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

“Innovations in Natural Products Chemistry - An Interdisciplinary Approach to Understanding Nature’s Chemical Library”

Madison, Wisconsin
July 13–17, 2019

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

Local Organizers

Melany Puglisi, Co-Chair (Chicago State University)
Tim Bugni, Co-Chair (University of Wisconsin - Madison)
Skylar Carlson (Smithsonian Marine Station)
Jim Glover (University of Iowa)
Chris Ireland (University of Utah)
Jason Kwan (University of Wisconsin – Madison)
Brian Murphy (University of Illinois at Chicago)
Nicholas Oberlies (University of North Carolina at Greensboro)
Jimmy Orjala (University of Illinois at Chicago)
Christine Salomon (University Of Minnesota)
Michael Thomas (University of Wisconsin – Madison)

Scientific Committee

John MacMillan, Chair (UC Santa Cruz)
Cindy Angerhofer (Aveda), Joseph Betz (NIH)
Guy Carter (Biosortia Pharmaceuticals), John Cardellina (ReevesGroup),
Kirk Gustafson (National Cancer Institute), Craig Hopp (NCCIH)
Ikhlas Khan (University of Mississippi), A. Douglas Kinghorn (The Ohio State University),
Barry O’Keefe (NCI-Frederick), Phil Proteau (Oregon State University)
Eric Schmidt (University of Utah), Ben Shen (The Scripps Research Institute-Florida)

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.
GENERAL SPONSORS

The Estate of Gerry and Lynn Brady
(President’s Opening Reception Sponsor)

SESSION SPONSORS

ASP Foundation by generous donations for the

David Slatkin Symposium

– David Slatkin Symposium

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UW–Madison School of Pharmacy pharmaceutical scientists are improving health and changing lives with new discoveries to help cure the world’s diseases.

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Providing pharmaceutical and biopharmaceutical product development and drug delivery consulting and laboratory services

Drug Discovery Services
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Driving translational drug discovery and development through the design and synthesis of novel small molecule based therapeutics

pharmacy.wisc.edu
**ASP Award Winners 2019**

**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

**Matt Suffness Young Investigator Award**
Amy L. Lane, University of North Florida

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**Kilmer Prize**
Abu Bakar Siddique, University of Louisiana at Monroe

**Undergraduate Research Award**
Julia Asay, University of California San Diego
Ashley Fukuchi, University of Hawaii at Hilo
Itzel Lizama-Chamu, University of Illinois, Chicago
Samuel Tanoeyadi, Oregon State University

**Student Research Award**
Taylor A. Lundy, University of Kentucky

**Student Travel Award**
Julia Austin, University of Illinois at Chicago
Omer I. Fantoukh, University of Mississippi
Jacklyn M. Gallagher, University of North Carolina at Greensboro
Laura Ioca, University of Illinois at Chicago
Sonja L. Knowles, University of North Carolina at Greensboro
Logan W. MacIntyre, University of Prince Edward Island
Carla Menegatti, University of Sao Paulo
Shogo Mori, University of Kentucky
Emily Paris, University of California San Diego
Sara P. Puckett, University of Connecticut

**Research Starter Grant**
Stephen Eric Nybo, Ferris State University

**Active Member Travel Grant**
Osayemwenre Erharuyi, University of Benin
C. Benjamin Naman, Ningbo University
Holly A. Showalter (Johnson), Waukee Community Schools

**D. John Faulkner Travel Award**
Mohamed Ibrahim, University of Mississippi

**David Carew Student Travel Award**
Daniel Shin, Seoul National University

**Jerry McLaughlin Student Travel Award**
Skylar Carlson, Smithsonian Marine Station
Seoung Rak Lee, Sungkyunkwan University

**Lynn Brady Student Travel Award**
Munhyun Bae, Harvard Medical School
Sunghee Bang, Duksung Women's University
George F. Neuhaus, Oregon State University
Choon Yong Tan, Ohio State University

**Waqar Bhatti Student Travel Award**
Angela Sester, TU Dortmund University

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**2019 Arthur E. Schwarting Award**

**2019 Jack L. Beal Award**
The American Society of Pharmacognosy Annual Meeting
60th Anniversary

“Innovations in Natural Products Chemistry
An Interdisciplinary Approach to Understanding Nature’s Chemical Library”

Madison, Wisconsin
July 13–17, 2019

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*

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**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
Posters #’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*

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**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

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**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

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**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
<table>
<thead>
<tr>
<th>Time</th>
<th>Session/Activity</th>
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<tbody>
<tr>
<td>12:10 PM – 12:30 PM</td>
<td>Andrés Mauricio Caraballo Rodríguez (University of California)</td>
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<td>Molecular Signatures From Ants’ Ecosystems - Susceptibility of Fungal Gardens to Mycoparasites</td>
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<td>10:30AM – 10:50 PM</td>
<td>William (Bill) Gerwick (Scripps Institution of Oceanography, UCSD)</td>
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<td>Nature’s Combinatorial Assembly-Line Biosynthesis Produces Vatiamides A-F</td>
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<td>10:50 AM – 11:10 PM</td>
<td>Amy E. Wright (Florida Atlantic University)</td>
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<td>Discovery of Marine Natural Products that Reduce the Levels of the Nodal Protein Survivin in Cancer Cells</td>
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<td>11:10AM – 11:30 PM</td>
<td>Néstor M. Carballeira (University of Puerto Rico)</td>
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<td>New Insights into the Biological Activity of Marine Methoxylated Fatty Acids</td>
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<td>11:30 AM – 11:50 PM</td>
<td>Christopher Thornburg (Leidos Biomedical Research, Inc.)</td>
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<td>NCI Program for Natural Product Discovery: Psammoclemolide, A Bioactive Sesterterpene from a Marine Sponge of the Chondropsidae Family</td>
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<td>11:50 AM – 12:10 PM</td>
<td>Yuta Kudo (Tohoku University)</td>
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<td>Identification of New Analogs and Putative Biosynthetic Intermediates of Tetrodotoxin Aimed at Elucidating its Biosynthetic Pathway and Structure Activity Relationship</td>
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<td>12:10 PM – 12:30 PM</td>
<td>Xiao Liang (University of Florida, Gainsville)</td>
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<td>Discovery, Total Synthesis and SAR of a Novel Quorum Sensing Signaling Molecule from a Marine Cyanobacterium</td>
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<td>12:30 PM – 2:00 PM</td>
<td>Fellows Meeting – Invitation Only – Hall of Fame Room</td>
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<td>12:30 PM</td>
<td>Afternoon and Evening on your own</td>
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<td>1:30 PM – 4:00 PM</td>
<td>Bitters Boot Camp (Ticketed)</td>
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<td>Meet at Level 4 – Bus Loading Area</td>
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<td>2:00 PM – 4:00 PM</td>
<td>Build a Wisconsin Cheese Board (Ticketed)</td>
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<td>Meet in the Lobby of The Concourse Hotel</td>
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<tr>
<td>2:00 PM – 4:30/5:00 PM</td>
<td>Stroll Down State Street (Ticketed)</td>
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<td>Meet in the Lobby of The Concourse Hotel</td>
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**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

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**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**

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**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**

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**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/
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

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.

In 1928, Alexander Fleming’s discovery of penicillin G from the fungus Pe- nicillium notatum 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 agents is depleted while resistance continues to develop to existing antibio-

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 Staphylococcus aureus will serve as an illustrative example of this concept.
Plenary Speakers

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.

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 gnarus and Collinsella aerofaciens forms the basis of this talk. R. gnarus, 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 plasmagens, 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, CUBE BIN AND GLYCOALKALOIDS TO RESPECTIVELY TREAT UROLITHIASIS, ERECTILE DYSFUNCTION AND CUTANEOUS LEISHMANIASIS.
Jairo Kenapp 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%. *Capiara* species leaf extract, rich in galloyquinic acids, has undergone 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. *Cubebum*, a dibenzyl butyrolactolic lignan, was initially isolated from *Zanthoxylum naranjillo* Engl (Rutaceae) 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. *Cubebun* inhibited phosphodiesterase 5 in vitro and induced erection in mice model (tadalafil, as positive control). Patents were applied and issued in USA, Europe and Japan.

Leishmaniasis is caused by a protozoan parasite from over 20 *Leishmania* species. An estimated 700000 to 1 million new cases and 20000 to 30000 deaths occur annually. The glycoalkaloids solasoline 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 Bardenette
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, Phyllanthusins, inspired by prior natural product isolated from *Phyllanthus poitanei* 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

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*PL-08* Englerin A Analogues

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**PL-07**

**PL-08**

**PL-09**

**PL-10**

**Plenary Speakers**
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 modiﬁes 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.
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 Deep 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.

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 cytokine 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 anti-cancer 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.

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 pharmaceutical 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 spectroscopic 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 Cameroonians 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.

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 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, Ria Khandpur, Caroline Golightly, Carmen Hoffbeck, Sara Locklear, Nicole Lopanik
1Department of Biology, Randolph-Macon College, Ashland, VA 23005, USA, 2Biology Department, Linfield College, McMinnville, OR 97128, USA, 3School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA, 4School 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 undergraduates, 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 Desulfuvibrio 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 algae, 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-DMPA. 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 active tubulin-binding conformation of the drug. Prof. Kingston also oversaw natural product discovery efforts in collaboration with scientists from Surname 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 reserve. 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
‘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 worldwide. Indeed, few in the field have not had occasion to reference the authoritative, periodic reviews on the current state of natural products drug...
FENUGREEK, GUT MICROBIOTA, AND RESILIENCY TO WESTERN DIETS
Annadora Bruce-Keller1, Jacqueline Stephens1, Allison Richard1, J. Michael Salbaum1, David Ribnicky2
1Pennington Biomedical Research Center, Baton Rouge, LA, USA,
2Department 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/lower 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 metagenomic/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, 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 resiliency in today's complex environment.

MULTOMIC SIGNATURES OF MICROBIAL METABOLITES FOLLOWING PREBIOTIC FIBER SUPPLEMENTATION
Brittany Lee-McMullen, Samuel Lancaster, Charles Abbott, Daniel Hornburg, Jennifer 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 human health. 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. Stevens1, Adrian F. Gombart1,3, Claudia S. Maier4, Thomas O. Metz5, and Ryan D. Bradley6  
1Linus Pauling Institute, Departments of 2Pharmaceutical Sciences, 3Biochemistry & Biophysics, and 4Chemistry, Oregon State University, Corvallis, OR 97331, USA, 5Integrative Omics Group, Pacific Northwest National Laboratory, Richland, WA 99352, USA, 6College 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).
A-01
CHEMOMETRICS: NOVEL APPROACHES FOR INVESTIGATING SECONDARY METABOLITES IN MEDICINAL PLANTS
Elizabeth M. Mudge1, Michael Chan1, and Paula N. Brown4
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. Leg
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 nocardioazidine 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 nocardioazidine A as a precursor to nocardioazidine B. 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.

