel bioactive compounds is steadily declining. Hence, detection of known natural compounds at an early stage in the drug discovery process is essential to efficiently identify new drug leads and to reduce time, effort, and cost. Molecular networking is a well-suited strategy to perform this task. In the present work, 352 plant extracts and 176 microorganism extracts from the NCI Natural Products Repository (NPR) have been screened against human pancreatic (HPAC), and thyroid (MDA-T32) cancer cells. The methanolic extract of *Cupressus arizonica* plant from the NCI Natural Product Repository (NPR), showed cytotoxic activity against HPAC and MDAT-32 cells with IC_{50} values of 17.42 ± 1.08 μg/mL and 13.91 ± 2.11 μg/mL, respectively. The extract was analyzed by LC-HRMS using the LC:Dionex/Thermo UltiMate 3000 system coupled with a Q-Exactive mass spectrometer. Molecular Networking (GNPS) platform allowed us to analyze, connect, and network based on fragmentation patterns to discover pockets of potential new bioactive molecules and categorize structurally similar compounds in clusters.

P-177B – Ogechukwu Chukwuemerie

In-Silico Identification of Antisickling Phytochemicals from Justicia Secunda

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Medicinal agents used to prevent the pathological episodes leading to sickling of erythrocytes in sickle cell conditions are becoming a research concern. Studies have shown that the leaves of *Justicia secunda* exhibit anti-sickling activity. *In-silico* methods and *in vitro* data are used to create models which are tested in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization. Protein/receptors MetAP2, Carbonmonoxy hemoglobin and ribonucleotide reductase that when inhibited

could reduce the pain episode in sickle cell anemia disease were idetified. The 3D structures of the receptors were obtained from PDB with the identification code 7A12, 6XDT and 5TUS respectively. Existing phytochemical constituents of *Justicia secunda* were obtained from literature. The phytocompounds were screened for drug-likeness, toxicity and bioavailability. The proteins and phytochemicals were duly prepared for molecular docking simulations (MDS). After validation of docking protocols, MDS was implemented in AutoDock-Vina®, with virtual screening scripts. Frontrunner phytochemicals were screened for bioactivity prediction on the Molinspiration Chemoinformatics web. The preliminary results obtained show that twelve of the phytochemical constituents of *Justicia secunda* had good druglike property, violated no lipinski's rules, verber rules, MDDR-like rule, and had negative prediction for mutagenic, tumorigenic, reproductive effect and irritant effects. The molecular docking simulations that will aid determination of the inhibition constant of the frontrunner phytochemicals against MetAP2, Carbonmonoxy hemoglobin and ribonucleotide reductase are in process. The predicted bioactivity determinations are also in progress.

P-178 – Jonathan Jeyaraj

Molecular Networking Driven Discovery of Potent Cytotoxic Natural Products from *Aspergillus* sp. (Trichocomaceae)

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The screening of 352 plant and 176 microbial extracts sourced from samples in the National Cancer Institute's Natural Products Repository led to the discovery of a potent cytotoxic *Aspergillus* sp. extract. Biological evaluation of the *Aspergillus* sp. fungal extract using the sulforhodamine B (SRB) assay against the human cancer cell lines HPAC (pancreatic) and MDA-T32 (thyroid) resulted in IC_{50} values 3.47 μg/mL and 10.54 μg/mL, respectively. These results support the well documented research of several different species of *Aspergillus* shown to produce chemically diverse bioactive natural compounds with activities that include cytotoxic, antimicrobial, antiviral, and anti-inflammatory effects. To isolate, identify, and structurally characterize potential novel anticancer natural products bioactive-based molecular networking of the active *Aspergillus* sp. extract and fractions was utilized. LC-ESI-MS/MS was carried out with the Thermo Q-Exactive Orbitrap and Vanquish-H UHPLC to perform molecular networking. The Global Natural Products Social Molecular Networking (GNPS), MZmine 3, and other *in silico* tools were used for data analysis. Acknowledgments, Program project grant supplement 3P01CA125066-12S2, funded by NCI-NIH.

P-179 – Christina Davidson

Removal of Polyphenols from Plant Extracts to Increase the Drug Discovery Efficiency

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Polyphenols, such as flavonoids and phenolic acids, are common compounds found in plant extracts. In the drug discovery process, these compounds act as Pan-assay interference compounds (PAINS) and often cause false-positive readout in cellular and *in vitro* assays. Their abundance in standard plant extracts can mislead many HTS results and suppress the detection of other minor bioactive compounds which have the potential to become drug leads. The goal of the present method is to remove polyphenols from polar plant fractions after solid-phase extraction (SPE) using Polyvinylpolypyrrolidone (PVPP) resin to enrich the minor compounds and to increase the confidence levels on HTS results. The phenolic content of the fractions removed in this method was evaluated using HPLC-QTOF to assess the efficiency of this method. All polyphenols were completely removed from the polar SPE fractions successfully, and the remaining compounds were enriched. This method will allow for efficient removal of polyphenols from SPE plant extract fractions and facilitate the investigation of less abundant bioactive compounds with increased confidence on screening results in the drug discovery process.

P-180 – T'ea Cameron

Freshwater Fungi as a Source for Antimicrobial Drug Leads

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New antibiotic drug leads are needed. In particular, a continuous challenge is the rise of hospital acquired infections, often caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. Historically, fungi have been a valuable source of such leads, and the goal of this study was to examine fungi from freshwater habitats. New compounds have been identified from this group, but it is still largely unexplored, as they comprise less than 1% of the 135,000 total fungi described. Thus, in this study, we identify freshwater fungi that produced antibiotic drug leads. Over 150 freshwater fungal strains were extracted and prioritized via dereplication techniques and bioassay-guided fractionation against MRSA and *A. baumannii*. Extracts with antibacterial activity were selected for further study.

P-181 – Manuel Rangel Grimaldo

Improving the Production of Wheldone, a Secondary Metabolite Produced by a Co-Culture of *Xylaria flabelliformis* and *Aspergillus Fischeri*

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Fungi naturally grow in competitive environments, and as such, they have evolved the ability to combat rival organisms. Combative conditions are hypothesized to activate biosynthetic gene clusters, resulting in the generation of secondary metabolites that modulate the growth of the competing organism. As such, co-culturing fungi, forcing them to compete for limited resources, may present a practical strategy to stimulate the biosynthesis of novel chemical diversity. In a previous study, we reported the biosynthesis of wheldone, a compound that was found only in the co-culture of *Xylaria flabelliformis* and *Aspergillus fischeri.* This new metabolite demonstrated cytotoxic activity in the low micromolar range against a panel of cancer cell lines. However, the low yields and the inherent difficulties of the co-culture are obstacles in the large-scale production of wheldone, and lack of materials hampers further pharmacological studies. Several strategies were explored to both enhance the co-culture fermentation conditions and improve the isolation yields. This study demonstrates significant improvements in the production of wheldone via fungal-fungal co-culture and offers tips that can be used to enhance secondary metabolite production in such systems.

P-182 – Thanh-Hau Huynh

New Macrolides with Diverse Starting Units from an Insect-Associated Kitasatospora Sp

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Four new 18-membered macrolides (1-4) bearing bicyclic spiro-bis-tetrahydropyran ketal functionality with diverse starting units were discovered by the chemical analysis of Kitasatospora sp. The planar structures were elucidated based on the combinational analysis of the UV, MS, and NMR data. The stereochemistry was assigned by J-based configuration analysis, ROESY correlations, and chemical derivatizations (acid hydrolysis, methylation, methanolysis, reduction, and the modified Mosher's method). 1 showed potent cytotoxicity against human cancer cell lines at submicromolar IC50 values.

P-183 – Quan Khong

Brevianamides A1 and A2: Cytotoxic Oxygenated Diketopiperazines from *Penicillium brevicompactum*

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Merkel cell carcinoma (MCC) is a rare, aggressive skin cancer that is frequently linked to polymavirus infection. The crude extract of a *P. brevicompactum* fungus showed potent cytotoxicity against the virus-positive cancerous cell line WaGa (IC50 < 0.5 μg/ml). Bioassay-guided isolation led to the immunosuppressant mycophenolic acid as the major active component along with two new cytotoxic oxygenated diketopiperazines, brevianamides A1 and A2. The absolute configurations of the new compounds were determined by ECD calculation and Marfey's method. Brevianamide A1 was cytotoxic against the Merkel cell carcinoma (MCC) cell lines Waga, MKL, UISO, and MCC13 with IC50 values at 6.5, 5.7, 6.3, and 4.5 μM, respectively. Further evaluation of brevianamide A1 in the NCI-60 Human Tumor Cell Lines Screen showed moderate antiproliferative activities with the average GI50 value of 6.4 μM. Brevianamide A1 underwent gradual decomposition under mild acidic conditions (0.1% formic acid or 0.1% trifluoroacetic acid) and the underlying chemical mechanism was investigated.

P-184 – Rohitesh Kumar

A Systematic Approach for the Identification of New Bioactive Compounds from the NCI Natural Product Prefractionated Library

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Fast, efficient, and economical approaches are needed to identify new chemical entities. Housing more than 200,000 unique extracts, with >500,000 fractionated samples derived from plant, marine and microbial organisms, the National Cancer Institute (NCI) Program for Natural Product Discovery (NPNPD) strives to develop methodologies for rapid isolation and identification of active principals. As such, an automated, high-throughput robotics platform separates extracts into 7 fractions, followed by HPLC, where 1 mg of the active fraction is further chromatographed into 22 sub-fractions in a 96-well, plate ready format for high throughput screening (HTS). The spectral fingerprint of active sub-factions (NMR, LC-MS/MS and FTIR) are

then obtained for fast dereplication and scale-up prioritization. Here, we present this systematic approach for natural product prefractionated library screening, hit prioritization, and eventual identification of previously unreported bioactive molecules.

P-185 – Venkat Macherla

Anti-Inflammatory Sulfated Withanolide Analogs with Reduced Cytotoxicity from *Withania Somnifera*

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During our platform technology development to index nature's untapped chemical space for new drug discovery applications, we found several compounds from a well-known medicinal plant, Ashwagandha (*Withania somnifera*). These compounds include a novel sulfated Withanolide analog, a known sulfated Withanolide, and many known non-sulfated withanolides. We selected all bioactive fractions from the fraction library of *Withania somnifera* extract via screening for inhibition of cell signaling within an IL-1⍟-driven HEK293 reporter cell line, isolated all major compounds from each bioactive well, and identified their structures using 1D and 2D-NMR and literature references. Both Withanolides with Michael acceptor functionality and sulfated Withanolides without Michael acceptor functionality showed significant inhibition in the IL-1⍟⍟ driven HEK293 reporter assay, but only the sulfated Withanolides displayed reduced cytotoxicity in the parental HEK293 cell line. The cytotoxicity caused by these molecules might be a result of the Michael acceptor functionality which is prone to interacting with many undesired proteins through cysteine, amine, and alcohol functional groups. The sulfated Withanolides with reduced cytotoxicity have the potential to move on to the next level of drug development.

P-186 – Brian Murphy

Discovery of New Antibiotic Demethoxytetronasin Using Novel Solid Support Assay

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For over a century nutrient agar has provided an accessible medium on which microbial growth inhibition could be detected. As a result, agar-based bioassays have been critical to both academic researchers working with microorganisms and the pharmaceutical industry's global effort to discover drugs. Despite the utility of simple agar assays to researchers around the globe, several limitations have prevented their widespread adoption in advanced high-throughput compound discovery and dereplication campaigns. To address a list of specific shortcomings, we developed the dual-sided agar plate assay (DAPA), which exists in 96-well plate format, allows microorganisms to compete on opposing sides of a solid support in individual wells, is amenable to high-throughput screening and automation, is reusable, and is low-cost. Herein we validate the use of DAPA as a tool for drug discovery and show its utility to discover new antibiotic natural products. From the screening of 217 bacterial isolates on multiple nutrient media against 3 pathogens, 55 hits were observed, 9 known antibiotics were dereplicated directly from agar plugs, and a new antibiotic demethoxytetronasin (1) was isolated from a *Streptomyces* sp. found in sediment between two tectonic plates in Nesgja, Iceland. These results demonstrate that DAPA is an effective, accessible, and low cost tool to screen, dereplicate, and prioritize single bacterial colonies in the front end of antibiotic discovery pipelines.

P-187 – Roberta O'Connor

Mining Natural Products for Activity Against *Cryptosporidium* and Other Apicomplexan Parasites

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Cryptosporidium is a waterborne gastrointestinal parasite of worldwide distribution. The parasite is a leading cause of diarrhea in children under five, and a common opportunistic infection of immunocompromised patients. There are no effective therapeutic options to treat cryptosporidiosis in susceptible populations, thus identification of new, effective compounds is a medical imperative . To address this need, we screened natural product libraries derived from marine organisms and subterranean fungi for anti-parasitic activity. While these screens are still ongoing, we have already identified several potent anti-cryptosporidial compounds, some with activity against multiple apicomplexan parasites such as *Plasmodium falciparum* and *Toxoplasma gondii*. In addition to EC50s, therapeutic indices and pharmacodynamic parameters, compounds are characterized as to their specific activity against parasite life cycle stages, time to kill, efficacy in parasite-organoid cultures and animal models, and identification of molecular

targets. Natural products from these remote sources are proving to be a source of highly effective, broad spectrum anti-parasitics with unique activities and structures, providing viable candidates for the drug development pipeline.

