60th Anniversary of the The American Society of Pharmacognosy Annual Meeting

"Innovations in Natural Products Chemistry - An Interdisciplinary Approach to Understanding Nature's Chemical Library"

Madison, Wisconsin

July 13 – 17, 2019

The American Society of Pharmacognosy Annual Meeting

60th Anniversary

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The organizing committee of the 2019 American Society of Pharmacognosy welcomes you to Madison, Wisconsin! The Madison Concourse Hotel is located in the center of the city within steps of the Wisconsin State Capitol. Sessions are at the Monona Terrace just a short walk from the Concourse Hotel. We have worked hard to make this a dynamic and inclusive meeting. Our plenary, invited and contributed talks include topics from many areas of natural products chemistry and speakers from diverse backgrounds. Excellent workshops are available on Saturday on timely and innovative areas of emerging technologies in natural products. This year's meeting also features a lunch session on Monday "Breaking the Bias Habit". We included three special symposia sponsored by the American Chemical Society, National Institutes of Health National Center for Complementary and Integrative Health and the ASP Foundation. Reconnect with friends and meet new colleagues at the opening reception on the roof of the Monona Terrace. Younger members will convene at the SETT for an evening of bowling, pool and table games on Sunday. On Sunday evening other members can take advantage of the "Dine Around the Square". Stop by the registration desk Saturday or Sunday to sign up for a table at one of the local restaurants. All events will feature local flavors! We hope you enjoy the conference and Madison.

We want to hear from you #ASP2019

ORGANIZING COMMITTEE

Melany Puglisi, Co-Chair
Tim Bugni, Co-Chair

ASP 2019

Madison, Wisconsin, July 13–17, 2019

ASP 2019 Organizers

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Meeting Planning and Registration

Laura Stoll (The 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 of 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 to Nadja Cech (nadja_cech@uncg.edu), co-chair of the ASP diversity 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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Norman R. Farnsworth Research Achievement Award

Geoffrey A. Cordell, University of Illinois, University of Florida, Natural Products Inc.

2018 Varro E. Tyler Prize

Guido Pauli, Ph.D., University of Illinois Chicago

2019 Varro E. Tyler Prize

Rudolf Bauer, University of Graz, Austria

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Amy L. Lane, University of North Florida

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Undergraduate Research Award

Julia Asay, University of California San Diego
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Samuel Tanoeyadi, Oregon State University

Research Starter Grant

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Active Member Travel Grant

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Student Research Award

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Carla Menegatti, University of Sao Paulo
Shogo Mori, University of Kentucky
Emily Paris, University of California San Diego
Sara P. Puckett, University of Connecticut

2019 Arthur E. Schwarting Award

Robert L. Bertrand**,** Mona Abdel-Hameed, and **John L. Sorensen.*** Lichen Biosynthetic Gene Clusters. Part I. Genome Sequencing Reveals a Rich Biosynthetic Potential. J. Nat. Prod. 2018, 81(4), 723–731. (DOI: 10.1021/acs.jnatprod.7b00769) AND Robert L. Bertrand, Mona Abdel-Hameed, and **John L. Sorensen**.* Lichen Biosynthetic Gene Clusters Part II: Homology Mapping Suggests a Functional Diversity. J. Nat. Prod. 2018, 81(4), 732–748. (DOI: 10.1021/acs.jnatprod.7b00770).

2019 Jack L. Beal Award

Aleš Machara,* Jan Kriváne k, Klára Dolejšová, Jana Havlíková, Lucie Bednárová, Robert Hanus, Pavel Majer, and Pavlína Kyjaková.* Identification and Enantiodivergent Synthesis of (5Z,9S)-Tetradec-5-en-9-olide, a Queen-Specific Volatile of the Termite *Silvestritermes minutus*. J. Nat. Prod. 2018, 81(10), 2266-2274. (DOI: 10.1021/acs.jnatprod.8b00632).

The American Society of Pharmacognosy Annual Meeting

60th Anniversary

"Innovations in Natural Products Chemistry
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Program Schedule

Saturday July 13, 2019

8:00 AM – 7:30 PM Registration – ***Level 4 – Registration Area 3 and 4***

9:00 AM – 4:00 PM Executive Committee Meeting (Invitation Only) – ***Level 4 – Hall of Ideas H, I***

9:00 AM – 12:00 PM AMWS1 – ***Level 4 - Meeting Rooms K,L,O,P***
Chair: Jason Kwan
"A Hands-on Global Natural Product Social Molecular Networking Workshop for Beginners and Advanced Users" with Pieter Dorrestein (University of California, San Diego)

1:00 PM – 4:00 PM PMWS1 – ***Level 4 – Meeting Rooms K,L,O,P***
Chair: Jason Kwan
"Expanding the Structure Elucidation Toolbox with Anisotropic NMR Parameters" with Robert Williamson (University of North Carolina, Wilmington) and Roberto Gil (Carnegie Mellon University)

6:00 PM – 9:00 PM **President's Opening Reception** - ***Level 6 - Rooftop – (Ticketed Event)***
Supported by the American Society of Pharmacognosy Foundation through a generous donation from the Estate of Gerry and Lynn Brady

Sunday July 14, 2019

7:00 AM – 5:00 PM Registration – ***Level 4 – Registration Area 3 and 4***

7:15 AM – 8:15 AM Continental Breakfast – ***Level 4 Grand Terrace***

Level 4 – Ballroom A - D
Welcoming Remarks and Announcements

8:15 AM – 8:30 AM **Melany Puglisi**, (Chicago State University) and **Tim Bugni**, (University of Wisconsin at Madison)

8:30 AM – 8:45 AM **Steve Swanson** (Dean, University of Wisconsin at Madison College of Pharmacy) and **Susan Mooberry** (ASP President)

Level 4 – Ballroom A - D
Symposium I - Innovations in Natural Products Chemistry
Chair: Tim Bugni

8:45 AM – 9:30 AM PL-01
Nancy Keller (University of Wisconsin at Madison)
Neil Kelleher (Northwestern University)
Fungal Secondary Metabolism: Regulation, Function and Drug Discovery

9:30 AM – 10:15 AM PL-02
Pieter Dorrestein (University of San Diego, Scripps Institution of Oceanography)
Creating the Global Natural Product Social Molecular Networking Infrastructure for the Community and by the Community-a Historical and Future Perspective

10: 15 AM – 10:45 AM Break – **Level 4 *Grand Terrace***

10:45 AM – 11:30 AM PL-03
Nadja B. Cech (University of North Carolina at Greensboro)
Metabolomics as a Tool for Antimicrobial Drug Discovery

11:30 AM – 12:00 PM I-01
Jaclyn M. Winter (University of Utah)
Exploring the Chemical Potential of Great Salt Lake Microorganisms

12:00 PM – 12:30 PM I-02
Harinantenaina Liva Rakotondraibe (The Ohio State University)
Mining New and Antiproliferative Compounds from Untapped Natural Product Sources

12:00 PM – 3:00 PM Exhibitor Set Up – ***Level 4 Grand Terrace***

12:30 PM – 1:30 PM Lunch on your own

12:30 PM – 2:00 PM Journal of Natural Products Editorial Board Meeting (Invitation Only)
Meeting Rooms K, L

12:30 PM – 4:00 PM Presenters for Poster Session I Set up Posters - ***Exhibit Hall B***

Level 4 – Ballroom A - D
Symposium II - Cancer
Chair: James Lyles

1:30 – 2:15 PM PL-04
Jason M. Crawford (Yale University)
Structure Elucidation of Colibactin and its DNA Interstrand Crosslink Product

2:15 – 3:00 PM PL-05
John A. Beutler (National Cancer Institute, National Institutes of Health)
Englerins: A Long, Strange Trip from East Africa to Kidney Cancer Drug Candidate

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Hall of Ideas E - G
Session S-PM1 - ***Journal of Natural Products Symposium***
"Heroes of the Journal of Natural Products"
Chair: Nadja Cech (University of North Carolina at Greensboro)
Sponsored by the American Chemical Society

3:00 – 3:20 PM S-01
Nadja B. Cech (University of North Carolina at Greensboro)

3:20 – 3:30 PM S-02
Mario Figueroa (Universidad Nacional Autónoma de México)
*Honoring **Rachel Mata***

3:30 – 3:40 PM S-03
Lesley-Ann Giddings (Middlebury College)
*Honoring **David Newman***

3:40 – 3:50 PM S-04
Brian T. Murphy (University of Illinois at Chicago)
*Honoring **David George Ian Kingston***

3:50 – 4:00 PM S-05
Kerry Leigh McPhail (Oregon State University)
*Honoring **Gordon Mitchell Cragg***

4:00 – 4:50 PM Questions and Answers with the "Heroes"

4:50 – 5:00 PM Closing Remarks
Marcy Balunas (University Of Connecticut)

Hall of Ideas H - J
Session S-PM2 – Drug Discovery
Chair: Gina Porras-Brenes

3:00 – 3:20 PM C-01
Anam Shaikh (University of Texas Southwestern Medical Center)
Elucidating the Mechanism of Selective Cytotoxicity in NSCLC Cell Lines and Target Identification of Ikarugamycin

3:20 – 3:40 PM C-02
Jonathan Bisson (Center for Natural Products Technologies)
What Have We Learned from a Few Thousand Years of Natural Products Research?

3:40 – 4:00 PM C-03
Marilia Valli (University of Sao Paulo)
Chemoinformatic Characterization of the Natural Products Database from Brazil

4:00 – 4:20 PM C-04
Rosemary Ann Dorrington (Rhodes University)
A Molecular Networking Approach to Characterizing Pyrroloiminoquinone Production by Latrunculid Sponge Species

4:20 – 4:40 PM C-05
Angela I. Calderón (Auburn University)
An Interdisciplinary Approach to Study the Potential of Açaí-Anticancer Drug Interactions

4:40 – 5:00 PM C-06
Serge Alain Fobofou Tanemossu (Harvard Medical School)
Chemical Novelty from Hypericum Species (St. John's Wort)

5:00 PM – 7:00 PM **Poster Session I** - **Level 1** - ***Exhibit Hall B***
Poster #'s P-001 – P-149, Chair: Brian Murphy
(Please take posters with you at the end of the session. ASP is not responsible for lost or damaged posters)

5:00 PM – 8:00 PM Exhibitor Set-Up – ***Level 4 - Grand Terrace***

7:00 PM Buses Depart for The Sett – The University of Madison for the Young Members Event

7:30 PM – 11:00 PM **Young Members Event** (Dinner - Ticketed Event)
The Sett – The University of Madison
1308 W. Dayton Street
Madison, Wisconsin 53715

Directions to The Sett - University of Madison

7:30 PM **Dinner on the Square**

Monday July 15, 2019

7:45 AM – 5:00 PM Registration – ***Level 4 Registration Area 4***

7:45 AM – 8:45 AM Continental Breakfast – ***Level 4 Grand Terrace***

8:15 AM – 3:45 PM Exhibition – ***Grand Terrace***

Level 4 – Ballroom A - D
Symposium III – Microorganisms
Chair: Melany Puglisi

8:45 AM – 9:30 AM PL-06
Helen E. Blackwell (University of Wisconsin at Madison)
Chemical Tools to Intercept and Interrogate Bacterial Communication Pathways

9:30 AM – 10:15 AM PL-07
Jon Clardy (Harvard Medical School)
Molecular and Mechanistic Studies in the Gut Microbiome

10:15 AM – 10:45 AM Break - ***Level 4 Grand Terrace***

11:00 AM – 2:30 PM Presenters for Poster Session II Set up Posters - Exhibit Hall B

Level 4 – Ballroom A - D
Symposium IV – NIH: *Diet and Gut interactions*
Sponsored by NIH
Chair: Craig Hopp

10:45 AM – 11:05 AM S-06
Annadora J. Bruce-Keller (LSU Pennington Biomedical Research Center)
Fenugreek, Gut Microbiota, and Resiliency to Western Diets

11:05 AM – 11:25 AM S-07
Diana E. Roopchand (Rutgers)
Proanthocyanidin Metabolites Produced by Commensal Gut Microbes May Promote Metabolic Resilience

11:25 AM – 11:45 AM S-08
Michael Snyder (Stanford University)
Multiomic Signatures of Microbial Metabolites Following Prebiotic Fiber Supplementation

11:45 AM – 12:05 PM S-09
Hang Xiao (University of Massachusetts Amherst)
Microbiota-Mediated Biotransformation of Polymethoxyflavones: Key for their Anti-Inflammatory Activities in the Colon

12:05 PM – 12:25 PM S-10
Jan Frederik Stevens (Oregon State University)
Interactions between Gut Microbiota and Xanthohumol from Hops (Humulus lupulus) and their Impact on Host Health

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Exhibit Hall A
Breaking the Bias Habit - Lunch Session (Ticketed)
Chair: Tawnya McKee

12:30 PM – 1:30 PM PL-08
Jennifer Sheridan (Women in Science Leadership Institute, University of Wisconsin – Madison)
Breaking the Bias Habit®: Promoting Gender Equity

Level 4 - Hall of Ideas E - G
Session M-PM1 – Microorganisms
Chair: Caryn Wadler

1:30 PM – 2:00 PM I-03
Kevin Joseph Tidgewell (Duquesne University)
G.I.T. (G-protein, Ion Channel, and Transporter) Ligand Discovery from Natural Sources

2:00 PM – 2:30 PM I-04
Laura M. Sanchez (University of Illinois at Chicago)
Measuring Metabolites in Human Health and Microbial Systems

2:30 PM – 2:50 PM C-07
Chambers Connor Hughes (Scripps Institution of Oceanography, UCSD)
Brominating the Secondary Metabolome

2:50 PM – 3:10 PM C-08
Chung Sub Kim (Yale University)
Characterization of Autoinducer-3 Biosynthesis in E. coli

3:10 PM– 3:30 PM C-09
Bin Zhou (University of Illinois at Chicago)
Modification and SAR of Anti-TB Ruf I and II Mixtures Based on Ruf I and ClpC1-NTD-wt Co-Crystals

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Level 4 - Hall of Ideas H - J
Session – M-PM2 – Chemical Ecology
Chair: Daniel May

1:30 PM – 2:00 PM I-05
Frank C. Schroeder (Cornell University)
Toward Comprehensive Annotation of Metazoan Metabolomes: C. elegans as a Model

2:00 PM – 2:30 PM I-06
Grace Lim-Fong (Randolph Macon College)
Flexibility of a Defensive Symbiosis Across Biogeographical Gradients

2:30 PM – 2:50 PM C-10
Benjamin (BJ) Philmus (Oregon State University)
1,2,4-Triazine Natural Products. Biosynthesis Investigations and Genome Mining of an Interesting Structural Class of Compounds

2:50 PM – 3:10 PM C-11
Skylar Carlson (Smithsonian Marine Station at Fort Pierce)
Water-Soluble Chemical Cues for Coral Larvae: Strategies for Isolating Water-Soluble Drug Leads

3:10 PM – 3:30 PM C-12
Emily Mevers (Harvard Medical School)
Pyonitrins A-D: Chimeric Antifungal Agents Produced by Pseudomonas protegens

3:30 PM – 5:30 PM **Poster Session II – *Level 1 - Exhibit Hall B***
Poster #'s P-150 – P-300, Chair: James Gloer
(Please take posters with you at the end of the session. ASP is not responsible for lost or damaged posters)

6:15 PM Bus Transportation to The Wisconsin Institute for Discovery

7:00 PM – 10:00 PM **An Evening at The Wisconsin Institute for Discovery** *(Dinner - Ticketed Event)*
Discovery Building
330 North Orchard Street
Madison, WI 53715

Directions to the Discovery Building

10:00 PM Join us after the Event at:
The University of Madison – Memorial Union Terrace
800 Langdon Street
Madison Wisconsin, 53706

Directions to the Memorial Union Terrace

Tuesday July 16, 2019

7:45 AM – 12:30 PM Registration – ***Level 4 Registration Area 4***

7:45 AM – 8:45 AM Continental Breakfast – ***Level 4 Grand Terrace***

7:45 AM – 12:30 PM Exhibition – ***Level 4 Grand Terrace***

Level 4 – Hall of Ideas E-J
Symposium V – Pharmacology
Chair: John MacMillan

8:45 AM – 9:30 AM PL-09
Jairo Kenupp Bastos (Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP)
The Potential of Galloyl Quinic Acids, Cubebin and Glycoalkaloids to Respectively Treat Urolithiasis, Erectile Dysfunction and Cutaneous Leishmaniasis

9:30 AM – 10:00 AM I-07
Joanna Burdette (University of Illinois at Chicago)
Natural Product Drug Discovery and Target Identification for Ovarian Cancer

10:00 AM – 10:30 AM Break - ***Level 4 Grand Terrace***

Level 4 – Hall of Ideas E - G
T-AM1 - David Slatkin Younger Members Symposium
Chair: Skylar Carlson
Sponsored in part by the ASP Foundation and Chicago State University (through generous donations)

10:30AM – 10:50 PM C-13
Angela Sester (TU Dortmund University)
Generation of Myxochelin-Derived Lipoxygenase Inhibitors in a Genetically Modified Myxococcus xanthus Strain

10:50AM – 11:10 PM C14
Ikenna E. Ndukwe (Merck)
Bruker Award
Anisotropic NMR – A Useful Tool for Natural Product Structure Characterization and Verification

11:10 AM – 11:30 PM C-15
Alanna Condren (University of Illinois at Chicago)
Biofilm Inhibition Alters Specialized Metabolism and Increases Virulence

11:30 AM – 11:50 PM C-16
Joseph M. Egan (Simon Fraser University)
Bruker Award
MADByTE: A Structure Driven Approach to Untargeted NMR Metabolomics of Natural Product Extracts

11:50 AM – 12:10 PM C-17
Emily Rue (Oregon State University)
Isomeric Identification of Procyanidins Using Ultrahigh Pressure Liquid Chromatography-Tandem Mass Spectrometry

12:10 PM – 12:30 PM C-18
Andrés Mauricio Caraballo Rodríguez (University of California)
Molecular Signatures From Ants' Ecosystems - Susceptibility of Fungal Gardens to Mycoparasites

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Level 4 – Hall of Ideas H - J
Session T-AM2- Marine Natural Products
Chair: Chris Ireland

10:30AM – 10:50 PM C-19
William (Bill) Gerwick (Scripps Institution of Oceanography, UCSD)
Nature's Combinatorial Assembly-Line Biosynthesis Produces Vatiamides A-F

10:50 AM – 11:10 PM C-20
Amy E. Wright (Florida Atlantic University)
Discovery of Marine Natural Products that Reduce the Levels of the Nodal Protein Survivin in Cancer Cells

11:10AM – 11:30 PM C-21
Néstor M. Carballeira (University of Puerto Rico)
New Insights into the Biological Activity of Marine Methoxylated Fatty Acids

11:30 AM – 11:50 PM C-22
Christopher Thornburg (Leidos Biomedical Research, Inc.)
NCI Program for Natural Product Discovery: Psammoclemolide, A Bioactive Sesterterpene from a Marine Sponge of the Chondropsidae Family

11:50 AM – 12:10 PM C-23
Yuta Kudo (Tohoku University)
Identification of New Analogs and Putative Biosynthetic Intermediates of Tetrodotoxin Aimed at Elucidating its Biosynthetic Pathway and Structure Activity Relationship

12:10 PM – 12:30 PM C-24
Xiao Liang (University of Florida, Gainsville)
Discovery, Total Synthesis and SAR of a Novel Quorum Sensing Signaling Molecule from a Marine Cyanobacterium

12:30 PM – 2:00 PM Fellows Meeting – Invitation Only – ***Hall of Fame Room***

12:30 PM Afternoon and Evening on your own

1:30 PM – 4:00 PM **Bitters Boot Camp** (Ticketed)
Meet at Level 4 – Bus Loading Area

2:00 PM – 4:00 PM **Build a Wisconsin Cheese Board** (Ticketed)
Meet in the Lobby of The Concourse Hotel

2:00 PM – 4:30/5:00 PM **Stroll Down State Street** (Ticketed)
Meet in the Lobby of The Concourse Hotel

Wednesday July 17, 2019

8:00 AM – 3:30 PM Registration – ***Level 4 Registration Area 4***

8:00 AM – 9:00 AM Continental Breakfast – ***Level 4 Grand Terrace***

8:00 AM – 11:00 AM Exhibition

11:00 AM – 3:00 PM Exhibition Dismantling

Level 4 – Ballroom A,B,C,D
Award Symposium I
Chair: Bindesh Shrestha and Marcy Balunas

9:00 AM – 9:45 AM A-01
Paula Brown (Phytoanalytics)
Water's Award Lecture
Chemometrics: Novel Approaches for Investigating Secondary Metabolites in Medicinal Plants

9:45 AM – 10:30 AM A-02
Amy L. Lane (University of North Florida)
Matt Suffness Young Investigator's Award Lecture
Unlocking the Treasure Trove of Biosynthetic Pathways from the Sea

10:30 – 11:00 AM Break - ***Level 4 Grand Terrace***

Level 4 – Ballroom A,B,C,D
Award Symposium II
Chair: John Cardellina

11:00 AM - 12:00 PM A-03
Guido F. Pauli (University of Illinois at Chicago)
2018 Varro Tyler Prize Award Lecture
Tyler, Taylor & Tales: The Complexity of Reductionism

12:00 PM - 1:15 PM Lunch on your own

12:00 PM – 1:15 PM ASP Ambassadors Meeting (Invitation Only) –
Level 4 Meeting Rooms M and N

Level 4 – Ballroom A,B,C,D
Award Symposium III
Chair: John Cardellina and Cindy Angerhofer

1:15 PM – 2:15 PM A-04
Rudolph Bauer (University of Gratz)
2019 Varro Tyler Prize Award Lecture
The Echinacea Story: A Scientific Life Dedicated to a North American Medicinal Plant

2:15 PM – 3:15 PM A-05
Geoffrey A. Cordell (University of Illinois, University of Florida, Natural Products Inc.)
Norman R. Farnsworth Research Achievement Award Lecture
Continuing Adventures on a Heterocycle

3:30 PM – 5:30 PM ASP Business Meeting – ***Meeting Rooms K,L,O,P***

6:00 PM – 7:00 PM **Closing Reception** – ***Grand Terrace***

7:00 PM – 10:00 PM **Closing Ceremony and Banquet – *Ballrooms A,B,C,D***
(Ticketed Event)

Thank you for your participation in the 2019 ASP Meeting!

See you at the 2020 International Congress of Natural Products Research in San Francisco
http://icnpr2020.org/

Plenary Speakers

PL-01

FUNGAL SECONDARY METABOLISM: REGULATION, FUNCTION AND DRUG DISCOVERY

Nancy P. Keller
Department of Medical Microbiology and Immunology, Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, U.S.A

npkeller@wisc.edu

One of the exciting movements in microbial sciences has been a refocusing and revitalization of efforts to mine the fungal secondary metabolome. The magnitude of biosynthetic gene clusters (BGCs) in a single filamentous fungal genome combined with the historic number of sequenced genomes suggests that the secondary metabolite wealth of filamentous fungi is largely untapped. Mining algorithms and scalable expression platforms have greatly expanded access to the chemical repertoire of fungal-derived secondary metabolites. Here I discuss new insights into the transcriptional and epigenetic regulation of BGCs and the ecological roles of fungal secondary metabolites in warfare, defence and development. In particular I will illustrate new approaches in mining cryptic and algorithm 'invisible' BGCs.

References

Keller NP Nature Reviews Microbiology. 17(3):167-180.
Lim FY, et al. MBio May 29;9(3). pii: e00785-18.

PL-02

CREATING THE GLOBAL NATURAL PRODUCT SOCIAL MOLECULAR NETWORKING INFRASTRUCTURE FOR THE COMMUNITY AND BY THE COMMUNITY-A HISTORICAL AND FUTURE PERSPECTIVE

Pieter Dorrestein
University of California - San Diego, La Jolla, CA, USA

In this lecture I will describe why and how GNPS was established, the challenges we faced, the impact of GNPS on the natural product community emerging tools and what the future will bring.

PL-03

METABOLOMICS AS A TOOL FOR ANTIMICROBIAL DRUG DISCOVERY

Nadja B. Cech
Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412, USA.

In 1928, Alexander Fleming's discovery of penicillin G from the fungus *Penicillium nototum* sparked the antibiotic revolution. Since that time, intense interest has been focused on natural products as sources of antimicrobial agents. Antimicrobial drug discovery from natural products has been fruitful; of the five major classes of antibiotics used clinically today, four are based on molecules produced by nature. However, with the exception of Teixobactin (also a natural product), the last three decades have been characterized by relatively little progress in the development of new antibiotics. We face an impending crisis whereby the pipeline for new antimicrobial leads is depleted while resistance continues to develop to existing antibiotics at an alarming rate. Successful antimicrobial drug discovery efforts have employed fractionation-based approaches in phenotypic assays. Leads in these assays are typically prioritized in two ways (1) because of interesting chemistry (i.e. new structures) or (2) because of strong potency. A disadvantage of these approaches is that they ignore a critically important consideration, that of mechanism of action. We have recently shown that mass spectrometry metabolomics studies of bacteria can be employed to provide mechanistic insight in antimicrobial screening efforts. The development of new anti-virulence leads against a clinically relevant strain of Methicillin-resistant *Staphyolcoccus aureus* will serve as an illustrative example of this concept.

PL-04

STRUCTURE ELUCIDATION OF COLIBACTIN AND ITS DNA INTERSTRAND CROSSLINK PRODUCT.

Mengzhao Xue,[1,] Chung Sub Kim,[1,2,*] Alan R. Healy,[1,2] Kevin M. Wernke,[1] Zhixun Wang,[1] Madeline C. Frischling,[1] Emilee E. Shine,[1,4] Weiwei Wang,[5,6] Seth B. Herzon,[1,3,#] and Jason M. Crawford[1,2,4#]*
*[1]Department of Chemistry, Yale University, New Haven, CT 06520, USA. [2]Chemical Biology Institute, Yale University, West Haven, CT 06516, USA. [3]Department of Pharmacology, Yale School of Medicine, New Haven, CT 06520, USA. [4]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06536, USA [5]Department of Molecular Biophysics & Biochemistry, Yale School of Medicine, New Haven, CT 06520, USA. [6]W. M. Keck Biotechnology Resource Laboratory, Yale School of Medicine, New Haven, CT 06510, USA. #Correspondence to: jason.crawford@yale.edu, seth.herzon@yale.edu; * Equal contribution.*

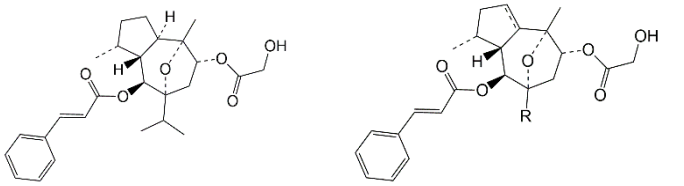
Select strains of *Escherichia coli* and *Klebsiella pneumoniae* produce the virulence factor and cytotoxin known as colibactin. Colibactin is derived from its prodrug form known as precolibactin. Pathogenic strains of adherent invasive *E. coli* (AIEC) carrying the colibactin pathway (*clb*+) cause tumor formation in at least four distinct mouse models examined to date and are thought to be responsible for initiating colorectal cancer in humans. Through a combination of genome editing, stable isotope labeling, DNA alkylation, and chemical synthesis studies, we characterized the structures of precolibactin, colibactin, and its DNA interstrand crosslink product from DNA exposed to *clb*+ *E. coli*. The structure of colibactin was confirmed by comparison to a synthetic standard. The standard led to an identical DNA interstrand crosslink as the natural materials. An uncharacterized peptidase ClbL represented the final critical step in precolibactin biosynthesis was responsible for converting DNA alkylators into DNA interstrand crosslinkers. These studies present the structure, mode of action, and biosynthesis of (pre)colibactins that account for all known biosynthesis and cancer cell biology studies for this important pathway.

PL-05

ENGLERINS: A LONG, STRANGE TRIP FROM EAST AFRICA TO KIDNEY CANCER DRUG CANDIDATE

John A. Beutler[1], Sarka Tumova[2], Cody Peer[3], David J. Beech[2], William J. Chain[4], Antonio M. Echavarren[5].
[1]Molecular Targets Program, [3]Clinical Pharmacology Program, National Cancer Institute, Frederick, MD 21702 USA, [2]Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Leeds, UK LS2 9JT, [4]Department of Chemistry & Biochemistry, University of Delaware, Newark, DE 19716, [5]Institute of Chemical Research of Catalonia, 43007 Tarragona, Spain

Englerin A, a sesquiterpene diester discovered as a potent and selective inhibitor of renal cancer cell growth from the Tanzanian plant *Phyllanthus engleri*, has two major limitations as a clinical candidate. First, it is highly toxic when administered intravenously and second, it is hydrolyzed by stomach acid to englerin B, which is inactive, and thus is not orally bioavailable. The intravenous toxicity has been linked to agonism of TRPC4/5 ion channels. Analogue synthesis in the Chain and Echavarren laboratories has led to compounds which are less potent at TRPC4-containing channels and which have reduced toxicity to mice, while maintaining the NCI 60 potency and selectivity of the natural product.



Englerin A Analogues

PL-06

CHEMICAL TOOLS TO INTERCEPT AND INTERROGATE BACTERIAL COMMUNICATION PATHWAYS

Helen E. Blackwell
Department of Chemistry, University of Wisconsin–Madison, 1101 University Ave., Madison, WI 53706, USA.

Many bacteria communicate using small organic molecules and peptides to monitor their population densities in a process called "quorum sensing." At high cell densities, bacteria use this signaling network to switch from an isolated, nomadic existence to that of a multicellular community. This lifestyle switch is significant; only in groups will pathogenic bacteria turn on virulence pathways and grow into drug-impervious communities called biofilms that are the basis of myriad chronic infections. In turn, certain symbiotic bacteria will only colonize their hosts and initiate beneficial behaviors at high population densities. Our research is broadly focused on the design, synthesis, and characterization of non-native ligands that can intercept quorum sensing and provide new insights into its role in host/microbe interactions. These molecules provide a novel approach to study quorum sensing with both spatial and temporal control in a range of settings. We have developed a series of efficient synthetic methods that provide us with straightforward access to these ligands. In addition, we have applied our quorum sensing antagonists and agonists *in vitro* and *in vivo* to investigate quorum sensing as an anti-infective target. In this talk, I will introduce quorum sensing and motivate why I believe chemists are poised to make unique contributions to this research field. Thereafter, I will go on to introduce my lab's research approach, highlight our recent results, and outline our future goals.S-06

PL-07

MOLECULAR AND MECHANISTIC STUDIES IN THE GUT MICROBIOME

Jon Clardy
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

While multiple studies have established correlations between bacterial members of the human gut microbiome and host health or disease, few address the molecular and mechanistic basis that underlies the association. We used a functional phenotypic assay using murine dendritic cells, extracts from single isolate bacterial cultures, and assays for cytokine production to begin systematically screening members of the gut microbiome, especially those with statistical correlations with disease. Our studies on *Ruminococcus gnavus* and *Collinsella aerofaciens* forms the basis of this talk. *R. gnavus*, which is strongly linked with Crohn's Disease, produces a pro-inflammatory complex polysaccharide, a glucorhamnan, that was characterized both structurally and functionally. *C. aerofaciens* produces plasmalogens, members of an unexpected but widespread lipid family

PL-08

BREAKING THE BIAS HABIT®: PROMOTING GENDER EQUITY

Jennifer Sheridan
University of Wisconsin-Madison

This interactive talk is an introduction to the concept of implicit (or unconscious) gender bias, treating the application of such biases as a *habit of mind*. Attendees will learn how to uncover their own biases, discover some underlying concepts and language used in the psychological and social psychological literature to describe such processes, and learn evidence-based strategies for reducing the application of these biases in their own actions.

PL-09

THE POTENTIAL OF GALLOYL QUINIC ACIDS, CUBEBIN AND GLYCOALKALOIDS TO RESPECTIVELY TREAT UROLITHIASIS, ERECTILE DYSFUNCTION AND CUTANEOUS LEISHMANIASIS.

Jairo Kenupp Bastos
School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo. Avenida do Café S/N, Ribeirão Preto, SP, Brazil, 14040-903

Renal stone disease, also known as urolithiasis, is common with a recent overall estimated prevalence rate of 15%, with a five-year recurrence rate of up to 50%. *Copaifera* species leaf extract, rich in galloyl quinic acids, has underwent pre-clinical and phase I clinical evaluation under ANVISA guidelines (Brazilian FDA). The tri-substituted 3,4,5-tri-*O*-galloylquinic acid methyl ester was synthesized and evaluated in the fly model (*Drosophila melanogaster* malpighian tubule model) for investigation of its mechanism of action, which might be in part related to annexin A1 inhibition.

Erectile dysfunction affects approximately 40% of men at age 40 and nearly 70% of men at age 70. Cubebin, a dibenzyl butyrolactolic lignan, was initially isolated from *Zanthoxylum naranjillo* Engler (Rutacea) and was accidentally found to induce erection in mice. It was isolated in larger amounts from the commercial seeds of *Piper cubeba* L. (Piperaceae) and evaluated in toxicological pre-clinical assays in rats. Cubebin inhibited phosphodiestarase 5 in vitro and induced erection in mice model (tadalafil, as positive contro)l. Patents were applied and issued in USA, Europe and Japan.

Leishmaniasis is caused by a protozoa parasite from over 20 *Leishmania* species. An estimated 700000 to 1 million new cases and 20000 to 30000 deaths occur annually. The glycoalkaloids solasonine and solamargine at 10 μM each significantly reduced parasite *Leishmania mexicana* counts in infected macrophages and dendritic cells more efficiently than sodium stibogluconate. A standardized *Solanum lycocarpum* glycoalkaloid extract topical formulation significantly reduced lesion sizes and parasite counts recovered from lesions in a mice model.

PL-10

NATURAL PRODUCT DRUG DISCOVERY AND TARGET IDENTIFICATION FOR OVARIAN CANCER

Joanna Burdette
University of Illinois at Chicago

High grade serous ovarian cancer (HGSOC) is a lethal gynecological malignancy with a need for new therapeutics and prevention strategies. Our research program focuses on evaluating the mechanism of action and identifying new natural compounds that can be used either as prevention of treatment of this deadly disease. First, we developed potent synthetic analogs of the class, Phyllanthusmins, inspired by prior natural product isolated from *Phyllanthus poilanei* as part of a collaborative, multi-investigator project. The most potent analog, PHY34, demonstrated nanomolar potency in HGSOC cell lines *in vitro* and displayed cytotoxic activity through

late-stage autophagy inhibition and activation of apoptosis. PHY34 was readily bioavailable through intraperitoneal administration *in vivo* where it significantly reduced HGSOC tumor burden. Strategies were developed to identify the biological target by using photo affinity and biotin-tagged versions of PHY34 and found that this modies nuclear/cytoplasmic transport. This class of compounds holds promise as a potential, novel chemotherapeutic and demonstrates the effectiveness of targeting the nucleocytoplasmic transport as a viable strategy for combating the disease. Next we focused on verticillin A, for its anticancer properties and mode of action in HGSOC cells. Verticillin A is a epipolythiodioxopiperazine (ETP) alkaloid that is isolated from several terrestrial and marine filamentous fungi and has been shown to be cytotoxic in several cancer cell lines including OVCAR3, OVCAR4, OVSAHO, and Kuramochi. RNA-Seq analysis was performed with OVCAR8 cells treated with Verticillin A and the data found an upregulation of apoptosis signaling pathway and oxidative stress response and downregulation of cancer stemness signaling pathways. A proteomic histone profiling performed in OVCAR8 cells indicated that Verticillin A caused epigenetic modifications with global changes in histone methylation and acetylation marks. Verticillin also rapidly induces formation of reactive oxygen species and DNA double strand breaks that along with epigenetic regulation trigger cell death. Encapsulation of verticillin into nanoparticles allowed for effective reduction of tumor burden in vivo. Thus, our study identifies Verticillin A as a novel epigenetic modifier in ovarian cancer cells and indicates therapeutic potential for treatment of HGSOC. Lastly, we focus on the identification of new phytoprogestins, which can be used to prevent ovarian cancer. Phytoprogestin compounds are identified by bioassay-guided fractionation using a luciferase reporter assay. The compounds are tested for their agonist or antagonist effects. Apigenin and kaempferol were both found to be mixed agonists and had in vivo progestin-like effects by inhibiting genistein induced uterine proliferation. Surprisingly, irilone isolated from red clover did not have agonist nor antagonist effects but potentiated progesterone mediated signaling without degrading the receptor. A natural product that potentiates progesterone mediated signaling has not been identified before, and this could be particularly useful for women who are afflicted with progesterone resistant gynecological diseases. Together these represent strategies for the reduction and/or prevention of ovarian cancer.

Invited Speakers

I-01

EXPLORING THE CHEMICAL POTENTIAL OF GREAT SALT LAKE MICROORGANISMS

Guangwei Wu[1], Zhuo Shang[2], Christopher A. Kauffman[1], Inho Yang[2], William Fenical[2], and Jaclyn M. Winter[1]
[1]Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT, 84112, [2]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, San Diego, CA, 92093

Biological pressures can influence the chemical diversity of secondary metabolites and microorganisms isolated from extreme environments have proven to ideal resources for drug discovery efforts and for characterizing novel biosynthetic pathways. The Great Salt Lake, also recognized as America's Dead Sea, is an endorheic hypersaline lake in Utah. Recently, we started a natural product drug discovery program aimed at interrogating halophilic bacteria isolated from this unique environment. From our initial isolation campaign, we successfully isolated ten new actinomycetes and sequenced and assembled all ten genomes. Genome mining revealed a wealth of biosynthetic clusters in the Great Salt Lake actinomycetes that have little similarity to other biosynthetic clusters in public databases. Fermentation studies with two of these strains led to the isolation and elucidation of the bonnevillamides and salinipeptins, which are new classes of linear nonribosomal heptapeptides and ribosomally synthesized and post-translationally modified peptides, respectively. The bonnevillamides and salinipeptins contain unprecedented amino acid building blocks and the discovery and characterization of these new chemical entities, as well as their corresponding biosynthetic machinery will be discussed.

I-02

MINING NEW AND ANTIPROLIFERATIVE COMPOUNDS FROM UNTAPPED NATURAL PRODUCT SOURCES

Harinantenaina L. Rakotondraibe
College of Pharmacy, Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, Ohio 43210, USA

Natural products are the ultimate sources of many medicinally important compounds and have been used frequently for decades in studies as chemical probes and for the validation of new pharmacological targets in drug discovery. Since many of the anti-infective and cytotoxic chemotypes and pharmacophores that are present in each family of natural products seem to have been identified already, investigation of new and unexplored natural product sources is needed to discover unique bioactive natural products with novel chemotypes and pharmacophores for new generations of anticancer and anti-infective drugs. In the laboratory of the author, emphasis is placed on the secondary metabolites of liverworts and their endophytes, microbial associates of U.S. endemic lichens, and endemic Malagasy plants to discover compounds active against recently identified drug-resistant diseases, including cancer. Bioactive marine organisms such as algae, cyanobacteria and fungi can form endemic lichens in coastal regions. These newly formed, restricted and highly stressed marine lichens survive symbiotically under unique conditions and can be sources of new bioactive secondary metabolites with chemotypes and pharmacophores different from those already discovered. Results obtained in the search for bioactive metabolites from fungal associates of liverworts and U.S. endemic lichens will be presented. Moreover, a recently developed one-dimensional NMR-based dereplication method utilizing Total Spectroscopy Scanning to assist mass spectrometry, detect minute amount of known compounds, and prioritize new and active compounds, will be also discussed.

I-03

G.I.T. (G-PROTEIN, ION CHANNEL, AND TRANSPORTER) LIGAND DISCOVERY FROM NATURAL SOURCES

Kevin J. Tidgewell
Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15219

Natural products chemistry has provided compounds for the discovery and characterization of many pharmacologically relevant classes of receptors, especially in the realm of central nervous system functioning. G-protein coupled receptors, ion channels, and transporters (GITs) are important drug targets due to their involvement in biological processes that span central and peripheral systems. GIT ligand discovery can utilize both orthosteric and allosteric binding sites and the extracellular expression of these targets can be used for metabotropic signaling or to cross biological membranes. GITs can be difficult to work with because crystal structures of the native proteins are rare, their binding and desensitization pathways are complex, and traditional pharmacological assays can require multi-milligram quantities for full characterization of molecules. Despite these shortcomings, the Tidgewell lab employs strategies to investigate marine cyanobacteria and terrestrial plants for GIT ligands which can modulate biological processes. By employing a variety of bioassays in combination with modern spectrometric techniques we are systematically exploring extracts for GIT modulators. We have found that marine cyanobacteria are consistent producers of GIT ligands and are pursuing isolated compounds for efficacy against cancer, depression, anxiety, pain, and comorbid disease states. We are also exploring Cameroonian plants used ethnopharmacologically for the treatment of inflammation and pain to better understand and find treatments for chronic pain. While GIT ligand discovery is difficult, the benefits for human health and scientific understanding of biological processes is worth the effort.

I-04

MEASURING METABOLITES IN HUMAN HEALTH AND MICROBIAL SYSTEMS

Laura Sanchez
University of Illinois at Chicago, Chicago, IL USA

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 to study these cues and gradients in cheese rind-derived microbial co-cultures and biofilm forming Gram negative microbes. However, the idea that there may be chemical gradients or chemical cues in the human body has been under explored, especially in terms of cancer progression and metastasis. Many cancers tend to colonize specific tissues, and it remains unclear why specific cancers colonize specific organs. My lab has begun to rethink the origins of cancer based on our knowledge of chemical ecology in microbial based systems and nature and have developed a novel platform to study the chemical gradients and chemical cues found in specific organ microenvironments to facilitate a new understanding of primary and secondary metastases in cancer. In order to study the chemistry in specific microenvironments, we first had to develop an IMS platform to visualize the molecule maps that small molecules occupy in mammalian cell cultures. IMS has previously been used to create small molecule maps in fresh frozen tissue sections, spheroids, and microbial cultures. However, these static experiments cannot capture the early chemical signaling events that likely occur in events such as metastasis. Therefore, we have developed an innovative experimental design to complement the existing strengths of IMS to study these chemical cues with intact spatial referencing in living organs and cells. Taking advantage of known strengths of imaging on agar for bacteria, we have adapted it into low melting agarose for mammalian

tissues and cells. This is the first application of IMS to 3D ovarian cancer metastasis, and the technique is not only specific to detection of chemical exchange in cancer, but can be applied to many biological questions of spatial exchange or origins.

I-05

TOWARD COMPREHENSIVE ANNOTATION OF METAZOAN METABOLOMES: *C. ELEGANS* AS A MODEL

Frank C. Schroeder
BTI and Cornell University, Ithaca NY

How can we complement genomics and proteomics of animal model organisms such as *C. elegans*, Drosophila or mouse with a comprehensive structural and functional annotation of the corresponding metabolomes? Growing evidence suggests that small molecules of largely undetermined structure play important roles in the biology of microorganisms, animals, and their mutual interactions, affecting key physiological pathways that regulate lifespan, development, and metabolism, with estimates for the number of unique metabolites reaching as high as several 10,000 in a single species. Our goal is to develop scalable approaches for structure elucidation and linking newly identified small molecule metabolites with genotypes and probable biological functions.

In this lecture, I will present structures and functions of several novel families of small molecule signals we recently identified in the nematode *C. elegans*. We found that, using simple building blocks from conserved primary metabolism and a strategy of combinatorial assembly, *C. elegans* and other nematode species create complex molecular architectures to regulate almost every aspect of their life history. The resulting signaling molecules can be active at femtomolar concentrations, changing behavior, development, of lifespan by modulating conserved insulin or nuclear hormone receptor signaling. The discovery of new types of modular, primary metabolism-derived signaling molecules in *C. elegans* provides a strong incentive for a comprehensive re-analysis of metabolism in higher animals, including humans.

I-06

FLEXIBILITY OF A DEFENSIVE SYMBIOSIS ACROSS BIOGEOGRAPHICAL GRADIENTS

Grace Lim-Fong[1], Ria Khandpur[1], Caroline Golightly[1], Carmen Hoffbeck[2], Sara Locklear[3], Nicole Lopanik[3]
[1]Department of Biology, Randolph-Macon College, Ashland, VA 23005, USA, [2]Biology Department, Linfield College, McMinnville, OR 97128, USA, [3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA, [4]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

Intimate and persistent associations, that is, symbioses, have been forged independently multiple times during evolutionary history, and these partnerships have changed the evolutionary trajectories of both host and symbiont. Some hosts, such as the bryozoan *Bugula neritina* (Linnaeus), depend on their bacterial symbiont for chemical defense. While many of these defensive symbioses have been delineated and described at the species level, life history and interpopulation variations in symbiotic frequency are less well understood. Here, we observed the symbiotic frequency in wild populations of *B. neritina* across a latitudinal gradient and correlated symbiotic frequency with *in situ* predation pressure. *Bugula neritina* was also raised from larvae in mesocosms at two temperatures representing latitudinal extremes. Our field study revealed that symbiotic frequency was significantly higher and predation pressure was marginally higher at lower latitudes. These field observations were corroborated by the mesocosm experiments, where sibling larvae of *B. neritina* raised at higher temperatures had higher symbiont titers than those raised in cooler water. Taken together, these results suggest that environmental factors, such as water temperature, can "fine-tune" the host-symbiont dynamic, and this flexibility allows *B. neritina* populations to thrive across biogeographical gradients.

Session Speakers

S-02

ACS "HEROES" SYMPOSIUM: PROFESSOR RACHEL MATA

Mario Figueroa and Rachel Mata
Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México

Professor Rachel Mata received her B.Sc. in Pharmacy from the Universidad Central de Venezuela and her M.Sc. and Ph.D. degrees from the School of Pharmacy and Pharmacal Sciences at Purdue University. In 1985, she joined the Department of Pharmacy of the School of Chemistry at UNAM as a full-time faculty, and has remained there to the present, where she became Professor Emerita in 2016. Dr. Mata has had a long and distinguished career in the natural products' field. She has conducted extensive studies on Mexican medicinal plants to promote their rational use, demonstrating exemplary leadership in this area for many years. Part of her work has resulted in the generation of medicinal plants' monographs, which follow the WHO guidelines and have been included in the Mexican Herbal Pharmacopeia. She has also made important contributions to the discovery of many structurally different bioactive compounds from plants and fungi. An important element of Dr. Mata's career as a scientist is her dedication and ability to train and inspire students. The stimulating interaction with the eager, enthusiastic, and unprejudiced young minds of hundreds of undergraduate and graduate students, as well as postdoctoral fellows, has been her greatest professional satisfaction, and many of her trainees have gone on to their own stellar careers in the U.S., Mexico, and other countries in Latin America.

The outcome of her scientific work has resulted in the publication of more than 200 research papers in peer-reviewed journals, including over 50 in the *Journal of Natural Products*, book chapters, patents and other types of publications. Dr. Mata has participated in several national and international committees, organizations and advisory boards; she was President of the Phytochemical Society of North America (1996-1997), Fellow of the American Society of Pharmacognosy (2014-present), and long-term editorial advisory board member of the Journal of Natural Products. She has also received a number of awards and prizes over the years from her academic institution (UNAM) as well as from other organizations, including most recently the Norman R. Farnsworth Research Achievement Award from the American Society of Pharmacognosy (2014). In summary, through her leadership and contributions to natural products research and education, she is an inspiration to the younger generation of scientists in Pharmacognosy.

S-03

ASP HEROES SYMPOSIUM: HONORING DAVID NEWMAN

Lesley-Ann Giddings
Department of Chemistry & Biochemistry, Middlebury College, Middlebury, VT 05753, USA

As a scientist and historian, David Newman has been instrumental in keeping natural products at the forefront of drug discovery for almost 60 years. Initially, David worked as an organic chemist synthesizing and characterizing pyrroles, photographic dyes, and pyrophoric metal halides before spending two years as an "external graduate student" with John Postgate, FRS and graduating with his DPhil in 1968. He later spent 20 years in industry studying the effects of drugs on oxygen transport in erythrocytes as well as isolating/optimizing the production of microbial natural products. There were several firsts; the amino acid sequence of *Desulfovibrio desulfuricans* rubredoxin, the isolation of a terrestrial antibiotic (chloramphenicol) from a marine invertebrate (*Lunatia heros*), as well as a method devised to detect vancomycin-class antibiotics, leading to aricidin. In 1991, David brought his expertise in natural products to the National Cancer Institute, where he spent 24 years establishing partnerships with industry and academia to evaluate the bioactivity of extracts from collections of 15,000+ marine invertebrates, 1000+ marine alga, 65,000 plants, and 30,000+ fungi. Some of this work has been published in the Journal of Natural Products (JNP). Notably, these collaborations led to the large-scale production / development of several drug candidates; wortmannin (>60 grams), halichondrin B (300 mg from 1 metric ton of sponge), leading to the development of eribulin with Eisai, and geldanamycin (>3 kg) for the production of 17-AAG and 17-DMAP. From 1997 to 2016, David published extensive analyses of the state of natural product drug discovery in the JNP, resulting in >15,000 citations. With 21 patents and 213 publications / >19,000 citations (*h* index 45), David is being honored for shaping the scientific legacy of JNP through his written perspectives and scientific efforts to find novel therapeutics.

S-04

PROFESSOR DAVID KINGSTON: A LIFETIME OF SERVICE TO NATURAL PRODUCTS RESEARCH

Introduction by: Brian T. Murphy
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612

Professor David Kingston is a rare example of a scientist who is a leader in multiple sub-disciplines of natural products research, as he ran successful synthetic and discovery programs. Spanning several decades, his group pioneered studies on the anticancer drug Taxol, providing a meticulous description of the effects of changes in structure on bioactivity. Further, studies carried out by his group were critical toward determining the bioactive tubulin-binding conformation of the drug. Prof. Kingston also oversaw natural product discovery efforts in collaboration with scientists from Suriname and Madagascar as part of an NIH-funded International Cooperative Biodiversity Groups program. These international partnerships sought not only to discover bioactive natural products from host country organisms, but also to train scientists in the process of discovery and resource conservation. With a talented team of collaborators, the many accolades of these projects are: >85 peer-reviewed articles, establishment of a host-country bioassay lab, training of several host-country scientists, construction of new school buildings, and conservation of 1.6 million acres of land as a nature preserve. Professor Kingston contributed much of this research – greater than 131 articles and 12 book reviews – to the Journal of Natural Products (JNP). He was Associate Editor (1983-1998) and served on the Editorial Advisory Board (1998-2018) and Honorary Advisory Board (2018-2023) of JNP. He served as ASP President (1988-1989), and received the society's highest honor, the Norman R. Farnsworth ASP Research Achievement Award, in 1999.

S-05

HONORING GORDON CRAGG

Kerry L. McPhail[1]
[1]*Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA.*

In 2004, Dr. Gordon Cragg received the National Institutes of Health Award of Merit for "Inspirational leadership and dedication to international collaborative research in biodiversity and natural products drug discovery." This accolade, received as he retired from his fifteen-year term as Chief of the Natural Products Branch in the Developmental Therapeutics Program at the National Cancer Institute (NCI), is just one of many awards that recognize his extensive accomplishments and service in natural products drug discovery and development. It aptly describes why his name is known across multiple generations in natural products research communities world-wide. Indeed, few in the field have not had occasion to reference the authoritative, periodic reviews on the current state of natural products drug

discovery co-authored with Dr. David Newman, and numerous book chapters addressing anti-cancer natural products. Dr. Cragg published his first scientific journal article in 1962 on extracts of the plant *Leonotis leonuris* in the South African Journal of Chemistry, and most recently, in 2017, co-edited a special issue of the Journal of Natural Products in honor of Professor Phil Crews, co-chaired an NIH Expert Review Panel and traveled to Brazil to deliver an NIH training seminar on technology transfer. In the intervening decades, after gaining an international education, he held several academic positions, which included working closely with Professor George R. Pettit, until joining the NCI in 1984. He played a key role in the development of anti-cancer and antiviral agents such as Taxol®, michellamine B, calanolides and conocurvone, and worked tirelessly to establish policies for international collaborations in natural products drug discovery, traveling extensively worldwide for this purpose. It is remarkable that someone who has contributed so much in promoting international, multidisciplinary, multi-institutional collaboration to advance the discovery and development of effective new pharmaceutical drugs, continues to give his time and boundless energy as an NIH Special Volunteer, a position he has held since 2005 to the present day.

S-06

FENUGREEK, GUT MICROBIOTA, AND RESILIENCY TO WESTERN DIETS

Annadora Bruce-Keller[1], Jacqueline Stephens[1], Allison Richard1, J. Michael Salbaum[1], David Ribnicky[2]
[1]Pennington Biomedical Research Center, Baton Rouge, LA, USA,
[2]Department of Plant Biology, New Brunswick, NJ, USA

Fenugreek (*Trigonella foenum-graecum*) is used in many parts of the world for diabetes, cardiovascular disease, and depression. While the mechanism(s) of these actions is not known, it is becoming increasingly clear that gut microbiota are key players in homeostasis and also mediate true first-pass metabolism of dietary compounds. Furthermore, the high protein and fiber content of fenugreek seeds is particularly suited to modify intestinal bacteria and offset the dysbiotic effects of high fat/low fiber Western-style diets. Based on these observations, experiments were designed to determine the extent to which fenugreek induces physiologic resiliency via changes to intestinal microbiota

To this end, male C57Bl/6 mice were given open source high fat (HFD) or Western-style (WD) diets supplemented with/without 2% (w/w) fenugreek for 12 weeks. Mice were subjected to a battery of metabolic, behavioral, and metegenomic/metabolomics analyses to map all effects of fenugreek – beneficial and/or adverse – on diet-induced physiological decline. Data show that fenugreek significantly alters intestinal microbial populations under all diet conditions, and reverses key diet-induced changes to gut microbiota. Indeed, fenugreek specifically reversed the actions of diet on numerous taxa in a manner that predicted specific aspects of behavioral and metabolic resiliency. Finally, while no direct toxicological effects of fenugreek were found under any diet conditions, data suggest that the beneficial effects of fenugreek supplementation were differentially impacted by the choice of diet (WD versus HFD). While these data support a role for gut bacteria in beneficial responses to fenugreek, key additional data are needed to confirm and identify mechanisms by which fenugreek-microbiota interactions drive physiologic benefits. First, the impact of gut microbiota on the beneficial profile of fenugreek needs to be unequivocally established. Further, whether fenugreek-microbiota interactions alter the gut metabolome directly via unmasking/generation of otherwise-inaccessible botanical phytochemicals; or indirectly via altered metabolism of Western/high fat diets should be resolved. Finally, identification of intestinal and blood-based metabolites that mediate fenugreek-based physiologic resiliency is needed to accelerate the translation of these findings. Current studies combining conventional and germ-free mice, an adaptive microbiome transplantation paradigm, and a series of cutting-edge in silico analyses are underway to meet these needs. It is hoped that completion of these studies will improve understanding how gut microbiota balance the interactions of adverse and beneficial dietary elements, leading to novel but rational therapies that promote overall physiologic resilience in today's complex environment.

S-07

PROANTHOCYANIDIN METABOLITES PRODUCED BY COMMENSAL GUT MICROBES MAY PROMOTE METABOLIC RESILIENCE

Diana E. Roopchand
Department of Food Science, NJ Institute of Food, Nutrition, and Health, Rutgers University, New Brunswick, NJ 08901.

Studies suggest that consumption of dietary polyphenols can lower the risk of developing chronic diseases such as metabolic syndrome and type-2 diabetes. Mechanisms behind these important public health observations remain to be elucidated as many bioactive polyphenols, such as proanthocyanidins (PACs), are poorly absorbed. Compared to high-fat diet (HFD)-fed controls, C57BL/6J mice fed HFD supplemented with grape polyphenols (GP) developed a bloom of *Akkermansia muciniphila* in association with attenuated symptoms of metabolic syndrome. PACs, the main class of polyphenols in the GP extract, were sufficient to induce this bloom. PACs can be biotransformed by colonic bacteria to more bioavailable microbial metabolites (MM), which may mediate the health benefits of dietary polyphenols. To establish cause-effect relationships, longitudinal microbiome-wide association studies (MWAS) coupled to *in vitro* microbiology and gnotobiotic mouse experiments will be used to identify human and murine bacterial isolates or consortia responsible for production of PAC-derived MMs. Cell-based assays and a HFD-induced gnotobiotic model of obesity will then be used to test the hypothesis that MMs contribute to metabolic homeostasis. Successful completion of this proposal will contribute knowledge towards precision nutrition approaches for promoting metabolic resilience, which may include prebiotics, probiotic commensal bacteria, and/or postbiotic microbial metabolites.

S-08

MULTIOMIC SIGNATURES OF MICROBIAL METABOLITES FOLLOWING PREBIOTIC FIBER SUPPLEMENTATION

Brittany Lee-McMullen, Samuel Lancaster, Charles Abbott, Daniel Hornburg, Jeniffer Quijada, Michael Snyder
Stanford University, Stanford, CA, USA

Prebiotic fiber supplementation has been correlated with a number of positive health outcomes. The gut microbiota metabolizes these fibers releasing a range of metabolites that are physiologically beneficial to their human host. Mechanistic evidence linking specific microbiota with the metabolites they produce, and the downstream biological effects is largely unknown. To investigate this further, we assembled a cohort of individuals and performed longitudinal multiomic analysis during prebiotic supplementation and washout. We have a dataset including metagenomics, transcriptomics, proteomics, metabolomics, and lipidomics to characterize the systemic alterations that occur in the microbiome and host while taking prebiotic fiber supplementation. By integrating these datasets, we have generated a unique biological signature, including the microbiome and microbial-derived metabolites, that influence host biological activity. Through this study we have gained an immense amount of information about the physiological changes during prebiotic fiber supplementation and can begin to truly understand these beneficial health outcomes from fiber at a mechanistic level.

S-09

MICROBIOTA-MEDIATED BIOTRANSFORMATION OF POLYMETHOXYFLAVONES: KEY FOR THEIR ANTI-INFLAMMATORY ACTIVITIES IN THE COLON

Hang Xiao

Department of Food Science, University of Massachusetts, Amherst, MA 01003

Dietary flavonoids and microbiota in the colon interact in a reciprocal manner as bacteria can metabolize flavonoids to various metabolites, and flavonoids can modulate microbiota composition and associated biofunctions. This interaction, which remains to be elucidated, is anticipated to have a significant impact on the risk of several diseases such as colitis. A detailed understanding of this interaction will facilitate the development of dietary flavonoid-based strategies for preventing these diseases. Consumption of citrus fruits and their components has been found to associate inversely with inflammation-related chronic diseases. Polymethoxyflavones (PMFs), a unique class of citrus flavonoids, displayed potent anti-inflammatory properties in the colon in our animal studies. We found that gut microbiota mediated the production of an array of colonic metabolites of PMFs after their oral administration in mice, and these metabolites possessed much stronger anti-inflammatory effects than their parental PMFs. Furthermore, we identified strains of PMF-metabolizing bacteria from human stool and found that dietary PMFs modulated the abundance and metabolic functions of these fecal bacteria in mice with colitis. Overall, our results provided a solid basis to utilize this unique interaction between citrus PMFs and gut microbiota in the prevention of colonic inflammation and associated diseases.

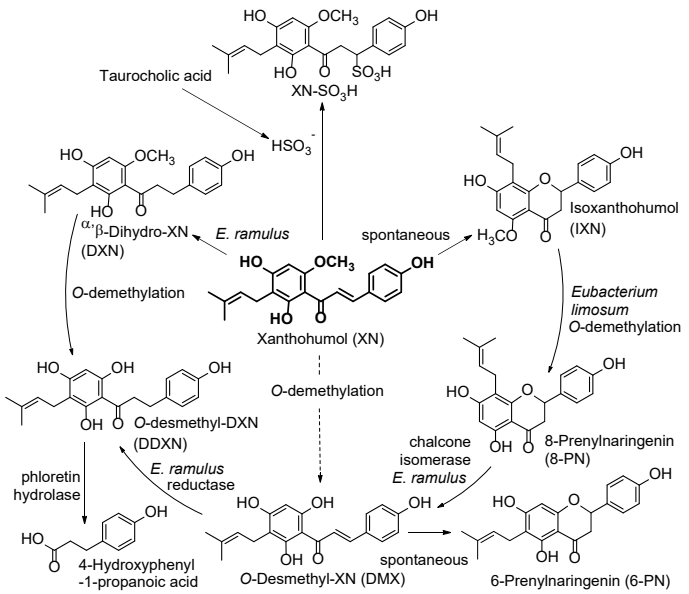
S-10

INTERACTIONS BETWEEN GUT MICROBIOTA AND XANTHOHUMOL FROM HOPS (*HUMULUS LUPULUS*) AND THEIR IMPACT ON HOST HEALTH

Jan F. Stevens[1,2], Adrian F. Gombart[1,3], Claudia S. Maier[4], Thomas O. Metz[5], and Ryan D. Bradley[6]

[1]Linus Pauling Institute, Departments of [2]Pharmaceutical Sciences, [3]Biochemistry & Biophysics, and [4]Chemistry, Oregon State University, Corvallis, OR 97331, USA, [4]Integrative Omics Group, Pacific Northwest National Laboratory, Richland, WA 99352, USA, [6]College of Naturopathic Medicine & School of Graduate Studies, National University of Natural Medicine, Portland, OR 97201, USA

Gut microbiota mediate the physiological impacts of many natural products. We have elucidated the gut microbial pathways of xanthohumol (XN) metabolism (see scheme). Oral administration of XN and its metabolite, α,β-dihydro-XN, to high fat-fed C57BL/6J mice profoundly altered the composition of the gut microbiome of high fat-fed mice. Oral XN improved glucose/lipid metabolism and decreased pro-inflammatory cytokine levels in this mouse model. These and other data provide a strong rationale for testing the effects of XN on gut inflammation in humans. We are conducting two prospective clinical trials with XN (24 mg q.d.): one with 24 adults and one with 24 adults diagnosed with Crohn's Disease (Funding by NCCIH grants R01AT009168 and R01AT010271).



Award Winners

A-01

CHEMOMETRICS: NOVEL APPROACHES FOR INVESTIGATING SECONDARY METABOLITES IN MEDICINAL PLANTS

Elizabeth M. Mudge[1], Michael Chan[1], and Paula N. Brown[1]
[1]Centre for Applied Research & Innovation, BC Institute of Technology, Burnaby, BC V5G3H2

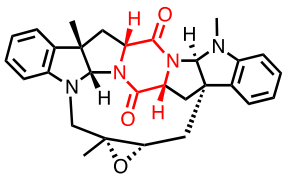
Plants produce a myriad of phytochemicals that allow them to grow, adapt and thrive in their environments. Parsing, organizing and interpreting this plethora of information can be a daunting task. Consequently, the vast majority of approaches used to study plant chemistry are reductionist, only targeting specific compounds or compound classes and often selected based on ease of detection or isolation. Such approaches, while still providing invaluable insights and information, only explore a fraction of the chemical information available. Chemical profiling coupled with chemometric analyses offers alternative means through which chemical information from a plant can be investigated and allows researchers to study plant metabolism in novel and innovative ways. A chemometric study data set can contain vast amounts of information and key factors in using the data effectively are experimental design, availability of reference materials, sample preparation and the selection of statistical analyses performed. This presentation will discuss and demonstrate some recent approaches employed to interpret plant secondary metabolite data seta for authentication of botanicals, phytochemical discovery and improving the safety and efficacy of botanical-based products.

A-02

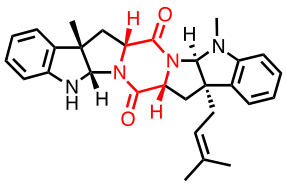
UNLOCKING THE TREASURE TROVE OF BIOSYNTHETIC PATHWAYS FROM THE SEA

Amy L. Lane
Chemistry Department, University of North Florida, Jacksonville, FL 32224

Microbial genomes encode a treasure trove of biosynthetic pathways that yield structurally intriguing bioactive natural products. Characterization of these pathways facilitates practical applications of natural products, provides tools for chemoenzymatic syntheses, and offers insights into microbial ecology and evolution. My research program aims to unlock the treasure trove of biosynthetic potential of marine bacteria. This presentation will highlight our findings for pathways in which cyclodipeptide synthases (CDPSs) catalyze 2,5-diketopiperazine (DKP) assembly from aminoacyl-tRNAs. Although CDPSs are broadly distributed across bacterial phyla, fewer than ten multistep biosynthetic pathways that include CDPSs have been experimentally characterized to date. We experimentally established the nocardioazine biosynthetic pathway, in which two CDPSs catalyze assembly of *cyclo*(L-Trp-L-Trp) DKP. The stereochemistry of this DKP is then inverted, followed by prenylation and methylation to yield nocardioazine B as a precursor to nocardioazine A. We also applied enzymes from this pathway to develop a biosynthetic approach for generating DKPs from unnatural amino acids to expand the breadth of DKP diversity. Together, our results highlight Nature's aptitude for chemical synthesis and showcase biosynthetic approaches for expanding Nature's chemical library.



nocardioazine A



nocardioazine B

A-03

TYLER, TAYLOR & TALES: THE COMPLEXITY OF REDUCTIONISM

Guido F. Pauli
PCRPS and Department of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612

Natural product (NP) research is inherently complex matter as NPs are biosynthesized by multipart machineries, embedded into intricate matrices, and expressed as metabolomic mixtures. Whether NPs are sources of new drugs, biological tools, applied as health products directly: science focuses on reductionist approaches to resolve the underlying complex questions at the chemistry-biology interface. The privilege of becoming associated with the pharmacognostic legacy of *Varro E. Tyler* is an opportunity to highlight research outcomes and new concepts that connect with his long-term vision for the rationalization of medicinal plant use. One focus involves the means by which chemical standardization of widely used botanical health products can be expanded to multiple bioactive markers. Analogous to *Taylor* series in mathematics, tools such as 2D NMR barcoding and qNMR quantitation can help approximate a more holistic description of complex systems such as herbal extracts. At the same time, these advanced tools challenge reductionist approaches by providing new insights into the major impact of minor constituents (residual complexity), and by demonstrating the significance of recognizing multiple components as Nature's highly common blueprint for bioactive principles. Withstanding the temptations of overly reductionist approaches to NP complexity, and following Y'ing principles, has been producing evidence that calls into question widely published *Tales* that oversimplify NP bioactivity and discovery. Discussed examples include the danger of equating whole plants with single constituents (*Curcuma* vs. curcumin), quandaries generated by a small array of overstudied NPs (IMPs), and chemical knock-out materials opening new opportunities.

A-04

THE ECHINACEA STORY: A SCIENTIFIC LIFE DEDICATED TO A NORTH AMERICAN MEDICINAL PLANT

Rudolf Bauer
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitaetsplatz 4, 8010 Graz, Austria

Echinacea species are originating from North America and have been used by the indigenous people to treat wounds and infections. Only in the 20th century, they became known as a medicinal plants in Europe, where they started to be researched and developed into medicinal products.

Echinacea preparations are now globally used as herbal immunomodulators, in particular for the treatment of the common cold. Clinical studies have been performed with variable outcome, because the preparations differ in their composition, due to the use of various species, plant parts, and extraction methods. Most frequently, roots of *Echinacea angustifolia* and *E. pallida,* and aerial parts of *E. purpurea* are used. Their constituents and pharmacological effects have been intensively studied.

Polysaccharides, glycoproteins, alkamides and caffeic acid derivatives have been considered as most relevant constituents of echinacea.

Recently, it could be demonstrated that Echinacea alkamides bind to cannabinoid receptors and thereby trigger effects on the immune system. Pharmacokinetic studies have shown that Echinacea alkamides are rapidly absorbed after oral application. Therrefore, they must be considered as highly relevant for activity.

In conclusion, with more than 1200 scientific publications, Echinacea has become one of the best researched medicinal plants.

A-05

CONTINUING ADVENTURES ON A HETEROCYCLE

Geoffrey A. Cordell
Natural Products Inc., Evanston, IL, USA and Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL, USA

A reflective and nostalgic journey chronicling some of the people, places, and heterocyclic compounds encountered in over 50 years in the world of the natural product sciences, concluding with some thoughts on (eco)pharmacognosy and the scientific challenges ahead.

Contributed Speakers

C-01

ELUCIDATING THE MECHANISM OF SELECTIVE CYTOTOXICITY IN NSCLC CELL LINES AND TARGET IDENTIFICATION OF IKARUGAMYCIN

Anam F. Shaikh[1,2], Elizabeth A. McMillan[1], Chensu Wang[1], Michael A. White[1], John B. MacMillan[1,2]
[1]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA, [2]Department of Chemistry, University of California Santa Cruz, Santa Cruz, CA, 95064, USA

The MacMillan laboratory has established a large library of natural products from our collection of marine-derived bacteria for oncology drug discovery, including a large-scale screen to identify natural products that could be used for personalized therapy in lung cancer. We utilized a collection of 180 non-small cell lung cancer (NSCLC) cell lines characterized for mutations and other molecular annotations to identify a natural product called ikarugamycin that shows potent and selective activity. From this screen, we identified that ikarugamycin showed activity against around one-third of the initial NSCLC cell lines screened with IC_{50} values from 0.090 μM to 1 μM. Through a parallel high throughput screen conducted in HeLa cells, we were able to demonstrate that ikarugamycin activates transcription factor EB (TFEB), a master regulator of lipid catabolism. Further investigation of these results demonstrated that ikarugamycin-induced activation of TFEB occurs through induction of calcium efflux from the ER, which results in activation of AMPK. The exact target that leads to increased calcium efflux from the ER is unknown. Herein, we seek to characterize the mechanism of cell death and specific molecular target of ikarugamycin that leads to TFEB activation, as well as the mechanism of selective cytotoxicity in NSCLC cell lines.

C-02

WHAT HAVE WE LEARNED FROM A FEW THOUSAND YEARS OF NATURAL PRODUCTS RESEARCH?

Jonathan Bisson,[1] James G. Graham,[1] and Guido F. Pauli.[1]
[1] Center for Natural Products Technologies (U41 AT008706) University of Illinois at Chicago, Chicago, IL 60612, USA.

From the Ebers Papyrus to the latest issue of JNP, much of the world's knowledge of Natural Products (NP) is shared in "text" form. As technological advances fostered new growth in NP research, initiatives to encode various forms of NP information into databases emerged. Until fairly recently it has been feasible for rooms (real or virtual) of people to annotate current NP data into these resources, but current explosive growth in NP communications has demonstrated this approach as anachronistic. As manual data entry and curation of entered data are beyond recourse for most research groups, new approaches are needed. Recent advances in text-mining and machine-learning that provide for (semi)-automated data annotation and entry, allow for assisted curation through rational integration of human and digital assets.

Our program is currently combining a number of key resources: the 200,000 manually-curated NP citations found in the NAPRALERT database; >500,000 abstracts and citation references extracted from CrossRef and PubMed; ~200,000 structures from UNPD and PubChem; along with millions of taxonomic entries from Wikidata. Subsequently, our ***PH****armacognosy* ***O****ntology* (PHO) will be employed to train Bayesian and Machine-Learning models. We envision the evolution of a machine-learning-assisted data entry and curation process- one capable of keeping pace with the rising tide of NP data and distinguishing meaningful data from noise.

C-03

CHEMOINFORMATIC CHARACTERIZATION OF THE NATURAL PRODUCTS DATABASE FROM BRAZIL

Fernanda I. Saldívar-González[1], Marilia Valli[2], Adriano D. Andricopulo[3], Vanderlan S. Bolzani[2], José L. Medina-Franco[1]
[1]School of Chemistry, Department of Pharmacy, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico; [2]Nuclei of Bioassays, Biosynthesis and Ecophysiology of Natural Products (NuBBE), Department of Organic Chemistry, Institute of Chemistry, Sao Paulo State University - UNESP; [3]Laboratory of Medicinal and Computational Chemistry (LQMC), Institute of Physics of Sao Carlos, University of Sao Paulo - USP

Data repositories have been important tools for drug discovery. Our research efforts have been engaged in developing NuBBE Database (NuBBE$_{DB}$, http://nubbe.iq.unesp.br/portal/nubbedb.html), the first database of natural products from Brazilian biodiversity. A chemoinformatic analysis of NuBBE$_{DB}$ was performed with physicochemical properties revealing that this database comprises a focused chemical space, within the space of traditional and drug-like physicochemical properties, and similar to approved drugs. We found a few scaffolds that are exclusive of NuBBE$_{DB}$, i.e. could not be found in other databases, and could be used as a starting point for the identification of new agents with therapeutic activity. This analysis shows that NuBBE$_{DB}$ has a large percentage of drug-like compounds and support quantitatively that this is a promising source of molecules for drug discovery and medicinal chemistry. This innovative and unique database has a collaborative work with the Chemical Abstracts (CAS-ACS) aiming at a comprehensive database of natural products in Brazil. Promoting the successful use of Brazilian biodiversity, would be a demonstration that technological innovation is achievable, supporting economic growth without a negative impact in the environment.