A-03
TYLER, TAYLOR & TALES: THE COMPLEXITY OF REDUCTIONISM
Guido F. Paoli
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 mult part machinery, embedded into intricate matrices, and expressed as metabonomic 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 (Carcuna 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, alkaloids 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. Therefore, 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. Shaikh1,2, Elizabeth A. McMillan1, Chensu Wang1, Michael A. White1, John B. MacMillan1,2

1Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA; 2Department 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 IC50 values from 0.90 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,2 and Guido F. Pauli1

1Center 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 communication 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 PHIrmacognosy Ontology (PHIO) 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. Saldivar-González1, Maríalia Valli1, Adriano D. Andricopulo1, Vanderlan S. Bolzani2, José L. Medina-Franco1

1School of Chemistry, Department of Pharmacy, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico; 2Nuclei of Bioassays, Biosynthesis and Ecophysiology of Natural Products (NuBBE), Department of Organic Chemistry, Institute of Chemistry, Sao Paulo State University - UNESP; 3Laboratory 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 developed in developing NuBBE Database (NuBBE), http://nubbe.iq.unesp.br/portal/nubbedb.html, the first database of natural products from Brazilian biodiversity. A chemoinformatic analysis of NuBBE 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, 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 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. Dorrington1, Jarro-Charles J. Kalinski1, Shirley Parker-Nance1 and Samantha C. Waterworth1

1Department of Biochemistry and Microbiology, Rhodes University, PO Box 94, Grahamstown 6140, South Africa; 2Box 94, Grahamstown 6140, South Africa.

Marine sponges of the family, Latrunculidae, 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 T. favus, T. pedunculata, two recently described species, T. nguni and T. 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 Ryi Banks reef producing pyrroloiminoquinone suite resembling those observed for T. favus and T. nguni, but with no detectable tsitisikamamines. 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 tsitsikammin-mines present in *C. bellae* sponges.

### C-05

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

Angela I. Calderón¹, Yihee Zhang¹, Satyanarayana R. Ponugula², Jingjing Qian¹, Richard A. Hansen¹

¹Department of Drug Discovery and Development, Auburn University, Auburn, AL 36849, ²Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL 36849, ³Department 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 acai 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 acai extracts were investigated. Specifically, the parallel artificial membrane permeability assay (PAMPA) model was utilized to simulate intestinal filtration of passively diffused constituents of acai extracts. These were subsequently screened for *in vitro* liver CYP3A4 inhibition and induction. The passively absorbable portion of a methanol acai 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 spectrometry (LC-MS) data. Subsequently, five highly permeable acai compounds were characterized by tandem mass spectrometry.

### C-06

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

Serge A.T Fobofou¹, Katrin Franke², Ludger Wessjohann¹, Jon Clardy³

¹Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, MA 02115, USA, ²Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, 06120 Halle (Saale), Germany, ³Leibniz Institute-DMZ German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany

![Hypericum extract](image)

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 *Hypericums* collected in Africa. Some of these compounds exhibit biological activities. Compounds 1 and 2 are two examples of previously undescribed compounds isolated from *H. roperianum* and *H. lanceolatum*, respectively. Compound 1 represents the first dimeric coumarin isolated from this genus, while 2 is an example of polycyclic polyphenylated acyl-phloroglucinol (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 92039-0204

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 bromoaranes 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,2,5 Regan Russell,1,3 Vanessa Sperandio,1,4 and Jason M. Crawford2,3,4

¹Department of Chemistry, Yale University, New Haven, CT 06520, USA, ²Chemical Biology Institute, Yale University, West Haven, CT 06516, USA, ³Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA, ⁴Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA, ⁵Department 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 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 enterohemorrhagic *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,¹ Larry L. Klein,³ 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. Pauli²,³

¹Dept of Med Chem & Pharmacognosy, Institute for Tuberculosis Research, ²Center for Biochemical 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 MIC₉₀ of <0.2 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 MIC₉₀ 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 crude. 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. tuberculosis protease chaperone. A series of reactions were performed, which led to the isolation and purification of 20 modified ruf-derivatives. 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 (ROI C) are also bioactivity related motifs. Compound 1, the methylated derivative of rufs I and II, is the most active with an MIC₉₀ 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. Fennwick,² Brenda T. Shaffer,² Qing Yang,² Joyce E. Loper¹, Steven E. Ealick,² Benjamin Philmus²

¹Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA. ²Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA. ³Department 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 allelochemical produced by the cyanobacterium Nostoc spongiforme. Here we present our labs 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**

Skyler Carlson¹, Jennifer Sneed¹, Sarath Gunasekera¹, Danielle L. Dixon³, Valerie J. Paul¹

¹Smithsonian Marine Station, Fort Pierce, FL, 34949, USA. ²College 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 Hydroclathrus boergesii. Polar molecules present a number of challenges; most instrumentation is designed to identify and isolate compounds retained by octadeclsilane (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 Movers, Josep Sauri, Eric J. N. Helfrich, Matthew Henke, Ken Barns, Tim S. Bugni², David Andes², Cameron R. Currie, Jon Claridy², ¹Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, ²Structure Elucidation Group, Process and Analytical Research and Development, Merck & Co, Inc., Boston, MA 02115, ³Pharmaceutical Sciences Division, School of Pharmacy, University of Wisconsin—Madison, Madison, WI 53705, ⁴Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, ⁵Department 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 MYCOCHELIN- DERIVED LIPOXYGENASE INHIBITORS IN A GENETICALLY MODIFIED MYXOCOCCUS XANTHUS STRAIN**

Angela Sester, 1, 2 Lea Winand, 1 Simona Pace, 3 Oliver Wers, 4 and Markus Netter 4

1 Department of Biochemical and Chemical Engineering, TU Dortmund University, 44227 Dortmund, Germany; 2 Leibniz Institute for Natural Product Research and Infection Biology e.V., 07745 Jena, Germany; 3 Chair of Pharmaceutical and Medicinal Chemistry, Friedrich-Schiller-University, 07743 Jena, Germany

The catechol siderophores mycochelin A and B are potent inhibitors of the enzyme human Lipoxygenase (5-LO) with IC50 values comparable to the FDA-approved anti-asthmatic drug zileuton. Recently, we expanded the mycochelin 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 mycochelin 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-15**

**BIOFILM INHIBITION ALTERS SPECIALIZED METABOLISM AND INCREASES VIRULENCE**

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

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-14**

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

Ikenna E. Ndukwu 1, Yizhou Liu 2, Yu-hong Lam 1, Edward R. Sherer 1, R. Thomas Williamson 1, 3, Johan Isaksson 1, Kirk Gustafson 1, Till Maurer 1, Mikhail Reibarkh 1, and Gary E. Martin 1

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. Current address: Department of Chemistry, University of North Carolina Wilmington, Wilmington, NC 28403.

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, 1H-1H 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 brefussin 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 13C NMR, provides sufficient molecular differentiation to allow unambiguous characterization of a pair of diastereomers.

**C-16**

**MADBYTE: A STRUCTURE DRIVEN APPROACH TO UNTARGETED NMR METABOLICOMICS 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-
C-17

MENSACARCIN INDUCES MITOCHONDRIAL TOXICITY AND INHIBITS GLUCOSE UPTAKE IN MELANOMA

Elizabeth Kaweesi¹, Grace Davis², David Brown², Sandra Losgen¹
¹Department of Chemistry, Oregon State University, Corvallis, OR 97331, ²Department 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 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 IC₅₀ 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 IC₅₀ of 1μM.

C-18

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

Andrés Mauricio Caraballo-Rodríguez¹, Kathleen E. Kyle¹, Sara P. Puckett¹, Ricardo R. da Silva², Justin J. van der Hoof²,¹, Madeleine Ernst¹, Marcy J. Balunas¹, Jonathan L. Klassen², Pieter C. Dorrestein¹
¹Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA; ²Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA.

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 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. Moss¹, Grant Seiler², Tiago F. Leão¹, Gabriel Castro-Falcón¹, Lena Gerwick¹, Chambers C. Hughes¹, and William H. Gerwick¹,²,³
¹Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, ²Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, ³Skaggs 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 alkylated lipopeptides and their brominated analogs, from the cyanobacterium Moerua 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 α-METHOXYLATED FATTY ACIDS

Néstor M. Carballeira
Department of Chemistry, University of Puerto Rico, Rio 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 biophysical 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, Tanja Grkovic, Jason R. Evans, John R. Britt, Michelle Ahalt-Gottholm, Melinda G. Hollingshead, David J. Newman, Jerry M. Collins, and Barry R. O'Keefe

1Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, Maryland 21702. 2Data Management Services, Inc., Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute, Frederick, Maryland 21702. 3Biological Testing Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702. 4Natural 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-hydroxybutyryl 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 antitumor 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 and Mari Yotsu-Yamashita

1Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, 6-3 Aramaki-Aza-Aoba, Aoba-ku, Sendai, Miyagi 980-8578, Japan. 2Graduate School of Agricultural Science, Tohoku University, 468-1 Aramaki-Aza-Aoba, Aoba-ku, Sendai, Miyagi 980-8578, 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 news, suggesting a monoterpenoid 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 Li, 1,2 Susan Matthew, 1,2 Qi-Yin Chen, 1,2 Jason C. Kwan, 1 Valerie J. Paul 1 and Hendrik Luesch 1,2

1Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610, USA, 2Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville, Florida 32610, USA, 3Smithsonian 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.
IDENTIFICATION OF UNIQUE BACTERIAL METABOLITES THROUGH MULTIVARIATE STATISTICAL ANALYSIS.
Ali Shahbandi1, Derick D. Jones Jr.1, Daniel A. Todd1, Nadja B. Cech1
1Department 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.

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°C for 2 h to solubilize cell wall, and then with α-amylase (Termamyl 120L) at 93°C 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).

SMALL MOLECULE ACCURATE RECOGNITION TECHNOLOGY (SMART) TO DENOISE 2D NMR SPECTRA OF NATURAL PRODUCTS
Chen Zhang, Shilin Zhu2, Raphael Reher1, Brendan Duggan1, Kelsey Alexander1, Garrison W. Cottrell1, William H. Gerwick1,2,3
1‘Scripps Institution of Oceanography, 2Department of Computer Sciences and Engineering, 3Skaggs 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.

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

Edyta M. Grzelak,1 Wei Gao,2, Mary P. Choules3, Geping Cai2, Baoojie Wang,1 Yuehong Wang,1 James B. McAlpine1, Jinhua Cheng1, Hanki Lee3, Joo-Won Suh3,4 Guido F. Pauli2,5, Scott G. Franzblau2, Sang-Hyun Cho6 and Birgit U. Jaki1,3,4

1Institute for Tuberculosis Research1,7 and Dept. of Med. Chem. & Pharmacognosy,2 College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA; Center for Nutraceutical and Pharmaceutical Materials3 and Div. of Bioscience & Bioinformatics, College of Natural Science4, 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.

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

Edyta M. Grzelak1, Yang Liu2, J. Brett Friesen3,4, David C. Lankir1, Dejan Nikolić1, Shao-Nong Chen3, James B. McAlpine1, Joo-Won Suh3,4, Seung Hwan Yang5, Jinhua Cheng1, Hanki Lee3, Jin-Yong Kim1, Sang-Hyun Cho6, Guido F. Pauli2,5, Scott G. Franzblau2, and Birgit U. Jaki1,3,4

1Inst. for TB Research, 7Dept. Med. Chem. & Pharmacognosy, COP, UIC, Chicago, IL, 60612, 2Center for Nutraceutical & Pharmaceutical Materials, 3Div. 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 countersection separation (CCS) of compounds with anti-Mycobacterium tuberculosis (M. tb) activity from actinomycete culture extracts. It applies TLC-based bioautography2 and the GUSS fresh 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
**P-009**

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

Andrew N. Lowell1, Zachary A. Kohanov1, Harrison O. Miller1, and Jacob C. Chappell1

1Department 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.