P-188 – Quanbo Xiong

Rebirthing Industrial Natural Products Discovery Through 21st Century Technology for Infectious Crop Diseases

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Corteva Agriscience™, the largest pure-play agricultural biotech in the world, is leveraging advances in genomics and metabolomics to reinvigorate industrial Natural Products discovery. Over the last twenty years most major multinational corporations have abandoned Natural Products discovery due to a failure to identify novel bioactive scaffolds. Yet in that same time Corteva successfully advanced multiple Natural Product and Natural Product-inspired agrichemicals to market. The most recent examples of UK2A-based fungicides Inatreq™ and Adavelt™ demonstrate the enormous value proposition of Natural Products for industrial biotechnology at large. However, solutions to access novel scaffolds and avoid rediscovery are necessary for continued growth. To address this need, Corteva is deploying high-throughput integrated genomics and metabolomics to analyze biosynthesis and discover novel Natural Products to deliver sustainable agrichemicals against crop pathogens and ensure the global food supply amid a changing climate and growing population.

P-189 – Andrea Stierle

Berkeleylactone A, a Potent, Novel Macrolide Antibiotic Produced in Co-Culture by Two Extremophilic Fungi Isolated from an Acid Mine Waste Lake in Butte, Montana

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In 1995 the Stierle Lab launched an investigation of the secondary metabolites of the extremophilic fungi isolated from the metal-rich, highly acidic waters of the Berkeley Pit. This toxic lake is a legacy of one of Montana's richest copper mines and ground zero of one of the largest EPA Superfund sites in North America. This research has led to the discovery of a range of novel structure types including berkeleydione and berkelic acid, both of which showed potent activities in the NCI 60 cell line screen. Most recently, co-culture experiments with Berkeley Pit fungi led to the cryptic biosynthesis of Berkeleylactone A, a novel 16-membered macrolide, which exhibits potent activity (<1 μg/mL MIC) against gram-positive bacteria, particularly those already resistant to other antibiotics. These include methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus anthracis*. It does not induce resistance in target bacteria and is thought to act by a novel mechanism of action. Berkeleylactone A and synthetic efforts to generate analogues with enhanced *in vivo* and *in vitro* activities will be described.

P-190 – Alex Swystun

Metallophore Production from Deep Arctic Marine Sediment Derived Bacteria Using Single Cell Culturing and High-Throughput Bioassay Methods

Alex Swystun[1], Margaret Walter[2], Kevin Kiser[2], Patrick Erwin[2], R. Thomas Williamson[1], Blake Ushijima[2], Wendy K. Strangman[1]. Department of Chemistry and Biochemistry[1], Department of Biology and Marine Biology[2], University of North Carolina Wilmington, Wilmington, NC 28403

Metal ions play many important roles in environmental chemical ecology and human health. Acquisition of these often-scarce metals is critical for microbial survival and fitness. As a strategy for metal acquisition, many species of bacteria rapidly produce low molecular weight, metal-chelating natural products called metallophores. When metallophores bind to metals, their structures often change dramatically; this molecular structure change can be critical for bioactivity and future pharmaceutical applications. Using a series of deep arctic marine sediment cores and traditional culturing techniques, a preliminary extraction library of 47 isolates was generated and screened for metallophore production in agar using a modified Chrome Azurol-S metallophore production disk diffusion assay. To increase assay efficiency, the assay was modified further from agar to liquid. To increase bacterial diversity and improve culturing and time efficiency, a microfluidic single-cell bacterial sorter was incorporated to isolate and culture individual bacterium from some of these cores. These pure micro-cultures were then assayed for production of metallophores using an automated liquid handling system with the goal of discovering new metallophores specific to a variety of transition metals including iron, zinc, copper, manganese, and cobalt. Metal-specific chelators will be characterized and screened in their apo- and metal-bound states in a panel of bioassays to assess potential bioactivity to determine pharmaceutical potentials.

P-191 – Brice Wilson

Natural Products as a Source of Chemical Diversity in Cyclic AMP Dependent Protein Kinase A (PKA) Inhibitor Discovery

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cAMP dependent protein kinase A (PKA) enzymatic activity is a central cellular signaling node underlying many fundamental physiological processes. PKA dysregulation is associated with a number of human diseases including Cushing's syndrome, Carney Complex disorders, and certain cancers. This cohort of PKA dependent diseases includes fibrolamellar hepatocellular carcinoma (FLHCC), a rare largely incurable liver cancer uniquely driven by the overexpression of an oncogenic fusion between an HSP40 family member, *DNAJB1*, and the catalytic domain of PKA alpha, *PRKACA*. This fusion protein (J-PKAcα) is enzymatically active and drives FLHCC tumor growth, making it an attractive target for inhibitor discovery. We implemented **a high throughput screening campaign of >140,000 fractionated natural products extracts to discover pharmacophores evaluated for J-PKAcα inhibition**, fusion specificity, direct binding, intracellular activity, and x-ray crystallography. This screen revealed several previously unreported PKA inhibitors which may form the foundation of future medicinal chemistry efforts to optimize these scaffolds for translational applications.

P-192 – Priscilla Winder

Screening the Harbor Branch Oceanographic Institute Enriched Fraction Library for Inhibitors of Survivin

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Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins that is overexpressed in nearly all types of cancer cells and expressed only during cell division in normal cells. It is involved in apoptosis, the regulation of the cell cycle, cellular stress response, and metastasis formation. A cell-based high content imaging screening assay to identify novel inhibitors of survivin was established using DLD-1 colorectal adenocarcinoma and the A549 non-small cell lung carcinoma cell lines. Nearly 3,000 fractions from Harbor Branch's marine natural products enriched fraction library were screened for their ability to downregulate the expression of survivin in DLD-1 and/or A549. Bioassay guided fractionation of the active fractions led to the identification of numerous known and new marine derived compounds that decrease the levels of survivin.

P-193 – Khaled Shaaban

University of Kentucky Natural Product Repository: A Collection of Diverse Microbial Natural Products

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Natural products remain a major inspiration and source for drug leads and bioactive probes. We seek to explore the microbial diversity (and corresponding biosynthetic potential) of untapped terrestrial microbes from environments. As part of our ongoing effort, we isolated microbes from soil samples and traditional medicinal plants endophytes from diverse collection sites in Appalachian Kentucky (including caves, underground and surface coal mines and subterranean drilling sites), Maryland (USA), New Mexico (USA), Pakistan (including sites in the Himalayans and Cholistan desert), China (Kubuqi desert), Egypt (including collection sites in western desert, phosphate mines and the Red Sea) and Brazil (primarily the Pantanal region). Culturable microbes were dereplicated and prioritized based on capacity for novel secondary metabolite production. Cumulatively, this program has led to the accumulation and deposition of >2,200 non-redundant microbial isolates (bacteria and fungi), >4,000 corresponding fractionated extracts and >620 pure bacterial/fungal metabolites (nearly half of which are exclusive to the UK collection). This CPRI natural product repository represents broad chemical diversity [angucycline(on)es, anthracycline(on)es, coumarins, terpenes, macrolides, peptides, phenazines, glycosides, xanthones, indoles). The corresponding repository has been made available to collaborators with novel biochemical, cell-based and/or animal-model based assays access to the repository and active hits from this broad collaborative effort have been advanced to preclinical studies and probe development studies in the areas of cancer, infectious disease, neurodegenerative diseases, regeneration and substance use disorders.

P-194 – Natchanun Sirimangkalakitti

A New Tetracyclic Bromopyrrole-Imidazole Derivative through Direct Chemical Diversification of Substances Present in Natural Product Extract from Marine Sponge *Petrosia* (*Strongylophora*) sp.

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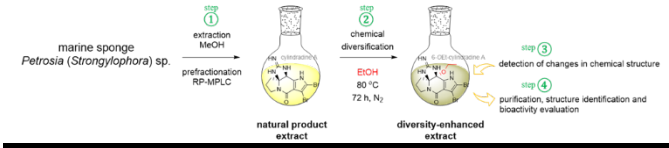
Chemical diversification of substances present in natural product extracts can lead to a number of natural product-like compounds with a better chance of desirable bioactivities. The aim of this work was to discover unprecedented chemical conversion and produce new compounds through a one-step reaction of substances present in the extracts of marine sponges. In this report, a new unnatural tetracyclic bromopyrrole-imidazole derivative, *rac*-6-*O*Et-cylindradine A (**1**), was created from a chemically diversified extract of the sponge *Petrosia* (*Strongylophora*) sp. We also confirmed that **1** originated from naturally occurring (-)-cylindradine A (**2**) via a new reaction pattern. Moreover, (-)-dibromophakellin and 4,5-dibromopyrrole-2-carboxylic acid, as well as **2**, were reported

4,5-dibromopyrrole-2-carboxylic acid, as well as **2**, were reported herein for the first time in this genus. Studies on the possible reaction mechanism and bioactivities were also conducted. The results indicate that the direct chemical diversification of substances present in natural product extracts can be a speedy and useful strategy for the discovery of new compounds.



P-195 – Keyara Piri

Tricyclic Diterpenoids as Potential Androgen Receptor N-Terminal Domain Antagonist

Keyara Piri, Inderpal Sekhon, Seiji Shinkawa, Guanglin Chen, Qiao-Hong Chen

Prostate cancer is one of the deadliest cancers among men in the United States, responsible for roughly 34,700 deaths in 2022. Even with currently available treatments, the disease can develop into castration-resistant prostate cancer (CRPC), which can continue to progress when the transcriptional activity of the androgen receptor (AR) reactivates. AR, therefore, remains to be the viable therapeutic target for CRPC. Deadly CRPC can likely be treated by targeting another functional domain on the AR. Tricyclic aromatic diterpenoid QW07 was reported to block the transcriptional activity of AR NTD (N-terminal domain). However, QW07 does not show selective suppression of AR-positive cell proliferation over the AR-negative one. This study aims to develop tricyclic aromatic diterpenoids as potential NTD AR antagonists. We envision the target tricyclic diterpenoids that can be synthesized through chemical manipulation of commercially available dehydroabietylamine can selectively suppress AR-positive prostate cancer cell proliferation by interacting with AR NTD. Twelve derivatives have been designed by adding different chemical moieties to the C-18 position. These compounds have been synthesized and characterized by interpreting their ^{1}H NMR, ^{13}C NMR, high-resolution mass spectroscopy, and infrared spectroscopy data. These derivatives have been evaluated for their anti-cancer potency on AR-positive prostate cancer cell lines (LNCaP, 22Rv1, and VCaP) using AR-negative cell models (PC-3 and DU145) as a comparison. The antiproliferative data from WST-1 bioassay indicated that dehydroabietylamine derivatives can suppress AR-positive prostate cancer cell proliferation.

P-196 – Jin Yi Tan

Partnering with Community Centers to Perform "Environment to Bioassay" Antibiotic Discovery

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With the growing threat of antimicrobial resistance, there is an urgent need to discover new antibiotics. Microorganisms are a major source of antibacterial drugs, but a typical pipeline employed for microbial drug discovery is highly time consuming, labor intensive, and often results in re-isolation of known antibiotics. For the past few years, we have created innovate methods to improve earlier stages of the antibiotic discovery process. This includes a mass spectrometry-based bioinformatics tool (IDBac) to minimize redundancy of NP production between isolates, and a dual sided agar plate assay (DAPA) which allows microorganisms to compete on opposing sides of a solid support in individual wells. To integrate these advances into a single pipeline, we developed a new *Environment to Bioassay* antibiotic discovery approach that combines high-throughput robotics with DAPA and IDBac to rapidly select, screen, and prioritize antibiotic-producing bacteria. This framework allows us to accomplish many stages of the microbial drug discovery pipeline directly from bacterial cell mass grown on multiwell plates in a semi-automated fashion and offers an advantage in terms of scale and capacity. This project has been integrated with educational outreach efforts in partnership with community centers from underserved areas of Chicago, such as the James Jordan Boys & Girls Club. There are three central components to the program – field work, applied science experiments, and environmental literacy – with the goal of inspiring students from marginalized backgrounds to become the next cohort of university STEM majors. Overall, we aim to demonstrate 1) the feasibility and efficiency of a new antibiotic discovery approach, and 2) that graduate-level research can be successfully integrated with community partnerships.