C-04

A MOLECULAR NETWORKING APPROACH TO CHARACTERIZING PYRROLOIMINOQUINONE PRODUCTION BY LATRUNCULID SPONGE SPECIES

Rosemary A. Dorrington[1], Jarmo-Charles J. Kalinski[1], Shirley Parker-Nance[2] and Samantha C. Waterworth[3].
[1]Department of Biochemistry and Microbiology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa. [2]Elwandle Node, South African Environmental Observation Network, Port Elizabeth, South Africa. [3]Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, WI, USA.

Marine sponges of the family, Latrunculiidae, are prolific producers of pyrroloiminoquinone alkaloids, a compound class exhibiting promising bioactivities that includes discorhabdins tsitsikammamines and makaluvamines. The aim of this study was to profile the secondary metabolite reservoir of six latrunculid sponge species to identify potentially new bioactive pyrroloiminoquinone compounds. We profiled chemical extracts from more than 80 specimens representing *Tsitsikamma favus, Tsitsikamma pedunculata,* two recently described species, *Tsitsikamma nguni* and *Tsitsikamma michaeli* as well as *Cyclacanthia bellae*, all endemic to the southeast coast of South Africa and the Antarctic deep water sponge, *Latrunculia apicalis*. LC-MS/MS driven molecular networking reveal that the closely related *T. nuguni* and *T. favus* sponges exhibit similar chemical profiles, containing tsitsikammamines and brominated C-series discorhabdins. *T. michaeli* sponges collected from different sites in Algoa Bay exhibited location specific chemotypes, with sponges collected from Riy Banks reef producing pyrroloiminoquinone suites resembling those observed for *T. favus* and *T. nguni*, but with no detectable tsitsikammamines. Contrarily, samples of *T. michaeli* collected at Evans Peak reef produced a distinct suite of pyrroloiminoquinones, including several new discorhabdins. Surprisingly, the shallow-water encrusting sponge *C. bellae* and deep-water *L. apicalis* sponges produce closely related pyrroloiminoquinone suites, including a plethora of known and potential-

ly new sulfur-bridged discorhabdins, with trace amounts of tsitsikammamines present in *C. bellae* sponges.

C-05

AN INTERDISCIPLINARY APPROACH TO STUDY THE POTENTIAL OF AÇAÍ-ANTICANCER DRUG INTERACTIONS

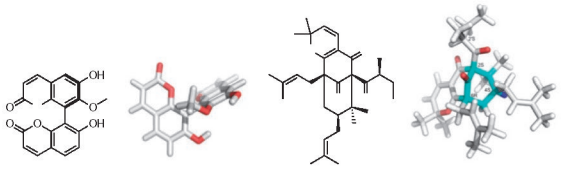
Angela I. Calderón[1], Yilue Zhang[1], Satyanarayana R. Pondugula[2], Jingjing Qian[3], Richard A. Hansen[3]
[1]Department of Drug Discovery and Development, Auburn University, Auburn, AL 36849, [2]Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL 36849, [3]Department of Health Outcomes Research and Policy, Auburn University, Auburn, AL 36849.

A serial, cross-sectional study conducted using data from the National Health and Nutrition Examination Survey from 1999-2014 showed stable overall use of Botanical Dietary Supplements (BDS) among a nationally representative sample of U.S. cancer patients and generated preliminary data on popular BDS in the U.S. FDA Adverse Event Reporting System (FAERS) that were involved in Adverse events (AEs) when used concomitantly with anticancer drugs. The examination of AE reporting patterns for concomitant BDS and anticancer drugs using FAERS suggested açaí for risk signals for AEs. In an attempt to decrease the general discrepancy between *in vivo* and *in vitro* botanical-drug interaction in our study, the passive diffusion absorption profiles of açaí extracts were investigated. Specifically, the parallel artificial membrane permeability assay (PAMPA) model was utilized to simulate intestinal filtration of passively diffused constituents of açaí extracts. These were subsequently screened for *in vitro* liver CYP3A4 inhibition and induction. The passively absorbable portion of a methanol açaí extract exhibited inhibition and induction effects on CYP3A4 suggesting the potential to produce botanical-drug interactions. The chemical composition of the extract was further investigated using the chemometric tool Mass Profiler Professional (MPP) on liquid chromatography-mass spectroscopy (LC-MS) data. Subsequently, five highly permeable açaí compounds were characterized by tandem mass spectrometry.

C-06

CHEMICAL NOVELTY FROM *HYPERICUM* SPECIES (ST. JOHN'S WORT)

Serge A.T Fobofou[1,2], Katrin Franke[2], Ludger Wessjohann[2], Jon Clardy[1]
[1]Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, MA 02115, USA. [2]Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, 06120 Halle (Saale), Germany. [3]Leibniz Institute-DSMZ German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany



LC-MS and NMR metabolomics were combined with multivariate data analysis to prioritize *Hypericum* species for novel compound discovery. We could isolate and characterize 23 new natural products from 3 prioritized *Hypericum*s collected in Africa. Some of these compounds exhibit biological activities. Compounds **1** and **2** are two examples of previously undescribed compounds isolated from *H. roeperianum* and *H. lanceolatum*, respectively. Compound **1** represents the first dimeric coumarin isolated from this genus, while **2** is an example of polycyclic polyprenylated acylphloroglucinol (PPAP).

C-07

BROMINATING THE SECONDARY METABOLOME

Gabriel Castro-Falcon, Grant S. Seiler, and Chambers C. Hughes
Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0204

Reactivity-guided isolation is an innovative method for the discovery of specific natural products (NPs) in complex extracts. It identifies metabolites based on their reactivity and has the potential to transform the way in which natural products chemists uncover certain classes of NPs. Specifically, the approach entails the covalent labeling of targeted NPs using chemoselective reagents or "probes" with prominent UV/vis properties and a distinct bromine isotopic signature (^{79}Br:^{81}Br 1:1) and is tied to the well-established practice of using reactive bromoarenes such as 4-bromobenzoyl chloride to yield crystalline material amenable to X-ray crystal structure analysis. Despite the fact that it does not directly furnish unaltered NPs, the method provides profound insight into the chemical space of an extract. It is a precise examination into the presence and abundance of a specific functionality or structural moiety that can be directly coupled to genome-mining studies. The method promises to de-orphan a large number of BGCs and lead to the discovery of new NPs in a chemocentric manner. Here, we will discuss the design and application of probes that target NPs with electrophilic moieties (α,β-unsaturated carbonyl groups, epoxides, β-lactones, β-lactams), conjugated alkenes, enediynes, isocyanides, terminal alkynes, phosphonates, and amino groups.

C-08

CHARACTERIZATION OF AUTOINDUCER-3 BIOSYNTHESIS IN *E. COLI*

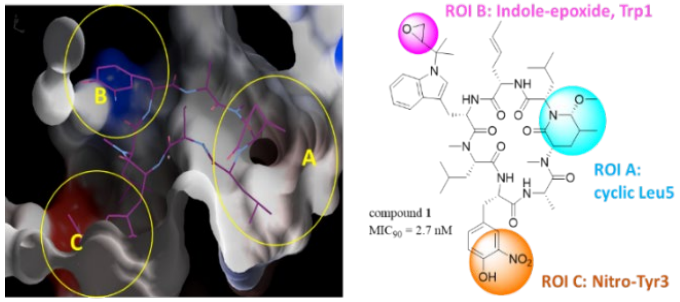
Chung Sub Kim,[1,2] Yick Chong Lam,[1,2] Alexandra Gatsios,[1,2] Regan Russell,[3,4] Vanessa Sperandio,[3,4] and Jason M. Crawford[1,2,5,#]
[1]Department of Chemistry, Yale University, New Haven, CT 06520, USA. [2]Chemical Biology Institute, Yale University, West Haven, CT 06516, USA. [3]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [4]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. [5]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06536, USA. #Correspondence; Presenting Author: chungsub.kim@yale.edu

Escherichia coli is a well-studied model organism that has served as a transformative biological resource, not only for unraveling central basic mechanisms of molecular genetics and cell biology, but also for its widespread applications in molecular biology, microbiology, and biotechnology. Natural variants of *E. coli* are among the first to colonize the human intestinal tract after birth and are estimated to reside in about 90% of the population. Pathogenic strains fall within eight known pathotypes and cause a variety of severe infections, such as meningitis, hemorrhagic colitis, pneumonia, urinary tract infections (UTIs), hemolytic uremic syndrome, colorectal cancer, and others with about 165,000 infections being reported annually in the United States alone. Despite the bacterium's expansive biomedical importance, a molecular understanding of the signaling systems that *E. coli* use to regulate virulence and quorum sensing is incomplete. Here, we characterized the structure and biosynthesis of the elusive autoinducer-3 (AI-3), providing molecular resolution to a microbiology problem that has spanned nearly 20 years. We show that AI-3 is produced across pathogenic, probiotic, and commensalistic strains, and the small molecule is biosynthesized by a gene that is essential for growth. Finally, we demonstrate that AI-3 activates virulence genes in enterohemmorhagic *E. coli* (EHEC).

C-09

MODIFICATION AND SAR OF ANTI-TB RUF I AND II MIXTURES BASED ON RUF I AND CLPC1-NTD-WT CO-CRYSTALS

Bin Zhou,[1,2] Cele Abad-Zapatero,[3] Larry L. Klein,[2] Gauri Shetye,[2] Nina M. Wolf,[2] Hyun Lee,[1,3] James B. McAlpine,[1,2] Shao-Nong Chen,[1,2] Sang-Hyun Cho,[2] Scott G. Franzblau,[2] and Guido F. Pauli[1,2]*
[1]Dept of Med Chem & Pharmacognosy, [2]Institute for Tuberculosis Research, [3]Center for Biomolecular Sciences, College of Pharmacy, Univ of IL at Chicago, IL 60612, USA.



Rufomycins (ruf; syn. ilamycins) represent a family of promising, highly potent anti-TB cyclic heptapeptides. The extract of *Streptomyces sp.* strain MJM3502 with an MIC_{90} <0.20 µg/mL was identified as a hit from the high-throughput screening (HTS) of ~7,000 actinomycete cultures. Subsequently, two major components, rufs I and II, were identified as the potential anti-TB leads with MIC_{90}s of ~0.01 µM. Scaled-up culturing led to the isolation of ~5 g of a ruf II + I mixture (80:20) from 28 g crudes. Semi-synthetic structure modification focused on Regions of Interest (ROIs A-C) of rufs I and II, based on three binding "pockets" in the high-resolution (1.4 Å) X-ray structure of co-crystals of ruf I and ClpC1-NTD-wt, the binding domain of the *M. tb.* target protease chaperone. A series of reactions were performed, which led to the isolation and purification of 20 modified ruf-analogs. This led to new structure-activity relationship (SAR) insights and identified the cyclohexane ring of leucine (ROI A) is the most important bioactivity- determining motif. The indole-epoxide ring of tryptophan (ROI B) and the nitro and hydroxyl groups of tyrosine (POI C) are also bioactivity related motifs. Compound **1**, the methylated derivative of rufs I and II, is the most active with an MIC_{90} as low as 2.7 nM.

C-10

1,2,4-TRIAZINE NATURAL PRODUCTS. BIOSYNTHESIS INVESTIGATIONS AND GENOME MINING OF AN INTERESTING STRUCTURAL CLASS OF COMPOUNDS.

Khaled H. Almabruk[1], Michael K. Fenwick[2], Brenda T. Shaffer[3], Qing Yang[3], Joyce E. Loper[3], Steven E. Ealick[2], Benjamin Philmus[1].
[1]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA. [2]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA. [3]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA.

Triazine containing natural products constitute a small set of known metabolites including toxoflavin, a virulence factor produced by the plant pathogenic bacterium *Burkholderia glumae* and nostocine A, an alleochemical produced by the cyanobacterium *Nostoc spongiaeforme*. Here we present our lab's findings involving the biosynthetic route used by *Pseudomonas protegens* Pf-5 to form the 1,2,4-triazine ring through an intermediate common to other flavin biosynthetic pathways. By screening microbial genomic sequences available publically, we have also identified multiple biosynthetic gene clusters that have a common core of genes. We postulate that triazines are synthesized via a common intermediate that is then diversified into a larger chemical space than previously appreciated.

C-11

WATER-SOLUBLE CHEMICAL CUES FOR CORAL LARVAE: STRATEGIES FOR ISOLATING WATER-SOLUBLE DRUG LEADS

Skylar Carlson[1], Jennifer Sneed[1], Sarath Gunasekera[1], Danielle L. Dixson[2], Valerie J. Paul[1].
[1]Smithsonian Marine Station, Fort Pierce, FL, 34949, USA, [2]College of Earth, Ocean, and Environment, University of Delaware, Lewes, Delaware, 19716

Chemical ecology is natural products drug discovery in the wild. Looking for molecules responsible for an interaction in the environment has been a challenging application of the natural products chemistry tool kit. Recruitment, settlement, and metamorphosis and the role that small molecule chemical signaling plays in these processes on coral reefs has presented many challenges. The most difficult has been extracting polar molecules from seawater – a complex, salt laden matrix. Coral reefs have been declining rapidly in recent decades. A hallmark of this decline is the shift from coral dominated to more algae and cyanobacterial dominated coral reefs. Interrogating the chemical cues indicative of these distinct habitats has led our interdisciplinary research team to the Mesoamerican barrier reef in Belize. Our understanding of chemical cues driving recruitment has thus far been limited to seawater soaked with individual organisms from the reef. Over the last three years, we have utilized natural product chemistry to analyze the molecular composition of these seawater soaks. From this data, we are working to isolate individual molecules responsible for the attraction of *Porites astreoides* larvae to the crustose coralline algae *Hydrolithon boergesenii*. Polar molecules present a number of challenges; most instrumentation is designed to identify and isolate compounds retained by octadecylsilane (C18) derivatized HPLC columns with a UV chromophore. The molecules we have identified during this project are neither. The techniques presented are a summation of the efforts to identify polar molecules that answer an ecological question but can aid polar drug discovery efforts.

C-12

PYONITRINS A-D: CHIMERIC ANTIFUNGAL AGENTS PRODUCED BY *PSEUDOMONAS PROTEGENS*

Emily Mevers[1], Josep Saurí[2], Eric J. N. Helfrich[1], Matthew Henke[1], Ken Barns[3], Tim S. Bugni[3],
David Andes[4], Cameron R. Currie[5], Jon Clardy[1]. [1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, [2]Structure Elucidation Group, Process and Analytical Research and Development, Merck & Co., Inc., Boston, MA 02115, [3]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin—Madison, Madison, WI 53705, [4]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, [5]Department of Bacteriology, University of Wisconsin—Madison, Madison, WI 53705

Bacterial symbionts frequently provide chemical defenses for their hosts, and such systems can provide discovery pathways to new antibiotics. A recent investigation into an insect-associated *Pseudomonas protegens* strain led to the discovery of a family of small naturally-occurring antifungal agents. Semi-crude mixtures containing these new chimeric metabolites exhibited efficacy in an *in vivo* murine candidiasis model. Bioactivity-guided fractionation led to the pyonitrins, highly complex aromatic metabolites in which ten of the twenty carbons are quaternary, and seven of them are contiguous. The *P. protegens* genome revealed that the production of the pyonitrins is likely the result of a spontaneous reaction between biosynthetic intermediates of two well-studied *Pseudomonas* metabolites, pyochelin and pyrrolnitrin. The combined discovery of the pyonitrins and identification of the responsible biosynthetic gene clusters revealed an unexpected biosynthetic route that would have prevented a potentially useful therapeutic agent from being discovered by bioinformatic analysis alone.

C-13

GENERATION OF MYXOCHELIN-DERIVED LIPOXYGENASE INHIBITORS IN A GENETICALLY MODIFIED *MYXOCOCCUS XANTHUS* STRAIN

Angela Sester[†,‡] Lea Winand,[†] Simona Pace,[§] Oliver Werz,[§] and Markus Nett[†]

[†]Department of Biochemical and Chemical Engineering, TU Dortmund University, 44227 Dortmund, Germany, [‡]Leibniz Institute for Natural Product Research and Infection Biology e.V., 07745 Jena, Germany, [§]Chair of Pharmaceutical and Medicinal Chemistry, Friedrich-Schiller-University, 07743 Jena, Germany

The catechol siderophores myxochelin A and B are potent inhibitors of the enzyme human 5lipoxygenase (5-LO) with IC_{50} values comparable to the FDA-approved anti-asthmatic drug zileuton. Recently, we expanded the myxochelin biosynthetic pathway in Myxococcus xanthus by genetic engineering, so that it gives rise to a derivative featuring an imidazoline moiety. We then attempted to further increase the structural diversity of the myxochelin family by exploiting the substrate promiscuity of the involved pathway enzymes. Feeding studies revealed that selected aryl carboxylic acids can be introduced into the biosynthesis. In total, 14 previously not described analogues were generated and structurally characterized by NMR and MS analyses. A subset of 12 derivatives, which were produced in multi-milligram amounts, were evaluated together with their parental molecules for their inhibitory activity towards human 5-lipoxygenase. In this way, new data on the structure-activity relationship in this natural product family was obtained.

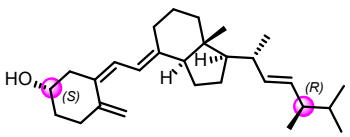
C-14

ANISOTROPIC NMR – A USEFUL TOOL FOR NATURAL PRODUCT STRUCTURE CHARACTERIZATION AND VERIFICATION

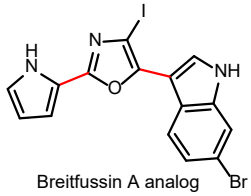
Ikenna E. Ndukwe[1], Yizhou Liu[2], Yu-hong Lam[3], Edward R. Sherer[3], R. Thomas Williamson[1,4], Johan Isaksson[5], Kirk Gustafson[6], Till Maurer[1], Mikhail Reibarkh[1], and Gary E. Martin[1,7]

[1]Analytical Research & Development, Merck & Co., Inc., Rahway, NJ. [2]Analytical Research and Development, Pfizer, CT 06340. [3]Modelling and Informatics, Merck & Co., Inc., Kenilworth, NJ. [4]Current address: Department of Chemistry, University of North Carolina Wilmington, Wilmington, NC 28403. [5]Centre for Research-based Innovation on Marine Bioactivities and Drug Discovery (MABCENT), University of TromsØ, N-9037 TromsØ, Norway. [6]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702-1201. [7]Current address: Department of Chemistry and Biochemistry, Seton Hall University, South Orange, NJ 07079.

Nuclear Magnetic Resonance (NMR) spectroscopy is a very powerful technique for the characterization of natural products; fundamental NMR parameters are sensitive reporters of the nuances of molecular structure. In some cases, molecular scaffolds of natural products can be readily predicted or deduced from a variety of measured NMR data, including chemical shifts and *J*-coupling constants, ^{1}H-^{1}H connectivity networks and heteronuclear one-bond and long-range correlations. However, some structural features such as proton-deficient parts of the molecule, chiral centers separated by flexible linkers and pseudo-enantiomers still represent a significant challenge for conventional NMR methods. Ambiguity of measured NMR parameters in such cases can sometimes lead to incorrectly assigned structures being reported in the literature. Anisotropic NMR, which includes Residual Dipolar Coupling (RDC) and, more recently, Residual Chemical Shift Anisotropy (RCSA), offers the possibility to stem the tide of incorrectly assigned structures being reported in the literature. Recent advances in anisotropic NMR demonstrates its utility both for the orthogonal structure validation and *de novo* stereochemistry determination of molecules. Herein, we explore the utilization of anisotropic NMR for unambiguous characterization of natural products with diverse molecular features. In particular, the relative stereochemistry of two remote stereocenters of vitamin D2, a highly flexible molecule, was unambiguously assigned whereas the planar features of a brietfussin analog was leveraged for the constitutional determination of the proton-deficient central oxazole ring. We also show that in some cases, RCSA data alone, acquired via one-dimensional ^{13}C NMR, provides sufficient molecular differentiation to allow unambiguous characterization of a pair of diastereomers.



Vitamin D2



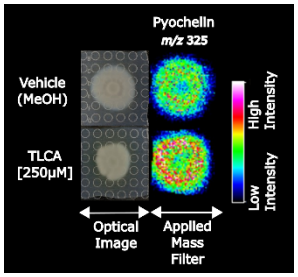
Breitfussin A analog

C-15

BIOFILM INHIBITION ALTERS SPECIALIZED METABOLISM AND INCREASES VIRULENCE

*Alanna Condren[1], Lisa Kahl[2], Manuel Banzhaf[3], Lars Dietrich[2] and Laura Sanchez[1]**

[1]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612. [2]Department of Biological Sciences, Columbia University, New York, NY, 10027. [3]Institute of Microbiology & Infection and School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK.



Taurolithocholic acid (TLCA) was previously shown to have biofilm inhibition activity *in vitro* against two Gram negative bacterial pathogens. We hypothesize that the presence of TLCA induces these pathogens to alter their natural product production, leading to the observed bioactivity. We have investigated the impacts of TLCA on the clinically relevant pathogen, *Pseudomonas aeruginosa* strain PA14. Our studies show that TLCA alters PA14 colony morphology and using imaging mass spectrometry we observed corresponding natural product production confirming our hypothesis. We observed an increase in pyochelin production suggesting that PA14 has increased virulence. A *Galleria mellonella in vivo* model confirmed TLCA causes PA14 to enter a hypervirulent state. Taken in tandem with previous literature, our work promotes that future investigations of biofilm inhibitors must consider how these inhibitors alter the chemical environment within a bacterial biofilm which, in this case, lead to undesired side effects.

C-16

MADBYTE: A STRUCTURE DRIVEN APPROACH TO UNTARGETED NMR METABOLOMICS OF NATURAL PRODUCT EXTRACTS

Joseph M. Egan[1], Roger G. Linington[1]

[1] Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada, V5A 1S6

NMR based metabolomics approaches often focus around biomolecules, comparing known compound spectra against complex mixture spectra to verify or quantify metabolites of interest. These approaches, although important in the study of biological systems, are of limited use in natural prod-

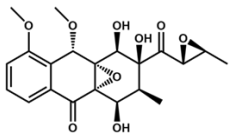
uct drug discovery efforts, where the focus is on the discovery of new molecules. However, the direct structural information afforded from an NMR based approach makes it an attractive compliment to MS based approaches. We have developed the MADByTE (**M**etabolomics **A**nd **D**ereplication **By** **T**wo-dimensional **E**xperiments) platform to utilize features from orthogonal 2D NMR experiments to determine scaffold structure information from multiple metabolites in a complex extract, simultaneously. Comparison of the resulting substructure information across samples from a natural product library offers the opportunity to determine the structure features and distribution of unknown compounds. This allows for prioritization of novel molecules without the need for a database of standards.

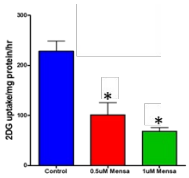
C-17

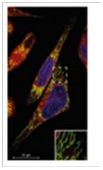
MENSACARCIN INDUCES MITOCHONDRIAL TOXICITY AND INHIBITS GLUCOSE UPTAKE IN MELANOMA

Elizabeth Kaweesa[1], Grace Davis[2], David Brown[2], Sandra Loesgen[1]
[1]Department of Chemistry, Oregon State University, Corvallis, OR 97331, [2]Department of Human Nutrition, Foods, and Exercise, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Mensacarcin is a stereogenic complex polyketide with potent anti-tumor activity produced by a soil-dwelling *Streptomyces bottropensis*. The US National Cancer Institute (NCI) 60 human tumor cell line anticancer drug screen reveals mensacarcin's cytostatic properties in almost all tested cell lines with distinct cytotoxic properties in melanoma cell lines at an average IC_{50} of 0.5 -1μM. Fluorescence studies show that mensacarcin co-localizes in mitochondria and impairs mitochondrial function. Further experiments using the Seahorse XF analyzer show a reduction in acidification rate, an indicator of decreased glycolytic activity in melanoma cells treated with mensacarcin. Next, we were able to verify glucose uptake inhibition in melanoma and a normal muscle cell line. Lastly, *in vitro* combinatorial treatment studies with mensacarcin and vemurafenib (Zelboraf®) exhibits synergistic effects and promisingly, mensacarcin is active in three vemurafenib resistant cell lines with an IC_{50} of 1μM.







C-18

MOLECULAR SIGNATURES FROM ANTS' ECOSYSTEMS - SUSCEPTIBILITY OF FUNGAL GARDENS TO MYCOPARASITES

Andrés Mauricio Caraballo-Rodríguez[1], Kathleen E. Kyle[2], Sara P. Puckett[3], Ricardo R. da Silva[1], Justin J. J. van der Hooft[1,4], Madeleine Ernst[1], Marcy J. Balunas[3], Jonathan L. Klassen[2], Pieter C. Dorrestein[1].
[1]Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA; [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA; [3]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA, [4]Bioinformatics Group, Department of Plant Sciences, Wageningen University, 6708 PB, Wageningen, The Netherlands.

Many species of ants farm fungal gardens as a way to access nutrients they are not able to produce by themselves. Then, any potential threat such as fungal pathogens needs to be controlled. After detecting peptaibols (fungal peptides) from ants' ecosystems we hypothesized that fungal gardens were susceptible to opportunistic pathogens. By setting up experiments inoculating fungal gardens with mycoparasitic strains, we demonstrated the susceptibility of *T. septentrionalis* fungal gardens to *Trichoderma* strains. This susceptibility was stronger in the absence of ants, consistent with previous reports describing fungus grooming and weeding by other attine species to protect their fungus garden from pathogens. Furthermore, the production of peptaibols was successfully reproduced under laboratory conditions by *Trichoderma* strains, implicating peptaibols as potential virulence factors of this mycoparasite. Thus, this investigation confirmed *Trichoderma* fungi as active and virulent mycoparasites in *T. septentrionalis* fungus garden ecosystems. Testing the ability of *T. septentrionalis* to control the pathogen spread and the impact of peptaibols on ants' behavior is in progress. This example nicely illustrates the importance of capturing molecular signatures from natural ecosystems to reveal multipartite interactions that do occur in nature.

C-19

NATURE'S COMBINATORIAL ASSEMBLY-LINE BIOSYNTHESIS PRODUCES VATIAMIDES A-F

Nathan A. Moss[1], Grant Seiler[1,2], Tiago F. Leão[1], Gabriel Castro-Falcón[1], Lena Gerwick[1], Chambers C. Hughes[1], and William H. Gerwick[1,2,3]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, [2]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093

Hybrid type I PKS/NRPS biosynthetic pathways typically proceed in a collinear manner wherein one molecular building block is enzymatically incorporated in a sequence that is coincident with gene arrangement. Here, genome mining combined with use of a fluorogenic azide-based click probe led to the discovery and characterization of vatiamides A-F, three structurally diverse alkynylated lipopeptides and their brominated analogs, from the cyanobacterium *Moorea producens* ASI16Jul14-2. These derive from a unique combinatorial non-collinear 90 kb biosynthetic pathway in which an upstream PKS cassette interacts with three separate cognate NRPS partners. This is facilitated by a series of promiscuous inter-module PKS-NRPS docking motifs possessing identical amino acid sequences. This interaction confers a unique capacity for combinatorial biosynthesis in a microbial system.

C-20

DISCOVERY OF MARINE NATURAL PRODUCTS THAT REDUCE THE LEVELS OF THE NODAL PROTEIN SURVIVIN IN CANCER CELLS

Esther A. Guzmán, Peter J. McCarthy Tara P. Pitts, Jill C. Roberts, Kirstie R. Tandberg, Priscilla L. Winder, and Amy E. Wright
Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946 USA

The nodal protein survivin has been identified as an important target for intervention in a number of cancers including colon, lung and breast cancers. It plays key roles in many cancer supporting processes including: inhibiting apoptosis; supporting mitosis and metastasis; conveying drug and radiation resistance through changes in the DNA repair response; inducing angiogenesis, and maintaining stem cell populations. Survivin has been demonstrated to play a role in the aggressiveness of many cancers and its expression correlates with poor prognosis. A number of approaches to antagonize survivin's multiple functions have been explored including vaccination, use of single amino acid mutants, ribozymes, siRNA and small molecule inhibition. Even with these successes, many have significant clinical drawbacks and there remains a need for additional small molecules that reduce the activity of survivin both for potential therapeutic use and to better understand the biology of survivin. In the current project a high content imaging assay was developed to detect compounds that reduce the levels of

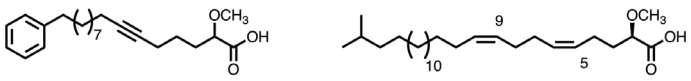
survivin in the A549 non-small-cell lung adenocarcinoma or DLD-1 colon carcinoma cell lines at concentrations that are not cytotoxic. Assay of 3000 fractions from the Harbor Branch Oceanographic Institute (HBOI) Marine Natural Products Peak Library and 100 compounds from the HBOI Pure Compound library identified 7 pure compounds and 173 fractions that reduce levels of survivin in A549 or DLD-1 cancer cells. The presentation will describe the assay, screening results, identification of active compounds and progress towards full characterization of the biological activity of the active compounds.

C-21

NEW INSIGHTS INTO THE BIOLOGICAL ACTIVITY OF MARINE A-METHOXYLATED FATTY ACIDS

Néstor M. Carballeira
Department of Chemistry, University of Puerto Rico, Río Piedras campus, 17 Ave Universidad STE 1701, San Juan, Puerto Rico 00925 (USA)

α-Methoxylated fatty acids are a particularly rare form of lipids that are most frequently found in marine organisms, most notably in sponges and related microorganisms. The role of the α-methoxylation in these lipids is poorly understood, but its presence imparts unusual properties to the fatty acid, which could result in improved biological and/or medicinal properties. For example, changes in pH, increased solubility in water, additional hydrogen bond acceptor (HBA) sites, unusual biophysical properties, changes in the rate of fatty acid metabolism, among others, could make these acids more cytotoxic to cancer cells or even make them better antimicrobial agents. In this presentation, we will survey our most recent results in this field, with emphasis on novel structures characterized by our group from Caribbean sponges. Bioinspired new synthetic analogs were also synthesized with enhanced biomedical properties and these will also be presented. Of importance has been the role of these lipids as topoisomerase IB inhibitors, antimicrobial properties against clinical isolates of multidrug resistant *Staphylococcus aureus* (MDRSA), and toxicity towards the lung A549 cell line and neuroblastomas.



C-22

NCI PROGRAM FOR NATURAL PRODUCT DISCOVERY: PSAMMOCLEMOLIDE, A BIOACTIVE SESTERTERPENE FROM A MARINE SPONGE OF THE CHONDROPSIDAE FAMILY

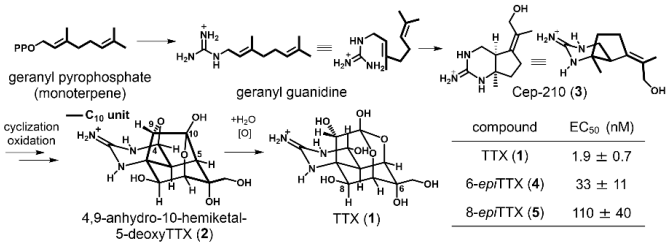
Christopher C. Thornburg[1], Tanja Grkovic[1], Jason R. Evans[2], John R. Britt[1], Michelle Ahalt-Gottholm[3], Melinda G. Hollingshead[3], David J. Newman[4], Jerry M. Collins[4], and Barry R. O'Keefe[4].
[1]*Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, Maryland 21702.* [2]*Data Management Services, Inc., Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, Maryland 21702.* [3]*Biological Testing Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702.* [4]*Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702.*

The NCI Program for Natural Product Discovery (NPNPD) is a newly launched, national program to advance natural product discovery technologies and facilitate the discovery of structurally defined, validated lead molecules ready for translation. In this work, self-organizing map (SOM) technology was used to explore the biological space of crude natural product extracts tested in the NCI-60 human tumor cell lines screen. This bioinformatics-guided approach aided the prioritization of chemistry efforts and led to the identification of several distinctive regions on the NCI-60 SOM that were selected for further evaluation in the NCI in vivo hollow fiber assay against a panel of twelve tumor cell lines. Notably, an organic solvent extract of the marine sponge *Psammoclema* sp. collected near the island of Mauritius showed efficacy in the hollow fiber assay against LOX IMVI (melanoma), NCI-H23 (non-small cell lung), OVCAR-3 (ovarian) and SF-295 (CNS) tumor cells. Bioassay-guided fractionation of the *Psammoclema* sp. extract led to the identification a new sesterterpene, psammoclemolide (**1**), containing a γ-hydroxybutenolide moiety and a β-hydroxybutyrl group. Psammoclemolide exhibited potent antiproliferative activity against tumor cells in the NCI-60 screen (GI_{50} = 0.66 μM, TGI = 2.0 μM, LC_{50} = 7.2 μM) and is currently being evaluated for possible antitiumor efficacy. Overall, this project demonstrates that the integrated processes described here can facilitate drug discovery by providing a platform to rapidly process extracts, analyze screening data, isolate and characterize active molecules.

C-23

IDENTIFICATION OF NEW ANALOGS AND PUTATIVE BIOSYNTHETIC INTERMEDIATES OF TETRODOTOXIN AIMED AT ELUCIDATING ITS BIOSYNTHETIC PATHWAY AND STRUCTURE ACTIVITY RELATIONSHIP

Yuta Kudo[1,2] *and Mari Yotsu-Yamashita*[2]
[1]*Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, 6-3 Aramaki-Aza-Aoba, Aoba-ku, Sendai, Miyagi 980-8578, Japan.* [2]*Graduate School of Agricultural Science, Tohoku University, 468-1 Aramaki-Aza-Aoba, Aoba-ku, Sendai, Miyagi 980-8572, Japan.*



Tetrodotoxin (TTX; **1**), a potent neurotoxin, blocks voltage-gated sodium ion channels (Na_v). TTX has been found in various marine and terrestrial animals, but its biosynthesis is still unknown. Identification of novel TTX analogs would be significance in elucidation of its biosynthesis and structure activity relationship. With our LC-MS guided screening strategy, we discovered TTX analogs and putative biosynthetic intermediates (such as **2** and **3**) from the toxic newts, suggesting a monoterpene origin of TTX in terrestrial. Also, the Na_v blocking activities of TTX epimers (**4** and a new analog **5**) were examined.

C-24

DISCOVERY, TOTAL SYNTHESIS AND SAR OF A NOVEL QUORUM SENSING SIGNALING MOLECULE FROM A MARINE CYANOBACTERIUM

Xiao Liang[1,2] *Susan Matthew,*[1] *Qi-Yin Chen,*[1,2] *Jason C. Kwan,*[1] *Valerie J. Paul*[3] *and Hendrik Luesch*[*,1,2]
[1]*Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610, USA,* [2]*Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville, Florida 32610, USA,* [3]*Smithsonian Marine Station, Fort Pierce, Florida 34949, USA*

Quorum sensing (QS) is a means of intercellular communication adopted by different bacterial species to coordinate bacterial behavior, which is mediated by diffusible signaling molecules named autoinducers. Many clinically relevant pathogenic bacteria use QS to regulate biological processes

associated with virulence. Therefore, there has been a significant interest in developing modulators of QS to manipulate bacterial behavior that is responsible for its interactions with the host.

Marine cyanobacteria have been a valuable source for the discovery of natural products with novel structures and unique modes of action. A novel compound named doscadenamide with unique structural skeleton was isolated from the marine cyanobacterium *Moorea bouillonii* and its structure was elucidated using a combination of 1D and 2D NMR techniques. The total synthesis of doscadenamide was developed to provide sufficient material for thorough biological investigation as well as to allow for the generation of a focused library for structure–activity relationship study. Moreover, the configuration of doscadenamide was validated by comparison of the NMR spectra of four synthetic diastereomers and the natural product. Our preliminary biological investigation revealed that doscadenamide exhibits QS activating activity, which could be a starting point to develop unique superagonists to modulate bacterial virulence related signaling process. Doscadenamide and its analogs or derivatives could represent a new class of chemical probes to understand QS and its role in host/bacteria interactions.

Poster Presentations

P-001

IDENTIFICATION OF UNIQUE BACTERIAL METABOLITES THROUGH MULTIVARIATE STATISTICAL ANALYSIS.

Ali Shahbandi[1], Derick D. Jones Jr.[1], Daniel A. Todd[1], Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, NC 27402

Identification of unique bacterial metabolites is often hindered by the complexities of the nutrient rich growth media. Liquid chromatography coupled to mass spectrometry is an excellent tool for detecting secondary metabolites, however the most abundant peaks on the chromatographic profiles are often ions from the complex growth media or commonly produced metabolites. In this study, we used multivariate statistical analysis to identify unique metabolites for 11 different strains of bacteria including: Enterococcus faecalis, Listeria monocytogenes, Staphylococcus lugdunensis, Staphylococcus saprophyticus, 3 strains of Staphylococcus epidermidis, and 3 strains of Staphylococcus aureus. The ultimate goal of this study is to develop a library of unique metabolites that can be used for bioassay development, drug discovery, or for a quick species identification.

P-002

SOLUBILIZATION OF POLYSACCHARIDE AND FUNCTIONAL COMPONENTS BY ENZYME TREATMENT FROM *PLATYCODON GRANDIFLORUM* AND *CODONOPSIS LANCEOLATA*

*Dong-Geon Nam, Mina Kim, Pereum Im, Jeong-sook Choe, Ae-Jin Choi**
Division of Functional Food & Nutrition, Department of Agrofood Resources, National Institute of Agricultural Sciences, Wanju, 55365, Republic of Korea

The objectives of this study were to characterize the physicochemical properties of *Platycodon grandiflorum* (Korean name, Doragi) and *Codonopsis lanceolata* (Korean name, Deoduk), and to optimize condition of extract processing for increasing the solubilization efficiency of saponins and polysaccharides disintegration. The effect of enzyme treatment depending on sample types was significantly higher in the hot-air dried powder (HDP) than freeze-dried powder (FDP). The optimum condition of enzyme treatment was with Pectinex Ultra SP-L + Celluclast 1.5L + Viscozyme L (PCV) at 50℃ for 2 h to solubilize cell wall, and then with α-amylase (Termamyl 120L) at 93℃ for 2 h to disintegrate starch. In HDP group, water soluble indexes of *Platycodon grandiflorum* and *Codonopsis lanceolata* treated with PCV increased 1.4 and 1.6 times more compared to control (CON, 50.53%, 41.41%) while total flavonoids contents increased 1.7 and 7 times compared to CON (0.24%, 0.02%). The total contents of indicator components such as Platycodin D, Polygalacin, and Platyconic acid A from *Platycodon grandiflorum* was 1.02%, increasing 1.3 times more compared to CON (0.76%) with a significant difference ($p<0.001$).

P-003

SMALL MOLECULE ACCURATE RECOGNITION TECHNOLOGY (SMART) TO DENOISE 2D NMR SPECTRA OF NATURAL PRODUCTS

Chen Zhang[1], Shilin Zhu[2], Raphael Reher[1], Brendan Duggan[3], Kelsey Alexander[1], Garrison W. Cottrell[2], William H. Gerwick[1,3]
[1]Scripps Institution of Oceanography, [2]Department of Computer Sciences and Engineering, [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093, USA

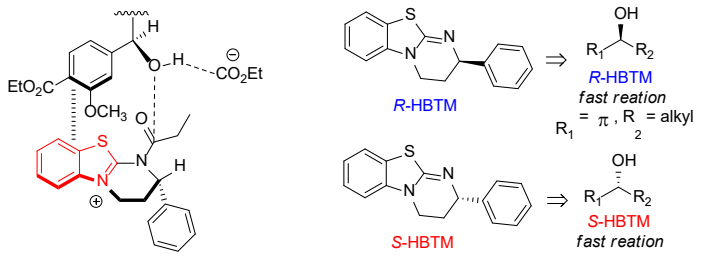
We present the 2D NMR spectra denoising function of the Small Molecule Accurate Recognition Technology (SMART), a system that integrates the benefits of 2D NMR with advances in deep learning to enhance and improve the efficiency of natural products discovery. This tool is highly effective in both assisting natural products discovery efforts even in presence of spectral noise. To effectively achieve this goal, we first generated 2D spectra white noise using a program that was previously used to simulate noise in electrical circuits. Next, a deep Convolutional Neural Network (CNN) with contrastive loss was trained on a dataset containing over 20,000 noisy HSQC spectra as the training set. This resulted in a fast convergence of the training result. To demonstrate the denoising function of SMART, noisy HSQC spectra of several newly isolated compounds of scarce amount were denoised with signals preserved, thereby facilitate the structural elucidation for natural products of low quantity. In addition, because other types of noise, impurities, or solvent effects are often seen in experimental HSQC spectra, we investigated the robustness of the SMART to recognize HSQC spectra in the presence of other types of noise or artifacts.

P-004

SENSITIVE, SIMPLE, AND COST/TIME-EFFECTIVE METHOD TO DETERMINE THE ABSOLUTE CONFIGURATION OF A SECONDARY ALCOHOL USING COMPETING ENANTIOSELECTIVE ACYLATION COUPLED WITH LC/MS

Seoung Rak Lee, Jae Sik Yu, Seulah Lee, Mun Seok, Jo, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

We present a simple, optimized, new chemical derivative method that utilizes competing enantioselective acylation followed by LC/MS analysis, and demonstrate its successful application to determine the absolute configuration of a secondary alcohol in natural products with multiple reactive functional groups. This new development relies on the enantiomeric pair of the HBTM (homobenzotetramisole) catalysts exhibiting adequate kinetic resolution for acylation of secondary alcohol, and then fast reaction was quantitatively confirmed via LC/MS as the characterization technique for the enantioselective transformations.



P-005

PHENOTYPIC SCREENING. OF ACTINOMYCETE CULTURE EXTRACTS.AS AN IMPORTANT FACTOR IN ANTI-*MYCOBACTERIUM TUBERCULOSIS* DRUG DISCOVERY.

*Edyta M. Grzelak[a], Wei Gao[a,b], Mary P. Choules[a,b], Geping Cai[a,b], Baojie Wan[a], Yuehong Wang[a], James B. McAlpine[a,b], Jinhua Cheng[d], Hanki Lee[d], Joo-Won Suh[c,d], Guido F. Pauli[a,b], Scott G. Franzblau[a], Sang-Hyun Cho[a,b] and Birgit U. Jaki[a,b]**

*Institute for Tuberculosis Research[a] and Dept. of Med. Chem. & Pharmacognosy[b], College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA; Center for Nutraceutical and Pharmaceutical Materials[c] and Div. of Bioscience and Bioinformatics, College of Natural Science[d], Myongji University, Cheoin-gu, Gyeonggi-do, Republic of Korea *Address correspondence to Birgit U. Jaki, bjaki@uic.edu*

Slow growth and safety limitations of *Mycobacterium tb* (*M. tb*) were the reason that whole-cell phenotypic screening campaigns for tuberculosis (TB) drug discovery were predominantly performed with the surrogate strains, *M. bovis* (BCG) or *M. smegmatis*. Thus, it can be assumed that many potentially useful TB drug leads could have been missed.

The essential part of a TB drug discovery platform established at the UIC Institute for Tuberculosis Research (ITR) is the direct high-throughput screening against virulent *M. tb* H37Rv using a luxABCDE reporter. The hits selected in primary screening are further subjected to extensive biological and chemical profiling to facilitate early dereplication and prioritization for bioassay-guided fractionation. The strategy for finding new molecules with significant anti-*M. tb* activity includes the development of biological assays, as well as innovations in isolation and identification of the active principles.

The workflow will be presented on the example of a whole-cell phenotypic high-throughput screening of a library containing 200,000 soil-derived actinomycete culture extracts that led to the discovery of the new anti-*M. tb* cyclic peptide, ecumicin.

P-006

TARGETED SEPARATION OF ANTI-*MYCOBACTERIUM TUBERCULOSIS* COMPOUNDS FROM ACTINOMYCETE EXTRACTS

Edyta M. Grzelak[1], Yang Liu[2], J. Brent Friesen[1,2], David C. Lankin[2], Dejan Nikolic[2], Shao-Nong Chen[1,2], James B. McAlpine[1,2], Joo-Won Suh[3,4], Seung Hwan Yang[3], Jinhua Cheng[3], Hanki Lee[3], Jin-Yong Kim[4], Sang-Hyun Cho[1], Guido F. Pauli[1,2], Scott G. Franzblau[1], and Birgit U. Jaki[1,2]

*[1]Inst. for TB Research, [2]Dept. Med. Chem. & Pharmacognosy, COP, UIC, Chicago, IL, 60612, [3]Center for Nutraceutical & Pharmaceutical Materials, [4]Div. of Biosciences & Bioinformatics, Col. Nat. Sci., Myongji University, Gyeonggi-Do 449-728, Republic of Korea *Address correspondence to Birgit U. Jaki, bjaki@uic.edu*

The reported new strategy involves countercurrent separation (CCS) of compounds with anti-*Mycobacterium tuberculosis* (*M. tb*) activity from actinomycete culture extracts. It applies TLC-based bioautography[1] and the GUESS[2] method for the selection of the optimal CCS solvent system. The two major advantages are: (i) the search for bioactives is accomplished without the need for reference or known compounds; this establishes a chemically untargeted and biologically fully targeted approach. (ii) CCS can be performed directly without the need for multiple partitioning experiments and *in vitro* assays. The selected optimal CCS solvent system accelerates the CCS procedure, because it is based exclusively on the "K value sweet spot" of the active compounds. Results from the ethyl acetate actinomycete extract from *Streptomyces* sp will be presented to demonstrate the methodology.

References

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2. Friesen & Pauli *J Liq Chromatogr Rel Technol.* 28, 2777-2806 (2005)

P-007

ANTI-INFLAMMATORY AND CARBOLYTIC ENZYMES INHIBITORY ACTIVITIES OF MONOGLYCOSIDE CUCURBITANE-TYPE TRITERPENES FROM *MOMORDICA CHARANTIA*

Wilmer H. Perera[1], Siddanagouda R. Shivanagoudra[1], Jose L Perez[1], Yuxiang Sun[2], G.K. Jayaprakasha[1,], Bhimanagouda S. Patil[1,*]*

[1] Vegetable and Fruit Improvement Center, Department of Horticultural Sciences, Texas A&M University, 1500 Research Parkway, Suite A120, College Station, TX 77845

[2] Department of Nutrition and Food Sciences, Texas A&M University, College Station, TX 77845

Bitter melon belongs to the Cucurbitaceae family, and various parts of the plant species have been extensively used in developing countries as a traditional remedy to treat diabetes mellitus. Several *in vitro* and *in vivo* studies have shown that extracts of bitter melons have potential anti-diabetic properties. Bitter melon has received much attention due to its cucurbitane-type triterpenoids and their various biological activities. In the present study, 23-O-β-D-allopyranosyl-5β,19-epoxycucurbitane-6,24-diene, karaviloside VI, karaviloside VIII, 25ξ-isopropenylchole-5(6)-ene-3-O-β-D-glucopyranoside, momordicoside A, momordicoside L and kuguaglycoside C were isolated from the acetone crude extract of Indian cultivar of *Momordica charantia*. Chemical structures were determined based on comprehensive HRESIMS, 1D and 2 D NMR experiments (DQCOSY, TOCSY, HMQC, HMBC, and NOESY). All compounds exhibited differential inhibition of α-amylase and α-glucosidase comparable to acarbose. The structure-activity relationship was established using molecular docking studies, where purified compounds were able to bind to the active sites of the proteins. Additionally, the purified compounds showed differential suppression of pro-inflammatory and anti-inflammatory genes in lipopolysaccharide-activated macrophage RAW 264.7 cells.

P-008

QUALITY EVALUATION OF DOPING TARGET CRUDE DRUGS BY LC-TOF/MS

Tadahiro Yahagi[1], Toshiyuki Atsumi[2], Junya Watanabe[1], Megumi Furukawa[1], Zhigang Yang[3], Keiichi Matsuzaki[1]

[1]Laboratory of Pharmacognosy, School of Pharmacy, NIHON University, 7-7-1 Narashinodai, Funabashi, Chiba 2748555, Japan, [2]Laboratory of Pharmacognosy, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1, Yoshino, Nobeoka, Miyazaki 8828508, Japan, [3]School of Pharmacy, Lanzhou University, 199 West DonggangRoad, Lanzhou, Gansu 730020, P.R.CHINA

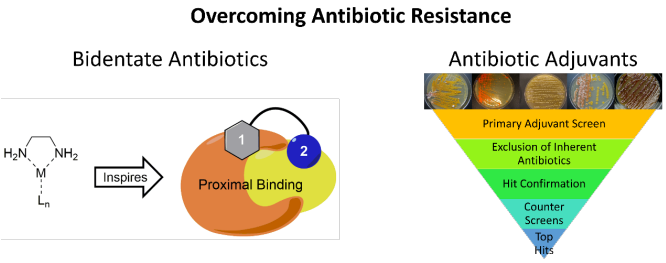
As we move toward the Tokyo Olympics in 2020, there is a growing awareness of anti-doping. Some of the crude drugs used in Kampo medicine are those containing a doping component, such as ephedrine and higenamine. Ephedrine and higenamine are classified as S6 STIMULANTS and S3 BETA-2 AGONISTS respectively, and are prohibited list by the World Anti-Doping Agency. In textbooks and the internet, Pinellia Tuber has been reported to contain ephedrine and Clove to contain higenamine. However, there is little evidence to support this information, and questions remain about the biosynthetic interpretation. In this study, we analyzed the doping target crude drugs by LC-TOF/MS monitoring: ephedrine (*m/z* 148 $[M+H-H_2O]^+$) and higenamine (*m/z* 272 $[M+H]^+$). As a result, ephedrine wasn't detected in Pinellia Tubar (n=56, Detection limit of ephedrine: 0.5 ppb). Similarly, higenamine wasn't detected in Clove (n=23, Detection limit of higenamine: 0.5 ppb). These results suggested that Pinellia Tubar and Clove may contain no doping component.

P-009

OTHER OPTIONS TO OVERCOME RESISTANT BACTERIA: BIDENTATE ANTIBIOTICS AND SCREENS FOR ANTIBIOTIC ADJUVANTS

Andrew N. Lowell[1], Zachary A. Kohanov[1], Harrison O. Miller[1], and Jacob C. Chappell[1]*
[1]Department of Chemistry, Virginia Tech (Virginia Polytechnic Institute & State University), Blacksburg, VA 24061.

Approaches that can augment existing treatments for infection are a compelling avenue of exploration in antibiotic development. Toward that end, we are undertaking a multipronged strategy to create bidentate antibiotics (an advanced iteration of hybrid antibiotics) as well as adapting a phenotypic screen to identify antibiotic adjuvants from natural product sources. Medicinal chemistry studies to derivatize potential parent antibiotics will be presented as precursors to formal creation of a covalently linked bidentate antibiotic. Progress towards optimization of a multicomponent transcription/translation-based screen for adjuvants will be discussed.

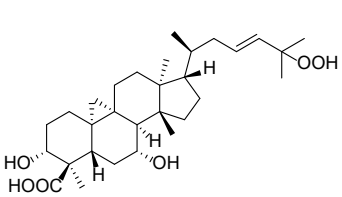


P-010

HYDROPEROXY-CYCLOARTANE TRITERPENOIDS FROM THE LEAVES OF *CASTANEA SATIVA* INHIBIT QUORUM SENSING IN *STAPHYLOCOCCUS AUREUS*

Akram M. Salam[1†], Gina Porras-Brenes[2†], James T. Lyles[2], John Bacsa[3], Cassandra L. Quave[2]
[1]Program in Molecular and Systems Pharmacology, Laney Graduate School, Emory University, Atlanta, Georgia, USA; [2]Center for the Study of Human Health, Emory University, Atlanta, GA; [3]Emory X-ray Crystallography Center, Atlanta, GA; †Co-first authors.

Methicillin-resistant *Staphylococcus aureus* (MRSA) presents one of the most serious infectious disease concerns worldwide. This bacterium exerts its pathogenicity via virulence factors, the production of which is regulated by quorum sensing (QS) through the Agr system. From an enriched extract of *Castanea sativa* leaves that inhibits QS, reverse-phase HPLC was used to isolate two novel hydroperoxy-cycloartane triterpenoids, **1** and **2**. Their structures were confirmed by X-ray, NMR and MS. Both cycloartanes demonstrate evidence of inhibiting the Agr system, confirmed by a reduction in *agr* expression in a reporter strain assay (IC_{50} = 16 μM) and reduction of δ-toxin outputs.



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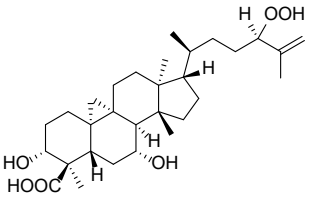


2

P-011

METABOLIC PROFILING OF ACTINOBACTERIAL ISOLATE FROM NEPAL

Niraj Aryal[1], Prajwal Rajbhandari[2], Harald Gross[1]
[1]University of Tübingen, Pharmaceutical Institute, Department of Pharmaceutical Biology, 72076, Tübingen, Germany
[2]Research Institute of Bioscience and Biotechnology (RIBB), Nakkhu-4, Lalitpur, Nepal

Actinomyces are known for producing diverse secondary metabolites. Here we attempt the screening of actinomycetes from unique ecological niches and high altitudes of Nepal followed by their chemical profiling.

Despite of genomic data accumulation, identification of molecules remains a central question in analytical chemistry, in particular for natural product research and untargeted metabolomics. In our work, investigations of secreted metabolites of *Streptomyces* WL006 and compound annotation using molecular networking and SIRUS 4 platform are presented. We have identified several compound class and potentially new derivatives.

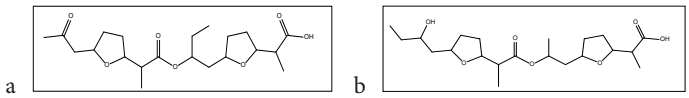


Fig 1: (a) Bonactin and its (b)New Bonactin derivative

P-012

DISCOVERY OF BOTANICAL NATURAL PRODUCTS TO COMBAT THE EMERGING MULTIDRUG-RESISTANT FUNGAL PATHOGEN *CANDIDA AURIS*

François Chassagne[1], Gina Porras-Brenes[1], James T. Lyles[1], Kate Nelson[2], Joan Shang[1], Cassandra L. Quave[1,2]
[1]Center for the Study of Human Health, Emory University, Atlanta, GA 30322, USA. [2]Department of Dermatology, Emory University, Atlanta, GA 30322, USA.

Candida auris is an emerging multidrug-resistant (MDR) fungal pathogen with mortality rates >35% for invasive infections. Although just discovered in 2009, *C. auris* has become an emerging global threat. Clinicians have documented strains with resistance to all three classes of antifungal drugs. We screened the Quave Natural Products Library (QNPL), which is composed of > 1,900 extracts from > 600 botanical species used in traditional medicine for infectious and inflammatory disease, in a tiered approach to identify novel anti-*Candida* leads. We first explored the QNPL at 16 μg/mL for growth inhibitory activity by broth microtiter assay against *C. albicans* and identified extract 429 as a potent inhibitor. The extract underwent liquid-liquid partitioning and fractionation by reverse-phase HPLC. Follow-up dose-response tests of extract 429 and its fractions against MDR *C. albicans* and non-albicans *Candida* (NAC) species obtained from the CDC Antibiotic Resistance Isolate Bank, including *C. auris*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* revealed MICs ≤ 2 μg/mL; the range of activity against *C. auris* isolates was from MIC of 0.125 - 2 μg/mL. Cytotoxicity was assessed against human keratinocytes (HaCaTs) by LDH assay (IC_{50} > 32 μg/mL), yielding a therapeutic index > 250. The chemical characterization of the most active fractions and their respective biological data will be presented.

P-013

COMMERCIAL QUANTITIES OF THE CYTOTOXIC COMPOUND, CYTOCHALASIN D FROM STATIC CULTURES OF A GROUP OF ENDOPHYTIC FUNGI

Emmanuel K. Oppong
Department of Chemistry Education, University of Education Winneba, P. O. Box 25, Winneba, Ghana

Cytochalasin D is a cytotoxic compound used in cellular research and drug development. It inhibits actin polymerization and induces depolymerisation of actin filaments formed during platelet shape change. Cytochalasin D is produced from molds often in mixtures with other cytochalasins. The similarity in structures makes it difficult to purify. Yields from the synthesis of the compound is not economical, hence the high cost of the cytotoxic compound. Static cultures of a group of endophytic fungi: RAR 5-6, XMR 12-17, RAJ and XGR 12-5 collected from Thailand were sub-cultured in 250 ml conical flask each for three weeks. All the fungi developed fruiting bodies. The cultures in the conical flasks ware each transferred into ten 2.0 l Flat bottomed flask bottles and allowed to grow for 8 weeks. The yields obtained were 6.51, 6.30, 3.65 and 5.32 g for RAR 5-6, XMR 12-71, RAJ 17-32 and XGR 12-5 respectively. TLC studies of the different crude extracts indicated that the four endophytic fungi growing in culture medium produced the same compounds. The crude extract obtained from RAR 5-6 was dissolved in warm ethyl acetate and left overnight. A white solid (0.90 g) precipitated from the solution. The solid was removed by filtration and recrystallized from the same solvent to give cytochalasin D, white needles (720 mg) mp (266-267 °C) (lit.9, 267 °C) m/z 507 (M+) $[\alpha]_D^{23}$ -13.5° (c =1.0, in dioxane). The production of pure cytochalasin D from these endophytic fungi could be a source of cheap commercial quantities of this important cytotoxic compound to enhance cellular research.

P-014

ANTIBIOTICS FROM ANTS: MOLECULAR DEFENSES FROM SPECIALIZED INSECT MICROBIOMES

Preston Chang, Krithika Rao, Rose Kim, Lauren Longo, Elisabeth Lawton, Ananya Koneti, Keon Rabbani, and Ethan Van Arnam.
Keck Science Department of Claremont McKenna, Pitzer, and Scripps Colleges; Claremont, CA

Defensive symbioses are ecological partnerships built around a type of pharmaceutical exchange; a host animal is protected by antibiotics or other defensive molecules produced by symbiotic microbes. Now appreciated to be widespread in nature, defensive symbioses are largely untapped sources of novel bioactive molecules with therapeutic potential for human use and are ideal systems to understand antibiotic use in nature. Toward these aims of antibiotic discovery and contextualizing ecological antibiotic use, we have characterized the molecular defenses from symbiotic bacteria associated with North American *Trachymyrmex* fungus-growing ants. Across four ant species, we recovered Actinobacteria with antibacterial and antifungal activity against ecologically-relevant pathogens. Activity-guided fractionation revealed two active antibiotics. One is a putatively-novel antibiotic produced by symbiotic bacteria recovered from multiple colonies of the desert-inhabiting New Mexican ant *Trachymyrmex smithi*. A different molecule, the thiopeptide antibiotic GE37468, is deployed by bacteria from *Trachymyrmex septentrionalis* ants on Long Island, New York at the far northern limit of their range. Intriguingly, this molecule closely resembles thiopeptide antibiotics produced by other animal symbionts, including members of the human microbiome. These thiopeptides may comprise a privileged class of host-compatible defensive molecules, and as such, have encouraging therapeutic potential.

P-015

BIOSYNTHETIC MECHANISM OF THE ANTIBIOTIC CAPURAMYCIN

Erfu Yan, Steven Van Lanen[1]
[1]College of Pharmacy, University of Kentucky, Lexington, KY, USA

Purpose:
Natural products have played an important role in the discovery of antibacterial agents since the introduction of penicillin in the 1940s. Until 2012, natural products or derivatives of natural products contributed about 75% of the total FDA-approved antibacterial agents. However, the discovery of novel antibiotics has dramatically decreased over the last few decades while infectious disease, and notably tuberculosis (TB), remains a major threat to global health. Thus, the discovery and development of new antibiotics are urgently needed. Capuramycin, was a kind of nucleoside antibiotics discovered in screening programs for new antibiotics in 1980s. Capuramycin-type antibiotics include A-500359s, A-503083s, and A-102395. The biosynthetic gene cluster and pathway for A-500359 and A-503083 have been identified and characterized. But the biosynthesis mechanism for A-102395 has not been fully resolved. The function of several of the gene products is difficult to predict based solely on sequence analysis, which is perhaps not unexpected since the structure of the capuramycin family of antibiotics consists of several novel chemical features. In this study, we have been finished the process of identifying several gene products CPR36, and 37, 38 and 12, which act as the function of aryl carrier protein, actinomycin synthetase, 4-amino-4-deoxychorismate synthase and ADC lyase, respectively. These key proteins initiate and participate the biosynthesis of an aniline-containing component of A-102395. LC-MS analysis, protein mass spectrometry, native protein electrophoresis, PPi exchanging assay, and malachite green assay were applied for product identification and characterization and the enzymes functionality.

Methods:
We have been finished the process of identifying several gene products with unknown function. As a beginning, we have cloned and expressed a bunch of genes such as SVP, CPR36, and 37, which are proposed to be a snake venom phosphodiesterase, Aryl carrier protein, actinomycin synthetase I respectively. It is expected that these key proteins initiate and participate the biosynthesis of an aniline-containing component of A-102395. LC-MS analysis, protein mass spectrometry, native protein electrophoresis, PPi exchanging assay, and malachite green assay were applied for product identification and characterization and the enzymes functionality.

Results:
In a traditional NRPS pathway, the chemistry is normally that the A domains catalyze a two-step, ATP-dependent reaction that involves the activation of the carboxylate group of the amino acid substrate to form an aminoacyl-AMP intermediate and then the transfer of the amino acid to the Phosphopantetheine arm of the neighboring thiolation domain. In our research, we proposed cpr37 is adelation domain protein based on the bioinformatics analysis. It initiates the paba and ATP to form a paba-AMP intermediate, meanwhile, the carrier protein is catalyzed to add the Phosphopantetheine arm of the CoA to its serine residue with the functionality of snake venom phosphodiesterase.

The high-resolution measurements of the masses of substrates and intermediates bound to phosphopantetheinylated (holo) carrier proteins was achieved by using protein mass spectrometry. Also, native protein electrophoresis also confirmed that there exist two different conformations between the holo and apo form of acyl carrier protein. The activity of the Cpr37 was tested with the 20 proteinogenic amino acids using the carboxylic acid–dependent ATP-[32]PPi exchange assay. Several amino acids were activated, however, the preferred substrate was L-lysine. This data consistent with the H^3 labeled PABA accumulation data which shows a steady amount increase of PABA as reaction time.

Conclusion:
In summary, we have established the biosynthetic gene cluster for cpr36 was identified aryl carrier protein. SVP act as a phosphodiesterase to transform

cpr36 to another active holo form of carrier protein. Finally, cpr37 worked as adelation domain to load PABA to the carrier protein, which is a critical advancement for interrogating the biosynthesis of the unusual chemical components of this family of antibiotics.

Methods:

1 Cloning of the A-102395 Gene Cluster—Amycolatopsis sp. SANK 60206 genomic DNA was partially digested with Sau3AI to give DNA fragments that were dephosphorylated with bacterial alkaline phosphatase and ligated into BamHI-digested cosmid vector SuperCos1 (Stratagene, Cedar Creek, TX). The ligation products were packaged with Gigapack III Gold packaging extract as described by the manufacturer (Stratagene), and the resulting recombinant phage was used to transfect Escherichia coli XL-1 Blue MR.

Cloning, Overexpression and Purification of Capuramycin Genes

The cpr36, cpr37, svp genes were amplified from SANK 60206 genomic DNA. The primers used for PCR were designed to introduce NdeI and BamHI restriction sites into the PCR products. PCR products from reactions using Vent DNA polymerase were subsequently incubated with Taq DNA polymerase and cloned into the pCR2.1 TA-vector from Invitrogen. Clones containing inserts were isolated by blue-white screening and subcloned into the pET3a vector (Novagen) using the NdeI and BamHI restriction sites. The genes were subsequently subcloned using the NdeI and BamHI restriction sites into the Pet30a vector (Novagen) for expression as a 6×His-tagged fusion. The downstream expression and purification of protein were applied with Q-Sepharose Fast Flow column and followed by manufacturer's instructruction (Pharmacia).

HPLC and Maldi Mass Spectrum Analysis

HPLC and Maldi Mass Spectrum were performed according to manufacturer's procedure. A 5 µm Alltech Alltima (4.6 mm × 150 mm) column was used to separate reaction mixtures on an Agilent 1100 HPLC system. Isocratic elution with 5% acetic acid in water was employed, and the absorbance at 220 nm was monitored. Peaks were identified by comparing retention times and m/z values for molecule weight.

Ppi assay

Add all reaction ingredients as following 5 mM sodium [32P]pyrophosphate , 100 mM TES, 25 mM $MgSO_4$, 10 mM DTT,10 mM ATP into 1.5 ml Eppendorf tubes, and different amino acids with the concentration of 5 mM. After running the reaction at 30 or 37 C in a water bath, for 20 to 30 min. Stop the reaction by adding 0.5 ml of charcoal suspension. Centrifuge at 15000rpm for 5min and suck out the liquid.

Carboxylic acid-dependent ATP-[32P]PPi exchange assays. A 100 lL reaction contained 50 mM Tris-HCl, pH 8, 5 mM MgCl2, 0.2 mM b-mercaptoethanol, 2 mM ATP, 0.05 mM PPi containing 1 3 106 dpm of [32P]PPi (Perkin-Elmer),10 Nm Cpr37 enzyme, and the indicated amino acid or carboxylic acid substrate. Reactions were performed at 308C for 20 min and terminated by the addition of 5 volumes cold 1% (w/v) activated charcoal and 4.5% (w/v) tetrasodium pyrophosphate in 7% (v/v)perchloric acid. Precipitate was collected with glass fiber filtersunder vacuum and washed consecutively with 40 mM sodium pyrophosphate in 1.4% (v/v) perchloric acid (400 mL), water (400 mL), and ethanol (200 mL). Filter papers were added to 10 mL scintillation fluid and radioactivity quantified. Data are reported as the average of three reactions.

P-016

A CUSTOM, REUSABLE 3D-PRINTED BIOASSAY PLATE FOR THE DISCOVERY OF ANTIBIOTICS FROM MICROORGANISMS UNDER MULTIPLE CULTIVATION CONDITIONS.

Jeongho Lee[1], Rui Ma[2], Linh Nguyen[1], Sanghyun Cho[2], Scott G. Franzblau[2], and Brian T. Murphy[1]

[1]Department of Medical Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607, USA, [2]Institute for Tuberculosis Research, University of Illinois at Chicago, Chicago, IL 60607

OSMAC (one strain-many compounds) is a well-known approach used to trigger the upregulation of microbial natural product biosynthetic gene clusters (BGCs) via multiple cultivation parameters. However, since academic research programs often lack the resources of their industrial counterparts – automation, personnel, capital – this approach has been somewhat underutilized. Here we report the design of a customized, 3D-printed multi-well bioassay plate. This plate is autoclavable and therefore reusable, and utilizes opposing sides of a single agar plug to allow a test organism to combat a growing pathogen, avoiding technical complications that are associated with plating both microorganisms on the same surface. As a result, growth inhibition analysis is more reliable. This method can be used with a diverse range of microorganisms including fast/slow-growing bacteria or fungi. To evaluate this method, five antibiotic-producing ATCC strains were tested against *P. aeruginosa* GFP (ATCC 10145GFP) and results were compared with a traditional agar overlay method. Additionally, 52 actinomycetes were tested in up to 18 cultivation conditions and 8 strains exhibited inhibition against *P. aeruginosa*. Results from this process, including ongoing methodological challenges, will be discussed.

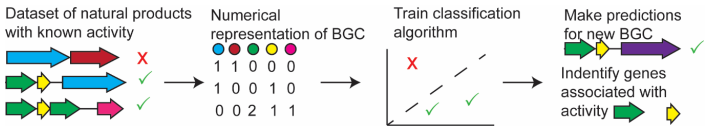
P-017

A MACHINE LEARNING BIOINFORMATICS TOOL FOR PREDICTING NATURAL PRODUCT BIOACTIVITY

Allison Walker[1], Jon Clardy[1]

[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA.

We developed a machine learning-based bioinformatics tool that predicts natural product bioactivity using the natural product's biosynthetic gene cluster. We trained three commonly used classifiers, logistic regression, support vector machines, and random forests, on binary classification problems to predict the presence or absence of certain bioactivities. For this initial study we focused on different antimicrobial activities. All classifiers performed well, with balanced accuracies of at least 57% and as high as 79%. Our tool will allow researchers to take advantage of the increasing amount of genetic data to prioritize bacteria that are most likely to produce natural products with desirable activities. We also determined that some biosynthetic genes are highly associated with certain activities, linking molecular features to bioactivity.



P-018

RECONSTITUTION AND GLYCOSYLATION OF ACYL CARRIER PROTEIN-BOUND POLYKETIDES

Auday A. Eida,[1] Mostafa E. Abugrain,[1] Corey J. Brumsted,[2] and Taifo Mahmud[1,2,]*
[1]Department of Pharmaceutical Sciences and [2]Department of Chemistry, Oregon State University, Corvallis, OR 97331-3507 (USA)

Glycosylation is a common modification reaction in natural products biosynthesis and has been known to be a post assembly line tailoring process in glycosylated polyketide biosynthesis. Here, we show that in pactamycin biosynthesis glycosylation can take place on an acyl carrier protein (ACP)-bound polyketide intermediate. Using *in vivo* gene inactivation, chemical complementation, and *in vitro* pathway reconstitution we demonstrate that the 3-aminoacetophenone moiety of pactamycin is derived from 3-aminobenzoic acid by a set of discrete polyketide synthase proteins (PtmS, PtmI, and PtmK) via a 3-[3-aminophenyl]3-oxopropionyl-ACP intermediate. This ACP-bound intermediate is then glycosylated by a broad-spectrum *N*-glycosyltransferase, PtmJ, providing a sugar precursor for the formation of the aminocyclopentitol core structure of pactamycin. This is the first example of glycosylation of a small molecule while tethered to a carrier protein. Additionally, we demonstrate that PtmO is a hydrolase that is responsible for the release of the ACP-bound product to a free β-ketoacid that subsequently undergoes decarboxylation.

P-019

MECHANISTIC STUDY OF TWO CYANOBACTIN HETEROCYCLASES TRUD AND PATD

Wenjia Gu, *Debosmita Sardar, Eric. W. Schmidt*
Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112 USA

Many azol(in)e heterocyclic natural products exhibit diverse biological activities and have high therapeutic values. In Ribosomally synthesized and post-translationally modified peptide (RiPP) biosynthesis, two heterocyclases TruD and PatD show high sequence identity, but catalyze different amino acid residues to form azolines: Cys for TruD while Cys/Thr/Ser for PatD. How these two similar enzymes catalyze diverse azoline formation is still under investigation. Here we will look into the ATP usage of these two enzymes, and identify the key residues that account for the catalytic differences. Understanding the mechanism of these two enzymes has potential applications for protein engineering and synthetic biology.

P-020

THE UNTAPPED POTENTIAL OF NATURAL PRODUCT BIOSYNTHESIS IN LICHEN FUNGI: OPPORTUNITIES AND CHALLENGES.

Robert L. Bertrand, Mona Abdel-Hameed, and John L. Sorensen.
Department of Chemistry, University of Manitoba, Winnipeg, Mb, Canada, R3T 4C8

Lichen fungi remain one of the most underdeveloped sources of novel bioactive natural products. This is in part this is due to challenges that result from the slow growth of the symbiotic organism. Our research program has been focused on examining the biosynthetic potential of the lichen *Cladonia uncialis*. We have carried out the sequencing, assembly and annotation of the genome and as a result, we have identified approximately 50 biosynthetic gene clusters that code for small molecule biosynthesis. A large number of these clusters have a gene that codes for a polyketide synthase. Numerous accessory genes, such as methyl transferases and hydroxylases, flank each of these polyketide synthase genes. Based on homology to genes characterized from non-lichen fungi we have been able to propose function for some of these gene clusters. This genome sequencing has revealed the rich biosynthetic potential of lichen fungi.

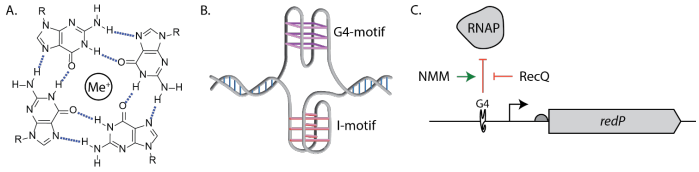
More recently, we have been using the filamentous fungus *Aspergillus oryzae* as platform for heterologous expression of biosynthetic genes. We have focused on the gene cluster involved in usnic acid biosynthesis, as well as other polyketide synthases. This talk will outline the challenges that we have encountered in heterologous expression of biosynthetic genes from *C. uncialis* in *A. oryaze*. This talk will present some of the success and challenges that we have had with the *A. oryaze* platform. A strategy for overcoming some of the challenges observed with heterologous expression will also be described.