**Overcoming Antibiotic Resistance**

**P-011**

**METABOLIC PROFILING OF ACTINOBACTERIAL ISOLATE FROM NEPAL**

Niraj Aryal1, Prajwal Rajbhandari2, Harald Gross3

1University of Tübingen, Pharmaceutical Institute, Department of Pharmaceutical Biology, 72076, Tübingen, Germany
2Research Institute of Bioscience and Biotechnology (RIBB), Nakhu-4, Lalitpur, Nepal

Actinomycetes 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 Chassagne1, Gina Porras-Brenes2, James T. Lyles3, Kate Nelson4, Joan Shang5, Cassandra L. Quave6

1Center for the Study of Human Health, Emory University, Atlanta, GA 30322, USA. 2Department 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 (IC50 > 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 were 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+) [α] D -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-015**

**BIOSYNTHETIC MECHANISM OF THE ANTIBIOTIC CAPURAMYCIN**

Erifu Yan, Steven Van Lanen  
1College 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. Capuramyacin, was a kind of nucleoside antibiotics discovered in screening programs for new antibiotics in 1980s. Capuramyacin-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 capuramyacin family of antibiotics consists of several novel chemical features. In this study, we have 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 I respective. 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.

**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, Aaryl carrier protein, actinomycin synthetase I respectively. 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 carbamoyl group of the amino acid substrate to form an aminocyl-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.

**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 carbamoyl group of the amino acid substrate to form an aminocyl-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 intermediate 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 carbamoyl 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-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
P-016
A CUSTOM, REUSABLE 3D-PRINTED BIOASSAY PLATE FOR THE DISCOVERY OF ANTIBIOTICS FROM MICROORGANISMS UNDER MULTIPLE CULTIVATION CONDITIONS.
Jeongho Lee1, Rui Ma2, Linh Nguyen1, Sanghyun Cho2, Scott G. Franzblau2, and Brian T. Murphy1
1Department of Medical Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607, USA, 2Institute 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 Walker1, Jon Clardy1
1Department 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 classes. 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
Auyad A. Eida,1 Mostafa A. Abuagrain,1 Corey J. Brumsted,1 and Taifo Mahmud1,2,*
1Department of Pharmaceutical Sciences and 2Department 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 vitro gene inactivation, chemical complementation, and in vitro pathway reconstitution we demonstrate that the 3-aminoacetophenone moiety of pactamycin is derived from 3-amino-benzoic acid by a set of discrete polyketide synthase proteins (PtmS, PtmL, 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. oryzae. This talk will present some of the success and challenges that we have had with the A. oryzae 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 Smustad, 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 ADENYLYLATION DOMAINS – PROMISING TOOLS TO METHYLATE NONRIBOSOMAL PEPTIDES
Shogo Maru1, Allan H. Pang1, Taylor A. Lundy1, Atefeh Garzan1, Oleg V. Tsodikov2, and Sylvie Garneau-Tsodikova1
1Department 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 adenyllylation (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, thiochondrinine 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.**

Snegha 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, Shogo Mori, and Sylvie Garneau-Tsodikova
1Department 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 Anieboh and John B. MacMillan
Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064 USA

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**

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

1Division of Pharmacy Practice and Science and 2Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, USA. 3Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, USA. 4Institute 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. 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, IC50 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.


**P-027**

**ANTIPROLIFERATIVE ACTIVITY OF A LIBRARY OF STEROIDS AND OPTIMIZED CHEMICAL DERIVATES.**

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

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. In our continuing effort to identify bioactive compounds with potential cancer therapeutic properties, the antiproliferative activity of a library of compounds (sulfotransferase inhibitors) was evaluated in a panel of cancer cells. In the present study, the sulforhabdamine 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 synthesized 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.


**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**


Division of Pharmacy Practice and Science, College of Pharmacy, The Ohio State University, Columbus, OH, USA.

Facultad de Química, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico.

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. 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 vitro 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.


**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% isopropl alcohol) was found to inhibit growth against all three cancer cell lines. After a second round of separation using a mono- or lytic 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. Fermantle1, Shengxin Cai1,4, April L. Riserger1,2, Tanja Grkovic2, Barry O’Keefe3, Robert Cichewicz1,4 and Susan L. Mooberry1,2.
1Department of Pharmacology, 2Mays Cancer Center, UT Health San Antonio, San Antonio, TX, 78229. 3Department of Chemistry and Biochemistry and 4Natural Products Discovery Group, University of Oklahoma, Norman, OK, 73019. 5Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. 6Natural Products Branch, Division of Cancer Treatment and Diagnosis, and 7Molecular 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. Riserger1,2, Corena V. Grant1, Shengxin Cai1,4, Tanja Grkovic2, Barry O’Keefe3, Robert Cichewicz1,4 and Susan L. Mooberry1,2.
1Department of Pharmacology, 2Mays Cancer Center, UT Health San Antonio, San Antonio, TX, 78229. 3Department of Chemistry and Biochemistry and 4Natural Products Discovery Group, University of Oklahoma, Norman, OK, 73019. 5Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702. 6Natural Products Branch, Division of Cancer Treatment and Diagnosis and 7Molecular 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, dehydrofalconinol. 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 dehydrofalconinol 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 (Thymelaeaceae), 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 delayed gefitinib-driven resistance in NSCLC by targeting AXL degradation.

P-034

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

Zeinab Y. Al Subeh1, Aaron Colby2, Cedric J. Pearce1, Mark Grinstaff1, and Nicholas H. Oberlies1
1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27412, USA. 2Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA.

Lung cancer is the leading cause of cancer death worldwide. 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 Weiss1, Dimitry Kovalchick2, Omer Murik1, Assaf Sukiennik1, Aaron Kaplan1, and Shmuel Carmeli1
1Plants and Environmental Sciences, the Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, Jerusalem 9190401, Israel; 2Raymond and Beverly Sackler School of Chemistry and Faculty of Exact Sciences, Tel Aviv University, Tel Aviv 69978, Israel, Metabomed Ltd, Yavne 81220, 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 Neto1, Daniel Raifery1, Norberto Pederine Lopes1
1Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903, Brazil; 2School of Medicine, University of Washington, Seattle, WA, 98109, USA; 3Departamento 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 epidermic 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 Cleary1, Gordon Lu1, Emily Pierce2, Rachel Dutton2, Laura Sanchez2
1Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612; 2Division 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 Pseudomonas 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-Trans experiments to build a picture of key bioprocesses that underlie microbial interactions.

P-038
A CONSERVED PEPTIDE FAMILY OF NATURAL PRODUCTS IS INVOLVED IN FLAGELLAR MOTILITY AND BIOFILM FORMATION IN PROTEOBACTERIA
Laura P. Ioca1, Jennifer Diaz2, Sylvia Kunakom2, Laura M. Sanchez2, Roberto G. S. Berlinc1, Alessandra S. Eustaquio3, Roberto G. S. Berlinc1
1Instituto de Química de São Carlos, Universidade de São Paulo; 2Department 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.2 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-
P-039
THE ACID ROCK DRAINAGE MICROBIOME AT ELY COPPER MINE IN VERSHIRE, VT AND ITS POTENTIAL TO PRODUCE NOVEL METABOLITES
Katherine Morrillo1, George Chlipala1, Kevin Kantsman2, Stefan Green2, Kieran Bhave1, Heather Driscoll3, Holly Peterson1, Laurent Assiama1, Benjamin Paquin1, Mark Maier-Wells-Cline2, Lesley-Ann Giddings2
1Department of Chemistry & Biochemistry, Middlebury College, Middlebury, VT 05753, USA, 2Research Resources Center, University of Illinois at Chicago, Chicago, IL, 60612, USA, 3Vermont Genetics Network/University of Vermont, Burlington, VT, 05405, USA, 4Department 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 Bradyrhizobiurn (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 STAPHYLOCCOCCAL VIRULENCE REGULATION USING METABOLOMICS
Lindsay K. Catesa1, Katrin Schuder1, Corey P. Parlet1, Alexander R. Horswill2, and Nadja B. Cech1
1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC, 27412, 2Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora CO, 80045, 3Department 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 down-regulated 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 SYMBIONS OF FUNGUS-GROWING ANTS
Manhuyng Bae1, and Jon Clardy1
1Department 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 (Escomopsis 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 Escomopsis fungus. Traditional bioassay-guided isolation led to the discovery of active and in-active form of new conocondin analogs—conocondins B (2) and C (3). This Escomopsis-produced conocondin 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. Kirk1, Alexa R. Sterling1, Erin M. Tully2, Jacob P. Strock1, Matthew J. Bertini1, Bethany D. Jenkins1
1Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, 2Department of Cell and molecular biology, URI, Kingston, RI 02881, 3Graduate 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. 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-045**

**MAKING A SPLASH AGAINST BRAIN EATING AMOeba WITH FUNGAL SECONDARY METABOLITES**

Kristof B. Canté, Tyler N. Graf, Christopher A. Rice, Laura Flores-Bocanegra, Dennis E. Kyle, Cedric J. Pearce and Nicholas H. Oberlies

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

*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. 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-OMe Tyr), N-methylthreonine (N-Me Thr), 3-methoxylalanine (3-O Me Ala), 3-hydroxyisocitrate (3-OH Isocitrate), 3,4-dimethylglutamine (3,4-DiMe Gln), 2,3-diaminobutyric acid (Dab) and 2-amino-2-butoenoic acid (Aba). In addition, a 2,3-dihydroxy-2,6,8-trimethyldeca-(4Z,6E)-dienoic acid (DHT-da) polyketide moiety was identified in I, while 2 contained a previously undescribed 2,6,8-trimethyldeca-(2E,4E,6E)-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-O Me Ala, D-3-O Me Tyr, N-Me Thr, (E)-4,6-dienoic acid (DHT-da). 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.