P-197 – Christopher Thornburg

NCI Program for Natural Product Discovery: Creating a Prefractionated Marine Aqueous Natural Product Library for High-Throughput Screening

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The NCI Program for Natural Product Discovery (NPNPD) is a national program to advance natural product discovery technologies and facilitate the discovery of structurally defined, validated lead molecules ready for translation. At the core of this program is the US National Cancer Institute's (NCI) Natural Product Repository's (NPR) diverse collection of plant, marine and microbial organisms, and subsequent ongoing production of a large, publicly available library of prefractionated natural product samples for screening. Notably, extracts in the NCI NPR are prepared from their respective source material utilizing both an aqueous (100% H_2O) and organic solvent (1:1 DCM/MeOH) extraction process, resulting in two sequential extracts per collected specimen. To date, over 550,000 partially purified natural

product samples have been generated from the organic solvent-based extracts contained within this collection, which are enriched in both non-polar and mid-polarity small molecules. However, as the NCI has long had a program investigating antiviral protein and peptide leads from NCI NPR aqueous extracts, such as cyanovirin-N (CV-N), scytoviron, griffithsin, and recifin A, the NPNPD has recently developed methods to include marine aqueous-based extracts in the fraction library for high-throughput screening (HTS). The details of these methods, which result in a protein- and peptide-rich fraction library, will be presented.

P-198 – Kirk Manfredi

Endophytes, Caves and Insects: Searching for Fungi that Produce Antimicrobial Metabolites

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Over the last several years this lab has been searching for fungi that produce antimicrobial compounds. Initially our work focused on isolating endophytes from native prairie plants. In 2020 we were fortunate to gain access to remote areas of Wind Cave National Park to collect soil and water samples which were then cultivated to grow any fungus associated with that environment. This past fall we began harvesting insects and using them as a source of fungus. Individual insects were captured and placed in petri dishes containing growth media. The insect would remain in the petri dish until its demise at which point any growing fungal colonies were isolated, purified, and cultivated. The cultivated samples were assayed for antimicrobial activity. Fungal samples that were active were identified through their ITS region the rRNA. This presentation will summarize the hit rate and species diversity between the three fungal sources. Additionally, we will evaluate the biological activity and present spectroscopic details of isolated compounds.

P-198B – Kamila Yuyama

Discovering the potential of the alkaloids from *Malouetia tamaquarina* (Apocynaceae) and its endophytes against Neglected Tropical Diseases (NTDs)

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Extracts from Amazonian plant *Malouetia tamaquarina* and their endophytes presented promising antiprotozoal activities against the parasites (Yuyama et al., ASP Meeting 2022). Two novel alkaloids (1 and 2) were isolated from the bark of *M. tamaquarina* and demonstrated significant activities against *Trypanossoma brucei rhodesiense* (IC50 of 2.3 and 20.4 mg mL $^{-1}$) and *Plasmodium falciparum* (IC50 of 0.6 and 7.5 mg mL $^{-1}$) respectively. Their chemical structures were established on the basis of 1D and 2D NMR analyses, as well as HRESIMS data. The isolation of the bioactive compounds from the endophytes is currently in progress.

P-199 – Ella Vardeman

The Effect of Caribbean Traditional Preparations of *Argemone mexicana* L. for Women's Health on the Vaginal Microbiota

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Despite numerous healthcare options available in New York City, medicinal plants are frequently used in immigrant communities as low-cost, culturally-relevant treatments for women's health concerns. Based on previous ethnobotanical studies, *Argemone mexicana* L. is popularly used by Dominican immigrants in New York City to treat gynecological infections. However, in commerce *A. mexicana* is sold and processed differently for this purpose. These differences in processing methods can alter the abundance of known antimicrobial alkaloids identified by UPLC-qToF-MS, such as berberine. Antibacterial screenings against pathogenic (*Gardnerella vaginalis*) and beneficial *(Lactobacillus* spp.) vaginal microbes showed that chemical variation related to processing correlates with bioactivity. Traditional preparations based on the ethnobotanical use of *A. mexicana* tested in co-culture assays demonstrated the influence of processing methods on the abundance of pathogenic and beneficial bacteria. Additionally, experiments quantifying bioactive alkaloids indicated how preparation methods influenced the relative abundance of antimicrobial alkaloids.

P-200 – Draco Kriger

Discovering Cyclopeptide Alkaloids from *C. Americanus*

Draco P. Kriger, Jonathan R. Chekan, University of North Carolina to Greensboro

Cyclopeptide alkaloids (CPAs) are a large class of natural products with a wide range of bioactivity ranging from anti-viral, sedative, and analgesic. They possess a characteristic ether linkage and an oxidative decarboxylation. Ceanothus americanus (New Jersey Tea) is a rich source of cyclopeptide alkaloids, producing at least 10 different analogs. These cyclic peptides have been known for decades but much of the biosynthetic pathways were unknown. Using a transcriptome mining approach, we linked many of the cyclic peptide alkaloids from C. americanus to their respective RiPPs precursor peptides. In addition to known CPAs, we predicted the presence of new CPAs. Isolation and structural characterization were completed to confirm the predictions from the transcriptome mining and resolve ambiguity around the structure of the original isolated molecules. These newly isolated CPAs will be screened for analgesic activity.

P-201 – Sung Chul Park

Novel Bioactive Prenylated Phenols Derived from Heterologous Expression of a *Pseudogymnoascus Destructans* Squalene Synthase Gene in *Aspergillus Nidulans*

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Filamentous fungi produce a wealth of uncharacterized natural products (NPs) that are often challenging to characterize due to cryptic expression in laboratory conditions. Previously we have had success in isolating novel NPs by expressing fungal artificial chromosomes (FACs) from a variety of fungal species into *Aspergillus nidulans*. Here we present a twist to FAC utility where we demonstrate that heterologous expression of a *Pseudogymnoascus destructans* FAC induced silent *A. nidulans* terpenes. Transformation of PdFAC1 into *A. nidulans* resulted in significant production of the host aspernidine-type metabolites compared to PdFAC1 free host extract. Of the total 9 prenylated phenolic compounds (**1**–**9**) isolated, three new aspernidine derivatives (**1**–**3**) were identified. Each of the 9 metabolites contained a farnesyl pyrophosphate (FPP) tail with different aromatic head, which could result in a significant difference in bioactivity. Nidulene A (**1**) showed antibacterial activity against gram positive *Staphyloccocus aureus* and gram negative *Escherichia coli*. PdFAC1 contains a squalene synthase that when deleted resulted in loss of production of these terpenes. We hypothesize that expression of the *P. destructans* native squalene synthase in *A. nidulans* increases the pools of FPP, the precursor to aspernidine synthesis in *A. nidulans.*

P-202 – Rahim Rajwani

Prediction, Detection, and Expression of Ribosomally Synthesized Post-Translationally Modified Peptides (Ripps)

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Advances in genome sequencing have revealed an abundance of uncharacterized natural products; however, metabolite discovery is yet limited by a lack of computational tools to predict final structures from DNA sequences, weak or no expression of target compounds, and their low-abundant ions not being selected for MS/MS acquisitions to facilitate identifications at scale. Here, we set out to address these challenges for RiPPs. First, we developed RiPPMaster, a computer program to compute all possible mature products for a given RiPP. Second, we cultured 56 genome-sequenced soil actinomycetes, encoding 191 RiPP gene clusters, into five different media and collected HRMS data at the MS1 level. We crossmatched RiPPMaster predicted masses with observed mass features and collected MS/MS spectra. The workflow led to the discovery of mature products for known RiPPs, (anantin, citrulassin, sapB, sapT, planosporicin), their variants, and novel RiPPs (lentzeapeptin). Examination of expression patterns of four peptide families (sapB, sapT, citrulassin and planosporicin) across culture conditions and strain genetic backgrounds revealed that increasing the number of culture conditions and/or strains increases the chances of discovery; however, the associations between expression and either culture condition or strain could not be generalized to other strains/conditions. Finally, we analyzed the effect of 800 diverse small molecules on RiPP expression and discovered novel inducers for sapB, planosporicin and lentzeapeptin. The current study provides novel computational and experimental workflows for sensitive detection of RiPPs and underscores the importance of further research into regulation of natural products to access them.

P-203 – Kelsey Alexander

Efficient DNA Extraction Methods of Filamentous Cyanobacteria for Genome Driven Drug Discovery

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Cyanobacteria are known producers of bioactive compounds. Genetic-guided approaches to isolation of new therapeutics is a growing technique to natural product discovery as sequencing costs decrease. Cyanobacteria can be challenging organisms to obtain quality DNA for sequencing. Cyanobacteria can have associated bacteria whose DNA gets extracted with the cyanobacteria DNA. Additionally, there are sheathes that can surround the cyanobacteria that make it harder to extract the DNA for sequencing. In this study, different methods of DNA extraction on cyanobacteria were evaluated for their percent cyanobacteria, read length, quality of assembly, number of biosynthetic gene clusters, length of time, and cost. Establishing efficient and effective methods of DNA extraction allows for better genomic driven drug discovery.

P-204 – Aleksandra Kruk

Tormentillae Tinctura Metabolites and Their Influence on Human Gut Microbiota Biodiversity *Ex Vivo*

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Tormentillae tinctura (TT) has been used for centuries to treat gastrointestinal tract ailments. Due to its tannin-reach composition, TT may have a beneficial effect on human gut microbiota (HGM) homeostasis. Due to these properties, TT could offer an opportunity for novel approaches in the therapy of Leaky Gut Syndrome. The research aimed to determine the mutualistic relationship between TT and HGM. TT metabolites were obtained by incubation of extract with human fecal slurries from 3 healthy donors. After incubation, the UHPLC-DAD-MSn analysis showed changes in the composition of TT. At the same time, 16S rDNA sequencing of HGM exhibits the presence of strains not previously observed in the control samples. Detailed sequencing results and qualitative analysis of TT metabolites will will be presented. The project has been funded by the Polish National Science Centre research grant Sonatina 5 2021/40/C/NZ7/00231.

P-204B – Sunghee Bang

Immunogen from Murine Gut Microbiome

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Gut bacteria play a pivotal role in human health. In particular, the homeostatic immunity, with its close correlations between immune systems and gut microbiota, is one of the most intriguing. However, the ways in which bacteria affect the host at a molecular level remain poorly understood. In order to harness correlations between the microbiome and immune response and ultimately to improve human health, we initially need to understand about the molecules and mechanisms driving host-microbiota interactions. Therefore, in current study, we focus on bacterial immunogen derived from gut microbiome and its mechanisms at a molecular levels.

P-205 – Alexa Lee

Identification of Natural Products from Bat-Associated Bacteria to Combat White-Nose Syndrome

Alexa Lee[1], Ashley Clements[1], Michael W. Mullowney[2], Nischala Nadig[3], Nicole Caimi[4], Neil Kelleher[5], Cheryl Andam[6], Andrea Porras-Alfaro[7], Diana Northup[4], Nancy Keller[3], Paris Salazar-Hamm[4], Lindsay Caesar[1]. [1]Dept. of Chemistry and Biochemistry, James Madison University, [2]Duchossois Family Institute, University of Chicago [3]Dept. of Medical Microbiology and Immunology, University of Wisconsin-Madison, [4]Dept. of Biology, University of New Mexico, [5]Dept. of Chemistry, Northwestern University, [6]Dept. of Biological Sciences, University of Albany, [7]Division of Environmental Biology, National Science Foundation

Bats play a crucial role in global biodiversity and support diverse ecosystems. Unfortunately, the fungal pathogen *Pseudogymnoascus destructans*, the causative agent of White-Nose Syndrome (WNS), is sweeping across the US, killing over 7 million bats in the last decade. Already two of the 47 North American bat species are endangered (4%), and another ten (21%) are affected by WNS with varying severity. Previous efforts surveyed >1000 bacterial isolates from bats captured in and around WNS-free caves in New Mexico and Arizona and found ~100 strains with anti-*P. destructans* activity, suggesting that bats' natural microbiome may provide some protection from infection. Currently, we are conducting a study on 18 of these strains using bioactivity, genomics, and metabolomics to identify antifungal compounds. Comparative molecular networking analysis revealed a molecular family unique to the most potent bioactive strains, and we have identified a plausible biosynthetic pathway for these metabolites. We are now working to purify predicted bioactive compounds for structure elucidation and bioactivity confirmation. Further experiments to identify additional bioactive molecules are also underway.