P-021

G-QUADRUPLEX MOTIFS CONTROL NATURAL PRODUCT BIOSYNTHESIS IN STREPTOMYCES

Dakota Snustad, Aleksandra Bajer, and Michael J. Smanski
Department of Biochemistry, Molecular Biology, and Biophysics and BioTechnology Institute, University of Minnesota – Twin Cities, Saint Paul, MN, 55108, USA

Genomes of actinomycete bacteria encode a wealth of natural product biosynthetic gene clusters (BGCs) that remain uncharacterized. Awakening silent natural product gene clusters is challenging due to the complex and multi-layered regulation that controls their transcription and translation. We have discovered a new regulatory mechanism to control natural product biosynthesis in the model species, *Streptomyces coelicolor.* Quadruplex G motifs present in promoters of key regulatory genes in BGCs repress transcription. An ortholog of the *Escherichia coli* helicase, RecQ, in the *S. coelicolor* genome is able to unwind quadruplex G motifs to activate gene expression. Here we characterize this novel regulon using the RED gene cluster of *S. coelicolor.*



P-022

INTERRUPTED ADENYLATION DOMAINS – PROMISING TOOLS TO METHYLATE NONRIBOSOMAL PEPTIDES

Shogo Mori[1], Allan H. Pang[1], Taylor A. Lundy[1], Atefeh Garzan[1], Oleg V. Tsodikov[1*], and Sylvie Garneau-Tsodikova[1*]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA.

Natural products (NPs) are very important sources of drugs. They are produced by biosynthetic enzymes including nonribosomal peptide synthetases (NRPSs). NRPSs are modular enzymes that contain multiple catalytic domains in each module, whose orchestrated actions synthesize complex peptidic NPs. Each domain normally has a specific function, but the adenylation (A) domains are sometimes found embedding another functional domain within their structures. Such A domains, called interrupted A domains, play an important role in tailoring the NP structures. We have now biochemically and structurally characterized interrupted A domains found in the biosynthetic pathway of thiocoraline and its analogous NP, thiochondrilline A. They contain unique *N*,*S*-dimethyl-L-Cys residues, whose dimethylation was proposed to be catalyzed by two different methylation (M) domains embedded in two different interrupted A domains. We identified the pathway for constructing the residue by a series of radiometric assays. We also solved the structure of one interrupted A domain that provided an insight into the mechanism of action of interrupted A domains. Using the

knowledge gathered by these studies, we engineered interrupted A domains from non-interrupted ones as well as a di-interrupted A domain from a mono-interrupted one. We are now investigating M domains that showed relaxed substrate promiscuity, which promises a high applicability of using a single M domain to engineer interrupted A domains with any substrates. All these studies guide our effort towards combinatorial biosynthesis of new methylated NPs.

P-023

CHARACTERIZATION OF A NOVEL PEPTIDE MACROCYCLASE.

Snigdha Sarkar, Eric W. Schmidt
Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112 USA

Cyclic peptides are largely resistant to proteolysis making them ideal drug candidates. Among families of ribosomally synthesized and posttranslationally modified peptide (RiPP) natural products, there are several known macrocyclases, all with distinct advantages and limitations. For this reason, it is important to continue searching for novel macrocyclases with different functions. Here, we purify and characterize a novel macrocyclase, which is capable of simultaneously cleaving short peptide fragments and ligating the cleaved ends to form cyclic products.

P-024

PROBING THE LIMITS OF ENGINEERING NONRIBOSOMAL PEPTIDE MULTIFUNCTIONAL ENZYMES

Taylor A. Lundy[1], Shogo Mori[1], and Sylvie Garneau-Tsodikova[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0596, USA

Nonribosomal peptides (NRPs) are a large class of natural products with therapeutic relevance. NRPs are biosynthesized by mega-enzymes called nonribosomal peptide synthetases (NRPSs) that use amino acids and their analogues as building blocks. NRPSs are modular and can be subdivided into catalytic domains (adenylation (A), condensation (C), and thiolation (T)) and auxiliary domains (*e.g.*, methyltransferase (M)). Adenylation domains are essential for the diversity of NRPs because they dictate the building blocks incorporated into the final structure. Nature has added more diversity to NRPSs by embedding auxiliary domains into A domains, creating interrupted A domains. The most common type of interruption is an M domain that occurs between either the a2-a3 or a8-a9 of the ten conserved motifs of A domains. We aim to emulate and expand what Nature has created and generate artificial interrupted A domains. To emulate Nature, we generated two fully bifunctional artificial interrupted A domains by inserting different noncognate M domains into a naturally occurring uninterrupted A domain. These engineered A domains were capable of selectively methylating the amino acid in accordance with the natural M domain specificity. To expand on what Nature has created, we added a backbone methylating M domain to an already interrupted A domain that naturally contains a side chain methylating M domain to create a trifunctional A domain that can adenylate as well as *N*- and *S*- methylate L-Cys. This provides an exciting proof-of-concept for generating interrupted A domains as future tools to modify NRPSs and increase their NP diversity.

P-025

HARNESSING NON-ENZYMATIC CHEMISTRY IN OXAZININ A BIOSYNTHESIS

Victor Aniebok and John B. MacMillan
Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064

Natural Products (NPs) that contain one or more non-enzymatic steps in their biosynthesis have expanded the chemical complexity with which to approach historical problems such as drug design. The chemistry behind these NPs and the mechanisms behind the non-enzymatic steps that help produce them can lead to important biological applications. Elucidating the mechanisms behind the formation of these NPs allows for better knowledge of the biological system and the production of potentially more potent analogs. In order to produce more potent analogs, the potentially non-enzymatic steps of oxazinin A have been studied via total synthesis of the natural product.

P-026

APOPTOSIS BY CORCHORUSOSIDE C AND EXPLORATION OF ITS MECHANISM OF ACTION IN BOTH DU-145 PROSTATE CANCER CELLS AND ZEBRAFISH

Gerardo D. Anaya-Eugenio,[1] Ermias Mekuria Addo,[2] Nathan Ezzone,[1] Joshua M. Henkin,[3] Tran N. Ninh,[4] Yulin Ren,[2] Djaja D. Soejarto,[3] A. Douglas Kinghorn,[2] Esperanza J. Carcache de Blanco[1]
[1]Division of Pharmacy Practice and Science and [2]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, USA. [3]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, USA. [4]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam

Currently, plants continue to be an important source of natural products with therapeutic potential.[1] In the present study, the antiproliferative effect and the mechanism of action of corchorusoside C (**1**), a cardenolide isolated from *Streptocaulon juventas* collected in Vietnam, was explored in DU-145 prostate cancer cells (80 nM). Compound **1** induced DU-145 cell shrinkage and cell detachment, but when evaluated in CCD-112CoN cells the cytotoxic activity was significantly reduced, IC_{50} 2.3 μM. A preliminary mechanistic study suggested that **1** inhibits activity and protein expression of NF-κB (p50 and p65), IKK (α and β), Bcl-2 and ICAM-1. Also, **1** increased the appearance of the sub-G1 phase population in DU-145 cells. In contrast, **1** induced upregulation of PARP-1, and caspases-3 and -7. ROS levels were increased post-treatment with **1**, and MTP decreased in a dose-dependent manner. Interestingly, **1** modulated caspases activity with non-differential morphological effects in a zebrafish model (*Danio rerio*). Thus, corchorusoside C (**1**) induces apoptosis in DU-145 cells and targets the same pathways both *in vitro* and *in vivo* in zebrafish.

Reference: 1. Mann, K. *Nat. Rev. Cancer* **2002**, *2*, 143–148.

P-027

ANTIPROLIFERATIVE ACTIVITY OF A LIBRARY OF STEROIDS AND OPTIMIZED CHEMICAL DERIVATES

Gerardo D. Anaya-Eugenio,[1] Xiuqin Ruan,[2] Appaso M. Jadhav,[2] Pui-Kai Li, [2] Esperanza J. Carcache de Blanco[1]
[1]Division of Pharmacy Practice and Science and [2]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

In the United States, cancer is the second cause of death and it is one of the most serious public health problems. This disease is defined as a com-

plex of diseases that occurs when cells of any part of the body grow out of control.[1] In our continuing effort to identify bioactive compounds with potential cancer therapeutic properties, the antiproliferative activity of a library of compounds (steroid sulfatase inhibitors) was evaluated in a panel of cancer cells. In the present study, the sulforhodamine B (SRB), mitochondrial transmembrane potential (MTP) and cell cycle assays were used. The results indicated that compound SI-10 showed selectivity and high level of cytotoxicity in HeLa cervical cancer cells with IC_{50} of 20 nM. Based on these preliminary results, sixteen analogs were synthetized and the new derivative SU-114-1 inhibited HeLa cell growth with an IC_{50} value of 1.1 μM. Furthermore, SI-10 compound induced loss of MTP and increased Hela cell population in sub-G1 phase at 0.5 and 5 μM. Further investigation is needed to determine the mechanism of action of SI-10 in Hela cells.

References 1. Siegel, R. L.; Miller, K. D.; Jemal, A. CA: Cancer J. Clin. **2018**, *68*, 7–30.

P-028

ANTIPROLIFERATIVE ACTIVITY OF THE XANTHOQUINODIN JBIR-99 FROM *PARENGYODONTIUM ALBUM* MEXU 30054 AND MECHANISM OF ACTION IN PC-3 HUMAN PROSTATE CANCER CELLS

Gerardo D. Anaya-Eugenio,[a] Daniela Rebollar-Ramos,[b] Maria del Carmen González,[c] Huzefa Raja,[d] Rachel Mata,[b] Esperanza J. Carcache de Blanco.[a]
[a]Division of Pharmacy Practice and Science, College of Pharmacy, The Ohio State University, Columbus, OH, USA. [b]Facultad de Química, [c]Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico, [d]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, N.C, USA.

The prostate cancer is the most common malignancy in men and the second leading cause of cancer-related deaths.[1] In this study the antiproliferative activity and the potential mechanism of action of the xanthoquinodin JBIR-99 (**1**) isolated from fungi *P. album* MEXU 30054 was evaluated. The cytotoxicity of **1** was evaluated in a panel of human cancer cells lines and CCD-112-CoN normal colon cells, using the SRB assay. PC-3 prostate cancer cells were used in biochemical assays; the protein expression levels were analyzed by western blot. The *in vivo* toxicity was determined using a zebrafish model. The results indicated that **1** showed cytotoxicity in all cancer cell lines but demonstrated relative selective potency against PC-3 cells. In CCD-112-CoN cells, **1** was non-cytotoxic at 100 μM. In PC-3 cells, **1** induced loss of MTP, production of ROS, and cell cycle arrest in S phase. The expression and activity of caspase-3 was increased, which correlates with the upregulation of NF-κB (p65) and IKKβ, and downregulation of PARP-1 and BCL-2. Finally, **1** did not cause any visible developmental toxicity in zebrafish at 50 μM. These results demonstrate xanthoquinodin (**1**) induces apoptosis in PC-3 prostate cancer cells by activation of both intrinsic and extrinsic apoptotic pathways.

References 1. Siegel, R. L.; Miller, K. D.; Jemal, A. CA: Cancer J. Clin. **2018**, *68*, 7–30.

P-029

INDUCTION OF APOPTOSIS ON TRIPLE NEGATIVE BREAST CANCER CELLS SPHEROIDS WITH MARINE NATURAL COMPOUNDS.

Esther A. Guzmán, Tara P. Pitts, and Amy E. Wright
Marine Biomedical and Biotechnology Research, Harbor Branch Oceanographic Institute at Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946.

Breast cancer is the second leading cause of cancer death among US women. Among its subtypes, triple negative breast cancer, comprising about 12% of breast cancers, is the most aggressive and difficult to treat. Cancer cells grown in spheroid conditions (3D-cultures) allow the cells to interact with each other and the extracellular matrix providing a better representation of the in vivo environment than two dimensional cultures. A small subset of samples from the HBOI peak library fractions were tested against MDA-MB-231 triple negative breast cancer (TNBC) cells grown in spheroid conditions using a multiparametric high content imaging assay. The same samples used in the spheroid assay were tested in a standard cytotoxicity assay. There are fractions that would have been called hits in both formats. However, there are fractions which exhibit very modest cytotoxicity in 2D cultures, while significant cytotoxicity was observed in the spheroid assay. This effort identified HB-395, a known marine natural compound active against TNBC cells when grown as spheroids (3D) but not when cells are grown in the traditional single layer method (2D). We believe this difference is due to the compound activating different transcriptomic and proteomic pathways, and we are in the process of testing this hypothesis using arrays. The data will increase the importance of using spheroids for screening and may help us predict what conditions are necessary to obtain a clinical response with this compound.

P-030

APPLYING PHYLOGENETICS TO IDENTIFY BIOACTIVE LAXAPHYCIN TYPE-B SECONDARY METABOLITES

Sullivan, Peter; Krunic, Aleksej; Chen, Wei-Lun; Burdette, Joanna; Orjala, Jimmy
University of Illinois at Chicago, College of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, 833 South Wood St. Chicago, IL 60612, United States

Upon extraction and fractionation of UIC 10484, a cyanobacterial strain collected in Indiana, USA, the material was subjected to three growth inhibition assays using human melanoma MDA-MB-435, breast adenocarcinoma MDA-MB-231, and ovarian adenocarcinoma OVCAR3 cell lines. Fraction 3 (40% isopropyl alcohol) was found to inhibit growth against all three cancer cell lines. After a second round of separation using a monolithic HPLC column, the subfractions were assessed for their bioactivity against the same three cancer lines. Dereplication applying phylogenetics and mass spectrometry on the active subfraction constituents was then carried out. Using the 16S rRNA sequence to perform the phylogenetic assessment, UIC 10484 was found to be closely related to the known laxaphycin secondary metabolite producer UIC 10045. Additionally, UIC 10339, which is another laxaphycin producer, is phylogenetically distinct from both UIC 10045 and 10484 highlighting the taxonomic variability in secondary metabolite production within the phylum. After analyzing the MS/MS data of the active components, it was confirmed that UIC 10484 produces novel laxaphycin type-B secondary metabolites. Laxaphycins B5 and B6 were elucidated based on 2D NMR and MS/MS data in addition to performing advanced Marfey's analysis to determine the stereochemistry of both cyclic compounds. Eleven of the 12 amino acids between the two are the same with B5 having a 3-hydroxyleucine while B6 instead has a leucine. As is consistent with all laxaphycin type-B secondary metabolites, both B5 and B6 have a long aliphatic β-amino acid residue. IC_{50} evaluations are currently under way.

P-031

NATURAL PRODUCTS AS IMMUNOTHERAPY ENHANCERS

Charles S. Fermaintt[1], Shengxin Cai[3,4], April L. Risinger[1,2], Tanja Grkovic[5], Barry O'Keefe[6,7], Robert Cichewicz[3,4] and Susan L. Mooberry[1,2].
[1]Department of Pharmacology and [2]Mays Cancer Center, UT Health San Antonio, San Antonio, TX, 78229. [3]Department of Chemistry and Biochemistry and [4]Natural Products Discovery Group, University of Oklahoma, Norman, OK, 73019. [5]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. [6]Natural Products Branch, Division of Cancer Treatment and Diagnosis, and [7]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD, 21702.

Immune checkpoint inhibitors have made a significant impact in the treatment of many cancers. However, there are limitations to their efficacy in part due to the actions of tumor-associated macrophages that can create a tumor-supportive phenotype, ultimately leading to a low treatment response and poor prognosis. Growing evidence suggests that agents which shift the gene expression profile of macrophages from a pro-tumor phenotype (M2) to an anti-tumor phenotype (M1) greatly augment the effectiveness of immune checkpoint inhibitors. This prompted us to initiate a screen to identify natural products that have the potential to elicit an anti-tumor immune signature in macrophages. We screened 4865 plant and fungal extracts for those that promoted the differentiation of human THP1 monocytes from a suspension to adherent phenotype using a variation on the colorimetric SRB assay and a total of 51 extracts were identified as hits. We further prioritized 9 taxonomically diverse extracts that promoted an M1-associated response, albeit with distinct immune signatures, that were subject to bioassay-guided fractionation. These efforts have led to the identification of potent bioactive compounds that will be further evaluated in tumor models and mechanistic studies.

P-032

USING GENOME-WIDE CRISPR-CAS9 SCREENS TO IDENTIFY THE MECHANISM OF ACTION OF NATURAL PRODUCTS

April L. Risinger[1,2], Corena V. Grant[1], Shengxin Cai[3,4], Tanja Grkovic[5], Barry O'Keefe[6,7], Robert Cichewicz[3,4] and Susan L. Mooberry[1,2]
[1]Department of Pharmacology [2]Mays Cancer Center, UT Health San Antonio, San Antonio, TX, 78229. [3]Department of Chemistry and Biochemistry [4]Natural Products Discovery Group, University of Oklahoma, Norman, OK, 73019. [5]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. [6]Natural Products Branch, Division of Cancer Treatment and Diagnosis and [7]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD, 21702.

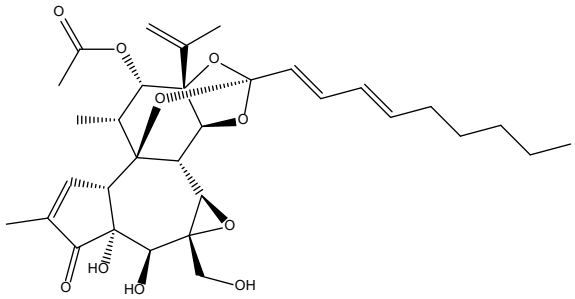
A common roadblock in the study of bioactive natural products is a detailed understanding of their mechanisms of action. We used a mechanism-blind approach to identify natural products that are selectively cytotoxic to cell lines representing molecularly defined subtypes of triple negative breast cancer (TNBC), which currently have no targeted treatment options. These efforts identified a series of polyacetylenes that are selectively cytotoxic to mesenchymal subtypes of TNBC. We utilized a pooled CRISPR-Cas9 genome-wide knockout screen to identify gene products that mediate the sensitivity of mesenchymal TNBC cells to our lead compound, dehydrofalcarinol. The sgRNA-mediated depletion of *HSD17B11* provided a survival advantage, indicating that this gene product, 17β-hydroxysteroid dehydrogenase type 11, was critical for the selective cytotoxic effects of this compound. The role of 17β-hydroxysteroid dehydrogenase type 11 in mediating the sensitivity of MDA-MB-231 cells to dehydrofalcarinol was further confirmed by siRNA knockdown. Our studies demonstrate that CRISPR-Cas9 genetic screens are an effective method to identify key mediators of the biological activities of natural products, which will facilitate a pathway-based understanding of novel bioactive compounds.

P-033

YUANHUADINE IN COMBINATION WITH EGFR-TKI CAN DELAY AND OVERCOME ACQUIRED RESISTANCE IN HUMAN NON-SMALL CELL LUNG CANCER CELLS

*Donghwa Kim, Duc-Hiep Bach, Yan-Hua Fan, Thi-Thu-Trang Luu, Ji-Young Hong, Hyen Joo Park, Sang Kook Lee**
College of Pharmacy, Natural Products Research Institute, Seoul National University, Seoul 08826, Korea

Epidermal growth factor receptor (EGFR) mutation is one of the major driver oncogenes in non-small cell lung cancer (NSCLC) and most frequently found in Asian patients. Recently, AXL has been reported to play a role in drug resistance mechanisms for many anti-cancer drugs, including erlotinib and cetuximab, in multiple cancers. In the present study, we demonstrate the involvement of AXL in the acquired resistance to gefitinib and osimertinib of EGFR-mutant NSCLC cells, then show the combination effect of an AXL degrader and EGFR-TKIs to overcome EGFR-TKIs-driven resistance in EGFR-mutant NSCLC cells. Yuanhuadine (YD), a natural antitumor agent from the flower of *Daphne genkwa* (Thymelaeceae), effectively suppressed the expression of AXL by accelerating protein degradation. YD combined with gefitinib or osimertinib synergistically inhibited the growth of resistant cells *in vitro* and suppressed tumor growth in a nude mouse xenograft model. Moreover, administration of YD with gefitinib effectively delayed gefitinib-driven acquired resistance in a long-term xenograft model. These findings suggest that the combination of YD with either gefitinib or osimertinib is a potentially effective treatment strategy for overcoming and delaying acquired resistance in NSCLC by targeting AXL degradation.



Yuanhuadine

P-034

EUPENIFELDIN-POLYMER LOADED THIN FILMS TO PREVENT LOCAL RECURRENCE OF LUNG CANCER

Zeinab Y. Al Subeh[1], Aaron Colby[2], Cedric J. Pearce[3], Mark Grinstaff[2], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27412, USA, [2]Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA, [3]Mycosynthetix, Inc., 505 Meadowlands Drive, Suite 103, Hillsborough, North Carolina 27278

Lung cancer is the leading cause of cancer death world widely. According to the American Cancer Society, the 5-year survival rate for lung cancer patients is only 23%. Even for patients who received curative surgical resection, 30-55% of those develop recurrence and die of their disease. Developing a prolonged-released drug delivery system that can be placed at the tumor site after surgical resection could significantly reduce the rate of tumor recurrence and improve the disease prognosis. Eupenifeldin, which is a fungal secondary metabolite, is reported to have high potency against

various cancer cell lines. We tested the ability of eupenifeldin to elute from polymer (poly(glycerol-co-E-caprolactone)-C18) thin films. Five different types (sets) of eupenifeldin-polymer loaded thin films were made using layer by layer technique. The amount of released eupenifeldin was monitored by submerging each film in phosphate buffer saline at 37 C for 90 days. Type 5 thin film, which was loaded with 1200 µg eupenifeldin and covered with two polymer layers on each face, maintained a significant and consistent release of eupenifeldin over 90 days.

P-035

SECONDARY METABOLITES FROM *AEROMONAS VERONII* STRAIN A134 ISOLATED FROM A *MICROCYSTIS AERUGINOSA* BLOOM

Gad Weiss[1], Dimitry Kovalerchick[2,3], Omer Murik[1], Assaf Sukenik[4], Aaron Kaplan[1], and Shmuel Carmeli[2]
[1]Plants and Environmental Sciences, the Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, Jerusalem 9190401, Israel, [2]Raymond and Beverly Sackler School of Chemistry and Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel, [3]Metabomed Ltd, Yavne 81220, Israel, [4]The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research, Migdal, Israel

Aeromonas veronii strain A134 was isolated from *Microcystis aeruginosa* colonies collected in Lake Kinneret. The *Aeromonas* culture media inhibited the growth of *M. aeruginosa* (strain MGK). The crude extract of a large-scale culture of *A. veronii* A134 was separated in few chromatographic steps to afford three new metabolites, 9-chlorolumichrome (**1**), veronimide (**2**), and veronipyrazine (**3**), along with the known lumichrome and several known diketopiperazines. The structures of the new compounds were established by analyses of the data from 1D and 2D NMR experiments and HRMS data of the compounds, as well as, a single-crystal x-ray analysis of synthetic **1**. The structure elucidation and proposed biogenesis of the new compounds are described below.

P-036

NATURAL PRODUCTS IN VERTEBRATES COMMUNICATION BY MASS SPECTROMETRY METABOLOMICS

Fausto Carnevale Neto[1,2], Daniel Raftery[2], Norberto Peporine Lopes[1]
[1]Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903, Brazil, [2]School of Medicine, University of Washington, Seattle, WA, 98109, USA, [3]Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil.

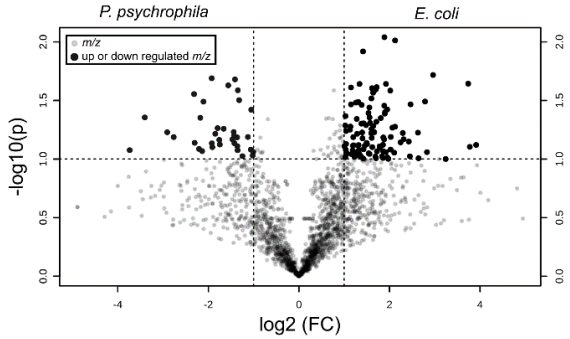
The existence of each organism is entirely dependent of the ecosystem. There is no life in complete isolation, and organisms have co-evolved to interact. One of the main modes of interactions is through chemical communication. Although many studies have described molecules responsible for chemical signaling, generating scientific knowledge and technological advancements, there is still an unexplored scenario to address the role of molecules in their natural - and complex – conditions. We investigated the diverse 'molecular landscape' in chemical communication of different vertebrates by using mass spectrometry-based metabolomics combined with molecular networking, a method that grouped related parent ions in different clusters based on similarities in MS/MS fragmentation patterns. The resulting clusters assisted the chemical identification based on systematic gas-phase fragmentation studies considering the MS/MS spectra. The approach led to the discovery of a new class of naturally water-soluble fluorophores from lymph and skin interstitial tissue, epidermis, and multicellular exocrine skin glands of amphibians, named hyloin. We have also revealed the chemical structure of natural water-soluble pigments secreted by modified epidermic glands of male South-American Howler Monkeys. The compounds have important ecological role on chemical communication, impact on animals' physiology, and raise question regarding the study of evolutionary biochemical pathways.

P-037

METABOLOMICS FOR IDENTIFICATION OF COMPOUNDS INVOLVED IN BACTERIAL-FUNGAL INTERACTIONS

Jessica Cleary[1], Gordon Luu[1], Emily Pierce[2], Rachel Dutton[2], Laura Sanchez[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612. [2]Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA

Multispecies microbiome systems have recently been closely linked to human, animal, and plant life processes. The growing field of metabolomics presents the opportunity to detect changes in overall metabolomic profiles of microbial species interactions. Using cheese rinds as a model system for studying complex microbiomes, this study aims to identify how metabolomic profiles change when microbes are grown in co-culture compared to isolated cultures and specifically which changes are important for bacterial-fungal interactions. To observe shifts in metabolomic profiles when fungi are cultured with different microbial partners, all fungi were grown alone and with *E. coli* and *Psuedomonas psychrophila*. LC-MS/MS performed on extracts provided metabolomic profiles of different growing partners. Statistical analysis of the data sets highlights metabolites that experience changes in abundance with different conditions and therefore are likely to play ecological roles in these interactions. We will couple the identification of these molecules with genomic information from RB-Tnseq experiments to build a picture of keystone processes that underlie microbial interactions.



Volcano plots display show fold change of *m/z* values vs. *m/z* values that are considered significant within the samples.

P-038

A CONSERVED PEPTIDE FAMILY OF NATURAL PRODUCTS IS INVOLVED IN FLAGELLAR MOTILITY AND BIOFILM FORMATION IN PROTEOBACTERIA

Laura P. Ióca[1,2], Jennifer Diaz[2], Sylvia Kunakom[2], Laura M. Sanchez[2], Roberto G. S. Berlinck[1], Alessandra S. Eustaquio[2], Roberto G. S. Berlinck[1].
[1]Instituto de Química de São Carlos, Universidade de São Paulo;
[2]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago.

Bacteria can colonize diverse environments as well as establish associations with plants, animals and fungi, playing important ecological.[1] *Pseudovibrio* spp. are usually found in healthy sponges worldwide.[2] We identified a biosynthetic gene cluster (BGC) that is conserved among 70% of all sequenced *Pseudovibrio* isolates, and it is also found in 4% of *Pseudomonas* genomes, a common plant and animal pathogen bacteria.[3] The conservation of this BGC among different proteobacteria suggests a relevant ecological function for the organisms that harbor it. In order to investigate its function, we de-

veloped a reverse genetics method for *Pseudovibrio brasiliensis*. We observed that the absence of this BGC affects bacterial motility. Bacterial motility is known to contribute to host colonization, however gaps remain in our knowledge regarding which metabolites mediate this process and how they do so. We are in the process determining the structures of the compounds produced by *P. brasiliensis*. We are also attempting to generate *Pseudomonas* knockout mutants to investigate the function of the BGC in this genus.

Funding: Fapesp, NACTS and startup UIC funds. **References:** [1]*Nature Rev. Microbiol.* 2010, 8:634; *Ann. Rev.Microbiol.* 2003, 57:249. [2]*Front. Mar. Sci.* 2018, 5:81; *Appl. Environm. Microbiol.* 2018, 84:e0 2516. [3]*Front. Cell. Infect. Mibrobiol.* 2017, 7:39.

P-039

THE ACID ROCK DRAINAGE MICROBIOME AT ELY COPPER MINE IN VERSHIRE, VT AND ITS POTENTIAL TO PRODUCE NOVEL METABOLITES

Katherine Morillo[1], George Chlipala[2], Kevin Kuntsman[2], Stefan Green[2], Kieran Bhave[1], Heather Driscoll[3], Holly Peterson[4], Laurent Asiama[1], Benjamin Paquin[1], Mark Maienschein-Cline[2], Lesley-Ann Giddings[1]
[1]Department of Chemistry & Biochemistry, Middlebury College, Middlebury, VT 05753, USA, [2]Research Resources Center, University of Illinois at Chicago, Chicago, IL, 60612, USA, [3]Vermont Genetics Network/University of Vermont, Burlington, VT, 05405, USA, [4]Department of Geology, Guilford College, Greensboro, NC, 27403, USA,.

Several copper mines in Vermont are on the Superfund National Priorities List due to having poor water quality, high metal concentrations, and high metal sulfide oxidation rates. Ely Copper Mine, a Superfund site in Vershire, VT, has been abandoned and accumulating acidic water or acid rock drainage (ARD) for >50 years. To understand how to develop site-specific strategies to remediate copper mines, we characterized the microbial community at Ely Brook, which drains Ely Copper Mine, to assess microbial diversity and the community's potential to produce metal-chelators, such metal resistance proteins and secondary metabolites. Water and sediment were collected in winter and summer, and DNA and cDNA were analyzed using shotgun metagenome and metatranscriptome sequencing, respectively. Our data show that Proteobacteria is the most dominant phylum, and diazotrophic *Bradyrhizobium* (4.0–8.8%), *Pseudomonas* (3.7–4.7%), and *Streptomyces* (2.5–5.2%) are the most abundant genera. Mostly terpenes and polyketides biosynthetic genes were identified from assembled contigs, and several metal resistance proteins, namely copper and zinc resistance proteins, were overexpressed. Significant differences in gene expression were observed in winter versus summer sediment, and metabolites produced by select microbes cultured under various growth conditions were characterized to show how seasonal variations affect metabolomes. Our data provide insight into the roles of microbes within acid rock drainage.

P-040

LET'S TALK ABOUT SEX (PHEROMONES)! IDENTIFYING THE ROLE OF LINEAR PEPTIDES IN STAPHYLOCOCCAL VIRULENCE REGULATION USING METABOLOMICS

Lindsay K. Caesar[1], Katrin Schilcher[2], Corey P. Parlet[3], Alexander R. Horswill[2], and Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC, 27412, [2]Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora CO, 80045, [3]Department of Microbiology, University of Iowa Carver College of Medicine, Iowa City IA, 52242

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global health concern due to its production of several virulence factors, many of which are acquired through horizontal gene transfer (HGT). Vancomycin is the gold standard treatment for severe MRSA infections; however, recent evidence suggests that some strains have acquired vancomycin-resistance from *Enterococcus faecalis* via HGT. One sex pheromone secreted by *S. aureus, staph*-cAM373, stimulates plasmid transfer from *E. faecalis* to *S. aureus.* To elucidate the mechanism by which *staph*-cAM373 is processed, its production was monitored in a clinically-relevant strain of MRSA and genetic mutants using both mass spectrometry and cellular aggregation assays. Increased virulence was demonstrated *in vivo* in strains containing a mutation of the *staph*-cAM373-encoding gene *camS*, implying a role for *staph*-cAM373 in virulence regulation. Using a list of peptides found in other Gram-positive bacteria, we identified four peptides that were downregulated in a deletion mutant that may act as virulence regulators. Metabolomics analysis is underway to identify additional peptide signals that may play a role in this process. Future investigations will be conducted to elucidate the role of these linear peptides in staphylococcal virulence regulation.

P-041

CHEMICAL WARFARE BETWEEN MICROBIAL SYMBIONTS OF FUNGUS-GROWING ANTS

Munhyung Bae[1], and Jon Clardy[1]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Recent chemical investigations into bacterial symbionts of fungus-growing ants have led to the discovery of secondary metabolites that play putative roles in maintaining the health of this complex system. Using a newly developed trans-well system, which was designed to uncover molecular interactions between microbes, we discovered a specific chemical interaction occurring between these bacterial symbionts and a fungal pathogen (*Escovopsis* sp.), which infects the ant nest. New analog of dentigerumycin, dentigerumycin F (**1**) which was constitutively produced by a *Pseudonocardia* strain, upregulated the production of a potent and selective antibacterial agent by the pathogenic *Escovopsis* fungus. Traditional bioassay-guided isolation led to the discovery of active and in-active form of new conocandin analogs—conocandins B (**2**) and C (**3**). This *Escovopsis*-produced conocandin B (active form) exhibit highly-potent inhibitory activity against *Pseudonocardia* sp. and drug resistant pathogen bacteria including MRSA and VRE and preliminary data suggest that these metabolites are inhibit the fatty acid biosynthesis enzyme FabH. From this study we have gained insights into the complex interactions occurring between fungus-growing ants, their symbiotic bacteria, and a pathogenic fungus.

P-042

FACTORS CONTRIBUTING TO INCREASED DOMOIC ACID PRODUCTION IN NARRAGANSETT BAY RHODE ISLAND

Riley D. Kirk[1], Alexa R. Sterling[2], Erin M. Tully[2], Jacob P. Strock[3], Matthew J. Bertin[1], Bethany D. Jenkins[2]
[1]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, [2]Department of Cell and molecular biology, URI, Kingston, RI 02881, [3]Graduate school of Oceanography, URI, Narragansett, RI.

Some marine diatoms in the genus *Pseudo-nitzschia* produce the neurotoxin domoic acid (DA). A toxin best known as the causative agent in Amnesic Shellfish Poisoning (ASP). This diatom is globally distributed and long-term data from Narragansett Bay (NB), Rhode Island have shown that diatoms in the genus *Pseudo-nitzschia* persist in NB. Despite the presence of *Pseudo-nitzschia*, domoic acid has only been recently detected during an algal bloom in the fall of 2016. The presence of this toxin-producing genus and the ability of the toxin to bioaccumulate in the food web drives a need to determine the factors that cause increased abundance of toxic species and/or increased production of toxin within the ecosystem. Additionally, sensitive analytical measurements to monitor DA levels in seawater are

needed. Through weekly sampling of NB, and analysis of factors such as dissolved nutrients, chlorophyll, temperature, salinity and genotyping, in conjunction with LC-MS/MS with MRM monitoring of DA in seawater, we have determined some potential driving factors of DA production. Domoic acid is present in the bay year-round but increased toxin production is inversely correlated with phosphate, temperature, and salinity. Further, recovery experiments of DA have led to a simple way to detect the toxin in dissolved seawater.

P-043

RE-ISOLATION AND RE-ANALYSIS OF FUNGAL SECONDARY METABOLITES IN SEARCH OF CHEMICAL DIVERSITY

Allison J. Wright, Sonja L. Knowles, Huzefa A. Raja, and Nicholas H. Oberlies
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC.

Due to their adaptability fungi are ever changing their metabolite profile, typically biosynthesizing a variety of secondary metabolites. Previously characterized fungal cultures were re-analyzed with the intention of re-isolating scaled up quantities of metabolites of interest. However, in doing so, there were several instances where fungal metabolites were isolated that were not observed previously. Biological activity was evaluated using a variety of previously unutilized bioassays. Based on recent antibacterial assay results several fungal strains that were not previously tested are now classified as encompassing antibacterial properties. The secondary metabolites produced by these specific fungal strains are being tested to determine which compounds are accountable for the antibacterial activity. Several fungal strains exhibited cytotoxic activity in melanoma, breast cancer, and ovarian cancer. The active fungal strains underwent purification and structure elucidation in order to evaluate the secondary metabolites that contribute to this activity. The fungal extracts have also been evaluated for anti-amoeba activity. Thus far, at least four compounds have been isolated that were not previously biosynthesized, along with new biological activities. In turn, re-isolation and re-analysis of fungal secondary metabolites can lead to the discovery of unsought yet interesting changes in metabolite profile and biological activity.

P-044

BIOSYNTHESIS OF NEW EPIPOLYTHIODIOXOPIPERAZINE ALKALOIDS ANALOGUES VIA PRECURSOR-DIRECTED BIOSYNTHESIS

Chiraz Soumia M. Amrine[1], Jessica L. Long[1], Joanna E. Burdette[2], Cedric J. Pearce[3], Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Department of Medicinal Chemistry and Pharmacognosy. University of Illinois at Chicago, Chicago, IL 60612, USA. [3]Mycosynthetix, Inc., 505 Meadowlands Drive, Suite 103, Hillsborough, NC 27278, USA.

Verticillins are members of the epipolythiodioxopiperazine (ETP) alkaloid class of fungal metabolites and are known as potent cytotoxic agents with IC_{50} values lower than 10 nM. Studies showed that verticillin A has activity as a selective histone methyl transferases inhibitor with important anticancer properties. However, a common challenge with natural products pertains to obtaining a suitable patent position. Precursor-directed biosynthesis was used to produce a series of "non-natural natural" epipolythiodioxopiperazine alkaloids. The biosynthesis of these verticillin analogues was monitored *in situ* via the droplet liquid micro-junction surface sampling probe (droplet probe), and a suite of NMR and mass spectrometry data were used for their characterization. All analogues demonstrated nanomolar IC_{50} values vs a panel of cancer cell lines. This approach yielded novel compounds that would be difficult to generate via synthesis.

P-045

MAKING A SPLASH AGAINST BRAIN EATING AMOEBA WITH FUNGAL SECONDARY METABOLITES

Kristóf B. Cank[1], Tyler N. Graf[1], Christopher A. Rice[2], Laura Flores-Bocanegra[1], Dennis E. Kyle[2], Cedric J. Pearce[3] and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27402, USA, [2]Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA, 30602, USA, [3]Mycosynthetix, Inc., Hillsborough, NC, 27278, USA

Naegleria fowleri, commonly known as "brain eating amoeba", is a free-living amoeba, and is responsible for primary amoebic meningoencephalitis (PAM). This is a very rare but severe human disease that is rapidly fatal leading to death in approximately one week or less. The infection typically occurs among healthy children and young adults due to recent freshwater exposure from recreational aquatic activities such as swimming and diving. The lack of effective treatments, as well as the 95% mortality rate creates an urgent need for new and more effective therapeutics. We have screened over 4000 fungal extracts in a single point assay at 50 µg/ml concentration. For elimination of cytotoxic fractions, we tested the samples against four different human cancer cell lines including melanoma, breast, ovarian, and lung carcinoma cell lines. To exclude the already known compounds, the active samples were evaluated by using our in-house developed UPLC-PDA-HRMS-MS/MS dereplication method. Bioactivity directed isolation and structure elucidation of secondary metabolites resulted in several compounds with notable activity against *Naegleria fowleri*. The characterization of additional fractions is currently ongoing. This study shows that the inherent structural diversity of fungal secondary metabolites indicates that fungi can be a promising source for new anti-*Naegleria* therapeutics.

P-046

CYCLIC DEPSIPEPTIDES FROM THE SPONGE *THEONELLA SWINHOEI* THAT INHIBIT RAS/RAF INTERACTION

Chang-Kwon Kim[1], Dongdong Wang[1], Emily Smith[1,2], Heidi R. Bokesch[1,2], Unwoo Kang[1], Carole A. Bewley[3], Curtis J. Henrich[1,2], Deborah K. Morrison[4], and Kirk R. Gustafson[1]
[1]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702-1201, USA, [2]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702-1201, [3]Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, [4]Laboratory of Cell and Developmental Signaling, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702-1201

Two new cyclic depsipeptides were isolated from the marine sponge *Theonella swinhoei* cf. *verrucosa*, collected from Papua New Guinea. The planar structures were determined by extensive 1D and 2D NMR and HRESIMS. These peptides contain four common amino acids Pro, Ala, Gly, Ser, together with seven nonproteinogenic amino acid residues: β-methoxytyrosine (β-OMeTyr), *N*-methylthreonine (*N*-MeThr), 3-methoxyalanine (3-OMeAla), 3-hydroxyleucine (3-OHLeu), 3,4-dimethylglutamine (3,4-DiMeGln), 2,3-diaminobutanoic acid (Dab) and 2-amino-2-butenoic acid (Aba). In addition, a 2,3-dihydroxy-2,6,8-trimethyldeca-(4*Z*,6*E*)-dienoic acid (DHtda) polyketide moiety was identified in **1**, while **2** contained a previously undescribed 2,6,8-trimethyldeca-(2*E*,4*E*,6*E*)-trienoic acid moiety N-linked to a terminal serine residue. The absolute configurations of the amino acids were established by the advanced Marfey's method. The amino acid constituents were identified as L-Pro, D-Ser, L-Ala, D-3-OMeAla, (2*S*,3*S*)-

Dab, (2*R*,3*R*)-3-OHLeu, (2*S*,3*R*)-*N*-MeThr, (2*S*,3*S*,4*R*)-3,4-DiMeGln and (2*R*,3*R*)-β-OMeTyr. These new peptides showed potent inhibition of Ras/Raf signaling pathway with IC_{50} values of 5.8 and 7.2 μM, respectively.

P-047

THE GOOD, THE BAD, AND THE ALGAE: NOVEL NATURAL PRODUCT DISCOVERY FOR BIOFUELS AND BIOSECURITY

Carolyn L. Fisher[1], Pamela D. Lane[1], Todd W. Lane[1]
[1]) Sandia National Laboratories, Livermore, CA

Microalgae mass culture systems are the most promising avenue for a renewable biofuel resource. However, outdoor algal production ponds are likely to succumb to unpredictable, devastating crashes due to algal grazing or infection, such as by *Chytridium sp.*, which drives up the economic barrier to biofuels. Separately, antimicrobial resistance is steadily increasing, leaving our soldiers, public, and healthcare system defenseless and in peril. We are interested in identifying novel natural products that support the biofuel economy, our public and military's health, and our nation's security.

We hypothesize that there are novel antimicrobial natural products waiting to be discovered within high-biomass, high-nutrient, non-sterile, marine, outdoor algal production cultures. To this end, we extracted saltwater samples from 75 diverse algal-bacterial culture systems with ethyl acetate and screened for antimicrobial susceptibility. We found 25 extracts that have antimicrobial properties against a panel of bacteria and/or fungi. We are currently screening these extracts against human pathogens and select agents (e.g. *Burkholderia sp.*) and algal parasites (e.g. *Chytridium sp*). Metabolomics, assay-guided fractionation, and NMR analysis will identify and characterize responsible metabolites with antimicrobial activity. Our goal is to identify novel therapeutics for biological threats affecting the public and our military as well as for microalgae production systems. Through these efforts, our work in identifying antimicrobials will support, enhance, and ensure our nation's energy and bio-security.

P-048

BIOPROSPECTING PUERTO RICO'S MARINE MICRO AND MACROALGAE FOR PHARMACEUTICALLY RELEVANT SECONDARY METABOLITES

Marie L. Matos-Hernandez[1], Wildelis Figueroa-Rodriguez[1], Kelsey L. Alexander[2], Raphael Reher[3], William H. Gerwick[3,4], C. Benjamin Naman[4], and Eduardo J. E. Caro-Diaz[1]
[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico - Medical Sciences Campus, San Juan, PR 00935. [2]Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA, USA. [3]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA. [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093. [5]College of Food and Pharmaceutical Sciences, Ningbo University, Zhejiang, China

Puerto Rico is home to a large and varied marine biodiversity, many of which produce unique natural products. In part, this rich biodiversity results from the island's location at the juncture of the Caribbean Sea and Atlantic Ocean, and represents an attractive resource for drug discovery efforts. We initially targeted the brown alga *Sargassum sp.* for natural products isolation given its widespread prevalence along the Puerto Rican coastline. Additionally, we have focused on marine cyanobacteria based on their well-described propensity to produce secondary metabolites that show biological activity in assays related to human disease. Recently, we embarked on a multi-institution exploration of Culebra Island in Puerto Rico and collected a variety of marine organisms for extraction and metabolite isolation for the potential of drug discovery. We present our initial findings and preliminary data for these collections along with our strategy for further development of these projects.

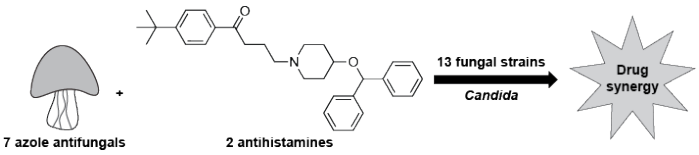
P-049

SYNERGISTIC COMBINATIONS OF AZOLES AND ANTIHISTAMINES AGAINST *CANDIDA* SPECIES *IN VITRO*

Emily K. Dennis[1] and Sylvie Garneau-Tsodikova[1]
[1] Pharmaceutical Sciences, University of Kentucky, Lexington, KY, 40536

Fungal infections are a major cause of skin and mucosal membrane diseases and if untreated can lead to invasive diseases. Immunocompromised individuals, such as those undergoing chemotherapy, are most susceptible to fungal infections. Azoles are the most prescribed antifungals, as they are broad-spectrum and orally bioavailable. However, it is estimated that 7% of *Candida* infections are resistant to azoles. One way to treat drug-resistant infections is to administer combinations of drugs to patients.

In this study, we explored combinations of 7 azole antifungals and 2 antihistamines (terfenadine and ebastine) against a panel of 13 *Candida* fungal strains to explore repurposing these antihistamines for treating fungal infections. We found 55 out of 91 combinations tested to be synergistic with azoles. To evaluate the efficiency of these combinations to inhibit fungal growth, we performed time-kill assays. We also investigated the ability of these combinations to disrupt a preformed biofilm. Finally, we tested the specificity of the combinations towards fungal cells by mammalian cytotoxicity assays. These findings suggest a potential new strategy for targeting drug-resistant *Candida* infections.



P-050

INTERACTIONS OF *LYTHRUM SALICARIA* L. EXTRACT WITH PIGLET GUT MICROBIOTA AND ITS POTENTIAL IMPACT ON ENTEROTOXIGENIC *E. COLI* INFECTIONS.

Temesgen H. Dadi[1], Wilfried Vahjen[1], Jürgen Zentek[1], Matthias F. Melzig[2], Sebastian Granica[3], Jakub P. Piwowarski[1,2,3]
[1]Institute of Animal Nutrition, Freie Universität Berlin, Germany, [2]Department of Pharmaceutical Biology, Freie Universität Berlin, Germany, [3]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland

Lythrum salicaria L. herb is an ellagitannin-rich plant material traditionally applied in human and veterinary medicine in treatment of diarrhoea and intestinal inflammation. The *ex vivo* anaerobic incubation of *L. salicaria* aqueous extract (LSH) with caecal (CE) and distal colon (DC) content obtained from 6-8 week old piglets has shown that LSH (2mg/mL) significantly modulated the composition of gut microbiota, without having impact on its diversity and metabolic activity. The metabolism of major LSH constituents- ellagitannins to bioavailable urolithins began in CE and continued in DC, however it was not observed for jejunal and ileal content. *In vitro* studies have shown LSH (100 μg/mL) and castalagin (100 μM) to inhibit growth of enterotoxigenic *E. coli* (IMT 0147:K89:K88) and its adhesion to small intestine epithelial cells (IPEC-J2) monolayers. Prolonged (6 day) incubation of IPEC-J2 cells in medium containing LSH (100 and 500 μg/mL) resulted in a significant increase of monolayer integrity, which correlated with elevated claudin 4 production.

Acknowledgment: The project was financially supported by Alexander von Humboldt Foundation.

P-051

ISOLATION OF NOVEL DIMERIC NAPHTHOQUINONES FROM FUNGAL STRAIN MSX53507

Jacklyn M. Gallagher[1], José Rivera-Chávez[2], Laura Flores-Bocanegra[1], Tyler N. Graf[1], Huzefa A. Raja[1], Blaise A. Darveaux[3], Cedric J. Pearce[3], Joanna E. Burdette[4], Nicholas H. Oberlies[1]

[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Institute of Chemistry, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Coyacán, Mexico City 04510, Mexico, [3]Mycosynthetix, Inc., Hillsborough, North Carolina 27278, USA, [4]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, llinois 60607, USA

Fungi have shown to be prolific producers of dynamic secondary metabolites, which exhibit diverse physiological activities. Specifically, naphthoquinones and their derivatives have long been of interest to medicinal and natural product researchers, due to biological activities such as cytotoxicity, antibacterial, antifungal, antiparasitic, and insecticidal. An established dereplication protocol, along with results from preliminary screening of fractions against cancer cell lines MDA-MB-435, MDA-MB-231, and OVCAR3 suggested further investigation of MSX53507 was needed. Bioactivity guided fractionation led to the isolation and elucidation of two known and four new compounds. Of the new compounds, two are an unprecedented, dimeric, 6-12' head-to-tail binding of some of the isolated monomeric naphthoquinones. The other is a new compound which has a bridged eight-member ring system in which the bridge creates two six-membered rings. Interestingly, fungal strain MSX53507 utilizes the same building block to generate some of the new chemotypes identified, and ultimately showcasing the broad and divergent biosynthetic pathways of fungi.

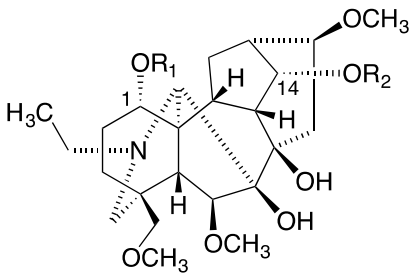
P-052

STRUCTURE-ACTIVITY RELATIONSHIPS AND EVALUATION OF ESTERIFIED DITERPENOID ALKALOID DERIVATIVES AS ANTIPROLIFERATIVE AGENTS

Koji Wada[1], Masuo Goto[2], Takahiro Shimizu[1], Nami Kusanagi[1], Kang-Po Li[2], Kuo-Hsiung Lee[2,3], Hiroshi Yamashita[1]

[1]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 7-15-4-1, Maeda, Teine, Sapporo 006-8585, Japan, [2]Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, USA, [3]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taiwan

Little information has been reported on the antiproliferative effects of the diterpenoid alkaloid constituents of *Aconitum* and *Delphinium* plants. C-1 and 14 esterifications of delcosine (**1**) were carried out to provide 46 new diterpenoid alkaloid derivatives (**2**–**29**, **34**, **2a**–**7a**, **9a**, **13a**, **13b**, **14a**, **14b**, **16a**, **17a**, **24a**, **35a**). Selected compounds (**3**–**14**, **16**–**29**, **3a**–**7a**, **9a**, **13a**, **13b**, **14a**, **14b**, **16a**, **17a**, **24a**, **35a**) were evaluated for antiproliferative activity against five human tumor cell lines including triple-negative breast cancer (TNBC) and P-glycoprotein (P-gp) overexpressing multidrug-resistant (MDR) subline. Several newly synthesized delcosine derivatives (**6**, **7**, **13**, **13a**, **13b**) showed substantial suppressive effects against all human tumor cell lines tested. In contrast, the natural alkaloids (**1**, **31**, **33**) showed no effect. Most of the active compounds were delcosine derivatives with two specific substitution patterns: C-1 and C-1,14. Particularly, 1-acyldelcosine derivative (**5**–**7**) displayed more potency compared with 1,14-diacyldelcosine derivatives (**5a**–**7a**). These acylated alkaloid derivatives caused accumulation of TNBC cells at sub-G1 within 24 h. 1-Acylation of **1** appears to be critical for producing antiproliferative activity in this alkaloid class and a means to provide promising new leads for further development into antitumor agents.



1 : $R_1 = R_2 = H$

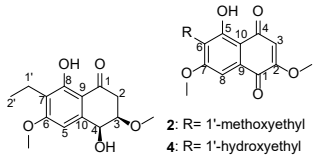
P-053

CYTOTOXIC NAPHTHOQUINONE DERIVATIVES ISOLATED FROM *PYRENOCHAETOPSIS* SP. (MSX63693)

Laura Flores-Bocanegra[1], Huzefa A. Raja[1], Joanna E. Burdette[2], Cedric J. Pearce[3], Nicholas H. Oberlies[1]

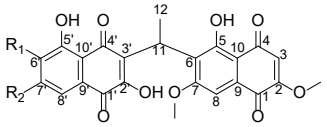
[1] Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412. [2] Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612. [3] Mycosynthetix, Inc. Hillsborough, NC 27278.

In order to discover new anticancer drug leads, our research group has been working on the study of filamentous fungi from the Mycosynthetix library. An organic extract from the fungus *Pyrenochaetopsis* sp. (strain MSX63693) displayed cytotoxic activity against the human cancer cells lines MDA-MB-435, MDA-MB-231 and OVCAR3 with about 14, 28 and 18 % of growth inhibition, respectively, when tested at 20 µg/mL. Bioassay-guide fractionation led to the isolation of three new naphthoquinone derivatives 3*R*,4*S*-7-ethyl-4,8-dihydroxy-3,6-dimethoxy-3,4-dehydronaphthalen-1(2H)-one (**1**), 5-hydroxy-6-[1-(methoxy)ethyl]-2,7-dimethoxy-1,4-naphthalendione (**2**) and the dimer 6-ethyl-3-[1-(5,8-dihydro-1-hydroxy-3,6-dimethoxy-5,8-dioxo-2-naphthalenyl)ethyl]-2,5-dihydroxy-7-methoxy-1,4-naphthalenedione (**3**), along with the known compounds **4**-**8**. Compounds **2** and **3** showed moderate cytotoxic activity, while the known compounds **6** and **8** were the most active.



1

2: R= 1'-methoxyethyl
4: R= 1'-hydroxyethyl
5: R= methyl
6: R= isopropyl acetate
7: R= ethyl



3: R_1= ethyl; R_2= methoxy
8: R_1= methyl ketone; R_2= hydroxy

P-054

SULFUR-CONTAINING ALKALOIDS FROM CULTURED FRESHWATER *DESMONOSTOC* SP. (CYANOBACTERIA).

Lydia Davis, Aleksej Krunic, Jonathan Bisson, Steven Kurina, Joanna E. Burdette, Jimmy Orjala

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA.

The cell and media extracts of cultured freshwater *Desmonostoc* sp. (UIC 10771), collected in Reykjavík, Iceland, showed antiproliferative activity against MDA-MB-435, MDA-MB-231 and OVCAR3 cell lines. Bioassay-guided fractionation of the extracts led to the identification of the known thiazole-containing cytotoxin, Aulosirazole, as well as two novel sulfur-containing alkaloids. Identification of these metabolites was carried

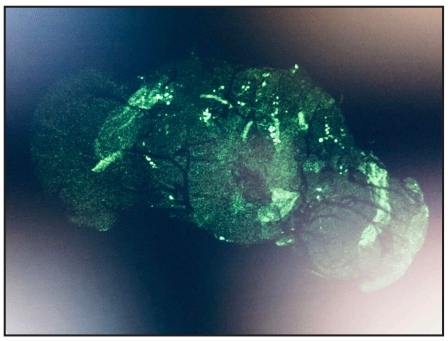
out by HRESIMS- and HRESIMS/MS-guided dereplication, confirmed by 1-D NMR experiments. Isolation and elucidation of the novel metabolites is ongoing and current research will be presented.

P-055

PHYTOCHEMICAL FRIENDS AND FOES - EVALUATION OF NEUROPROTECTIVE EFFECTS OF NOXIOUS SECONDARY PLANT METABOLITES IN A DROSOPHILA MODEL OF PARKINSON'S DISEASE

Urmila Maitra, Thomas Harding, Maggie Owens, and Lukasz Ciesla
Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487

Parkinson's disease (PD) is the second most common age-associated neurodegenerative disorder and is characterized by the loss of dopaminergic (DA) neurons. Emerging studies have identified the beneficial roles of phytochemicals against multiple chronic disorders, including neurodegenerative diseases. However, most of the reported findings are restricted to only a handful of phytochemicals. There is a dire need to develop a low cost, high throughput screening platform to identify phytochemicals that confer neuroprotection. We have developed a novel approach to identify neuroprotective phytochemicals that uses drug screening techniques (cellular membrane affinity chromatography and cell membrane coated superparticles) and a Drosophila model of PD. Using this approach, we have identified two groups of phytochemicals that are protective against PD pathogenesis by modulating the inflammatory response pathways. Here we will present our latest findings on a group of lipophilic phytochemicals that exert disease-preventive actions by engaging adaptive cellular response pathways in cells. We will also provide evidence that intermittent but not continuous pre-treatment with these compounds provide neuroprotective effects.



Brain of transgenic Drosophila melanogaster that expresses green fluorescent protein in dopaminergic neurons.

P-056

PHYTOCHEMICAL INVESTIGATION OF *MIMOSA PIGRA*

Mohammed Hawwal[1,2,3], Omer Fantoukh[1,2,3], Zulfiqar Ali [1], Ikhlas Khan[1,2].
[1]National Center for Natural Products Research, [2]Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, [3]Department of Pharmacognosy, College of Pharmacy, King Saud University, Alriyadh, Saudi Arabia

In Southern Brazil, more than 60 plants were screened for anti-dermatophyte activity, and dichloromethane fractions of methanolic extract of *Mimosa pigra* showed the lowest MIC values (1.9 µg/mL) without DNA destruction at 10 and 50 µg/mL of cell viability of human leukocytes. In relation, this research is aimed at isolating interesting secondary metabolites with promising antifungal activity and safe human toxicity profiles. Powdered leaves of *m. pigra* were extracted by percolation in methanol at room temperature and yielded crude extract after removal of solvent under reduced pressure. The crude extract was fractionated by VLC using reversed-phase C-18 silica with gradient elution of methanol-water (0:1 to 1:0), leading to 16 fractions. All of the fractions were tested against *C. albicans* and several of them showed promising anti-candida activity. The obtained fractions were subjected to repeated column chromatography over Sephadex LH-20, RP-18 silica, and normal phase silica to yield nine phenolic compounds. The isolated secondary metabolites will be tested against *C. albicans* to detairmin which secondary constituents responsible for the anti-candida activity.

P-057

OPTIMIZATION OF PRE-FRACTIONATION FOR INCREASED DETECTION OF GPCR LIGANDS FROM CYANOBACTERIA

Andrea L. Rague and Kevin J. Tidgewell
Department of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, United States

Marine cyanobacteria are known to produce anti-cancer, anti-biotic, and anti-viral compounds, however, the activity of cyanobacterial metabolites in the central nervous system (CNS) has not been well studied. Our lab has screened over 37 cyanobacterial extracts against a panel of GPCRs and the results confirm that these organisms produce metabolites which bind to a variety of CNS receptors. Although many of our extracts have promising activity, one challenge is to isolate *new* natural products amid the *known* major secondary metabolites. In order to increase the detection of minor compounds with CNS activity, there is a need to improve the standard method for cyanobacterial processing. The aim of this study was to analyze the biological data from our cyanobacterial fraction library and to develop a new fractionation method which increases the detection of activity from minor compounds. Our analysis revealed that the greatest number of hits come from the most polar third of fractions, and these fractions comprise 50% of the extracted mass, suggesting that the activity of minor compounds may be undetectable using this method. This data was used to develop a new fractionation method that would result in a more even mass distribution. As a proof of concept, a cyanobacterial sample was extracted and subjected to both the old and new fractionation protocol. The fractions obtained from the two methods indicate that the new method produces fractions with more evenly distributed masses. The expected benefits of even mass distribution are increased reproducibility of hits as well as increased detection of activity from minor compounds.

P-058

PROPOSAL OF MOLECULAR FUNCTIONS ENABLING DEVELOPMENT OF A DEFENSIVE *BURKHOLDERIA* SYMBIONT IN LAGRIA VILLOSA BEETLES

Samantha C Waterworth[1], Laura V Florez[2], Martin Kaltenpoth[2], Ian J. Miller[1], Jason C. Kwan[1]

The most ancient of symbioses between invertebrates and bacteria have been found to rely on the provision of certain functions that enable the host to survive, such as the production of essential amino acids. Some invertebrate symbionts, however, establish their symbiosis with the host through the production of defensive compounds that protect the host from predation and/or disease. In many of these cases the biosynthetic gene clusters encoding the defensive compounds have been identified but the producing bacterium cannot be cultured. Investigation into the bacterial symbionts associated with *Lagria villosa* beetles revealed the presence of a unculturable, symbiotic *Burkholderia gladioli* strain. The strain carried a reduced genome that included a hybrid NRPS/Trans-AT PKS gene cluster that produced a novel, protective, anti-fungal compound called lagriamide. Comparative genomics of binned genomes from metagenomics data will be presented,

proposing potential molecular mechanisms by which the symbiosis was established and had led to the unculturability of the strain.

P-059

MICROBIAL ASSOCIATES INFLUENCE HEALTH OF *TRACHYMYRMEX SEPTONTRIONALIS* FUNGUS GARDENS

Sara P. Puckett[1], Brendan P. Stewart[1], Rofina Johnkennedy[2], Jonathan L. Klassen[2], and Marcy J. Balunas[1,]*
[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269 USA. [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269 USA

Trachymyrmex septontrionalis ants are fungus-gardening ants that live along the Eastern seaboard of the United States. These ants have adaptively incorporated the actinomycete, *Pseudonocardia*, to benefit from the secondary metabolites produced to protect their gardens. However, the garden itself is comprised of many bacteria that may contribute to the garden's overall health through production of antibacterial compounds. The goal of this project is to determine the function of microbial associates within the fungus garden and their contribution to the garden's overall health.

Fungus garden isolates collected from gardens on Long Island were tested for bioactivity against a suite of bacterial pathogens. Initial bioactivity screenings resulted in two strains with strong growth inhibition of *Acinetobacter baumannii* and moderate inhibition of *Pseudomonas aeruginosa* and *Bacillus subtilis*. Using both liquid and solid media, JKS566 and JKS571 produced dark brown coloration when co-cultured with *B. subtilis*, as well as increased antimicrobial activity. Advanced chromatographic and spectroscopic techniques were employed to isolate and identify bioactive components.

P-060

FUNGAL FIGHT CLUB: INVESTIGATION OF INTERSPECIFIC CHEMICAL INTERACTIONS BETWEEN FUNGI USING *IN SITU* CHEMISTRY AND DELETION MUTANTS

Sonja L. Knowles[1], Huzefa A. Raja[1], Allison J. Wright[1], Ann Marie L. Lee[1], Lindsay K. Caesar[1], Nadja B. Cech[1], Matthew E. Mead[2], Jacob L. Steenwyk[2], Laure Ries[3], Gustavo H. Goldman[3], Antonis Rokas[2], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC; [2]Department of Biological Sciences, Vanderbilt University, Nashville, TN; [3]University of Sao Paulo, Brazil.

Fungi have evolved strategies to survive in complex environments, such as the ability to respond chemically to different environmental cues. This, coupled with genomic data indicating that numerous biosynthetic gene clusters are silent, allows us to take advantage of these traits by co-culturing to activate the untapped biosynthetic potential of fungi. This study triangulates information from biology/mycology, metabolomics and genomics, and natural products chemistry to uncover and activate silent gene clusters to search for unique secondary metabolites. To evaluate these, and to gain insight into the secondary metabolic arsenal fungi possess, we co-cultured *Aspergillus fischeri*, a genetically tractable fungus that produces a suite of mycotoxins, with *Xylaria cubensis*, a fungus that produces the fungistatic compound and FDA-approved drug, griseofulvin. The co-culture exposed the activation of several secondary metabolites that were not present in either monoculture. In order to identify which strains likely biosynthesized these metabolites, a combination of genomics and mass spectrometry was utilized. By employing the droplet probe, the spatial distribution of the secondary metabolites during the mono and co-cultures were assessed. To evaluate that secondary metabolites play an important role in defense and territory establishment, we co-cultured *A. fischeri* lacking the master regulator of secondary metabolism *laeA* with *X. cubensis*. We found that the reduced secondary metabolite biosynthesis of the ΔlaeA strain of *A. fischeri* eliminated the organism's ability to compete in co-culture and led to its displacement by *X. cubensis*.

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Fungi naturally grow in competitive environments, and to cope have evolved strategies, such as the ability to biosynthesize a wide array of secondary metabolites. Under standard laboratory culture conditions, the profile of the secondary metabolites that fungi are biosynthesizing is limited to the carbon sources that are in the media. Genomic data from fungi indicate that numerous biosynthetic gene clusters are silent, and thus, the true chemical potential of a fungal culture is perhaps unknown, or at least, unobserved. By taking advantage of the fact that fungi have evolved ways to survive in complex environments, as well as to respond chemically to different environmental cues, co-culturing is a way to activate the untapped biosynthetic potential of fungi. This study triangulates information from biology/mycology (fungal co-culture), metabolomics and genomics (to locate producer species), and natural products chemistry (isolation and elucidation of new chemistry) to uncover and activate silent gene clusters to search for unique secondary metabolites. To evaluate these, and to gain insight into the secondary metabolic arsenal fungi possess, we co-cultured *Aspergillus fischeri*, a genetically tractable fungus that produces a suite of mycotoxins, with *Xylaria cubensis*, a fungus that produces the fungistatic compound and FDA-approved drug, griseofulvin. Initially, the monocultures (standard laboratory culture conditions) of both *A. fischeri* and *X. cubensis* were analyzed to get a baseline understanding of the secondary metabolites biosynthesized. In co-culture, *A. fischeri* increased the production mycotoxins and *X. cubensis* activated several biosynthetic gene clusters. In order to identify which strains likely biosynthesized the activated secondary metabolites a combination of genomics and mass spectrometry was utilized. By utilizing the droplet probe, the spatial distribution of the secondary metabolites during the mono and co-cultures were assessed. To evaluate that secondary metabolites, play an important role in defense and territory establishment, we co-cultured *A. fischeri* lacking the master regulator of secondary metabolism *laeA* with *X. cubensis*. We found that the reduced secondary metabolite biosynthesis of the ΔlaeA strain of *A. fischeri* eliminated the organism's ability to compete in co-culture and led to its displacement by *X. cubensis*.

P-061

NCI PROGRAM FOR NATURAL PRODUCTS DISCOVERY: HIGH-THROUGHPUT ISOLATION OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS FROM A PRE-FRACTIONATED LIBRARY

Tanja Grkovic[1], Rhone K. Akee[1], Christopher C. Thornburg[1], Spencer K. Trinh[1], Jason R. Evans[2,3], John R. Britt,[1] and Barry R. O'Keefe[3,4]
[1]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, [2]Data Management Services Inc., Frederick, MD 21702, [3]Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD 21702. [4]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702.

The NCI Program for Natural Products Discovery (NPNPD) is a newly launched, priority program for the NCI that aims to generate a screening library of 1,000,000 fractions. Here we present a high-throughput, high capacity HPLC-based method for the isolation and identification of natural products sourced from the NPNPD prefractionated library. The procedure is designed as a follow up to initial screening of the fraction library and provide purified or semi-purified natural products in a 96 deep-well plate format. The methodology is reversed HPLC-based, where 1 mg of the hit fraction is chromatographically separated into 22 sub-factions and is capable of processing 40 samples in a single run to generate 880 sub-fractions in an assay ready-format. Examples of rapid isolation and identification of biologically active natural products from plant and marine biota will be presented.

P-062

USING MICROBIAL DRUG DISCOVERY TO BRIDGE BASIC AND TRANSLATIONAL TB RESEARCH IN NORTHERN VIETNAM

Linh Thuy Nguyen[1,3], Tuan Anh Tran[1,3], Lien Kim Thi Pham[2,4], Duc Anh Dang[2], Cuong Van Pham[1], Brian T. Murphy[3]
[1]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam; [2]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; [3]Department of Medicinal Chemistry and Pharmacognosy, Colleague of Pharmacy, University of Illinois at Chicago, Illinois, United States; [4]Institute of Tuberculosis Research, University of Illinois at Chicago, Illinois, United States.

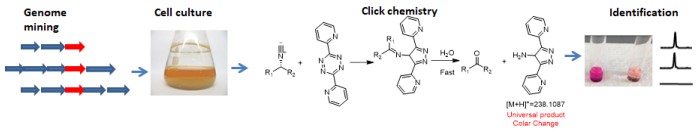
Marine bacteria have been a valuable source of novel secondary metabolites with a wide variety of biological activities, such as antimicrobial, antiviral and antitumor activities (William et al, Nat. Chem. Bio, 2006; János Bérdy, J. Antibiot, 2005). The East Sea of Vietnam, which covers an area of approximately three-million square kilometers, encompasses a multitude of microenvironments covering depths from 200 m to 5,000 m which ultimately select for distinct communities of microbial diversity. The marine biodiversity of the East Sea is considered to be one of the most extensive in the world, but remains poorly understood and unexplored. Through an ongoing NIH D43 program titled "Enhancing basic and translational TB research in northern Vietnam" (a collaboration between the University of Illinois at Chicago, Institute of Marine Biochemistry and the National Institute of Hygiene and Epidemiology in Hanoi, Vietnam; UIC, IMBC, NIHE), we aim to enhance basic and translational research capacity that focuses on the discovery and development of antibiotic leads for tuberculosis (TB). We are building a "smart" microbial strain library from East Sea samples using the bioinformatic pipeline IDBac (Chase M. Clark, Maria S. Costa et al, PNAS, 2018). This growing library will be a resource for our program at IMBC & NIHE, which includes natural product discovery, genome mining, and translational TB bioassay screening. In the future we aim to establish northern Vietnam as a translational screening center for TB, and will facilitate collaboration with institutions across and outside Vietnam.

P-063

COMBINING GENOME MINING AND CLICK CHEMISTRY FOR RAPID DISCOVERY OF NOVEL CYANIDES

Wenlong Cai[1], Yaobing Huang[1] and Wenjun Zhang[1,2]
[1]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720; and [2]Chan Zuckerberg Biohub, San Francisco, CA 94158

Isonitrile moiety containing natural products are uncommon, but they usually show intriguing bio-activities ranging from antibiotics, metal acquisition, detoxification and virulence. Currently there are two biosynthetic mechanisms known to generate isonitrile functionality: isonitrile synthases family (represented by IsnA), and non-heme Iron (II) dependent oxidase/decarboxylase family (represented by ScoE). Preliminary genome mining results indicated that there are abundant of putative secondary metabolite genes in microbes fall into either family, but the final products are still largely unknown. The isonitrile moiety is labile and has weak UV-Vis signatures, making purification and structural elucidation of novel cyanides quite challenging. As part of our novel cyanide discovery project, we have developed a fast, reliable and quantitative method for identification of cyanides from microbes. Tetrazines which selectively react with isonitrile and give a universal final product were chosen and optimized for a click chemistry identifying target natural products directly from culture medium. We have further developed a coupling reaction making it retractable to identity the molecular weight of intact natural product.



P-064

ALTERING THE REGIOSPECIFICITY OF C6-INDOLE PRENYLTRANSFERASES

*Ahmed R Aoun, Tae Ho Kim, Nagaraju Mupparapu, Christopher M Nguyen, Yu-Hsin Lin and Sherif I Elshahawi**
Department of Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, California 92618

Prenyltransferase (PT) biocatalysts are late-stage tailoring enzymes that modify natural products. PTs catalyze the attachment of prenyl moieties to natural product acceptors using pyrophosphate donors. This prenyl modification in small molecules leads to changes in structural and biological activities. Understanding the structural insights as well as the mechanisms by which PTs function allows them to be used as a unique approach towards drug development. PriB PT is an example of aromatic PTs that has been characterized previously as a l-tryptophan (l-Trp) C6 C-prenyltransferase (C6-PT). Analysis of PriB binding pocket, highlighted key residues that might play an important role in determining the regiospecificity of the enzyme and their mechanisms. Hence, we hypothesized that site-directed mutagenesis of one of these residues will alter the enzyme regiospecificity and/or its permissiveness leading to variation in the pharmacokinetics and biological activities of small molecules. Site-directed mutagenesis approach was used to engineer PriB PT as a model for C6-PTs and the purified mutant proteins were produced and purified. In vitro reactions of the purified engineered proteins have shown variability in regiospecificty and/or permissiveness toward substrate acceptors when compared to wild type. The long-term goal for this study is to utilize the permissiveness of PriB enzyme for drug diversification and determine the activity of the mutant PriB enzymes. This study will also shed some light on the mechanistic insights of aromatic C6-PTs.

P-065

MECHANISTIC INSIGHTS INTO SECONDARY METABOLITE PRODUCTION THROUGH INTER-SPECIES INTERACTION IN ACTINOBACTERIA

Deepa Acharya[1], Ian Miller[1], Doug Braun[1], Yusi Cui[1], Marc Chevrette[2], Mark Berres[3], Cameron Currie[2] Lingjun Li[1], Jason Kwan[1], Tim Bugni[1]
[1]School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, [3]Bioinformatics Resource Center, University of Wisconsin-Madison, 425 Henry Mall, Madison, WI 53706

Co-culture has proven to be a remarkable tactic to elicit the production of novel secondary metabolites in various Actinobacteria. Research in our laboratory found that a Rhodococcus sp. induces a Micromonospora sp. to produce an antibiotic, keyicin when co-cultured together. In this dissertation, the mechanism of interspecies interaction in bacteria was studied to understand how bacteria regulate their BGC activation. Multi-omics approaches were applied on keyicin producing Micromonospora sp. in monoculture and co-culture to determine the regulatory bottleneck for the corresponding BGC, kyc. Genome wide transcriptomics using RNA-Seq, proteomics using isobaric tagging snd metabolomics were utilized to elucidate the regulation of keyicin biosynthesis. Moreover, small molecule signaling was found to be key for keyicin production. Quorum sensing regulators like exogenously added acyl homoserine lactones and cyclic dipeptides isolated from the inducing Rhodococcus sp., led to keyicin production. The amalgamation of several omics techniques was powerful in tracking the process of biosynthesis of keyicin from the genome to the metabolite. These interdisciplinary approaches can be utilized to systematically study interspecies interaction, which will equip us with the knowledge to activate other similarly regulated BGCs.