**P-046**

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

Chang-Kwan Kim, Dongdong Wang, Emily Smith, Unwoo Kang, Carole A. Bewley, Curtis J. Henrich, Deborah K. Morrison, and Kirk R. Gustafson

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-OMe Tyr), N-methylthreonine (N-Me Thr), 3-methoxylalanine (3-O Me Ala), 3-hydroxyisocitrate (3-OH Isocitrate), 3,4-dimethylglutamine (3,4-DiMe Gln), 2,3-diaminobutyric acid (Dab) and 2-amino-2-butoenoic acid (Aba). In addition, a 2,3-dihydroxy-2,6,8-trimethyldeca-(4Z,6E)-dienoic acid (DHT-da) polyketide moiety was identified in I, while 2 contained a previously undescribed 2,6,8-trimethyldeca-(2E,4E,6E)-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-O Me Ala, D-3-O Me Tyr, N-Me Thr, (E)-4,6-dienoic acid (DHT-da). 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.
Dab, (2R,3R)-3-OHLeu, (2S,3R)-N-MeThr, (2S,3S,4R)-3-DiMeGln and (2R,3R)-β-O-MeTyr. These new peptides showed potent inhibition of Ras/Raf signaling pathway with IC₅₀ 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. Fisher¹, Pamela D. Lane¹, Todd W. Lane²
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 hypothesized 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. *Berkholderia 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-Hernandez¹, Wildelis Figueroa-Rodriguez¹, Kelsey L. Alexander¹, Raphael Reher³, William H. Gerwick¹,², C. Benjamin Naman¹, and Eduardo J. E. Caro-Diaz¹
¹Department of Pharmacognosy and Molecular Basis of Phytotherapy, ²Department of Pharmaceutical Biology, Freie Universität Berlin, Germany, ³Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico - Medical Sciences Campus, San Juan, PR 00935

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 product 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. Dennis¹ and Sylvie Garneau-Tsodikova¹
¹Pharmaceutical 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 7azole 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. Dadi¹, Wilfried Vahjen¹, Jürgen Zentek¹, Matthias F. Melzig¹, Sebastian Granica¹, Jakub P. Piwowarski‡,§
¹Institute of Animal Nutrition, Freie Universität Berlin, Germany, ²Department of Pharmaceutical Biology, Freie Universität Berlin, Germany, ³Department 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.

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P-051

ISOLATION OF NOVEL DIMERIC NAPHTHOQUINONES FROM FUNGAL STRAIN MSX53507
Jacklyn M. Gallagher1, José Rivera-Chávez1, Laura Flores-Bocanegra1, Tyler N. Graf1, Huzefa A. Raja1, Blaise A. Darveau2, Cedric J. Pearce2, Joanna E. Burdette3, Nicholas H. Oberlies4
1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA; 2Institute of Chemistry, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Coyacán, Mexico City 04510, Mexico; 3Mycosynthetix, Inc., Hillsborough, North Carolina 27278, USA; 4Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, Illinois 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 MSX35907 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 Wada1, Masuo Goto2, Takahiro Shimizu2, Nami Kasanagi3, Kang-Po Li4, Kuo-Hsiung Lee3, Hiroshi Yamashita5
1Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 7-15-4-1, Maeda, Teine, Sapporo 060-8585, Japan; 2Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, USA; 3Chinese 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-Acetylation 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.

P-053

CYTOTOXIC NAPHTHOQUINONE DERIVATIVES ISOLATED FROM PYRENOCHAETOPSIS SP. (MSX63693)
Laura Flores-Bocanegra1, Huzefa A. Raja1, Joanna E. Burdette2, Cedric J. Pearce3, Nicholas H. Oberlies4
1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27412; 2Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612; 4Mycosynthetix, Inc. Hillsborough, NC 27228.

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 cell 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. Biosay-guided fractionation led to the isolation of three new naphthoquinone derivatives 3R,4S,7-ethyl-4,8-dihydroxy-3,6-dimethoxy-3,4-dehydroanaphthalen-1(2H)-one (1), 5-hydroxy-6-[1-(methoxy)ethyl]-2,7-dimethoxy-1,4-naphthalenedione (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.

P-054

SULFUR-CONTAINING ALKALOIDS FROM CULTURED FRESHWATER DESMONOSTOC SP. (CYANOBACTERIA)
Lydia Davis, Alexzej Krunic, Jonathan Bissou, Steven Karina, 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 Reykjavik, Iceland, showed antiproliferative activity against MDA-MB-435, MDA-MB-231 and OVCAR3 cell lines. Biosay-guided fractionation of the extracts led to the identification of the known thiazole-containing cytotoxic, Aliosirazole, 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.

**P-056**

**PHYTOCHEMICAL INVESTIGATION OF MIMOSA PIGRA**

Mohammed Hawwak1,2, Omer Fantoukh1,2, Zulfiqar Ali 1, Ikhas Khan1,2.

1National Center for Natural Products Research, 2Department of Biomedical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, 3Department of Pharmacognosy, College of Pharmacy, King Saud University, Abiyadh, 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 determine 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. Waterworth1, Laura V. Florez2, Martin Kallenpoth3, Ian J. Miller1, Jason C. Kwan1

1Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, 2Department of Biology, University of North Alabama, 3Department of Pathology and Microbiology, The University of Mississippi Medical Center, Jackson, MS 39216

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* sp. 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 lagrimamide. 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, Brendan P. Stewart, Rofina Johnkenedy, Jonathan L. Klasser, and Marcy J. Balunas

1Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269 USA. 2Department 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 antibiotic 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, Huzefa A. Raja, Allison J. Wright, Ann Marie L. Lee, Lindsay K. Caesar, Nadia B. Ceci, Matthew E. Mead, Jacob L. Steenwyk, Laure Ries, Gustavo H. Goldman, Antonis Rokas, and Nicholas H. Oberlies

1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC; 2Department of Biological Sciences, Vanderbilt University, Nashville, TN; 3University 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 fungal 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. Under standard laboratory culture conditions, 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 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*.

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**P-061**

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

Tania Grkovic¹, Rhone K. Akee², Christopher C. Thornburg³, Spencer K. Trinh⁴, Jason R. Evans⁵, John R. Britṭ¹, and Barry R. O’Keefe¹,⁶,⁷,⁸

¹Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702; ²Data Management Services Inc., Frederick, MD 21702; ³Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD 21702; ⁴Molecular 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-fractions 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 Nguyen¹,², Tuan Anh Tran³, Lien Kim Thi Pham², Duc Anh Dang¹, Cuong Van Pham¹, Brian T. Murphy¹

¹Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam; ²National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; ³Department of Medicinal Chemistry and Pharmacognosy, Colleague of Pharmacy, University of Illinois at Chicago, Illinois, United States; ⁴Institute 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 Béresdy, 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 Cai¹, Yaobing Huang¹ and Wenjun Zhang¹,²

¹Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA 94720; ²Chan 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 Aoun¹, Tae Ho Kim, Nagaraju Mupparapu, Christopher M Nguyen, Yu-Hsin Lin and Sherif Elshahawi²

¹Department 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 permisiveness 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 regiospecificity and/or permisiveness toward substrate acceptors when compared to wild type. The long-term goal for this study is to utilize the permisiveness 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 Acharya1, Ian Miller1, Doug Braun1, Yusi Cui1, Marc Chevrette2, Mark Berres1, Cameron Currie1 Lingjun Li1, Jason Kwan1, Tim Bugni2

1School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, 2Bioinformatics 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 and 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 SACCHAROPEROXYDOLACTONICUM N1-4**

Jeffrey S. Li1, Colin C. Barber2, and Wenjun Zhang1,3

1Department of Chemical and Biomolecular Engineering, University of California Berkeley, Berkeley, CA 94720, USA, 2Department of Plant and Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA, 3Chan 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 saccharoperoxydolactonicum 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 Tran1,2 and Alessandra S. Eustáquio1

1Department of Medicinal Chemistry and Pharmacognosy, and Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA, 2Institute 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 PKCΩ**

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 PKCΩ 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 PKCΩ.
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 92039, United States

Antagonistic interaction between co-cultured organisms has been a productive method by which to activate normally silent secondary metabolite bio-synthetic 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 Leptolyngbya species (ASF and IS) 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 Sula Lagoon (IS). They 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, palmyramide A, leptochaelin, the fagaalumides, cryptomaldamide and curacin D. 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

1Department of Chemistry, 2Department of Cell Biology, Microbiology, and Molecular Biology, 3Department of Global Health, College of Public Health, University of South Florida, Tampa, FL 33620, USA, 4Department of Infectious Diseases, Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA, 5Department 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
1Department of Chemistry, University of South Florida 2Burnett 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 methyltransferase) 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, 2 Yiyi Yu, 2 Zhengyu Cao, 1 Weiyang Zhang, 1 Bin Zhang, 1 Ye Yuan, 1 Lijian Ding, 2 Shan He, 1 C. Benjamin Naman 1
1Li Dak Sum Marine Biopharmaceutical Research Center, Department of Marine Pharmacy, College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo, Zhejiang 315800, China. 2Department 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 CHEMEOGEOGRAPHICAL ANALYSES AND COMPARATIVE GENOMICS TO DERIVE NEW CHEMICAL INSIGHTS FROM THE MARINE CYANOBACTERIUM MOOREA BOUILLONII

Christopher A. Leber1, C. Benjamin Naman1, Lena Keller1, Eduardo J. E. Caro-Diaz1, Tiago F. Leão1, Nathan A. Moss1, Valsamma Joseph1, Jason S. Biggs1, Lena Gerwick1, and William H. Gerwick1

1Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, 8622 Kennel Way, La Jolla, CA 92039, USA, 2National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Fine Arts Avenue, Kochi 682 016, Kerala, India, 3University 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 Objective Relational Comparative Analysis (ORCA). These comparative analyses revealed 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 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

DEREPICATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH QUADRUPOLE-TIME-OFFLIGHT MASS SPECTROSCOPY AND ANTIVIRAL ACTIVITIES OF PHLOROTANNINS FROM ECKLONIA CAVA

Thi Phuong Du1, Hye Moon Cho1 and Won Keun Oh1

1Korea 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, 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 chloroformufoceckol A (12), which showed an EC50 value of 13.48 ± 1.93 μM, is a potential active antiviral component of E. cava.

P-075

ISOCOMARINS, DIKETOPIPERAZINES AND A NOVEL VALEROLACTAM DERIVATIVE FROM HUMICOLA FUSCOATRA, AN ENDOPHYTE FROM THE MARINE RED ALGA ASPARAGOPsis TAXIFORMIS

Dulce Silva1, Iatí Mendonça1, Alana Honório1, Victor Rufino1, Rafael Silva1, Nair Yokoya1

1Institute of Chemistry, São Paulo State University – UNESP, Araraquara, Brazil. 2Institute 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 alga 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 acetone-rich fraction (MeCNFr) showed strong anti-cholinesterase activity and cytotoxic activity against HCT-116 and MCF-7 tumor cell lines with IC50 of 9.8 and 10.3 μg/mL, respectively. Fractionation over C-18 silica-gel CC, eluted with a H2O-MeOH gradient yielded 7 fractions. Fractions 3 and 5 were purified by HPLC-DAD-UV (H2O-MeCN gradient, 65 to 100% MeCN) and afforded two isocoomarins, 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 1H-13C HMBC experiments, indicated its molecular formulae as C23H18NO5 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 Grigoroiu1, Zachary Golden1, Ernesto Marticorena1, Melany P. Puglisi1, Skylar Carlson2, Stanley Budzynski1 and Jason Kwan1

1Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628
2Smithsonian Marine Station at Fort Pierce, 701 Seaway Dr., Fort Pierce, FL 34949

3University 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 Caulerpa spp. from the Florida Keys. Minimal growth inhibition (8.4%) and growth inhibition (6.6 %) was observed. Subsequent bioassay-guided isolation 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 μg/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 H₂O partitions from C. racemosa, C. sertularioides and C. cupressoides and CHCl₃ partitions from C. sertularioides and C. cupressoides. Caulerpina 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-Awadhi1, Bowen Guo1, Mohammad A. Rezaei1, Jason C. Kwan1, Chenglong Li2, Tao Ye3, Valerie J. Paul4 and Hendrik Luersch2