P-206 – Maribel Okiye

Unveiling the Biological Potential of the Human Oral Microbiome

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The human oral microbiome typically contains over 700 different microbial species. These interactions can shape the microenvironment throughout the human body, as these interactions are paramount to maintaining oral and overall systemic health. Recent advances in technology, such as next-generation sequencing (NGS), have revealed the complexities of the oral microbiome, linking dysbiosis of the oral microbiome with several chronic ailments such as cardiovascular disease, diabetes, and rheumatoid arthritis. However, the role of microbial secondary metabolites in oral and systemic disease progression remains poorly understood. We conducted a metabolomics study on the human salivary secondary metabolome during the progression of early-stage periodontal disease (gingivitis). In this study, we sought to assess the changes in the oral secondary metabolome during disease progression by emulating dysbiosis of the oral microbiome through a twenty-one-day induction of gingivitis in twenty human subjects. Our study identifies three secondary metabolites, cyclo(L-Val-L-Pro) and cyclo(L-Pro-L-Tyr) known regulatory properties, indicating a specialized role for secondary metabolites in oral health maintenance. Surprisingly, we also uncovered a previously unknown metabolic lag that occurs during dysbiosis recovery of the oral cavity, which suggests either a lingering presence of signaling molecules for pathogenic microbe proliferation or a total oral metabolome modification following microenvironmental stress in the oral cavity. This work represents a high-resolution metabolomic landscape for understanding oral health during gingivitis that opens new opportunities for combating progressive periodontal disease and sepsis due to the translocation of oral microbes in the human body.

P-207 – Weronika Skowrońska

Hamamelidis Cortex Reduces the Inflammatory Response of Cells Involved in Wound Healing

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Hamamelidis cortex (*Hamamelis virginiana* L.) is a traditional herbal medicinal product used topically in the treatment of dermatological diseases, hemorrhoids, and oral cavity inflammation. We have analyzed the chemical composition of the extract, isolated the main constituents, and examined the effect of skin microbiota (SM) *ex vivo* cultures on changes in its chemical composition. The extract and the main compounds – gallotannins were investigated regarding their effect on the inflammatory response of cells involved in wound healing – neutrophils (PMNs), keratinocytes (HaCaT) and fibroblasts (NHDF) indicating inhibition of IL1β and IL8 secretion by PMNs, as well as IL6 and IL8 by HaCaT and NHDF. In contrast, ellagic acid – the product of extract's metabolism by SM *ex vivo* cultures, caused a significant increase in the release of IL6 and IL8 by HaCaT and NHDF. Project financed by the National Science Center in Poland, Preludium 20 grant no. 2021/41/N/NZ7/00602.

P-208 – Caitlin Winner

Uncovering Small Molecule Crosstalk in the Human Microbiome

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Naturally occurring functional small molecules have become a mainstay in modern medicine, first propelled by the discovery of penicillin in 1929. This elevated interest in bacterially produced small molecules that could serve as therapeutic agents led to what became known as the Golden Age of Antibiotic discovery, with over 20 different classes of antibiotics being brought to market. While the majority of these studies focused on soil microbes, studies today have shifted to other microbial communities. One such area that has gained attention in recent years is the human microbiome. Advances in DNA sequencing technology in the past decade have allowed for the rapid identification of microorganisms that comprise the human microbiome. With this influx of genomic data, numerous bioinformatics studies have been conducted and show that many microbiota have the biosynthetic capacity to produce functional small molecules. Further association studies have been able to correlate microbiota composition with human health, however the underlying mechanisms of these associations remain vastly underexplored. Uncovering these molecular mechanisms would greatly enhance our understanding of numerous diseases and how they can be more selectively treated. In the Mo lab, we are interested in studying human microbiome strains that have been correlated to human disease but lack a known molecular mechanism. Our goal is to isolate and characterize the small molecules that mediate these microbe-host interactions so that we can gain a better understanding of the role the microbiome plays in human health and disease.

P-209 – Yousong Ding

Discovery and Production of Marine Bioactive Molecules

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Natural product (NP) research faces significant challenges with the low rate of discoveries and limited chemical supply. However, recent statistical analyses have shown that organisms from unexplored sources produce compounds with little similarity to one another, presenting an opportunity for discovery. Furthermore, advancements in DNA sequencing techniques have revealed the vast potential of natural products hidden in microbial

genomes. Here, I will present the discovery and production of NPs from poorly explored marine microbes using multi-disciplinary approaches. Specifically, the first part of my presentation will cover the discovery of several natural products, such as lookeyolides and korormicins, from marine bacteria and fungi associated with devastating coral diseases. I will also discuss their biosynthetic logic. In the second part of my presentation, I will demonstrate the use of synthetic biology approaches, particularly cyanobacterial chassis and biocatalysis, for the production of marine natural product analogs and their synthetically challenging synthons (e.g., the polyketide moiety of anticancer apratoxins). Overall, our research suggests that the marine environment holds a wealth of untapped potential for the discovery of new bioactive compounds, and that multi-disciplinary approaches can help overcome the challenges facing NP research.

P-210 – Emma Smith

Investigation and Discovery of Natural Products from Pacific Coral Probiotics

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Vibrio coralliilyticus is a temperature-dependent pathogen responsible for mass coral mortality events throughout the Pacific. Isolating and identifying compounds that inhibit *V. coralliilyticus* can help to combat coral disease events caused by this pathogen, preventing the disastrous consequences of mass coral mortalities. *Pseudoalteromonas piscidia* (Y97), *P. umbrosa* (B95), *P. ardens* (R96), *P. obscura* (P94), and *Vibrio tetraodonis* (OCN044) subspecies *pristinus* are five probiotic coral-associated bacteria, discovered by collaborators, and inhibit the growth of *V. coralliilyticus* (OCN008). Three of these strains, P94, B95, and R96, are novel species; antiSMASH analysis of these strains indicates biosynthetic richness. This research aims to chemically characterize these novel species as well as to characterize antibiotic compounds and other natural products from all five probiotic strains. Lead compounds from Y97 and P94 active against *V. coralliilyticus* have been isolated and are being characterized. All five strains displayed mild antimicrobial activity when tested against multidrug-resistant *Pseudomonas aeruginosa* (PA9027). Future work includes further chemical investigation of novel strains as well as identifying lead compounds active against both *V. coralliilyticus* and *P. aeruginosa.*

P-211 – Mario Augustinović

Discovery of New Cyclic Peptides from a Marine Sediment-Derived Bacterium

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In 2021, nearly 250 million cases of malaria were reported worldwide, resulting in over 620,000 deaths. Almost 85% of all approved antimalarial drugs are derived from or inspired by natural products (NPs), overwhelmingly represented by plants. Bacteria, which contain biosynthetic machinery markedly dissimilar to plants, represent an untapped source of potential antimalarials. In this current study, we hypothesize that our bacterial library contains unique antimalarial NPs with activity against the chloroquine-resistant *P. falciparum* strain Dd2. To this end, bacterial NPs were screened against Dd2, with a positive hit rate of ~2%. An additional screening on the human hepatocarcinoma line HepG2 was performed in tandem to establish selectivity. Fractions of the strain H002, a *Streptomyces* sp. demonstrated 90% inhibition against *P. falciparum* while showing no effect on HepG2, implicating selectivity toward *P. falciparum*. Dereplication from a large scale regrowth determined that peptides structurally related to tyrocidines were present. Tyrocidines are a group of cyclic decapeptides that have previously reported antimalarial activity; however, we determined that the H002 metabolites contain a modification to the historically conserved structural motif. Previous work suggests that the proline residue is needed to impart activity, but our work suggests that modifications to the tyrocidines conserved motif does not lead to loss of activity. Fractions containing the modified cyclic peptides demonstrated reproducible bioactivity, and work is underway to isolate and confirm their structures.

P-212 – Eunah Jeong

Discovery of Fungal Phenolic UDP-Xylosyltransferase through Mass Spectrometry-Based Metabolic Profiling

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Microbial enzymes can catalyze various structural modifications of exogenous compounds, leading to a diverse array of structural analogs. Such enzymes could be utilized as powerful biocatalysts, however, tracking the enzymatic reactions on desired substrates make it a challenging task to discover novel biocatalysts from nature. Here, we suggest an untargeted-metabolomics analysis pipeline for enzyme discovery from microorganisms. Two major computational tools used in this pipeline, molecular networking and MassQL, enabled us to rapidly annotate chemical reactions in response to exogenous compounds. We analyzed multiple extracts from fungal cultures supplemented with various phytochemicals

using LC-MS/MS. The metabolic phenotyping revealed that *Lentinus brumalis* catalyzes *O*-xylosylation on diverse scaffolds. Genomic and transcriptomic analysis identified a candidate UDP-xylosyltransferase, and the target enzyme, named UGT66A1, was purified and confirmed as a UDP-xylosylase with a broad substrate spectrum. Additionally, it was observed that all *O*-xylosides had significantly reduced antifungal activity when compared to their aglycones, suggesting *O*-xylosylation may be a detoxification process of fungi. This study highlights the usefulness of mass spectrometry-based metabolic profiling in accelerating enzyme discovery.

P-213 – Robert Samples

OpenASAP: an Affordable 3D Printed Atmospheric Solids Analysis Probe (ASAP) Mass Spectrometry System for Direct Analysis of Solid and Liquid Samples

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Atmospheric Solids Analysis Probe (ASAP) mass spectrometry enables versatile direct sampling of solid and liquid samples but is limited by the high cost of commercial systems. We introduce OpenASAP, an open-source, affordable ASAP system for mass spectrometers that can be fabricated for under $20 using 3D printing. OpenASAP is adaptable to instruments from various manufacturers and can be produced with consumer-grade 3D printers. The probe allows rapid analysis without sample preparation, making it valuable for high throughput screening, investigating spatial localization and function of analytes in biological samples, and integrating mass spectrometry in teaching. We showcase its effectiveness by obtaining mass spectra of three natural product standards at levels as low as 10 ng/ml, detecting metabolites in complex microbial cultures, and directly sampling thin layer chromatography (TLC) spots.

P-214 – Courtney Kapczynski

Multi Year Investigation of Urban Agal Blooms in Northwest Florida Using LC-MS/MS

Courtney Kapczynski[1], Stuart Oerhle[2,3], Avery Tatters[4], Wendy Strangman[1], [1]University of North Carolina Wilmington; [2]Northern Kentucky University, [3]Waters Corporation, [4]United States Environmental Protection Agency

The occurrence of harmful algal blooms (HABs) is increasing in urban areas due to various natural and human-induced factors, including excess nutrients and warming waters. The complexity of identifying toxic HAB events is a challenge, as different algae species produce varying toxins, even within the same strain. In an effort to prevent the formation of harmful algal blooms, numerous methods have been developed to better understand their behavior. In the fall of 2021 and 2022, the EPA sampled 10 water retention and treatment ponds in Pensacola, FL. Recently developed chromatography methods were utilized to detect a wide range of HAB toxins. Two UPLC-MS-MS methods, reversed-phase and HILIC, were optimized using reference standards to identify and quantify these toxins, while untargeted metabolomics assessed the chemical diversity of natural products in these waters. These combined analyses will provide valuable insights concerning the presence of HAB toxins and other compounds over multiple years in a model system reflecting water treatment practices implemented throughout the Southeastern United States.

P-215 – Chiraz Soumia Amrine

Semi-Synthetic Derivatives of Verticillins through Acetylation of the C11 Hydroxy Group

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The verticillins, a class of epipolythiodioxopiperazine alkaloids (ETP) have gained attention due to their potent activity against cancer cells, noted both in vitro and in vivo. In this study, ester, carbonate, sulfonate and carbamate derivatives were synthesized using the isolated natural products from *Clonostachys rogersiana*. Semi-synthetic efforts were designed to explore the reactivity of the C11 and C11' hydroxy substituents. Verticillin H was used as a starting material to generate nine semisynthetic analogues. Likewise, verticillin A succinate was synthesized as a way of proving the successful application of these reactions on various ETP starting materials. The skills of synthetic chemistry to generate analogues were coupled with those of natural products chemistry to both monitor reaction progress (i.e., ^{1}H NMR) and purify leads (i.e., prep-HPLC). The synthesized compounds and their corresponding starting materials were screened for activity against a panel of breast and ovarian cancer cell lines: MDA-MB-435, MDA-MB-231, and OVCAR3.

P-216 – Charmaine Lindsay

Investigation of Precursor-derived Secondary Metabolites of *Penicillium arauntiacobrunneum* for Potential Cytotoxic Activity

*Charmaine A. Lindsay[1], Choon Y. Tan[1], Gerardo D. Anaya-Eugenio[1], Chad A. Rappleye[2], Richard W. Spjut[3], Esperanza Carcache de Blanco[1], A. Douglas Kinghorn[1], Harinantenaina L. Rakotondraibe[*1]. [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, [2]Department of Microbiology, The Ohio State University, Columbus, Ohio 43210, United States, [3]World Botanical Associates, Bakersfield, CA 93380, United States*

Cancer is the second leading cause of death worldwide and survival rate is especially low in late disease-stage diagnoses, due to poor responses to treatment. As such, there is a continued need for new therapeutic options to address this health care crisis. One avenue for novel compound discovery is isolation of small molecules from unusual sources. Mycobionts of U.S. endemic lichens meet this criterion based on their previously demonstrated ability to produce cytotoxic compounds. Furthermore, the production of novel and cytotoxic compounds can be even further enhanced by precursor-driven biosynthesis. Therefore, we proposed and tested the hypothesis that *Penicillium aurantiacobrunneum* (Trichocomaceae), a mycobiont associated with the U.S. endemic lichen *Niebla homalea* (Ramalinaceae), can produce antiproliferative compounds through precursor feeding experiments with *para*-F-DL-phenylalanine. The structures of the resulting fluorinated compounds and future work to assess their cytotoxic activity will be discussed.