P-066

INVESTIGATION OF SECONDARY METABOLISM IN THE INDUSTRIAL ANAEROBIC ORGANISM *CLOSTRIDIUM SACCHAROPERBUTYLACETONICUM* N1-4

Jeffrey S. Li[1], Colin C. Barber[2], and Wenjun Zhang[1,3]
[1]Department of Chemical and Biomolecular Engineering, University of California Berkeley, Berkeley, CA 94720, USA, [2]Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA, [3]Chan Zuckerberg Biohub, San Francisco, CA 94158, USA

Anaerobes, historically valued for their role in industrial fermentations, are increasingly looked to for natural products discovery. With a relatively large genome size of 6.6 Mb, *Clostridium saccharoperbutylacetonicum* N1-4 is a promising example of an anaerobic source of natural products, featuring seven biosynthetic gene clusters encoding putative polyketide/nonribosomal peptide secondary metabolites. We utilized a transcriptomics-guided approach to identify genes active in secondary metabolism. Comparative metabolomics of a genetic deletion mutant then led to the discovery of a novel compound. The biosynthesis of this compound was investigated using heterologous expression and *in vitro* studies. The biological role of this compound remains to be determined.

P-067

ATTEMPTS TO DEVELOP CRISPR-CAS12A FOR GENOME ENGINEERING OF *BURKHOLDERIA* SP.

Tuan Anh Tran[1,2] and Alessandra S. Eustáquio[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, and Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA, [2]Institute of Marine Biochemistry, and University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

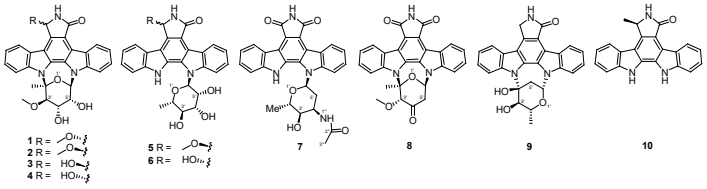
We report our attempts to apply CRISPR-Cas12a for genome engineering of *Burkholderia*, an emerging source of natural products (Kunakom & Eustáquio, *J Nat Prod*, in revision). We used the broad-host-range vector pSL2680 (Ungerer & Pakrasi, *Sci Rep* 2016) to construct a plasmid encoding a guide RNA to direct the Cas12a protein to the desired cut site. Repair homology arms were also ligated in the vector to achieve in-frame deletion of a transcriptional activator of spliceostatin biosynthesis (Eustáquio *et al.*, *PNAS* 2014). We tested various experimental conditions, including designing the plasmid for introducing either one cut or two cuts on the targeted DNA sequence. However, we have been unable to identify mutants with the desired, precise genome editing. Colony PCR and HPLC analyses indicated the possibility of off-target deletions or genome rearrangements, since a) the wild-type PCR band was absent in the clones obtained; b) the band that would indicate in-frame deletion was also absent, potentially indicating larger genome deletion than designed; and c) obtained clones were blocked in spliceostatin production as expected. CRISPR-Cas off-target activity has been reported along with studies to increase its specificity (Kocak *et al.*, *Nat Biotechnol* 2019). We are currently performing genome sequencing to investigate whether off-target effects indeed happened in our case.

P-068

IDENTIFICATION AND STRUCTURE–ACTIVITY RELATIONSHIPS OF MARINE-DERIVED INDOLOCARBAZOLES WITH INHIBITORY ACTIVITY AGAINST PKCΘ

Jinhui Wang, Chengdong Xu, Lele Qin, and Zhongjun Ma
Institute of Marine Biology and Pharmacology, Ocean College, Zhejiang University, Zhoushan 316021, China.

10 new indolocarbazoles (**1**–**10**) were isolated from a marine-derived actinomycete strain. The structures of these compounds were characterized on the basis of extensive 1D, 2D NMR and HRESIMS analysis. The absolute configurations were determined through single-crystal X-ray diffraction, ECD experiment, and comparison of their spectroscopic data. The newly isolated compounds together with other 57 known staurosporine derivatives were evaluated kinase inhibitory activity against PKCθ aimed to probe the preliminary structure–activity relationships. Finally, we found that aminopyranoside group similar to that of staurosporine was necessary for inhibiting the kinase of PKCθ.



P-069

CO-CULTURING MARINE CYANOBACTERIA WITH ANTAGONISTIC ORGANISMS LEADS TO ALTERED EXPRESSION OF BIOACTIVE SECONDARY METABOLITES

Aurora C. Guild, Evgenia Glukhov, and William H. Gerwick
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, United States

Antagonistic interaction between co-cultured organisms has been a productive method by which to activate normally silent secondary metabolite biosynthetic gene clusters. To this end, we performed co-culture experiments with non-axenic, tropical filamentous cyanobacteria in order to evaluate their influence on each other. As identified by 16S rRNA sequencing, two of the cultures were *Leptolynbya* species (ASF and ISB) while the others were *Moorea producens* (PAL and JHB) and *Moorea bouillonii*. All five cultures were isolated from original field collections made in shallow waters near Jamaica (JHB), Palmyra atoll (PAL), American Samoa (ASF), Papua New Guinea (PNG) and Sulawesi (ISB), and have been maintained in culture for between 4 and 24 years. These cultured cyanobacteria have previously been established as producers of a number of potent biologically active natural products such as hectochlorin, the jamaicamides[5], palmyramide A[3], leptochaelin[1], the fagaaluamides[4], cryptomaldamide[6] and curacin D[2]. These four cyanobacteria were co-cultured in a matrix comprised of all possible pairs, and then were further co-cultured with *Candida albicans* with the intention of up-regulating the production of an antifungal secondary metabolite. The crude extracts from each experiment were analyzed by mass spectrometry, MS2-based molecular networking, UV spectrophotometry, biological activity to cancer cells in culture, and antifungal activity bioassays.

P-070

DITERPENOIDS DERIVED FROM THE ANTARCTIC SPONGE *DENDRILLA MEMBRANOSA* DISPLAY POTENT ACTIVITY IN INFECTIOUS DISEASE SCREENING

Andrew J. Shilling[1], Alexandre M. Bory[1], Jessie Adams[2], Ala Azhari[3], Alison Roth[3], Christopher G. Witowski[1], J. Alan Maschek[1], Lindsey N. Shaw[2], John H. Adams[3], Dennis E. Kyle[4], Charles D. Amsler[5], James B. McClintock[5], Bill J. Baker[1].
[1]Department of Chemistry, [2]Department of Cell Biology, Microbiology, and Molecular Biology, [3]Department of Global Health, College of Public Health, University of South Florida, Tampa, FL 33620, USA, [4]Department of Infectious Diseases, Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA, [5]Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

The Antarctic sponge *Dendrilla membranosa* was found to contain diterpenoid secondary metabolites with promising activity in a screening campaign focused on *Leishmania donovani*, *Plasmodium falciparum,* and MRSA biofilm. In total, 13 natural products and 12 semi-synthetic derivatives were isolated or derived from this cold-water poriferan resulting in a library of 22 compounds, with 3 compounds being both isolated directly from the sponge as well as alternatively obtained via semi-synthetic routes. Eight of the 22 compounds within the library are previously unreported structures.

P-071

BIOASSAY-GUIDED ISOLATION OF MANGROVE FUNGAL SECONDARY METABOLITES ACTIVE AGAINST TUBERCULOSIS

Bingjie Yang,[1] Marisa Fuse,[2] Kyle Rohde,[2] Bill J. Baker[1]
[1]Department of Chemistry, University of South Florida
[2]Burnett School of Biomedical Sciences, University of Central Florida

Marine secondary metabolites are known for their structural diversity and bioactivity. They often present novel scaffolds that inspire drug discovery. Mangrove plants live at the marine margin and consequently harbor both terrestrial and marine endophytic fungi. The interaction between mangrove fungi and the mangrove plant can modify the way that plant responds to the environment, which leads to the consideration that mangrove fungi are possible sources to screen for new products. In this project, mangrove endophytic fungi are cultured using three different treatments. Two treatments utilize epigenetic modulators to promote the expression of latent biosynthetic pathways. These methods use 5-azacytidine as a DNMT (DNA methyltrasferase) inhibitor and sodium butyrate as a HDAC (Histone deacetylase) inhibitor. The third treatment is the control culture with no epigenetic modulator agents. Crude extracts prepared from each mature culture are fractionated, first using MPLC and subsequently by HPLC. Using *Mycobacterium tuberculosis* bioassays to guide each fractionation step resulted in the isolation of bioactive compounds that target the bacteria.

P-072

DISCOVERY OF NEW SOUTH CHINA SEA CYANOBACTERIAL METABOLITES LEADS TO THE CLARIFICATION OF AN OLD STRUCTURE AND A DEEPER INVESTIGATION OF ITS ASSOCIATED BIOACTIVITY

Fang Fang[1], Te Li[1], Yiyi Yu[2], Zhengyu Cao[2], Weiyan Zhang[1], Bin Zhang[1], Ye Yuan[1], Lijian Ding[1], Shan He[1], C. Benjamin Naman[1]
[1]Li Dak Sum Marine Biopharmaceutical Research Center, Department of Marine Pharmacy, College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo, Zhejiang 315800, China. [2]Department of Traditional Chinese Medicine Pharmacology, School of Chinese Traditional Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu 211198, China.

Marine bioprospecting was conducted around the South China Sea Paracel Islands during early 2017, with the permission of the local Chinese government and military entities that implement tight restrictions on who can visit or utilize the present maritime resources. The marine natural products that result from investigations of this niche ecosystem are considered to be difficult to access, and precious. The collected samples were extracted and then analyzed by LC-MS/MS untargeted metabolomics using Global Natural Products Social Molecular Networking (GNPS). The resulting molecular network and fragmentation library hit and miss list suggested which groups likely contain new and potentially unique molecules, accordingly providing directions for sample prioritization and targeted isolation research projects. Thus two new bioactive natural products were discovered from a cyanobacterial extract, tentatively identified as a member of *Oscillatoria*. After characterization of these compounds, the total synthesis was undertaken. Due to the structural similarities of a previously reported compound not isolated in this study, malevamide A, this 'old' molecule was also synthesized, and, obscurities in its reported structural configuration were clarified. A broad and deep biological activity testing is underway.

P-073

USING CHEMOGEOGRAPHICAL ANALYSES AND COMPARATIVE GENOMICS TO DERIVE NEW CHEMICAL INSIGHTS FROM THE MARINE CYANOBACTERIUM *MOOREA BOUILLONII*

Christopher A. Leber[1], C. Benjamin Naman[1], Lena Keller[1], Eduardo J. E. Caro-Diaz[1], Tiago F. Leão[1], Nathan A. Moss[1], Valsamma Joseph[2], Jason S. Biggs[3], Lena Gerwick[1], and William H. Gerwick[1]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, 8622 Kennel Way, La Jolla, CA 92093, USA, [2]National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Fine Arts Avenue, Kochi 682 016, Kerala, India, [3]University of Guam Marine Laboratory, UOG Station, Mangilao, GU 96923, USA

The filamentous cyanobacterium *Moorea bouillonii*, widely distributed across the western tropical Pacific and Indian Oceans, is known to be a prolific producer of natural products. However, *M. bouillonii* secondary metabolites have only been described from a limited array of locations, and a greater diversity of natural products could likely be accessed through the study of *M. bouillonii* across a wider geographic area. Extracts of twelve samples of *M. bouillonii* from six different regions were analyzed via LCMS, and the resultant chromatograms were compared using a data pipeline developed for **O**bjective **R**elational **C**omparative **A**nalysis (ORCA). These comparative analyses revealed a that samples clustered together according to geographical region. One MS1 feature found to be driving this clustering was present in high abundance in samples from Saipan, low abundance in samples from Guam, and was undetectable in other samples. Isolation and structure elucidation of this feature yielded a new compound with interesting structural moieties suggestive of unusual biosynthetic processes. Bioinformatics and comparative genomics identified a putative biosynthetic gene cluster.

P-074

DEREPLICATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROSCOPY AND ANTIVIRAL ACTIVITIES OF PHLOROTANNINS FROM *ECKLONIA CAVA*

Thi Phuong Doan[1], Hyo Moon Cho[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

Ecklonia cava is edible seaweed that is found in Asian countries, such as Japan and Korea, and its major components include fucoidan and phlorotannins. Phlorotannins that are isolated from *E. cava* are well-known to have an antioxidant effect and strong antiviral activity against porcine epidemic diarrhea virus (PEDV), which has a high mortality rate in piglets. In this study, the bioactive components were determined based on two different approaches: (i) bio-guided isolation using the antiviral activity against the H1N1 viral strain, which is a representative influenza virus that originates from swine and (ii) high-resolution mass spectrometry-based dereplication, including relative mass defects (RMDs) and HPLC-qTOF-MS/MS fragmentation analysis. The EC70 fraction showed the strongest antiviral activity and contained thirteen phlorotannins, which were predicted by dereplication. Ten compounds were directly isolated from *E. cava* extract and then identified. Moreover, the dereplication method allowed for the discovery of two new phlorotannins. The structures of these two isolated compounds were elucidated using NMR techniques and HPLC-qTOF-MS/MS fragmentation analysis. In addition, molecular modelling was applied to determine the absolute configurations of the two new compounds. The antiviral activities of seven major phlorotannins in active fraction were evaluated against two influenza A viral strains (H1N1 and H9N2). Six of the compounds showed moderate to strong effects on both of the viruses and phlorofucofuroeckol A (12), which showed an EC50 value of 13.48 ± 1.93 μM, is a potential active antiviral component of *E. cava.*

P-075

ISOCOUMARINS, DIKETOPIPERAZINES AND A NOVEL VALEROLACTAM DERIVATIVE FROM *HUMICOLA FUSCOATRA*, AN ENDOPHYTE FROM THE MARINE RED ALGA *ASPARAGOPSIS TAXIFORMIS*

Dulce Silva[1], Iatã Mendonça[1], Alana Honório[1], Victor Rufino[1], Rafael Silva[1], Nair Yokoya[2]
[1]Institute of Chemistry, São Paulo State University – UNESP, Araraquara, Brazil. [2]Institute of Botany, SMA, Sao Paulo, Brazil.

The marine environment is an under-explored source of microbes including bacteria, fungi, actinomycetes, cyanobacteria and diatoms, which are producers of several bioactive secondary metabolites with marked chemodiversity. In the search for new bioactive substances, a fungal strain identified as *Humicola fuscoatra* was isolated from the marine red algae *Asparagopsis taxiformis*, previously collected from Fortaleza beach, Ubatuba – SP. The fungal strain was grown on malt liquid medium for 21 days and then extracted with EtOAc. Its acetonitrile fraction (MeCNFr) showed strong anticholinesterase activity and cytotoxic activity against HCT-116 and MCF-7 tumor cell lines with IC_{50} of 9.8 and 10.3 **μg**/mL, respectively. Fractionation over C-18 silica-gel CC, eluted with a H_2O-MeOH gradient yielded 7 fractions. Frs. 3 and 5 were purified by HPLC-DAD-UV (H_2O-MeCN gradient, 65 to 100% MeCN) and afforded two isocoumarins, the diketopiperazine cycle(Phe-Pro), in addition to a novel valerolactam derivative, which has shown moderate cytotoxic activity. HRESIMS data associated to detailed NMR spectral analyses, including ^{1}H-^{15}N HMBC experiments, indicated its molecular formulae as $C_{12}H_{17}NO_4$, and established its structure as a novel dienone-valerolactam derivative. Such data corroborated the chemodiversity of marine-derived endophytic fungi and its potential as an interesting source for bioprospection.

P-076

CHEMICAL MEDIATION OF THE *CAULERPA* SPP. MICROBIOME

Elena Grigoriou[1], Zachary Golden[1], Ernesto Marticorena[1], Melany P. Puglisi[1], Skylar Carlson[2], Stanley Budzynski[1] and Jason Kwan[2]
[1]Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628
[2]Smithsonian Marine Station at Fort Pierce, 701 Seaway Dr., Fort Pierce, FL 34949
[3]University of Wisconsin, Madison, College of Pharmacy, 777 Highland Avenue, Madison, WI 53705

Thirteen strains of *Vibrio*, including known pathogens to benthic marine organisms, have been isolated from the surface of *Caulerpa cylindracea* in the Atlantic Mediterranean. High densities of microbial populations on the surface of the algae suggest an algal-bacterial association that may increase the fitness of *C. cylindracea*. The objective of this study was to explore the role of metabolites from *Caulerpa* spp. in the formation of the algal microbiome. A panel of 38 strains of surface-associated bacteria (SAB) isolated from *Caulerpa* spp. were used to screen the solvent partitions of common species of *Caulerpa* spp. from the Florida Keys. Minimal growth inhibition (8.4%) and growth inhibition (6.6 %) was observed. Subsequent bioassay-guided isolaton of the active extract from *C. sertularioides* against *Vibrio* sp. from the surface of *C. mexicana* yielded caulerpin and two derivatives that significantly promoted the growth of *Vibrio* sp. below natural concentration (1.8 ug/mL). Settlement assays conducted in the laboratory showed that caulerpin induced settlement of *Vibrio* sp. from the seawater. In addition,

30% of the unidentified SAB tested were induced to settle when exposed to the H_2O partitions from *C. racemosa*, *C. sertularioides* and *C. cupressiodes* and $CHCl_3$ partitions from *C. sertularioides* and *C. cupressiodes*. Caulperin and other metabolites may play a role in the chemical mediation of *Caulerpa* microbiome.

P-077

DISCOVERY, SYNTHESIS, PHARMACOLOGICAL PROFILING AND BIOLOGICAL CHARACTERIZATION OF BRINTONAMIDES A–E, NOVEL DUAL PROTEASE AND GPCR MODULATORS FROM A MARINE CYANOBACTERIUM

Fatma H. Al-Awadhi[1,2], Bowen Gao,[3] Mohammad A. Rezaei,[1] Jason C. Kwan,[1] Chenglong Li,[1] Tao Ye,[3] Valerie J. Paul[4] and Hendrik Luesch[1]
[1]Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kuwait University, Kuwait. [3]State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, China. [4]Smithsonian Marine Station, Fort Pierce, Florida.

Five novel modified linear peptides named brintonamides A–E (**1**–**5**) were discovered from a marine cyanobacterial sample collected from Brinton Channel, Florida Keys. The total synthesis of **1**–**5** in addition to two other structurally related analogues was achieved, which provided more material to allow rigorous biological evaluation and SAR studies. Compounds were subjected to cancer-focused phenotypic cell viability and migration assays and orthogonal target-based pharmacological screening platforms to identify their protease and GPCR modulatory activity profiles. The cancer related serine protease KLK7 was inhibited to similar extents with an IC_{50} near 20 μM by both representative members **1** and **4**, which differed in the presence or lack of the N-terminal unit. In contrast to the biochemical protease profiling study, clear SAR was observed in the functional GPCR screens, where five GPCRs were modulated by compounds **1**–**7** to varying extents. CCR10 was potently modulated by **4** with an IC_{50} of 0.44 μM. We performed *in silico* modeling to understand the structural basis underlying the differences in the antagonistic activity among brintonamides towards CCR10. Due to the significance of KLK7 and CCR10 in cancer progression and metastasis we demonstrated the ability of **4** at 10 μM to significantly target downstream cellular substrates of KLK7 in vitro, and to inhibit CCL27-induced CCR10-mediated proliferation and the migration of highly invasive breast cancer cells.

P-078

REARRANGED STAUROSPORINE AGLYCONE ANALOGUES FROM THE MARINE SPONGE *DAMIRIA* SP.

Trong D. Tran[1,▽], Laura K. Cartner[1,2], Heidi R. Bokesch[1,2], Curtis J. Henrich[1,2], Xin Wang[3], Barry R. O'Keefe[1], Kirk R. Gustafson[1]
[1]Molecular Targets Program, Center of Cancer Research, National Cancer Institute, Frederick, Maryland 21701-1201, USA; [2]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702-1201, USA; [3]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA; [▽]Current address: GeneCology Research Centre, Faculty of Science, Health, Engineering and Education, University of the Sunshine Coast, Maroochydore DC, Queensland 4558, Australia

A collection of the marine sponge *Damiria* sp. collected around Phuket Island, Thailand provided two new alkaloids, damarines A (**1**) and B (**2**) that represent unprecedented analogues of the aglycone moiety of staurosporine (**3**). The pentacyclic indolocarbazole core of staurosporine is derived from two tryptophan moieties that subsequently cyclize in a pseudosymmetric fashion. However, the two indoles in **1** and **2** have opposite orientations so the resulting indolocarbazole is asymmetric. This arrangement has never been observed before in a natural product. In addition, the oxopyrrole in staurosporine is replaced with aminoimidazole and oxoimidazole rings in **1** and **2**, respectively. The structures of **1** and **2** were established by extensive NMR analysis, including application of the LR-HSQMBC pulse sequence, a long-range heteronuclear correlation experiment that has particular utility for defining proton-deficient scaffolds.

P-079

APPLICATION OF NEW NMR METHODOLOGIES IN THE STRUCTURAL CHARACTERIZATION OF A NOVEL FAMILY OF ALKALOIDS FROM THE MARINE ASCIDIAN *POLYANDROCARPA* SP.

Xiangrong Tian[1,2], Dongdong Wang[1], Heidi R. Bokesch[1,3], Gary E. Martin[4], R. Thomas Williamson[5], Kirk R. Gustafson[1]
[1]Molecular Targets Program, Center of Cancer Research, National Cancer Institute, Frederick, MD 21701, USA; [2]Research & Development Center of Biorational Pesticide, College of Plant Protection, Northwest A&F University, Yangling 712100, China; [3]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA, [4]Seton Hall University, Department of Chemistry and Biochemistry, 400 South Orange Avenue, South Orange, NJ 07079, USA; [5]University of North Carolina at Wilmington, Department of Chemistry, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

A series of novel polyhalogenated pentacyclic alkaloids was isolated from an extract of the marine ascidian *Polyandrocarpa* sp. Assembly of the carbon-nitrogen framework was accomplished with the aid of heteronuclear correlation data measured using the 1,1-HD-ADEQUATE pulse sequence. This experiment is based on proton detection of one bond ^{13}C-^{13}C couplings, and its sensitivity is enhanced via BIRD-based homodecoupling of vicinal proton couplings. Thus, 1,1-HD-ADEQUATE provides correlations that are equivalent to 2-bond HMBC correlations, without the ambiguity over 2-bond versus 3-bond correlations in the HMBC experiment. This attribute is particularly useful for establishing the presence of nonprotonated carbons adjacent to protonated neighbors, that are not amenable to detection using H2BC. The new alkaloids contain both Cl and Br substituents, and assigning the sites of chlorination versus bromination is difficult based solely on ^{13}C chemical shift comparisons or calculations. The recently described bs-CLIP-HSQMBC experiment allows detection of the $^{35,37}Cl$ isotope effect on both protonated and nonprotonated carbons. This pulse sequence was crucial to the structure elucidation, as it unambiguously established the chlorination pattern in the new alkaloids.

P-080

A NOVEL, BORON-CONTAINING MACROLIDE AS A MOSQUITO-SPECIFIC TOXIN FOR INSECTICIDE DEVELOPMENT

Jocelyn Macho[1], Peng Fu[2], Kaitlyn Vian[1], Aswad Khadilkar[1], John Abrams[3], John MacMillan[1,2]
[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, [2]Department of Chemistry, University of Texas Southwestern Medical Center, [3]Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390

The rise of mosquito resistance to insecticides escalates the need for novel vector control efforts. As natural products (NPs) display a wide variety of biological activity, we systematically screened our library of NPs for compounds lethal to mosquitos but harmless against other organisms. NP-34, a novel, boron-containing macrolide from a marine-derived *Streptomyces* strain was discovered to be a selective mosquito toxin: in a cell-based assay

of mosquito cell lines, NP-34 showed a 80% kill rate at 50 nM, over fly, moth, and human cells. We aim to identify the mechanism of action and the target receptor(s) of NP-34 via click chemistry, and to explore potency through structure-activity relationship studies in efforts for NP-34 to be employed as an eco-friendly insecticide. We are currently isolating boron-containing macrolides from bacterial-fermentations and semi-synthetically functionalizing analogs in effort to reap the most toxic and most selective version of the NP. Additionally, since host species isolation results in miniscule quantities of NP-34, we are in efforts of heterologously expressing the sequenced biosynthetic gene cluster (BGC) in yeast to maximize production of these B-containing macrolides. To monitor and quantify production of these macrolides, 11-B NMR and ICP-MS methods are being developed.

P-081

TRUNCULINS AND CYCLIC IMINES FROM OKINAWAN MARINE ORGANISMS

Hiromi Hirade[1], Idam Hermawan[1] and Junichi Tanaka[1]
[1]Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

Two new cytotoxic compounds, trunculins X and Y, were isolated from a sponge *Sigmosceptrella* sp. With conventional spectroscopic analysis, their structures were found to be stereoisomers of trunculins, a group of norsesterterpenoid peroxides previously isolated from Australian sponges. The absolute configuration of trunculin X was solved by X-ray crystallography on a crystalline derivative, while that of trunculin Y was solved by analyzing spectral data of several derivatives. Trunculins X and Y showed cytotoxicity at a level of IC_{50} 3.2 and 0.5 μM against NBT-T2 cells, respectively.

A strain of the dinoflagellate *Vulcanodinium rugosum*, collected at Kabira Bay, was cultured. As its extract showed moderate antiviral activity against respiratory syncytial virus (RSV), it was separated to give two cyclic imines. One of them was identified as portimine, while the other was found to be new and named kabirimine. The absolute configuration of portimine was determined by X-ray crystallography. The planar structure of kabirimine and its relative stereochemistry for three partial structures, a spiro-ring, a tetrahydrofuran ring and an epoxide, were elucidated by spectroscopic analyses. The whole relative stereochemistry was elucidated by comparing calculated optical rotation values of all possible isomers with that of kabirimine. Portimine showed potent cytotoxicity at a level of IC_{50} <0.97 nM against HEp2 cells, while kabirimine exhibited moderate antiviral activity against RSV with IC_{50} 4.20 μM.

In this poster, we would like to discuss structures of the above natural products.

P-082

ACCELERATED NMR ANALYSIS OF AN IDIOSYNCRATIC METABOLITE, FATUAMIDE A, FROM THE AMERICAN SAMOAN CYANOBACTERIUM *LEPTOLYNGBYA* SP.

Kelsey L. Alexander[1], Brendan Duggan[2], C. Benjamin Naman[3,4], Arihiro Iwasaki[5], Tiago Leao[3], Eduardo Caro-Diaz[3], Evgenia Glukhov[3], Lena Gerwick[3] and William H. Gerwick[2].
[1]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093. [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093.[3]Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093. [4]Li Dak Sum Yip Yio Chin Kenneth Li Marine Biopharmaceutical Research Center, College of Food and Pharmaceutical Sciences, Ningbo University, Zhejiang, China [5]Department of Chemistry, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

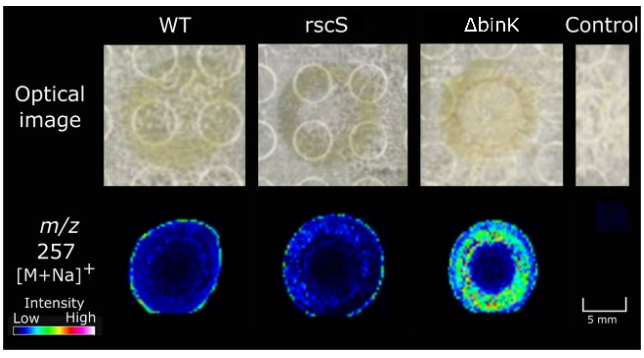
A collection of an American Samoan cyanobacterium *Leptolyngbya* sp. ASX-22JUL14-2 was laboratory cultured to provide an extract cytotoxic to NCI-H460 human lung cancer cells *in vitro*. After molecular networking, targeted isolation of a secondary metabolite from the cytotoxic fraction followed, and a combination of NMR and MS data suggested it was a hybrid PKS/NRPS cyclic peptide. Elucidation of its structure, which contained several idiosyncratic features, required the use of a variety of analytical techniques. 2D NMR, while of critical importance for natural products research, can be very time consuming. Therefore, NMR techniques such as ASAP (Acceleration by Sharing Adjacent Polarization) and NUS (Non Uniform Sampling) were used to enable more rapid data acquisition and expedited structure elucidation. Using these techniques we propose a provisional structure for this metabolite, and from genome sequence data, we propose a plausible biosynthetic pathway for fatuamide A.

P-083

IMAGING MASS SPECTROMETRY REVEALS COLONIZATION FACTORS DRIVING AN ANIMAL-MICROBE SYMBIOSIS

Katherine E. Zink[1], Denise Tarnowski[2], Phillip R. Lazzara[1], Terry W. Moore[1], Mark J Mandel[2], and Laura M. Sanchez[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60610, USA, [2]Department of Medical Microbiology and Immunology, University of Wisconsin - Madison, Madison, WI 53716, USA.

The Hawaiian bobtail squid, *Euprymna scolopes*, has co-evolved with a marine bacterium, *Vibrio fischeri*, in a lifelong symbiosis, providing a two-partner system from which to study host-microbe symbiosis. The squid is born free of symbionts, and selectively acquires a monoculture of *V. fischeri*, which is less than 0.1% of the surrounding bacteria. Using imaging mass spectrometry (IMS), we have detected specialized metabolites that differ between mutants. Differing levels of wrinkled phenotypes have been correlated to *in vivo* colonization capability: ΔbinK outcompetes wild type *V. fischeri* in the squid while *rscS* often cannot colonize. The *m/z* 257 $[M + Na]^+$ is only detected in ΔbinK, indicating it may aid the bacteria during squid colonization. Molecular networking via GNPS gives a strong match to the diketopiperazine His-Pro ($[M + H]^+ = 235.12$). Orthogonal analytical techniques are being employed to validate this assignment and characterize stereochemistry. Further work will explore whether the compound, produced in abundance in a strong colonizer, is an important colonization factor in establishing this animal-microbe symbiosis.



P-084

CRYPTIC NATURAL PRODUCT INDUCTION IN ACTINOMYCETES USING A MICROBIAL "INDUCER"

Libang Liang[1], Brad Haltli[1,2,3], Douglas H. Marchbank[1,3], Hebelin Correa[4], Russell G. Kerr[1,2,3]
[1]Department of Chemistry, University of Prince Edward Island, Charlottetown, PE, Canada, [2]Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada, [3]Nautilus Biosciences Croda, Charlottetown, PE, Canada

More than seventy percent of clinically important antibiotics were isolated from actinomycetes, mainly from terrestrial sources; however, natural products from marine actinomycetes are little studied, and they represent an immense reservoir for natural product discovery. A heat-killed "inducer" supplementation strategy was developed to turn on cryptic biosynthetic pathways in actinomycetes.[1] The strategy involves supplementing autoclaved "inducer" cultures to the fermentations of actinomycetes. This induction method was compared to a conventional co-culture induction method to assess reproducibility and effectiveness in turning on cryptic biosynthetic pathways. As examples, metabolomics analysis of the marine *Streptomyces* sp. RKBH-B178 fermentations led to the identification of a new biotransformation product PQS-GlcA, and upregulation of the new natural product hydrazidomycin D through the heat-killed "inducer" supplementation strategy, while live co-cultivation induced and upregulated two new natural product analogues from another marine strain *Streptomyces* sp. RKND-216. Further natural product screening of the latter strain resulted in identification of a new PK-NRP hybrid antibiotic Levesqamide (IC_{50} = 1.34 μM against *Mycobacterium tuberculosis*) and its biosynthetic gene cluster.

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P-085

USING FUNCTIONAL CHROMATOGRAPHY AND HPLC TO DETERMINE AND CHARACTERIZE NEW BIOACTIVE POLYKETIDE COMPOUNDS FROM CARIBBEAN MARINE SPONGE *PLAKORTIS HALICHONDRIOIDES*

Luis A. Amador[1], Francisco J. Jusino-Alamo[1], Andrew J. Ambrose[2], James J. La Clair[3], Eli Chapman[2] and Abimael D. Rodríguez[1]
[1]Department of Chemistry, University of Puerto Rico, P.O. Box 23346, UPR Station, San Juan, PR. 00931-3346. [2]Department of Pharmacology and Toxicology, College of Pharmacy University of Arizona. 1703 East Mabel Street, P.O. Box 21207, Tucson, AZ. 85721. [3]Department of Chemistry and Biochemistry, UC San Diego, 9500 Gilman Drive, La Jolla, CA. 92093.

A great variety of natural marine compounds have been reported isolated from different species of organisms of the *Plakortis* genus that exhibit important antibacterial, antifungal, antiplasmodial, antitumor activity, among others. Recently, functional chromatography has been described as an affinity purification technique of natural products, which allow to isolate molecules using resins linked to proteins that bind by affinity to target molecules. In this research, functional chromatography was used to isolate three new polyketide compounds from the marine sponge *P. halichondrioides* collected in 2006 in Isla Mona, Puerto Rico. Two fractions were identified, each one composed of a pair of compounds. Fraction 1 contained the known compound Plakortone G (**1a**) and the new compound 9,10-dihydroplakortone G (**1b**), this fraction was hydrogenated to obtain only compound **1b**. Fraction 2 was separated by HPLC obtaining the new compounds Spongosortin B (**2a**) and 11,12-dihydrospongosortin B (**2b**).

P-086

PRELIMINARY STUDY OF BIOASSAY GUIDED ISOLATION PRODUCTS OF THE MARINE SPONGE *MONANCHORA CLATHRATA*

Luis A. Amador[1], Jan Vicente[2] and Abimael D. Rodríguez[1]
[1]Department of Chemistry, University of Puerto Rico, P.O. Box 23346, UPR Station, San Juan, PR. 00931-3346. [2]Institute of Marine and Environmental Technology, Center fon Environmental Science, University of Maryland, 701 E Pratt St Suite 236, Baltimore, MD. 21202.

Marine organisms are an important source of natural products with great potential to be used as drugs in the treatment of different diseases. Secondary metabolites isolated from species of the genus *Monanchora* has been reported for exhibiting different types of activity against malaria and cancer. In the course of continue developing strategies to isolate and characterize marine natural products for purposes to medicinal chemistry, we have focused in the study of *Monanchora clathrata*. First in-house tests using this sponge threw an important toxic activity against *Cyprea tigris*. Thereby, we have found advances with *M. clathrata* extracts in the determination and chemical characterization of the compounds responsible of this bioactivity

P-087

EXTENDING THE SCOPE OF SECONDARY METABOLITES FROM MARINE CYANOBACTERIA OF THE GENUS *LEPTOLYNGBYA*

Yueying Li[1,2], Huashi Guan[2], William H. Gerwick[1]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA, [2]Key Laboratory of Glycoscience & Glycotechnology, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, P.R. China.

Marine cyanobacteria have been identified as rich sources of secondary metabolites. As a well-known genus under the cyanobacteria phylum, *Leptolyngbya* originated from a diverse range of ecological habitats such as marine, fresh water, swamp, rice field and have been implicated in many ecological phenomena. Three *Leptolyngbya* samples were collected from American Samoa and Panama. The samples were grown and purified under a light and temperature-controlled environment with semi-continuous dilution. Cultivation, chemical profiling, biological evaluation and bioinformatics integration in the secondary metabolite discovery were undertaken to develop a better understanding of this genus. By integrating the traditional marine natural product discovery processes with the MS/MS based molecular networking method, chemical profiles of these *Leptolyngbya* samples resulted in the isolation of 10 novel secondary metabolites named pagoamide A, fagaaluamide A-G and fagaaluamide H-I. Detailed HRESIMS, 1D- and 2D- NNR study, advanced Marfey's analysis and chemical hydrolysis and hydrazinolysis reactions were involved to help solve the stereochemistry. Fagaaluamide A was found to modulate neuronal function. Genetic analysis enabled taxonomic assignment and further biosynthetic studies.

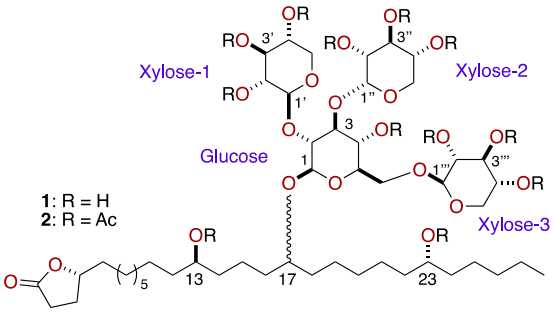
P-088

ABSOLUTE STEREOSTRUCTURE OF A COMPLEX GLYCOLIPID FROM A WEST AUSTRALIAN SPONGE

Mariam N. Salib[1], and Tadeusz F. Molinski[1,2]
[1]Department of Chemistry and Biochemistry, [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0358

An unusual glycolipid (**1**) was isolated from a marine sponge (93-07-67) collected in Western Australia, and its structure was elucidated via integrated spectroscopic analysis of the corresponding peracetate **2**, mass spectros-

copy, degradation, and derivatization. GC-EI-MS of persilylated OMe-glycosides and standards indicated the presence of α- and β- D-xylose and D-glucose. Analysis of $^{1}J_{CH}$, measured in a ^{13}C coupled HSQC experiment, showed α-glucose, α-xylose-3 and β-xylose-1 anomers, and nOe verified α-xylose-2. NMR analysis of **2** identified three 2° OH groups and a terminal butyrolactone. MS fragmentation established the C-17 glycosidic bond, and the remaining OH groups at C-13 and C-23. The absolute configuration of the lactone was assigned as 4*R* by comparison of the electronic circular dichroism (ECD) of the liberated aglycone against that of standard (*S*)-(−)-4-methylbutyrolactone. Acylation of the aglycone with (*R*)- and (*S*)-2-methoxy-2-(naphthalen-1-yl)acetic acid (NMA), and subsequent NMR analysis of the anisotropy of the tri-NMA ester signals supported the 13*R*,23*R*-**2** configuration. The remaining stereocenter at C-17 will be assigned by comparison against two NMA-derivatives of stereodefined standards.



P-089

NEW ANTI-INFLAMMATORY CEMBRANOIDS FROM THE RED SEA SOFT CORAL *SARCOPHYTON CONVOLUTUM*

Mohammed M.A. Ahmed [1,2,3], Mohamed A. Albadry [1, 3], Ehab A. Ragab [1], Elsayed M. El-Ghaly [3], Said K. Ismail[3], Shabana I. Khan [1,2], Amar G. Chittiboyina [1], Ikhlas A. Khan [1,2]

[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA. [2]Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, MS 38677, USA. [3]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Seven new cembranoids; sarcoconvolutumolides A-G (**1**-**7**), along with other seven known cembranoids (8-14) have been isolated from the soft coral *Sarcophyton convolutum*. The structures of the new compounds were elucidated by extensive spectroscopic data analysis including 1D-NMR, 2D-NMR and MS data. The absolute stereochemistry of the new compounds was assigned on the bases of modified Mosher's reaction and circular dichroism (CD) analysis. The total extract and the isolated compounds were found to be non-cytotoxic toward a panel of six tumor cell lines. However, the total extract possessed a good anti-inflammatoy activity via inhibition of iNOS in LPS-induced macrophages (RAW264.7 cells), while compounds **2** and 9 were showed moderate activity. As a result, this species could be a potential source of anti-inflammatory agents.

P-090

BIOSURFACTANTS FROM MARINE CYANOBACTERIA

Jakia J. Mehjabin[1], Julie G. Petitbois[1], Liang Wei[1], and Tatsufumi Okino[1,2]

[1]Graduate School of Environmental Science, Sapporo, 060-0810, Japan, [2]Faculty of Environmental Earth Science, Sapporo, 060-0810, Japan

Biosurfactants are promising amphipathic compounds derived from microorganisms and show a variety of activities such as anticancer, antimicrobial, antiadhesive and anti-biofilm activity against human pathogens. We searched for biosurfactants from marine cyanobacteria, which we have screened for bioactive compounds for a long time. The samples were collected near Kota Kinabalu, Malaysia. Some samples were cultured in our laboratory. Biosurfactants were isolated from the field collected *Moorea bouillonii* and cultured *Leptolyngbya* sp. The compounds from *M. bouillonii* were columbamide type compounds. The one from *Leptolyngbya* sp. contained a long methylene chain, olefinic methine, carbonyl and methoxy group. The isolated compounds were tested by oil displacement assay and Du Noüy ring method. The biosurfactant activities will be presented.

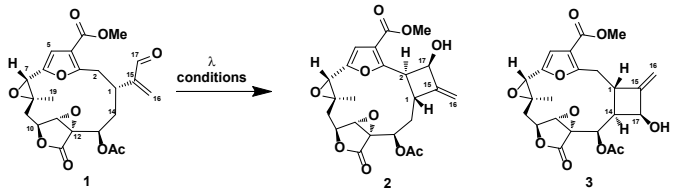
P-091

BIOMIMETIC SEMISYNTHESIS OF (+)-PROVIDENCIN FROM (-)–BIPINNATIN E

Paul D. Scesa, Lyndon M. West

Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, Florida 33431, United States.

A biomimetic semisynthesis of the marine diterpenoid (+)-Providencin (**2**) starting from the related diterpenoid (-)–Bipinnatin E (**1**) is described. Irradiation of **1** with UVA yields **2** via a photochemical Norrish-Yang cyclization, along with the yet unreported regioisomer **3** as the major product. Single crystal x-ray diffraction analysis of **1** allowed the determination of its absolute configuration and thus by chemical correlation the absolute configuration of **2**. This is the first successful synthesis of **2**, for which significant growth inhibitory activity toward cancer cell lines has been reported. These results support the proposed biosynthetic hypothesis describing the photochemical formation of **2**.



P-092

A NEW SESTERTERPENOID AND NEW STEROIDS FROM *MONANCHORA* SP. INHIBIT WNT/B-CATENIN SIGNALING IN COLON CANCER CELLS

Boram Park[1], Nguyen Quoc Tuan[1], Dong Wook Won[1], Sujung Im[1], and MinKyun Na[1,]*

[1]College of Pharmacy, Chungnam National University, 99 Daehak-ro, Yuseong, Daejeon 34134, Republic of Korea

Marine sponges are known as medicinal resources producing for novel bioactive compounds with various pharmacological potential. A marine sponge, a species of *Monanchora* (family Crambidae), was collected from deep waters off the Aleutian Islands of Alaska. Polycyclic guanidine alkaloids from the sponge *Monanchora* sp. were reported to have diverse bioactivities including cytotoxicity, antibiotic, antiviral, and anti-inflammatory effects. In the present study, a new spirocyclic ring-containing sesterterpenoid (**1**) and two new steroids (**2** and **3**) were isolated from a methanol extract of *Monanchora* sp. The chemical structures of new molecules (**1**-**3**) were elucidated on the basis of spectroscopic data analysis (1D and 2D NMR, HRMS). The absolute configuration of compound **1** was determined by comparison of the experimental and calculated ECD spectra. The stereo chemistry of compounds **2** and **3** was deduced from the computational chemical shifts analysis. Treatment with compounds **1**-**3** caused inhibition of Wnt/β-catenin signaling through the down-regulation of cytosolic β-catenin levels in HEK293-FL reporter cells. Compounds **1**-**3** inhibited proliferation of **β-catenin response transcription (CRT)-positive** colon cancer cells (HCT116 and SW480), which might be implicated in the down-regulation of Wnt/β-catenin signaling.

P-093

NEW BIOACTIVE SECONDARY METABOLITES FROM A *SYNOICUM* SP. ANTARCTIC TUNICATE

Sofia Kokkaliari[1], Charles D. Amsler[3], James B. McClintock[3], Alexander D. Crawford[2,4], Bill J. Baker[1,4]

[1] Department of Chemistry, University of South Florida, Tampa, Florida, USA, [2] Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway, [3] Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama, USA, [4] Institute for Arctic & Antarctic Biodiscovery, Medford, Oregon, USA

Marine organisms have attracted the interest of the scientific community in the past few decades, resulting in the discovery of new and novel compounds demonstrating activity against various targets. An area of great interest has been organisms native to Antarctica, due to the circumpolar current which has isolated the ecosystem and is the source of its great biodiversity. In our attempt to isolate new bioactive compounds, a phenotypic zebrafish bioassay was utilized to screen crude extracts. The morphological changes to developing zebrafish embryos were assessed. The Antarctic tunicate *Synoicum* sp. was identified through this process. Following further fractionation, 5 new and 7 known compounds were isolated. The compounds, which belong in the indole and indolone family of alkaloids, were further assessed for their bioactivity. A sequence of MPLC and HPLC purification procedures were performed and the new compounds were identified using 1D and 2D NMR.

P-094

ELUCIDATING THE BIOSYNTHESIS OF PENILUMAMIDE AND EXPLORING THE ECOLOGICAL ROLE OF ITS LUMAZINE BUILDING BLOCK

Stephanie Heard, Jaclyn Winter
University of Utah, Salt Lake City, UT, USA

Folate is an essential B vitamin that humans must acquire through their diet, but bacteria and fungi contain the genes required to synthesize this metabolite de novo. While folate metabolism in microorganisms is crucial, several natural products isolated from a wide variety of sources contain structural components similar to intermediates derived from the folate pathway. Many of these pterins serve as essential redox cofactors, but it has also been suggested that they possess immunomodulatory and anti-diabetic activities. We searched the literature for natural products containing structural components similar to pterins and found the lumazine-containing peptide penilumamide, which was isolated from a marine-derived Aspergillus sp. This small nonribosomal peptide contains an unprecedented 1,3-dimethyllumazine-6-carboxamide functional group coupled to methionine sulfoxide and anthranilic acid. The presence of this lumazine functional group is rare in natural products, making penilumamide an excellent candidate for biosynthetic interrogation and for revealing not only how this unprecedented building block is synthesized, but also to investigate the ecological role of the pterin-like core. The 33 Mbp genome of the penilumamide-producing fungal strain Aspergillus flavipes CNL-338 was sequenced and assembled, revealing 66 biosynthetic clusters of which 21 are nonribosomal peptide-related. Four nonribosomal peptide synthetases (NRPSs) were located adjacent to a suite of genes dedicated to pterin biosynthesis. The biosynthesis of the lumazine building block will be presented, as well as gene inactivation studies and reconstitution of the nonribosomal peptide synthetases.

P-095

STRUCTURE ELUCIDATION OF SPIRODACTYLONE, A POLYCYCLIC ALKALOID FROM THE SPONGE *DACTYLIA* SP., AND GENERATION FROM THE CO-METABOLITE DENIGRIN B

Unwoo Kang[1], Donald Caldwell[2], Laura K. Cartner[1,3], Dongdong Wang[1], Chang-Kwon Kim[1], Xiangrong Tian[1,4], Heidi R. Bokesch[1,3], Martin J. Schnermann[2], Kirk R. Gustafson[1]

[1]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA, [2]Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, [3]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, [4]Research & Development Center of Biorational Pesticide, College of Plant Protection, Northwest A&F University, Yangling 712100, China

Spirodactylone (**1**), an unprecedented polycyclic alkaloid, was isolated from an extract of the marine sponge *Dactylia* sp. The chemical structure was elucidated by extensive spectroscopic methods including LR-HSQMBC. Compound **1** represents a hexacyclic carbon skeleton bearing a unique tricyclic core comprised of indolizinone and cyclohexadienone moieties that are fused to provide a spiro ring system, which has never been reported from natural sources before. Oxidative treatment of the co-metabolite denigrin B (**2**) converted it into **1**, which confirmed the structure and provided insight into the probable biogenesis of spirodactylone (**1**).

P-096

NON-TARGETED HR-LC-MS/MS BASED GNPS PLATFORM FOR THE INVESTIGATION OF BIOACTIVE SECONDARY METABOLITES FROM SOME *AXINELLA* SPECIES

Xavier Siwe-Noundou, Shirley Parker-Nance and Rosemary A. Dorrington. Department of Biochemistry and Microbiology, Marine Natural Products Chemistry Laboratory, Rhodes University, PO Box 94 Grahamstown, 6140, South Africa

Natural products (marine and terrestrial) are known to be the main sources of foods, fragrances, insecticides etc., but more importantly are the principal sources of most of the active ingredients of medicines. It has been reported that more than 80% of available drugs are directly or inspired from natural products. Marine sponges are a rich source of diverse, bioactive secondary metabolites that are potential lead compounds for the development of new therapeutics. This is due to their unique chemical features *(e.g.* common and frequent occurrence of halogen substituents), as well as their targeted biological activities. The focus of this study is on the characterization of secondary metabolites of marine sponges of genus *Axinella* known to produce anti-cancer, antibacterial, anti-inflammatory and neuroprotective compounds, such as alkaloids, cyclopeptides, polyethers, triterpenoids, steroids, and diketopiperazines as well as phenolic compounds.

We investigated the secondary metabolites of some species including *Axinella loribellae*, *A. natalensis* and *A. vermiculata* collected in Algoa Bay South Africa. Crude chemical extracts were analysed by HR-LC-MS/MS (both positive and negative modes) and the resultant MS^2 data loaded into GNPS platform for identification of chemical constituents. The bromine and chlorine patterns observed from the LC-MS data indicated the presence of halogenated compounds. The HR-LC-MS/MS coupled to GNPS allowed the identification of a number of known compounds including: sodwanone H, sodwanone M, sodwanone O, sodwanone P, sodwanone R, sodwanone S and sodwanone V; in addition to potentially new halogenated compounds. We will present our progress in the bioactivity-guided isolation and characterisation of selected known and unknown halogenated compounds from these three sponge species.

P-097

UBIQUITIN-PROTEASOME MODULATING CEMBRANES FROM TAIWANESE MARINE SOFT CORALS

Chang-Yih Duh[1], Xue-Hua Ling[1], and Shang-Kwei Wang[2]
[1]Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung 80441, Taiwan; [2]Department of Microbiology and Immunology, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

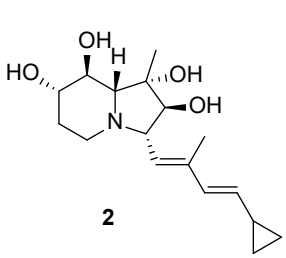
Marine soft corals have evolved unique characteristics in metabolic and physiological capabilities to produce secondary metabolites that may function in defense, food capture, interference competition. Soft coral-derived metabolites exhibit diverse biological activities such as cytotoxicity, inhibition of inflammatory reaction, anti-microbial and anti-viral activities. The ubiquitin-proteasome system (UPS) is a major intracellular, nonlysosomal proteolytic system that is involved in many important cellular pathways, including protein quality control, protein homeostasis, DNA repair, cell cycle progression, pathogen infection, transcriptional regulation, cellular differentiation, and immune modulation. Therapeutic drugs, Bortezomib, Carfilzomib and Ixazomib, target UPS have been licensed in treating multiple myeloma. Moreover, UPS inhibitors are demonstrated to attenuate the progression of neural degenerative disease. Therapeutic prospect of UPS inhibitor is promising and valuable. HCMV UL76 interacts with proteasome regulatory subunits of 26S proteasome. Fluorescence intensities and the phenotypic behaviors of UL76-aggresome are used as markers for proteasome inhibition. We have established a cell-based high-content drug screening assay direct-acting UPS. In total, we have assessed 30 cembranes isolated from Taiwanese soft corals. Four of them were shown to modulate EGFP-UL76 high-content profile in comparative to proteasome inhibitors MG132 and bortezomib.

P-098

CYCLIZIDINE- AND MEDERMYCIN-TYPE ANALOGUES FROM MARINE-DERIVED *STREPTOMYCES* SP.

Yongjun Jiang and Zhongjun Ma
Institute of Marine Biology and Pharmacology, Ocean College, Zhejiang University, Zhoushan 316021, China.

The group of cyclizidine-type alkaloids and medermycin-type naphthoquinones has grown very slowly since the first isolation. However, the unique structures and various biological activities of these compounds have attracted considerable attention from the chemical and biological communities. In the course of our efforts towards search for structurally new and bioactive natural products from marine-derived actinomycetes, eight new cyclizidine-type alkaloids (**1**-**8**), and four new medermycin-type naphthoquinones (**9**-**12**) were identified from the large culture of these strains.[2-3] Biological evaluations of all of the compounds showed that compounds **2**, **5**, **7**, **8** and **12** exhibited significant activity against PC3 and HCT116 human cancer cell lines. All the new medermycin-type analogues (**9**-**12**) showed antibacterial activity against *E. coil* and MRSA, and antifungal activity against *Candida albicans*. Interestingly, Compounds **2**, **5**, **7**, **8** and **12** exhibited moderate inhibition against ROCK2 protein kinase. The structures and their biological data will be presented.



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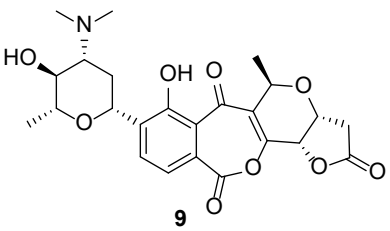


9

P-099

A HIGH-THROUGHPUT METHOD FOR OBTAINING MICROBIAL EXOMETABOLOMICS DATA USING A 3D PRINTED PLATFORM

Caroline Birer[1], Rosalie K. Chu[2], Christopher R. Anderton[2], Erik S. Wright[1]
[1]Department of Biomedical Informatics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15219, United States, [2]Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99354, United States

Microbial interactions are governed by an immense variety of small molecules. To date, our understanding of microbial communication is largely based on a small number of molecules used for quorum sensing. This limitation is partly explained by the low-throughput and high cost of most experimental approaches using mass spectrometry (MS). Here, we engineered a 3D printed device for eavesdropping on the exometabolome of pairs of microorganisms grown in interconnected environments. Our device can be used to easily co-culture microorganisms with or without additional stimuli (e.g. nutrients, drugs, etc.). Coupled with high-throughput robotics (e.g., Triversa Nanomate robot) and MS, the platform can be used to measure an exometabolome in 4 minutes at a cost of only US $2. We have validated our approach with mixtures of 27 known compounds at decreasing concentrations, where we were able to detect 79% and 17% of known compounds 10 nmol and 10 pmol, respectively. Furthermore, we applied the platform to study the exometabolome of common intestinal microorganisms in monoculture and co-culture during growth in the presence or absence of green tea. We have observed the secretion of a number of compounds by *E. coli* that are induced only in the presence of green tea. This approach enables us to process an immense number of samples, unlocking previously insurmountable problems such as decrypting the higher-order principles of inter-cellular communication. Interpreting the language of the microbiome may bring us one step closer to manipulating microorganisms by communicating through small molecules.

P-100

COMPARATIVE MOLECULAR NETWORKING STUDY OF A CYANOBACTERIAL BLOOM AND MARINE SPONGE SHOWS INTRIGUING OVERLAP IN CHLORINATED METABOLITES

Roberta Teta,[1] Gerardo Della Sala,[2] Germana Esposito,[1] Christopher W. Via,[3] Carmela Mazzoccoli,[2] Claudia Piccoli,[2,4] Matthew J. Bertin,[3] Valeria Costantino,[1] Alfonso Mangoni.[1]
[1]Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, via Domenico Montesano 49, 80131, Napoli, Italy, [2]Laboratory of Pre-clinical and Translational Research, IRCCS-CROB, Referral Cancer Center of Basilicata, Via Padre Pio 1, 85028 Rionero in Vulture, Italy,[3]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA, [4]Department of Clinical and Experimental Medicine, University of Foggia, viale Pinto 1, 71122 Foggia, Italy

The bloom-forming cyanobacterium *Trichodesmium thiebautii* and the marine sponge *Smenospongia aurea* have been prolific producers of chlorinated polyketides and hybrid polyketide peptides. A comparative analysis of cyanobacterium and sponge extracts using MS/MS-based molecular networking visualized the chemical space of chlorinated metabolites shared between the two organisms, revealed interesting differences in metabolite composition, and ultimately resulted in the isolation of six new chlorinated compounds (**1**-**6**), four of which were related to the previously characterized trichophycin B and named smenolactones A-D. The two other metabolites were analogs of the previously described compound conulothiazole B. Further investigation of the trichophycin/smenolactone compound class showed that two compounds (trichophycin B and smenolactone C) were

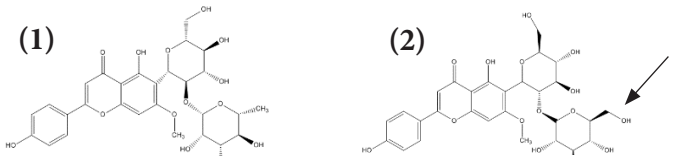
active against MCF-7 human breast cancer cells at sub-micromolar concentrations.

P-101

EXTENDING THE NIST TANDEM MASS SPECTRAL LIBRARY TO IDENTIFY METABOLITES MISSING FROM REFERENCE SPECTRAL LIBRARIES

Meghan C. Burke, William E. Wallace, Stephen E. Stein
Mass Spectrometry Data Center, National Institute of Standards and Technology, Gaithersburg, MD 20899

Natural products are complex mixtures with diverse chemical structures. Mass spectral library searching is used to assign chemical structures to mass spectra; however, identification requires the compound that generated a given query spectrum to be present in the reference spectral library. The recently developed NIST hybrid mass spectral library search extends the scope of mass spectral library searching by matching highly similar mass spectra for which fragment ions that differ by the difference in precursor mass, termed DeltaMass, are also allowed to match. Here, we demonstrate the ability of the hybrid search to identify compounds that are currently absent from the reference spectral library by searching the publicly available Global Natural Products Social Molecular Networking (GNPS) spectral library against the NIST 17 tandem mass spectral library. One example includes spectral assignment of **(1)** 2O-rhamnosyl swertisin, currently absent from the library, to **(2)** Spinosine with a DeltaMass of -15.99578 Da (position indicated with arrow). Results illustrate how the hybrid mass spectral library search can identify high quality spectra corresponding to metabolites that would otherwise remain unidentified.



P-102

CORRELATION OF *MT*SK INHIBITORY ACTIVITY WITH CHEMICAL CONSTITUENTS IDENTIFIED BY HIGH RESOLUTION MS: A BIOCHEMOMETRICS STUDY CASE IN *ALPINIA GALANGA*

Madison Patrick, *Yilue Zhang, Angela I. Calderón.*
Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849

Alpinia galanga, (galangal), has been reported to be active against *Mycobacterium tuberculosis*, and has been used traditionally to treat bacterial infections. 1´-s-1´Acetoxychavicol acetate (ACA) is a known antitubercular compound found in galangal. Based on the literature reports, hexane and dichloromethane (DCM) extracts of galangal were prepared and tested for inhibition of the *Mycobacterium tuberculosis* shikimate kinase (*Mt*SK). *Mt*SK catalyzes the fifth reaction of the *Mt*SK pathway to produce shikimate-3-phosphate (S3P). S3P production was measured using LC-MS Q-TOF. These extracts, along with ACA standard compound and rottlerin, a known SK inhibitor, were tested for their inhibitory activity against *Mt*SK at concentrations of 50 µg/mL and 50 µM, respectively. The hexane extract displayed the highest *Mt*SK inhibition of 47% whereas DCM extract, rottlerin and ACA were all categorized as inactive with *Mt*SK inhibition rates less than hexane extract. The screening results suggest that ACA, an antitubercular compound, may not work through this mechanism of action. A combination of Global Natural Products Social Molecular Networking and Mass Professional Profiler Software was used in the chemical profiling of biactives. Eight known compounds have been successfully identified as present in the extract. Two (acacetin and cirsimaritin) out of six of compounds have not been previously documented as being present in *A. galanga*. LC-MS-based chemical fingerprinting and profiling of the bioactives in the hexane extract is in progress to identify additional chemical constituents that inhibits synergistically *Mt*SK.

P-103

ANALYZING LARGE METABOLOMICS DATASETS USING REPEATED HIERARCHICAL CLUSTERING AND PRINCIPAL COMPONENT ANALYSES IN R

Shaurya Chanana[1], Chris S. Thomas[1], Fan Zhang[1], Tim S. Bugni[1]
[1]Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin, Madison, WI 53705, USA

Analyzing the metabolomics of large systems of microbes using untargeted approaches is strongly implicated in discovering new drugs, understanding ecological niches and habitats, evolutionary mechanisms and even personalized medicine. Thus, there is an urgent need for methods to broadly identify chemical trends in large collections of liquid chromatography-mass spectrometry (LC-MS) datasets. In order to address this need, we have developed an R script that uses repeated binary partitioning via hierarchical clustering analysis (HCA) to sort large datasets into smaller groups based on shared molecular features in an unsupervised fashion. The resulting sub-groups can be further analyzed by other multivariate analyses such as principal component analysis (PCA) to obtain further insights. We used this script to analyze 1,046 LC-MS samples of aquatic Actinobacteria and discovered three new analogs of Lomaitviticin, a small molecule with anti-cancer activity. In order to provide substantial cross-platform compatibility, the script is packaged into a Docker image. Our algorithm can be adapted to different workflows across different disciplines with minor modifications thus facilitating a collaborative workflow with reproducible results.

P-104

CHEMICAL AND BIOLOGICAL INVESTIGATION OF NATURAL PRODUCTS FROM *EPICOCCUM SORGHINUM*

Fang-Rong Chang[1,2,*], *Ching-Chia Chang[1], Chi-Ying Li[1], Yi-Hong Tsai[1], and Chien-Kei Wei[1]*
[1]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, [2] National Research Institute of Chinese Medicine Taipei, 112, Taiwan

In our current research, the subject endophyte *Epicoccum sorghinum* (Sacc.) Aveskamp was isolated from the stem of *Arundo donax* Linn. Its ethyl acetate extract was exhibited anti-inflammatory and cytotoxicity activities. One new compound, named Epicorepoxydon A (**1**), together with six known benzyl-skeleton derivatives (**2**–**7**), one known ethyl phenyl-skeleton derivative (**8**), seven known diketopiperazines (**9**–**15**), and one known steroid (**16**) were discovered through bioassay-guide fractionation. The structures of isolates were established by spectroscopic data, such as NMR and MS spectra. The relative configuration of new compound (**1**) was deduced by the NOESY spectrum. Moreover, its absolute configuration was determined by X-ray single crystal analysis.

Additionally, all isolates were evaluated various bioactivity assays, including cytotoxicity, anti-inflammatory, anti-platelet aggregation, anti-angiogenesis and free radical scavenging activities. Compounds **2** and **6** demonstrated cytotoxic activity against three human cancer cell lines (MDA-MB-231, HepG2, and A549). Compounds **4** and **6** showed anti-inflammatory activity. Compound **2** possessed anti-platelet aggregation activity. Compounds **2** and **6** exhibited anti-angiogenesis activity. And compounds **4**, **5**, **6** and **8** had free radical scavenging activity.

Furthermore, we proposed a biosynthesis pathway of polyketide secondary metabolites and investigated their structure-activity relationship (SAR) of key isolates from this fungus.

P-105

OPTIMIZATION OF TARGETED FUNGAL SECONDARY METABOLITE PRODUCTION USING LC-MS METABOLOMICS

Anne-Claire D. Limon[1], Ala Azhari[2], Dennis Kyle[3], Mario A. Rodriguez[4], Bill J. Baker[1]
[1]Department of Chemistry, University of South Florida, Tampa, FL, USA, [2]Department of Microbiology and Medical Parasitology, King AbdulAziz University, Jeddah, Saudi Arabia, [3]Department of Cellular Biology, Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA, USA, [4]Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Reynosa, Tamaulipas, Mexico.

Microorganisms, specifically fungi, have a significant ability to produce bioactive metabolites that can be used in the discovery and development of pharmaceuticals. Epigenetic regulation is a key mechanism to orchestrate the expression or suppression of gene activity in laboratory conditions; hence, manipulating these mechanisms offers new opportunities to express down-regulated secondary metabolite genes and has the potential to generate new potent and novel metabolites. Although, it is possible to mimic the fungi's natural environment, more evidence correlating specific biological variables with fungal growth is needed in order to optimize the culture's metabolite production yields. To identify a correlation, the fungus of interest was grown on five different media, each exposed to six variables: temperature, light, amount of fungus initially inoculated, pH, salinity and cultivation period. The extraction with ethyl acetate of 520 individual cultivation conditions led to crude extracts, then analyzed by coupled Liquid Chromatography-Mass Spectroscopy. The previous chemical investigation of an unidentified fungal endophyte from a La Encrucijada tropical mangrove of Tapachula (Chiapas, Mexico), treated with an epigenetic DNA methyl transferase inhibitor revealed the presence of a novel sesterterpene active against the *Leishmania donovani* parasite. The data analysis describes which variable or combination of variables is optimal for production of the compound of interest.

P-106

DISCOVERY OF NEW POLYKETIDE-NONRIBOSOMAL PEPTIDE HYBRID METABOLITES FROM GUT OF MUD DAUBER *AMMOPHILA SABULOSA INFESTA*

Joon Soo An[1], Dong-Chan Oh[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea

Insecta is the most diverse class of the Animalia kingdom on the Earth. Symbiotic bacterial communities in insect guts play important roles for hosts in various manners and they are now considered as under-investigated sources for bioactive compounds. We collected the mud dauber *Ammophila sabulosa infesta* specimens from the mount Gwanak in Seoul and isolated bacterial strain (*Streptomyces* sp. #NNI01) from the gut of the insect. LC/MS-based chemical profiling resulted in the discovery of new polyketide-nonribosomal peptide hybrid metabolites. We also discovered additional new secondary metabolites from the bacterial strain NNI01 upon cultivation with supplement of certain types of amino acids. The planer structures of these new compounds were elucidated mainly by NMR and MS spectroscopic analysis. The configurations of these metabolites were determined based on *J*-based configuration analysis, ROESY correlations, and DP4 probability calculation followed by multiple-step chemical reactions including ozonolysis, acid hydrolysis, and Marfey's derivatization.

P-107

INVESTIGATION OF THE CYTOTOXIC CONSTITUENTS OF THE MYCOBIONT *EPICOCCUM NIGRUM* ISOLATED FROM THE LICHEN *NIEBLA HOMALEA*

P. Annécie Benatrehina,[1] Choon Yong Tan,[1] Chad Rappleye,[2] L. Harinantenaina Rakotondraibe[1]*
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210. [2]Department of Microbiology, The Ohio State University, Columbus, OH 43210.

Mycobionts of the macrolichen, *Niebla homalea* (Ach.) Rundel & Bowler (Ramalinaceae) were isolated, and among them *Epicoccum nigrum* (Pleosporaceae, Ascomycota) was found to exhibit antiproliferative activity against the breast (MCF-7) and the ovarian (A2780) cancer cell lines. Fermentations of *E. nigrum* under various liquid and solid conditions were conducted, and interestingly, only the fungal extracts obtained from agar cultures were cytotoxic. *E. nigrum* is a known producer of azaphilone-type compounds, and preliminary chromatographic work on one of the above bioactive extract led to the isolation of acetosellin, a bulgarialactone-type azaphilonoid. ^{1}H-NMR-based chemical profiling of the cytotoxic extracts as well as the isolation and identification of the active constituents will be presented.

P-108

UNTARGETED LC-HRMS METABOLOMICS APPROACH FOR METABOLIC PROFILING OF ECHINOCANDIN B-PRODUCING *ASPERGILLUS PACHYCRISTATUS* NRRL 11440

Bruno Perlatti[1], Nan Lan[1], Yongying Jiang[2], Gerald F. Bills[1]
[1]Texas Therapeutics Institute, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77054, USA. [2]Institute for Applied Cancer Science, M.D. Anderson Cancer Center, Houston, TX 77054, USA.

Aspergillus pachycristatus NRRL 11440 is an industrially important strain used to produce the antifungal echinocandin B and is a sister species of the model organism *A. nidulans*. The two species share approximately 40 secondary metabolite gene clusters but diverge in about 10-15 gene clusters, including those for penicillin and echinocandin. Nevertheless, while over 100 metabolites have been described from *A. nidulans*, only a few metabolites, e.g., echinocandin B and sterigmatocystin, have been reported for *A. pachycristatus*. To better understand species-level differences in the *A. pachycristatus* metabolome relative to *A. nidulans*, ethyl acetate extracts of the NRRL 11440 wild type strain and three strains with disrupted metabolic regulator genes were grown in different media and subjected to untargeted UPLC-HRMS. Data were processed using the Global Natural Products Social Networking (GNPS) and manually curated using the *A. nidulans* metabolome as a proxy. Gene disruptions often profoundly affected metabolite expression. From hundreds of detected peaks, over 40 molecules were putatively identified. We report for the first time that *A. pachycristatus* produces many of the same metabolites as *A. nidulans* including fellutamides, emericellamides, aspernidines, triacetylfusarinines, aspercryptin, and others, from known and unknown biosynthetic pathways.

P-109

ISOLATION OF SECONDARY METABOLITES FROM ENDOPHYTIC FUNGUS ASSOCIATED WITH *DATURA INNOXIA*

Nighat Fatima[1,2], Anaya Eugenio, Gerardo David[1], Abdul Mannan[2], Irum Shahzadi[3], LengChee Chang[4], Esperanza J. Carcache de Blanco[1]*
[1]*Division of Pharmacy Practice and Science and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH,* United States, [2]*Department of Pharmacy* and [3]Department of Biotechnology, *COMSATS University Islamabad Abbottabad Campus, Pakistan,* [4]*Department of Pharmaceutical Sciences, the Daniel K. Inouye College of Pharmacy, University of Hawaii, Hilo, HI, United States*

Crude ethyl acetate extract (prepared by solid state fermentation using sabouraud dextrose agar) of an endophytic fungus isolated from the leaves of *Datura innoxia* was partitioned with hexane and ethyl acetate. The crude extract (CBF-DL-Cr), hexane (CBF-DL-H), ethyl acetate (CBF-DL-E) and aqueous (CBF-DL-A) partitions were used for initial cytotoxic screening against MCF-7, DU-145 and PC-3 cell lines. CBF-DL-Cr was found active in a panel of cancer cells with highest % inhibition in MCF-7 breast cancer cells (87.3%±17.4), followed by DU-145 prostrate cell line (85.1%±4.7). CBF-DL-H partition showed from 74-100% inhibition against DU-145, PC3 and MCF-7 cell lines. CBF-DL-E partition was found active in DU-145 and MCF-7 cell lines with 74 to 93.7% inhibition. CBF-DL-E partition was subjected to fractionation by using normal phase column chromatography. CBF-DL-E4 sub-fraction showed 100%±14.6, 76.3%±5.7, 100%±3.5 % inhibition against DU-145, PC3 and MCF-7, respectively. CBF-DL-E4 is undergoing bioassay-guided fractionation and a preliminary mechanism of action will be pursued for the active compounds isolated from this endophytic fungi active fraction.

P-110

CHARACTERIZATION OF NATURAL EPICOCCAMIDES FROM *EPICOCCUM NIGRUM* ASSOCIATED WITH *TAXUS FAUNA*

Nighat Fatima[1,2,3,6], Tawanun Sripisut[1,7], Ulyana Muñoz Acuña[2], Syed Aun Muhammad[4], Muneer Ahmed Qazi[5], Esperanza J. Carcache de Blanco[2], Safia Ahmed[5], Leng Chee Chang [1,*]
[1] *Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawaii, Hilo, HI,* [2]*Division of Pharmacy Practice and Science and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, United States,* [3]*Department of Pharmacy, COMSATS University Islamabad, Abbottabad, Pakistan,* [4]*Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan Pakistan,* [5]*Institute of Microbiology, Faculty of Natural Science, Shah Abdul Latif University, Khairpur, Pakistan,*[6]*Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan,* [7]*School of Cosmetic Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai, Thailand*

Bioassay-directed fractionation of the ethyl acetate extract from the endophytic fungus *Epicoccum nigrum*, led to the isolation and identification of a novel epicoccamide derivative, epiconigine (**1**) and a known compound, epicoccamide (**2**). Epicoccamide has an unusual structure due to three biosynthetically distinct subunits; glycoside, fatty and tetramic acids. The structures of compounds **1** and **2** were determined on the basis of spectroscopic methods including NMR and mass spectrometry. Compounds **1-2** were found active when evaluated for their cytotoxicity and NF-κB inhibition. Furthermore, docking analysis suggested that these compounds possess strong association with p50 homodimer and p50/p65 heterodimer target proteins responsible for NF-κB inhibition. Further study is worthy based on the activity of these active compounds.

P-111

TWO NOVEL CYCLIC DEPSIPEPTIDES ISOLATED FROM INSECT-SYMBIONT *STREPTOMYCES ALBIAXIALIS* ICBG1318.