1Department of Medicinal Chemistry, Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville.
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kuwait University, Kuwait.
3State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, China.
4Smithsonian 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 IC₅₀ 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 IC₅₀ 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 the differences in the antagonistic activity among brintonamides towards 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. Tran1,2, Laura K. Cartner1,2, Heidi R. Bokesch1,3, Curtis J. Henrich1,4, Xin Wang1, Barry R. O’Keefe1, and Kirk R. Gustafson1

1Molecular Targets Program, Center of Cancer Research, National Cancer Institute, Frederick, Maryland 21701-1201, USA; 2Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702-1201, USA; 3Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA; 4Current 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 tropyrranol 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 Tian1,2, Dongdong Wang3, Heidi R. Bokesch1,3, Barry R. O’Keefe1,2, Kirk R. Gustafson1

1Molecular Targets Program, Center of Cancer Research, National Cancer Institute, Frederick, MD 21701, USA; 2Research & Development Center of Biorational Pesticide, College of Plant Protection, Northwest A&F University, Yangling 712100, China; 3Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA; 4Smithsoniain University, Department of Chemistry and Biochemistry, 400 South Orange Avenue, South Orange, NJ 07079, USA; 5University 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 13C-13C 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 13C chemical shift comparisons or calculations. The recently described bs-CLIP-HSQMBC experiment allows detection of the 35,37Cl 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 Machu1, Peng Fei1, Kaitlyn Vian1, Aswad Khadilkar1, John Abrams1, John MacMillan1,2

1Department of Chemistry and Biochemistry, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, 2Department of Chemistry, University of Texas Southwestern Medical Center, and 3Department 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 macrocycles 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 macrocycles. To monitor and quantify production of these macrocycles, 11-B NMR and ICP-MS methods are being developed.

**P-081**

**TRUNCULINS AND CYCLIC IMINES FROM OKINAWAN MARINE ORGANISMS**

Hiromi Hirade1, Idam Hermawan1 and Junichi Tanaka2

1Department 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 *Siganoseptrella* sp. With conventional spectroscopic analysis, their structures were found to be stereoisomers of trunculins, a group of norsesterpenoid perylene oxides 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 IC₅₀ 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 tetrahydrofurain 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 IC₅₀ 0.97 nM against HEp2 cells, while kabirimine exhibited moderate antiviral activity against RSV with IC₅₀ 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. Alexander1, Brendan Duggan1, C. Benjamin Numan1,4, Aritiro Iwasaki2, Tiago Leao3, Eduardo Caro-Diaz2, Evgenia Glukhov1, Lena Gerwick1 and William H. Gerwick2

1Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093. 2Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093. 3Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093. 4Li Dak Sum Yip Yiu Chin Kenneth Li Marine Biopharmaceutical Research Center, College of Food and Pharmaceutical Sciences, Ningbo University, Zhejiang, China 2Department 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. Zink1, Denise Tarnowski2, Phillip R. Lazzara1, Terry W. Moore1, Mark J Mandel2, and Laura M. Sanchez1

1Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60610, USA. 2Department 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 monocolony 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: ∆binK outcompetes wild type *V. fischeri* in the squid while rscS often cannot colonize. The mass/charge 257 [M + Na]+ is only detected in ∆binK, 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 Liang1, Brad Halthi2,3, Douglas H. Marchbank1,3, Helbelin Correa1, Russell G. Kerr1,2,3
1Department of Chemistry, University of Prince Edward Island, Charlottetown, PE, Canada; 2Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada; 3Nautilus 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. 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 (IC50 = 1.34 μM against Mycobacterium tuberculosis) and its biosynthetic gene cluster.

References

P-086

PRELIMINARY STUDY OF BIOASSAY GUIDED ISOLATION PRODUCTS OF THE MARINE SPONGE MONANCHORA CLATHRATA
Luis A. Amadare1, Jan Vicente3 and Abimael D. Rodriguez4
1Department of Chemistry, University of Puerto Rico, P.O. Box 23346, UPR Station, San Juan, PR, 00931-3346. 2Institute of Marine and Environmental Technology, Center for 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 on the study of Monanchora clathrata. First in-house tests using this sponge throw 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 Li1, Huashi Guan1, William H. Gerwick2
1Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92039, USA; 2Key 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- NMR study, advanced Marfey’s analysis and chemical hydrolysis and hydroazinolysis 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. Salibi, and Tadeusz F. Molinski1,2
1Department of Chemistry and Biochemistry, 2Skaggs 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 spectro-
copy, degradation, and derivatization. GC-El-MS of persilylated OMe-glycosides and standards indicated the presence of α- and β-D-xylene and D-glucose. Analysis of 1H NMR measured in a 13C coupled HSQC experiment, showed α-glucose, α-xylene-3 and β-xylene-1 anomers, and NOE verified α-xylene-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 4R by comparison of the electronic circular dichroism (ECD) of the liberated aglycone against that of standard (S)-(−)-4-methylbutyrolactone. Acrilation 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 13R,23R-2 configuration. The remaining stereocenter at C-17 will be assigned by comparison against two NMA-derivatives of stereodefined standards.

**P-090**

**BIOSURFACTANTS FROM MARINE CYANOBACTERIA**

Jaka J. Mehjabin1, Julie G. Pettibone2, Liang Wei1, and Tatsufumi Okino3,4

1Graduate School of Environmental Science, Sapporo, 060-0810, Japan
2Faculty 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 Monarchora bouillonii and cultured Leptolyngbya sp. The compounds from M. bouillonii were cumbamibamide 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-089**

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

Mohammed M.A. Ahmed1,2,*, Mohammed A. Albadry1, Elsayed M. El-Ghaly1, Said K. Ismail1, Shabana I. Khan1,2, Amar G. Chittiboyina1, Ikhas A. Khan1,2

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

Seven new cembranoids; sarcocvonolutumolides 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 basis 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 1-3 showed moderate activity. As a result, this species could be a potential source of anti-inflammatory agents.

**P-092**

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

Boram Park1, Nguyen Quoc Tuan1, Dong Wook Won1, Sujung Im1, and MinKyun Na1,2

1College 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 sesterpenoid (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 spectroscoptic 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 stereochemistry 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 thedown-regulation of Wnt/β-catenin signaling.
P-093
NEW BIOACTIVE SECONDARY METABOLITES FROM A SYNOCICUM SP. ANTARCTIC TUNICATE
Sofia Kokkaliari1, Charles D. Amsler2, James B. McClintock3, Alexander D. Crawford4,1, Bill J. Baker1,4
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-dimethyl-lumazine-6-carboxamide functional group coupled to methionine sulfoxide and anthranilic acid. The chemical structure 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 DACTYLLA SP., AND GENERATION FROM THE CO-METABOLITE DENIGRIN B
Unwo Obabio, Donald Caldwell1, Laura K. Cartner2,3, Dongdong Wang4, Chang-Kwon Kim5, Xiangrong Tian1,4, Heidi R. Bokesch1,3, Martin J. Schnermann6, Kirk R. Gustafson1
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, Yanging 712100, China

Spiroactyline (1), an unprecedented polycyclic alkaloid, was isolated from an extract of the marine sponge Dactylla sp. The chemical structure was elucidated by extensive spectroscopic methods including LR-HSQC-BC. Compound 1 represents a hexacyclic carbon skeleton bearing a unique tricyclic core comprised of indolizine 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 spiroactyline (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.
University of Alabama at Birmingham, Birmingham, AL, USA

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, tripterpenoids, steroids, and diketopiperazines as well as phenolic compounds.

We investigated the secondary metabolites of some species including Axinella loriellae, A. natans 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 MS2 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: sodowanone H, sodowanone M, sodowanone O, sodowanone P, sodowanone R, sodowanone S and sodowanone 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 Duit^1^, Xue-Hua Ling^2^, 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, Carbilzomib 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 naphthoqui-nones 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 cyclizidi-ne-type alkaloids (1-8), and four new medermycin-type naphthoquinones (9-12) were identified from the large culture of these strains. Biological evaluations of all of the compounds showed that compounds 2, 11 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. coli* 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.

P-099  
**A HIGH-THROUGHPUT METHOD FOR OBTAINING MICROBIAL EXOMETABOLOMICS DATA USING A 3D PRINTED PLATFORM**  
Caroline Brier, Rosalie K. Chu, Christopher R. Anderton, Erik S. Wright  
Department of Biomedical Informatics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15219, United States; 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 evesdropping 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 grown 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**  
Robert A. Teta, Gerardo Della Sala, Germana Esposito, Christopher W. Viti, Carmela Mazzoccoli, Claudia Piccoli, Matthew J. Bertin, Valeria Costantino, Alfonso Mangoni  
^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 aures* 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 smenosclones A-D. The two other metabolites were analogs of the previously described compound conulothiazole B. Further investigation of the trichophycin/smenosclone compound class showed that two compounds (trichophycin B and smenosclactone 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 MTSK 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’-x’-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 (Mtsk). Mtsk catalyzes the fifth reaction of the Mtsk pathway to produce shikimate-3-phosphate (S3P). S3P production was measured using LC-MS Q-TOF. These extracts, along with ACA standard compound and rotterlin, a known SK inhibitor, were tested for their inhibitory activity against Mtsk at concentrations of 50 µg/mL and 50 µM, respectively. The hexane extract displayed the highest Mtsk inhibition of 47% whereas DCM extract, rotterlin and ACA were all categorized as inactive with Mtsk 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 MtSK.

P-103
ANALYZING LARGE METABOLOMICS DATASETS USING REPEATED HIERARCHICAL CLUSTERING AND PRINCIPAL COMPONENT ANALYSES IN R
Shaurya Chanana¹, Chris S. Thomas¹, Fan Zhang¹, Tim S. Bugn¹
¹Pharmaceutical 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 Lomaivitcin, 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 Chang¹*, Ching-Chia Chang¹, Chi-Ying Li¹, Yi-Hong Tsai¹, and Chien-Kei Wei²
¹Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, ²National 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**

Anne-Claire D. Limon, Ala Azhari, Dennis Kyle, Mario A. Rodriguez, Bill J. Baker

1Department of Chemistry, University of South Florida, Tampa, FL, USA, 2Department of Microbiology and Medical Parasitology, King AbdulAziz University, Jeddah, Saudi Arabia, 3Department of Cellular Biology, Center for Tropical & Emerging Global Diseases, University of Georgia, Athens, GA, USA, 4Instituto 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 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 metabolism 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 cultures 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 period. The extraction with ethyl acetate of 520 individual cultures 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 multisecondary metabolites, e.g., echinocandin B and sterigmatocystin, have been reported for Aspergillus pachycristatus (Ramalinaceae) were isolated, and among them Epicoccum nigrum (Penicillium, 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 acetocellin, a bulgarialactone-type azaphiloid. 1H-NMR-based chemical profiling of the cytotoxic extracts as well as the isolation and identification of the active constituents will be presented.