P-217 – Hoan Tam Pham

Total Synthesis and Biological Evaluation of Lyngbyapeptin A from a Marine Cyanobacterium

Hoan Tam Pham; Qi-Yin Chen; Hendrik Luesch, Department of Medicinal Chemistry and Center for Natural Products, Drug Discovery and Development (CNPD3), College of Pharmacy, University of Florida, Gainesville, Florida, USA

Lyngbyapeptin A is a linear modified tetrapeptide which was first discovered from the marine cyanobacterium Moorena bouillonii collected in Papua New Guinea and was later isolated from the same species found in Guam. The natural product contains one thiazole ring, a 3-methoxy-2-butenoyl moiety, and a high level of N-methylation. In previous research, lyngbyapeptin A did not show any significant cytotoxicity but was not rigorously investigated for other biological activities. Furthermore, it was prone to decomposition due to the unstable nature of the 3-methoxy-2-butenoyl moiety, hence preventing further testing. We achieved the total synthesis of lyngbyapeptin A featuring a series of peptide bond formation reactions, which provided more material for in-depth biological evaluation and enabled us to ascribe functions to this marine natural product.

P-218 – Yulin Ren

Glucose Transport Inhibition and Cancer Cell Cytotoxicity of the Fruits of *Aronia melanocarpa*

Gunnar Meyer[1], Pratik Shriwas[1–3], Tyler Frank[1], Kevin DeRose[1], Jizhou Lei[1], Jessica R. Grebenc[1], Ryan Slaughter[4,5], Gary Gao[4,5], Xiaozhuo Chen[2,3,6,7], A. Douglas Kinghorn[1], and Yulin Ren[1]. [1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, United States, [2]Department of Biological Sciences, [3]Edison Biotechnology Institute, [6]Molecular and Cellular Biology Program, and [7]Department of Biomedical Sciences, Ohio University, Athens, OH 45701, United States, [4]OSU South Centers, and [5]Department of Horticulture and Crop Science, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, OH 43210, United States

Black chokeberry, *Aronia melanocarpa* (Michx.) Elliott (Rosaceae), is a shrub native to North America, of which the fruits (Aronia berries) are gaining popularity in the food industry. These berries show multiple bioactivities potentially beneficial to human health, but their glucose transport inhibitory activity has not been reported. Herein, the chloroform-soluble extract of Ohio-grown Aronia berries and its fractions, along with ursolic acid isolated and several analogues, were tested in glucose uptake and cytotoxicity assays, against human A549 non-small cell lung cancer and HepG2 hepatoma cells. The results showed that the chloroform-soluble extract of Aronia berries and some of the fractions were active, with the cytotoxicity profiles being consistent with those observed for their glucose transport inhibitory activity. These indicate that Aronia berries could target cancer cell metabolism to mediate their cytotoxicity. However, all compounds tested did not show any obvious activities, indicating that ursolic acid and the analogues selected are not the major active components for the metabolism-targeted cytotoxicity of Aronia berries grown in Ohio.

P-219 – Yulin Ren

The Cytotoxic Cardiac Glycoside (–)-Cryptanoside A Isolated from the Stems of *Cryptolepis dubia* Collected in Laos

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A cardiac glycoside epoxide, (–)-cryptanoside A (**1**), was isolated from the stems of *Cryptolepis dubia* (Burm.f.) M.R. Almeida (Apocynaceae) collected in Laos, and its complete structure was confirmed by analysis of its spectroscopic and single-crystal X-ray diffraction data. This cardiac glycoside epoxide exhibited potent cytotoxicity against several human cancer cell lines tested, including HT-29 colon, MDA-MB-231 breast, OVCAR3 and OVCAR5 ovarian cancer, and MDA-MB-435 melanoma cells, with the IC_{50} values found to be in the range 0.1–0.5 μM. It also inhibited Na^+/K^+-ATPase activity and increased the expression of Akt and the p65 subunit of NF-κB but did not show any effects on the expression of PI3K. Molecular docking profiles showed that (–)-cryptanoside A (**1**) binds to Na^+/K^+-ATPase, and thus **1** may directly target Na^+/K^+-ATPase to mediate its cancer cell cytotoxicity.

P-220 – Yunlin Ren

Cytotoxic Leuconoxine-type Diazaspiroindole Alkaloid Isolated from *Cryptolepis dubia* and Its Analogues

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The leuconoxine-type diazaspiroindole alkaloids are a small group of monoterpene indole alkaloids that contain a pentacyclic unit with three contiguous quaternary stereogenic centers and have attracted wide interest. Of these, a new member, 6-chloro-6,7-dehydroleuconoxine (**1**), and a known analogue, 6,7-dehydroleuconoxine or melodinine E (**2**), were isolated from the stems of *Cryptolepis dubia* (Burm.f.) M.R. Almeida [syn.: *C. buchananii* Roem. & Schult.] (Apocynaceae) collected in Laos. The structure of **1** was determined by analysis of its spectroscopic data and by comparison of these data with those of **2**, of which the complete structure has been determined by analysis of its single-crystal X-ray diffraction data. Interestingly, when tested against a small panel of human cancer cell lines, compound **1** exhibited selective cytotoxicity toward OVCAR3 human ovarian cancer cells, indicating that the pentacyclic diazaspiroindole unit may be useful in the design of new anticancer agents.

P-221 – Lisa Rusali

Efforts Toward a Biomimetic Synthesis of the *Aristotelia* Alkaloids

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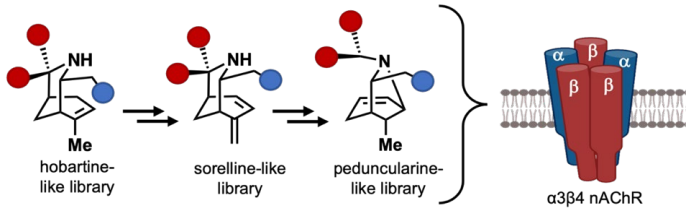
Antagonists for the α3β4 nicotinic acetylcholine receptor (nAChR) have been shown to reduce drug- seeking behavior and withdrawal symptoms in rodents, but existing α3β4 nAChR antagonists face a myriad of issues, including a lack of subtype selectivity, poor pharmacokinetic properties, and multiple off-target liabilities. Recently, several alkaloids isolated from *Aristotelia chilensis* were identified as α3β4 antagonists that are possess moderate selectivity over the α4β2 and α7 subtypes. This prompted us to consider whether the >30 *Aristotelia* alkaloids reported in the literature also have nAChR activity. Herein, we report our efforts to access the cores of several of these alkaloids using a biomimetic approach and a preliminary evaluation of their functional activity at the α3β4 nAChRs. This work will expand our understanding of the structure-activity relationships between the *Aristotelia* alkaloids and the nAChRs, which will, in turn, aid in developing highly potent and selective pharmacological probes to study the role of the α3β4 nAChR in substance use disorder.



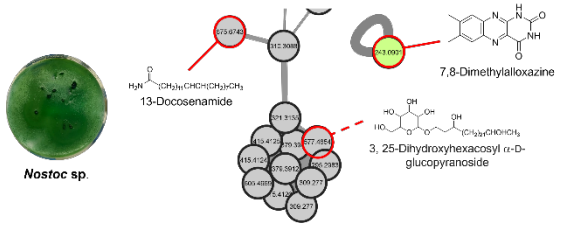
P-222 – Mario Figueroa

Bioprospection of *Nostoc* Species from Wetlands of Mexico

Mariano Jacome,[1] Eberto Novelo,[2] and Mario Figueroa.[1] [1] Facultad de Química and [2] Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México

Two *Nostoc* species isolated from wetlands, Yalahau in Quintana Roo and the Magdalena River in Mexico City, Mexico, were cultivated in liquid and solid BG11 and BG0 media. The metabolomic analysis of their methanolic extracts using HRESIMS data and the GNPS platform led to the identification of several metabolites, including pheophytin A, xanthurenic acid, 13-docosenamide, 7,8-dimethylalloxazine, and 3,25-dihydroxyhexacosyl α-D-glucopyranoside, among others. The antibacterial activity of the extracts against ESKAPE pathogens was also established. This work represents the first chemical and biological study of *Nostoc* species from Mexico. This work was supported by DGAPA-PAPIIT UNAM IN203923 (MF). MJ thanks

the fellowship from Subprograma 127 of Facultad de Química, UNAM.



P-223 – Nozomi Mosu

Analysis of Animal-Derived Rare Actinomycetes as a New Source of Bioactive Compounds

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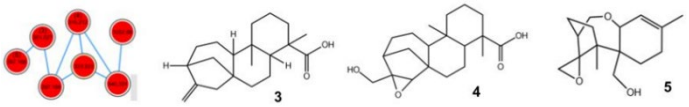
Rare actinomycetes (non-Streptomyces actinomycetes) have been attracting attention as potential resource for novel compounds. In this study, we focused on actinomycetes derived from feces, hairs, and gastrointestinal tracts of animals such as mammals and birds as a source of novel bioactive compounds. We isolated 32 species of actinomycetes from several animal samples such as goat, cattle, nutria, penguin, and orca. Although the majority of actinomycetes obtained from soil are Streptomyces spp., rare actinomycetes alone were isolated from the animal samples. Our result indicates that rare actinomycetes derived from mammals or birds may be a new source for bioactive compounds.

P-224 – Dulce Silva

Coculture of Endophytic Fungi *Humicola fuscoatra* and *Nemania bipapillata* Isolated from Marine Red alga *Asparagopsis taxiformis* Triggers Chemodiversity

Iatã C. Mendonça[1], Victor H.Rufino[1], Edelson Dias[1] (PG), Givaldo S. Silva[1], Lucas H. Moura[1], Yan Fernandes[1], Dulce Silva[1]. [1]NuBBE-Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais, Institute of Chemistry, UNESP - Sao Paulo State University, Araraquara, SP, Brazil

Microorganisms from marine ecosystems may be highlighted as relevant sources of unique natural products due to their high chemodiversity, which represents great potential for bioprospecting and source of value-added bioproducts. Several genes related to the biosynthesis of especial metabolites in microorganisms remain silenced under standard laboratory growth conditions. Some approaches have been used to trigger activation of silenced biosynthetic pathways such as coculture. In this work, two fungal strains, *Humicola fuscoatra* and *Nemania bipapillata,* were isolated from marine alga *Asparagopsis taxiformis*. After growth in Petri dishes, they were inoculated both separately and together in Erlenmeyer flasks containing malt growth medium. Filtration of the fermented broths yielded the aqueous filtrates which were subjected to partition with EtOAc and solvent evaporation to yield the extracts. The monoculture and coculture extracts were analyzed by UPLC-MS/MS and GNPS molecular networking platform, which provided annotation of chemical constituents produced only in coculture: alkaloid isoresespin (1), monoterpene gentiopicroside (2), and a cluster with two diterpenes, kaurenoic acid (3) and 17-Hydroxy-15,16-epoxykauran-18-oic acid (4), and a sesquiterpenoid, verrucarol (5), in addition to four further possible terpenes that did not match the platform library and might be isolated and identified in the next steps.



P-225 – Sean Romanowski

Burkholderia Bacterial Host Development Leads to Discovery of a Novel Lipopeptide

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Burkholderia are prolific producers of natural products (NPs). A major shortcoming in drug discovery is that NPs are usually produced in quantities too low for practical application. A critical need exists for the development of high-yielding, alternative hosts beyond E. coli for heterologous expression. Burkholderia sp. FERM BP-3421 produces autologous spliceostatins at up to 6 g/L, and heterologous expression of antibacterial capistruin was achieved in quantities 600-fold greater than with E. coli, making FERM BP-3421 a promising candidate for host development. Towards chassis development of FERM BP-3421 we utilized RNA-seq to obtain its transcriptome and identify highly expressed biosynthetic gene clusters (BGCs) with the ultimate goal of genome minimization to improve heterologous expression. 29 BGCs were predicted, one of which contained a highly expressed nonribosomal peptide synthetase gene cluster we named sel. Deletion of the sel BGC helped connect the BGC to the novel lipopeptide selethramide and to illuminate its function as a surfactant involved in promoting surface motility. We are currently testing spliceostatin and selethramide deletion mutants as improved chassis strains.