Carla Menegatti[1], Vitor Bruno Lourenzon[1], Weilan G. P. Melo[1], Diego Rodríguez Hernández[1], Fabio S. do Nascimento[2], and Mônica T. Pupo[1]
[1]*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil,* [2]*Faculty of Phylosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.*

Microbial symbionts impact their host environment with the ability to biosynthesize a large variety of bioactive natural products. The ancient and complex relationship established between Attini ants and fungi cultivated by them for food is one of the best-known symbiotic associations. However, there is a lack of studies on the microbiota of bees. We pursued efforts on the identification of bioactive natural products from insect symbionts. In this work we describe the isolation and structural identification of two novel cyclic depsipeptides from *Streptomyces albiaxialis* ICBG1318, isolated from the stingless bee *Melipona scutellaris. S. albaxialis* ICBG1318 showed high antimicrobial activity against the entomopathogenic fungus *Beauveria bassiana* and the bacterial entomopathogen - *Paenibacillus larvae*. The bacterium was cultured in ISP-2 agar. Bioassay-guided fractionation of the ethyl acetate extract by SPE and HPLC led to the isolation of two compounds. Analyses of HR-ESI-MS data, 1D and 2D NMR data allowed the structure determination of two novel analogous depsipeptides – VL1 and VL2. 1D and 2D NMR revealed the presence of six unusual amino acids: piperazic acid, hydroxyleucine, hydroxyglycine, methylglycine and two methylalanines. VL-1 and VL-2 have the molecular formula $C_{36}H_{61}N_7O_{12}$ and $C_{37}H_{63}N_7O_{12}$, respectively, determined by high-resolution ESI-TOF mass spectrometry. VL-1 and VL-2 displayed highly selective activity against the entomopathogenic *P. larvae,* which suggest a role in colony protection against this honeybee bacterial pathogen. The establishment of the absolute configuration of VL-1 and VL-2 is in progress.

P-112

ANALYSIS OF NATURAL PRODUCTS INVOLVED IN THE INTERACTIONS BETWEEN *PSEUDONOCARDIA* AND *ESCOVOPSIS* ASSOCIATED WITH *TRACHYMYRMEX* ANTS USING MOLECULAR NETWORKING

Carlismari O. Grundmann, Eduardo A. S. Junior, Weilan G. P. Melo, Norberto P. Lopes, and Mônica T. Pupo
School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, SP 14040900, Brazil

Microorganisms produce a range of secondary metabolites, which have several ecological functions and raise the interest of exploring their chemical potential. In different ecosystems, all living organisms generally are associated with microorganisms through obligate mutualistic relationships. This is the case for ants of the Attini tribe, which have developed over time a relationship of domestication and later mutualism with Basidiomycete fungi that are grown in fungal gardens and serve as the main food source of these ants. Several microorganisms, mainly the fungus of the genus *Escovopsis*, can parasitize the colonies of these farmer insects. As a defense strategy, attine ants have established symbiotic association with actinobacteria such as *Pseudonocardia* to produce substances with antimicrobial activity. Bacterial natural products in this interaction are still poorly explored and may present promising antibiotic activities, contributing for the design of new drugs. In this work, we describe the inhibition of two strains of *Escovopsis* at different levels due to the production of substances by the 39 *Pseudonocardia* strains, isolated from *Trachymyrmex* ants. LC-ESI-MS/MS data were acquired and analyzed by molecular networking using GNPS platform. Initial analysis of the obtained data allowed us to establish the best concentration of inoculum for cocultures experiments and to get information about metabolites possibly involved in the antifungal activity.

P-113

EXPLORING THE UNTAPPED BIOSYNTHETIC POTENTIAL OF A TERMITE-ASSOCIATED ANAEROBE

Colin Charles Barber[1], Jeffrey S. Li[1], Wenjun Zhang[2]
[1]University of California, Berkeley, Berkeley, CA 94720, [2]Chan-Zuckerberg Biohub, San Francisco CA, 94158

Aerobic microbes such as Actinobacteria, Firmicutes, and filamentous fungi have yielded a wealth of natural products, but only a handful of natural products have been isolated from anaerobes.[1] Nevertheless, genomic analyses indicate that anaerobes encode vast, unexpected biosynthetic potential.[2] Herein we describe the biosynthetic wealth of *Ruminiclostridium cellobioparum termitidis* CT1112, a cellulose-degrading gut resident of the wood-feeding termite, *Nasutitermes lujae*.[3] Gene cluster networking and homology-based analyses show *Rct* encodes eight unprecedented NRPS/PKS-containing gene clusters, many of which are expressed during planktonic growth. The *Rct* metabolome also includes several compounds with unique MS^2 fragmentation patterns. Our results indicate that *Rct* may yield several novel natural products.

P-114

USING IDBAC TO UNCOVER THE MICROBIAL AND NATURAL PRODUCT POTENTIAL OF FRESHWATER SPONGES

Chase M. Clark, Antonio Hernandez, Michael Mullowney, Laura M. Sanchez, and Brian T. Murphy
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612

Marine sponges and their associated microbiomes continue to provide a diverse set of medically-relevant natural products. However, there have been few studies on the potential of freshwater sponges to produce natural products (NPs). At least three sponge genera have been reported in the Great Lakes and, like other freshwater sponges, their microbiome diversity is poorly understood. In order to understand the capacity of Great Lakes sponges as sources of NPs, we wanted to first assess the extent of overlap in their cultivable microbiomes. To do this, 900 bacterial colonies were cultivated from the tissue of two *Eunapius fragilis* sponges collected less than a mile apart. Using IDBac we visualized pseudo-phylogenetic and NP-producing patterns and determined that while overlap exists between the two microbiomes, roughly half the isolates grouped according to an individual sponge. To study natural product production, we used IDBac to automate the reduction of the 900 isolates to a highly-diverse set of 160 for bioassay and dereplication studies. This ability to prioritize and illuminate the relationships between isolates, geographic location, methods of isolation, etc., fills a major unmet need in our drug discovery program and is educating our search for antimicrobial natural products from freshwater sponges. Details on this and efforts to discover novel antibiotics from these samples will be discussed.

P-115

A NEW SPIROBISNAPHTHALENE FROM A COPROPHILOUS *PREUSSIA* ISOLATE AND CLARIFICATION OF THE STRUCTURE OF A KNOWN SPIROBISNAPHTHALENE

Cody E. Earp,[1] Nan Lan,[2] Gerald F. Bills,[2] and James B. Gloer[1]
[1]Department of Chemistry, University of Iowa, Iowa City, IA, 52242, [2]Texas Therapeutic Institute, The Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, TX, 77054

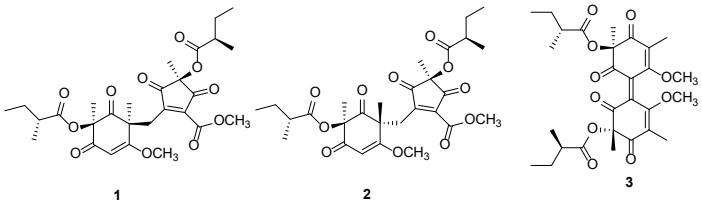
While studying coprophilous (dung-colonizing) fungi as sources of antifungal agents, strain TTI-0686 was obtained from rabbit dung collected in Colorado. Fermentation of this fungus led to production of a crude extract that showed activity against *Cryptococcus neoformans*, *Candida albicans*, and *Staphylococcus aureus*. Silica gel column chromatography, followed by reversed phase HPLC, afforded a known spirobisnaphthalene, along with a new analogue. Analysis of ^{1}H NMR and MS data led to literature reports describing two similar possible structures for the known metabolite, which were reported separately in the literature as spiro-mamakone A and spiropreussione A. Upon closer examination of the reports, it was noted that the two papers had used different NMR solvents. ^{1}H NMR data were obtained in both solvents for the isolated compound, and the results matched the data from both papers, indicating that the two literature reports had characterized the same compound. Further analysis by 2D NMR determined that the correct structure for this compound matches that originally reported for spiro-mamakone A. The structure of the new analogue was established by analysis of MS and 2D NMR data and by comparison with the data for spiro-mamakone A. Spiro-mamakone A showed activity against *C. neoformans* and *S. aureus*.

P-116

BEAUVETETRAONES A-C, PHOMALIGADIONE-DERIVED POLYKETIDE DIMERS FROM THE ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*

Seoung Rak Lee, Jae Sik Yu, Seulah Lee, Mun Seok, Jo, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

We report the isolation of two novel epimeric phomaligadione-derived polyketides, beauvetetraones A-B (**1**-**2**), from the entomopathogenic fungus *Beauveria bassiana*. Beauvetetraones A and B feature an unprecedented methylene-bridged phloroglucinol skeleton with a highly rearranged scaffold. In addition, a dimer of two phomaligadiones, beauvetetraone C (**3**), was isolated for the first time from a natural source and plausible biosynthetic pathway of beauvetetraone A-C was described in our study.



P-117

DONGHAESULFINS A AND B, DIMERIC BENZ[*A*] ANTHRACENE THIOETHERS FROM VOLCANIC ISLAND DERIVED *STREPTOMYCES* SP.

Munhyung Bae,[1] Joon Soo An,[1] Eun Seo Bae,[1] Jedo Oh,[1] So Hyun Park,[1] Yeonjung Lim,[2] Yeon Hee Ban,[3] Yun Kwon,[1] Jang-Cheon Cho,[2] Yeo Joon Yoon,[3] Sang Kook Lee,[1] Jongheon Shin,[1] and Dong-Chan Oh[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea, [2]Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea, [3]Department of Chemistry and Nanoscience, Ewha Womans University, Seoul 03760, Republic of Korea

The chemical analysis of a Streptomyces strain, from a Korean volcanic island, yielded new benz[a]anthracene dimers linked by a thioether bond. The structures of donghaesulfins A and B (**1** and **2**) were elucidated by spectroscopic analysis including energy-dispersive X-ray (EDX). Their configurations were determined by ROESY NMR data, DP4 calculations, the modified Mosher's method, and ECD calculations. Donghaesulfins A (**1**)

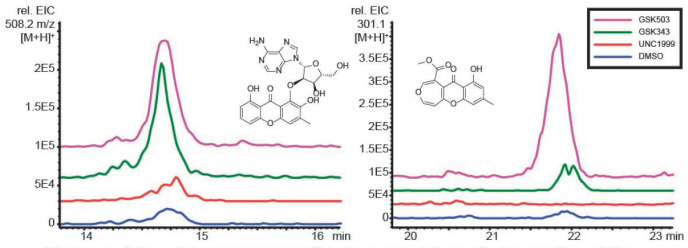
induced quinone reductase, whereas donghaesulfin B (**2**) displayed antiangiogenesis activity.

P-118

HISTONE METHYLTRANSFERASE INHIBITORS AS NEW EPIGENETIC TOOLS FOR THE ACTIVATION OF FUNGAL SECONDARY METABOLISM

Donovon. A. Adpressa[*], *George F. Neuhaus*[a], *Gisela González-Montiel*[a], *Lanelle R. Connolly*[b], *Michael Freitag*[b], *Sandra Loesgen*[a]
[*]*NMR Structure Elucidation, Merck & Co., Inc., Boston, MA 02115.*
[a]*Department of Chemistry,* [b]*Department of Biochemistry, Oregon State University, Corvallis, OR 97330*

It has recently been demonstrated that epigenetic modifications play a fundamental role in the regulation of fungal development and consequently the production of secondary metabolites. This study aims to demonstrate that small molecule histone methyltransferase inhibitors (HMTi) are able to affect secondary metabolism in filamentous fungi. While many chemical HDACi and DNAMTi are routinely used to access otherwise silent metabolites, chemical histone methyl transferase inhibitors have not been applied to the de-repression of fungal secondary metabolism. We performed mechanistic studies using the model organism *F. gramienarum*, which show that select HMT inhibitors succeed in de-repressing secondary metabolism similar to a genetic HMT knockout. Next we used HMTi to treat the non-sequenced ascomycete *Chalara* sp., effecting the production of otherwise silent xanthones. The isolation and characterization of silent natural products, HMTi screening assays, and biological activities will be discussed.



Extracted ion chromatograms for two natural products produced by *Chalara* sp. 6661 upon treatment with HMT inhibitors GSK503 (purple) & GSK343 (green)

P-119

BURKHOLDERIA SPP. FROM FUNGUS-FARMING ANT GARDENS INHIBIT FUNGAL GARDEN PATHOGEN *ESCOVOPSIS*

Daniel S. May, Charlotte Francoeur, Don Hoang, Cameron R. Currie
Department of Bacteriology University of Wisconsin, Madison, WI 53706

Fungus-farming ants (tribe: Attini) cultivate a mutualistic fungus as a food source. This fungus is in turn parasitized by the pathogenic fungus *Escovopsis*. In most cases, attine ants participate in a mutualistic relationship with an Actinomycete, *Pseudonocardia*, that produces metabolites that inhibit the growth of *Escovopsis*. Two ant genera have lost the ability to harbor *Pseudonocardia* on the exoskeleton; however, the fungal gardens of these ants are not overrun with *Escovopsis*, suggesting that other ways exist to control the pathogen infection. In human agriculture, *Burkholderia* have shown promise as biocontrol agents through the ability to produce antifungal secondary metabolites. We have isolated *Burkholderia* strains from attine fungal gardens, some of which inhibited the growth of *Escovopsis in vitro*. Extracts of the inhibitory *Burkholderia* strains were active against *Escovopsis* but not against other fungi. Genomic and metabolomic analyses of the inhibitory *Burkholderia* strains identified two secondary metabolites known to have antifungal properties. The production of both secondary metabolites was required for inhibition of *Escovopsis*, suggesting additive or synergistic activity. The production of antifungal metabolites by *Burkholderia* strains isolated from fungus gardens indicate other means of suppressing *Escovopsis* outside of the ant-*Pseudonocardia* mutualism.

P-120

DISCOVERY OF NEW SECONDARY METABOLITES FROM A FIRE ANT-ASSOCIATED BACTERIUM

Young Eun Du[1], *and Dong-Chan Oh*[1*]
[1]*Natural Products Research Institute, College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea*

Actinobacteria have been recognized as a great source of new biologically active compounds in history. During the chemical studies of actinomycetes isolated from insect ecosystems, we discovered a series of new compounds bearing a new carbon framework with a thioester functional group from a strain from the fire ants, *Formica yessensis*, collected near Namhansanseong Fortress, Republic of Korea. The structures of these new compounds were determined by 1D/2D NMR, HR-MS/MS, UV, and IR spectroscopic analysis. The relative configuration of the 5/6-membered bicycle in the molecule was elucidated by ROESY NMR correlation. The absolute configuration was determined by application of the PGME method onto its carboxylic acid functional group.

Keywords: Fire ants, *Formica yessensis*, PGME method, Thioester functional group.

P-121

ANTIBIOTIC POTENTIAL OF AQUATIC ACTINOBACTERIA FROM ICELAND

Erin Conley[1], *Jeongho Lee*[1], *Maria Sofia Costa*[1,2], *Rui Ma*[3], *Scott Franzblau*[3], *Sanghyun Cho*[3], *Brian T. Murphy*[1]
[1]*Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL, USA,* [2]*Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik, Iceland,* [3]*Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA*

Bacterial natural products have served historically as fruitful sources of antibiotics. However, the discovery of novel antibiotics has slowed drastically in recent decades, while rates of antibiotic resistance have increased exponentially. Generally, taxonomic distance has been shown to correlate positively with distinct natural product production. Thus, understudied bacterial genera have the capacity to yield new chemical space for antimicrobial leads. From a set of aquatic Actinobacteria from Iceland, we used matrix-assisted laser-desorption ionization/time-of-flight mass spectrometry (MALDI-TOF MS) and the IDBac bioinformatics platform to select a subset of bacterial isolates that exhibited distinct patterns of natural product production. Using a custom, reusable 3D-printed bioassay plate, we tested 30 bacterial isolates in 6 nutrient media conditions for antibiotic activity. With this assay, three bacterial isolates of the suborder *Micrococcineae* demonstrated growth inhibition of the prominent human pathogen *Pseudomonas aeruginosa*. Chromatographic and spectroscopic techniques will be used to purify and identify the compounds responsible for the observed antibiotic activity.

P-122

A NEW DECARESTRICTINE-DERIVED METABOLITE FROM A COPROPHILOUS ISOLATE OF *PENICILLIUM SACCULUM*

Dulamini I. Ekanayake,[1] Gerald F. Bills,[2] and James B. Gloer[1]
[1]Department of Chemistry, University of Iowa; Iowa City, IA 52242,
[2]Texas Therapeutic Institute, The Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, 1881 East Road, Houston, TX 77054

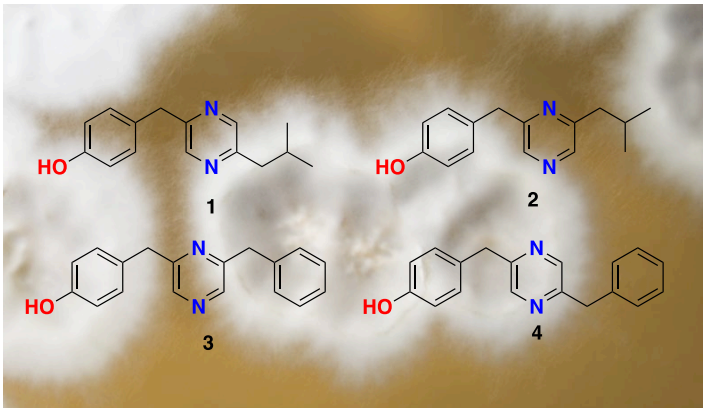
Studies of an isolate of *Penicillium sacculum* (TTI-0705) obtained from mule deer dung from Colorado led to identification of a new decarestrictine-derived metabolite, along with the known compound decarestrictine B. Decarestrictines are 10-membered lactones that are produced by various fungi including other species of *Penicillium*. Some of them are known to have inhibitory effects on cholesterol biosynthesis. The new analog consisted of a decarestrictine subunit linked to an unusual tricyclic tetrahydrofuroindole moiety. The structure of the new metabolite was assigned mainly by analysis of 2D NMR and HRESIMS data. Details of relative configuration of the new compound were elucidated on the basis of NOESY data, ^{1}H NMR comparison with related decarestrictines, and molecular modeling. Although the original extract showed antifungal activity against the human pathogenic fungi *Candida albicans* and *Cryptococcus neoformans*, as well as the bacterium *Staphylococcus aureus*, the new compound described here did not show significant activity in these assays.

P-123

NOVEL PYRAZINE COMPOUNDS FROM A BGC-RICH SOIL BACTERIUM *LENTZEA* SP.

Hong-Bing Liu, Gengxiang Zhao, Jack Davison, Shannon Ohlemacher, *and Carole A. Bewley*
Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0820, USA

In our antibacterial discovery projects three new pyrazine compounds (**1**-**3**) were isolated from the soil-derived bacterium *Lentzea* sp., along with one known analogue (**4**). Their structures were determined by 1D and 2D NMR spectroscopy and HRESIMS. Compounds **2** and **3** represent the first example of 2,6-disubstituted pyrazine compounds from nature. The genome sequence of this *Lentzea* sp. revealed an unusually large number of biosynthetic gene clusters including the proposed pathway for synthesis of this class of compounds.



P-124

FUNGAL METABOLISM OF LICOCHALCONE H

Yina Xiao, Ik-Soo Lee
College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea

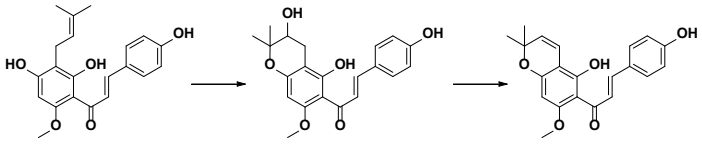
Licochalcone H (LH) is a chemical compound that is a positional isomer of licochalcone C (LC), a retrochalcone isolated from the root of *Glycyrrhiza spp*. Biological studies have suggested that it shows potential anti-cancer activity by inducing cell cycle arrest and apoptosis through the suppression of MATR3 in oral squamous cell carcinoma (OSCC) cells. Since microbial transformation is a useful approach to generate new bioactive metabolites from the substrates, the microbial transformation studies of LH were conducted to identify more metabolites which may enhance its potential anti-cancer activity. As a result of microbial transformations, the hydroxylated and glucosylated derivatives were obtained by the selected fungi. The production and structure elucidation of these derivatives will be presented in detail herein.

P-125

FUNGI-MEDIATED GENERATION OF MAMMALIAN METABOLITES OF XANTHOHUMOL

Fubo Han, Ik-Soo Lee
College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea

Xanthohumol (XN), a major prenylated flavonoid isolated from the female inflorescences (cones) of the hop plant (*Humulus lupulus*), has been used to add bitterness and flavor to beer. It has attracted lots of interest in recent times due to its potential health benefits. And, metabolism of XN and its derivatives in the human body is one of the most pressing issues for pharmaceutical scientists. Thus, the study for the development of in vitro microbial model for simulation of metabolism of XN in vivo was carried out. Different fungal strains were screened for their potential to transform XN to isoxanthohumol and other mammalian metabolites. Among the fungi screened *M. hiemalis* was the most active producer of the mammalian metabolites which included hydroxylated and cyclic dehydro-derivatives of XN. The results proved the potential of *M. hiemalis* in the production of mammalian metabolites of XN in large quantities and also as an in vitro model for metabolism studies of XN.



P-126

HOST DEFENSE AND REGULATION BY METABOLITES FROM BACTERIAL SYMBIONTS OF FUNGI

Joseph P. Gerdt[1], Emily Mevers[2], Jon Clardy[2]
[1]Department of Chemistry, Indiana University–Bloomington, 800 E. Kirkwood Ave., Bloomington, IN 47405, [2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115

Fungi play critical roles in human life. Some fungi cause deadly infections in humans, and others produce paradigm-shifting drugs. Some fungi are necessary for healthy forests and crops, whereas others cause devastating blights. This diverse kingdom of life is further elaborated by hidden interactions with bacteria. Certain bacteria are enriched in areas surrounding fungi, others ride along fungal surfaces, and some even reside within fungal cells. What impact do these bacteria have on their host fungi, and which metabolites drive these effects?

My research group is isolating bacteria from basidiomycete fungi in nature and uncovering metabolites that provide defense to the host fungus and/or elicit developmental changes in the host fungus. These metabolites not only play a vital role for the ecosystem but can also be co-opted for use as antimicrobials, pain modulators, and probes of conserved eukaryotic targets. This poster presents current progress in characterizing metabolites with the following profiles: antifungal, antibacterial, inhibition of bacterial virulence, modulation of human pain receptors, and inhibition of tumor growth.

P-127

CUAUTEPESTALORIN, A DIHYDROCHROMENE-OXOISOCHROMANE ADDUCT FROM *PESTALOTIOPSIS* SP. IQ-011

José Rivera-Chávez[1], Jade Zacatenco-Abarca[1], Jesús Morales[2], Blanca Martínez-Aviña[1], Simón Hernández-Ortega[1], and Enrique Aguilar-Ramírez[1]
[1]Departamento de Productos Naturales, Instituto de Química, Universidad Nacional Autónoma de México, 04510 Mexico City, Mexico, [2]CONACYT-Consorcio de Investigación, Innovación y Desarrollo para las Zonas Áridas (CIIDZA), Instituto Potosino de Investigación Científica y Tecnológica A. C., Camino a la Presa San José 2055, Lomas 4a sección, 78216 San Luis Potosí, Mexico

Multi-informative analysis of the bioactive extract from *Pestalotiopsis* sp., led to the isolation of a new 7,8-dihydrochromene-oxoisochromane adduct (**4**, cuautepestalorin), bearing a spiro-polycyclic ring system (6/6/6/6/6/6), along with its putative and new biosynthetic precursors, cytosporin M (**1**), cytosporin N (**2**), ox-opestalochromane (**3**), and the known benzophenone, pestalone (**5**). The structures of compounds **1**-**4** were elucidated using a set of spectroscopic (1D and 2D NMR) and spectrometric data (HRMS), while their absolute configuration was established using chiroptical methods (ECD) combined with TD-DFT calculations and X-ray crystallography. The α-glucosidase inhibitory properties of compounds **1**-**5** were evaluated *in vitro*. The results showed that compounds **3** and **4** inhibited α-glucosidase from *Saccharomyces cerevisiae* (αGHY), with IC_{50} values in the μM order, comparable with that of the positive control acarbose.

P-128

CYTOCHROMES P450 IN BACTERIAL NATURAL PRODUCTS BIOSYNTHESIS

Jeffrey D. Rudolf[1]
[1]Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

Cytochromes P450 (P450s) perform oxidative transformations on a wide range of structurally diverse molecules. P450s are essential for the biosynthesis of biologically active natural products; introduction of even one hydroxyl group, the most common P450-catalyzed reaction, can cause dramatic changes in compound solubility, biological activity, efficacy, and toxicity. Microbial P450s, and those from bacteria in particular, have been crucial for revealing their diverse roles in nature, expanding their catalytic repertoire, creating structural and mechanistic paradigms, and exposing their potential for biomedical and biotechnological applications. Despite this, our understanding of the true functional capacity of these enzymes is limited. For example, there are >16,000 putative P450 sequences from Streptomyces, the workhorse producers of natural products. Less than 250 (<1.5%) of these P450s have been functionally characterized and many of these remain orphan (natural substrate and function remains unknown) P450s. Our lab aims to utilize sequence similarity networks to discover P450s that catalyze novel reactions, functionalize unique scaffolds, and are important for natural products biosynthesis.

P-129

THE NATURAL PRODUCTS ATLAS: AN OPEN ACCESS PLATFORM FOR NATURAL PRODUCTS DISCOVERY

Jeffrey A van Santen, Roger G. Linington
Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

In the era of big data, access to a central repository of information on natural product structure and origin would be an invaluable tool for the natural products community. With the assistance of a large team of volunteers, we have developed a database platform of all known microbial natural products, that we call the Natural Products Atlas (NP Atlas). Our aim is to provide a community-driven resource for all data related to microbial natural products, including structures, names, points of origin, structural reassignments, total syntheses, and links to external databases such as MIBiG. Additionally, the ***npatlas.org*** website includes numerous tools for exploring natural product chemical space, mass-based dereplication, and much more. We have also developed an online data curation platform to enable seamless collaboration with volunteers. We will present our latest version of the database, demonstrate the functionality of the web-based tools, and discuss future development and goals of the resource.

P-130

AN IMPROVED DEREPLICATION STRATEGY FOR NOVEL ACTIVE MARINE NATURAL PRODUCTS DISCOVERY

Jia-Xuan Yan[1], Fan Zhang[1], Doug R. Braun[1], I-Wei Tsai[1], David R. Andes[2], Kenneth Barns[3], Gene E. Ananiev[3], and Tim S. Bugni[1]*
[1]Pharmaceutical Sciences Division, University of Wisconsin–Madison, Madison, WI, 53705, USA, [2]Department of Medicine, University of Wisconsin–Madison, Madison, WI, 53705, USA, [3]Small Molecule Screening & Synthesis Facility, UW Carbone Cancer Center, Madison, WI, 53705, USA

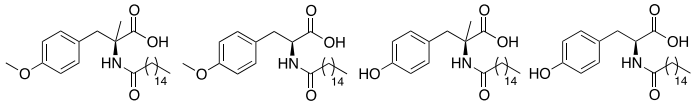
Drug resistant infectious diseases continue to threaten global health as well as contemporary medical practices. There is an urgent call for novel antibiotics discovery. Mining novel sources, such as the marine bacteria, will clear the way for chemical and biological novelties. Our lab employed LC/MS-principal component analysis (PCA) based strain selection followed by an automated, high-throughput LC/MS fractionation to generate marine bacterial natural product libraries for antimicrobial activities screening. Fractions with antibacterial or antifungal activities were analyzed by NMR and UHPLC/HRMS. The biological activity data and the spectroscopic data provided information for dereplication and further purification of active compounds. By utilizing this platform, a series of antibacterial compounds were discovered rapidly and their biosynthetic pathways were proposed. Overall, our result highlights the advantages of applying modern analytical techniques in marine natural product libraries to accelerate novel antibiotics discovery.

P-131

AN ICHIP-DOMESTICATED *ALTEROMONAS* SP. PRODUCES A UNIQUELY FUNCTIONALIZED *N*-ACYLTYROSINE

Logan W. MacIntyre[1], Marie J. Charles[2], Bradley A. Haltli[1,4], Douglas H. Marchbank[3,4] and Russell G. Kerr.[1,3,4]
[1]Department of Biomedical Sciences, [2]Department of Biology and [3]Department of Chemistry, University of Prince Edward Island, Charlottetown, PE, C1A 4P3, [4]Nautlius Biosciences, Charlottetown, PE, C1A 4P3.

It is well established that the isolation chip (ichip) allows for high-throughput *in situ* cultivation of bacteria while simultaneously affording pure cultures; however, reports of its use in natural product discovery are surprisingly sparse and to our knowledge limited to soil/sediment. The subject of this presentation is implementation of the ichip in the marine sponge *Xestospongia muta*. We emphasize isolation of a seemingly new bacterial species belonging to the genus *Alteromonas* that produces a new *N*-acylated amino acid. *N*-Palmitoyl-α,*O*-dimethyl-L-tyrosine (**1**) bears a methyl substituent at the α-position and exhibits Gram-positive antibacterial activity. Through an SAR experiment that includes synthetic analogues **2**-**4**, we conclude that the α-methyl imparts greater Gram-positive activity to *N*-acyltyrosines. As part of our ongoing efforts to characterize the biosynthesis of **1**, we will also discuss identification of proposed intermediates and putative biosynthetic genes.



P-132

ISOLATION AND CHARACTERIZATION OF NEW TETRACYCLIC MEROTERPENOIDS VIA BIOACTIVITY GUIDED FRACTIONATION

Mario Augustinović[1], Laura Flores-Bocanegra[1], Daniel A. Todd[1], Huzefa A. Raja[1], Steven Kurina[3], Cedric J. Pearce[2], Joanna E. Burdette[3], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, [2]Mycosynthetix Inc., 505 Meadowlands Dr. # 103, Hillsborough, NC [3]Department of Medicinal Chemistry and Pharmacognosy, The University of Illinois at Chicago, Chicago, Illinois

As part of an ongoing investigation for the isolation of novel anticancer drug leads from nature, fungal strain MSX62440 was found to possess micromolar inhibition activity against three cancer cell lines. Following large scale fermentation and extraction of MSX62440, three new meroterpenoids were subsequently isolated and characterized using bioactivity guided fractionation. Literature suggests only a handful of these compounds, known as the dasyscyphins, exist. These secondary metabolites contain indane ring system fused with a drimanyl moiety, leading to a set of tetracyclic sesquiterpenoids. NOESY correlations allowed for the assignment of relative configuration, while DFT calculations are ongoing for the determination of absolute configuration.

P-133

CHEMICAL AND BIOLOGICAL STUDIES OF ASCOMYCETES FROM CUATRO CIENEGAS BASIN, COAHUILA, MEXICO

Itzel R. Yeverino and Mario Figueroa
Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México 04510, México.

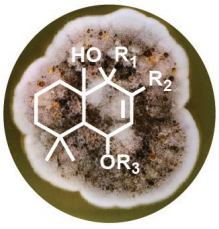
As part of our continuing search for novel antimicrobial compounds from unexplored habitats of Mexico, a series of 20 saprotrophic fungi were isolated from soil and sediment samples collected at the Cuatro Cienegas (CC) Basin, Coahuila, Mexico. Taxonomic diversity of fungal isolates was assessed by ITS barcoding. The organic ($CHCl_3$-MeOH) extracts from the axenic solid (moisture rice) cultures were tested against *E. coli*, *S. typhi*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *C. albicans*. Additionally, they were dereplicated against a database containing more than 300 fungal secondary metabolites via recording UPLC retention times, UV data, and HRESIMS-MS/MS spectra. Bioactive-guided fractionation (growth inhibition > 80% at 20 mg/mL and negative dereplication) of the scaled-up (10×) cultures led to the isolation of five butyrolactones (**1**-**5**) from the *Aspergillus* sp. (CC1-1); the cochliodinol (**6**) and a new derivative (**7**) from a fungus of the order Sordariales (CC9-6); and the ergochrome neosartorin (**8**) from the *Aspergillus* sp. (CC7-12). Their structures were elucidated using 1D and 2D NMR and HRMS data analysis, and compounds **6** and **7** were active against MDA-MB-435, MDA-MB-231 and Ovcar 3 cell lines (IC_{50} between 1.51 and 12.32 μM).To the best of our knowledge, this is the first report of chemical and biological studies of Ascomycetes isolated from the CC.

P-134

ANTIBACTERIAL DRIMANE SESQUITERPENES FROM *ASPERGILLUS USTUS*

George F. Neuhaus[1], Sandra Loesgen[1]*
[1]Department of Chemistry, Oregon State University, Corvallis, OR 97331, USA

Fungal natural products has helped to evolve modern medicine. In our quest for new antibiotics, we identified a culture of *Aspergillus ustus* that shows potent inhibitory activity against Gram-positive bacteria including *B. subtilis* (ATCC 49343), vancomycin-resistant *E. faecium* (ATCC 700221), and multidrug-resistant *S. aureus* (ATCC BAA-44). Chemical analysis led to the isolation of five new acylated drimane sesquiterpenes. Structure elucidation of the new compounds was performed using HRMS, 1+2D NMR techniques, and chemical derivatization. Relative configuration was determined using a NOE based analysis, while the absolute configuration was established by computation of chiroptical properties for comparison to experimental data. Two of the new compounds were found to contribute to the antibacterial activity. *Aspergillus ustus* has proven to be a rich source for chemical diversity and here we report the isolation of new antibacterial drimane sesquiterpenes.



P-135

AN EFFICIENT APPROACH FOR IDENTIFYING ANTAGONISM AMONG FUNGI SPECIES AND ANTIFUNGAL ACTIVITY

Airton Damasceno Silva[1], Alessandra R. P. Ambrozin[2], Renato L. Carneiro[1], Paulo C. Vieira[1,3]

[1]Departament of Chemistry, Federal University of São Carlos, São Carlos, SP, 13565-905, Brazil, [2]Institute of Science and Technology, Federal University of Alfenas, Poços de Caldas, SP, Brazil, [3]Departament of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14040-903, Brazil,

New antifungals are increasingly needed especially due to the emergence of resistant fungal strains. Traditional antifungal assays are laborious and require significant amounts of samples. In this paper we discuss a new proposal to evaluate antifungal activity and antagonism among fungal species. The main idea is to use experiments of fungal culture and co-culture, ^{1}H NMR data and chemometrics. The experiments were developed and proved by the accomplishment of fifty-seven co-culture and axenic cultures of six distinct fungi. The obtained data showed that this aproach proved to be an excellent way to obtain bioactive compounds, since it was able to predict the activity of four different extracts in a collection of sixty-three, which would be much more difficult and time consuming if applied randomly. The antifungal activities have been proven by standard in vitro assays.

P-136

GENOMIC APPROACH TO DISCOVER NEW PIPERAZIC ACID-CONTAINING SECONDARY METABOLITES BY UTILIZING AN ACTINOMYCETE DNA LIBRARY

Daniel Shin, Donghee Woo, Woong Sub Byun, Sang Kook Lee, Dong-Chan Oh

Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea

Starting from the recently reported genetic information of two key piperaizc acid biosynthesis enzymes, l-ornithine *N*-hydroxylase and N-N bond formation enzyme, we designed sets of oligonucleotides targeting the genes of these enzymes, separately. The designed primers were applied to the DNA library of 1,000 actinomycetes strains that we built, and dozens of strains were identified as potential producers of piperazic acid-containing compounds. By cultivating identified strains with humic acid, it was able to discover a series of new piperazic acid-containing cyclic peptides from a *Streptomyces* sp. PC5. The major compound PC5-A displayed anti-proliferative activity against various cancer cell lines. This result demonstrates that this DNA library-based genome mining is a useful tool to discover new natural products with a piperazic acid moiety.

P-137

ATYPICAL CHEMICAL ELICITORS FOR ACTIVATION OF SILENT BIOSYNTHETIC GENE CLUSTERS

Robert M. Samples[1,2], Marcy J. Balunas[1,2,]*

[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269, USA; [2]Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, CT 06269, USA

Microbial secondary metabolites or derivatives thereof constitute the majority of clinically important antibacterial and antifungal agents. With the rise of widespread resistance to these agents, the need for novel antimicrobials has become more urgent. Despite the large number of biosynthetically active strains and improved analytic tools available, discovery of novel compounds still poses numerous challenges in part due to silent biosynthetic gene clusters that are not active under typical laboratory cultivation conditions. Herein, we report the activation of silent and/or weakly active biosynthetic gene clusters in the tunicate-associated *Streptomyces* sp. PTY087I2, a granaticin producer, using media optimization and chemical elicitation. This approach resulted in significantly increased production of granaticin A as well as production of a suite of previously undetected metabolites. Novel strategies for activation of biosynthetically active gene clusters provides a promising approach for the discovery of bioactive microbial secondary metabolites.

P-138

NEW TOOLS FOR TARGETED CLONING AND OVER EXPRESSION OF BIOSYNTHETIC GENE CLUSTERS

Robert Stankey, Don Johnson, Joyanne MacDonald, Phil Brumm, and David Mead

Varigen Biosciences Corporation, 505 S. Rosa Rd. Ste. 15, Madison, WI, 53719

The genome sequencing revolution and development of biosynthetic gene cluster (BGC) prediction and analysis tools unlocked a wealth of new biosynthetic potential for further examination. Isolating a DNA clone for a BGC of interest for expression, refactoring, *etc*., can be a slow, expensive process due to their large size. Traditional cloning can take months to complete, and gene synthesis is expensive and stymied by GC-rich and/or repetitive sequence. Here we describe a rapid technique to directly clone large BGCs from genomic DNA without gels or agarose plugs. Using CRISPR-Cas9 on intact genomic DNA, we restricted regions flanking BGCs of interest. Linearized BAC vectors with overlaps matching the BGC cut sites were prepared, and the vector and restricted DNA assembled and transformed to *E. coli*. 31 BGCs from 27 Actinobacteria and fungal strains have been successfully captured, ranging from 12 to 96 kb (median = 49) in size. Starting from a cell pellet, this technique takes ~5 days to generate a BGC clone, which is directly ready for heterologous expression studies. To improve the success of heterologous expression a new *Streptomyces* BGC expression vector was developed which uniquely includes *two* inducible promoter elements, one flanking each side of the cloning site. The ACT (21 kb) and RED (33 kb) BGCs from *S. coelicolor* were cloned in both orientations of the pDualP vector and integrated into *S. lividans* ΔredΔact. We observed inducible production of the blue product of the ACT cluster and the red product of the RED cluster but not from the native promoters in this heterologous expression experiment. These results indicate that virtually any sequenced BGC can be cloned intact from complex genomes, and direct cloning to a dual-inducible heterologous expression vector can greatly accelerate downstream small molecule characterization.

P-139

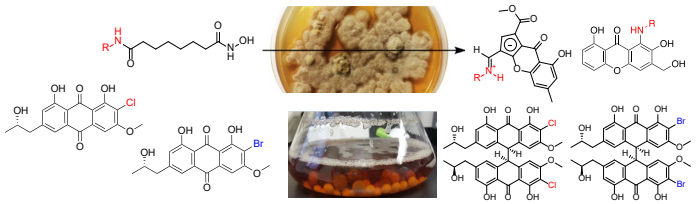
'UN-NATURAL' NATURAL PRODUCTS FROM FUNGI: FROM FUNGAL STRESS TO BIOTRANSFORMATIONS

Paige E. Mandelare, Elizabeth N. Kaweesa, Mahsa Khoshbakht, Jason Srey, Donovon A. Adpressa, Sandra Loesgen

Department of Chemistry, Oregon State University, Corvallis, OR 97330

Microorganisms produce genetically encoded secondary metabolites that have critical roles in intra- and inter-species communication, competition for resources, or defenses. Biosynthesis of these metabolites is normally tightly controlled, however in some cases, the process can be manipulated to produce unseen chemical entities with new bioactivities. Here we report new polyketides from endophytic ascomycete *Chalara* sp. which can incorporate various amines derived from HDAC inhibitor vorinostat analogues. *Aspergillus alliaceus* was shown to produce bianthrones under co-culture

conditions. All structures were determined by extensive NMR analyses and were tested in cytotoxicity and antimicrobial assays.



P-140

ANTIBIOTIC DISCOVERY IN AN AMYCOLATOPSIS SP. STRAIN ISOLATED FROM DESERT SOIL

Shannon I. Ohlemacher, Jack R. Davison, Hongbing Liu, Carole A. Bewley
Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA

Antibiotic resistance is a major public health threat that is further exacerbated by the dwindling number of antibiotics in the development pipeline. Natural products, also called secondary metabolites, from terrestrial and marine actinobacteria are the major source of antibiotics in clinical use. Actinomycete genomes routinely contain upwards of 25 biosynthetic gene clusters (BGCs) encoding secondary metabolites, but challenges remain in isolating novel actinomycete strains from the environment and expressing the BGCs under laboratory culture conditions. Towards discovering novel secondary metabolites with antibacterial activity, we isolated an actinomycete from desert soil, A09, and sequenced its genome with Nanopore technology. The 16S rRNA sequence revealed that A09 belongs to the genus *Amycolatopsis*. Twenty-five biosynthetic gene clusters representing at least 12 biosynthetic types were predicted in the genome by the antiSMASH algorithm. A09 was grown in nine media with varying nitrogen and carbon sources in an attempt to elicit expression of the BGCs. A09 produced secondary metabolites with anti-*Staphylococcus aureus* activity in five media. Two compounds were purified by high pressure liquid chromatography (HPLC) and identified by high-resolution tandem mass spectrometry (HR-MS/MS). Future work will include structure elucidation of additional antibacterial secondary metabolites by HR-MS/MS and NMR as well as treatment of A09 with subinhibitory concentrations of cytotoxic small molecules to induce expression of additional cryptic BGCs.

P-141

URBAN CYANOBACTERIAL BLOOMS OF EASTERN NORTH CAROLINA: CHEMODIVERSITY AND BIOACTIVITY-GUIDED COMPOUND DISCOVERY

Michael Recchia[1], Kylie Wright[1], Amy Grogan[1], Catharina Alves-de-Souza[1], and Wendy K. Strangman[1]
[1]Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Ln. Wilmington, North Carolina 28409

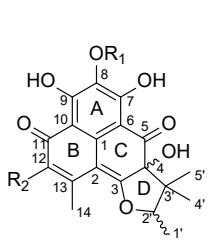
Stormwater retention ponds and related features are common and often mandated in many areas of the country that experience high rainfall. Although these ponds are important for flood and runoff mitigation, their high nutrient loads and warm temperatures can often support harmful cyanobacterial algal blooms (CHABs) known to produce a variety of toxins and other bioactive compounds. Semi-targeted UPLC-QToF based metabolomics analysis coupled with GNPS molecular networking enabled rapid evaluation of bloom metabolite profiles from Eastern North Carolina. A prolific local bloom in King's Highway Pond was highlighted for monitoring throughout the summer bloom season and across several years. Corresponding bioassays for inhibitors of serine proteases and toxicity against the planktonic grazer *Thamnocephalus platyurus* resulted in the discovery of new prenylated members of the aeruginosin class of linear peptides and new cyclic depsipeptide micropeptins containing rare sulfated glyceric acid side chains.

P-142

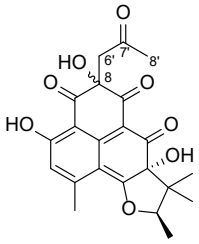
PHENALENONES FROM A MARINE-DERIVED FUNGUS *PENICILLIUM* SP.

Sung Chul Park[1], Elin Julianti[1], Sungjin Ahn[1], Donghwa Kim[1], Sang Kook Lee[1], Minsoo Noh[1], Dong-Chan Oh[1], Ki-Bong Oh[1], and Jongheon Shin[1,]*
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea

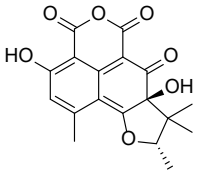
Six new phenalenone derivatives (**1**-**6**) along with five known compounds (**7**-**11**) of the herqueinone class were isolated from a marine-derived *Penicillium* sp. fungus. The absolute configurations of these compounds were assigned based on chemical modifications and their specific rotations. 4-Hydroxy-sclerodin (**6**) and an acetone adduct of triketone (**7**) exhibited moderate anti-angiogenetic and anti-inflammatory activities, respectively, while *ent*-peniciherqueinone (**1**) and isoherqueinone (**9**) exhibited moderate abilities to induce adipogenesis without cytotoxicity.



1 R^1 = Me, R_2=OH, 4*S*, 2'*S*
2 R^1 = H, R_2=OH, 4*S*, 2'*S*
3 R^1 = Me, R_2=H, 4*R*, 2'*R*



4 epimer at C-8
5



6

P-143

BIOGEOGRAPHY OF ATTINE ANT SYMBIONTS' SECONDARY METABOLITE

Taise T. H. Fukuda[1,2], Emily Mevers[1], Eric J. N. Helfrich[1], *Weilan G. P. Melo[2], Monica T. Pupo[2], Jon Clardy[1]*
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115 USA; [2]School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP 14040-903 BR.

Attine ants cultivate a fungus with which they maintain a permanent and obligatory mutualistic association – the ants feed the fungus and the fungus feeds the ants. To help prevent nest infection by parasites, especially by the specialized fungal pathogen *Escovopsis* sp., the ants have an association with symbiotic actinobacteria of the genus *Pseudonocardia* that produce protective secondary metabolites. Investigations of ants in a previously unstudied region, colonies from around Brazil including the Amazon Forest, Atlantic Forest and Brazilian Cerrado (Savanna), led to an interesting eco-

logical finding. Metabolomic analysis (HR-LCMS) on 50 *Pseudonocardia* strains representing four distinct collection sites revealed that a significant number of strains (90%) produced cahuitamycin and/or oxachelin, which are structural isomers. These two metabolites were only found in bacteria isolated from Brazilian samples. The same analyses were performed with 98 strains isolated from ants collected in Panama, and it was not possible to identify either the metabolites or their biosynthetic pathways. In phylogenetics analyses, it was possible to observe that the strains from Brazil and Panama form two separate clades. This shows that the production of a secondary metabolite can be associated with the evolution of ant-associated *Pseudonocardia*.

P-144

HIRSUTELLONES PKS-NRPS ANALOGUES FROM AN ENDOPHYTIC FUNGI OF BRUGUIERA GYMNORHIZA

Yi Sun[1,2], Zhiguo Liu[1,2], Fangbo Zhang[1,2]
[1]China Academy of Chinese Medical Sciences, Beijing, China, Beijing, China
[2]Institute of Chinese Materia Medica, Beijing, China

In this study, we isolated many endophytic fungi from the Tropical Medicinal Plants in Xishuangbanna, Yunnan Province, China. Based on the UPLC-MS/MS analysis, together with the cytotoxicity assay, we screened a number of secondary metabolites from the endophytic fungi. A target strain was found in the medicinal plant of *Bruguiera gymnorhiza* (L.) Lam and identified as *Xenoacremonium sinensis* sp. nov., which is a new strain. Its secondary metabolites were systematically isolated by various chromatographic techniques and identified as the hirsutellones analogues by spectroscopic analysis. Eight new PKS-NRPS hybrid compounds (Xenoacremones A-H) were obtained, which showed veryunique structural features such as 12- or 13-membered macrocyclic ether-containing 1,4-disubstituted phenyl and γ-lactam or succinimide moieties. Xenoacremone A--H showed cytotoxicity against A549, k562, and Hela tumor cells, and xenoaremone A could inhibit the expression of PI-3K/AKT signaling pathway.

P-145

DEFINING THE ROLE OF A BACTERIAL ENZYME OF UNKNOWN FUNCTION

Taylor Kornfuehrer[1] and Alessandra S. Eustáquio[1]
[1]Department of Medicinal Chemistry and Pharmacognosy and Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607

S-adenosyl-L-methionine (SAM) is a biochemical cofactor that is ubiquitous across all domains of life. It is the primary methyl donor in various methylation reactions involving DNA, RNA, and proteins. Under physiological conditions, the active form (*S,S*)-SAM spontaneously racemizes about its sulfonium center to (*R,S*)-SAM. The (*R,S*) form is inactive as a methyl donor and has been suggested to inhibit methyltransferases, which may lead to cellular damage over time.

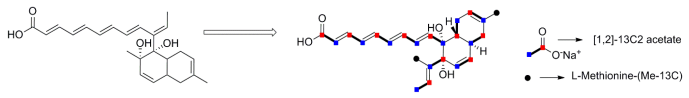
Cellular mechanisms for (*R,S*)-SAM remediation are not well understood, though yeast are known to use homocysteine *S*-methyltransferases (HMTs) for this purpose. However, not all bacteria contain HMTs, so it is likely (*R,S*)-SAM remediation occurs in bacteria that do not contain HMTs via a yet-to-be discovered enzyme. Previous studies identified a bacterial gene of unknown function, DUF62, that can hydrolyze SAM. However, these studies did not examine the differential hydrolysis of the (*S,S*) and (*R,S*) forms. We aim to test whether DUF62 is selective for one form of SAM over the other by performing HPLC separations of the diastereomers as previously reported and incubating the isolated compounds with recombinant DUF62 enzymes for comparative kinetic analysis.

P-146

STRUCTURAL REVISION OF CAMPAFUNGIN, AN ANTIFUNGAL POLYKETIDE FROM *PLENODOMUS ENTEROLEUCUS* (PLEOSPORALES), AND STRUCTURAL CHARACTERIZATION OF NEW ANALOGS

Bruno Perlatti[1], Connie B. Nichols[2], J. Andrew Alspaugh[2], and Gerald F. Bills[1]
[1]Texas Therapeutics Institute, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, Texas 77054, USA. [2]Departments of Biochemistry and Medicine, Duke University Medical Center, Durham, NC, 27710, USA.

Campafungin is a polyketide described in 2011 and recognized in the *Candida albicans* fitness test due to its antiproliferative and antihyphal activity. The mode of action was hypothesized as inhibition of a cAMP-dependent PKA pathway, albeit not fully elucidated (1). The proposed structure was an unnatural scaffold, but structural data were unavailable. Labeling studies with [Me-^{13}C]-L-methionine, [1-13C], [2-13C] and [1,2-13C] acetate enabled characterization of a tetraenoic acid fused to a trans-decalin core arising from a undecaketide precursor and was consistent with known PKS machinery. New analogs were also observed. A proposed biosynthesis, MIC, and structural characterization will be presented.



(1) Roemer, T. et al., Chemistry & Biology, 18, 148-164, 2011.

P-147

UNUSUAL BIOACTIVE REARRANGEMENT PRODUCTS FROM AQUEOUS PHOTOLYSIS OF PHARMACEUTICAL STEROIDS AND THEIR ENRICHMENT THROUGH DIURNAL LIGHT-DARK CYCLING

Nicholas C. Pflug,[1] Christopher J. Knutson,[1] Dalma Martinović-Weigelt,[2] Kathryn C. Breuckman,[3] Eleanor E. Meyer,[3] Dale C. Swenson,[1] Kristine H. Wammer,[3] David M. Cwiertny,[4] and James B. Gloer[1]
[1]Department of Chemistry, University of Iowa; Iowa City, IA 52242, [2]Department of Biology, University of St. Thomas; St. Paul, MN 55105, [3]Department of Chemistry, University of St. Thomas; St. Paul, MN 55105, [4]Department of Civil & Environmental Engineering, University of Iowa; Iowa City, IA 52242

In an ongoing effort to study the environmental fate of potent, endocrine-active steroid hormones, we encountered two unusual phenolic rearrangement products with a novel tetracyclic ring system upon aqueous photolysis of the pharmaceutical dienone steroids dienogest and methyldienolone. The structures were assigned by analysis of 2D NMR and HRMS data and proposed in a previous report. We now describe verification of these proposed structures by X-ray diffraction analysis of the lead compound, which we have called dienogestenol. Although dienogestenol is a minor primary photoproduct of dienogest, we also demonstrate here its enrichment in test samples across several simulated diurnal cycles due to the reversibility, of a competing photohydration reaction process. *In vitro* receptor transcriptional activation assays revealed that these unusual photoproducts exhibit progestogenic and androgenic activity, albeit with someone lower potency than their respective parent compounds (low-μM to sub-nM EC_{50} values). The unusual structure and atypical route to accumulation of dienogestenol may result in previously unrecognized adverse ecological consequences associated with dienogest release into the environment.

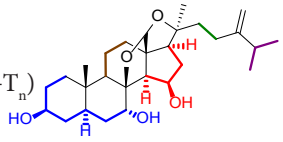
P-148

ONE-DIMENSIONAL TOTAL CORRELATION SPECTROSCOPY AS A DEREPLICATION TOOL FOR IDENTIFICATION OF NEW AND KNOWN SECONDARY METABOLITES

Choon Yong Tan, Harinantenaina L. Rakotondraibe
Division of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

Spin coupling networks are subsets of nuclei related to one another by direct or indirect spin-spin coupling within a molecule, and are detectable by one-dimensional total correlation spectroscopy (1D TOCSY). The exclusivity of spin coupling networks to a single molecule can be utilized as fingerprints to quickly differentiate and dereplicate known compounds with identical masses, as well as to elucidate structures of possible new compounds, in crude natural product extracts. Furthermore, spin coupling networks of compounds present in microgram quantities in mixtures could be detected by 1D TOCSY. The conceptualization of a TOCSY-based dereplication will be highlighted.

$$^{1}\text{H NMR of a compound} = \sum \text{1D TOCSY spectra } (T_1+T_2+T_3\ldots+T_n)$$

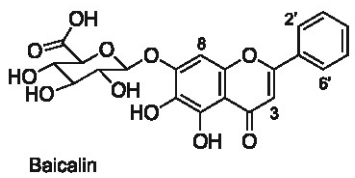


P-149

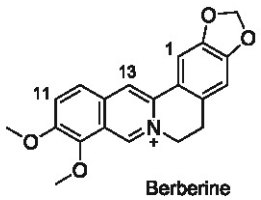
STRUCTURAL ANALYSIS OF THE COMPLEX OF BAICALIN AND BERBERINE IN AQUEOUS SOLUTION

Yoshinori Uekusa, Riina Tsutsumi, Kenjiro Nakamoto, and Fumiyuki Kiuchi
Faculty of Pharmacy, Keio University, Minato-ku, Tokyo 105-8512, Japan

Baicalin and berberine are the representative biologically-active constituents of crude drugs, Scutellaria root and Coptis rhizome/Phellodendron bark, respectively. These compounds are reported to form a 1:1 complex resulting in formation of yellow precipitates in decoctions of Kampo formulae which contain these crude drugs. Although an NMR study of this complex in methanol has been reported, little is known about the structural basis of this complex in an aqueous solution. In this study, we attempted to obtain structural information of the complex in aqueous solutions by using NMR spectroscopy. In an NMR titration experiment, significant chemical shift changes were observed in the signals of baicalin (H_{bai}-3, H_{bai}-8 and H_{bai}-2'/6') and berberine (H_{ber}-1, H_{ber}-11 and H_{ber}-13), indicating that these positions are involved in the interactions between these molecules. To obtain further information of the complex, we synthesized derivatives of the molecules such as baicalin methyl ester and 13-methylberberine and examined their complexes. These data will also be presented.



Baicalin



Berberine

P-150

DISCOVERY OF A STABLE VITAMIN C GLYCOSIDE AT HIGH CONCENTRATIONS IN APPLES (*MALUS* SPP.)

Alistair T. Richardson[1]
[1]*University of Otago, Department of Chemistry, PO Box 56, Dunedin, New Zealand.*

A stable glycoside of Vitamin C (ascorbic acid) has been identified in a wide variety of popular crops for the first time; including apples, pears, apricots and many others. The glycoside was isolated from crab apples (*M. sylvestris*) via acetylation and subsequent purification. Chemical synthesis of several Vitamin C glycosides, and their acetylation, facilitated the characterisation of this metabolite and provided material for further biological experiments. The structure of the naturally occurring glycoside was 2-O-β-D-glucopyranosyl-L-ascorbic acid. The distribution of this metabolite in apples and other crops was investigated using analytical LC-MS. Leaves of all crops were found to contain the glycoside, but only trace amounts were detected in fruit. The exception was crab apples in which the glycoside was present in extremely high concentrations (200-900mg/100 g FW) compared to typical Vitamin C levels in apples (5-30 mg/100 g FW). Closer investigation of various apple varieties revealed this metabolite was present in phloem and may therefore be involved in the transport of ascorbic acid from source to sink tissues.

P-151

UNTARGETED METABOLOMICS FOR DETECTING MARKERS OF VIRULENCE AND GROWTH IN *METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS* (MRSA)

Derick D. Jones Jr.[1]*, Lindsay K. Caesar*[1]*, Joshua Kellogg*[1]*, Daniel A. Todd*[1]*, Alexander H. Horswill*[2]*, Nadja B. Cech*[1]
[1]*Deparment of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412, USA.* [2]*Deparment of Immunology and Microbiology, University of Colorado Denver, Aurora, CO 80045 USA*

Drug resistant infections are an increasing problem world-wide, responsible for an estimated 700,000 annual mortalities. Current treatment modalities to combat infections of this type rely heavily on antibiotics. Unfortunately, the use of antibiotics accelerates the development of resistance. Severe drug resistant infections such as MRSA are often managed with "last resort treatments" which have side effects similar to chemotherapy, including, loss of appetites for months at a time, increased hospital stays, and, in extreme cases, septic shock. One potential alternative strategy for treating the most aggressive drug resistant infections, is by disarming the infections of its weapons of destruction, considered to be toxins or virulence agents. This approach is best known as an anti-virulence strategy. By silencing or inhibiting the production of toxins of this sort, it is possible to develop more targeted treatment approaches for these superbugs. In this project we have developed and optimized a method using ultra high-performance liquid chromatography coupled to high resolution mass spectrometry that can simultaneously identify and track multiple MRSA metabolites associated with virulence (toxin production) and growth. We employed this method to track metabolite production in MRSA cultures exposed to a fungal metabolite (ambuic acid) with anti-virulence activity. With this approach, we identified several features (ions) in the mass spectrometry datasets that are associated with either virulence or growth in MRSA. Experiments to solve the structures associated with these features are ongoing.

P-152

DESIGNING TURMERIC (*CURCUMA LONGA*) EXTRACTS FOR PURCUMIN, PURCUMINOIDS, AND NOCUMIM BIOASSAY CONFIGURATIONS

J. Brent Friesen[1,2], Yang Liu[1], Shao-Nong Chen[1], James B. McAlpine[1], and Guido F. Pauli[1]
[1]Center for Natural Product Technologies (CENAPT), PCRPS and Dept. of Med. Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA; [2]Department of Physical Sciences, Dominican University, River Forest, IL 60305, USA.

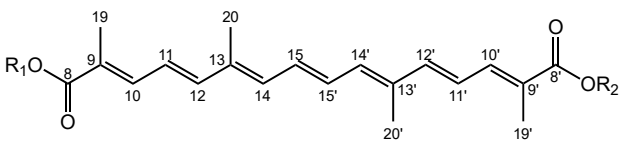
Preparations originating from *Curcuma longa* rhizomes are reported to produce numerous favorable therapeutic outcomes. Unfortunately, studies supporting these claims often fail to distinguish between the chemical makeup of turmeric rhizome, crude turmeric extract, curcuminoid-enriched turmeric extract (CTE), curcumin-enriched material, and curcumin as a single chemical entity. This study introduces the DESIGNER approach to Deplete and Enrich Select Ingredients to Generate Normalized Extract Resources for the production of a curcuminoid knockout extract ("nocumin"), as well as chemically well-defined curcuminoid mixtures. Liquid-only countercurrent separation (CCS) technology with optimized conditions was employed to fractionate the CTE into four key materials: the lipophilic "nocumin", highly pure curcumin ("purcumin"); a mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin that is free of other constituents ("purcuminoids"); and hydrophilic "nocumin". Chemical characterization of these materials utilized ^{1}H NMR, qHNMR, and HPLC to confirm chemical composition, assess residual complexity, and assign purity. The newly developed *C. longa* DESIGNER extracts are distinctive natural product preparations, which may be tested individually or in combination. The biological testing of these materials will help fill the gap between chemical composition and possible biological and therapeutic applications of materials derived from turmeric.

P-153

SOLUBILITY-ENHANCEMENT OF BERBERINE-BAICALIN COMPLEX BY CROCINS

Kazuki Okoshi, Yoshinori Uekusa, Yuji Narukawa, and Fumiyuki Kiuchi
Faculty of Pharmacy, Keio University, Minato-ku, Tokyo, 105-8512, Japan.

Baicalin and berberine have been reported to form a 1:1 complex and precipitate out from water decoction of Kampo formulae containing Scutellaria Root and Coptis Rhizome/Phellodendron Bark. We previously found that when Gardenia Fruit was present in the decoction, the amount of the precipitates largely decreased. In this study, through activity-guided fractionations, we identified all-*trans* crocin-1 (**1**) as the constituent that decreases the amount the precipitates from Gardenia Fruit. In an aqueous solution, a ternary complex consisting of baicalin, berberine and **1** was detected by MS analysis. In ^{1}H-NMR, chemical shift changes were observed in the polyene part protons of **1** in the presence of berberine-baicalin complex, whereas chemical shifts of the gentiobiosyl protons were unchanged. These observations indicated that the polyene part of **1** contributed to form the ternary complex through hydrophobic interactions, and hydrophilicity of the gentiobiosyl moiety may act to increase the solubility of the complex. Further analyses and discussion of the role of **1** will be presented.



1 ($R_1 = R_2$ = gentiobiosyl)□

P-154

HPTLC FOR ENSURING QUALITY DIETARY SUPPLEMENTS

Maged H. M. Sharaf
CAMAG Scientific Inc., Wilmington, NC 28401, USA

Under the U.S. Dietary Supplement Health and Education Act of 1994 (DSHEA), dietary supplement manufacturers are responsible to substantiate the safety of the dietary ingredients used in manufacturing their products. Manufacturers are also responsible for making true and not misleading representations or claims about their products. As part of DSHEA, the U.S. Congress gave the Secretary of Health and Human Services and the FDA by delegation, the express authority to issue regulations establishing current good manufacturing practice requirements (CGMPs) for dietary supplements. In 2007, the FDA has issued a final CGMPs rule establishing requirements for the production of dietary supplements. This rule requires manufacturers ensure that a dietary supplement contains what it is labeled to contain and is not contaminated with harmful or undesirable substances such as pesticides, heavy metals, or other impurities. The rule also requires manufacturers to use at least one scientifically valid test to ensure the identity, purity, quality, strength, and composition of dietary supplements, thus assuring consumers they are purchasing the type and amount of ingredients declared.

High-performance thin-layer chromatography (HPTLC) is an invaluable tool for the analysis of dietary ingredients and products, especially those based on botanicals. This presentation will highlight the use of HPTLC in ensuring the identity, purity, quality, strength, and composition of dietary ingredients and products and absence of adulteration.

P-155

DYNAMICS OF THE ISOFLAVONE METABOLOME OF TRADITIONAL PREPARATIONS OF *TRIFOLIUM PRATENSE* L.

Gonzalo R. Malca-Garcia, Daniel Zagal, James Graham, Dejan Nikolić, J. Brent Friesen, David C. Lankin, James McAlpine, Shao-Nong Chen, and Guido F. Pauli
UIC/NIH Center for Botanical Dietary Supplements Research, Dept. of Med. Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

Red clover has been reported to be helpful for alleviation of menopausal symptoms, premenstrual syndrome, mastalgia, high cholesterol, and other conditions. We investigated the chemical differences of traditional preparations of infusions, decoctions, and tinctures, and evaluated the chemical variability in a traditional red clover tincture over time. For this purpose, eight isoflavone aglycones as well as two glucosides, ononin and sissotrin, were used as marker compounds. Quantitative NMR (qHNMR), LC-MS-MS, and UHPLC-UV methods were used to identify and quantitate the major phenolic compounds found within each extract. The percentages of biochanin A and formononetin were 0.01 & 0.27% in infusions and 0.09 & 0.51% in decoctions, respectively. In tinctures after 24 hours, the same compounds yielded were present at 1.89 & 2.22%, respectively. Both infusion and decoction showed elevated concentrations of isoflavonoid glucosides, such as ononin and sissotrin, than the 45% ethanolic tinctures. Marked dynamic chemical changes ("dynamic residual complexity") of the red clover tincture was observed over time (one-month), with biochanin A and formononetin reaching peak concentrations at around six days. This provides clues as to why different formulation methods (infusions, decoctions, and tinctures) have evolved traditionally to treat different health conditions. Moreover, the outcomes show that tinctures, taken over a period of time, are dynamic medicinal formulations that allow for time-controlled release of bioactive compounds.

P-156

TARGETED AND UNTARGETED APPROACHES TO STUDY THE EFFECTS OF STORAGE CONDITIONS ON STABILITY OF *HYDRASTIS CANADENSIS* (GOLDENSEAL)

Manead Khin,[1] Lindsay K. Caesar,[1] Joshua J. Kellogg,[1] and Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC 27412

Hydrastis canadensis, commonly known as goldenseal, is a perennial herb that is native to southeastern Canada and eastern United States. The extract of goldenseal is bioactive due to the alkaloids present and has been used as a folk medicine for treatments of infection. However, these types of medicinal plants may also face degradation if not stored properly. The purpose of the study is to analyze the stability of known and unknown metabolites of goldenseal during exposure to different storage conditions, using untargeted metabolomics and mass spectrometry. The research project focuses on identifying the chemical changes in the content of goldenseal using under different temperature conditions (40°C ± 5°C as high temperature, 20°C ± 5°C as room temperature, and 4°C ± 5°C as low temperature) or different light : dark cycles (16 hours : 8 hours, 12 hours : 12 hours, and 0 : 24 hours), or different sample conditions (powdered version of the roots, and actual roots). This shelf-life project is a six-month study, where each month is treated as one-time mark.

P-157

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR THE ANALYSIS OF LIME FLOWERS (*TILIAE FLOS*) PREPARATIONS.

Natalia Melnyk[1], Oleg Koshovyi[1], Agnieszka Bazylko[2], Maria Ziaja[3], Sebastian Granica[2]
[1]Department of Pharmacognosy, National University of Pharmacy, Kharkiv, Ukraine, [2]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland [3]Department of Natural Sciences, University of Rzeszów, Rzeszów, Poland

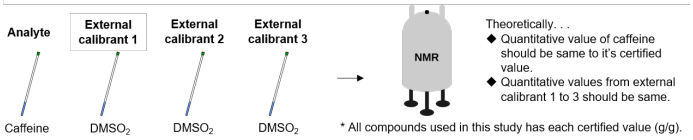
Tiliae flos commonly known as lime flower is a popular medicinal plat material used in the form of infusion. *Tiliae flos* has a monograph in the European Pharmacopoeia 9.0 (EP9.0) which defines lime flower as whole dry flowers obtained from *T. plathyphyllos* L., *T. cordata* Mill., *T. x vulgaris* Hayne or their mixture. The monograph also mentions two other species *T. americana* L. and *T. tomentosa* Moench which should not be included in medicinal plant material according to EP9.0. Although linden flower is a well-known traditional plant material reports on its chemical composition are still limited. There are no studies showing the differences in the chemical composition of extracts prepared from lime flowers collected from different *Tilia* species. The aim of the current study was to develop a fast and sensitive HPTLC method for the discrimination between extracts prepared from flowers of five chosen *Tilia* species listed above. The best resolution was obtained on HPTLC silica plates using THF:CH_2Cl_2:HCOOH:CH_3COOH:H_2O 9:9:4:2:3 as mobile phase. After spraying with Natural Product's Reagent significant differences in phenolics profile were shown. Hierarchical key for the identification of lime species based on their chemical composition was developed.

P-158

THE PRACTICALITY OF EXTERNAL CALIBRATION QUANTITATIVE NMR OF NATURAL PRODUCTS

Yuzo Nishizaki,[1, 2] Shao-Nong Chen,[1] David C. Lankin,[1] and Guido F. Pauli.[1]
[1] University of Illinois at Chicago, Chicago, IL 60612, USA, [2] National Institute of Health Sciences, Kawasaki, Kanagawa 210-9501, Japan.

Quantitative NMR (qNMR) is a reliable tool for quantitative analysis in the field of natural products research. Most natural products are isolated in very limited quantities, and their distinct structures made them unavailable commercially. Therefore, the use of external calibration (EC) techniques is most appropriate for their subsequent spectroscopic (NMR) analyses. In contrast, hyphenated chromatography requires internal calibration (IC) methods, which are incompatible with biological evaluation as added IC contaminates the NP. Although the EC method is generally regarded as being less accurate and of lower precision than the IC method, only few reports have examined the contributing factors in detail using. The present study demonstrates that the principle sources affecting the analytical results are: **(1)** probe tuning, **(2)** accuracy of 90° pulse width calibration, and **(3)** the precision of the NMR tube diameter. The results serve as a practical guide for boosting EC method accuracy and demonstrates that accurate purity determination is practical for any NP.



P-159

THE CURCUMIN-GLUCURONIDE DECONJUGATION CAPACITY OF BONE IS UNAFFECTED BY COMMON AGE-RELATED PERTURBATIONS TO THE BONE MARROW MICROENVIRONMENT.

Andrew Kunihiro[1], Paula B. Luis[2], Julia Brickey[3], Jennifer B. Frye[3], Claus Schneider[2], Janet L. Funk[1,3]
[1]Department of Nutritional Sciences, University of Arizona, Tucson, AZ, [2]Department of Pharmacology, Vanderbilt University, Nashville, TN, [3]Department of Medicine, University of Arizona, Tucson, AZ

Curcuma longa L.-derived curcumin (C) prevents osteoclast-mediated bone resorption in humans and pre-clinical models of osteoporosis and osteolytic bone metastasis (BMETs) despite primarily circulating as curcumin-glucuronide (GC). We have documented the bioactivity of C, but not G-C, in blocking pro-osteolytic pathways and the capacity of bone to deconjugate GC delivered following oral ingestion, a β-glucuronidase (GUSB) mediated process. To extend these findings, effects of clinically relevant bone milieu perturbations on GUSB and ingested C metabolism were determined. Despite reports of GUSB regulation by sex hormones and adipose cell replacement of GUSB-positive bone marrow accompanying aging and menopause, GUSB activity and deconjugation of GC in bone were minimally affected by age, sex or ovariectomy (OVX). GC levels were higher in the proximal tibial metaphysis, a common site of bone loss in OVX mice and osteolytic BMET-bearing mice that contained sufficient GUSB to deconjugate the majority of local GC. In T-cell deficient nude mice bearing GUSB-negative BMETs, neither GUSB levels nor the deconjugation capacity of bone were altered compared to tumor-free nude or WT mice. Extending our prior observation that C but not GC blocks the formation of bone resorbing osteoclasts, bioactivity of quercetin, but not quercetin-glucuronide, was also confirmed. These findings suggest that activation of glucuronidated bone-protective polyphenols within bone is preserved despite perturbations associated with common, age-related resorptive bone diseases.

P-160

ANTI-DIABETIC EFFECTS OF WILD *GLYCINE SOJA* SEED EXTRACT ON TYPE II DIABETIC MOUSE MODEL

Yun Mi Lee[1], Eunjung Son[1], Seung-Hyung Kim[2], Ho Kyoung Kim[1], Dong-Gyu Jang[3], Dong-Seon Kim[1]*
[1]Herbal Medicine Research Division, Korea Institute of Oriental Medicine, 1672 Yuseong-daero, Yuseong-gu, Dajeon 34054, Republic of Korea. [2]Institute of Traditional Medicine and Bioscience, Daejeon University, Daejeon 300-716, Republic of Korea[i]. [3]Daejeon Bio Venturetown 306-7, Yuseong-daero 1662, Yuseong-gu, Daejeon 34054, Republic of Korea

This study was designed to estimate anti-diabetic effects of *Glycine soja* seeds extract on type 2 diabetic mouse model and hepatocytes in relation to energy metabolism. Oral administration of *Glycine soja* extract at the doses of 150 and 300 mg/kg/day for 6 weeks showed significant anti-diabetic effects on diabetes mellitus type 2 (T2DM) of *db/db* mice. *Glycine soja* extract lowered significantly blood levels of glucose, HbA1c, insulin, IGF-1 and leptin, and increased adiponectin level. It up-regulated phosphorylation of AMPK, and down-regulated expression of GLUT2 in liver tissues of mice while up-regulated expression of GLUT4 in muscle tissues of mice. Moreover, *Glycine soja* extract increased glucose uptake by hepatocytes and recovered insulin responsiveness, which had been attenuated by palmitate treatment, through restoring AKT phosphorylation and peroxisome proliferator-activated receptor gamma (PPARγ) DNA binding activity. On the whole, treatment of *Glycine soja* extract is thought to lower blood glucose level by regulating energy metabolism and reducing insulin resistance in T2DM.

P-162

GENDER DIFFERENCES IN BEHAVIORAL DEFICITS AND RESPONSE TO *CENTELLA ASIATICA* WATER EXTRACT AND CONSTITUENT COMPOUNDS IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE.

Amala Soumyanath[1], Maya Caruso[1], Donald Matthews[1], Kirsten Wright[1], Armando Alcazar Magana[2,4], Claudia S. Maier[2], Jan F. Stevens[3,4], Nora Gray[1], Joseph Quinn[1,5]
[1]Department of Neurology Oregon Health and Science University, Portland, OR, 97239, USA; Departments of [2]Chemistry and [3]Pharmaceutical Sciences and [4]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA; [5]Department of Neurology, Veterans Affairs Portland Health Care System Center, Portland, OR 97239, USA.