**P-106**
**DISCOVERY OF NEW POLYKETIDE-NONRIBOSOMAL PEPTIDE HYBRID METABOLITES FROM GUT OF MUD DAUBER AMMOPHILA SABULOSA INFESTA**

Joon Soo An, Dong-Chan Oh

1Natural 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 specimens from the mount Gwank in Seoul and isolated bacterial strain (Streptomyces sp. *NN101*) 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 NN101 upon cultivation with supplement of certain types of amino acids. The planar 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 MYCOBIOT BEYOND**

P Amnéric Benatrehina, Choon Yong Tan, Chad Rappleye, L. Harinantenaina Rakotondraibe

1Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, 2Department of Microbiology, The Ohio State University, Columbus, OH 43210.

Myco-bionts of the macrolichen, Niebla homalea (Ach.) Rundel & Bowler (Ramalinaceae) were isolated, and among them Epicoccum nigrum (Penicillium, 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 acetocellin, a bulgarialactone-type azaphiloid. 1H-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, Nan Lan, Yongying Jiang, Gerald F. Bills

1Texas Therapeutics Institute, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, TX 77054, USA, 2Institute 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 Platform (GNP) 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 identified, 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 acetocellin, a bulgarialactone-type azaphiloid. 1H-NMR-based chemical profiling of the cytotoxic extracts as well as the isolation and identification of the active constituents will be presented.
P-109

ISOLATION OF SECONDARY METABOLITES FROM ENDOPHYTIC FUNGUS ASSOCIATED WITH DATURA INNOXIA

Nighat Fatima1,2, Anaya Eugenio, Gerardo David1, Abdul Mannan3, Iram Shahzadi1, LengChee Chang2, Esperanza J. Carcache de Blanco2,*
1Division of Pharmacy Practice and Science and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, United States, 2Department of Pharmacy and 3Department of Biotechnology, COMSATS University Islamabad Abbottabad Campus, Pakistan, 4Department 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 sa-bouraud 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% ±2.2, 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 fungus active fraction.

P-110

CHARACTERIZATION OF NATURAL EPICOCCAMIDES FROM EPICOCCUM NIGRUM ASSOCIATED WITH TAXUS FAUNA

Nighat Fatima1,2,3, Tawanun Sripisut3, Ulyana Muñoz Acuña3, Syed Aun Muhammad1, Muneer Ahmed Qazi1, Esperanza J. Carcache de Blanco2, Safa Ahmed1, Leng Chee Chang2,*
1Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawaii, Hilo, HI, 2Division of Pharmacy Practice and Science and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, United States, 3Department of Pharmacy, COMSATS University Islamabad, Abbottabad Campus, Pakistan, 4Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan Pakistan, 5Institute of Microbiology, Faculty of Natural Science, Shah Abdul Latif University, Khairpur, Pakistan, 6Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan, 7School of Cosmetic Science, Mae Fah Luang University, Taisad, Muang, Chiang Rai, Thailand

Bioassay-directed fractionation of the ethyl acetate extract from the endophytic fungus Epichocum nigrum, led to the isolation and identification of a novel episcocamide derivative, episcocigine (1) and a known compound, episcocamide (2). Episcocamide has an unusual structure due to three biosynthetically distinct subunits: glycoside, fatty and tetratic 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-kB inhibition. Furthermore, docking analysis suggested that these compounds possess strong association with p50 homodimer and p50/p65 heterodimer target proteins responsible for NF-kB inhibition. Further study is worthy based on the activity of these active compounds.

P-111

TWO NOVEL CYCLIC DEPSIPETIDES ISOLATED FROM INSECT-SYMBIOTIC STREPTOMYCES ALBIAXIALIS ICBG1318.

Carla Menegatti1, Vitor Bruno Lourenzo1, Weilan G. P. Melo1, Diego Rodriguezn Hernandez2, Fabio S. do Nascimento0, and Mônica T. Pupo1
1School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil, 2Faculty of Phyloscience, 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 albiauxialis ICBG1318, isolated from the stingless bee Melipona scutellaris. S. albiauxialis 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, hydroxyeleucine, hydroxyglycine, methylglycine and two methylalanes. VL-1 and VL-2 have the molecular formula C14H21N6O9 and C13H20N6O17, 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 TRACHYMURMEX ANTS USING MOLECULAR NETWORKING

Carlsimari 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. 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 Pseudomonadida 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 Pseudomonadida 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, Jeffrey S. Li, Wenjun Zhang

1University of California, Berkeley, Berkeley, CA 94720, 2Chan-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. Nevertheless, genomic analyses indicate that anaerobes encode vast, unexpected biosynthetic potential. Herein we describe the biosynthetic wealth of Ruminoclostridium cellobioparum termiditis CT1112, a cellulose-degrading gut resident of the wood-feeding termite, Nasutitermes lujac. 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 MS2 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 cultured 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 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 F. Earp, Nan Lan, Gerald E. Bills, and James B. Gloer

1Department of Chemistry, University of Iowa, Iowa City, IA, 52242, 2Texas 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 1H 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 spiro-preussione A. Upon closer examination of the reports, it was noted that the two papers had used different NMR solvents. 1H 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, BEAUVIERIA 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 Sang 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, beavetetraones A-B (1-2), from the entomopathogenic fungus Beauveria bassiana. Beavetetraones A and B feature an unprecedented methylene-bridged phloroglucinol skeleton with a highly rearranged scaffold. In addition, a dimer of two phomaligadiones, beavetetraone C (3), was isolated for the first time from a natural source and plausible biosynthetic pathway of beavetetraone 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, Joon Soo An, Eun Seo Bac, Jedo Oh, So Hyun Park, Yeonjung Lim, Yeon Hee Ban, Yun Kwon, JangCheon Cho, Yeo Joon Yoon, Sang Kook Lee, Jongheon Shin, and Dong-Chan Oh

1Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea, 2Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea, 3Department 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 antangiogenesis activity.

**P-118**

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

Donovan, A. Adpressa1, George F. Neuhaus1,1, Gisela González-Montiel2,1, Lannelle R. Connolly1, Michael Freitag1, Sandra Loesger1

1NMR Structure Elucidation, Merck & Co., Inc., Boston, MA 02115.
2Department of Chemistry, 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 DNAMT inhibitors are 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. graminearum*, 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.

**P-119**

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

Daniel S. May, Charlotte Francoeur, Don Huang, 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 Dg1, and Dong-Chan Oh1

1Natural 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 bicyclic molecule 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 Conley1, Jeongho Lee1, María Sofia Costa2,3, Rui Ma1, Scott Franzblau1, Sanghyun Cho1, Brian T. Murphy1

1Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL, USA, 2Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik, Iceland, 3Institute 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 *Micrococcales* 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 DEARESTRICTINE DERIVED METABOLITE FROM A COPROPHILOUS ISOLATE OF PENICILLIUM SACCUUM**

Dulamini I. Ekanayake, Gerald F. Bills, and James B. Gloer

Departments of Chemistry, University of Iowa, Iowa City, IA 52242, and Texas A&M University, College Station, TX 77843, USA

Studies of an isolate of Penicillium saccatum (TTI-0705) obtained from mule deer dung from Colorado led to identification of a new dearestricine derived metabolite, along with the known compound dearestricine B. Dearestricines 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 dearestricine subunit linked to an unusual tricyclic tetrahydrofuroidole 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, 1H NMR comparison with related dearestricines, 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-124**

**FUNGAL METABOLISM OF LICOCHALCONE H**

Yina Xia, 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 is an area of active research, particularly in view of its potential health benefits. Thus, the study for the development of an 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 isoaxanthohumol 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, Emily Mevers, and Jon Clardy

Departments of Chemistry, Harvard Medical School, 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-OXISOCHROMANE ADDUCT FROM PESTALOPIOPSIS SP.**

José Rivera-Chávez1, Jade Zacatenco-Abarca1, Jesús Morales1, Blanca Martínez-Aviña1, Simón Hernández-Ortega1 and Enrique Aguilar-Ramírez1

1Departamento de Productos Naturales, Instituto de Química, Universidad Nacional Autónoma de México, 04510 Mexico City, Mexico, 2CONACYT-Consortio 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-oxisochromane adduct (4, cuautepestalorin), bearing a spiro-polycyclic ring system (6/6/6/6/6/6), along with its putative and new biosynthetic precursors, cytopsorin M (1), cytopsorin N (2), ox-ostepachromane (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* (aGHY), with IC50 values in the μM order, comparable with that of the positive control acarbose.

**P-128**

**CYTOCHROMES P450 IN BACTERIAL NATURAL PRODUCTS BIOSYNTHESIS**

Jeffrey D. Rudolf1

1Department 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-131**

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

Logan W. Machtypré1, Marie J. Charles2, Bradley A. Haltli3,4, Douglas H. Marchbank1,4 and Russell G. Kerr1,3,4

1Department of Biomedical Sciences, 2Department of Biology and Environment, 3Department of Chemistry, University of Prince Edward Island, Charlottetown, PE, C1A 4P3, 4Nautilus 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-acetylated amino acid. N-Palmitoyl-a-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-acetytyrosines. As part of our ongoing efforts to characterize the biosynthesis of 1, we will also discuss identification of proposed intermediates and putative biosynthetic genes.

![Natural Product](image1)

**P-132**

ISOLATION AND CHARACTERIZATION OF NEW TETRACYCLIC MEROTERPENOIDS VIA BIOACTIVITY GUIDED FRACTIONATION

Mario Augustinović1, Laura Flores-Bocanegra1, Daniel A. Todd1, Huzefa A. Raja1, Steven Kurina1, Cedric J. Pearce2, Joanna E. Burdette1, and Nicholas H. Oberlies1

1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 2Mycosynthiks Inc., 505 Meadowlands Dr. # 103, Hillsborough, NC 3Department 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 (CHCl3-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 (IC50 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. Neushaw1, Sandra Loesgen2

1Department 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, 1D-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.
AN EFFICIENT APPROACH FOR IDENTIFYING ANTAGONISM AMONG FUNGI SPECIES AND ANTIFUNGAL ACTIVITY
Airton Damasceno Silva1, Alessandra R. P. Ambrozini1, Renato L. Carneiro1, Paulo C. Vieira1,2,3
1Department of Chemistry, Federal University of São Carlos, São Carlos, SP, 13565-905, Brazil; 2Institute of Science and Technology, Federal University of Alfenas, Poços de Caldas, SP, Brazil; 3Department 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, 1H 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 approach 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.

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 piperazic acid biosynthesis enzymes, L-ornthine 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.

ATYPICAL CHEMICAL ELICITORS FOR ACTIVATION OF SILENT BIOSYNTHETIC GENE CLUSTERS
Robert M. Samples1,2, Marcy J. Balunas1,2,3
1Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269, USA; 2Department 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. PTY08712, a granatycin producer, using media optimization and chemical elicitation. This approach resulted in significantly increased production of granatycin 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.

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 into 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. codicolor were cloned in both orientations of the pDualP vector and integrated into S. lividans ΔredAa1. 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.

‘UN-NATURAL’ NATURAL PRODUCTS FROM FUNGI: FROM FUNGAL STRESS TO BIOTRANSFORMATIONS
Paige E. Mandelare, Elizabeth N. Kawaesa, Mahsa Khoshbakht, Jason Srey, Donovan 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
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.
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 Sun1,2, Zhiguo Liu1,2, Fangbo Zhang1,2

1China Academy of Chinese Medical Sciences, Beijing, China, Beijing, China
2Institute 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 gymniriza (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 very unique structural features such as 12- or 13-membered macrocyclic ether-containing 1,4-disubstituted phenyl and γ-lactam or succinimide moieties. Xenoacremon A--H showed cytotoxicity against A549, k562, and Hela tumor cells, and xenoaremon A could inhibit the expression of PI-3K/AKT signaling pathway.