P-226 – Yern Hyerk Shin

Revisiting Coley's Toxins: Immunogenic Cardiolipins from *Streptococcus pyogenes*

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Streptococcus pyogenes (Sp), a gram-positive and human pathogenic bacterium causing streptococcal diseases such as strep throat, scarlet fever, rheumatic fever, puerperal fever, and streptococcal toxic shock syndrome, is one of the key ingredients of Coley's toxin, an early form of cancer immunotherapy ~100 years ago. To discover immunogenic metabolites from the bug, a cell extract of S. pyogenes was analyzed and subjected to a bioactivity-guided fractionation resulting in the identification of an immunogenic 18:1/18:0/18:1/18:0 cardiolipin (CL), SpCL-1 (**1**). Cellular assays of **1** with murine and human BMDCs (bone marrow-derived dendritic cells) showed TLR2/TLR1-dependent signaling whereas acyl chain-switched (**2**) showed no significant activity in our assays.

P-227 – Swarnali Chatterjee

Exploring the Neuroprotective Potential of Selected Dietary Soft Electrophiles

Swarnali Chatterjee, Urmila Maitra, Bianca McCarty, Qiaoli Liang, Lukasz Ciesla, The University of Alabama, Tuscaloosa AL 35487

Neurodegenerative diseases and other age-associated chronic disorders pose significant medical challenges as there is currently no effective and sustainable cure available. Epidemiological data suggest that a regular dietary intake of flavonoids and omega-3 fatty acids enhances cognitive capacity in both animal models and humans and delays the onset of age-related neurological diseases. We hypothesize that flavonoids and other dietary phytochemicals may mimic the physiological effects of omega-3-derived pro-resolving and anti-inflammatory molecules and are involved in the process of resolution of neuroinflammation. Additionally, we propose that the anti-inflammatory and neuroprotective activity of these dietary molecules is directly related to their soft electrophilic properties. Using various analytical techniques, we have traced these molecules in complex biological matrices and studied their anti-inflammatory effects. Our preliminary results suggest that certain dietary soft electrophiles, such as gardenin A, thymoquinone, and vitamin E vitamers, enhance the production of lipid pro-resolving molecules and confer neuroprotection in a paraquat-induced *Drosophila* model of Parkinson's disease (PD). Our previous research also indicated that gardenin A in particular is a very promising neuroprotective agent. We have recently shown that gardenin A provides neuroprotection in a mammalian model of PD. Using ultra-high performance liquid chromatography with high resolution mass spectrometry we traced gardenin A and its metabolites in mice brains confirming its brain bioavailability. In conclusion, our study sheds light on the potential of certain dietary soft electrophiles as neuroprotective agents, which could lead to the development of new preventive and treatment options for neurodegenerative diseases and other age-related chronic disorders.

P-228 – Perihan Gürbüz

Screening of Selected Phenolic Compounds for BBB Specific Artificial Membrane Permeability Capabilities and Neuroprotective Effects Via In Vitro GSK-3β, CK-1δ, and AChE Inhibition

Perihan Gürbüz[1], Sengül Dilem Doğan[2], Miyase Gözde Gündüz[3], Loreto Martínez-González[4,5], Concepción Pérez[6], Ana Martinez[4,5] [1]Department of Pharmacognosy, Faculty of Pharmacy, Erciyes University, Kayseri, Türkiye, [2]Department of Basic Sciences, Faculty of Pharmacy, Erciyes University, Kayseri, Türkiye, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye, [4]Centro de Investigaciones Biologicas, CSIC, Ramiro de Maetzu 9, 28040 Madrid, Spain, [5]Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain, [6]Medicinal Chemistry Institute-CSIC, Juan de la Cierva 3, 280 06 Madrid, Spain

One of the main obstacles to the treatment of diseases of the central nervous system (CNS) is the drug's penetration of the blood-brain barrier (BBB) at therapeutic concentrations. In order to explore the capacity of some phenolic compounds derived mainly from Asteraceae, Apiaceae and Cistaceae plants to penetrate into the brain, we used the Parallel Artificial Membrane Permeability Assay (PAMPA-BBB). The in vitro permeabilities (Pe) of commercial drugs through lipid membrane extract together with phenolic compounds were determined and described. The anti-Alzheimer effects of CNS+ compounds were examined by their inhibitory capacity towards glycogen synthase kinase (GSK-3β), casein kinase 1 (CK-1δ) and acetyl-cholinesterase (AChE) enzymes associated with AD. Most of the CNS+ compounds strongly inhibited GSK-3β therefore, molecular docking studies of these compounds were carried out in the active sites of GSK-3β to predict the most appropriate binding modes and support the experimental data.

P-229 – Won-Kyung Cho

Antiviral Effect of Isoquercitrin against Influenza A Viral Infection via Modulating Hemagglutinin and Neuraminidase

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Isoquercitrin (IQC) is a component abundantly present in many plants and is known to have various medicinal effects. In this study, we demonstrate that IQC exhibits strong anti-influenza A virus infection by modulating hemagglutinin (HA) and neuraminidase (NA) activities. We used green fluorescent protein-tagged Influenza A/PR/8/34, A/PR/8/34, and HBPV-VR-32 to evaluate the anti-Influenza viral effect of IQC. The results of fluorescence microscopy and fluorescence-activated cell sorting analysis showed IQC significantly represses GFP expression by IAV infection, dose-dependently. Consistently, IQC strongly inhibited cytopathic effects by H1N1 or H3N2 IAV infection. Immunofluorescence analysis confirmed that IQC represses the IAV protein expression. Time-of-addition assay showed that IQC inhibits viral attachment and entry and exerts a strong virucidal effect during IAV infection. Hemagglutination assay confirmed the inhibitory effect of IQC on IAV HA. Further, IQC potently reduced the NA activities of H1N1 and H3N2 IAV. In conclusion, IQC prevents IAV infection at multi-stages via virucidal effects, inhibiting attachment, entry, and viral release. Our results indicate that IQC could be developed as a potent antiviral drug to protect against influenza viral infection.

P-230 – Ju Hye Yang

Indigo Pulverata Levis Alleviates Inflammatory Responses in DNCB-Induced Atopic Dermatitis-Like Mice Model

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The Indigo Pulverata Levis extract (CHD) is used in traditional Southeast Asian medicine; however, its beneficial effects on atopic dermatitis (AD) remain uninvestigated. Therefore, we investigated the therapeutic effects of CHD in 2,4-dinitrochlorobenzene (DNCB)-induced BALB/c mice model. We evaluated immune cell infiltration, skin thickness, and the serum IgE and TNF-α levels in DNCB-induced AD mice. Moreover, we measured the expression levels of pro-inflammatory cytokines, the mitogen-activated protein kinase (MAPK), and the nuclear factor-kappa B (NF-κB) in the mice skin cells. Our in vivo results revealed that CHD reduced the dermal and epidermal thicknesses and inhibited immune cell infiltration. Furthermore, it suppressed the proinflammatory cytokine expression and MAPK and NF-κB phosphorylations in the skin tissue and decreased serum IgE and TNF-α levels. Based on these results, we suggests that CHD is a potential drug candidate for AD treatment.

P-231 – Tamam El-Elimat

Homoisoflavonoids with Cytotoxic Activity from the Bulbs of *Bellevalia Desertorum*

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Ten compounds (1-10) were identified from the bulbs of *Bellevalia desertorum* Eig & Feinbrun (Asparagaceae). Compound 7 was a new homoisoflavonoid analogue. The structures of the isolated compounds were elucidated using a series of spectroscopic and spectrometric techniques, principally HRESIMS, 1D-NMR (^{1}H and ^{13}C-NMR) and 2D-NMR (COSY, edited-HSQC, and HMBC), while absolute configurations were assigned using ECD spectroscopy. The isolated compounds (1-10) were tested for cytotoxic activities against a panel of human cancer cell lines.

P-232 – Tamam El-Elimat

Cytotoxic Colchicinoids from *Colchicum tuviae* Feinbrun (Colchicaceae)

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Thirteen compounds were isolated from the methanolic extract of the aerial parts of Colchicum tuviae Feinbrun (Colchicaceae). Of these, three were new and ten were known. The structures of the isolated compounds were elucidated using a series of spectroscopic and spectrometric techniques, principally HRESIMS, 1D-NMR (^{1}H and ^{13}C-NMR) and 2D-NMR (COSY, edited-HSQC, and HMBC). The cytotoxic activities of the isolated compounds (1-13) were evaluated using the OVCAR3 (ovary) and MDA-MB-435 (melanoma) cancer cell lines. Compound **2** was the most potent against the OVCAR3 cell line, with IC_{50} value of 16.7 nM. Whereas compounds 7 and 8 were equipotent on MDA-MB-435 cell line with IC_{50} values of 14.2 nM.

P-233 – Kyungha Lee

A Systemic Analysis on the Chemical Diversity of Monoterpene Indole Alkaloids in 275 Species of Apocynaceae, Loganiaceae, and Rubiaceae

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Monoterpene indole alkaloids (MIAs) are crucial plant specialized metabolites derived from tryptophan and secologanin. MIAs have been considered one of the most interesting classes for their structural diversity and physiological activity. In this study, we performed systemic analysis for the chemical diversity and distribution patterns of MIAs in Apocynaceae, Loganiaceae, and Rubiaceae, which are plant families known as MIA-producing taxa. To this end, the alkaloids-rich fractions of 275 species in three families were prepared for performing untargeted LC-MS/MS analysis. The MS/MS molecular networking analysis revealed that each scaffold of MIAs exhibited distribution patterns limited to certain genera or families. *Uncaria scandens* (Rubiaceae) was prioritized as a target for a large-scale purification, due to the occurrence of many genus-specific metabolites. Eight compounds were isolated and identified as strictosamide (**1**), mitraphylline (**2**), uncarine A (**3**), uncarine C (**4**), uncarine F (**5**), uncarine E (**6**), lyaloside (**7**), and 5(*S*)-5-carbomethoxystrictosidine (**8**). Although all of these compounds were previously known compounds, compounds **2**–**6** were compounds specifically found only from the genera *Mitragyna* and *Uncaria*. We expect that our investigation on MIAs will contribute to find previously unknown MIAs and expand our knowledge on distribution and diversity of MIAs.

P-234 – Fatimah I. Qassadi

Extraction, Evaluation and Identification of Bioactive Products from Natural Plant Based Extracts: Antimicrobial Activities and Phytochemical Characterization

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Antimicrobial resistance has become one of the most important and pressing healthcare challenges of the present time [1]. Natural products, such as those from plants, may provide new classes of antibiotics to combat antibiotic resistance. *Aegle marmelos* (or Bael) and *Tinospora cordifolia* (or Guduchi or Giloy) are extensively used in Ayurvedic medicine as a treatment for a range of conditions including gastrointestinal symptoms such as diarrhoea [2, 3]. This study investigated the in vitro inhibitory effects of solvent extracts from leaves of *A. marmelos* and stems of *T. cordifolia* on clinical isolates of food poisoning strains of *Escherichia coli* (Enteroaggregative *Escherichia coli* (EAEC), and Enteroinvasive *Escherichia coli* (EIEC)), and on non-pathogenic *Escherichia coli (O157:H7 and DH5α pUC19).* We employed LC-MS-based untargeted metabolomics to identify bioactive metabolites from plant extracts. There was a significant inhibition of the growth of pathogenic *E. coli* strains by methanol and 70% ethanol extracts of *A. marmelos* and *T. cordifolia*, respectively, with IC50 values ranging between 1.76 ± 0.25 -6.34 ± 2.93 mg/ml. The ethanolic extracts of both plants had some statistically significant activity at high concentrations on O157:H7 though not on DH5α pUC19. Phytochemical profiling of the most active extracts was conducted using a dereplication approach with a Dionex UtiMate 3000 high-performance liquid chromatography (HPLC) system coupled to a Q-Exactive plus hybrid quadrupole-Orbitrap mass spectrometer equipped with heated electrospray ionization (HESI) sources and database searching. Chemical analysis revealed several coumarins, alkaloids, sterols, and flavonoids, mainly flavonol glycoside derivatives, which were tentatively identified in both plant extracts. *T. cordifolia* extract contains high levels of antimicrobial quinones such as benzoquinones, naphthoquinones, anthraquinones, and polycyclic quinones. The antimicrobial metabolites found in the extracts of *A. marmelos* and *T. cordifolia* appear to have potential as candidates for novel antimicrobial drug development.

P-235 – Yu kyung Choi

Plantago lanceolata as a Potential Treatment for Reflux Esophagitis

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Plantago lanceolata L. is a cosmopolitan species belonging to Plantaginaceae. A variety of biological activities have been reported in several studies for *P. lanceolata* extract. Reflux esophagitis (RE) is a common type of gastroesophageal reflux disease, and its incidence continues to increase. This study verifies the effects of the *P. lanceolata* 70% EtOH extract (PLE) on esophageal tissue, against oxidative stress and inflammation in acute RE-induced rats. The total polyphenol and flavonoid contents of PLE were 109.91 ± 1.51 mg gallic acid equivalent/g and 87.84 ± 0.58 mg rutin equivalent/g, respectively. The major compound of PLE, verbascoside, was quantified by HPLC analysis to be 97.31 mg/g. PLE showed concentration-dependent antioxidant activities in DPPH and ABTS radical scavenging and FRAP assays. PLE treatment in LPS-induced RAW 264.7 cells significantly decreased the production of reactive oxygen species and nitric oxide. Additionally, PLE suppressed NF-κB phosphorylation and downregulated the expression of inflammatory proteins, including iNOS and COX-2. In a rat model of RE, PLE inhibited the inflammatory response of TNF-α and IL-1β through regulation of the MAPKs pathway. Moreover, PLE increased the expression of claudin, one of the tight junction proteins, to preserve the physical barrier function. Overall, our findings suggest that PLE has the potential as a natural treatment option for RE.