Centella asiatica (CA) water extract (CAW; 2–10 mg/mL) administered in drinking water, improved memory in wild type (WT) and 5XFAD mice, an Alzheimer's disease model. This study compared 5xFAD mice fed purified rodent diet AIN-93M (control), or AIN93M with CAW (1%), or comparable amounts of CA triterpenes (TTs; 0.045%) or caffeoylquinic acids (CQAs; 0.016%). Memory assessed as contextual fear response (CFR), and hippocampal gene expression were measured after 4 and 5 weeks treatment respectively. Compared to WT mice, 5XFAD males showed significantly less (-39%; p=0.003) CFR freezing, whereas the decrease was non-significant (-19%; p=0.283) in 5xFAD females. Treatment with CAW, TTs, or CQAs improved CFR ($p<0.05$) in 5xFAD males, but not females. In 5xFAD mice, synaptophysin gene expression was increased ($p<0.01$) by TTs in females only, and by CQAs in both males and females. Increased expression ($p<0.05$) of antioxidant response element genes was only observed in 5xFAD females treated with CAW (Nrf2) or CQAs (Nrf2 and Ho-1). These data provide evidence for gender differences in 5xFAD pathology and in response to CAW, TTs and CQAs.

P-163

ANTI-INFLAMMATORY ACTIVITIES OF OPHIOPOGONIS RADIX ON HYDROGEN PEROXIDE-INDUCED CELLULAR SENESCENCE OF NORMAL HUMAN DERMAL FIBROBLASTS

Yumi Kitahiro, Makio Shibano
Department of Natural Products research, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan

Ophiopogonis Radix (Ophiopogon root), which nourishes the yin, has been used in clinical practice to promote fluid secretion and to moisturize the lungs and skin in traditional Chinese and Japanese (Kampo) medicine. To evaluate this traditional medicinal effect, we investigated the anti-inflammatory effect of Ophiopogonis Radix on senescent cells. The results indicated that methanol extracts and the main constituents of *O. japonicus* (compound **1**: metylophiopogonanone A, compound **2**: metylophiopogonanone B, compound **3**: ophiopogonanone A compound **4**: ophiopogonin B) are significantly downregulated the expression of interleukin (IL)-6 and IL-8, which were enhanced by senescent normal human dermal fibroblasts. Moreover, the methanol extracts and compound **1**–**4** decreased IL-6 production in a strong and concentration-dependent manner by the ELISA method. In addition, in traditional Japanese herbal medicine including Ophiopogonis Radix also had anti-inflammatory effects.

P-164

ACCELERATION OF NATURAL PRODUCT RESEARCH BY INTEGRATED APPROACH BASED ON MS AND NMR ANALYSIS

Hyunwoo Kim[1], Chen Zhang[1], Garrison W. Cottrell[2], and William H. Gerwick[1,3]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, La Jolla, California 92037, United States of America. [2]Department of Computer Science and Engineering, University of California, San Diego, La Jolla, California 92093, United States of America. [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093, United States of America

Investigation of novel secondary metabolites from natural products is one of the major goals of natural product researchers. Isolation of single compounds from natural products is carried out through several steps such as sample collection, extraction, separation, isolation and structure elucidation, all of which require much time and resources. However, sometimes these efforts result in the rediscovery of known compounds. To overcome this inefficiency, many strategies have been developed such as genome mining or compound dereplication using MS, NMR, UV or other analytical approaches. These approaches generally precede isolation steps and are widely used in natural product research. In this presentation, newer methods using MS/MS molecular networking with GNPS or NMR using an HSQC-based artificial intelligence recognition system (SMART), will be described. These strategies have the potential to improve the efficiency of natural product discovery programs.

P-165

A FLIPR ASSAY FOR DISCOVERY OF $GABA_A$ RECEPTOR MODULATORS OF NATURAL ORIGIN

M. Teresa Faleschini[1], Anne Maier[2], Sarah Fankhauser[2], Katharina Thasis[2], Simon Hebeisen[3], Matthias Hamburger[1], Veronika Butterweck[2,4]
[1] University of Basel, Pharmacenter, Klingelbergstrasse 50, 4056 Basel, Switzerland, [2] University of Applied Sciences Northwestern Switzerland, Institute for Pharma Technology, School of Life Sciences, Hofackerstrasse 30, 4132 Muttenz, Switzerland, [3] B'Sys GmbH, Benkenstrasse 254, 4108 Witterswil, Switzerland, [4] Zeller Medical AG, Seeblickstrasse 4, 8590 Romanshorn, Switzerland

A Fluorometric Imaging Plate Reader (FLIPR) assay in 96-well microtiter format utilizing CHO cells stably transfected with $GABA_A$ receptors of $\alpha_1\beta_2\gamma_2$ subunit composition was validated for rapid screening of plant extract libraries and efficient localization of active compounds in extracts. Validation was performed with pure compounds and extracts known to contain allosteric $GABA_A$ receptor modulators. A protocol for HPLC-based activity profiling was developed, whereby separations of 0.4 to 1.2 mg of extracts on an analytical HPLC column were found to be sufficient for the sensitivity of the bioassay. The protocol successfully localized the activity of known GABAergic natural products, such as magnolol in *Magnolia officinalis*, valerenic acid in *Valeriana officinalis*, and piperine in *Piper nigrum* extract. EC_{50} values of compounds (magnolol: 4.81 ± 1.0 µM, valerenic acid: 12.56 ± 1.2 µM and piperine: 5.76 ± 0.7 µM) were found to be comparable or lower than those reported using *Xenopus* oocyte assays.

P-166

BIOACTIVE SMALL MOLECULE DISCOVERY THROUGH METABOLITE LABELING AND LIVE CELL BINDING ASSAYS

Nicholas Zill[1], Samantha Marinkovitch[1], Wenjun Zhang[1,2]
[1]Department of Chemical and Biomolecular Engineering, University of California, Berkeley, Berkeley, CA, 94704, USA, [2]Chan Zuckerberg Biohub, San Francisco, CA, 94158, USA

Deuterium Adduct Bioactivity Screening (DABS) is a novel approach to screening for broadband bioactivity in a variety of cell lines. This methodology pairs the untargeted deuteration of complex cell extracts with cell binding assays in order to detect extended residency of small molecules inside of targeted cell lines. The development and application of this assay will be presented, specifically with its use in elucidating human microbiota secondary metabolite production.

P-167

PHYTOCHEMICAL COMPOSITION AND BIOACTIVITY OF INFUSIONS FROM *BISTORTAE RHIZOMA*.

Karolina A. Pawłowska[1], Katarzyna Jankowska[1], Marta Dudek[1,2], Sebastian Granica[2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland [2]Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland

Bistortae rhizoma is the pharmacopoeial plant material acquired from *Persicaria bistorta.* Although it exhibits astringent (*adstringens*) and antidiarrheal (*antidiarrhoicum*) properties, aqueous extracts are used mostly topically on account of high contents of tannins. Research on phytochemical composition of *Bistortae rhizoma* showed contents of gallotannins, procyanidins (catechin oligomers), triterpenoids, coumarins, steroids, fatty acids.

The aim of this study was to isolate and identify main compounds from aqueous extract of *Bistortae rhizome* and evaluate their anti-inflammatory activity using human neutrophil's model.

The extraction of rhizome was carried out with hot water, as the plant material is used in aqueous preparations. Raw extract was subsequently evaporated and fractionated by liquid-liquid extraction with diethyl ether, ethyl acetate and *n*-butanol. Fractions were separated using column chromatography (using Diaion HP-40, silica gel and Toyopearl HW-40F) and preparative HPLC. The result was the isolation and identification of 30 compounds. Their structures were established based on 1D/2D NMR experiments and MS^n analyses. Obtained compounds were classified as derivatives of gallic acid and flavan-3-ol, as well as chlorogenic acid. The main compounds of the extract were chlorogenic acid and procyanidin B3. Raw extract and isolated compounds were tested as anti-inflammatory agents. Extract and some of compounds at concentrations (12.5, 25,50 and 100 µg/ml or 6.25, 12.5 and 25 µM, respectively) were able to decrease the production of IL-8, IL-1β and TNF-α, from LPS stimulated neutrophils. Present results are the first report treating comprehensively chemical composition and anti-inflammatory activity of bistort rhizome.

P-168

HUMULUS LUPULUS (HOPS) AND ITS BIOACTIVE COMPOUNDS MODULATE ESTROGEN BIOSYNTHESIS THROUGH *CYP19A1* (AROMATASE) AND/OR *AKR1C3*

Amanda C. Maldonado, Atieh Hajirahimkhan, Shao-Nong Chen, Guido F. Pauli, Judy L. Bolton, Birgit M. Dietz
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago (IL)

Menopausal women have a substantially higher risk of developing estrogen receptor positive (ER+) breast cancer. Since the Women's Health Initiative reported that hormone therapy correlated with increased risk of breast cancer, many women have turned to botanical dietary supplements (BDS) such as *Humulus lupulus* (hops) to seek relief from menopausal symptoms. Little is known about the biological effects that these botanicals may have in modulating key pathways responsible for estradiol/estrone production. Aromatase (*CYP19A*1) and aldo-keto reductase (*AKR1C3*) are two enzymes responsible for key steps in the biosynthesis of 17-estradiol () in breast tissue. Downregulation of *CYP19A1* and *AKR1C3* may decrease biosynthesis and, thus, the risk for breast carcinogenesis. This study analyzes the *in vitro* effects of hops and its bioactive markers, xanthohumol (XH), 8-prenylnaringenin (8-PN), and 6-prenylnaringenin (6-PN), in modulating these enzymes in MCF-7:WS8 and MCF-7 cells overexpressing AKR1C3 (MCF-7:1C3). qRT-PCR quantified changes in *CYP19A*1 and *AKR1C3* mRNA expression. Hops and pure 6-PN significantly upregulated *CYP19A1*, while XH significantly upregulated *AKR1C3*, respectively. However, 8-PN was previously shown to act as a potent inhibitor of *CYP19A1* activity in a cell-free assay. Western Blot and In-Cell Western analysis was used to confirm relative protein expression of *AKR1C3* and *CYP19A1*. This study highlights the importance of elucidating the relative risk/benefit ratio of botanical supplements for women's health. [NIH P50 AT00155 and ACS PF-18-049-01-NEC]

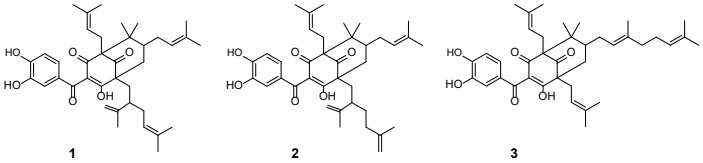
P-169

THE BOTANICAL SOURCES OF NORTHER BRAZILIAN RED PROPOLIS

Gari V. Ccana-Ccapatinta[1], Jennyfer A. Aldana, Matheus H. Tanimoto[1], and Jairo K. Bastos[1]
[1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, 14040-903 Ribeirão Preto, Brazil

The Brazilian red propolis (BRP) constitutes an important commercial asset for norther Brazilian beekeepers. Apiaries devoted to the production of BRP are located around native populations of the fabaceous species *Dalbergia ecastophyllum* which offers a red exudate that is collected by bees to

produce the red propolis. The role of *D. ecastophyllum* as the main botanical source of BRP has been previously confirmed. However, in addition to the flavonoids, isoflavonoids and chalcones, which are also present in *D. ecastophyllum* exudates, samples of BRP are reported to contain substantial amounts of polyprenylated benzophenones whose botanical source could be attributed to a Clusiaceae family member. A botanical survey along the surrounding flora of some apiaries located in Canavierias, Bahia state, norther Brazil, lead to the identification of two resin producing Clusiaceae, *Clussia* sp. and *Symphonia globulifera*. HPLC-DAD metabolic fingerprint comparison of BRP with *Clusia* sp. and *S. globulifera* resins supports the contribution of *S. globulifera* as an additional botanical source of BRP. Guttiferone E (**1**), xanthochymol (**2**) and oblongifolin B (**3**) were identified as main continents in BRP and *S. globulifera* resin. The importance of *S. globulifera* for BRP production will be discussed.



P-170

CERTIFICATION OF GINSENOSIDES IN *PANAX GINSENG* C.A. MEYER RHIZOME, LEAF, EXTRACT, AND SUPPLEMENT REFERENCE MATERIALS

Hugh V. Hayes, Walter B. Wilson, Catherine A. Rimmer
Chemical Sciences Division, Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

Ginsenosides are main constituents of the *Panax* plant family, and they have been reported to be a major source of ginseng bioactivity. The root of *Panax ginseng* (Asian ginseng) is the primary source of ginsenosides in traditional processing for medicinal practices; however, the use of leaves has been reported in commercially available ginseng products. Since various plant parts differ in phytochemical makeup, it is critical to identify ginsenoside compositions and concentrations in other parts of the pant to better investigate potential supplement contamination or adulteration.

The work presented here shows the method development for ginsenoside value assignment in candidate Standard Reference Materials 3384 (*Panax Ginseng* C.A. Meyer Rhizome), 3385 (*Panax Ginseng* C.A. Meyer Root Powder Extract), and 3388 (Ginseng-Containing Solid Oral Dosage Form). A liquid chromatography/tandem mass spectrometry method was developed for the separation and identification of ginsenosides after sonication extraction under basic conditions. Accurate means of identifying ginsenoside compositions and concentrations in various complex sample matrices is imperative for the phytochemical determination of specific *Panax* species for use in dietary supplement products and research.

P-171

BOTANICAL DIETARY SUPPLEMENTATION STANDARD REFERENCE MATERIALS AT THE NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY

Hugh Hayes[1], Charles Barber[1], Adam Kuszak[2], Melissa Phillips[1], Catherine Rimmer[1], and Laura Wood[1]
[1]Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. [2]Office of Dietary Supplements, National Institutes of Health, Rockville, MD 20852.

The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health, Office of Dietary Supplements (NIH, ODS) has developed suites of Standard Reference Materials (SRMs) for natural product dietary supplements. SRMs, such as those for *Ginkgo biloba* (SRMs 3246 - 3249) and Green Tea *Camellia sinensis* (SRMs 3254 – 3257), include value assignments for targeted organic and/or inorganic compounds with biological activities and/or used for supplement product standardization. SRM use promotes experimental rigor and supports manufacturing quality control efforts. With two-thirds of adults in the U.S. reporting supplement use, reference materials are critical for research on health effects and for ensuring consumer safety. NIST and NIH, ODS are working to develop additional reference materials including those focused on botanical identity and safety. Currently available RMs and future plans will be presented.

P-172

NATURAL PRODUCT ANALYSIS: HOW CAN INTERLABORATORY COMPARISONS HELP WITH RESEARCH AND PRODUCT QUALITY?

Hugh Hayes[1], Charles Barber[1], Adam Kuszak[2], Melissa Phillips[1], Catherine Rimmer[1], and Laura Wood[1]
[1]Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. [2]Office of Dietary Supplements, National Institutes of Health, Rockville, MD 20852.

In 2007 the National Institutes of Health, Office of Dietary Supplements (NIH-ODS) and the National Institute of Standards and Technology (NIST) began a quality assurance program for dietary supplement laboratories. The program was designed to help the community improve analytical procedures and establish individual laboratory measurement proficiency. The QAP focused on the determination of nutritional elements, toxic elements, water-soluble vitamins, fat-soluble vitamins, fatty acids, and marker compounds in natural products intended for oral consumption. Over time, the program has added studies for the determination of contaminants such as mycotoxins, pesticides, phthalates, and furans as well as determination of authenticity and adulteration. Specific studies, observations, and community improvements will be presented.

P-173

BAICALEIN IS A PHYTOHORMONE THAT SIGNALS THROUGH THE PROGESTERONE AND GLUCOCORTICOID RECEPTORS

Julia R. Austin[1], Brenna Kirkpatrick[1], Joanna E. Burdette[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, IL, 60612.

Many women turn to herbal supplements for the treatment of their ailments. In the US, herbal supplements are an $8 billion industry, although it is unclear what active compounds certain herbal supplements contain. Previous studies have shown that herbal supplements contain compounds that modify steroid signaling, and specifically our lab has identified molecules in herbal supplements that interact with and modify progesterone receptor signaling, termed phytoprogestins. Progestins are typically used in the treatment of various gynecological diseases. Due to promiscuous binding to multiple steroid receptors, off-target effects are associated with many synthetic progesterone-based therapies. Based on structures from previously identified phytoprogestin compounds and the literature, we evaluated three other flavones, for their progesterone-like activity. Of the compounds tested, baicalein was the only compound that had an effect on progesterone signaling as a progesterone antagonist. Baicalein was then tested for its glucocorticoid activity and was found to be a glucocorticoid receptor (GR) agonist. Importantly, baicalein is a major component of skullcap, which is an herbal supplement used for anti-inflammatory applications. GR activation is a well-known molecular target for anti-inflammatory molecules. We investigated if baicalein modified GR signaling. It was shown that baicalein induced GR target genes, stabilized GR, and inhibited migration. In summary, baicalein is a phytohormone that blocks progesterone receptors and

acts as a GR agonist. If future experiments confirm that baicalein directly signals through GR, this will connect baicalein with a well-accepted cellular target for anti-inflammation and anti-tumor action, GR.

P-174

METABOLOMIC PROFILING TO GAUGE UNCERTAINTY AND AUTHENTICITY OF KRATOM (*MITRAGYNA* SP.) COMMERCIAL PRODUCTS

Joshua J. Kellogg,[1] E. Diane Wallace,[1] Nicholas H. Oberlies,[1] and Nadja B. Cech[1]

[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC 27412

Kratom [*Mitragyna* sp. (Rubiaceae)], known in Southeast Asia for its anesthetic and pain-reducing properties, was first described in scientific literature a century ago. Its recent surge in popularity has increased scrutiny of kratom commercial products. Questions remain about the efficacy, potency, and potential toxicity of kratom supplements, which has translated into debates and conflicting media coverage on the public health response to kratom usage across the United States. To explore the chemical makeup of commercial kratom products, we examined 53 commercial samples of kratom from five different suppliers, as well as leaves harvested from two plants. Metabolomics analysis used both unsupervised (principal component analysis) and supervised (partial least square-discriminant analysis) statistical modeling approaches to examine the similarity of the commercial samples. The resulting distribution of samples revealed a subsection of commercial samples with significantly higher concentrations of the indole alkaloids rhynchophylline and rotundifoline, suggesting chemical and possibly taxonomic variances between samples. Resolving the ambiguity of constituent species is essential considering the various alkaloids present could possess different toxicity and efficacy profiles, resulting in significant differences for the user.

P-175

ABSORPTION AND METABOLISM OF IRILONE IN THE CACO-2 CELL MODEL

*Jialin Liu, Shao-Nong Chen, Guido Pauli, Richard B. van Breemen**

Linus Pauling Institute, College of Pharmacy, Oregon State University, Corvallis,OR 97331, UIC/NIH Center for Botanical Dietary Supplements Research, Chicago, IL 60612

Red clover (*Trifolium pratense* L.) is a popular isoflavone-containing botanical dietary supplement conventionally used in hormone replacement therapy for menopause, irilone is an important isoflavone from red clover. The intestinal absorption and metabolism of irilone were investigated *in vitro* using the human intestinal Caco-2 cell culture model (1) to understand the mechanism of its high oral bioavailability. The effects of irilone on the permeabilities of other isoflavones in red clover (daidzein, formononetin, biochanin A, and genistein) and their metabolites were also studied. In addition, how other constituents in the red clover extract affect the absorption and metabolism of irilone was investigated to explain bioavailabilities observed in clinical trials.

(1) van Breemen RB, Li Y. *Expert Opin Drug Metab Toxicol*. 2005 Aug;1(2):175-85.

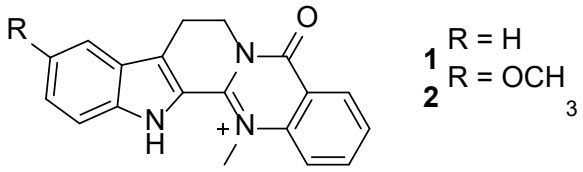
P-176

HIGH AFFINITY HERG AND LOW AFFINITY $CA_V1.2$ BLOCKERS DEHYDROEVODIAMINE AND HORTIAMINE IN DECOCTIONS OF THE TCM DRUG EVODIAE FRUCTUS

Jakob K. Reinhardt[1], Igor Baburin[2], Stanislav Andranovits[2], Steffen Hering [2], and Matthias Hamburger[1]

[1] University of Basel, Pharmacenter, Klingelbergstrasse 50, 4056 Basel, Switzerland, [2] University of Vienna, Department of Pharmacology and Toxicology, 1090 Vienna, Austria

Most herbal drugs used in Traditional Chinese Medicine (TCM) are considered safe based on their use over centuries. However, we found the major alkaloids dehydroevodiamine (**1**) and hortiamine (**2**) in Evodiae fructus (fruits of *Evodia rutaecarpa*) to be potent blockers of I_{Kr} (rapid delayed rectifier current) with proarrhythmic effects *in vitro* and *in vivo* (rabbits and Beagle dogs). For a better assessment of possible risks associated with the use of *Evodia*, aqueous decoctions were prepared according to TCM procedures from a range of herbal drug samples, and extracted alkaloids were quantified by LC-MS. Considering dosage recommendations of the Chinese Pharmacopoeia, a daily intake of 0.9-11.7 mg of **1** and 0.11-1.8 mg of **2** was calculated. The effect of these decoctions on action potentials in stem-cell derived cardiomyocytes, and the effects on HERG (IC_{50} of I_{Kr} inhibition <1 μM) and $Ca_v1.2$ (IC_{50} of I_{Ca} inhibition >50 μM) channels expressed in HEK 293 cells was determined. The intake of significant amounts of I_{Kr} blocking alkaloids **1** and **2**, together with their comparably low potency inhibition of $Ca_v1.2$ suggests a high risk for pro-arrhythmic effects such as Torsade de Pointes.



P-177

BIOFLAVONOID COMPOSITION – UP446 REDUCED CELLULAR OXIDATIVE STRESS AND ATTENUATED HYPEROXIA-COMPROMISED MACROPHAGE PHAGOCYTIC FUNCTION BY REDUCING HMGB1 RELEASE

Mesfin Yimam[1], Mei Hong[1], Ping Jiao[1], Teresa Horm[1], Qi Jia[1,] Lin L Mantell[2], Xiaojing Yang[2], Katelyn Dial[2], Alexander Gauthier[2], Mosi Lin[2]

[1]Unigen Inc., 2121 South State Street, Suite 400, Tacoma, WA 98405, USA, [2] Laboratory of Pulmonary Toxicology, College of Pharmacy, St John's University, 8000 Utopia Parkway, Jamaica, NY 11439

Particulates generated from environmental air pollution are known to exert exogenous oxidative stress to biological systems through generation of reactive oxygen species (ROS) that could lead to compromised host defense and inflammation-induced lung injury. ROS and High-mobility group box protein 1 (HMGB1) play key roles in pathogenesis of lung injury. It is a well characterized damage-associated with oxidative stress induced HMGB1 known to compromise functions of alveolar macrophages and stimulate secretion of proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. Collectively, these factors could result in detrimental pathological changes in lung injury. Natural products with significant anti-inflammatory and anti-oxidant properties could be applied to overcome pollution-triggered lung injury. Hence, we tested UP446, a compound comprised primarily of baicalin from *Scutellaria baicalensis* Georgi and (+)-catechin from the heartwoods of *Acacia catechu* in UV-induced ROS generation in human immortal keratinocyte, hydrogen peroxide-induced DNA damage in human skin fibroblast cells, and

hyperoxia-induced HMGB1 release from cultured murine macrophages. While UP446 produced statistically a significant reduction in free radical generation (29% - 45% reduction) and protection from DNA damage (36.8% reduction), perhaps the most clinically relevant activity in relation to lung injury was observed through reduction of HMGB1. UP446 resulted in dose-dependent statistically significant reductions (75.9% - 89.7%) the hyperoxia-induced HMGB1 release in RAW 264.7 cells when tested at 3.7, 11.1, and 33.3 µg/ml. UP446also attenuated hyperoxia-compromised macrophage phagocytic function of RAW 264.7 cells.

P-178

CELLULAR FREE RADICAL SCAVENGING AND DNA PROTECTING EFFECTS OF SOLIPRIN™ AND ALOEWHITE™

Ping Jiao[1], Feifei Long[2], Lidia Brownell[1], Mei Hong[1], Teresa Horm[1], Mesfin Yimam[1], Hanshuo Zhang[2], Qi Jia[1]
[1]Unigen Inc., 2121 South State Street, Suite 400, Tacoma, WA 98405, USA, [2] Genoarray Biotech, 199 Dongping Street, Suzhou 215123, China.

The oxidative stress induced by ultraviolet radiation, toxic agents, microbial insults or many other environmental factors is considered as one of the major contributors to skin hyperpigmentation and photoaging. Over production of reactive oxygen species by UVA & UVB can cause severe damage to lipids, proteins, and nucleic acids in skin cells leading to adverse effects on the structure and function of the skin. Natural products could slow or protect these deleterious processes by interfering at multiple pathways. To test this hypothesis, we evaluated several natural compounds and thousands of plant extracts from our plant library for reduction of reactive oxygen species (ROS) in human immortal keratinocyte exposed to UVA & UVB irradiation (280-500 nm) by a solar simulator. Hits from this assay were subjected to a secondary cellular assay for confirmation in DNA damage protection induced by 30% hydrogen peroxide in human skin fibroblasts. Cell viability from both assays was evaluated under oxidative stress and co-cultured with natural compounds. Two compositions - Soliprin™ (75% catechin and 15% baicalin combination) and AloeWhite™ (>95% Aloesin) have been confirmed reducing UVA&B induced ROS in keratinocytes and protecting DNA damage in fibroblasts caused by oxidative stress. Human safety and efficacy studies of both Soliprin™ and AloeWhite™ will be presented and discussed with potential as anti-skin aging & even skin tone bioactives in cosmetics.

P-179

HYDROLYZED *EPIMEDIUM* EXTRACT AND ICARITIN PROMOTE NON-GENOTOXIC ESTROGEN METABOLISM

Ryan T. Hitzman[†], Gonzalo R. Malca-Garcia[†‡], Tareisha L. Dunlap[†], Yu Tang[†‡], Shao-Nong Chen[†‡], Judy L. Bolton[†], Guido F. Pauli[†‡], Birgit M. Dietz[†*]*
[†]UIC/NIH Center for Botanical Dietary Supplements Research, [‡]Center for Natural Product Technologies; Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago

Epimedium species have been used for millennia in Traditional Chinese Medicine. Some studies have shown that *Epimedium* exhibits beneficial effects in post-menopausal bone loss due to its estrogenic properties, yet other investigations do not support estrogenic actions of *Epimedium* on breast and uterine tissue. The present study investigates the effect of *Epimedium* on AhR/ER crosstalk and, thus, chemical estrogen carcinogenesis in more detail. AhR induced P450 1A1 metabolizes estradiol (E_2) into non-genotoxic 2,3-OH-E_2, while the constitutively active P450 1B1 metabolizes E_2 to 3,4-OH-E_2, which upon further oxidation to its quinone may form genotoxic DNA adducts. Preferential activation of P450 1A1 and 2-hydroxylated estrogen metabolites are associated with reduced breast cancer risk. Moreover, the increased P450 1A1 activity has potentially chemopreventive effects in postmenopausal women with positive ER status. To analyze the influence of *Epimedium* on AhR and AhR/ER crosstalk, a methanolic *E. sagittatum* extract, an auto-hydrolyzed extract, extracts hydrolyzed with snailase enzyme, and pure icaritin (a prenylflavonoid aglyone) were evaluated. Results indicated that, while the methanolic extract had no AhR activity, snailase-treated extracts and icaritin, also found in snailase-treated extract, significantly activated AhR and degraded ERα, suggesting a positive influence on estrogen metabolism. Hydrolyzed *E. sagittatum* extracts likely increase the consistent bioavailability of icaritin and, therefore, can potentially enhance the efficacy and safety of *Epimedium* botanicals. [1F31AT010090-01; P50 AT0001555]

P-180

PHYTOCHEMICAL CHARACTERIZATION OF *ROSA MULTIFLORA* THUNB. (ROSACEAE) IN JAPAN AND SOUTH KOREA, WITH A FOCUS ON THE BIOACTIVE FLAVONOL GLYCOSIDE "MULTIFLORIN A"

Yumi Kitahiro[1], Hiroshi Ikeda[2], Hyoung-Tak Im[3], Makio Shibano[1]
[1]Department of Natural Products research, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan, [2]The University Museum, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, [3]Department of Biology, Chonnam National University, Gwangju 61186, South Korea

Dried achene or anthocarpous accessory fruits of *Rosa multiflora* Thunb., Rosae fructus ("Eijitsu" in Japanese), has been used in clinical practice to improve constipation within traditional Japanese medicine. Recently, it has been claimed that the efficacy of this crude drug is decreasing, and multiflorin A, the purgative component, was not detected within the tested samples. In order to clarify the causes of this issue, we investigated *Rosa* section *Synstylae* (Rosaceae), including *R. multiflora*, growing in Japan and South Korea with a focus on secondary metabolite multiflorin A.

We recognize that there are two chemotypes based on the occurrence (Type I) or absence (Type II) of multiflorin A. The chemotype of *Rosa* section *Synstylae* (Rosaceae) plants collected in Japan (excluding Tsushima Island) were all classified as Type I with exception of two species, *R. luciae* and *R. sambucina*. On the other hand, both Type I and Type II were detected within Rosae fructus obtained from *R. multiflora* collected in South Korea.

P-181

ROLE OF ROSEMARY EXTRACT AS A GASTROINTESTINAL PROTECTANT IN DSS MODEL OF COLITIS

Bhaskar Vemu, Jacob Veenstra, Mirielle Nauman, Xiaopei Tong, Jeremy Johnson
Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Rosemary (Rosmarinus officinalis) extract is a rich source of polyphenolic compounds including the diterpenes carnosol and carnosic acid that is often utilized as a natural food preservative. In addition to the natural food preservation properties there is also evidence that rosemary extract can improve gastrointestinal health. The multi-functional protein sestrin 2 has been identified as being under expressed in colon related diseases including colitis. Using HCT116 and SW480 we evaluated rosemary extract as a modulator of sestrin 2. Next, a dextran sodium sulfate (DSS) colitis model was used to test the efficacy of a standardized rosemary extract for improving disease outcomes. Mice were pre-treated with rosemary extract followed by exposure to the gastrointestinal irritant DSS. Rosemary extract was found to improve the disease activity index (intestinal length, weight, fecal blood, consistency and weight loss) and inflammatory markers compared to control mice ($P < 0.05$). On preliminary analysis of the samples, it was observed that the high dose rate has reversed the DSS induced decrease in Sesn2

protein. These results suggest that rosemary extract can prevent damage to the colon following exposure to DSS. On preliminary analysis of the samples, it was observed that the high dose rate has reversed the DSS induced decrease in Sesn2 protein. Further studies would be required to establish the changing molecular dynamics of Sesn2 and tight junction proteins as a function of rosemary exposure.

P-182

CYTOTOXIC AND NON-CYTOTOXIC CARDIAC GLYCOSIDES CHARACTERIZED FROM *STREBLUS ASPER*

Yulin Ren[1], Qingwei Tan[1], Wei-Lun Chen[2], Jinhong Ren[2,3], Pratik Shriwas[4,5,6], Chunhua Yuan[7], Tran Ngoc Ninh[8], Hee-Byung Chai[1], Xiaozhuo Chen[4,5,6,9], Djaja D. Soejarto[2,10], Michael E. Johnson[2,3], Joanna E. Burdette[2], and A. Douglas Kinghorn[1]

[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA. [2]Department of Medicinal Chemistry and Pharmacognosy and [3]Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA. [4]Department of Biological Sciences, [5]Edison Biotechnology Institute, and [6]Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701, USA. [7]Campus Chemical Instrument Center, The Ohio State University, Columbus, OH 43210, USA. [8]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. [9]Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA. [10]Science and Education, Field Museum of Natural History, Chicago, IL 60605, USA

The cytotoxic (+)-strebloside and a new non-cytotoxic analogue, (+)-17-hydroxystrebloside, along with other compounds, have been purified from the combined flowers, leaves, and twigs of *Streblus asper* collected in Vietnam. The C-14 hydroxy and the C-19 formyl groups were found to be important for (+)-strebloside to mediate its cytotoxicity toward HT-29 cells, but the presence of the C-17 hydroxy group resulted in such activity being abolished. A Na^+/K^+-ATPase inhibition assay showed that (+)-strebloside inhibited Na^+/K^+-ATPase, but (+)-17-hydroxystrebloside did not. Docking profiles demonstrated that (+)-strebloside binds to Na^+/K^+-ATPase through its functional C-14 hydroxy and the C-19 formyl groups. The binding between (+)-strebloside and (+)-17-hydroxystrebloside and Na^+/K^+-ATPase is similar, but the C-17 hydroxy group of the latter compound is surrounded by several hydrophobic residues of Na^+/K^+-ATPase, which may affect the binding pose and contribute to its lack of activity.

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NA^+/K^+-ATPASE-TARGETED CYTOTOXICITY AND PRELIMINARY STRUCTURE-ACTIVITY RELATIONSHIP OF DIGOXIN

Yulin Ren[1], Wei-Lun Chen[2], Jinhong Ren[2,3], Ellen J. Sass[4], Pratik Shriwas[5,6,7], Xiaozhuo Chen[5,6,7,8], David M. Lucas[1,4], Michael E. Johnson[2,3], Joanna E. Burdette[2], and A. Douglas Kinghorn[1]

[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA. [2]Department of Medicinal Chemistry and Pharmacognosy and [3]Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA. [4]Division of Hematology, Department of Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210, USA. [5]Department of Biological Sciences, [6]Edison Biotechnology Institute, [7]Molecular and Cellular Biology Program, and [8]Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA

Digoxin showed potent cytotoxicity toward human HT-29 colon cancer cells, and the C-12 and C-14 hydroxy groups and the C-17 lactone ring are important in the mediation of such an activity. However, the C-3 glycosyl residue seems not necessary for such an effect. Interestingly, a new analogue, 20,22-dihydro-21-hydroxydigoxin synthesized from digoxin, was found to be inactive in this cytotoxicity assay. A Na^+/K^+-ATPase inhibition assay showed that digoxin inhibited Na^+/K^+-ATPase, but 20,22-dihydro-21-hydroxydigoxin did not. Docking profiles indicated that digoxin binds to Na^+/K^+-ATPase through its functional C-12 and C-14 hydroxy groups. The binding between digoxin and 20,22-dihydro-21-hydroxydigoxin and Na^+/K^+-ATPase is similar, but the lactone moiety of the latter compound is surrounded by several hydrophobic residues of Na^+/K^+-ATPase, which may affect the binding pose and contribute to its lack of cytotoxicity.

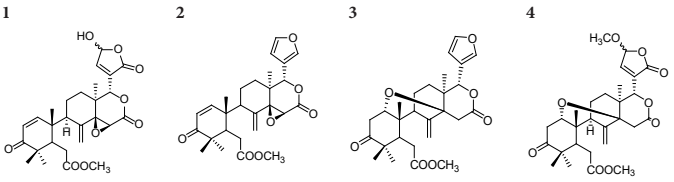
P-184

LIMONOIDS IN *ENTANDROPHRAGMA ANGOLENSE*

Isoo Youn,[1] Junfei Zhou,[1] Samiya Papa,[1] Xiao Guo,[2] Zaijie Jim Wang,[2] Chun-Tao Che[1]

[1]Department of Medicinal Chemistry and Pharmacognosy, [2]Department of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Sickle cell disease is a genetic disorder which deteriorates red blood cells into a sickle form with hemoglobin S, resulting in vaso-occlusive crises and causing anemia and pain. In the course of searching for potential anti-sickling and analgesic substances from natural source, an 80% MeOH extract of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae), an African medicinal plant allegedly useful for treating sickle cell disease in folk medicine, exhibited sedative activity in animal models. The extract was partitioned and further separated on Sephadex LH-20, reverse-phase C-18 silica gel, and semi-preparative HPLC. Up till now, four limonoids have been isolated. They were identified to be deethyl andirolide S (**1**), andirobin (**2**), and methyl angolensate (**3**), and 6-deacetoxydomesticulide D 21-methylether (**4**). Compound **1** is a new structure and **2** is identified for the first time in the *Entandrophragma* genus.



P-185

TIME ON TARGET: EFFICIENT PREPARATIVE LC GRADIENTS FOR PURIFICATION OF NATURAL PRODUCTS FROM CALIBRATION OF ANALYTICAL SYSTEMS

Jack Silver
Teledyne Isco, Lincoln, NE, USA

Preparative HPLC (high performance liquid chromatography) is widely used to purify natural products. One bottleneck in the purification process is method development. Significant time can be required to produce an efficient preparative purification method that resolves the bioactive compound from impurities and minimizes both time and solvent usage. This work extends a simple method of calibrating analytical HPLC systems to match the preparative HPLC system using the existing scouting gradients typically employed by a research group. After the calibration is complete, the determined delay volume is applied to the scouting gradient. This delay volume encompasses any dwell volumes, column volumes, mixing volumes, solvent mis-proportioning, and other corrections required to match the analytical system to the preparative system. After completing the above calibration step, the user simply enters the retention time of the desired compound from the analytical HPLC scouting run into their preparative

HPLC to generate a focused preparative method. This method was demonstrated for reverse phase chromatography, and is now extended to normal phase silica gel purifications. The ability to calculate methods for both normal and reverse phase differentiates this technique from other techniques using the linear solvent strength model, which is limited to reverse phase.

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DE-GREENING NATURAL PRODUCT CHEMISTRY: A LIQUID/LIQUID METHOD TO SELECTIVELY REMOVE CHLOROPHYLLS FROM BOTANICAL CRUDE EXTRACTS

Seon Beom Kim[1], Jonathan Bisson[1], J. Brent Friesen[1,2], Guido F. Pauli[1], and Charlotte Simmler[1]
[1]Center for Natural Product Technologies, PCRPS and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA, [2]Physical Sciences Department, Dominican University, River Forest, IL 60305, USA

Chlorophyll (Cphyl) pigments, present in crude extracts (CE) from photosynthetic organisms such as cyanobacteria, algae, and plants, frequently act as (pan-bio-)assay interfering compounds (PAINs) that precipitate in cell culture media and affect fluorescence readouts. We developeda reapid and reproducible method for Cphyl clean-up based on liquid/liquid partition using Centrifugal Partition Chromatography. In this method, the upper phase of the biphasic solvent system, composed of hexanes/EtOAc/MeOH/Water (5:5:5:5, v/v) is used as the stationary phase to capture the Cphyls from various CEs. In less than 40 min per injection, CEs can be separated ("de-greened") from their Cphyl components during mobile phase elution, leading to the production of a Cphyl Knock-Out Extract (KOE). The phytochemical profile preservation of the KOEs compared to the initial CEs, the chemical composition of the recovered Cphyl fractions, as well as the reproducibility of the cleanup method were evaluated by HPTLC, UHPLC-UV/MS, ^{1}H-NMR, and mass recovery.

P-187

EVALUATION OF STANDARDIZED SMILAX CHINA L. FOR SKIN WHITENING TREATMENT

Eunjung Son[1], Yun Mi Lee[1], Ho Kyoung Kim[1], Dong-Seon Kim[1]*
[1]Herbal Medicine Research Division, Korea Institute of Oriental Medicine, 1672 Yuseong-daero, Yuseong-gu, Dajeon 34054, Republic of Korea

Melanin, secreted by melanocytes, is the primary skin pigment in the basal layer of the epidermis. Numerous studies have been conducted on new skin-whitening agents to identify safer and more effective natural products. In a previous *in vitro* study, we discovered that *Smilax china* Linne root extract, which contains oxyresveratrol and dioscin, is a good candidate for skin whitening. The aim of the present study is to evaluate the safety and efficacy of a preparation containing SSC (Standardized *Smilax china* root extract) as a topical skin-whitening agent. Twenty-two healthy females applied a topical treatment containing 0.3% SSC on their faces for 4 weeks. The brightening effect was evaluated using a chromameter, and the effect of the treatment on hypermelanosis was evaluated by a Mexameter® and a questionnaire. A cream formula with 0.3% SSC increased skin lightness after 2 weeks (1.27% – 1.45%) and 4 weeks (2.81% – 3.35%). The melanin index value decreased after 2 and 4 weeks of application, and more than 72.7% of the subjects reported improvements. The SSC-containing cream caused no adverse reactions and effectively whitened skin; thus, SSC may be a useful active ingredient in cosmetic formulations for skin whitening.

P-188

NO CLINICALLY RELEVANT INTERACTIONS OF ST. JOHN'S WORT EXTRACT ZE 117 LOW IN HYPERFORIN WITH CYTOCHROME P450 ENZYMES AND P-GLYCOPROTEIN

Catherine Zahner[1], Esther Kruttschnitt[1], Julia Uricher[1], Michael Lissy [2], Martin Hirsch [2], Simon Nicolussi [1], Stephan Krähenbühl [3], Veronika Butterweck[1] and Jürgen Drewe [1]
[1]Max Zeller Soehne AG, Romanshorn, Switzerland, [2]Nuvisan GmbH, Neu-Ulm, Germany, [3]Division of Clinical Pharmacology & Toxicology, University Hospital, Basel, Switzerland.

Hypericum perforatum L. (St. John's wort) is used to treat mild-to-moderate depression. Its potential safety risks are pharmacokinetic drug interactions *via* cytochrome P450 enzymes and P-glycoprotein, presumably caused by hyperforin. In a phase I, open-label, non-randomized, single-sequence study, the low-hyperforin *Hypericum* extract Ze 117 was investigated using a drug cocktail in 20 healthy volunteers. No pharmacokinetic interactions of Ze 117 were observed for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and P-glycoprotein. AUC and C_{max} of the used probe drugs showed 90%-confidence intervals of the geometric mean ratios of the drugs taken together with Ze 117 *vs.* probe drug alone well within the predefined bioequivalence range of 80% to 125%. Though Ze 117 did not induce dextromethorphan metabolism by CYP2D6, it weakly increased dextromethorphan AUC ratio (mean 147.99, 95% CI 126.32-173.39) but not the corresponding metabolic ratio. Ze 117 does not show clinically relevant pharmacokinetic interactions with important CYPs and P-glycoprotein.

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IN VITRO ANTIOXIDANT & ANTIMICROBIAL ACTIVITY OF MODERN BOTANY™ PRODUCTS AND SELECTED NATURAL PRODUCT INGREDIENTS

Young M [1], Levielle G [1], Jackson S. J[2]
[1]Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland. [2]Quality Department, Modern Botany Ltd, Main Street Schull, Co. Cork, Ireland. P8I P292

The project aim was to investigate the antimicrobial and antioxidant of selected Modern Botany™ products and their ingredients. Modern Botany™ oil from different manufacturing sources was tested for antioxidant activity and results showed that the Irish manufactured Modern Botany Oil exhibited 80% DPPH radical scavenging activity, compared to UK manufactured product which had a DPPH of 73%. Selected ingredients such as *Borago officinalis* (Borage), *Calendula officinalis* (Marigold), and *Linum usitassimum* (Flaxseed) (diagram 1, table 1), also exhibited significant antioxidant activity and would warrant further analysis. These tests of Modern Botany™ products suggest that further testing is warranted for antimicrobial and antioxidant activity.

Diagram 1:

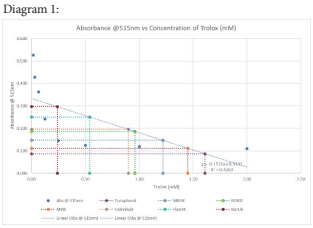

Table 1 – % DPPH scavenging and Trolox equivalence (mM) for Modern Botany Oil & Selected Ingredients

	Average ABS (Con)	Average Abs (Sam)	StdDev (Sample)	%DPPH (0.05mM)	Trolox equivalent (mM)
Tocopherol	0.544	0.086	0.020	84	1.61
MB(IE) Oil (new)	0.544	0.111	0.003	80	1.45
MB(IE) Oil (old)	0.544	0.118	0.004	78	1.40
MB UK Oil	0.544	0.147	0.025	73	1.22
Borage (IE)	0.544	0.186	0.016	66	0.96
Calendula	0.544	0.196	0.030	64	0.90
Flax(UK)	0.544	0.25	0.086	54	0.54
Flax(IE)	0.544	0.297	0.090	45	0.24
Borage (UK)	0.544	0.395	0.033	27	-0.40
Rosewood	0.544	0.453	0.065	17	-0.79
Chamomile	0.544	0.486	0.038	11	-1.0

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IN VITRO ANTIBACTERIAL ACTIVITY OF MODERN BOTANY™ PRODUCTS AND SELECTED NATURAL PRODUCT COSMECEUTICAL INGREDIENTS

Young M [1], Levielle G[1], Jackson S. J[2]
[1]Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland. [2]Quality Department, Modern Botany Ltd, Main Street Schull, Co. Cork, Ireland. P8I P292

This aim of this project was to investigate the antimicrobial activity of selected Modern Botany™ products and their ingredients used in cosmeceutical preperations. Modern Botany™ deodorant was tested for antimicrobial activity and showed bactericidal activity against both *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermis (S. epidermis*), two bacteria commonly found on skin. The deodorant also showed activity against *Escherichia coli (E. coli*) see (diag 1). These tests of Modern Botany™ products suggest that further testing is warranted for antimicrobial activity.

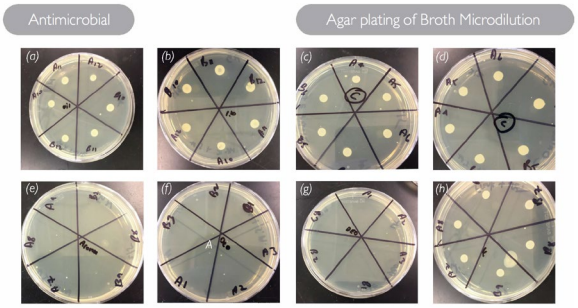

Diagram 1. a) MB oil in *S. aureus*, b) MB oil in *S. epidermis*, c) *S. aureus* control, d) *S. epidermis* control, e) Aroma in *S. aureus*, f) Deo in *S. aureus*, g) Deo in *S. epidermis*, h) Frankincense in *S. epidermis*

P-190

IDENTIFICATION OF GROWTH INHIBITING AND RESISTANCE MODIFYING BOTANICALS FOR CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII*

Micah Dettweiler[1], Monique Salazar[1], Marco Caputo[2], James T. Lyles[2], Cassandra L. Quave[1,2]
[1]Department of Dermatology, Emory University, Atlanta, GA, [2]Center for the Study of Human Health, Emory University, Atlanta, GA

Acinetobacter baumannii is a pathogenic bacterium and an emerging threat particularly associated with antibiotic resistance and nosocomial infections. In this study, we identify botanical extracts with growth inhibitory and resistance modifying activity (RMA) against carbapenem-resistant *A. baumannii*. A total of 1,476 extracts from 600 species of plants and macrofungi contained in the Quave Natural Products Library (QNPL) were screened at 256 µg/mL for growth inhibition of *A. baumannii*; of these, 24 extracts from 18 species inhibited growth above 80%. Extracts achieving this threshold were subjected to bioassay-guided fractionation and dereplication to identify bioactive fractions. To test for synergy, QNPL extracts were screened in combination with ¼ MIC meropenem, and extracts with an activity increase > 70% in combination were tested in checkerboard assays with meropenem. One extract exhibited significant synergy (FIC Index ≤ 0.5), dropping the MIC to carbapenem from 512 to 128 µg/mL, a 4-fold drop. We are actively pursuing isolation of bioactive compounds responsible for the RMA and growth-inhibitory activities.

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NATURE PRODUCT LEADS FOR DRUG RESISTANT HUMAN AND PLANT PATHOGENS

Mohamed Ali M. Ibrahim: Recipient of 2019 D. John Faulkner Travel Award
National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677

Our ongoing drug discovery research has revealed many fascinating stories that resulted in the development of numerous natural product drug leads against several challenging human and plant diseases and highlights the importance of the conservation of terrestrial natural resources (**Ibrahim et al., PNAS 2013, 110, 16832-16837**). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a destructive pathogen with a high mortality rate. More than 50% of *S. aureus* infections around the world are caused by MRSA. A group of active metabolites named "platanosides", (**Ibrahim et al., 2015 patent US8633166B2**), were isolated from *Platanus occidentalis*, commonly called American sycamore. The isolated metabolites were shown to prevent the growth of MRSA on surfaces and systemically. The in vitro anti-MRSA activity indicated that changing the olefinic geometry of the *p*-coumaroyl units greatly affects the MRSA activity. American sycamore is significant to the forest products industry and holds good potential as a dedicated biofuels crop grown on short rotations in plantations. However, the growth and productivity of sycamore plantations is hampered by bacterial leaf scorch disease (BLS) caused by *Xylella fastidiosa*. A potential ecological link has been suggested between the isolated platanosides and these serious diseases that harm many crucial American crops. Genomic DNA was isolated from sycamore leaves and subjected to PCR for DNA barcoding. Using the PCR method, the presence of *Xylella* was confirmed in all BLS-symptomatic sycamore samples. Validating this ecological link and developing these detection tools will ultimately facilitate selection of elite BLS-resistant families of sycamore as well as remedies to control *X. fastidiosa*-caused diseases.

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ANTIBACTERIAL ACTIVITIES OF METABOLITES FROM *VITIS ROTUNDIFOLIA* (MUSCADINE) ROOTS AGAINST FISH PATHOGENIC BACTERIA

Kevin K. Schrader[1], Mohamed A. Ibrahim[2,3], Howaida I. Abd-Alla[2], Charles L. Cantrell[1] and David S. Pasco[3]
[1]United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, National Center for Natural Products Research, University, MS 38677, USA. [2]Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Division, National Research Centre, Dokki, Giza 12622, Egypt. [3]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Enteric septicemia of catfish, columnaris disease and streptococcosis, caused by *Edwardsiella ictaluri*, *Flavobacterium columnare* and *Streptococcus iniae*, respectively, are the most common bacterial diseases of economic significance to the pond-raised channel catfish *Ictalurus punctatus* industry. Certain management practices are used by catfish farmers to prevent large financial losses from these diseases such as the use of commercial antibiotics. In order to discover environmentally benign alternatives, using a rapid bioassay, we evaluated a crude extract from the roots of muscadine *Vitis rotundifolia* against these fish pathogenic bacteria and determined that the extract was most active against *F. columnare*. Subsequently, several metabolites were isolated from the root extract. Among the isolated compounds, (+)-hopeaphenol (**2**) and (+)-vitisin A (**3**) were found to be the most active (bacteriostatic activity only) against *F. columnare*, with 24-h 50% inhibition concentrations of 4.0 ± 0.7 and 7.7 ± 0.6 mg/L, respectively, and minimum inhibitory concentrations of 9.1 ± 0 mg/L for each compound which were approximately 25X less active than the drug control florfenicol. Efficacy te-

sting of **2** and **3** is necessary to further evaluate their antibacterial potential against columnaris disease.

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A PROPOSED PIPELINE FOR SCREENING NATURAL PRODUCTS AS CNS DRUG CANDIDATES

Riley D. Kirk, Shelby Johnson, Joe Christian, Navindra Seeram, Matthew J. Bertin
Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881

The opioid crisis in America has created the need for pharmaceutical development of new, non-opioid analgesics. Compounds isolated from marine cyanobacteria have shown activity on opioid receptors as well as other neuro-pharmacological targets. Herein, a pipeline for screening natural products as CNS drugs that are permeable through the BBB and active on CNS receptors has been developed. A library of new-to-science pure compounds isolated from *Trichodesmium thiebautii* was evaluated using this workflow. Compounds were screened *in silico* for drug-likeliness using SWISS ADME, then assessed as potential ligands for neuro-relevant targets such as GPCR ligands using SWISS target predictor. One major obstacle in neuropharmaceutical development is passing the blood-brain barrier (BBB); The permeability assay PAMPA was used to predict the passive permeability across this membrane using both LC-MS/MS with MRM monitoring as well as the unique UV chromophores of the compounds. Murine microglia cells (BV2) were used to evaluate each compounds ability to reduce nitric oxide production via the Griess assay as well as reduce pro-inflammatory cytokines via ELISA analysis. These procedures identified the depsipeptide, unnarmicin D, as a potent anti-inflammatory with BBB permeability. In planned studies, unnarmicin D will be subjected to specific cell-based assays measuring cAMP modulation in response to G-protein activation.

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BIODIVERSITY AND ANTITUMOR ACTIVITIES OF ENDOPHYTES OF *BROUSSONETIA PAPYRIFERA* L.

Huang Baokang[1] and Yu yaru[2]
[1] College of Pharmacy, Second Military Medical University, Shanghai 200433, PR China,[2] School of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou 350108, Fujian, PR China E-mail:hbkcn@163.com

Broussonetia papyrifera (L.) Vent. Belongs to Moraceae family and is widely distributed in China. Its dried and ripe fruits are used as "Chushizi" for medicinal purposes. It has been recorded by the Chinese Pharmacopoeia and possesses multiple medical functions. As a fast-growing tree species, *Broussonetia papyrifera* has the characteristics of rapid growth, strong stress resistance and barren tolerance. It can be used for ecological control or ecological restoration. Endophytic fungi were isolated and identified from the different parts of *Broussonetia papyrifera* in different habitats, which showed its rich biodiversity. Eight strains belong to *Aspergillus*, *Apiospora*. and *Eyronellaea* were resistant to heavy metal zinc (>200 mmol/L). The fermentation products of Endophytic Fungi from *Chaetomium globousm* and *Alternaria* sp. have in vitro anti-tumor activities against SMMC-7721, A-549 and other cancer cell lines. The main active components include Altersolanol-A, C, isochaetoglobosin D, chaetoglobosin G, etc. This project is supported by NSFC (No. 81773831), and the results indicate that it can be used for ecological remediation of heavy metal contaminated soils by using the tolerance or hyperconcentration characteristics of *Broussonetia papyrifera* and its endophytic fungi.

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EXPLORING THE ANTIMICROBIAL ACTIVITY FROM ENDOPHYTES ISOLATED FROM NATIVE NORTH AMERICAN PRAIRIE PLANTS

Kirk P. Manfredi[1] Bridget Shoemaker[1], Michael Walter[2] and Laura F. Walter[3]
[1]Department of Chemistry and Biochemistry, University of Northern Iowa, Cedar Falls, IA 50614; [2]Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614; [3]Tallgrass Prairie Center, University of Northern Iowa, Cedar Falls, IA 40614

For the past 25 years our lab has been interested in the secondary metabolites produced by native prairie plants from the midwestern US. We have previously assayed prairie plant extracts for antimicrobial, anti-viral, anticancer, antioxidant, and immune enhancement activity. Though we have isolated some compounds with interesting structure and moderate activity, we have yet to identify any compounds with sufficient activity to warrant drug development. Since plants are known to harbor asymptomatic microbes (endophytes) we began an investigation into these organisms found in the seed and stems of native prairie plants. In this presentation we give the results of our initial investigation into the secondary metabolites of endophytic fungi isolated from native prairie plants. We have isolated endophytes from both the seeds of the flowering plants and their vascular tissue. We have also compared the endophytic profile from seeds isolated from wild plants collected in the field to seeds produced by the UNI Tallgrass Prairie Center for native prairie restoration. The results of the antimicrobial (fungal and bacterial) assays from fungal isolates that could be cultured on rice will be presented.

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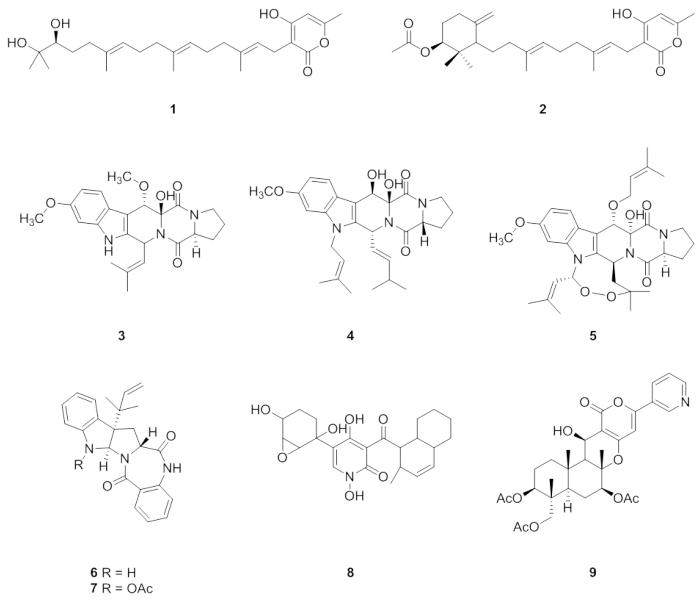
A NEW MERODITERPENOID FROM THE CULTURES OF AN ENDOPHYTIC FUNGUS, *NEOSARTORYA FISCHERI* JS533

Sunghee Bang[1], Ji Hoon Song[2], Soonok Kim[3], Ki Sung Kang[2], and Sang Hee Shim[1,]*
[1] College of Pharmacy, Duksung Women's University, Seoul 01369, South Korea, [2] College of Korean Medicine, Gachon University, Seongnam 13120, South Korea, [3] National Institute of Biological Resources, Incheon 22689, South Korea

Glehnia littoralis has been used as a traditional medicine for the treatment of stroke, however, the secondary metabolites produced by endophytic fungus from this plant have not been studied thus far. Therefore, a new meroditerpenoid, sartorypyrone E (**1**) along with eight known compounds (**2**-**9**), were isolated from ethyl acetate extract of the cultures of an endophytic fungus. The fugal strain *Neosartorya fischeri* (JS553) was obtained from a halophyte *Glehnia littoralis*, which was collected from Suncheon bay, South Korea. The planar structures of these compounds were elucidated by combined spectroscopic studies including 1D- and 2D-NMR, HR-MS, and UV and comparison with previously reported literature. The relative and absolute configurations of sartorypyrone E (**1**) were determined by ROESY and modified Mosher's method. Among the isolates, fischerin (**8**), exhibited

significant neuroprotective effect in HT22 cells by inhibition of ROS, Ca^{2+} influx and MAPKs (JNK, ERK, and p38) phosphorylation.



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NEW B-RESORCYCLIC ACID LACTONE DERIVATIVES FROM A HALOPHYTE-ASSOCIATED FUNGUS, COLLETOTRICHUM GLOEOSPORIOIDES JS419

Sunghee Bang[1], Changyeol Lee[1], Soonok Kim[2], and Sang Hee Shim[1,]*
[1]College of Pharmacy, Duksung Women's University, Seoul 01369, South Korea,[2]National Institute of Biological Resources, Incheon 22689, South Korea

A variety of bioactive secondary metabolites have been reported from plant-associated microorganism, especially endophytes. Endophytes are bacterial or fungal microorganisms that colonize in inter- and/or inter-cellularly for its life cycle within tissues of the host plant, causing no negative effects. In our chemical research for bioactive secondary metabolites, an endophytic fungus *Colletotrichum gloeosporioides* JS419, which was isolated from a halophyte *Sueda japonica* Makino collected in a swamp of Suncheon, was found to produce new polyketides. Their chemical structures were elucidated by interpretation of NMR and HRESIMS data, together with comparison of previously reported literature. In result, we isolated five new β-resorcyclic acid lactone derivatives (**1**-**6**, **8**, **13**-**15**) with two known compounds (**7**, **9**-**12**, **16**-**20**).

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ETHNOBOTANICAL AND PHYTOCHEMICAL STUDIES ON *GAULTHERIA* BERRIES FROM SOUTHWEST CHINA

Binsheng Luo[1], Chunlin Long[1,2,] and Edward J. Kennelly[3,*]*
[1]College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China, [2]Key Laboratory of Ethnomedicine (Minzu University of China), Ministry of Education, Beijing 100081, China, [3]Department of Biological Sciences, Lehman College, City University of New York, Bronx, NY 10468, USA,

The Asian and North American genus Gaultheria has blue-colored edible berries, and is a close relative of highbush blueberries (Vaccinium). Gaultheria is used in many areas for its medicinal, ornamental, ecological, and commercial values. Initial ethnobotanical investigations in southwest China found many examples of local indigenous people consuming Gaultheria berries as wild foods, including Gaultheria yunnanensis, Gaultheria griffithiana, Gaultheria longbracteolata, and Gaultheria semi-infera. A nutritional analysis found that G. longbracteolata and G. semi-infera have higher protein content (1.08g/100g, 0.76g/100g), fiber (7.6g/100g, 8.9g/100g), and lower calories (282kJ/100g, 249kJ/100g) than some blueberries. Additionally, G. griffithiana and G. longbracteolata have high vitamin C content (22.3mg/100g, 21.5mg/100g) and procyanidin content(290mg/100g, 500mg/100g) contributing to their strong antioxidant activity. HPCL-PDA analysis of the methanol extract from Gaultheria species showed high flavone content in some species. More HPLC and LC-MS analyses are being conducted to further characterize the chemical composition in Gaultheria berries, and antioxidant capacity.

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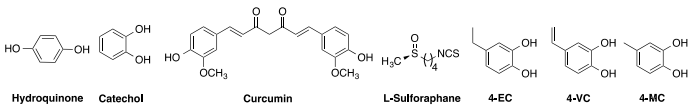
DIETARY/MEDICINAL PLANTS AND NRF2 ACTIVATION

*Xiaohua Wu, Shugeng Cao**
Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, HI, USA

According to a CDC report, 70% of annual deaths are due to chronic diseases. Disease prevention, through which individuals with risk factors for a disease are treated in order to prevent a disease from occurring, is a very important factor in our health efforts. The demand value of prevention always exceeds that of treatment. The transcription factor Nrf2 is a master regulator of oxidative stress defense in the human body. As Nrf2 modulates the expression of a large number of cyto-protective genes, it plays a critical role in the prevention of many diseases. Transient activation of Nrf2 by low dose of activators in dietary supplements or herbal medicines is beneficial on human health although Nrf2 in some cancers is constitutively over-expressed. Plant secondary metabolites, hydroquinone, catechol, curcumin and L-sulforaphane are well-known Nrf2 activators. Utilizing the Nrf2–ARE assay, we screened some dietary plants or herbal medicines used in Hawaii, Pacific Islands, and (South) East Asia. Results showed that *Barleria lupulina* (an herbal medicine used in South Asia, Southeast Asia and South China), *Morinda citrifolia* (known as Noni in Hawaii), and a few Chinese herbs obviously activated Nrf2. We identified some Nrf2 activators including 4-ethyl catechol (4-EC), 4-vinyl catechol (4-VC), and 4-methyl catechol (4-MC) from *Barleria lupulina* and *Morinda citrifolia*.



P-200

SELECTED SNP MARKER LOCI FOR LYCOPENE CONTENT ASSOCIATED WITH FLESH COLOR IN WATERMELON

Geung-Joo Lee, Saminathan Subburaja
Dept of Horticulture, Chungnam National University, Daejeon 34134, Korea

Recent interest has focused on different flesh colors in watermelon due to one of rich resources for the antioxidant cis-isomeric lycopene, one of the carotenoids. The formation of lycopene is a major step in carotenoid biosynthesis, in which lycopene β-cyclase (LCY-B) and lycopene ε-cyclase (LYC-E) enzymes are involved in the formation of lycopene, and LYC-B is involved in the formation of β-carotene. This study is to elucidate genetic relationship among watermelon genotypes with red, yellow or orange flesh color from the whole genome resequencing data. An elevated level of lycopene was noted in all red flesh watermelon lines ranging from 333 to 477 μg/g, while orange-fleshed watermelons have previously been reported to contain mainly β-carotene (91~171 μg/g), with traces of lycopene and phytoene. In the present study, we selected 2369 SNPs with lower PIC values (0.1-0.38) to discriminate 24 genotypes that exhibited different flesh colors. A cluster analysis indicated that red flesh genotypes with a high lycopene content was separated from the non-red flesh inbred lines, such as yellow or orange with a low lycopene content. We randomly selected several SNPs on

protein coding genes that presented polymorphism between red flesh and non-red flesh types. Results revealed that these SNP-carrying genes presented preferential and stage-specific expression between red and yellow genotypes. The selected SNP-linked to red flesh loci were further validated, and those SNPs were converted into cleavage amplified polymorphic sequence (CAPS) markers which allows marker-assisted selection of watermelons with high lycopene content.

P-201

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY METHODS FOR THE DETERMINATION OF UROLITHINS AND THEIR GLUCURONIDES.

Meike Tröger[1], Alexander Weng[1], Matthias F. Melzig[1], Sebastian Granica[2], Jürgen Zentek[3], Wilfried Vahjen[3], Jakub P. Piwowarski[1,2,3]
[1]Department of Pharmaceutical Biology, Freie Universität Berlin, Germany, [2]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland [3]Institute of Animal Nutrition, Freie Universität Berlin, Germany.

Urolithins – a group of bioavailable natural polyphenols – are produced from ellagitannins and ellagic acid by the gut microbiota. They are considered responsible for the health-promoting effects of ellagitannin-rich food products and medicinal plants. The HPTLC methods for qualitative and quantitative determination of urolithin A, iso-urolithin A, urolithin B and their respective glucuronides using silica gel and RP18-coated plates were developed. Quantification was carried out using fluorescence densitometry. Elution pattern, characteristic fluorescence excitation spectra and relative response factors referred to umbelliferon were determined, what allows distinct identification and quantification of urolithins without authentic reference substances. The methods were validated in terms of linearity, sensitivity, recovery and precision. The developed methods were found to be relatively fast, easy accessible, sensitive (LOQ<10 ng), precise and accurate for the determination of urolithins in various biological samples. Acknowledgment: The project was financially supported by Alexander von Humboldt Foundation.

P-202

MODULATION OF MAPK AND NFKB PATHWAYS BY HUMAN GUT MICROBIOTA METABOLITES OF ELLAGITANNINS.

Aneta Bobowska[1], Matthias F. Melzig[2], Sebastian Granica[1], Agnieszka Filipek[1], Jakub P. Piwowarski[1,2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland, [2]Department of Pharmaceutical Biology, Freie Universität Berlin, Germany.

Ellagitannins present in various natural products are metabolized by human gut microbiota to urolithins, bioavailable molecules of small size. Following absorption in the gut, urolithins rapidly undergo phase II metabolism. Thus to fully evaluate their biological activity, the *in vitro* studies should be conducted for their glucuronide conjugates. The aim of the study was to determine the influence of urolithins and their respective glucuronides on MAPK and NFκB pathways after LPS stimulation of THP-1-derived macrophages. Urolithin A (40 μM) inhibited p38 MAPK phosphorylation and induced ERK1/2 phosphorylation, while its glucuronide remained inactive. The impact on NFκB p65 nuclear translocation was in contrast more pronounced for urolithin glucuronides than for the respective aglycones. Urolithin A was the most active urolithin in terms of inhibiting the inflammatory response. Phase II metabolism was shown to significantly alter urolithins' pharmacological properties. Acknowledgment: The project was financially supported by a Polish Ministry of Science and Higher Education research grant, Iuventus Plus [IP2015 062274].

P-203

GUT MICROBIOTA TRANSFORMATIONS OF INFUSIONS FROM PLANT MATERIALS USED IN URINARY TRACT'S DISEASES.

Dominik Popowski[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1,2], Sebastian Granica[2]
[1]Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland [2]Institute for Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Urinary tract diseases (UTDs) mostly infections are affecting 150 million people each year worldwide. While treating UTD, novel medicine struggles with the issues of multi-drug resistance of uropathogens and their recurrence. Searching for a therapy as an alternative to antibiotics or as a supporting therapy pre or post to antibiotics administration may led to reconsideration of testing and usage of traditional methods, based on plant-origin medical remedies.

Human intestinal microbiome influences nutrition, immunological functions and bioavailability of xenobiotics. It can also perform biotransformation of compounds before their absorption. Describing gut microbiota influence on the form of constituents of natural products used in the UTDs treatment is important task in the process of investigating mechanisms of their action.

Presented study focuses on phytochemical characterization of infusions of medicinal plants used traditionally in treatment of UTDs (i. a. *Filipendula ulmaria*, *Ononidis spinosa*, *Polygonum aviculare*) and investigating the influence of gut metabolism on the constituents of the extracts. Gut-microbiota biotransformation of the extracts was performed by incubation the extracts with faecal samples. Different times of incubation (from 2 to 24h) were tested to describe the process. UHPLC-DAD-MS^n was used for the phytochemical screening and analysis of the post-incubation media. Various metabolites, that could be responsible for therapeutic activity, were detected (i. a. aglycons, urolithins).

P-204

CHEMICAL AND BIOLOGICAL INVESTIGATION OF IKARUGAMYCIN

David Delgadillo[1], Elizabeth A. McMillan[2], Michael A. White[2], and John B. MacMillan[1].
[1]Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA 95064, USA, [2]Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390, USA.

Ikarugamycin (IKA) is a previously isolated polycyclic tetramate macrolactam natural product, which has been shown to possess several different biologically relevant activity profiles. A chemistry-driven *de novo* discovery strategy recently identified IKA as a potent and selective inhibitor of cellular proliferation amongst Non-Small Cell Lung Cancer (NSCLC) cell lines. However, a detailed characterization of IKA and several analogs has yet to be performed. Here we describe the chemical characterization and biological cytotoxicity profiling of IKA and its' analogs against several NSCLC cell lines. Biological evaluation of all IKA analogs revealed that the double bond within the 5-6-5 ring moiety is crucial to selective antiproliferative activity against NSCLC HCC44, H23, and Calu-1. All selective compounds exhibited an IC_{50} value within the 0.09-1.00 μM range. The structures of the IKA analogs were determined by spectroscopic methods including UV-Vis, HRMS, 1D and 2D NMR techniques. This work aims to further investigate the relationship between IKA analogs and the effect that structural diversity may have on the potency and selectivity of its' antiproliferative properties in relationship to NSCLC.

P-205

NOVEL BROMINATED VINYLIC FATTY ACIDS EFFECTIVELY INHIBIT THE LEISHMANIA TOPOISOMERASE IB ENZYME MEDIATED BY HALOGEN BOND FORMATION

Néstor M. Carballeira[1], Denisse Alequín[1], Leilani M. Lotti[1], Victorio Jauregui Matos[1], Leonardo L. G. Ferreira[2], Adriano D. Andricopulo[2], Rosa M. Reguera[3], Yolanda Pérez-Pertejo[3], and Rafael Balaña-Fouce[3]
[1]Department of Chemistry, University of Puerto Rico, San Juan, PR. [2]Laboratory of Medicinal and Computational Chemistry, University of Sao Paulo, Sao Carlos, Brazil. [3]Department of Biomedical Sciences, University of León, Campus de Vegazana, León, Spain.

Marine sponges and anemones have provided most of the naturally occurring halogenated vinylic fatty acids (FA) known to date. Red algae such as *A. taxiformis* and *Bonnemaisonia nootkana* biosynthesize interesting brominated and chlorinated FA. Some of these compounds exhibit antimicrobial activity but their antiprotozoal activity remains unexplored. Our research group have been actively studying the antileishmanial activity of novel FA of marine origin, specifically targeting the leishmania topoisomerases (*L*TopIB). We hypothesized that FA with a brominated vinylic functionality could be potent TopIB inhibitors for the potential of forming halogen bonds with either the enzyme or the DNA. With this end goal in mind, we synthesized novel halogenated vinylic FA and tested their potential as *L*TopIB and hTopIB (human) inhibitors along with their antileishmanial activity towards *L. infantum* amastigotes. We report that the brominated analogs efficiently inhibit the topoisomerases as well as displaying antiparasitical activity towards *L. infantum* amastigotes.

P-206

METABOLOMIC PROFILING OF COFFEE PULP BY-PRODUCTS WITH ANTI-INFLAMMATORY PROPERTIES USING HIGH RESOLUTION MASS SPECTROMETRY

Darren Dumlao[1], Scott Harder[1], Randy Arnold[1], Baljit Ubhi[1], Marialuce Maldini[2]
[1]SCIEX, Redwood City, CA, [2]SCIEX, Milano, Italy. Mariateresa.maldini@sciex.com

OMICS is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in various -omes. Metabolomics, as a methodology for measuring small-molecule metabolite profiles has become an important component of systems biology. Because of the comprehensive nature of metabolite measurement and the capacity to detect subtle changes in a large dataset, metabolomics has found a broad application.

Coffee is the second largest traded food commodity in the world. Beyond roasted seeds, most of the original fruit, and in particular the pulp, is discarded as waste, with severe environmental and economic consequences in many developing countries. Pulp coffee by-products have been recently reported to possess an interesting anti-inflammatory activity[1], thus a metabolomics profiling of the such phytocomplexes is needed to determine compounds potentially responsible for the high biological activity. In this context, High Resolution Mass Spectrometry is required to identify and characterize with high confidence secondary metabolites occurring in a complex sample.

The TripleTOF® systems can collect high resolution MS/MS spectra at high MS/MS acquisition rates and have excellent low mass sensitivity, making the ideal instruments for metabolomics workflows.

In addition, improved, easy to use software, methods and libraries custom-designed for untargeted and targeted customer applications are available. The breadth of data acquisition capabilities is been improved by SWATH® Acquisition, MRMHR acquisition, information dependent high-resolution MS acquisition (IDA), and high-speed MS/MS scanning.

[1] Magoni et al. "Valorizing coffee pulp by-products as anti-inflammatory ingredient of food supplements acting on IL-8 release"; Food Research International 112 (2018) 129–135.