**P-145**

**DEFINING THE ROLE OF A BACTERIAL ENZYME OF UNKNOWN FUNCTION**

Taylor Kornfuehrer1 and Alessandra S. Eustáquio1

1Department 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 Perlatti1, Connie B. Nichols2, J. Andrew Alspaugh3, and Gerald F. Bills1

1Texas Therapeutics Institute, The Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, Houston, Texas 77054, USA.
2Departments 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-1-13C]-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,1 Eleanor E. Meyer,1 Dale C. Swenson,1 Kristine H. Wammer,1 David M. Ciwertny,1 and James B. Gierer1

1Department of Chemistry, University of Iowa; Iowa City, IA 52242,
2Departments of Biochemistry and Medicine, 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 photoprocess of dienogest, we also demonstrate here its enrichment in test samples across several simulated diurnal cycles due to the reversibility, of a competing photohydroxylation reaction process. In vitro receptor transcriptional activation assays revealed that these unusual photoproducts exhibit progesterogenic and androgenic activity, albeit with some lower potency than their respective parent compounds (low-μM to sub-nM EC50 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.
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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.

\[ ^1H \text{ NMR of a compound} = \sum \text{1D TOCSY spectra (T} \_1+T \_2+T \_3+\ldots+T \_n) \]

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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 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\_n-3, H\_n-8 and H\_n-2/6') and berberine (H\_n-1, H\_n-11 and H\_n-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.

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DISCOVERY OF A STABLE VITAMIN C GLYCOSIDE AT HIGH CONCENTRATIONS IN APPLES (MALUS SPP.)
Alistair T. Richardson
1University 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., Lindsay K. Caesar, Joshua Kellogg, Daniel A. Todd, Alexander H. Horswill, Nadja B. Cech
1Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412, USA. 2Department 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), PCPRS 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-liquid 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 Naruskawa, 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 polypeptide 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 polypeptide 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.
P-156
TARGETED AND UNTARGETED APPROACHES TO STUDY THE EFFECTS OF STORAGE CONDITIONS ON STABILITY OF HYDRASTIS CANADENSIS (GOLDENSEAL)
Mansad Khin,1 Lindsay K. Caesar,1 Joshua J. Kellogg,1 and Nadja B. Cech1
1Department 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 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 Melnyk1, Olek Koshovyi1, Agnieszka Bazylko1, Maria Ziaja1, and Sebastian Granica1
1Department of Pharmacognosy, National University of Pharmacy, Kharkiv, Ukraine; 2Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland; 3Department of Natural Sciences, University of Rzeszów, Rzeszów, Poland

Tiliae flos commonly known as lime flower is a popular medicinal plant 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. platyphyllos 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:CH3Cl:HCOOH:CH3COOH:H2O 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 PRACTICABILITY OF EXTERNAL CALIBRATION QUANTITATIVE NMR OF NATURAL PRODUCTS
Yazo Nishizaki,1,2 Shao-Nong Chen,3 David C. Lankin,1 and Guido F. Pauli1
1University of Illinois at Chicago, Chicago, IL 60612, USA; 2National 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 Kanihiro1, Paula B. Luis1, Julia Brickey1, Jennifer B. Frye1, Claus Schneider2, Janet L. Fusk1,3
1Department of Nutritional Sciences, University of Arizona, Tucson, AZ; 2Department of Pharmacology, Vanderbilt University, Nashville, TN; 3Department 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 G-C, in blocking pro-osteolytic pathways and the capacity of bone 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.
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ANTI-DIABETIC EFFECTS OF WILD GLYCINE SOJA SEED EXTRACT ON TYPE II DIABETIC MOUSE MODEL
Yun Mi Lee1, Eunjun Son1, Seung-Hyung Kim1, Ho Kyong Kim1, Dong-Gyu Jang1, Dong-Seon Kim2
1Herbal Medicine Research Division, Korea Institute of Oriental Medicine, 1672 Yuseong-dao, Yuseong-gu, Daejeon 34054, Republic of Korea.
2Institute of Traditional Medicine and Bioscience, Daejeon University, Daejeon 300-716, 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 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 p eroxisome proliferator-activated receptor gamma (PPARY) 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.

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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 Soumyanath1, Maya Caruso1, Donald Matthews1, Kirsten Wright2, Armando Alcazar Magana3, Claudia S. Maier3, Jan F. Stevens3, Nora Gray4, Joseph Quinn5
1Department of Neurology Oregon Health and Science University, Portland, OR, 97239, USA; Departments of Chemistry and Pharmaceutical Sciences and Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA; 2Department 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 Kitahira, 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: ophiopogonane 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.

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ACCELERATION OF NATURAL PRODUCT RESEARCH BY INTEGRATED APPROACH BASED ON MS AND NMR ANALYSIS
Hyunwoo Kim1, Chen Zhang2, Garrison W. Cottrell3, and William H. Gerwick2,3
1Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, La Jolla, California 92037, United States of America.
2Department of Computer Science and Engineering, University of California, San Diego, La Jolla, California 92093, United States of America. 3Skaggs 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.
A FLIPR ASSAY FOR DISCOVERY OF GABA_\textsubscript{A} RECEPTOR MODULATORS OF NATURAL ORIGIN

M. Teresa Faleschini\textsuperscript{1}, Anne Maier\textsuperscript{1}, Sarah Fankhauser\textsuperscript{2}, Katharina Thasis\textsuperscript{1}, Simon Hebeisen\textsuperscript{1}, Matthias Hamburger\textsuperscript{1}, Veronika Butterweck\textsuperscript{1,2,4}

\textsuperscript{1} University of Basel, Pharmacare, Klingelbergstrasse 50, 4056 Basel, Switzerland, \textsuperscript{2} University of Applied Sciences Northwestern Switzerland, Institute for Pharma Technology, School of Life Sciences, Hofackerstrasse 30, 4132 Muttenz, Switzerland, \textsuperscript{4} B'Sys GmbH, Berkenstrasse 254, 4108 Witterswil, Switzerland, \textsuperscript{2} Zeller Medical AG, Seeblickstrasse 4, 8590 Romanshorn, Switzerland

A Fluorometric Imaging Plate Reader (FLIPR) assay in 96-well microtitre format utilizing CHO cells stably transfected with GABA\textsubscript{A} receptors of α,β,γ, 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\textsubscript{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, valeric acid in Valeriana officinalis, and piperine in Piper nigrum extract. EC\textsubscript{50} values of compounds (magnolol: 4.81 ± 1.0 μM, valeric 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.

BISTORTAE RHIZOMA

THE BOTANICAL SOURCES OF NORTHER BRAZILIAN RED PROPOLIS

Gari V. Ceana-Capatinha\textsuperscript{1}, Jennyfer A. Aldana, Matheus H. Tanimoto\textsuperscript{1}, and Jário K. Bastos\textsuperscript{2}

\textsuperscript{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 polyphenylated 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, northern Brazil, lead to the identification of two resin producing Clusiaceae, *Clusia* 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 compartments 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 plant 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 Hayes1, Charles Barber2, Adam Kuszak3, Melissa Phillips1, Catherine Rimmer1, and Laura Wood1

1Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. 2Office 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 Hayes1, Charles Barber1, Adam Kuszak2, Melissa Phillips1, Catherine Rimmer1, and Laura Wood1

1Chemical Sciences Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA. 2Office 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. Austin1, Brenna Kirkpatrick1, Joanna E. Burdette2

1Department 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 phytoprogesterins. 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 phytoprogesterin 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**

**METABOLOMOMIC PROFILING TO GAUGE UNCERTAINTY AND AUTHENTICITY OF KRATOM (MITRAGYNA SP.) COMMERCIAL PRODUCTS**

Joshua I. Kellogg, E. Diane Wallace, Nicholas H. Oberlies, and Nadja B. Cech

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 in-dole 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 Paula, 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 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 bioavailability observed in clinical trials.


**P-176**

**HIGH AFFINITY HERG AND LOW AFFINITY Ca_{1,2} BLOCKERS DEHYDROEVODIAMINE AND HORTIAMINE IN DECOCTIONS OF THE TCM DRUG EVODIAE FRUCTUS**

Jakob K. Reinhardt, Igor Baburin, Stanislav Andranovits, Steffen Hering, and Matthias Hamburger

1 University of Basel, Pharmcenter, 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_{Ca}, (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_{Ca} inhibition <1 µM) and Ca_{1,2} (IC_{50} of I_{Ca} inhibition >50 µM) channels expressed in HEK 293 cells was determined. The intake of significant amounts of I_{Ca} blocking alkaloids 1 and 2, together with their comparably low potency inhibition of Ca_{1,2} suggests a high risk for pro-arrhythmic effects such as Torsade de Pointes.

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**BIOFLAVONOID COMPOSITION – UP446 REDUCED CELLULAR OXIDATIVE STRESS AND ATTENUATED HYPEROXIA-COMPROMISED MACROPHAGE PHAGOCYTIC FUNCTION BY REDUCING HMGB1 RELEASE**

Mesfin Yimam, Mei Hong, Ping Jiao, Teresa Horns, Qi Jia, Lin L Mantell, Xiaojing Yang, Katelyn Dial, Alexander Gauthier, and Mosi Lin

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...
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CELLULAR FREE RADICAL SCAVENGING AND DNA PROTECTING EFFECTS OF SOLIPRIN™ AND ALOEWHITE™

Ping Jiao, Feifei Long, Lidia Brownell, Mei Hong, Teresa Horn, Mesfin Yimam, Hanshao Zhang, Qi Jia

1Unigen Inc., 2121 South State Street, Suite 400, Tacoma, WA 98405, USA, 2Genoarray 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.

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PHYTOCHEMICAL CHARACTERIZATION OF ROSA MULTIFLORA THUNB. (ROSACEAE) IN JAPAN AND SOUTH KOREA, WITH A FOCUS ON THE BIOACTIVE FLAVONOL GLYCOSIDE “MULTIFLORIN A”

Yumi Kitahiro*, Hiroshi Ikeda*, Hyoug-Tak Im*, Makio Shibano1

1Department of Natural Products research, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan, 2The University Museum, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, 3Department of Biology, Chonnam National University, Gwangju 61186, South Korea

Dried achene or anthocarpous accessory fruits of Rosa multiflora Thund., 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 pungent 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.

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HYDROLYZED EPIedium EXTRACT AND ICARITIN PROMOTE NON-GENOTOXIC ESTROGEN METABOLISM


1UIC/NIH Center for Botanical Dietary Supplements Research, 2Center 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 (E2) into non-genotoxic 2,3-OH-E2, while the constitutively active P450 1B1 metabolizes E2 to 3,4-OH-E2, 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 aglycone) 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 AT001555]

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ROLE OF ROSEMARY EXTRACT AS A GASTROINTESTINAL PROTECTANT IN DSS MODEL OF COLITIS

Bhaskar Vemu, Jacob Veenestra, Miirielle 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.