P-236 – Ryan Cohen

Benchmarking DFT Methods for NMR Structure Elucidation of Natural Products

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Density functional theory (DFT) benchmark studies of ^{1}H and ^{13}C NMR chemical shifts often yield differing conclusions, likely due to non-optimal test molecules and non-standardized data acquisition. To address this issue, we carefully selected and measured ^{1}H and ^{13}C NMR chemical shifts for 50 structurally-diverse small organic molecules containing atoms from only the first two rows of the periodic table. Our NMR dataset, DELTA50, was used to calculate linear scaling factors and to evaluate the accuracy of 73 density functionals, 40 basis sets, 3 solvent models, and 3 gauge referencing schemes. The best performing DFT methodologies for ^{1}H and ^{13}C NMR chemical shift predictions were WP04/6-311++G(2d,p) and ωB97X-D/def2-SVP, respectively, when combined with the polarizable continuum solvent model (PCM) and gauge-independent atomic orbital (GIAO) method. Geometries should be optimized at the B3LYP-D3/6-311G(d,p) level including the PCM solvent model for the best accuracy. Predictions of 20 organic compounds and natural products from a separate probe set had root-mean square deviations (RMSD) of 0.07 to 0.19 for ^{1}H and 0.5 to 2.9 for ^{13}C. Maximum deviations were less than 0.5 and 6.5 ppm for ^{1}H and ^{13}C, respectively.

P-237 – Cody Earp

Use of Biosynthetic Gene Clusters and Biochemometrics to Identify Stemphone Analogues

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Traditionally, a bioactivity guided approach is used to guide the selection of samples to pursue. Advancements have been made in correlating biosynthetic gene clusters (BGC) with the secondary metabolite they produce. By taking advantage of this, a correlative metabologenomic data set was generated for various fungal isolates. However, this resulted in thousands of potential leads. To prioritize which leads should be followed, bioactivity was used as a filter by using biochemometrics to predict active constituents. From there, we used the meabologenomic dataset to identify the BGC most likely to be responsible for their production. From this analysis, a promising mass of 589 *m/z* was identified in three *Aspergillus* spp. and was predicted to have cytotoxic activity based on the products of similar clusters. The extracts of these three cultures underwent further purification and three related compounds were isolated, two of which matched the predicted mass. Upon elucidating the structures, these compounds were identified as analogues of three known meroterpenoids, stemphones B, E, and G. Analogues B and G matched the predicted mass and were active against two cancer cell lines.

P-238 – Jae Sang Han

Isolation of Sesquiterpene Lactone Dimers from the Roots of *Aucklandia Lappa* Guided By LC-MS/MS Based Molecular Networking

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Aucklandia lappa, belongs to the family Asteraceae, is widely distributed in Korea, China, and India. The roots of A. lappa referred to as "Mok-Hyang" in Korea, have been used as traditional medicine to treat various ailments, including asthma, coughs, and dermatitis. During the preliminary bioactivity screening, the n-hexane and dichloromethane-soluble fractions were showed potent inhibitory activity against the production of nitric oxide in LPS-induced RAW264.7 cell. To predict the bioactive components in this fraction, molecular networking (MN) based on LC-MS/MS data was conducted using the GNPS web platform. In the molecular network, sesquiterpene lactone monomer and dimer were mainly distributed in n-hexane and dichloromethane-soluble fractions. As a result, four undescribed sesquiterpene dimers **1-4** along with six known sesquiterpenes **5-10** were efficiently isolated and their structure were elucidated by various spectroscopic methods, such as 1D-NMR, 2D-NMR, HRESIMS, and ECD calculation. In addition, all isolated compounds were tested for their inhibitory effect against the nitric oxide production in LPS-induced RAW264.7 cells and showed significant inhibitory effects with IC_{50} value ranging from 0.3 to 22.5 μM.

P-239 – Sangwook Kang

Epoxinnamide: An Epoxy Cinnamoyl-Containing Nonribosomal Peptide from an Intertidal Mudflat-Derived *Streptomyces* sp.

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Cinnamoyl-containing nonribosomal peptides (CCNPs) form a unique family of actinobacterial secondary metabolites and display various biological activities. A new CCNP named epoxinnamide **(1)** was discovered from intertidal mudflat-derived *Streptomyces* sp. OID44. The structure of **1** was determined by the analysis of NMR data along with a mass spectrum. The absolute configuration of **1** was assigned by the combination of advanced Marfey's method, $^3J_{HH}$ and ROESY analysis, DP4 calculation, and genomic analysis. The putative biosynthetic pathway of epoxinnamide (**1**) was identified through the whole-genome sequencing of *Streptomyces* sp. OID44. In particular, the thioesterase domain in the NRPS biosynthetic gene cluster was proposed as a bifunctional enzyme, which catalyzes both epimerization and macrocyclization. Epoxinnamide (**1**) induced quinone reductase (QR) activity in murine Hepa-1c1c7 cells by 1.6-fold at 5 μM. It also exhibited effective antiangiogenesis activity in human umbilical vein endothelial cells (IC_{50} = 13.4 μM).

P-240 – Byung Sun Min

Anti-osteoclastogenic Activities of Indole Alkaloids Isolated from the Grass of *Hordeum vulgare* var. *hexastichon*

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The alkaloid fraction of Hordeum vulgare var. hexastichon grass (HVA) significantly inhibited RANKL-induced osteoclast formation and protected mice from LPS-induced bone loss. A phytochemical investigation of HVA afforded nine indole alkaloids, including one new compound [hordeumin A (**1**)] and eight known analogues (**2**–**9**). Of them, four (**1**, **2**, **4**, and **5**) were anti-osteoclastogenic compounds. Of these four, compound **5** significantly suppressed RANKL-induced osteoclast formation, actin ring formation, and bone resorption in a concentration-dependent manner. It also suppressed the RANKL-induced NF-κB and MAPK signaling pathways and the activation of c-Fos and NFATc1. Compound **5** also reduced the expression levels of osteoclast-specific marker genes, including TRAP, CtsK, DC-STAMP, OSCAR, and MMP9.

P-241 – Byung Sun Min

Anti-inflammatory Lignans from the Roots of *Asarum heterotropoides* var. *mandshuricum*

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Asarum heterotropoides var. mandshuricum F. Maekawa (Aristolochiaceae) root extract led to the isolation and characterization of one new ferulic acid glucose ester (**1**) and nine known lignans (**2**–**10**). Their structures were elucidated using extensive spectroscopic methods, including 1D and 2D NMR, and MS spectra. The anti-inflammatory effects of the isolated compounds were investigated via their inhibition against NO production in LPS-stimulated RAW264.7 mouse macrophage cells. Among them, compound **7** showed the most effective inhibitory activity against NO production and expression of iNOS and COX-2 protein in an exceedingly dose-dependent manner. In addition, further study revealed that the mechanism of anti-inflammatory activity of the most active lignan (**7**) might be associated with the inhibition of ERK and NF-κB phosphorylation.

P-242 – Sara Neiheisel

Facilitating the Identification of Secondary Metabolites in a *Centella asiatica* Extract Using 1D Selective Gradient TOCSY NMR Spectroscopy

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Centella asiatica is a perennial pennywort that has been utilized in traditional medicine systems in China and India for centuries. More recently, it has been popularized in the United States as a botanical supplement for cognitive enhancement and neuroprotective effects. Although C. asiatica shows preclinical evidence of its therapeutic potential, lack of consistency in Centella products limits validation of its medicinal benefits in human studies. To complement standardization and quality control efforts, a comprehensive isolation and NMR analysis of C. asiatica compounds is being performed from authenticated C. asiatica provided by the BENFRA Botanical Dietary Supplements Research Center (NIH/NCCIH U19AT010829). The ongoing isolation has yielded marker metabolites, including ursane and oleanane-type triterpenoids as well as caffeoylquinic acids. The isolated compounds will be examined using selective one-dimensional TOCSY (S1DT), providing characteristic proton spin networks to streamline identification of individual compounds within a complex extract. This project aims to integrate the developed spin-network database with mass spectrometric data from BENFRA. Structural insights of metabolites and results of NMR-TOCSY experiments will be discussed. This work was supported by the pilot project 1R03AT011872-01, funded by NIH/NCCIH, USA.

P-243 – Daniel Zagal

Chirality Challenges in Metabolomic Characterization of Natural Products

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One of the challenges in elucidating molecular structures for metabolomic characterization of natural products is chirality. Enantiomers cannot be differentiated by conventional (non-chiroptical) analytical techniques; however, their biological activity may be vastly different. Additionally, it is difficult to determine the complexity of extracts accurately as multiple stereoisomers of "one" compound may be present. As evidence of this phenomenon, a previously unreported compound from silymarin, the crude extract of Silybum marianum fruits, was identified and characterized. The ^{1}H NMR spectrum featured chemical shifts and coupling constants that were very close to those of taxifolin, a common plant flavonol similar to quercetin, but with stereocenters at positions 2 and 3 in the C-ring. The difference in coupling constants between protons at positions 2 and 3 of 11.2 vs 2.5 was key to elucidating the structure as epitaxifolin, the 2RS/3SR diastereomer of taxifolin. Ongoing optical rotation experiments of isolated taxifolin and epitaxifolin will determine whether they exist as a racemic mixture in the plant. A lack of bulk optical rotation would confirm this finding, implying that up to 4x as many flavanolignans as previously thought are present in silymarin. Furthermore, it raises the bar for meaningful metabolomic standardization of S. marianum botanicals. This finding serves as a reminder for the myriad of complexities that are to be taken into account in the field of botanical dietary supplement research.

P-244 – Aysegul Caskurlu

Humulene Sesquiterpenoids from Endemic *Ferula Brevipedicellata*

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Ferula plants are traditional medicines and sources of structurally distinct sesquiterpenes, coumarins, and monoterpenes, which collectively have been associated with therapeutically relevant bioactivities. F. brevipedicellata Peşmen ex Sağıroğlu & H. Duman occurs endemically in Turkey. Its constituents and bioactivities have not been studied yet. We now report its first systematic phytochemical investigation. While Ferula species are known to contain different classes of sesquiterpenes, humulenes are relatively rare, yet represent a major proportion of the CH_2Cl_2 extract of F. brevipedicellata. Isolates include the previously described humulene sesquiterpenes, kurubaschic acid angelate and kurubaschaldehyde benzoate, which were structurally dereplicated by spectroscopic methods as well as quantum-mechanics driven ^{1}H iterative functionalized Spin Analysis (HifSA) for confirmatory relative stereochemical assignments. Characterization of three new compounds also involved crystal X-ray diffraction analysis for absolute stereochemical reference. A new methodology enables the definitive identification and distinction of angelic vs. tiglic acid using high-resolution long-range ^{1}H,^{1}H J coupling patterns. The outcomes support the distinct chemotaxonomical placement of F. brevipedicellata in the genus.

P-245 – Ermias Mekuria Addo

Dereplication and Isolation of Secondary Metabolites from the Two Selected Lichens *Mobergia calculiformis* and *Niebla Josecuervoi*

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Lichens are the result of the symbiotic partnerships between fungi and macroalgae or cyanobacteria, and are known to produce unique and bioactive secondary metabolites such as usnic acid. However, some of these metabolites can constitute up to 30% of the dry mass of the lichen, and thus might present a challenge in identifying and isolating minor compounds that can be new and of biological interest. Hence, in the current study, LC-MS^2 and NMR spectroscopy, especially 1D selective methods such as 1D-TOCSY and 1D-NOESY, were used to initially identify compounds and guide the isolation process from the lichens Mobergia calculiformis and Niebla josecuervoi. Data obtained from the LC-MS^2 acquisitions were further analyzed by Feature-Based Molecular Networking, from the Global Natural Products Social Molecular Networking (GNPS) platform, which enabled the annotation of several compounds such as norstictic, salazinic and usnic acids. The results obtained both from the molecular networking and NMR analysis for each species will be described. This work was supported by program project P01 CA125066, funded by NCI, NIH, Bethesda, MD, USA

P-246 – So-Ri Son

Unusual Anti-Oxidative Meroterpenoids from the Roots of *Patrinia Scabra*

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In our ongoing investigation on the potential pharmacological effects of P. scabra roots, a 70% aqueous EtOH extract inhibited ROS generation in TNF-α-induced human dermal fibroblasts (HDFs). To identify bioactive compounds, four unusual meroterpenoids conjugated sesquiterpene with monoterpene (**1**–**4**) were isolated via LC-MS guided isolation. The structures of the compounds were determined using a combination of spectroscopic techniques, chemical derivatization, quantum chemical calculations, and X-ray crystallography. All isolates were then evaluated for their ability to inhibit ROS production in TNF-α-induced HDFs. Our findings suggest that potential anti-oxidative properties of P. scabra roots could be attributed to the presence of these novel meroterpenoids.