P-207

IDENTIFICATION OF ADULTERATION IN BOTANICAL SAMPLES WITH UNTARGETED MASS SPECTROMETRY METABOLOMICS

*Authors: E. Diane Wallace[1], Daniel A. Todd[1], Joshua J. Kellogg[1], Nadja B. Cech[1]**
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC 27402

According to the 2017 Council for Responsible Nutrition survey, botanicals makeup 39% of the total dietary supplement usage in the United States. The use of dietary supplements in general has increased by 8% since 2015, thus with a raise in botanical dietary supplement usage, there is a need to ascertain the authenticity and chemical composition of such products. This can be a health issue for consumers as well as possibly a much less effective product. A method for adulteration detection of botanicals utilizing untargeted mass spectrometry based metabolomics was standardized and implemented on multiple instrument platforms, including LC-UV, as well as Q-ToF and Orbitrap mass spectrometers. The outlier limitation was determined by combining two different plant species, Hydrastis canadensis L. (Ranunculaceae) and Coptis chinensis Franch. (Ranunculaceae), in different percentages to emulate different levels of adulteration. The methodology was effective on all instrument platforms, but the sensitivity varied. The detection limit at which the adulterated sample was statistically an outlier was measured using Hotelling's T^2 95% confidence interval. The outlier limitation for LC-UV, Thermo Q Exactive Plus, and Waters Synapt G2 Q-ToF were 50%, 10%, and 50% adulteration respectively. Each platform resulted in successful adulteration determination showing this method is viable across multiple platforms and detectors. A targeted analysis was also performed to show the contrast of a more quantitative approach and the reproducibility of the different mass analyzers.

P-209

IDENTIFYING NATURAL PRODUCT QUORUM SENSING INHIBITORS USING *CASTANEA* SPP. AS A MODEL SYSTEM FOR COMPOUND ACTIVITY MAPPING

James T. Lyles[1], Sandeep Voleti[2], Micah Dettweiler[3], Akram Salam[4], and Cassandra L. Quave[1,3]
[1]Center for the Study of Human Health, Emory University, Atlanta, GA 30322, [2]Department of Biology, Emory University, Atlanta, GA 30322, [3]Department of Dermatology, Emory School of Medicine, Atlanta, GA 30322, [4]Molecular and Systems Pharmacology, Emory University, Atlanta, GA 30322

Antibiotic resistant bacteria continue to be a worldwide health concern. Botanically based traditional medicine practices offer one reservoir of underexplored therapies to address hypervirulent bacterial strains. An initial screen of Italian plants used in traditional medicine identified quorum sensing (QS) inhibitors from *Castanea sativa.* Metabolomic analysis and compound activity mapping permits the study of synergistic bioactivity. To this end, leaves from six *Castanea* species and a backcross *Castanea* hybrid were analyzed by LC-MS and screened against *Staphylococcus aureus* for anti-QS activity. The MS data was processed with MZmine 2 to identify features. The biological activity was merged with the MS features by calculating activity and cluster scores in GraphPad Prism. The MS features and activity plot were visualized using the R network analysis tool xMWAS. Then, the model was dereplicated using public natural products databases. Features associated with the anti-QS bioactivity of *Casatanea* spp. were identified. Additionally, results were compared to compounds identified by bioactivity

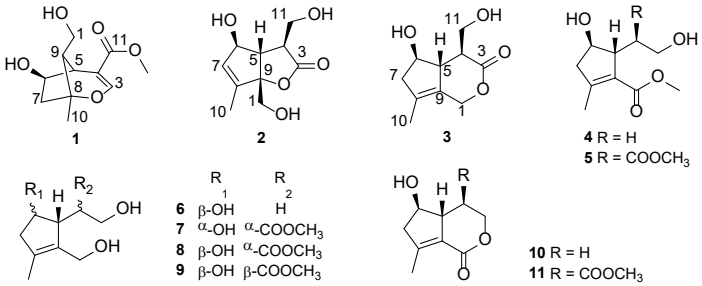
guided fractionation. This model serves as a proof of concept for the identification of plant metabolites responsible for anti-QS and other bioactivities by compound activity mapping.

P-210

IRIDOIDS FROM THE LEAVES AND BARKS OF *PSYDRAX SUBCORDATA*

Junfei Zhou, Brian Guo and Chun-Tao Che*
Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Psydrax subcordata (syn. *Canthium subcordatum,* Rubiaceae) is a medicinal plant in central and western Africa. From the leaves and barks of this plant, ten new iridoids (**1**–**10**) together with a known compound (**11**) have been isolated. The structures were elucidated based on spectroscopic analyses including the use of circular dichroism exciton chirality method for the determination of absolute configuration.

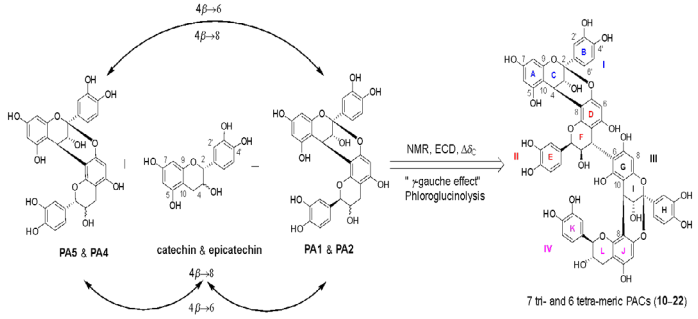


P-211

TRI- AND TETRAMERIC PROANTHOCYANIDINS WITH DENTAL ACTIVITIES FROM *PINUS MASSONIANA*

*Bin Zhou[1], Yvette Alania[2], Mariana C. dos Reis[2], Rasika S. Phansalkar[1], Joo-Won Nam[1,3], James McAlpine[1], Shao-Nong Chen[1], Ana K. Bedran-Russo[2], and Guido F. Pauli[1]**
[1]Dept Med Chem & Pharmacognosy, Coll Pharmacy and [2]Dept Restorative Dentistry, Coll Dentistry, U of IL at Chicago, IL 60612, USA; [3]College of Pharmacy, Yeungnam Univ, Gyeongbuk 712-749, ROK

In order to find the most bioactive, dentin enhancing proanthocyanidins (PACs) and explore their structure–activity relationships (SARs), a scaled-up phytochemical isolation from *Pinus massoniana* (pine) bark was carried out. Besides four common A-type dimers epicatechin-(2β→O→7,4β→8)-catechin (PA1, **2**), epicatechin-(2β→O→7,4β→8)-epicatechin (PA2, **4**), epicatechin-(2β→O→7,4β→8)-*ent*-catechin (PA4, **5**), and epicatechin-(2β→O→7,4β→8)-*ent*-epicatechin (PA5, **6**), seven trimers (**10**–**16**) and six tetramers (**17**–**22**) were isolated. The structures of all these tri- and tetrameric PACs were unambiguously assigned by NMR, electronic circular dichroism (ECD) data, the ^{13}C "γ-gauche effect", differential chemical shifts ($\Delta\delta_C$) relative to four stereo-chemically defined dimers (terminal unit II), and phloroglucinolysis combined with MS and chiral HPLC. Among the 13 PACs, eight were new, and the structure of one (**11**) was revised based on new evidence. Dental bioassay study confirmed our hypothesis that tri- and tetrameric PACs showed potential activities, and the different 4→8/4→6 linkages or terminal configurations did not make obvious difference in bioactivities. This study establishes *P. massionia* PAC library with solid structural information and in amounts that enable systematic dentin SAR studies.



P-212

INVESTIGATION OF COVALENT REVERSIBILITY OF CRM1-KPT INHIBITOR BINDING

Duy Vo[1], Yuh Min, Chook[2], John B. MacMillan[1]
[1]Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA, USA, [2]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

The secondary metabolite Leptomycin B (LMB) has been shown to be a potent inhibitor of the nuclear export receptor CRM1 in human, but with significant toxicity issues. Inhibition of CRM1 is a potential therapeutic target because imbalance in cytosolic level of certain growth-regulatory proteins transported by CRM1 has been observed in various cancer cell lines. Previous work has clarified that CRM1 inhibition by LMB is through an irreversible covalent inhibition while second generation inhibitors were designed though a slowly reversible covalent inhibition, leading to compounds with greatly improved tolerability that are in Phase I/II clinical trials. Chemically, the mechanism of action can be attributed to the Michael Addition between active site's cysteine and inhibitor. This work aims to understand how the atomic features of an inhibitor can be tuned and what effects they have on the reversibility of CRM1 inhibition. Third generation CRM1 inhibitors were designed with specific electronic nature at the C-α position in order to increase acidity of the α-proton and potentially enhance the reversible Michael Addition and deconjugation from CRM1.

P-213

MASS SPECTROMETRY UTILIZATION FOR THE DISCOVERY OF NEW TREATMENTS AGAINST ANTIBIOTIC RESISTANT GRAM-NEGATIVE BACTERIAL INFECTIONS

Heather L. Winter[1], Laura Flores Bocanegra[1], William J. Crandall[1], Fridah Rotich[2], Madeline Tillman[1], Cedric J. Pearce[3], Nicholas H. Oberlies[1], Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412, [2] Davidson College, Davidson, North Carolina, 28035, [3] Mycosynthetix, Inc, Hillsborough, NC, 27278.

In recent years, a concerning emergence of highly antibiotic-resistant and challenging to treat pathogens has resulted in an immediate need for the discovery of new antimicrobials and the reevaluation of known compounds that may have hidden antimicrobial properties. Fungi are established producers of a complex array of secondary metabolites demonstrating a broad range of bioactivities. Our team screened a fungal natural product library, consisting of 2,508 extracts, against a highly antibiotic-resistant Gram-negative strain of *Acinetobacter baumannii*, resulting in the discovery of 18 active lead extracts. Utilization of bioassay-guided fractionation, mass spectrometry-based dereplication and metabolomics has aided in deciphering the extract chemical profiles. Several known compounds have been

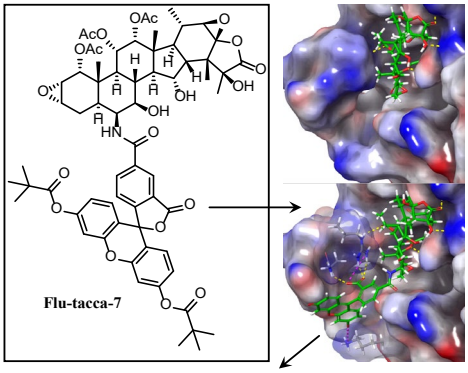
identified from active extracts and fractions, however literature holds no precedence for reported activity against Gram-negative pathogens. With minimum inhibitory concentration values comparable to known antibiotics, our lead compounds serve as unique scaffolds in the pursuit of candidates for alternative treatments to traditional classes of antibiotics.

P-214

ELUCIDATING AND OPTIMIZING THE BINDING OF COVALENT MICROTUBULE STABILIZING TACCALONOLIDES

Lin Du,[1,2] Samantha Yee,[3] and April L. Risinger.[3]
[1]Department of Chemistry and Biochemistry [2]Institute for Natural Products Applications and Research Technologies, The University of Oklahoma, Norman, OK. [3]Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX.

Many successful and effective therapeutics bind covalently to their drug targets, including aspirin, β-lactams, omeprazole, and clopidogrel. However, the irreversible nature of their binding prompts safety concerns due to potential for off-target reactivity and urges novel approaches to evaluate their target specificity. The taccalonolide microtubule stabilizers circumvent clinically relevant forms of taxane resistance due to their ability to bind covalently to β-tubulin. An amalgamation of chemical, computational, and biological approaches were employed to evaluate taccalonolide binding, including the synthesis and imaging of fluorescent probes that serendipitously improved drug binding, advanced covalent docking using COVDOCK, and a new cellular target validation method based on single-residue mutations and immunoblotting. Our cell-based method to identify critical taccalonolide-β-tubulin binding residues is the first comprehensive study of the binding site of any microtubule-stabilizing agent in a targeted manner and paves the way for future efforts to develop this class of natural products as new cancer therapeutics. Additionally, the carefully optimized taccalonolide-fluorescein probes provide superior biochemical properties compared to commercial taxane-based probes for detection and imaging of cellular tubulin.



New binding site on β-tubulin

P-215

BLOOD PRESSURE EFFECTS AND CHEMISTRY OF BLACK AND WHITE TEA FROM VIETNAM

Lucy Noon[1], Ceri L. Davies[1], and Laura M. Bystrom[1]
[1]School of Sciences and Social Sciences, Bath Spa University, Newton Park, Bath, BA2 9BN UK

Tea (*Camellia sinensis*) is one of the most widely consumed beverages in the world. Increasing evidence suggests that drinking tea can protect against cardiovascular disease. Such health effects have been attributed to the theanine, caffeine and polyphenol content of tea, all of which can affect blood pressure. This study aimed to evalaute the blood pressure effects, as well as the theanine, caffeine, and total phenolic content of black and white tea obtained from a traditional community in Vietnam. Theanine and caffeine content were measured using high-performance liquid chromatography (HPLC), and total phenolics were assessed by the Folin-Ciocalteu assay. Twenty four healthy subjects were provided with one cup of black or white tea for fourteen days. Blood pressure measurements were taken before the intervention and on day 14. Black tea was found to contain significantly higher theanine and caffeine concentrations than white tea (p=0.001). Black tea also showed a trend towards higher total phenolic content. Neither tea had significant effects on blood pressure. However, a small non-significant reduction in systolic and diastolic blood pressure was found in both experimental groups.

P-216

DESIGN, SYNTHESIS, *IN SILICO* AND BIOLOGICAL CHARACTERIZATION OF NOVEL FLAVONOL AND PYRAZOLE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS: EVIDENCE IN HUMAN SKIN CANCER CELLS

*Tithi Roy[1], Sergette Banang-Mbeumi[1], Pratik Basnet[2], Mario Sechi[3], Siva Murru[2], Jean Christopher Chamcheu[1,]**
[1]College of Pharmacy and [2]College of Art, Education & Science, University of Louisiana at Monroe, LA, USA and [3]Department of Chemistry and Pharmacy, University of Sassari, Italy.

One strategy towards meeting the urgent treatment hurdles associated with increased cancer progression and mortality is to synthesize and develop novel, safe and low-cost analogues of bioactive compounds with known anticancer health-benefits, found in plant kingdom that are regularly consumed by humans. Here, 22 new flavonol- and pyrazole-based derivatives were synthesized by microwave assisted methods, characterized by spectroscopy and various analytical techniques and, along-side curcumin and fisetin as references, were then evaluated for *in-vitro* anticancer activity against human melanoma (A375), and non-melanoma skin cancer (NMSC: UW-BCC1 and A431) cells, versus normal keratinocytes/melanocytes. Pre-treatment with all compounds (0.1-80 μM) exhibited significant decrease in cell growth/viability with minimal effects on normal cells. Four of these compounds (i.e. 6, 10, 13 and 18) displayed low micromolar anticancer activity with over 2-4-folds potency matched to references; 10>13>6>18>curcumin>fisetin. By immunoblotting, the potent analogues markedly modulated wound closure and colony formation, induced apoptosis, and mechanistically modulated deregulated molecular targets Akt, p90RSK, p70S6K, STAT3, EGFR, and ERK1/2 in melanoma and NMSC cells. Furthermore, *in silico* analysis of interactions at the ATPase site of Akt, mTOR, and p70S6K, revealed similar binding mode (6,10,18) and different behavior (13) relative to fisetin. Based on these pilot findings, bioactive flavonols (6,10,18) and pyrazole (13) are identified as novel leads to further develop potent anticancer agents with accent on skin cancers.

P-217

ANTIMALARIAL XANTHONE FROM LEAF EXTRACT OF *ANTHOCLEISTA VOGELII* PLANCH (LOGANIACEAE)

Alaribe C.S[1]., Adesegun S[2]., Bamiro J[3]., Shode. O.F[4] and Coker H.A.B[1]*
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria, [2]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria, *[3]Dept of Obsterics & Gynaecology Research Laboratory,College of Medicine,LUTH,Nigeria, [4] Dept of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, Durban. South Africa*

Anthocleista vogelii Planch (Loganiaceae) is a medicinal plant used in Nigeria for management of malaria and other ailments. Phytochemical analysis of the petroleum ether leaf extract of the plant using thin layer and column chromatography led to isolation of two xanthones identified using

IR, LC-MS, 1D and 2D NMR spectroscopy as decussatin (1-hydroxy-3,7,8-trimethoxyxanthone) and swertiaperennin (1,8-dihydroxy-3,7-dimethoxy-xanthone). The crude extract demonstrated significant ($P < 0.05$) dose dependent decrease in the level of parasitaemia in mice infected with *P. berghei* parasite using *in vivo* suppressive antimalarial screening model. Decussatin however demonstrated some level of suppressive activities. Swertiaperennin is yet to be investigated due to low yield. The crude extract and decussatin demonstrated good iron chelating ability which may be involved in its antimalarial activity. The present study demonstrated antimalarial activity of the extract.

KEY WORDS: Parasitaemia, Decussatin, Suppressive, Swertiaperennin and Anthocleista vogeliiPlanch (Loganiaceae)

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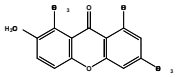
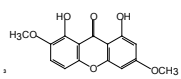

Decussatin


swertiaperennin

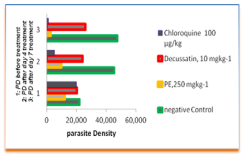


Fig 1: Illustration of the Parasite Density inhibition of Chloroquine, Crude extract, PE, Decussatin and the negative control

P-218

RESOLUTION OF OPTICALLY ACTIVE A-HYDROXY KETONES IN PSEUDO-SOLID-PHASE BY MECHANO-BIOCATALYSIS

Shuvendu Das[1,2], Changan Li[2], Shih Yang[1], Leonard MacGillivray[2], Mark Arnold[1,2]

[1]Center for Biocatalysis & Bioprocessing, Coralville, IA, USA, [2]The University of Iowa, Iowa City, IA, USA

Optically active α-hydroxy ketones are very important molecules for many drugs, inhibitors, and natural products. Specifically, benzoins and furoins are used as urease inhibitors and building blocks of different heterocyclic drug products and other organic compounds. These compounds are generally synthesized as racemic mixtures via benzoin condensation by using a catalyst such as cyanide, thiamine, and chiral thiazolium & triazolium salts. These racemic compounds can be directly resolved into its pure *R*-enantiomer via a lipase catalyzed selective acetylation reaction of the *S*-enantiomer. Generally, the lipase catalyzed acetylation reaction is carried out in organic solvents such as tetrahydrofuran (THF) over 24-72 hours. We would like to offer a game changing approach where the use of toxic organic solvents is eliminated or reduced, and the total time of reaction is decreased significantly. To accomplish this, we propose to carry out the reaction in the pseudo-solid-phase while controlling mechanical forces by grinding with a ball mill. Preliminary findings demonstrate the feasibility of this solvent-reduction (90% v/v less solvent) approach. In this preliminary work, the lipase reaction was almost completed in 30 minutes when using a mortar and pestle, thereby requiring less time and eliminating the THF solvent by replacing with only 10% (v/v) DMSO. Currently, we are optimizing reaction conditions for both enantioselective acetylation of benzoin and furoin in a ball mill. In this presentation, pseudo-solid-phase reaction conditions will be detailed, and results of these reactions will be presented. Our final goal is to extend this mechano-biocatalytic approach in other areas of biocatalysis for the improvement of green chemistry.

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(-)-OLEOCANTHAL PREVENTS BREAST CANCER RECURRENCE AFTER PRIMARY TUMOR SURGICAL EXCISION AND NEOADJUVANT THERAPIES IN ORTHOTOPIC NUDE MOUSE MODELS

Abu Bakar Siddique[1], Nehad Ayoub[2], Afsana Tajmim[1], Sharon A. Meyer[1], Khalid El Sayed[1]

[1]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana 71201, [2] Department of Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid 22110, Jordan

Several million women are living with breast cancer (BC) and have been worried about future recurrence and surviving cancer-free. BC recurrence represents a challenge for survivors who had their primary tumors surgically excised and/or completed radiation, neoadjuvant, or adjuvant chemotherapy. Current BC treatment poses high degree of morbidity and mortality risks, including lack of ability to reduce the recurrence risk. About 70% of patients with advanced disease will subsequently suffer tumor recurrence, which clearly justifying the need to discover novel recurrence inhibitors. (-)-Oleocanthal (OC) is a naturally occurring phenolic secoiridoid in extra-virgin olive oil (EVOO). Recently, OC has increased prominent attention due to its documented potent bioactivities against inflammation, Alzheimer's disease and several cancers. (-)-Oleocanthal already exerted exceptionally potent in vivo efficacy in multiple athymic nude mouse BC xenograft models. This study reports the novel ability of daily oral OC 10 mg/kg treatment to significantly prevent HER2+/ER+ BC recurrence in BT-474 nude mouse xenograft model. Interestingly, OC treatment significantly suppressed recurred tumor growth in both HER2+/ER+ BC (BT-474 cells) and triple negative BC (TNBC, MDA-MB-231 cells) in nude mouse recurrence tumor models. Further, OC 10 mg/kg treatment after completion of lapatinib neoadjuvant regimen significantly prevented BT-474 BC cells recurrence in nude mouse xenograft model. Significant reduction of the human BC recurrence marker CA 15-3 level was observed in OC-treated mice sera at the experiment end, which further confirmed OC recurrence inhibitory potential. Upregulation of E-cadherin and downregulation of vimentin along with activated c-Met and HER2, were significantly reversed in OC-treated mice recurred BC tumors, compared to vehicle control. Results highlight OC future potential as novel first-in-class natural product BC recurrence preventer.

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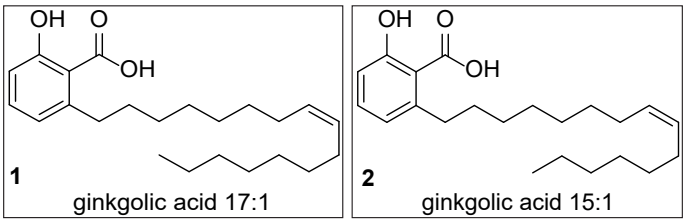
QUANTIFICATION OF ANTIMICROBIAL GINKGOLIC ACIDS BY MASS SPECTROMETRY BASED ON EXTRACTION METHOD

William J. Crandall[1], Heather L. Winter[1], Nadja B. Cech[1]

[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412.

Natural products are a historically utilized resource of unique drug candidates due to their production of diverse and complex bioactive compounds. Extraction of these compounds is important in exploratory analyses of natural products for biological activity against human disease states and pathogens. Historically, Ginkgo Biloba has been used as an herbal remedy with claims to boost immune health, increase blood flow, and preserve memory. Ginkgo Biloba contains unique compounds, referred to as Ginkgolic Acids **1**-**2**, that have demonstrated the ability to inhibit the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA). Optimization of extraction procedures can be employed to maximize the quantity of Ginkgolic Acids isolated from Ginkgo Biloba leaves. Mass spectrometry has been utilized in the quantifications of these pure compounds within the plant extracts. Comparison of extracted plant material to pure standards can be used in the assessment of extraction method effectiveness. A variety of maceration techniques will be analyzed in respect to extraction duration,

solvent composition, and temperature to maximize the quantity of bioactive compounds extracted.



P-221

ISOLATION OF *T. CRISPA* AND *T. SINENSIS* TO ADDRESS QUALITY CONCERNS FOR *TINOSPORA* DIETARY SUPPLEMENTS

Abidah Parveen[1,3], Omer Fantoukh[1], Zulfiqar Ali[2], Yan Hong Wang[2], Vijayasankar Raman[2], Ikhlas A. Khan[1,2]
[1]Department of Biomolecular Sciences, Division of Pharmacognosy, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, 38677, USA, [3]Abbottabad University of Science & Technology, Havelian, KPK, Pakistan.

Phytochemical study of *Tinospora crispa* Miers ex Hook.f. & Thomson and *Tinospora sinensis* (Lour.) Merr. (Family Menispermaceae) was performed to elaborate the chemical diversity and identify secondary metabolites that can be used as chemical/biological markers from plants used in dietary supplements. The study of the stems led to the isolation of nineteen compounds including one new from *T. crispa* and fifteen metabolites from *T. sinensis* including two new compounds. Chemical structures were elucidated by 1D and 2D NMR spectroscopy and confirmed by HRESIMS. A chemical fingerprinting method was developed by UPLC-PDA-MS and validated to distinguish *T. crispa* from *T. sinensis* and other closely related *Tinospora* species qualitatively and quantitatively to address quality concerns of *Tinospora* related dietary supplements.

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MYXOBACTERIAL PERCEPTION AND TRANSFORMATION OF EXOGENOUS ENVIRONMENTAL QUORUM SIGNALS

Barbara I. Adaikpoh[1], D. Cole Stevens[1]
[1]Department of BioMolecular Sciences, University of Mississippi, Oxford, MS 38677

The need to improve discovery of unusual bacterial specialized metabolites has necessitated exploration of less investigated and rare microorganisms including Myxobacteria, the gliding Gram-negative bacteria. Currently, over 600 unique and structurally diverse compounds have reportedly been identified via genomic and metabolomics based studies of Myxobacteria but these methods limit the scope of metabolic capacity of the bacteria especially when chemical interactions, which generally occur between microbiomes and their natural environment, are considered. Most Myxobacteria are known predators recently reported to exhibit morphological changes, which promotes predation, in response to the presence of prey in culture media. We hypothesize that Myxobacteria cultivation conditions that induce predation through supplementation with QSMs will result in production of specialized metabolites not produced under typical cultivation. MS/MS datasets were obtained from extracts of *Archangium sp*. strain Cb G35 exposed to a set of QSMs from bacterial, fungal and plant origin. Using the Global Natural Products Social Molecular Networking (GNPS) platform to provide a visual representation of the QSM-induced change in metabolic space, we observed the generation of new specialized metabolites with molecular weights ranging from 200-1000 Da, belonging to various molecular families and an interesting disappearance of certain native metabolites depicting a switch on/off in biosynthesis. Furthermore, these new specialised metabolites were produced as characteristic responses to each QSMs providing a basis for comparison. Identification of these metabolites would also reveal compounds never reported from this genus of Myxobacteria.

P-223

MULTIFUNCTIONAL REAGENTS FOR IDENTIFICATION AND ISOLATION OF NATURAL PRODUCTS FROM CRUDE EXTRACTS

Gabriel Castro-Falcón, Grant S. Seiler, Dongyup Hahn, Daniela Reimer and Chambers C. Hughes
Scripps Institution of Oceanography, University of California, San Diego. La Jolla, CA, 92037.

We have designed and implemented various reagents to query crude extracts for natural products with particular moieties or functional groups. These multifunctional reagents incorporate a reactive portion that chemoselectively binds to a chosen scaffold in natural products, as well as portions that can aid the process of detection, isolation and structural elucidation. The latter includes conspicuous chromophores and fluorogenic-moeities for UV/Vis detection, Br/Cl atoms and ionizing and fragmenting units for MS and MS^2 detection, chromatographically enrich-able components like perfluorinated alkyl chains, and crystallizing units for X-ray analysis. We have found that thiol, nitroso aryl and tetrazine reagents react chemoselectively with natural products containing electrophilic moieties (β-lactams, β-lactones, enones and epoxides), conjugated double bonds (dienes, trienes and polyenes) and isonitriles, respectively, even from crude extracts. These types of reagents are tools that can be used to systematically identify natural products with a specific functional group.

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WAUKEE ASPIRING PROFESSIONAL EXPERIENCE (APEX): AN INNOVATIVE TEACHING PROGRAM FOR HIGH SCHOOL STUDENTS TO PERFORM SCIENTIFIC RESEARCH PROJECTS FOR BUSINESSES AND ACADEMICS

Holly Showalter, Ph.D. Research Instructor
Waukee Aspiring Professional Experience (APEX), Waukee Innovation and Learning Center, 295 SE Ashworth Road, Waukee, IA, 50263.

Waukee APEX is a program in the Waukee Community School District in the Des Moines, IA area that has three goals: 1) Students perform value added projects for business and academia, 2) Students explore career options through job shadowing, informational interviews, project work, and 3) Increase the future workforce development for the state of Iowa. There are no GPA requirements or prerequisites to join the course, and students earn college credit. In addition to lessons in PCR and gel electrophoresis, students design projects such as seed germination improvement, plant tissue culture, or plant DNA extraction with a local company, Kemin Industries. Students sign confidentiality agreements, keep laboratory notebooks and present the data to the business partner while being coached by the APEX instructor. The business supplies all project materials, but owns the data and the intellectual property. The student gains real world experience so they can secure internships or undergraduate research if they so choose. Other projects with zebrafish embryos and murine cell culture are being conducted with Iowa State University and Des Moines University, respectively. Projects have been presented at local scientific conferences to give the students experience in creation of the poster along with answering questions. The course also has flexibility for the student to design their own project using the equipment available in the lab. In addition to scientific skills, each student also completes a resume and LinkedIn profile to put them ahead of their peers in college. Professional skills such as commu-

nication, collaboration and accountability are also fundamentals that are coached throughout the semester.

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TETRAHYDROQUINOLINES FROM *ALLOMYRINA DICHOTOMA* AND THEIR INHIBITORY EFFECTS ON LPS-MEDIATED HUMAN ENDOTHELIAL CELLS VIA NF-KB PATHWAY

*InWha Park[1], Wonhwa Lee[2], Joonseok Oh[3], and MinKyun Na1**
[1]College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea, [2]Aging Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea, [3]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States.

Globally, the most commonly consumed insects are beetles (Coleoptera), *Allomyrina dichotoma* L. (family Scarabeidae, order Coleoptera), a rhinoceros beetle, is mainly distributed in Korea, Japan, China, and Taiwan. The biological acitivities of *A. dichotoma* larvae have been reported to include the hepatoprotective, anticancer, anti-obesity, and antioxidant. However, the anti-inflammatory effects of small molecule from the larvae have not yet been studied. In current study, three new tetrahydroquinolines (**1**-**3**) were isolated from the larvae of *A. dichotoma*. The structures were elucidated by interpretation of NMR spectroscopic and mass spectral data. Their relative configurations were determined by analyses of the ^{1}H-^{1}H coupling constants and the NOE cross-peaks, as well as the computational chemical shifts calculation followed by DP4 analysis. Anti-inflammatory effects of **1**-**3** were evaluated in human endothelial cells. New compounds could stabilize vascular barrier integrity on LPS-induced vascular inflammation via inhibition of the NF-kB pathway.

P-226

BRAZILIAN BROWN PROPOLIS AS A NATURAL SOURCE AGAINST *LEISHMANIA AMAZONENSIS*

Victor Pena Ribeiro[1], Caroline Arruda[1], Jennyfer Andrea Aldana Mejía[1], Ana Carolina Bolela Bovo Candido[2], Lizandra Guidi Magalhães[2], Jairo Kenupp Bastos[1]
1- School of Pharmaceutical Sciences of Ribeirão Preto of the University of São Paulo, Brazil, 2- University of Franca, Brazil.

Leishmaniasis is a serious health problem around the world. The treatment available causes high toxicity and persistent side effects. Therefore, the discovery of new and safe natural-derived therapeutic agents against leishmaniasis is important. Thus, the aim of this work was to investigate the chemical composition and leishmanicidal properties of a brown propolis produced in the city of Monte Verde-MG. For this purpose, the chemical profile of the extract and its fractions were obtained by HPLC-UV-DAD, and the major compounds were isolated with chromatography techniques and analyzed by NMR for structural determination. The activity against *Leishmania amazonensis* promastigote and amastigote forms were evaluated at concentrations of 1.56, 3.12, 6.25, 12.5, 25 and 50 μg/mL with amphotericin B as positive control. Cytotoxicity experiments were also performed against normal (CHO-k1) and tumor cell lines (AGP01 and HeLa) using XTT colorimetric method. Phenolic compounds, flavonoids and terpenoids were identified in brown propolis. The major compounds were identified as follows: *p*-coumaric acid (24.6%) for methanolic fraction, and artepelin-C (29.2%) for ethyl acetate fraction. Propolis was neither cytotoxic against normal cell lines (CHO) nor cytotoxic against HeLa cell lines, with IC_{50} > 100 μg/mL, whereas it showed a potential against the AGP01 cell line with IC_{50} < 10 μg/mL. In the determination of the leishmanicidal activity, the IC_{50} was 2.8 μg/mL against amastigote forms and 3.9 μg/mL against promastigote forms, showing a promising activity against *Leishmania amazonensis*.

P-227

BIOAVAILABILITY OF ARTEMISININ DELIVERED ORALLY AS DRIED LEAVES OF *ARTEMISIA ANNUA:* INHIBITION OF HEPATIC METABOLISM AND DIFFERENCES IN ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Matthew Desrosiers[1], Alexis Mitteleman[2], and Pamela J. Weathers[1]
[1]Dept. Biology and Biotechnology; [2]Dept. Biomedical Engineering, Worcester Polytechnic Institute, Worcester MA.

Artemisia annua L. is a promising therapeutic candidate for treating malaria as it is the main producer of the antimalarial drug, artemisinin (AN). Semisynthetic derivatives of AN are the main component of artemisinin combination therapies (ACTs), the global treatment for malaria. ACTs are often unavailable or too expensive, thus alternatives are needed. Efficacy of dried leaf *A. annua* (DLA) has been demonstrated in clinical trials and enhanced bioavailability of AN from DLA has been shown, however the mechanism underlying this enhanced bioavailability is poorly understood. First pass metabolism of AN in the liver plays a major role in determining bioavailability. Here, absorption, distribution, metabolism, and excretion (ADME) studies showed that compared to male rats, females had significantly more AN in several organs after 1 hour regardless of AN delivery method. We also used human liver microsomes to determine if DLA extracts and phytochemicals inhibit the cytochrome P450 isoforms (CYPs) responsible for AN metabolism. We showed that methanolic DLA extracts had an IC_{50} about 4-fold lower than pure AN indicating other phytochemicals in DLA inhibit CYPs. Experiments investigating individual phytochemicals effects on both CYP2B6 and CYP3A4 are ongoing to determine which phytochemicals have the strongest inhibitory activity. Together these results help explain the greater bioavailability of AN from *per os* consumption of dried *A. annua* leaves vs. pure AN.

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ANTIDIABETIC *IN VITRO* AND *IN VIVO* EVALUATION OF CYCLODIPEPTIDES ISOLATED FROM *PSEUDOMONAS FLUORESCENS* IB-MR-66E

Mariana Lozano-González[1], Berenice Ovalle-Magallanes[1], Manuel Rangel-Grimaldo[1], Susana de la Torre-Zavala[2], Lilia Noriega[3], Claudia Tovar-Palacio[3], Armando Tovar-Palacio[3], and Rachel Mata[1]
[1]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico. [2]Instituto de Biotecnología, Universidad Autónoma de Nuevo León, Monterrey, Mexico. [3]Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Mexico City 14080, Mexico

Three cyclodipeptides [Cyclo (L-Pro-L-Leu) (**1**); Cyclo (L-Pro-L-Val) (**2**) and Cyclo (L-Pro-L-Phe) (**3**)] were isolated from *Pseudomonas fluorescens* IB-MR-66e. The structures were established by spectral means and corroborated by synthesis. The antidiabetic potential of compounds **1**-**3** was explored *in vivo*, *in vitro* and *in silico*. The three peptides showed important inhibitory activity against α-glucosidase enzyme. Further analysis *in vivo* using a sucrose tolerance test corroborated that compounds **1** and **3** (1-30 mg/kg) significantly reduced the postprandial state. Peptide **1** (1-30 mg/kg) also reduced the postprandial peak after a glucose challenge and exhibited significant hypoglycemic during an insulin tolerance test. Altogether, these results suggest that **1** exerts its antidiabetic action throughout a mechanism involving α-glucosidase inhibition, and other mechanisms yet to be established. Since compound **1** does not affect insulin secretion but improves its utilization, it might be a good candidate for further investigation for drug development. Moreover, the presence of DKPs in food products might be useful for preventing diabetes or its progression upon their consumption. The fact that compound **1** decreased Akt phosphorylation in muscle and mitochondrial respiration linked to ATP production highlights also its potential as an antitumor agent.

P-229

IN VITRO HEPATOTOXICITY OF *PETASITES HYBRIDUS* EXTRACT (ZE 339) DEPENDS ON THE CONCENTRATION, INTRINSIC CYTOCHROME ACTIVITY OF THE CELL SYSTEM AND THE SPECIES USED

Kristina Forsch[1], Verena Schöning[1], Greta Marie Assmann[2], Christin Moser[1], Beate Siewert[1], Veronika Butterweck[1], Jürgen Drewe[1]
[1]Max Zeller Söhne AG, CH-8590 Romanshorn, Switzerland, [2]University of Konstanz, D-78464 Konstanz, Germany

Ze 339, a CO_2 extract prepared from the leaves of *Petasites hybridus,* possesses antispasmodic and anti-inflammatory effects and is proven to be effective in the treatment of allergic rhinitis. To study possible hepatotoxic effects of Ze 339, its main constituents and metabolites, a series of *in vitro* investigations were performed. Furthermore, different reconstituted fractions of the extract were examined in three *in vitro* test systems using hepatocytes: Two human cell lines, with lower and higher intrinsic activity of cytochrome P450 enzymes (HepG2, HepaRG) as well as a rodent cell line with high intrinsic activity (H-4-II-E) were used. Metabolic activity, assessed by the WST1 assay, was chosen as indicator of cytotoxicity. To assess potential bioactivation of Ze 339 compounds, metabolic experiments using S9 fractions from rats, dogs and humans, and isolated cytochromes (human/rat) were performed and the formation of reactive metabolites was assessed by measuring cellular concentrations of glutathione and glutathione disulphide. Apoptotic behavior was examined by determining caspase activity of the extrinsic and the intrinsic pathway. Modification of mRNA expression of genes involved in adaptive, cellular defense mechanisms was examined by quantitative real-time polymerase chain reaction (RT-PCR) in HepaRG cells. Our data revealed that the cytotoxicity of Ze 339, its single constituents and main metabolites depends on the concentration, the intrinsic cytochrome activity of the cell system and the species used (rat > dog > human).

P-230

THERAPEUTIC EFFECTS OF KIOM-2015E, ACER PALMATUM THUMB., ON BENZALKONIUM CHLORIDE-INDUCED DRY EYE IN A MOUSE MODEL

Yeon Hee Kim[1,2], Eun Hee Park[1,3], Won-Kyung Cho[1], and Jin Yeul Ma[1]
[1]Korean Medicine (KM) Application Center, Korea Institute of Oriental Medicine (KIOM), Daegu, Republic of Korea, 41062, [2] Institute of New Drug Research, Myungmoon Bio, Daegu, Republic of Korea, 41059, [3] Korean Drug CO.,LTD., Seoul, Republic of Korea, 06300

Acer palmatum thumb. has been used to treat various diseases such as hemostasis, traumatic bleeding hepatic disorders, and poor eyesight in Asia. In this study, we demonstrate the therapeutic effect of Acer palmatum thumb. Leaves (KIOM-2015E) on dry eye. The efficacies of the water extracts (KIOM-2015EW) and 25% ethanol extracts (KIOM-2015EE) of Acer palmatum Thumb. Leaves were evaluated using a benzalkonium chloride-induced dry eye mouse model. Both KIOM-2015EE and KIOM-2015EW markedly increased tear production from at 4 day of treatment, and decreased lissamine green staining score, TUNEL positive cells and inflammatory indexes. The topical treatment of KIOM-2015EE showed best improvements in decreasing ocular surface staining scores, inflammation, dead cells and increasing tear production than other groups in dose-dependently. Additionally, KIOM-2015E significantly reduced NF-κB activation in BAC-treated cornea. The topical treatment showed much better effect than oral administration, and KIOM-2015EE was more effective than KIOM-2015EW. In conclusion, KIOM-2015E improves the clinical symptoms via the inhibition of inflammatory responses and alleviates dry eye-related signs. Taken together, our results indicate that KIOM-2015E has potential to be develop as a therapeutic agent to treat dry eye.

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EMBRACING MODES FOR QUALITY AND SAFETY ANALYSIS FOR SAFER BOTANICAL PRODUCTS AND AUTHENTIC PLANT SAMPLES

Abidah Parveen[1,2,3], Omer Fantoukh[1,2], Jane Manfron Budel[4], Mir Tahir Maqbool[2], Zulfiqar Ali[2], Vijayasankar Raman[2], Ganesh Babu NM[5], Jianping Zhao[2], M. Khalid Ashfaq[2], Yan-Hong Wang[2], Ikhlas A. Khan[1,2]
[1]Department of Biomolecular Sciences, Division of Pharmacognosy, [2]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA, [3]Department of Pharmaceutical Sciences, Abbottabad University of Science and Technology, Havelian, Abbottabad District, KPK, Pakistan, [4]Department of Pharmaceutical Sciences, State University of Ponta Grossa, UEPG, PR 84030-900, Brazil, [5] The University of Trans-disciplinary Health Sciences and Technology, Bengaluru-560064, Karnataka, India.

Overlapping geographical occurrence, history of traditional use, confusion in species identification and morphological resemblances among various species of *Tinospora* are some considerations that necessitate the importance of qualitative analysis for efficient quality control. Phytochemical investigation was performed and thirty-four compounds were isolated from both *T. crispa* and *T. sinensis*. Furanoditerpenoids isolated from *T. crispa* were evaluated for their role on the liver in murine model under LPS induced health compromised conditions. Studies for morpho-anatomy of authentic materials of three species of *Tinospora*, namely *T. cordifolia, T. crispa* and *T. sinensis* were conducted by light microscopy. Chromatographic profiles of the commercial products were obtained and compared by HPTLC to establish a cost effective methods to assess the quality of the products.

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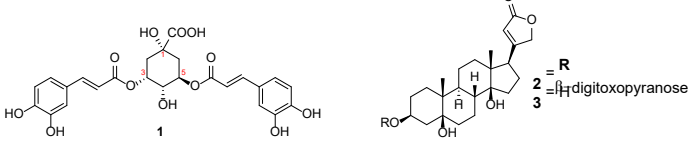
BIOASSAY-GUIDED ISOLATION OF CYTOTOXIC COMPONENTS FROM THE STEMS OF *STREPTOCAULON JUVENTAS*

Ermias Mekuria Addo,[1] Joshua M. Henkin,[2] Gerardo D. Anaya-Eugenio,[1] Tran Ngoc Ninh,[3] Yulin Ren,[1] Harinantenaina L. Rakotondraibe,[1] Esperanza J. Carcache de Blanco,[1] 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 Medicinal Chemistry and Pharmacognosy, 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 of Natural History, Chicago, IL 60605.

During the phytochemical investigation of the stems of the Vietnamese medicinal plant *Streptocaulon juventas* (Lour.) Merr. (Apocynaceae), several cytotoxic cardenolides and their glycosides as well as a lignan, a phenol and two dicaffeoyl quinic acid esters (e.g., 3,5-dicaffeoylquinic acid, **1**) were isolated. Some of the cardiac glycosides were tested against HeLa, MCF-7, and DU-145 cells, for which all of them showed potent activity [e.g., periplogenin digitoxoside (**2**) with IC_{50} values of 19.2, 96.1, and 172.9 nM, respectively], suggesting that the cardiac glycosides are the secondary metabolites responsible for the observed cytotoxic activity of *S. juventas*. Furthermore, with the exception of periplogenin (**3**), the isolated cardenolides were also toxic to zebrafish embryos at 50 μM.



P-233

JAUNDICE: SURVEY OF TRADITIONAL REMEDIES AND IN-VITRO LIPID PEROXIDATION IN THE PROGRESSION OF LIVER INJURY AT THE CELLULAR LEVEL

Adeyoyin Adeniji
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, College of Medicine Campus, PMB 12003, Suru-Lere, Lagos-Nigeria

Jaundice affects the liver. Tissue damage and endotoxemia enhances the formation of free oxygen radicals and reactive oxygen metabolites which increase lipid peroxidation through the accumulation of hydrophobic bile acids. In this study, nineteen plants were recorded during an ethnobotanical survey for jaundice therapy: *Alstonia boonei* (Apocynaceae), *Cajanus cajan* (Leguminosae: Papilionoideae), *Calliandra portoricensis* (Mimosaidaea), *Celastrus paniculatus* (Celastraceae), *Cochlospermum tinctorium* (Cochlospermaceae), *Curcuma longa* (Zingiberaceae), *Curculigo pilosa* (Hypoxidaceae), *Cymbopogon citratus* (Poaceae), *Enantia chlorantha* Oliv. (Annonaceae), *Gossypium barbadense* (Malvaceae), *Kigelia africana* (Bignoniaceae), *Lawsonia inermis* (Lythraceae), *Lophira alata* (Ochnaceae), *Mangifera indica* (Anacardiaceae), *Morinda lucida* (Rubiaceae), *Phyllanthus amarus* (Euphorbiaceae), *P. muellerianus* (Euphorbiaceae), *Rauwolfia vomitoria* (Apocynaceae), and *Sarcocephalus latifolius* (Rubiaceae). Free radical scavenging activity was evaluated using 2,2-Diphenyl-1- picrylhydrazyl (DPPH) radicals and inhibition of lipid peroxidation was determined with the thiobarbituric acid (TBA) method in two polyunsaturated fatty acid (PUFA) models of *Clarias gariepinus* Burchell (Clariidae) and *Scomber japonicus* Houttuyn (Scombridae) fish homogenates. Antioxidant activity varied from 1.53% to 80.95%. As the concentration of extract increased, the absorbance increased, while TBARS value decreased as 6.7305 x10-5 to 1.0384x10-5 (mg/tissue) and 8.2304x10-5 to 5.4100x10-5 (mg/tissue) in *C. gariepinus* and *S. japonicus* fish models, respectively. This indicated the mopping of the free radicals produced during jaundice. Thus, TBARS determination provided a measure of membrane lipid peroxidation and might be a direct assessment of the progression of liver injury at the cellular level.

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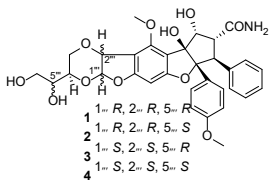
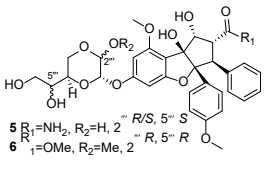
ISOLATION AND CHARACTERIZATION OF UNPRECEDENTED FLAVAGLINES FROM *AGLAIA PERVIRIDIS*

Garima Agarwal,[1] *Steven Kurnia,[2] Gerardo Anaya Eugenio,[1] Tran Ngoc Ninh,[3] Joanna E. Burdette,[2] Djaja D. Soejarto,[2,4] Esperanza J. Carcache de Blanco,[1] L. Harinantenaina Rakotondraibe,[1] and A. Douglas Kinghorn[1]*
[1] Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210; [2] College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612 [3] Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam; [4] Science and Technology, Field Museum, Chicago, IL 60605.

Cyclopenta[*b*]benzofurans, specific to the *Aglaia* spp., have garnered much interest as potential anticancer, anti-infective, and antiviral agents[2] owing to their apoptotic and translation factor inhibition activity.[1] As part of our continuing efforts to discover potential anticancer agents, the aqueous extract of *Aglaia perviridis* Hiern roots was found to contain four new flavaglines with a novel carbon skeleton (**1**-**4**) and one new compound (**5**) related to silvestrol (**6**). Furthermore, LC-MS/MS was used to create an in-house library for rapid dereplication and identification of new rocaglate derivatives.





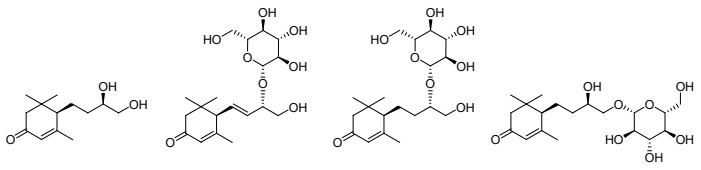
1. Pan, L. et al. *Nat. Prod. Res.* **2014**, *31*, 924-939. **2.** Muller, C. et al. *Antiviral Res.* **2018**, *150*, 123-129.

P-235

NEW MEGASTIGMANES FROM *OPUNTIA HUMIFUSA* CLADODES

Mun Seok Jo, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim.
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Opuntia humifusa, known as devil's tongue, has been used as Korean folk medicine to treat wounds and inflammation of the digestive and urinary tracts. Four new megastigmanes (**1**-**4**), and thirteen known compounds (**5**-**17**) were isolated through the phytochemical investigation of the MeOH extract of *O.humifusa* cladodes. The structures of the new compounds were determined through 1D and 2D NMR spectroscopic data analysis and HR-MS, and their absolute configurations were established by ECD measurement and computational NMR chemical shift calculations followed by DP4+ analysis as well as the application of Snatzke's method. All the isolated compounds were evaluated for their inhibitory effects on nitric oxide (NO) production in LPS-activated RAW 264.7 macrophages.

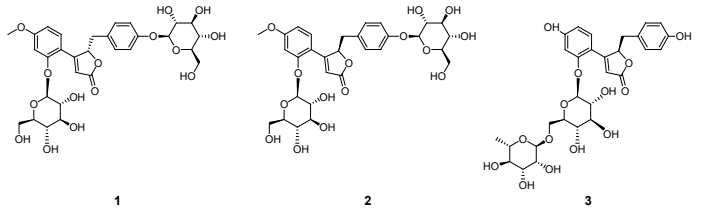


P-236

NEW 1,3-DIPHENYL PENTANE NORLIGNANS FROM *PUERARIA LOBATA* ROOTS

Mun Seok Jo, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Three new 1,3-diphenyl pentane norlignans (**1**-**3**), together with a known norlignan (**4**) and five known isoflavonoids (**5**-**9**) were isolated from the MeOH extract of *Pueraria lobata* roots. The structures of the new compounds were determined through the combination of 1D and 2D NMR spectroscopic experiments, and HR-MS as well as quantum chemical ECD calculations. All the isolated compounds were evaluated for their inhibitory effects on nitric oxide (NO) production in LPS-activated RAW 264.7 macrophages.

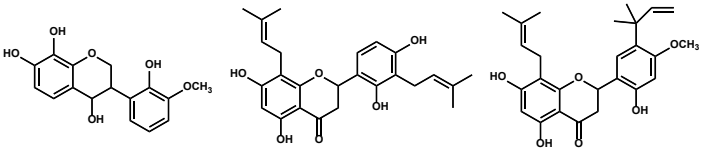


P-237

MULTIDRUG RESISTANCE IN FUNGI. PHENOLIC METABOLITES OF *DALEA* SPP. AS DIRECT INHIBITORS, AND AS PROBES OF EFFLUX TRANSPORTER SELECTIVITY

Gil Belofsky[1], Nicholas Hansen[1], Nicholas Ragland[1], Trevor Schultz[1], Brendan Wasmund[1], Rafael Cordova-Gamez[1], Anna Kolaczkowska[2], and Marcin Kolaczkowski[3]

[1]Department of Chemistry, Central Washington University, Ellensburg, WA, 98926, USA, [2]Department of Biochemistry and Molecular Biology, University of Environmental and Life Sciences, PL-50-375, Wroclaw, Poland, [3]Department of Biophysics, Wroclaw Medical University, PL-50-368, Wroclaw, Poland



Multidrug resistant (MDR) bacteria, like MDR *Staphylococcus aureus*, are now the subject of intense scientific scrutiny. Perhaps more alarming is the increased prevalence of MDR in fungi, since there are fewer effective antifungal antibiotics, and most of these are of the same chemical class of azoles. Recent news headlines drew attention to *Candida auris*, a new pathogenic, infectious MDR yeast species with high patient mortality. We report here the results of ongoing studies of the isolation, characterization, and activity of new and known phenolic metabolites toward engineered yeast strains of *Candida* and *Saccharomyces* that under- and overexpress MDR transport proteins. The assays reveal growth inhibition (cell killing), inactivation of specific transporters (e.g., *Candida* proteins Cdr1, Snq2, Yor1, and *Saccharomyces* Pdr5, Snq2, Yor1), synergistic, and antagonistic activities. The differing activities of the test compounds may lead to new insights into mechanisms of fungal MDR and suggest ways to combat these phenomena.

P-238

APPLICATION OF 1D-NMR AS A DEREPLICATION METHOD DURING THE PHYTOCHEMICAL INVESTIGATION OF *WARBURGIA UGANDENSIS* FOR MOSQUITO REPELLENT COMPOUNDS

P. Annécie Benatrehina,[1] Edna Alfaro Inocente,[2] Eliningaya Kweka,[3] Peter M. Piermarini,[2] L. Harinantenaina Rakotondraibe[1]

[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210. [2]Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH, 44691. [3]Tropical Pesticides Research Institute, Arusha, Tanzania.

Previous studies on the root bark of *Cinnamosma fragrans* (Canellaceae) led to the identification of the 1,4 unsaturated dialdehyde, cinnamodial, as the major mosquito (*Aedes aegypti*) toxic and repellent compound from this plant. To further conduct structure-activity relationships investigation, *Warburgia ugandensis*, belonging to the same family, was selected as a source of naturally-occurring drimane sesquiterpene derivatives. Through selective 1D-TOCSY NMR, known drimane sesquiterpenes and possible new compounds were detected in a crude extract of *W. ugandensis*. Among these, warbuganal showed toxicity comparable to cinnamodial against the *Ae. aegypti* larvae and adult mosquito. The application of 1D-TOCSY as a dereplication tool for metabolomics profiling of extracts of *W. ugandensis* facilitating the isolation of compounds of interest is described.

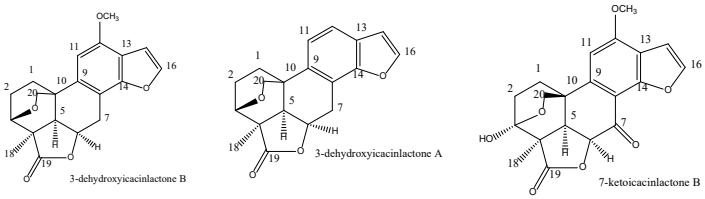
P-239

FURTHER 17-*NOR*-PIMARANES FROM *ICACINA TRICHANTHA*

Brian Guo[1], Ming Zhao[1,2], Bhaskar Vemu[3], Jeremy J. Johnson[3], and Chun-Tao Che[1]

[1]Department of Medicinal Chemistry and Pharmacognosy, [3]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA, [2]Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China.

Icacina trichantha Oliv. (Icacinaceae) is a shrub found in the forest and jungle regions of southern Nigeria. In rural areas, the large tuber of the plant is consumed for nourishment during famine and prepared in alcoholic extracts for use in the local traditional medicinal system. Reported ethnomedicinal uses include treatment of poisonings, malaria, and soft tumors. Our group has continued to conduct phytochemical analysis of the species, leading to further discovery of novel pimarane diterpenes (shown below). We also report the cytotoxic activity of several isolated compounds in human colorectal cancer HCT-116 and human prostate cancer PC-3 and 22Rv1 cell lines.



P-240

GLYCOSMIS OVOIDEA, A RUTACEOUS PLANT WITH CANCER CELL CYTOTOXICITY AND ZEBRAFISH NF-κB INHIBITORY ACTIVITIES

Peter J. Blanco Carcache[1], Nicole Eggers-Woodard[1], Tran Ngoc Ninh[2], Gerardo D. Anaya-Eugenio[1], Yulin Ren[1], Djaja D. Soejarto[3,4], A. Douglas Kinghorn[1]

[1]College of Pharmacy, The Ohio State University, Columbus, OH 43210, [2]Institute of Ecology and Biological Resources, Vietnam Academy of Science of Technology, Hanoi, Vietnam, [3]College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612, [4]Science and Education, Field Museum of Natural History, Chicago, IL, 60605

Glycosmis ovoidea Pierre is a Vietnamese native plant of the family Rutaceae for which there do not appear to have been any prior phytochemical investigations. The dried *G. ovoidea* combined stems, fruits, and leaves chloroform partition exhibited cytotoxicity against the HT-29 and MCF-7 cancer cell lines, with reversal of NF-KB p65 levels in tumor necrosis factor (TNF)-treated zebrafish (*Danio rerio*). This extract of *G. ovoidea* has afforded, thus far, a potent flavonoid cytotoxic agent, 5,3'-dihydroxy-3,6,7,8,4'-pentamethoxyflavone (**1**), a coumarin with a previously unassigned absolute configuration, six known coumarins inclusive of kimcuogin (**2**), and one new coumarin compound. The flavonoid (**1**) was tested against HeLa cells and displayed potent cytotoxicity (IC_{50} = 2 nM). All compounds isolated have been evaluated in the present investigation against a panel of cancer cell lines, and **2** showed synergizing effects on the cytotoxicity of **1**. Additionally, five of the *G. ovoidea* compounds have been investigated *in vivo*, using a zebrafish model, of which compound **1** displayed evidence of developmental toxicity.

Reference:

1. Blanco Carcache, P. J.; Tan, C.Y.; Czarnecki, A. A.; Ninh, T. N.; Fernandes, N. F.; Anaya-Eugenio G. D.; Ren, Y.; Soejarto, D. D.; Rakotondraibe, H. L.; Burdette, J. E.; Kinghorn, A. D. Poster presented at the 59th

Annual Meeting of the American Society of Pharmacognosy, Lexington, KY, July 21-25, 2018.

P-241

INHIBITION OF CHLOINESTERASE BY ALKALOIDS FROM THE RHIZOMES OF *COPTIS CHINENSIS*

Thao Quyen Cao[1], Jeong Ah Kim[2], Jae Sue[3], Byung Sun Min[1]
[1] College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea, [2]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea, [3]Department of Food and Life Science, Pukyong National University, Busan 48513, Republic of Korea.

Coptis chinensis has been used as a medicinal herb in traditional oriental medicine. In this study, chemical investigation of a water extract of *C. chinensis* identified two new quaternary protoberberines (**1**, **2**), a new tricyclic amide (**3**), together with five known compounds. Their chemical structures were elucidated by analysis with 1D and 2D NMR and high-resolution mass spectroscopy, as well as by comparison with those reported in the literature. Compounds **4**, **5**, and **7** showed potent inhibition against acetylcholinesterase (AChE) with IC_{50} values of 1.1, 5.6, and 12.9 μM, respectively. Compounds **2** and **4** showed inhibition of butyrylcholinesterase (BChE) with IC_{50} values of 11.5 and 27.8 μM, respectively. The kinetic activities were investigated to find out the type of enzyme inhibition involved. The types of AChE inhibition shown by compounds **5** and **7** were noncompetitive; BChE inhibition by compound **2** was also noncompetitive.

P-242

INHIBITION OF PTP1B BY STILBENE DERIVATIVES FROM THE RHIZOMES OF *RHEUM UNDULATUM*

Manh Tuan Ha[1], Jeong Ah Kim[2], Byung Sun Min[1]
[1]College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea, [2]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

A phytochemical study on the methanol extract of Korean rhubarb (*R. undulatum* L.) led to the isolation of nine stilbene derivatives (**1**–**9**) and one flavonoid (**10**). All structures were elucidated based on a comprehensive analysis of spectroscopic data. Compound **1** (5-methoxy-*cis*-rhapontigenin) was elucidated as a new compound, while compound **2** (5-methoxy-*trans*-rhapontigenin) was isolated from a natural source for the first time. Among the isolated compounds, stilbene derivatives (**7**–**9**) showed a strong inhibitory effect on protein tyrosine phosphatase 1B (PTP1B) with IC_{50} values ranging from 4.25 to 6.78 μM, which was significantly higher than that of the positive control, ursolic acid (IC_{50} = 11.34 μM). Furthermore, for the first time, kinetic analysis and molecular docking simulations were performed in order to understand the inhibition type as well as the interaction and binding mode of the active stilbenes (**7**–**9**) with PTP1B. Our results showed that the types of PTP1B inhibition were noncompetitive for α-viniferin (**8**) and mixed for piceatannol (**7**) and δ-viniferin (**9**). Docking simulations of these stilbenes demonstrated negative binding energies and close proximity to residues in the binding pocket of PTP1B.

P-243

TWO NEW ALKALOIDS OF NIRAM (A NATURAL DYE FROM POLYGONUM TINCTORIA) AND THEIR ANTI-INFLAMMATORY ACITIVITIES

Dong Hyun Kim[1], Kyoung Jin Park[1], Joon Min Cha[1], Tae Hyun Lee[1], Youn Sung Choi[1], Kang Ro Lee[1]
[1] Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

NIRAM was obtained by fermentation of the aerial parts from *Polygonium tinctoria*. *P. tinctoria* has been traditionally used as natural blue dye in Korea. NIRAM was reported to have anti-inflammation activity, and used for the treatment of atopic dermatitis. Previous phytochemical investigations on *P. tinctoria* reported flavonoid and alkaloid compounds. In a continuing search for bioactive constituents from Korean medical sources, we investigated EtOH extract of NIRAM. The investigation of EtOH extract of NIRAM resulted in the purification of seven alkaloid compounds (**1**-**7**), including two new compounds (**1**-**2**). The structures of new compounds were elucidated by 1D and 2D NMR (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC), IR, UV, and HRESIMS data. The absolute configurations of the new compounds were determined by ECD data analysis. Isolated compounds (**1**-**7**) were tested for their inhibitory effects on NO production in LPS-activated BV-2 cells. Compounds **1, 3,** and **5** showed potent inhibitory effects on nitric oxide production in LPS-activated BV-2 cells, with IC_{50} values of 22.87, 6.65, and 14.17 μM.

P-244

ANTIPLASMODIAL ACTIVITY OF PULCHERRIN A, I AND J FROM *CAESALPINIA PULCHERRIMA*

Osahon K. Ogbeide[1], Vincent O. Imieje[2], Osayemwenre Erharuyi[2], Irene Oseghale[2], Joseph B. Owolabi[1,3], Sammer Yousuf[4], Iqbal M. Choudhary[4], Abiodun Falodun[2]
[1]Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, [3]Department of Chemistry, School of Sciences, The Federal University of Technology, Akure, Nigeria, [4]International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.

Nature has been a reliable treasure of remedial agents and contemporary medicines for many millennia. *Caesalpinia pulcherrima* (**CP**) has been used for the management of many diseases including malaria. The phytochemical investigation of **CP** led to the isolation of Compounds **1**-**3**. These compounds were evaluated using the human malaria parasite; *P. falciparum*. Secondary antiplasmodial investigation of compounds **1**-**3** revealed significant inhibition of parasites growth in D6 and W2 clones with IC_{50} ranging from 5.36 - 11.27 μM. Compounds **1**-**3** could be lead bioactive compounds for use as chemotherapeutic agents particularly against D6 and W2 *P. falciparum*.

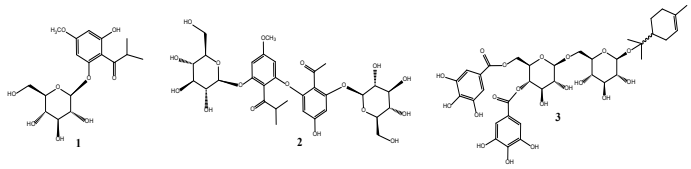
P-245

PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *CALLISTEMON CITRINUS*

Ereny M Abdelmalek[1,3], Fazila Zulfiqar[1], Mahmoud A Ramadan[3], Faten M Darwish[3], Mahmoud H Assaf[3], Samir A Ross[1,2]*
[1]National Center for Natural Products Research, [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

Callistemon citrinus (Myrtaceae) is a medicinal plant commonly known as crimson bottlebrush, which is indigenous to Australia and widely distribut-

ed in Asia and South America. The plant has been used as a traditional medicine for the treatment of gastrointestinal disorders, cough, bronchitis and infectious diseases. It is reported that the aqueous and alcoholic extracts of this plant possess antimicrobial, antioxidant, anti-inflammatory and antidiabetic activities. These finding motivated us to investigate phytochemical constituents from this plant and evaluate their biological activities. Different groups of compounds were isolated, from the methanolic extract of *C. citrinus* leaves including acylphloroglucinols, chromone glycosides, triterpenes, flavonoids and gallates. Compounds 1, 2 and 3 are new. Structure elucidation was achieved by means of 1D and 2D NMR spectroscopic and mass spectrometric techniques. The biological activity of these compounds will be discussed in the poster.

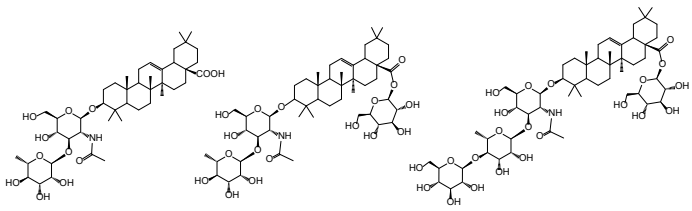


P-246

NEW TRITERPENOID GLYCOSIDES FROM *MASSULARIA ACUMINATA*

Fazila Zulfiqar[1], Samir A. Ross[1, 2], Zulfiqar Ali[1], Ikhlas A. Khan[1, 2]
[1]National Center for Natural Products Research, [2]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Massularia acuminata (Rubiaceae) is a tropical plant native to western Africa and locally known as pako ijebu or orin ijebu in Nigeria. The stem of the plant is traditionally used as a chewing stick for oral hygiene and the decoction/infusion for aphrodisiac in Nigeria. Phytochemical screening of the aqueous stem extract of *M. acuminata* revealed the presence of alkaloids, saponins, anthraquinones, phenolics, flavonoids, and tannins. However, there is no phytochemical investigation has been reported. A number of dietary supplements containing stem extract of *M. acuminata* are available in the market, claiming to have aphrodisiac potential and increase of testosterone level in the male. These findings prompted us to investigate *M. acuminata* as a part of our enduring efforts to explore phytochemical constituents of the medicinal plants which can be used as chemical/biological markers. Phytochemical investigation of *M. acuminata* stem yielded three new and three known triterpenoid glycosides. Their structures were elucidated on the basis of extensive analysis of spectroscopic data including 1D and 2D NMR and HRESIMS.

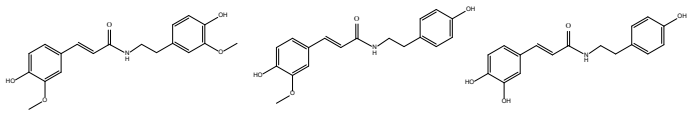


P-247

CHEMICAL CONSTITUENTS OF *LIMONIUM LEPTOPHYLLUM*

Duaa Eliwa[1,3], Fazila Zulfiqar[1], Abdel-Rahim Ibrahim[3], Amal Kabbash[3], Mona El-Aasr[3], Samir A. Ross[1,2]
*[*1]National Center of National Products Research, [2]Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA, [3]Depatment of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta 31527, Egypt.*

The genus *Limonium* Mill (*Plumbaginaceae*) known as Halophytes includes over 300 species world-wide and has a subcosmopolitan distribution in Europe, Asia, Africa, Australia and North America. Many *Limonium* species were well known in folk medicine in treatment of fever, arthritis, acute gastrointestinal diseases and as astringent. More recently, several species of the *Limonium* genus have been shown to have potential scientific, pharmacological, and medical uses as anti-inflammatory, antibacterial, antiviral and cytotoxic activity. Previous phytochemical studies of *Limonium* revealed the presence of different classes of biologically active compounds. The phytochemical analysis of methanol extract of the aerial parts of *Limonium leptophyllum* (Schrenk) resulted in the isolation and identification of flavonoids, N-trans-feruloyl-3-methoxytyramine, N-trans-caffeoyltyramine, N-trans-feruloyltyramine and gallates. Their structures were identified by spectroscopic methods including ^{1}H NMR,^{13}C NMR, 2D NMR and HRESIMS.



P-248

ARABIDAEA CHICA VERLOT: CHALLENGES ENCOUNTERED FROM CROP TO HERBAL MEDICINE

Núbia C. A. Queiroz, [1,2] Michelle P. Jorge,[1] Ilza M. O. Sousa,[1,2] Carmen S. P. Lima,[2] Maria Christina de Miranda Matias,[1] Ana Cristina Dal Rio,[2] Eduardo Baldon Pereira,[2] Victória Hahn Kakas Galassi,[1] João Ernesto de Carvalho,[1] Tais Freire Galvao,[1] Mary Ann Foglio[1]
[1] Faculty of Pharmaceutical Sciences, State University of Campinas, Sao Paulo, Brazil [2] Faculty of Medical Sciences, State University of Campinas, Sao Paulo, Brazil

Arrabidaea chica (Humb. & Bonpl.) Verlot is within the list of plant species recommended for studies by the unique system of health (RENISUS) in Brazil. The species is rich in anthocyanins with previous reports describing healing and antiulcerogenic activity in preclinical studies[1]. The crude standardized extract dried by atomization favored calcaneus tendons healing in animal model, as well as the reduction of cutaneous ulcerations, evaluated in experimental *in vivo* healing experimental model with semisolid basis incorporated extract[1,2]. The extract decreased 96% of the area of the wound in dermal ulcer models of male Wistar rats. In diabetic Wistar rats, *A. chica* extract decreased the ulcerated area by 85%.Those results prompted a randomized clinical trial of *Arrabidaea chica* formulation for oral mucositis in patients with head and neck cancer[3].

[1]Jorge M.P. et al. / Journal of Ethnopharmacology 118 (2008) 361–366; [2]A.A. Aro et al. / Life Sciences 92 (2013) 799–807; A.A. Aro et al. / Injury, Int. J. Care Injured 44 (2013) 884–892; [3]Queiroz N.C.A., et al. BMJ Open 2018;8:e019505. doi:10.1136/bmjopen-2017-019505

P-249

ARTEMISININ-BASED COMBINATION THERAPY BY TRANSDERMAL ROUTE AS THE POTENTIAL FOR IMPROVED OPTIONS IN MALARIA TREATMENT

Fabiana Volpe Zanutto[1,2], Fonseca-Santos, Bruno[3], Marlus Chorilli[3], Mary Ann Foglio[2]
[1]Faculty of Pharmaceutical Sciences, State University of Campinas, Sao Paulo, Brazil. [2]Graduate School of Bioscience and Technology of Bioactive Products, Institute of Biology, University of Campinas, Sao Paulo, Brazil. [3]UNESP- Univ Estadual Paulista, Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brazil.

Artemisinin discovered is one of the most important for malaria treatment [1,2]. These derivatives in association with aryl-amine-quinolones provide the best option for malaria treatment [3]. Artemether (ART) and Lumefantrine (LUM) come under BCS class II (poor aqueous solubility and high permeability) and these drug molecules possess low oral bioavailability due to improper dissolution and incomplete absorption [4]. New ART-LUM formulation may eliminate all the shortcomings and may lead to enhance the bioavailability due to increase solubility of these drugs across the skin, avoiding the first-pass metabolism. Permeation studies demonstrated the best outcome was achieved with ART-LUM system, with 2.5% of ART and LUM, after 24 h released 2279 ± 295 μg/cm^2 ART and 94 ± 13 μg/cm^2 LUM of drug permeation. The results presented here show the first transdermal delivery system containing the ART-LUM association.

[1]Phillips, M. A. *et al.* Malaria. (2017). doi:10.1038/nrdp.2017.50;
[2]WHO, W. H. O. WHO Model List of Essential Medicines. (2017);
[3]WHO, W. H. O. *World malaria report 2018*. (2018);
[4]Prabhu, P. *et al.* Nanostructured lipid carriers of artemether–lumefantrine combination for intravenous therapy of cerebral malaria. *Int. J. Pharm.* **513**, 504–517 (2016).

P-250

NCI PROGRAM FOR NATURAL PRODUCTS DISCOVERY: AUTOMATING THE PRODUCTION OF AN HTS FRIENDLY NATURAL PRODUCTS LIBRARY

Jason R. Evans[2,3], Spencer K. Trinh[1], Matthew R. Harris[1], John R. Britt,[1] Tanja Grkovic[1], and Barry R. O'Keefe[3,4]
[1]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, [2]Data Management Services Inc., Frederick, MD 21702, [3]Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD 21702. [4]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702.

The US National Cancer Institute's Natural Product Repository is one of the world's largest, most diverse collections of natural products containing over 230,000 unique extracts derived from plant, marine and microbial organisms that have been collected from biodiverse regions throughout the world. With the aim of making natural product libraries more amenable to HTS, we have initiated the prefractionation of the Repository extracts using an automated, high-throughput robotics platform capable of generating a library of 1,000,000 fractions. Here we present a visual tour of the robotics used in the fraction production process: from raw material to plated fraction.

P-251

A HALOPHYTIC NEW ZEALAND SPINACH [*TETRAGONIA TETRAGONOIDES* (PALL.) KUNTZE] PREVENTING OBESITY AND HYPERURICEMIA

Geung-Joo Lee[1], Young-Sil Lee[2], Seung-Hyung Kim[3], Dong-Seon Kim[2]
[1]Dept of Horticulture, Chungnam National University, Daejeon 34134, Korea, [2]Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Dajeon 34054, Korea, [3]Institute of Traditional Medicine and Bioscience, Daejeon University, Daejeon 34520, Korea

Obesity is a serious public health problem, which is associated with the development of metabolic disorders, such as type 2 diabetes, hyperlipidemia, fatty liver, hypertension, and cardiovascular disease. New Zealand spinach (NZS) that is growing along the oceans around the Korean peninsula has been used as an herbal medicine, but no report is available on its effects on obesity, lipid accumulation, or uric acid metabolism. In this study, we examined the anti-obesity and anti-hyperuricemic effects of NZS and their underlying mechanisms in high-fat diet (HFD)-induced obese mice. Mice were fed the normal fat diet (NFD); high-fat diet (HFD); HFD with 75, 150, or 300 mg/kg NZS extract; or 245 mg/kg Garcinia cambogia (GC) extract. NZS decreased body weight gain, total white adipose tissue (WAT), liver weight, and size of adipocytes and improved hepatic and plasma lipid profiles. With NZS, the plasma levels of the leptin and uric acid were significantly decreased while the levels of the adiponectin were increased. Furthermore, NZS decreased the expression levels of adipogenesis-related genes and xanthine oxidoreductase (XOR), which is involved in uric acid production, while increasing that of proteins associated with fatty acid oxidation. Results indicated that NZS exerts anti-obesity, anti-hyperlipidemia, and anti-hyperuricemic effects in HFD-induced obese mice, which are partly explained by regulation of lipid metabolism-related genes and proteins, and decreased expression of XOR.

P-252

TRITERPENOID ACIDS FROM *SCHINUS TEREBINTHIFOLIA* ATTENUATE VIRULENCE IN *S. AUREUS*

Gina Porras[1†], Huaqiao Tang[1,2†], James T. Lyles[1], John Bacsa[3], Cassandra L. Quave[1,4]*
[1]Center for the Study of Human Health, Emory University. [2]Department of Pharmacy, Sichuan Agricultural University, Chengdu, China. [3]X-ray Crystallography Center, Emory University. [4]Department of Dermatology, Emory University School of Medicine, Atlanta, GA, USA. [†]Co-first authors.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has caused a wide array of diseases that are significant causes of morbidity and mortality worldwide. Interference with bacterial virulence has emerged as an attractive approach for developing new anti-infective drugs. Quorum sensing controlled virulence factors include secreted toxins responsible for extensive damage to host tissues and evasion of the immune system response. *Schinus terebinthifolia* fruit extract was investigated as a potential source of quorum quenching compounds against *S. aureus* in our previous research. The present study describes the bio-guided isolation of three active compounds, 3-oxo-olean-12-en-28-oic acid (**1**), 3-oxotirucalla-7,24*Z*-dien-26-oic acid (**2**) and 3α-hydroxytirucalla-7,24*Z*-dien-27-oic acid (**3**) and their identification by MS, NMR and X-ray. They showed strong transcriptional inhibitory activity against all *S. aureus* accessory gene regulator (*agr*) alleles in the absence of growth inhibition (IC_{50} 2-6 μM). Selective quorum quenching activity was further supported by their ability to inhibit production of δ-toxin and lack of cytotoxicity against a human keratinocyte cell line (HaCaT). To the best of our knowledge, this is the first time that the anti-QS activity of tirucallane-type triterpenoids (**2** and **3**), was demonstrated. The findings of this study suggest that **1**-**3** have potential as candidates for anti-virulence strategies against MRSA infection.

P-253

TARGETED SCALE-UP PURIFICATION OF IRILONE FROM RED CLOVER (*TRIFOLIUM PRATENSE L.*) BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

Hyun Young Park, Seon Beom Kim, Gonzalo R. Malca-Garcia, Shao-Nong Chen, Guido F. Pauli
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA

Irilone is one of minor isoflavones in Red Clover (*Trifolium pratense* L.), recently shown to increase PRE/Luc activity induced by progesterone. As it is unavailable commercially, the aim was to purify irilone in sufficient for an in vivo animal study. Centrifugal partition chromatography (CPC) is an efficient preparative technology that was used for the targeted enrichment of irilone fraction using the optimized biphasic solvent system, **H**exane/**E**thyl acetate/**M**ethanol/**Wat**er (HEMWat 4/6/4/6). Starting with clinical extract, the irilone yield was 1.6% with >50% purity in one-step as shown by HPTLC and UHPLC. In a second orthogonal CCS step, HEMWat 3/7/5/5 was the best of five trial biphasic solvent systems for achieving, purification of irilone in high purity. The structure of the isolated irilone was confirmed by UV and NMR spectroscopy, and the purity was determined by UHPLC and qNMR methods. The two-step CPC methodology achieved a total recovery of irilone of >95% based on its content in the extract. The purified irilone has the quality of a reference standard for quantitation and is available at 100-mg scale for in vivo evaluation.

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TRAIL-SENSITIZING ACTIVITY OF C-27-CARBOXYLATED TRITERPENOIDS ISOLATED FROM *ASTILBE RIVULARIS*

Sujung Im[1], InWha Park[1], Hee Sun Byun[2], Gang Min Hur[2], and MinKyun Na[1*]
[1]*College of Pharmacy, Chungnam National University, 99 Daehak-ro, Yuseong, Daejeon 34134, Republic of Korea,* [2]*Department of Pharmacology and Physiology, College of Medicine, Chungnam National University, 266 Munhwa-ro, Daejeon 35015, Republic of Korea*

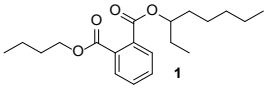
The plant *Astilbe rivularis* (Saxifragaceae) is known to be a resource of C-27-carboxylated oleanolic acids (C27OAs). Despite many recent advances, targeting of TNF-related apoptosis-inducing ligand (TRAIL) as a cancer therapy has limited success in many clinical trials, in part due to inactivation of death inducing signaling complex (DISC)-mediated caspase-8 signaling cascade in highly malignant tumors such as glioblastoma. In this study, we have isolated several C27OAs from *A. rivularis*. Screening of C27OAs derived from *A. rivularis* for TRAIL-sensitizing activity identified 3β-hydroxyolean-12-en-27-oic acid (1), 3β,6β,7α-trihydroxyolean-12-en-27-oic acid (2), and 3β-trans-*p*-coumaroyloxy-olean-12-en-27-oic acid (3) as novel TRAIL sensitizers. It is interesting that these C27OAs did not affect cell death induced by TNF and other death receptor (DR) type connections such as Fas or DNA damaging factors. Our results identify the C27OAs as new TRAIL sensitizers targeting the upstream DISC assembly of DR5, and provide a rationale for further development of C27OAs for facilitating TRAIL-based chemotherapy in glioblastoma patients.

P-255

PHYTOCHEMISTRY, ANTIOXIDANT AND HYPOGLYCAEMIC EFFECT OF *ELEUSINE CORACANA* LINN SEED EXTRACTS

Irene O. Oseghale[1], Vincent O. Imieje[1], Osayemwenre Erharuyi[1], Chidimma Iheanacho[2], Abiodun Falodun[1]
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.* [2]*Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria.*

Eleusine coracana commonly called finger millet is used in Northern Nigeria in the management of diabetes. The present study investigated the phytochemical constituents, the antioxidant and antidiabetic activities of extracts and fractions of *Eleusine coracana* seeds (ECS). Important phytochemicals and essential minerals were found present in ECS. The 50% Ethyl acetate:Methanol fraction of ECS had significant ferric reducing antioxidant power (79.02 ± 0.01 mMFSE/g) and Radical scavenging activity (IC_{50} = 29.65 **µ**g/mL). The extracts and fractions of ECS significantly reduced the average blood sugar level in diabetic experimental rats compared to control. GC-MS analysis of ECS active fraction revealed the presence of 1,2-benzenedicarboxylic acid butyl-2-ethylhexyl ester (**1**), ethyl oleate (**2**), and other compounds which may play a role in blood glucose lowering effect of ECS.



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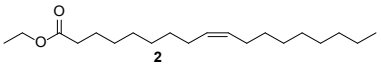


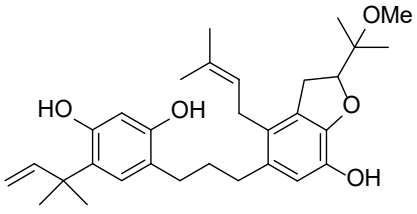
2

P-256

A NEW 1,3-DIPHENYLPROPANE ISOLATED FROM *BROUSSONETIA KAZINOKI*

Jisu Park, Ik-Soo Lee
College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea

Broussonetia kazinoki (Moraceae) is a temperate deciduous woody plant distributed throughout East Asia including Korea, Japan and China. Previous phytochemical studies on *B. kazinoki* have been reported on the isolation of alkaloids, 1,3-diphenylpropanes, and flavonoids. It also has been demonstrated that the extracts of the plant show diverse biological activities such as antifungal, antihyperglycaemic, antinociceptive, antioxidant and anti-inflammatory effects. Moreover, extract of *B. kazinoki* has been used as a cosmetic ingredient with skin whitening effect due to potent anti-tyrosinase activity. In this study, one new 1,3-diphenylpropane (**1**) was isolated from the root bark of *B. kazinoki*, together with a known isoprenylated 1,3-diphenylpropane (**2**) and three known flavans (**3**-**5**). The structure of **1** was elucidated by 1D and 2D NMR techniques and MS analyses.



1

P-257

NEW ANTICANCER METABOLITES FROM STAHLIANTHUS THORELII

Nham-Linh Nguyen[1,2], Hoa Thanh Vo[1,2], Wen-Chi Wei[2], Yu-Chi Lin[2], Zhi-Hu Lin[2], Mei-Chuan Chen[1], and Yao-Haur Kuo [1,2,3*]*

[1] Ph.D. Program in Clinical Drug Development of Herbal Medicine, School of Pharmacy, Taipei Medical University, Taipei 11031, Taiwan. [2] Division of Materia Medica Development, National Research Institute of Chinese Medicine, Taipei 11221, Taiwan. [3] Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan

In folk medicine, *Stahlianthus thorelii* has been used to treat the diseases relating to inflammation, ulcer, and cancer etc. We present here that three new phenoloc compounds, stahlithoroides A–C (**1**–**3**), along with five known analogues (**4**–**8**), have been isolated and characterized from the ethanolic extract of the rhizomes of *S. thorelii*. The structures of the new compounds were elucidated by NMR and MS data, while the relative configuration of **1** and **2** were further determined by X-ray crystallographic analysis. Compounds **1**–**6** were evaluated for cytotoxicity against 4 human tumor cell lines (A549, MCF-7, WiDr, and HepG2) with anticancer mechanism studies employing NF-κB and PDL-1 activities. Moreover, the quantification of two major compounds (**4** and **5**) by using HPLC-DAD was also validated.

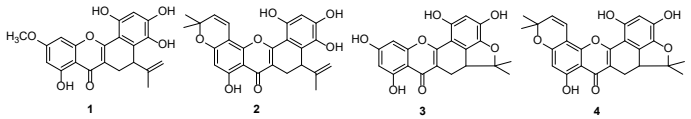
P-258

COMPETITIVE A-GLUCOSIDASE INHIBITORS FROM *ARTOCARPUS ELASTICUS*

Janar Jenis[1], Aizhamal Baiseitova[2], Ki Hun Park[2]*

[1]The Research Center for Medicinal Plants, al-Farabi Kazakh National University, Almaty, 050040, Kazakhstan, [2]Division of Applied Life Science (BK21 plus), IALS, Gyeongsang National University, Jinju, 52828, Republic of Korea

We found out that dihydrobenzoxanthones from *Artocarpus elasticus* were a good lead structure for α-glucosidase inhibition. All compounds (**1**-**6**) showed a significant enzyme inhibition toward α-glucosidase with IC_{50}s of 7.6 ~ 25.4 μM. Before to now competitive inhibitors of α-glucosidase haven't been reported from natural phenolic compounds. Competitive inhibition was thoroughly studied by different analyses. All tested dihydrobenzoxanthones (**1**-**4**) were established as competitive reversible simple slow-binding inhibitors. Binding affinity presented dose-dependence and correlated to inhibitory potencies (IC_{50}). Molecular modelling experiments showed that all inhibitors have sufficient hydrophobic interaction with F157 and R312 and arrayed the same direction in the active site. In addition, compounds **1** and **5** were established to be new compounds named as artoindonesianin W and artoflavone B, respectively.



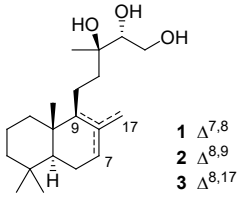
P-259

ANTI-*LISTERIA* DITERPENOIDS FROM *MADIA ELEGANS* FROM NORTH AMERICA

Jason A. Clement[1], Sung Ryeol Park[1], Matthew Todd[1], Fangyuan Zhang[2], and Zuyi Huang[2], Yanhong Liu[3]

[1]Natural Product Discovery Institute, Baruch S. Blumberg Institute, Doylestown PA, 18902, USA, [2]Department of Chemical Engineering, Villanova University, Villanova, PA 19085, USA. [3]Molecular Characterization of Foodborne Pathogens, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA.

Listeriosis is a serious bacterial infection caused by the food-borne pathogen *Listeria monocytogenes*. There are about 1,600 cases of listeriosis every year in the U.S.A., but of those about 260 die every year. Plants extracts used as additives in packaging might be an alternative to synthetic preservatives for prevention of the growth of *L. monocytogenes* in meat and dairy products. From recent screening of a library of nearly 1,800 crude liquid partitioned plant extracts, an extract of *Madia elegans* was chosen for further investigation. From the dichloromethane-soluble portion of a methanol extract of whole plant material, compounds **1**-**3** were isolated as a mixture. These compounds gave MIC values against *L. monocytogenes* on the order of 90 μM. These results suggest that *M. elegans* might be a candidate for further study as a natural preservative to prevent listeriosis.



P-260

PHYTOPROGESTINS FROM RED CLOVER, DOGWOOD, HOPS, WILD YAM, AND BLACK COHOSH MODULATE PROGESTERONE SIGNALING

Jeongho Lee, Julia R. Austin, Matthew Dean, Joanna E. Burdette, and Brian T. Murphy

Department of Medicinal Chemistry and Pharmacognosy and Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA

The use of botanical dietary supplements is becoming increasingly popular for the alleviation of hormonal-based conditions such as hot flashes, premenstrual syndrome, and fertility. Estrogen and progesterone receptors (ER and PR) play an essential role in these processes. However, despite the fact that many therapies used to alleviate gynecological conditions act through PR-mediated mechanisms, few studies have investigated or identified any herbal natural product components that act on this receptor, particularly when comparing literature focused on estrogenic constituents. In the current study, we used a progesterone response element (PRE)-luciferase reporter assay to identify phytoprogestins from five plants, red clover (*Trifolium pratense*), dogwood (*Cornus officinalis*), hops (*Humulus lupulus*), wild yam (*Dioscorea villosa*), and black cohosh (*Actaea racemosa*). Bioassay guided fractionation led to isolate nine phytoprogestins, which modulated PR activity.

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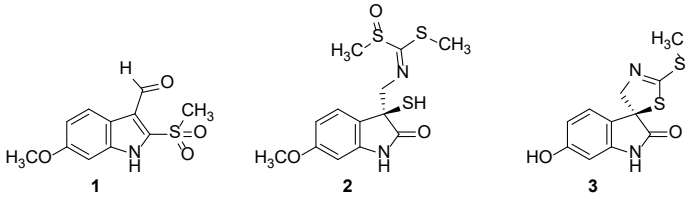
NEW SULFUR-CONTAINING INDOLE ALKALOIDS FROM TURKMEN *CAPPARIS HERBACEA* AND THEIR NEUROPROTECTIVE EFFECT AGAINST GLUTAMATE-INDUCED HT22 CELL DEATH

Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Mun Seok Jo, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Genus *Capparis* is used as traditional medicine for various purposes in the Middle East, but in-depth research regarding the chemical constituents and its biological properties are yet to be studied. Phytochemical investigation of the roots of *C. herbacea* was carried out with a comparative LC/MS based analytical approach to search for new bioactive molecules. As a result, three new sulfur-containing indole alkaloids (**1-3**) and known alkaloids (**4-6**) were identified and characterized by 1D, 2D-NMR, HR-MS, and quantum chemical ECD calculation. All isolated compounds were tested for neuroprotective effect against glutamate-induced HT22 cell death. This study is the first to report the chemical investigation of *C. herbacea*.

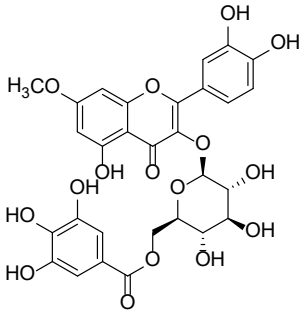


P-262

NEW CHEMICAL CONSTITUENTS FROM OMANI *WOODFORDIA UNIFLORA* AND THEIR BIOLOGICAL PROPERTIES

Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Mun Seok Jo, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Woodfordia uniflora, a slender tall shrub distributed region near Sub-Saharan Africa, East Africa and Middle East. No research has been previously done and the lack of information with regards to its chemical constituents prompted the phytochemical investigation on this plant. LC/MS-guided isolation led the identification of three new compounds (**1-3**), including one flavonoid glucoside derivative (**1**), one catechin derivative (**2**), and one synthetic catechin derivative (**3**). The known compounds were identified as six flavonoid glucoside derivatives (**4-9**) and three catechin derivatives (**10-12**). All the compounds were characterized by 1D, 2D-NMR, HR-MS, and quantum chemical ECD calculation. All the isolates were teste for their biological properties.



P-263

EVALUATING THE EFFECT OF OREGANO ESSENTIAL OIL AND CARVACROL ON SESTRIN 2 EXPRESSION IN HCT 116 COLON CELLS

Jacob Veenstra[1], Bhaskar Vemu[2], Mirielle Nauman[1] Brian Guo[1], CT Che[1], Jeremy Johnson[2]
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607, [2]Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, IL 60607

The purpose of this study was to determine the effect of oregano essential oil (OEO) and its major component, carvacrol, on sestrin 2 expression and regulation of mTORC1. Sestrin 2 is a multi-functional protein regulated by Nrf2 transcriptional activity. Sestrin 2 has been shown to regulate the activity of mTORC1, a determining factor in cell growth and inflammation. Phytochemicals found in Mediterranean herbs were evaluated for promoting sestrin 2 protein expression in HCT 116 colon cells. Our lab identified carvacrol, the major phytochemical component of OEO, as an inducer of sestrin 2. This study used OEO and carvacrol treatment in HCT 116 cells to determine the expression of sestrin 2. Western blot analysis was performed to determine the effect of sestrin 2 on mTORC1 phosphorylation and effect on its downstream targets. OEO treatment on HCT 116 cells for 24 hours showed a significant increase in sestrin 2 expression. Immunoblot with OEO also revealed that increasing sestrin 2 expression decreased phosphorylated p70S6K1, suggesting an inhibitory effect on mTOR activity. This study suggests that OEO and carvacrol are effective inducers of sestrin 2 and mTORC1 inhibition. Sestrin 2 induction could be a possible mechanism for preventing or treating certain gastrointestinal diseases associated with inflammation.

P-264

TARGETED ISOLATION OF PHOSPHODIESTERASE-4 INHIBITORY SELAGINELLIN ANALOGUES USING MS/MS-BASED DEREPLICATION ENHANCED BY *IN SILICO* ANNOTATION STRATEGY

Sunmin Woo[1], Jinwoong Kim[1], Sang Hyun Sung[1], and Kyo Bin Kang[2]
[1]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Korea, [2]College of Pharmacy, Sookmyung Women's University, Seoul 04310, Korea.

Selaginellins, unique pigments of the genus *Selaginella*, were recently reported as potent phosphodiesterase-4 (PDE4) inhibitors. We applied a MS/MS molecular networking-based dereplication strategy enhanced by *in silico* structural annotation, in order to prioritize selaginellin derivatives in *S. tamariscina*. Even without any previous isolated compounds or reported reference spectra of selaginellins, we could prioritize and isolate selaginellin derivatives. As a result, we could isolate ten previously unknown derivatives containing two unusual 1*H*,3*H*-dibenzo[*de*,*h*]isochromene analogues named selariscins A (**1**) and B (**2**). Some isolates showed PDE4 inhibitory activity with IC_{50} values in the range of 2.8–33.8 μM, and their binding modes were suggested by a molecular docking study.

P-265

CHEMICAL CHARACTERIZATION OF ALKALOIDS FROM KRATOM (*MITRAGYNA SPECIOSA*)

Laura Flores-Bocanegra, Diane Wallace, Daniel A. Todd, Joshua J. Kellogg, Tyler N. Graf, Nadja B. Cech, Nicholas H. Oberlies
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412.

Mitragyna speciosa, commonly known as kratom, has been used in traditional medicine mainly to relieve pain. It is finding growing use in the USA, although serious controversy exists on where it helps to stave or to enhance opioid addiction. While the chemistry of this plant has been investigated thoroughly, the characterization data in the literature are not always complete, especially for 2019 standards. Our team is working on a comprehensive study of the metabolism of the kratom alkaloids. However, very few of the nearly 50 compounds described from this plant are available commercially, and even for the ones that are, the quality of the standards is not always ideal. As such, we have started a project to isolate and characterize the kratom alkaloids, with an emphasis on modern spectroscopic and spectrometric techniques. In particular, this includes comparisons between theoretical and empirical ECD spectra. In addition, we developed a chromatographic method useful to identify and quantify these compounds in the context of kratom extracts.

P-266

QUASSINOID GLYCOSIDES MAY CONTRIBUTE TO THE ANTICANCER PROPERTIES OF FRUCTUS BRUCEAE IN VIVO

Yuan Xu[1], Mingming Xu[1], Lin Zhang[1], Zhenhua Zhu[1], Sheng Guo[1], Shulan Su[1], Jianming Guo[1], Chun-Tao Che[2], Zhi-Xiu Lin[3], Ming Zhao[1], Jin-Ao Duan[1]
[1]Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, China, [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, United States, [3]School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, China

Bruceoside A, an abundant quassinoid glycoside in Fructus Bruceae, was chosen for the pharmacokinetic study. It is the first case report on the pharmacokinetic study of quassinoid glycosides so far. A sensitive, accurate, and repeatable UHPLC–MS/MS method was developed for the determination of bruceoside A and its major metabolite. The results showed bruceoside A could be transformed into the potent anticancer component brusatol *in vivo*, rather than its direct deglycosylated metabolite bruceosin. And the intestinal bacteria were proposed to take a potential role during such transformation. Based on the present study, it could be concluded that the quassinoid glycosides possessing weak activities *in vitro* could do contribution to the anticancer properties of Fructus Bruceae *in vivo* via transforming into more active metabolites.

P-267

DEFINING THE ROLE OF XANTHONES FROM THE MANGOSTEEN FRUIT IN PROMOTING ANDROGEN RECEPTOR DEGRADATION

Mirielle Nauman[1,2], Bhaskar Vemu[2], and Jeremy Johnson[1, 2]
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago College of Pharmacy, Chicago, IL 60612, [2]Department of Pharmacy Practice, University of Illinois at Chicago College of Pharmacy, Chicago, IL 60612.

The purpose of this study is to determine how isoprenylated xanthones disrupt androgen receptor functionality in prostate cancer cells. Xanthones are a class of chemical compounds isolated from the purple mangosteen fruit (*Garcinia mangostana*) native to Southeast Asia with α-mangostin as the most common xanthone. Androgen receptor (AR) degradation represents an important strategy to overcome the rising drug resistance to FDA approved anti-androgens used in prostate cancer. Here we present data showing that xanthones not only interact with the androgen receptor, but also disrupt AR functionality and possibly degrade AR. Efficacy and mechanism of action studies were performed in two different prostate cancer cell lines (22Rv1 and LNCaP). Immunoblot data reveals a dose and time dependent decrease in AR, coupled with an increase in chaperone proteins, in response to xanthone treatments. Further analysis of post-translational modifications identified a reduction in the phosphorylation profile of AR. These actions coupled with upregulation of stress response elements inhibit the nuclear translocation of AR. This leads to an inhibition of transcription of downstream genes that are necessary for cell growth and proliferation. Our results suggest that α-mangostin promotes AR degradation by inhibiting nuclear translocation, which could be effective in drug resistant prostate cancer cases.

P-268

CHEMICAL CONSTITUENTS AND IN-VITRO ANTI-CANCER ACTIVITY OF *LEPTADENIA PYROTECHNICA*

Khasawneh, Mohammad A.[1]; Elwy, Hanan M.[2]; Hamza, Alaaeldin A.[2]
[1]United Arab Emirates University, Al-Ain, United Arab Emirates, [2]National Organization for Drug Control & Research, Cairo, Egypt

The aim of this study is to isolate the chemical constituents of the *Leptadenia pyrotechnica* (LP) (Forssk.) Decne and their biological evaluation. LP is a plant belonging to the family of Asclepiadaceae and is widespread in tropical Africa, Asia and the Mediterranean region and in the sandy plains in the Western Gulf countries. The hexane extract of LP whole plant was subjected to open column chromatography and was found to contain significant amounts of betulinic acid, lupenone, botulin and lupeuol along with other minor triterpenoids. Structures for the isolated compounds were confirmed by NMR techniques and high resolution MS. GC-MS analysis of LP hexane extract revealed the presence of derivatives of linoleic acid (octadecadienoic), diterpenes (phytol), triterpenes (squalene), and lupeol as the major constituents. *In-vitro* biological screening of the ethanolic and hexane LP extract revealed significant cytotoxic effect against MCF-7 human breast cancer cell line.

Key words: *Leptadenia pyrotechnica*; betulinic acid; lupenone; botulin; lupeuol

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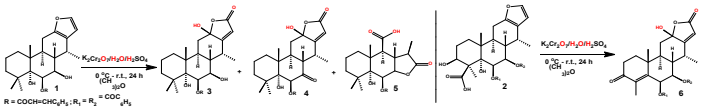
DERIVATIZATION OF CASSANE DITERPENOIDS FROM *CAESALPINIA PULCHERRIMA* (L.) SW. AND EVALUATION OF THEIR CYTOTOXIC AND LEISHMANICIDAL ACTIVITIES

Osayemwenre Erharuyi[1], Achyut Adhikari[2], Abiodun Falodun[1], Rehan Imad[2], Iqbal M. Choudhary[2,3]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, [2]H.E.J. Research Institute of Chemistry and Dr. Panjwani Centre for Molecular Medicine and Drug Research, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan, [3]Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21412, Saudi Arabia.

Oxidation of the cassane diterpenoids 6β-cinnamoyl-7a-hydroxyvouacapen-5α-ol (**1**) and pulcherrimin A (**2**), which were isolated from the roots of *Caesalpinia pulcherrima*, yielded four new derivatives **3–6**. Compounds **1-6** were tested for their cytotoxic activity against five cancer cell lines. Their leishmanicidal activity was also investigated. Compound **4** showed cytotoxic activity against all five cell lines and was more active than the parent compound **1**. Compound **6** showed significant leishmanicidal activity. The oxidation of **1** and **2** as well as the biological activities of **1-6** will be presented.



P-270

TWO NEW LIGNANS AND A NEW STILBENE FROM *SECURINEGA SUFFRUTICOSA*

Kyoung Jin Park[1], Joon Min Cha[1], Tae Hyun Lee[1], Dong Hyun Kim[1], Youn Sung Choi[1], Kang Ro Lee[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkawn University, Suwon 16419, Republic of Korea.

As a part of our ongoing search for bioactive constituents from Korean medicinal sources, *Securinega suffruticosa* twigs were studied. *S. suffruticosa* (Euphorbiaceae) is a dioecious shrub widely distributed in the mountainous areas of Korea, Mongolia, mainland China, and Russia. Previous phytochemical investigations have led to the isolation of flavonoids, other phenolic compounds, neurodegenerative alkaloids, and cytotoxic diterpenoids. MeOH extract of *S. suffruticosa* twigs was subjected to solvent-partitioning to yield *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH soluble fractions and repeated column chromatographic purification of the *n*-hexane-, $CHCl_3$-, and EtOAc-soluble fractions afforded two new lignans (**1**-**2**) and a new stilbene derivative (**3**), along with twenty-three known ones. The chemical structures of the new compounds (**1**-**3**) were elucidated by extensive NMR methods (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC). The isolates (**1**-**26**) were tested for their cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines in vitro using the SRB bioassay. Also, the effects of the isolated compounds on nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated murine microglia BV2 cells and for their neuroprotective effects via induction of nerve growth factor (NGF) in C6 glioma cells were evaluated.

P-271

LABDANE DITERPENE GLYCOSIDES OF *PINUS KORAIENSIS* TWIGS AND THEIR BIOLOGICAL ACTIVITIES

Kyoung Jin Park[1], Joon Min Cha[1], Tae Hyun Lee[1], Dong Hyun Kim[1], Youn Sung Choi[1], Kang Ro Lee[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkawn University, Suwon 16419, Republic of Korea

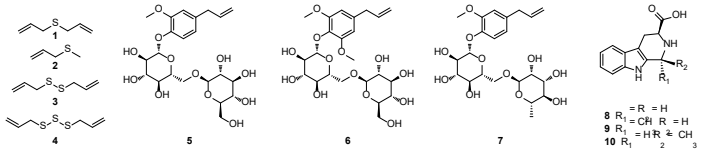
Pinus koraiensis Siebold et Zucc., belonging to the Pinaceae family, is mainly distributed in Korea, China, and Japan. The seed crop of this plant is well known as a food supplement, rich in phenolic constituents with antioxidant activity. Several bioactive diterpenes and phenolic compounds have been isolated from the pinecones of this plant. However, few phytochemical investigations on twigs of *P. koraiensis* have been performed. An extended phytochemical investigation of the twigs of *P. koraiensis* afforded ten new diterpenes (**1**-**10**) and a known compound (**11**). The chemical structures of the new compounds (**1**-**10**) were established using diverse NMR techniques (^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY), HRMS data analysis, and chemical methods. All the purified compounds (**1**-**11**) were evaluated for their cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15), for their anti-inflammatory activity through the measurement of nitric oxide (NO) production levels in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cell line, and for their neuroprotective effects via induction of nerve growth factor (NGF) in C6 glioma cells.

P-272

CHEMICAL CONSTITUENTS AND BIOACTIVE POTENTIAL OF GARLIC (*ALLIUM SATIVUM* L.)

Su Cheol Baek, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Mun Seok Jo, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

In our continuing search for new bioactive constituents from Korean natural resources, phytochemical investigation of garlic(*Allium sativum* L.) led to the isolation of ten known compounds including organosulfur compounds (**1**-**4**), eugenol diglycosides (**5**-**7**), and β-carboline alkaloids (**8**-**10**). The structures of the compounds were determined using NMR spectroscopic data and LC/MS analysis as well as computational NMR chemical shift calculations followed by DP4$^+$ analysis. The isolated compounds were evaluated for their cytotoxicity against cervical cancer cells and effects on adipogenic differentiation.



P-273

ALBANIAN FLORA AS A POTENTIAL SOURCE FOR NEW THERAPEUTIC AGENTS

Entela Hodaj-Çeliku[1,2,3], Olga Tsiftsoglou[3], Erjon Mamoci[2], Sokol Abazi[3], Dimitra Hadjipavlou-Litina[4], Diamanto Lazari[3], Jon Clardy[1]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115. [2]Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania, [3]Canadian Institure of Technology, Tirana, Albania, [4]School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece

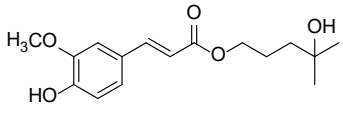
In the course of our investigation into bioactive natural products we have investigated the chemical constituents and the biological activity of the leaves and seeds of *Centaurea vlachorum* Hartvig, collected in 2011 from Mt. Kunora of Lura in Albania. Phytochemical investigations into the aerial parts and seeds of *C. vlachorum* led to the isolation of eleven compounds, including five sesquiterpene lactones, two flavonoids, two indole alkaloids and two dibenzylbutyrolactone lignans. The isolation and structure elucidation these metabolites will be presented. Moreover, the isolated compounds were tested for their free radical scavenging activity using the following in vitro assays: (i) interaction with the free stable radical of DPPH, (ii) inhibition of linoleic acid peroxidation with the dihydrochloric acid of 2,2-Azobis2-amidinopropane (AAPH) and their inhibitory activity towards soybean lipoxygenase was evaluated, using linoleic acid as substrate. The chemataxonomic significance of these compounds is summarized.

P-274

NEW PHENYLPROPANOID DERIVATIVES CONJUGATED ALIPHATIC ALCOHOL FROM THE ROOTS OF INDIAN GINSENG (*WITHANIA SOMNIFERA*)

Su Cheol Baek, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Mun Seok Jo, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Withania somnifera, commonly known as Indian ginseng, is popular as a functional food because of its diverse purported therapeutic efficacies. Chemical investigation of the MeOH extract of *W. somnifera* roots combined with LC/MS-based analysis resulted in the identification of two new phenylpropanoid derivatives (**1**-**2**) and seven known compounds (**3**-**9**). The structures of the isolated new compounds were determined by spectroscopic data, including 1D and 2D NMR and HR-MS measurement. The isolated compounds were evaluated for their inhibitory effects on nitric oxide (NO) production in LPS-activated RAW 264.7 macrophage.



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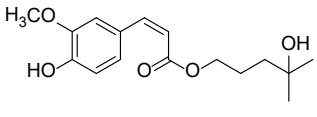


2

P-275

TWO NEW ECDYSTEROIDAL GLYCOSIDES FROM *SPHENOCENTRUM JOLLYANUM*

Temitayo O. Ajayi[1,2], Radhakrishnan Srivedavyasasri[1], Emmanuel E. Nyong[2,3], Micheal A. Odeniyi[4], Jones O. Moody[2], Samir A Ross[1,5,]*
[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Department of Pharmacognosy Faculty of Pharmacy, University of Ibadan, Nigeria. [3]Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria. [4]Department of Pharmaceutics, Faculty of Pharmacy, University of Ibadan, Nigeria. [5]BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University MS 38677, USA.

Sphenocentrum jollyanum is a sour tasting plant which belongs to the family Menispermaceae. It is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. It has found use as chewing sticks, relief for constipation, cough medicine, for sickle cell disease, rheumatism and other inflammatory conditions. Pharmacological activities include the use as antimalarial, antiviral, anti-angiogenic, analgesic, aphrodisiac and vermifuge. It has also been used in treatment of jaundice, breast engorgement, tumors, and dressing chronic wounds. The crude methanol extract of *S. jollyanum* root exhibited 98% and 80% antimicrobial activity against *Aspergillus fumigatus* Pinh and Vancomycin resistant *enterococcus* (VRE) at a concentration of 200 µg/mL, with IC_{50} 11.45 and 12.95 µg/mL, respectively. The ethyl acetate fraction of methanol extract showed *in-vitro* antimicrobial activity against *A. fumigatus* Pinh at 83% with IC_{50} of <8 µg/mL. The phytochemical investigation of ethyl acetate fraction yielded six compounds, which were identified by their NMR, IR and MS spectral analyses as two new ecdysteroidal glycosides, and four known ecdysteroids: polypodoaurein, polypodine B, ecdysterone, and 20, 26-dihydroxyecdysone.

P-276

CHEMISTRY AND BIOLOGY OF *SILENE RUBELLA* GROWING IN EGYPT

Ismail A. Hussein[1,2], Atef A. El-Hela[2], Abd-elsalam I. Mohammad[2], Radhakrishnan Srivedavyasasri[1], Samir A Ross[1,3,]*
[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo 11371, Egypt. [3]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

The genus *Silene* L. (Caryophyllaceae) is one of the largest genera of flowering plants in the world consists of about 700 species. In Egypt, 29 species of *Silene* are distributed in Suez area, Aqaba Gulfs, Sinai coastal plains, the Nile Valley, Oases and Gebel Elba Massive. The ethanolic extract of *S. rubella* showed moderate antibacterial activity against *Streptococcus aureus,* Vancomycin resistant *enterococcus* (VRE), *Bacillis subtilis* and *Salmonella typhimurium* and antiviral activity against HAV-10. It also showed moderate activity towards colon carcinoma cell line HCT-116 with IC_{50} 23.8 µg/ml. Phytochemical investigation of the aerial parts of *S. rubella* yielded eighteen compounds (**1** -**18**). The isolated compounds were identified by their NMR, and MS spectral data analyses as: apigenin (**1**), diosmetin (**2**), kaempferol (**3**), luteolin (**4**), myricetin (**5**), quercetin (**6**), isovitexin (**7**), rutin (**8**), vicinin 2 (**9**) (R)-naringin (**10**), (S)-naringin (**11**), chlorogenic acid (**12**), betulinic acid (**13**), oleanolic acid (**14**), ursolic acid (**15**), spinasterol (**16**), ecdysterone (**17**), and D-pinitol (**18**). All of these compounds are reported for the first time from this species. Compounds **13** and **14** exhibited potent activity towards VRE. Compounds **1**-**6**, exhibited moderate to potent activity against K562 Human Leukemia cells.

P-277

SCREENING ENDOLICHENIC FUNGI FOR ANTIMICROBIAL METABOLITES

Sabiba S. Hossain, Joshua J. Kellogg, Emily D. Wallace, Nadja B. Cech
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC 27402

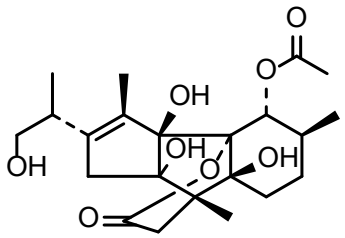
Endolichenic fungi are a diverse group of fungi that live asymptomatically in the thallus of lichens, and are a potential source of bioactive natural products. Over the past decade there has been a noticeable increase in publications on endolichenic fungal metabolites that fall into a variety of chemical structure classes and are potent against a diverse array of biological targets. The goal of this study was to investigate endolichenic fungi isolated from North Carolinian lichens for bioactive metabolites using an untargeted metabolomics profiling approach with liquid-chromatography-mass spectrometry (LC-MS). Fungi cultures were isolated from multiple lichen samples and grown on rice media. Cultured endolichenic fungi were extracted and the LC-MS data was collected for each fungus. As part of this study, the bioactivity against methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated for an extract from each isolate. One extract, numbered G1025, inhibited MRSA with an MIC of 50 ug/mL. Efforts to identify the active antimicrobial compounds in this extract are ongoing.

P-278

ISOLATION AND ELUCIDATION OF A NEW DITERPENE FROM THE BARKS OF *CINNAMOMUM CASSIA*

Suk Woo Chang[1], *Joon-pyo Hong[1], Dae Sik Jang[1]*
[1]Department of Life and Nanopharmaceutical Sciences, Graduate School, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Korea.

Cinnamomum cassia (Lauraceae) has been used as a traditional oriental medicine for the treatment of gastritis, diabetes and blood circulation disturbance in Korea, Japan, and China. Repeated chromatography of a hot water extract of the barks of *C. cassia* led to the isolation and characterization of one new diterpene (**1**) together with seven diterpenes (**2**-**8**) having previously known chemical structures. The structure of the new compound **1** was determined by interpretation of spectroscopic data, particularly by 1D and 2D-NMR studies. The isolation and structural eludication of compound **1** is described herein.



1

P-279

ISOLATION AND STRUCTURE ELUCIDATION OF ISOQUINOLINE ALKALOIDS FROM *ASIMINA TRILOBA* AND THEIR CYTOTOXICITY AND NEUROTOXICITY EVALUATIONS

Taghreed A. Majrashi[1,2,3], *Shabana Khan[1,2], Nicole Ashpole[2], Fazila Zulfiqar[1], Amar G. Chittiboyina[1], Zulfiqar Ali[1], and Ikhlas A. Khan[1,2]*
[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [2]Department of BioMolecular Sciences, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]College of Pharmacy, King Khalid University, Abha, Saudi Arabia

Annonaceae plants are important in folk medicine, and they have been used to treat various tumors. Regardless the growth in their popularities and their potential effects on health support (especially in their anticancer activities), studies have shown the development of atypical parkinsonisms, in people who consume Annonaceous products continuously. The potential risks of neurodegeneration associated with chronic consumption of plants of the Annonaceae family emphasize the need for additional studies to determine and identify the neurotoxic compounds in the edible annonaceous plants and compare their risk to their benefits in defeating cancer. The phytochemical investigation of the alkaloids from *Asimina triloba* twigs yielded one new aporphine glycoside along with seven known isoquinoline alkaloids. Some alkaloids along with three extracts were evaluated for their anticancer potential in four human solid tumor cell lines (SK-MEL, KB, BT-549, and SK-OV-3) and for their neurotoxicity in rat cortical neurons. The extracts and the alkaloids showed interesting cytotoxicity results. However, some of the extracts and alkaloids displayed a significant decrease in neurons viability as their concentrations increased.

P-280

HYDANTOIN DERIVATIVES FROM THE ROOTS OF *ARMORACIA RUSTICANA* AND THEIR NEUROPROTECTIVE ACTIVITIES

Tae Hyun Lee[1], *Kyoung Jin Park[1], Joon Min Cha[1], Dong Hyun Kim[1], Youn Sung Choi[1], and Kang Ro Lee[1]*
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea.

The roots of *Armoracia rusticana* (horseradish) have been widely used as a condiment in the world. The pungent flavor of horseradish is to be due to the sulfur-containing compounds deriving from hydrolysis of glucosinolates in Brassicaceae family. As part of the search for bioactive constituents from Brassicaceae family, the roots of *A. rusticana* (4 kg) were investigated. The roots were extracted with 80% MeOH, and partitioned with hexane, $CHCl_3$, EtOAc, and BuOH. The repeated column chromatography and purification of $CHCl_3$ fraction resulted in the isolation of five unique hydantoin constituents (**1**-**5**), together with three known compounds (**6**-**8**). The structures of new compounds (**1**-**5**) were elucidated by spectroscopic means (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC) and HRESIMS data. The absolute configurations of the compounds (**1**-**5**) were assigned by comparing with electronic circular dichroism (ECD) data analysis. Neurotrophic and anti-neuroinflammatory activities for the compounds (**1**-**8**) are in progress.

P-281

DEVELOPMENT OF A BUILDING BLOCK STRATEGY TO CLASSIFICATION, IDENTIFICATION, AND METABOLITE PROFILING OF OLEANANE TRITERPENOIDS IN *GYMNEMA SYLVESTRE* USING UHPLC-QTOF/MS

Ha Thanh Tung Pham[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

Gymnema sylvestre is a popular Ayurevedic medicinal plant for the treatment of diabetes mellitus which has oleanane triterpenoids as a major class of bioactive metabolites. In this study, a targeted, biosynthesis-inspired approach using UHPLC-qTOF/MS was implemented to elaborate the whole chemical profile for the standardization of Vietnamese *G. sylvestre* variety. The known compounds reported from the literature were first dissected to identify the building blocks of biosynthetic intermediates and the construction rules synthesizing the oleanane triterpenoids of the plant. These blocks were recombined to build up a theoretical virtual library of all reasonable compounds complying the deduced construction rules. Various techniques, including relative mass defect filtering, multiple key ion analysis, mass fragmentation analysis, and comparison with standard references were applied to determine the existence of these predicted compounds. Conventional isolation and structure elucidation of 6 selective new compounds were carried out to identify new building blocks and validate the assignments. Consequently, 119 peaks of oleanane triterpenoids were quickly assigned, among them 77 peaks are predicted to be new compounds by their molecular formulae and mass fragmentation behaviors. All identified metabolites were then classified in different layers to analyze their logical relationships and construct a multilayer chemical profile of oleanane triterpenoids. This new approach is expected to be used, with much practicality, for massive structural characterization and for exploring the biosynthetic relationships among various compounds in medicinal plants.

P-282

SCALE-UP AND INCREASED PRODUCTIVITY FOR PURIFICATION OF *SECO*-IRIDOIDS FROM *CENTAURIUM ERYTHRAEA* RAFN. USING CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)

Tsvetelina Mandova[1], Frédéric Dijoux[1]
[1]Gilson Purification, Saint Avé, France.

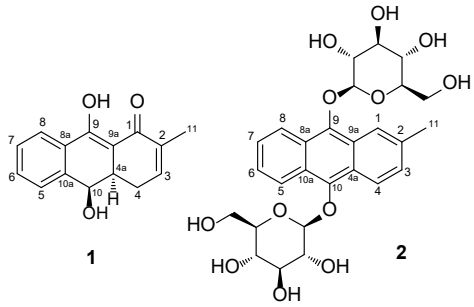
Centrifugal Partition Chromatography (CPC) is a powerful separation technique that leverages the unique mechanism of hydrostatic CCC (CounterCurrent Chromatography) columns and two-phase (biphasic) solvent systems. One phase, the stationary phase, is maintained in the column chambers by centrifugal force, while the other phase (mobile phase) is pumped through the column. The geometry of the CPC column, including parameters such as the size and shape of the cells and ducts, has a strong impact on the productivity and efficiency of the devices. We measured the productivity of a range of different CPC volume columns, from 250 mL up to 1000 mL, using the resolution between two benzenediols as a metric. Based on this analysis the separation performance of the CPC was confirmed by further scaling up to a 6L twin cell column. Three major *seco*-iridoids were separated from the common centaury plant (*Centaurium erythraea* Rafn., Gentianaceae). Separation was monitored using DAD and MS analysis, and for one of the most enriched fractions of the experiment, swertimarin accounted for 231mg/g, compared to the crude extract where swertiamarin was quantified as 8.72mg/g.

P-283

DIHYDROANTHRAQUINONES FROM *RUBIA PHILIPPINENSIS*

Quan Trong Khong[1], Joonseok Oh[2], InWha Park[1], Nam Giang Pham[1], and MinKyun Na[1]
[1]College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea, [2]Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States

Two new dihydroanthraquinones (**1**-**2**) were isolated from the untapped plant *Rubia philippinensis*. Their structures were built on the basis of spectroscopic and chemical data analysis. Compound **1** possesses an intramolecular hydrogen bonding, sufficiently robust to transfer heteronuclear magnetization via a nonbonded interaction, which was further assessed using VT, EXISDE, and PANIC experiments. The stereochemistry of **1** was first attempted by the Mosher's esterification and then accomplished by ECD data. Compound **2** is the first example of dihydroanthraquinone diglucosides reported from *Rubia* genus and its glucopyranose moieties were unequivocally analyzed using enzymatic hydrolysis followed by chiral derivatization and LC analyses.



P-284

ANTIBACTERIAL COMPOUNDS FROM THE AUSTRALIAN FRUIT OF *CORDYLINE MANNERS-SUTTONIAE*

Trong D. Tran,[1] Malin Olsson,[2] Nahid Choudhury,[2] David J. McMillan,[2] Jason K. Cullen,[3] Peter G. Parsons,[3] Paul V. Bernhardt,[4] Craig M. Williams,[4] Paul W. Reddell,[5] and Steven M. Ogbourne[1]
[1]GeneCology Research Centre, University of the Sunshine Coast, Queensland, Australia. [2]School of Health and Sports Sciences, University of the Sunshine Coast, Queensland, Australia. [3]QIMR Berghofer Medical Research Institute, Queensland, Australia. [4]School of Chemistry and Molecular Biosciences, University of Queensland, Queensland, Australia. [5]QBiotics Limited, Yungaburra, Queensland, Australia.

Australia is one of the most megadiverse countries in the world, home to an estimated 700,000 species, many of which are endemic and some under threat of extinction. Despite accounting for only 0.3% of the Australian continent, Queensland's tropical rainforests are internationally recognized as a global biodiversity hotspot. This unique ecological resource is also a unique and relatively untapped source of novel and new natural products. Utilising the plants of this eco-region, an in-house extract library (EcoLogic™) was created and screened against six ESKAPE pathogens whose infections are a growing public health burden. We identified an active extract from the fruit of *Cordyline manners-suttoniae*. Bioactivity guided fractionation of the active extract led to the isolation of 10 new and one known compounds. The most active compound inhibited the Gram-positive bacteria *Staphylococcus aureus* with MIC_{75} values that were comparable to those of the antibiotic, chloramphenicol. The structural elucidations of these compounds using NMR, X-ray diffraction analysis and/or chemical derivatization, and their structure activity relationships will be presented.

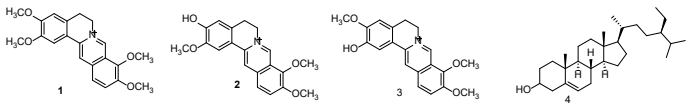
P-285

ANTIPROTOZOAL ACTIVITY OF ISOQUINOLINE ALKALOIDS ISOLATED FROM *ENANTIA CHLORANTHA*

Vincent O. Imieje[1,2], Ikhlas I. Khan[2,3], Babu Tekwani[3], Shabana I. Khan[2], Abiodun Falodun[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, 300001, Nigeria, [2]National Center for Natural Products Research, Institute of Pharmaceutical Sciences, University of Mississippi, 38677, USA,[3]Department of Bio-Molecular Sciences, School of Pharmacy, University of Mississippi, 38677, USA.

There is urgent need for the search of new antiprotozoal drugs due to the emergence of resistance strains against currently used ones. *Enantia chlorantha* (**EC**) has been used in Nigeria ethnomedicine in the treatment of several diseases including malaria. The fractionation of crude methanol extract of **EC** led to the isolation of compounds **1-4**. The compounds were significantly active in inhibiting *Plasmodium falciparum* parasites with IC_{50} values ranging between 1.7464 and >11.487 µM against D6 and W2 strains. Compounds **1** and **2** showed significant inhibition of *T. brucei* with IC_{50} and IC_{90} values of 15.19 µM and 23.69 µM, respectively. The isolated compounds could be used as leads in the development of affordable and potent antiprotozoal drugs.



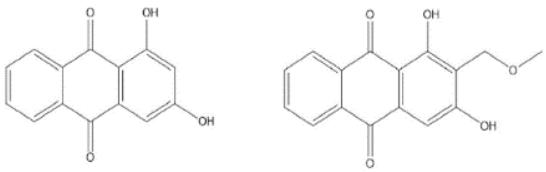
P-286

CYTOTOXIC PROPERTIES OF THE ANTHRAQUINONE DERIVATIVES ISOLATED FROM THE ROOTS OF *RUBIA PHILIPPINENSIS*

DongWook Won, Khong Trong Quan and MinKyun Na*

College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea.

Rubia philippinensis (Rubiaceae) has been reported to have anti-cancer, anti-inflammatory, antioxidant effects. In this study, the cytotoxicity potential of five anthraquinone derivatives isolated from the roots of *R. philippinensis*. Anthraquinone derivatives showed cytotoxic activity against various cancer cell lines. Significant activity of the compounds **4** and **5** was observed against the breast cancer cell line MDA-MB-231 with IC_{50} values of 14.65 ± 1.45 and 13.03 ± 0.33 µM, respectively. Compounds **4** (xanthopurpurin) and **5** (lucidin-ω-methyl ether) for high selective toxicity at lower concentrations without showing toxicity towards normal cells, confirming that compounds **4** and **5** may have the potentiality to be developed as anticancer leads.



Compound 4 Compound 5

P-287

EFFECTS OF CYCLOCARYA PALIURUS LEAVES ON ENDOCRINE AND IMMUNE FUNCTION IN TYPE II DIABETES RATS WITH YIN DEFICIENCY SYDROME

Jingxia Wang[1], Junkai Yao[1], Lu Fu[1], Leilei Wang[1], Lan Jia[1], Liang Meng[1], Ji'an Hua[1], Wei Li[1].

[1]Beijing University of Chinese Medicine, 11 Bei San Huan Dong Lu, Beijing 100029, China

Cyclocarya paliurus leaves (CPL) is a traditional Chinese medicine which could stimulate saliva and reduce thirst. Previous studies indicate that CPL has an excellent hypoglycemic effect on Type Ⅱ diabetes (T2D) rats. This study aimed to explore the effects of CPL on T2D with yin deficiency syndrome. The rat model was established by high-fat diet feeding for 8 weeks, combined subcutaneous injection of thyroxine (T4) 1 week (0.2 mg/kg), and single intraperitoneal injection of streptozotocin (30 mg/kg). Compared with the model group, the levels of thiiodothronine (T3), rT3, T4, interleukin-1β, tumor necrosis factor-α, cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), total protein, albumin and uric acid in serum were significantly decreased in CPL treated group, and the levels of cortisol, immunoglobulin G (IgG), IgM, complement 3 (C3) and C4 in serum were markedly increased in CPL treated groups. These results suggest CPL could inhibit the secretion of thyroid hormone, pro-inflammatory factors and energy metabolism, and promote the secretion of COR and immunoglobulins. It might be related to the regulatory effect of CPL on cyclic nucleotides. Thereby, CPL could correct the disorder of endocrine-immune system in diabetes rats with Yin deficiency to restore the balance of Yin and Yang.

P-288

ANTICOAGULANT AND ANTI-DRY EYE SYNDROME EFFECTS OF SWORD BEAN(*CANAVALIA GLADIATIA*) POD EXTRACT

Pureum Im*, Jeong Yeon Im, Ae-Jin Choi and Jeong-Sook Choi

Division of Functional Food & Nutrition, Department of Agrofood Resources, National Institute of Agricultural Sciences, Wanju 55365. Republic of Korea.

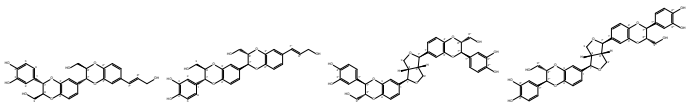
Sword bean were named because they look similar to sword. It is a subtropical crop and has been eaten as a vegetable in Africa and Asia. Because of the health benefits of sword been pod, cultivation and consumption have increased recently. Therefore, we studied the nutrition, antioxidant and physiological effect of sword bean pod. The characteristic nutrition was potassium(1,675 mg/100g). It is higher than banana.(1,448 mg/100g). sword bean pod was extracted by boiled water (100°C, 6hours) and freeze dried to analysis physiological effect. The results of aPTT(activated partial thromboplastin time) test were 25.3 sec in 100 ㎍/㎖(Aspirin: 30.7 sec). Also the results of mucous cell(Human conjunctival epithelial cells) recovery test were 140.46% in 100 ㎍/㎖(CMC0.5%: 145%). In conclusion, this study provides basic information of sword bean pod and physiological effects of its extract. It is expected that further research on the health functionality and utilization technology of sword bean pod could help human health.

P-289

ISOLATION AND STRUCTURE CHARACTERIZATION OF SPECIFIC BACTERIAL Β-GLUCURONIDASE INHIBITORS FROM NONI (*MORINDA CITRIFOLIA* L.) FRUIT

Fei Yang, Wenjun Zhu, Shi Sun, Qing Ai, Paba Edirisuriya and Kequan Zhou.*
Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48202, USA

Two sesquineolignans, (7*S*,8*S*,7'*R*,8'*R*)-isoamericanol B (**1**) and americanol B (**2**), and two dineolignans, moricitrin A (**3**) and B (**4**) were isolated from Noni (*Morinda citrifolia*) fruit powder, **2**, **3** and **4** were new compounds, the absolute configuration of **1** was determined for the first time. **1**-**4** showed potent inhibition against gut bacterial β-Glucuronidase (GUS) with IC_{50} as low as 0.6 μM, while showed none or weak effects on digestive enzymes α-amylase, *α*-glucosidase and lipase, suggesting that **1**-**4** were specific inhibitors against bacterial GUS with minimized gastrointestinal side effects, they could be promising candidates for alleviating irinotecan-induced diarrhea.



1 (IC_{50} 6.9 μM) **2** (IC_{50} 4.0 μM) **3** (IC_{50} 0.6 μM) **4** (IC_{50} 0.9 μM)

P-290

SEASONAL VARIATION OF MAIN COMPOUNDS AND ANTIOXIDANT COMPOUNDS CONTENT OF *RUDBECKIA LACINIATA* VAR. *HORTENSIA*

Ji Yeong Kim, Byung Soon Hwang, So Eun Park, Mi Jang, Gi Chang Kim, In Guk Hwang, Young Hee Park
Department of Agrofood Resources, National Academy of Agricultural Science, Rural Development Administration, Wanju 55365, Korea

Rudbeckia laciniata var. hortensia(RLH) belong to Compositate family are widespread in Korea and its young leaves consumed as foods. This plant have been traditionally used as herbal medicine and reported various physiological activities like induction of apoptosis in human cancer cell. Ethanol extract of RHL was measured by mass-spectometry and main compounds were identified. The main compounds were chlorogenic acid(**1**), neochlorogenic acid(**2**), cryptochlorogenic acid(**3**), isochlorogenic acid a(**4**), isochlorogenic acid b(**5**), isochlorogenic acid c(**6**). The six main compounds contents of RLH extract collected from May to September in 2018 were measured by ultra pressure liquid chromatography. In addition, total polyphenol and flavonoid contents were measured using colorimetric method as antioxidant component. The results showed that chlorogenic acid content, one of main compounds of RLH extract was ranged from 20.5 mg/g to 35.4 mg/g and reached at highest in autumn. Total polyphenol and total flavonoid content of RLH also varied with a range of 844.9 ~1530.8 mg/100g and 614.0 ~ 967.6 mg/100g, subjectively and similar tendency was found by collected month.

P-291

SIMULTANEOUS QHNMR QUANTIFICATION OF GERANIOLOIDS, HERBACETINOIDS, AND ROSAVINS FROM A *RHODIOLA ROSEA* COUNTERCURRENT SEPARATION FRACTION

Yu Tang,[1] Dejan Nikolić,[1] Daniel Zagal,[1] J. Brent Friesen,[2] James B. McAlpine,[1] David C. Lankin,[1] Guido F. Pauli,[1] and Shao-Nong Chen[1]
[1]UIC/NIH Center for Botanical Dietary Supplements Research, University of Illinois at Chicago, Chicago, IL 60612, United States, [2]Physical Sciences Department Dominican University, River Forest, IL 60305, United States

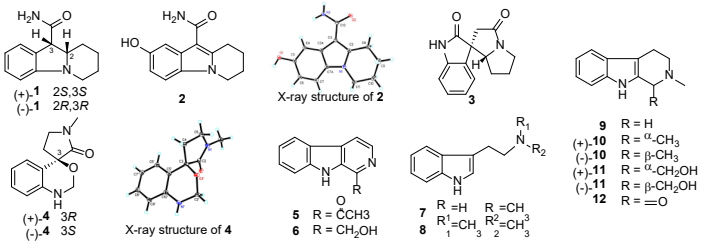
Rhodiola rosea rhizome extracts are regarded to contain adaptogens that exert anti-depressive, anti-fatigue, and general "anti-oxidant" effects. The main classes of designated bioactives in alcoholic extracts are monoterpenoids, flavonoids, phenylpropanoids, and phenylethanoids. In our study of *R. rosea*, a series of geranioloids, herbacetinoids, and rosavins were isolated and identified. To build standardization protocols, we developed a qHNMR method for the quantification of markers from these 3 classes in crude extracts. As the high proanthocyanidin (PAC) and fatty acid (FA) content in *R. rosea* interfered with qHNMR (and HPLC-UV) integration, a countercurrent separation (CCS) method was developed to remove PACs and FAs prior to quantification. The content of geranioloids, herbacetinoids, and rosavins was at last determined as 4.67, 1.85, and 4.74% w/w, respectively. Thereby, the demonstrated loss-free characteristic of CCS in tandem with qHNMR enables the poly-targeted chemical standardization of *R. rosea* for its putative bioactive compounds.

P-292

INDOLE AND Β-CARBOLINE ALKALOIDS FROM THE FRUITS OF *FLUEGGEA VIROSA*

Zhen-Long Wu[1,2,], Ming-Tao Xu[1], Qi-Fang He[1], Ying Wang[1], Wen-Cai Ye[1], and Chun-Tao Che[2]*
[1]Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China, [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612

Twelve tryptophan derived alkaloids were isolated from the fruits of *Flueggea virosa* (Roxb. ex Willd.) Voigt, including two new fused tricyclic indole alkaloids (**1** and **2**), and a new spirooxindole alkaloid (**3**). Compounds **4**–**12** are known indole or β-carboline alkaloids, all isolated from the *Flueggea* genus for the first time. Their structures and absolute configurations were elucidated by comprehensive spectroscopic analysis, computation, as well as X-ray diffraction. The discovery of these alkaloids illustrates the participation of tryptophan in alkaloids biosynthesis in the *Flueggea* genus, besides the common precursors lysine and tyrosine.

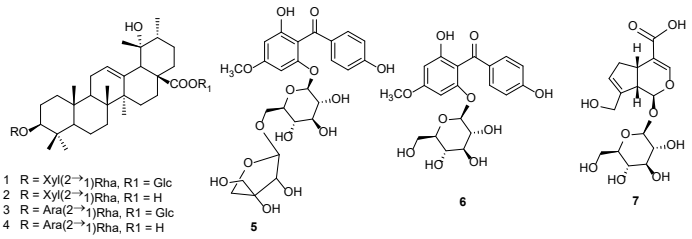


P-293

FADOGIA AGRESTIS: A PHYTOCHEMICAL STUDY AND SCREENING FOR ANTI-INFECTIVE ACTIVITIES OF AN AFRICAN TRADITIONAL HERB

Ahmed Galal Osman[1], Zulfiqar Ali[1], Omer Fantoukh[1,2], Vijayasankar Raman[1], and Ikhlas Khan[1,2]
[1]National Center for Natural Products Research, [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Fadogia agrestis is utilized for reducing fever, alleviating kidney pain, and induction of diuresis in the African traditional medicine. This herb possesses potential aphrodisiac properties and showed growth inhibitory effect against malaria parasite. Four triterpenoid glycosides (**1**-**4**), two benzophenone glycosides (**5** and **6**), and one iridoid glucoside (**7**) were isolated and characterized from the dried roots of *F. agrestis*. Compounds **6** and **2**, exhibited inhibitory effect against *Trypanosoma brucei* with IC_{50} 9.4 µg/mL for **6** and IC_{50} 8.3 µg/mL, IC_{90} 9.2 µg/mL for **2**.



P-294

NMR CHARACTERIZATION OF NEW MERODITERPENOIDS FROM THE RED ALGA *DELISEA* SP.

Dongdong Wang,[1] Heidi R. Bokesch,[1,2] Josep Saurí,[3] Kirk R. Gustafson,[1] and John A. Beutler[1]
[1]Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA; [2]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA; [3]Structure Elucidation Group, Process and Analytical Research and Development, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, MA 02115, USA

Red macroalgae are well-known producers of bioactive secondary metabolites, including isoprenoid and phenolic metabolites. This exploration of the red alga *Delisea* sp. led to the isolation of six new halogenated diterpene-phenols and diterpene-benzoic acids (**3**-**8**), together with callophycols A (**1**) and B (**2**). The chemical structures of the six new compounds (**3**-**8**) were characterized by extensive analysis of their NMR and mass spectroscopic data. The assignments of bromine and chlorine atoms in callophycols A and B were on the basis of ^{13}C chemical shift predictions and empirical data. The band-selective HSQC and CLIP-HSQMBC experiments, which can visualize the $^{35,37}Cl$ isotope effect on ^{13}C nuclei, were applied to provide unequivocal support for the halogen assignments in compounds **1**-**8**.

P-295

EFFECT OF SAMPLE DEGASSING ON ^1H NMR CHEMICAL SHIFTS OF AN N-CONTAINING NATURAL PRODUCT

Daniel Zagal, Gonzalo R. Malca-Garcia, Bin Zhou, G. Joseph Ray, David C. Lankin, James B. McAlpine, Shao-Nong Chen and Guido F. Pauli
UIC/NIH Center for Botanical Dietary Supplements Research and PCRPS, Dept. of Med. Chem. & Pharmacognosy, College of Pharmacy, UIC, Chicago, IL 60612, USA.

By default, solution NMR analysis of NPs is performed in non-degassed samples that contain atmospheric oxygen. As a paramagnetic nucleus, oxygen affects nuclear relaxation (T_1 and T_2) and, thus, signal intensities and lineshape. The aim of this work was to investigate whether sample degassing of NMR samples might be beneficial for improving the resolution of typically complex NP spectra such as alkaloids. Using the widely applied NMR standard, strychnine, for a proof-of-concept, a stock solution of known concentration was divided in order to prepare degassed (D) and non-degassed (ND) samples of near identical concentrations contained in 5 mm NMR tubes. The D sample was degassed using argon and maintained under an argon atmosphere. In this instance, sample degassing produced a separation of overlapping "multiplets" for hydrogens in the vicinity of nitrogen atoms. The signals of hydrogens in alpha position to the amine showed the greatest chemical shift difference (Δδ) of 0.312 ppm. The signals of aromatic hydrogens exhibited the least Δδ of 0.006 – 0.029 ppm. As expected, T_1 and T_2 increased by 0.648 s and 0.696 s, respectively. Because all coupling constants remained unchanged in the D sample, the separation of the signals was useful for the analysis of its overlapping multiplets via full spin analysis (HiFSA). However, rather than being a resolution enhancement per se, sample degassing presented itself as a pH effect due to the associated CO_2 removal, which affects the dispersion of the spectra of N-containing NPs more than the actual line resolution.

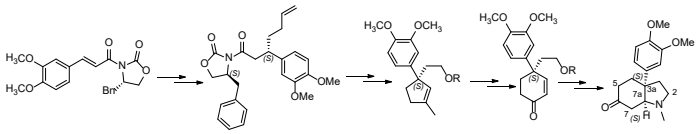
P-296

SYNTHETIC STRATEGY FOR *CIS*-3A-ARYLOCTAHYDROINDOLE SCAFFOLD: CONCISE SYNTHESIS OF (-) MESEMBRINE

Mohamed A. Albadry[1,2,3], Sateesh C. Rotte[3], Pradeep B. Lasonkar[3], [2] Amar G. Chittiboyina[3], and Ikhlas A. Khan[2,3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt [2]Departments of BioMolecular Sciences, Division of Pharmacognosy, and [3]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Mesembrine is a naturally occurring benzylphenethylamine-type alkaloid which has been isolated from the South African plant *Sceletium tortosum,* family Aizoaceae, known as Kanna. This plant has been used as a stimulating by South African natives to enhance wellbeing. In addition to mesembrine, several related alkaloids bearing aryloctahydroindole moiety have been isolated and characterized. Mesembrine displayed a potent serotonin reuptake inhibitory activity in the nanomole range with probable usefulness in the treatment of depression and anxiety. Regardless, many synthetic trials have been published around 40 routes toward mesembrine synthesis; It is still of interest to synthetic chemists due to its thought-provoking chemical features such as a *cis*-3a-aryloctahydroindole moiety with *syn* configuration at 3a and 7a in the bridged bicyclic ring. By employing Michael addition early in the synthesis on *α-β* unsaturated carbonyl of 3,4-dimethoxycinnamic acid, the desired stereogenic center at carbon 3a was created, afterward, intramolecular alkylidene C-H insertion and aldol

reactions to complete the *aryloctahydroindole* scaffold. The detailed synthetic transformations will be deliberated.



P-297

HIGHLIGHTS FROM A WORKSHOP ON ENHANCING NATURAL PRODUCT CLINICAL TRIALS

Guido Pauli[1], Adam Kuszak[2], Naomi Fukagawa[3], Gregory Bloss[2], Mahtab Jafari[4], Michael A Walters[5], Bruce Barrett[6], Frederic Bushman[7], Steven Casper[8], Floyd Chilton[9], Christopher Coffey[10], Mario Ferruzzi[11], Craig Hopp[2], Mairead Kiely[12], Daniel Lakens[13], John MacMillan[14], David Meltzer[15], Jeffrey Paul[16], Kathleen Pritchett-Corning[17,18], Sara Quinney[19], Sharon Ross[2], Harold Seifried[2], Kenneth Setchell[20], Nisha Sipes[21], Jacqueline Stephens[22], D Lansing Taylor[23], Herve Tiriac[24], Dan Xi[2], Freddie Ann Hoffman[25], Barbara C Sorkin[2]
[1]University of Illinois at Chicago, Chicago, IL, USA, [2]National Institutes of Health, Bethesda, MD, USA, [3]United States Department of Agriculture (USDA), Agricultural Research Service, Beltsville, MD, USA, [4]University of California, Irvine, Irvine, CA, USA, [5]University of Minnesota, Minneapolis, MN, USA, [6]University of Wisconsin, Madison, WI, USA, [7]University of Pennsylvania, Philadelphia, PA, USA, [8]U.S. Food and Drug Administration, College Park, MD, USA, [9]University of Arizona, Tucson, AZ, USA, [10]University of Iowa, Iowa City, IA, USA, [11]North Carolina State University, Raleigh, NC, USA, [12]University College Cork, Cork, Ireland, [13]Eindhoven University of Technology, Eindhoven, Netherlands, [14]University of California, Santa Cruz, Santa Cruz, CA, USA, [15]University of Chicago, Chicago, IL, USA, [16]Drexel Graduate College of Biomedical Sciences, College of Medicine, Evanston, IL, USA, [17]University of Washington, Seattle, WA, USA, [18]Harvard University, Cambridge, MA, USA, [19]Indiana University, Indianapolis, IN, USA, [20]Cincinnati Children's Hospital, Cincinnati, OH, USA, [21]National Institute of Environmental Health Sciences, Durham, NC, USA, [22]Louisiana State University, Baton Rouge, LA, USA, [23]University of Pittsburgh, Pittsburgh, PA, USA, [24]University of California, San Diego, La Jolla, CA, USA,[25]HeteroGeneity, LLC, Washington, DC, USA

Good practices for enhancing reproducibility and clinical relevance of research that informs the design of randomized, controlled clinical trials (RCT) of natural products (NP), as well as the design and conduct of the NP RCT themselves, were discussed by a broad range of experts and stakeholders at a workshop held at the NIH on September 13-14, 2018; highlights of those discussions will be presented.

RCT, when appropriately powered, are generally considered the gold standard for assessing the benefit-harm profile of an intervention. The resources required, and high cost of RCT (often ≥ $20M per trial) demands optimization of each trial to maximize utility of the information obtained. A number of large RCT of botanicals and other NP supported by NIH have not shown evidence of benefit versus a placebo control. These outcomes may provide as important an evidence base for guiding private and public health choices as studies supporting benefit, but are too often unpublished. Regardless of the outcome, the translational value of an RCT depends on the rigor and clinical relevance of the supporting data. Interpretation of RCT results is greatly enhanced when evidence for a causal, molecular mechanism of action is sufficient to inform NP quality control and pharmacokinetic studies. Strong mechanistic evidence may also enable assessment of bioavailability and activity at the site(s) of action.

Discussions spanned good practices across the continuum of NP research, including in leveraging ethnobotanical and epidemiological data and in the appropriate use of a wide variety of *in silico*, *in vitro* and *in vivo* models. One session addressed considerations for translational validity of preclinical experimental models as they inform the design of clinical trials. Several speakers discussed approaches to elucidating how gastrointestinal microbiota modulate the biological effects of ingested xenobiotics. Good practices for assessing bias in the literature and for prioritizing RCT are also highlighted. Greater adherence to these practices is expected to increase benefit from investment in NP RCT.

Supported by: FDA, NIH, USDA.

P-298

TRANSLATIONAL STUDIES ON *CENTELLA ASIATICA* – PRODUCT DEVELOPMENT AND REGULATORY APPROVALS FOR HUMAN CLINICAL STUDIES

Amala Soumyanath[1], Kirsten Wright[1], Armando Alcazar Magana[3,5], Maya Caruso[1], Nora Gray[1], Donald Matthews[1], Randall Woltjer[2], Ronald M. Laethem[6], Caroline L. Moseley[6], Troy T. Banks[6], Claudia S. Maier[3], Jan F. Stevens[4,5], Joseph Quinn[1,7]
Departments of [1]Neurology and [2]Pathology, Oregon Health and Science University, Portland, OR, 97239, USA; [3]Departments of Chemistry and [4]Pharmaceutical Sciences and [5]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA; [6] BioIVT, 2810 Meridian Parkway, Suite 100, Durham, NC 27713, USA; [7]Department of Neurology, Veterans Affairs Portland Health Care System Center, Portland, OR 97239, USA.

Our preclinical studies on *Centella asiatica* (CA) strongly support its potential as a phytotherapeutic agent against cognitive decline, through influences on antioxidant response, mitochondrial activity, and synaptic density. Based on these data, we have initiated Phase I clinical studies (NCT03929250) of CA. However, translation from preclinical models to optimized clinical trials presents particular challenges for botanicals. Using a robust scientific approach, we have identified an optimal dose range and manufactured a CA interventional product with a matching placebo for testing in humans. Notably, when a therapeutic outcome of a botanical product is pursued, approval must be obtained from the Food and Drug Administration for clinical evaluation as an "Investigational New Drug" (IND) in addition to Institutional Review Board and funder approvals. The presentation will describe our experience obtaining these approvals including required preclinical studies showing the product's safety, lack of effect on cytochrome P450 isoenzymes, detailed chemical characterization and stability.

P-300

THE UNIQUE SYMBIOTIC RELATIONSHIP BETWEEN THE AMERICAN SYCAMORE TREE AND A KEY ENDOPHYTE *BACILLUS AMYLOLIQUEFACIENS* INVOLVES THE EXPRESSION OF RAPAMYCIN.

M. Chris James[1], Xiaoyan Wang[2], Theodore Leininger[3], Amala Dass[4] and Mark T. Hamann[2]
[1]BioFactura, 435 Progress Dr., Ste. Z, Frederick, MD 21701, USA, [2]Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC 29425, [3]US Forest Service, 1515 Dickey, Stoneville, MS 38776.

In an effort to better understand the mechanism by which the phytopathogenic bacterium called *Xylella fastidiosa* (Xf) causes leaf scorch and cell death in plants 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 is highly regulated 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 promotes 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. Furthermore, the results of this study suggests that a plant based diet may result in exposure to low levels of rapamycin which could support biological resilience in humans based on its interaction with mTOR.

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