P-247 – Xiao Wang

i-HMBC: Unequivocal Identification of Two-Bond Heteronuclear Correlations in Natural Products at Nanomole Scale

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HMBC is an essential NMR experiment for determining multiple bond heteronuclear correlations in small to medium-sized organic molecules, including natural products, yet its major limitation is the inability to differentiate two-bond from longer-range correlations. There have been several attempts to address this issue, but all reported approaches suffer various drawbacks, such as restricted utility and poor sensitivity. Here we present a sensitive and universal methodology to identify two-bond HMBC correlations using isotope shifts, referred to as i-HMBC (isotope shift detection HMBC).[1] Experimental utility was demonstrated at the sub-milligram / nanomole scale with only a few hours of acquisition time required for structure elucidation of several complex proton-deficient natural products, which could not be fully elucidated by conventional 2D NMR experiments. Because i-HMBC overcomes the key limitation of HMBC without significant reduction in sensitivity or performance, i-HMBC can be used as a complement to HMBC when unambiguous identifications of two-bond correlations are needed.

P-248 – Daniela Rebollar-Ramos

Quality Control of Natural Amino Acids with Benchtop qH and 2D NMR

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Natural amino acids (AAs) are widely used in healthcare as nutrients/infusions, dietary supplements, and as drug excipients. As dietary supplementation, infusion, and excipient usage goes along with relatively high oral or i.v. doses, rigorous quality control measures and purity requirements are paramount for these compounds. While LC methodology is widely used for these purposes, the lack of UV chromophores and unfavorable chromatographic properties produce intrinsic challenges. Herein we demonstrate how qualitative and quantitative 1D ^{1}H and 2D HSQC benchtop (60 MHz) NMR methodology can assess the identity and the purity of some of the most challenging AAs and is a viable alternative for quality control. Experimental parameters such as sample amount, number of scans, relaxation delay and acquisition time were modified to improve the overall quality of the spectra. Comparison of purity values with those from high-field (600 MHz) measurement instills confidence in the benchtop NMR approach.

P-249 – Dongdong Wang

NMR Characterization of 2-Amino Imidazole Alkaloids from the Marine Sponges *Clathrina Darwinii* and *Leucetta Chagosensis*

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Calcareous sponges are well-known producers of bioactive secondary metabolites. Bioassay-guided isolation of the marine sponges Clathrina darwinii and Leucetta chagosensis led to the isolation of a group of 2-amino imidazole alkaloids with potent anti-

fungal activity. Their chemical structures were characterized by extensive analysis of the NMR spectroscopy and mass spectrometry data. The $^{1}H–^{13}C$ and $^{1}H–^{15}N$ long-range heteronuclear single quantum multiple bond correlation (LR-HSQMBC) and optimized heteronuclear multiple bond correlation (HMBC) experiments, which can visualize the four- and five-bond $^{1}H–^{13}C$ and $^{1}H–^{15}N$ heteronuclear correlations, were applied to provide unequivocal support for the full assignments for the naamidines.

P-250 – Weimao Zhong

LCMS-guided Discovery, Structural Characterization, and Biosynthesis of Ureido Peptidic Natural Products from Marine *Microbulbifer* spp. Bacteria

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Nonribosomally synthesized peptides are a large group of natural products with an extremely broad range of structural and functional diversities, including more than 20 clinically used drugs, such as antibiotics (penicillin, vancomycin), antitumor pharmaceuticals (bleomycin), and immunosuppressants (cyclosporine). Microbulbifer sp. bacteria represent an untapped reservoir of chemically diverse and biologically active secondary metabolites. Using mass spectrometry, we detected the presence of peptidic natural products in obligate marine Microbulbifer sp. bacteria isolated from the commensal microbiome of Floridian marine sponges. Herein, we reported the LCMS-guided discovery, isolation, structural characterization, and biosynthesis of a new group of ureido hexapeptides, termed bulbiferamides from four Microbulbifer strains. Their planar structures were established by comprehensive 1D and 2D NMR spectroscopy and MS/MS fragmentation analyses. All the configurations of component amino acids were determined to be L by Marfey's method. Notably, bulbiferamides feature an unprecedented group of ureido peptides cyclized by a rare N-aminoacylated Trp indole linkage. Genome sequencing identifies biosynthetic gene clusters encoding production of the bulbiferamides. Their biosynthetic pathways were proposed. The amino acid specificity of Phe adenolation (A) domain of bulbiferamide A was genetically and biochemically determined.

P-250B – Yurui Xie

Two new Phenolic Glycosides from *Paris polyphylla* var. *yunnanensis*

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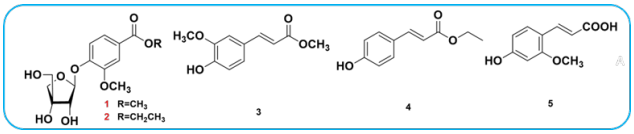
Paris polyphylla Smith var. yunnanensis (Franch.) Hand Maz. is used as one of the main raw materials of more than 80 kinds of traditional Chinese patent medicines. It is used to treat furuncles, swollen pharynx and throat, snakebite, sprain, and convulsion. Modern research has found that its extract has good biological activities. Compounds **1**-**5** were obtained by using column chromatography and semi-preparative HPLC. Their structures were established as 4-O-β-D- apiofuranoside-3-hydroxymethyl benzoate (**1**), 4-O-β-D-apiofuranoside-3-hydroxyethyl benzoate (**2**), methyl ferulate (**3**), trans-p-hydroxycinnamic acid ethyl ester (**4**), 2-methoxy-4-hydroxy cinnamic acid (**5**). Compounds **1** and **2** are new compounds that have not been reported.



P-251 – Rose Campbell

Impacts of Bacterial Metabolites on Coral Symbionts as Clues to Understanding Coral Disease

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Coral reefs are a critical ecosystem to the health and biodiversity of the Earth. Unfortunately, numerous coral diseases have ravaged reefs in recent years, wiping out over 80% of coral cover in the Caribbean in just 40 years. Most diseases have no clear single pathogen and are believed to be caused by polymicrobial coinfections that disrupt the microbiome's balance. Several diseases have been shown to directly harm the endosymbiotic dinoflagellate, which produces up to 90% of the coral's nutrients, and this likely is a key step in disease progression. However, much is yet to be understood about the progression of these diseases and the key metabolites involved in the signaling that ultimately leads to the death of the coral. Recently, we have begun investigating the interactions between known pathogens, bacterial symbionts, and the endosymbiotic dinoflagellates, seeking to understand the chemical exchanges that occur in this system and lead to dysbiosis. One approach has been to treat the dinoflagellates with chemical extracts of both probiotic and pathogenetic bacterial strains and subsequently monitor changes in the dinoflagellate's health and metabolomic profile. Thus far, several bacterial extracts appear to shift the dinoflagellate metabolome or have had a detrimental impact on the growth of the dinoflagellate. Work is on-going to understand the individual metabolites involved in these observed changes.

P-252 – Herma Pierre

Development of First-Generation Eupenifeldin Semi-Synthetic Analogues via Hydroxy Group Modifications

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Eupenifeldin is a bistropolone meroterpenoid that exhibits potent (nM) cytotoxicities against a variety of cancer cell types despite its less-than-ideal drug properties. To improve the drug-like properties of eupenifeldin, 29 semisynthetic analogues were generated, namely via semisynthetic modifications at the tropolonic and aliphatic hydroxy groups. The compounds were assessed against human melanoma (MDA-MB-435) and high-grade serous ovarian (OVCAR3) cancer cell lines, and many of the analogues showed cytotoxicities comparable to or greater than eupenifeldin. One analogue retained nM activity against both cancer cell lines and was the only analogue generated that improved the solubility of eupenifeldin (solubility increased 40-fold). These characteristics make this semisynthetic analogue a prime candidate for further pharmacological assessment.

P-253 – Paul Boudreau

Peer-Review: A Method to Increase Graduate Student Funding Applications and Success

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The National Science Foundation Graduate Research Fellowship Program (NSF-GRFP) has a long established and well-known bias towards a small cadre of wealthy schools which routinely receive most of the fellowships every year, while University of Mississippi students rarely receive any of these awards. Overcoming this bias is something that our students cannot be expected to achieve alone. University of Mississippi students do not have ready access to prior successful applications or peers who have expertise in demystifying the application process. Tackling both of these problems at once, we developed an NSF-GRFP Grant Writing Workshop to run in the Fall semester. Faculty members provided walkthroughs on the funding mechanism, formulating research aims, how to develop a personal statement, and common application pitfalls. After students write a draft of each component of the proposal they shared it with the faculty team and peer mentors who were previous applicants as part of a peer-review exercise. This Workshop was designed not just to help each crop of applying students, but to develop institutional knowledge about the NSF-GRFP that can be passed down generationally across different student classes at the University of Mississippi. In our first iteration of the workshop we had four student applicants, of whom two were successful! By rerunning the Workshop for future funding cycles, we hope that this past success will help encourage more students to participate, but also help overcome the "someone like me" problem where our students believe the NSF-GRFP is not designed for them. Knowing that this problem compounds for students traditionally excluded from the research enterprise, we are also hoping that future iterations of our Workshop can also help to tackle inequities in graduate school participation and success.

P-254 – Lesley-Ann Giddings, Brian Murphy and Christine Salomon

Equity in Action: The ASP Summer Research Fellowship Program

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One critical challenge facing STEM fields in the US, which is reflected within the ASP, is a lack of racial and ethnic representation among our membership, leadership, award recipients, and meeting speakers. To correct for the underrepresentation of Black, Indigenous and Latinx (BIL) students in our field, the ASP DEI and Executive Committees, ASP Foundation, and ASP Fellows collaborated to develop the Summer Research Fellowship (SRF) program. The program offers 1) a 2.5 month stipend for students to engage in research under a mentor in the natural product sciences; 2) 11 weekly online professional development workshops focused on science communication as well as exposure to graduate programs and careers in natural products; and 3) a special online ASP webinar for students to present their research to an international audience. The first two cohorts (2021-2022) were highly successful, with seven students accepted into graduate or professional programs and several others receiving prestigious research fellowships and awards (remainder of the cohorts are still engaged in undergraduate studies). Importantly, most students in the first cohorts are continuing to pursue research in the field of natural products chemistry. Students in the third cohort (2023) have begun their research, participating in the weekly workshops and preparing for their presentations. All eight current SRF students are attending the 2023 ASP meeting and presenting posters, so please be sure to visit their posters and welcome them. The DEI committee is seeking additional support through grants to continue the successful SRF program.

P-255 – ASP Younger Members Committee

Natural Product Careers and Opportunities

ASP Younger Members Committee

The ASP membership possesses a diverse range of highly sought-after skills. Nonetheless, navigating the digital era to identify the next career move can be an intimidating challenge, as a single competitive position may attract hundreds of applicants. The ASP annual meeting serves as an invaluable platform to facilitate face-to-face connections and discussions about employment opportunities with other members. This space is reserved for the assortment of academic, industrial, and government positions offered by companies and academics actively seeking natural product talent. Stop by and explore the current available positions, and discover the key individuals to engage with for networking for future career opportunities. ARE YOU LOOKING FOR STUDENTS, POSTDOC's, EMPOLYEES?? – bring your printed job ad and post it or email it to scarlson1@pacific.edu and we will post it for you!

P-256 Young Eun Du

Structure Elucidation and Bioactivities of Bacteria-Derived Compounds Separated from Unique Habitats

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Investigating the secondary metabolites produced by bacteria associated with unique habitats, such as insect or extreme environments has become a promising strategy for discovering novel bioactive compounds. Formicins A–C (1–3) were discovered from Streptomyces sp. SFA33, associated with wood ant (Formica yessensis). Two new secondary metabolites, svalbamides A (4) and B (5), were isolated from a culture extract of Paenibacillus sp. Serratiomycin (6), revised serratiomycin (7) and three new derivatives, serratiomycins D1–D3 (8–10), were discovered from a Serratia sp. strain isolated from the exoskeleton of a long-horned beetle. The structures of these compounds were elucidated based on 1D/2D NMR and UV spectroscopy with MS/MS analysis. The absolute configurations were determined by applying the phenylglycine methyl ester (PGME) method, advanced Marfey's method, methanolysis and subsequent Mosher's method. Some compounds showed significant anticancer activities on human triple negative breast cancer (TNBC) cells and antibacterial activities against Staphylococcus aureus and Salmonella enterica.

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See you in Poland for ICNPR2024!