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CYTOTOXIC AND NON-CYTOTOXIC CARDIAC GLYCOSIDES CHARACTERIZED FROM STREBLUS ASPER

Yalin Ren1, Qingwei Tan1, Wei-Lun Chen2, Jinhong Ren1,2, Pratik Shriwas3,5,6, Chunhua Yuan1, Tran Ngoc Ninh3, Hee-Byung Chai2, Xiaozhuo Chen4,6,9, Dijaja D. Soejarto2,10, Michael E. Johnson2,3, Joanna E. Burdette1, and A. Douglas Kinghorn1

1Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA. 2Department of Medicinal Chemistry and Pharmacognosy and Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA. 3Department of Biological Sciences, 4Edison Biotechnology Institute, and 5Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701, USA. 6Campus Chemical Instrument Center, The Ohio State University, Columbus, OH 43210, USA. 7Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. 8Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA. 9Science and Education, Field Museum of Natural History, Chicago, IL 60605, USA.

The cytotoxic (+)-strebloside and a new non-cytotoxic analogue, (+)-17-hydroxy-strebloside, 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-hydroxy-strebloside 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 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 cytotoxicity.

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NA’/K’-ATPASE-TARGETED CYTOTOXICITY AND PRELIMINARY STRUCTURE-ACTIVITY RELATIONSHIP OF DIGOXIN

Yalin Ren1, Wei-Lun Chen2, Jinhong Ren1,2, Ellen J. Sass3, Pratik Shriwas3,5,6, Xiaozhuo Chen4,6,9, David M. Lucas3,4, Michael E. Johnson2,3, Joanna E. Burdette1, and A. Douglas Kinghorn1

1Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA. 2Department of Medicinal Chemistry and Pharmacognosy and Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA. 3Division of Hematology, Department of Internal Medicine, College of Medicine, The Ohio State University, Columbus, OH 43210, USA. 4Department of Biological Sciences, Edison Biotechnology Institute, Molecular and Cellular Biology Program, and 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 hydrophilic residues of Na’/K’-ATPase, which may affect the binding pose and contribute to its lack of cytotoxicity.

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LIMONOIDS IN ENTANDROPHRAGMA ANGOLENSE

Iso Yoon1, Junfei Zhou1, Samiya Papa1, Xia Guo2, Zaijie Jim Wang3, Chun-Tao Che1

1Department of Medicinal Chemistry and Pharmacognosy, 2Department 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.D.C. (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 andiroside S (1), andirobin (2), and methyl angolensate (3), and 6-deacetoxydomesticulide D 21-methylether (4). Compound 2 is a new structure and 2 is identified for the first time in the Entandrophragma genus.

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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 Kim1, Jonathan Bisson1, J. Brent Friesen1,2, Guido F. Pauli1, and Charlotte Simmler1
1Center for Natural Product Technologies, PCPRES and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA; 2Physical Sciences Department, Dominican University, River Forest, IL 60305, USA
Chlorophyll (Cphyl) pigments, present in crude extracts (CE) from photo-synthetic 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 developed a rapid 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/EtOH/MeOH/Water (5:5:5:5, v/v) is used as the stationary phase to capture the Cphyls from various CE. 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 physico-chemical 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, 1H-NMR, and mass recovery.

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EVALUATION OF STANDARDIZED SMILAX CHINA L. FOR SKIN WHITENING TREATMENT
Eunju Kang1, Yun Mi Lee1, Ho Kyoung Kim1, Dong-Soon Kim1*
1Herbal Medicine Research Division, Korea Institute of Oriental Medicine, 1672 Yuseong-daero, Yuseong-gu, Daejeon 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.

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NO CLINICALLY RELEVANT INTERACTIONS OF ST. JOHN’S WORT EXTRACT ZE 117 LOW IN HYPERFORIN WITH CYTOCHROME P450 ENZYMES AND P-GLYCOPROTEIN
Catherine Zahner1, Esther Kruttisch2, Julia Uricher3, Michael Lissy4, Martin Hirsch2, Simon Nicollus5, Stephan Krähenbühl1, Veronika Butterweck1 and Jürgen Drewes1
1Max Zeller Soehne AG, Romanshorn, Switzerland; 2Navisan GmbH, Neu-Ulm, Germany; 3Division 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 Cmax 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 M1, Levielle G1, Jackson S2
1Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland; 2Quality Department, Modern Botany Ltd, Main Street Schull, Co. Cork, Ireland. P81 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 usitissimum (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:
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**IN VITRO ANTIBACTERIAL ACTIVITY OF MODERN BOTANY™ PRODUCTS AND SELECTED NATURAL PRODUCT COSMECEUTICAL INGREDIENTS**

Young M¹, Leivelle G², Jackson S. F³
¹Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork, Ireland. ²Quality Department, Modern Botany Ltd, Main Street Schull, Co. Cork, Ireland. P81 P292

This aim of this project was to investigate the antimicrobial activity of selected Modern Botany™ products and their ingredients used in cosmeceutical preparations. 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](Image)

**Antimicrobial**

![Diagram](Image)

**Agr. plating of Broth Microdilution**

**P-190**

**IDENTIFICATION OF GROWTH INHIBITING AND RESISTANCE MODIFYING BOTANICALS FOR CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII**

Micah Dettweiler¹, Monique Salazar², Marco Caputo³, James T. Lyles², Cassandra L. Quave¹-²
¹Department of Dermatology, Emory University, Atlanta, GA, ²Center 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 ½ 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, 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.

**P-192**

**ANTIBACTERIAL ACTIVITIES OF METABOLITES FROM VITIS ROTUNDIFOLIA (MUSCADINE) ROOTS AGAINST FISH PATHOGENIC BACTERIA**

Kevin K. Schrader¹, Mohamed A. Ibrahim²,³, Howaida I. Abd-Alla², Charles L. Cantrell⁴ and David S. Pasco⁴
¹United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, National Center for Natural Products Research, University, MS 38677, USA. ²Chemistry of Natural Compounds Department, Pharmaceutical and Drug Industries Division, National Research Centre, Dokki, Giza 12622, Egypt. ³National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Enteric septicemia of catfish, columnaris disease and streptococcus, 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 E. 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 E. 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.

**P-193**

**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-likeness using SWISS ADME, then assessed as potential ligands for neuro-relevant targets such as GPCR ligands using SWISS target predictor. One major obstacle in neuro-pharmacological 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 compound’s 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.

**P-194**

**Biodiversity and Antitumor Activities of Endophytes of Broussonetia Papyrifera L.**

Huang Boa-kang and Yu yaru

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:hhkcn@163.com

*Broussonetia papyrifera* (L.) Vent. Belongs to Moraceae family and is widely distributed in China. It is known 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, A. apispora, and E. elonella were resistant to heavy metal zinc (>200 mmol/L). The fermentation products of Endophytic Fungi from Chaetomi-um globosum 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.

**P-195**

**Exploring the Antimicrobial Activity from Endophytes Isolated from Native North American Prairie Plants**

Kirk P. Manfredi1 Bridget Shoemaker1, Michael Walter1 and Laura F. Walter1  
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 50614

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, anti-cancer, 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.

**P-196**

**A New Meroditerpenoid from the Cultures of an Endophytic Fungus, Neosartorya Fischeri JS553**

Sunghée Bang, Ji Hoon Song, Soonok Kim, Ki Sung Kang, and Sang Hee Shim

1 College of Pharmacy, Duksum 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 isolated from native prairie plants from the midwestern US. We have previously assayed prairie plant extracts for antimicrobial activity against columnaris disease.

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, anti-cancer, 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.
significant neuroprotective effect in HT22 cells by inhibition of ROS, Ca²⁺ influx and MAPKs (JNK, ERK, and p38) phosphorylation.

![Chemical structures](image)

**P-197**

**NEW B-RESORCYCLIC ACID LACTONE DERIVATIVES FROM A HALOPHYTE-ASSOCIATED FUNGUS, COLLETOTRICHUM GLOEOSPORIOIDES JS419**

Sunhee Bang¹, Changyol Lee¹, Sooark Kim¹, and Sang Hee Shim¹,²

¹College of Pharmacy, Duksum Women’s University, Seoul 01369, South Korea; ²National 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 Suaeda 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-resorcylic acid lactone derivatives (1-6, 8, 13-15) with two known compounds (7, 9-12, 16-20).

**P-198**

**ETHNOBOTANICAL AND PHYTOCHEMICAL STUDIES ON GAULTHERIA BERRIES FROM SOUTHWEST CHINA**

Binseng Luo¹, Chunlin Long¹,²,³ and Edward J. Kennelly¹*

¹College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China, ²Key Laboratory of Ethnomedicine (Minzu University of China), Ministry of Education, Beijing 100081, China, ³Department 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 high-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. HPLC-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.

**P-199**

**DIETARY/MEDICINAL PLANTS AND NRF2 ACTIVATION**

Xiaohua Wu, Shugeong 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**

Geun-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 (LCY-E) enzymes are involved in the formation of lycopene, and LCY-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. Influx and MAPKs (JNK, ERK, and p38) phosphorylation.
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 Tröger1, Alexander Weng1, Matthias F. Melzig1, Sebastian Granica2, Jürgen Zentek1, Wilfried Vahjen1, Jakub P. Piwowarski2,3

1Department of Pharmaceutical Biology, Freie Universität Berlin, Germany, 2Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland, 3Institute 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 NFκB PATHWAYS BY HUMAN GUT MICROBIOTA METABOLITES OF ELLAGITANNINS.

Aneta Bobowska1, Matthias F. Melzig1, Sebastian Granica1, Agnieszka Filipiak, Jakub P. Piwowarski2,3

1Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland, 2Department 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 Popowski1, Karolina A. Pawłowska1, Jakub P. Piwowarski2,3, Sebastian Granica1

1Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Poland, 2Institute 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 lead 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.e. *Filipendula ulmaria*, *Ononis 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-MS² 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 Delgadillo1, Elizabeth A. McMillan1, Michael A. White1, and John B. MacMillan1

1Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA 95064, USA, 2Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX 75390, USA.

Ikarugamycin (IKA) is a previously isolated polyyclic 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 bound within the 5-6-5 ring moiety is crucial to selective antiproliferative activity against NSCLC HCC44, H23, and Calu-1. All selective compounds
NOVEL BROMINATED VINYLIC FATTY ACIDS EFFECTIVELY INHIBIT THE LEISHMANIA TOPOISOMERASE IB ENZYME MEDIATED BY HALOGEN BOND FORMATION

Néstor M. Carballera1, Denisse Ale paganí1, Leilani M. Lotti1, Victorio Jauregui Matos1, Leonardo L. G. Ferreira2, Adriano D. Andricopulo2, Rosa M. Reguera3, Yolanda Pérez-Pe rtejo4, and Rafael Baláñ a-Fouca5
1Department of Chemistry, University of Puerto Rico, San Juan, PR.
2Laboratory of Medicinal and Computational Chemistry, University of Sao Paulo, Sao Carlos, Brazil. 3Department of Biomedical Sciences, University of Leon, Campus de Vegazana, Leon, Spain.

Marine sponges and anemones have provided most of the naturally occurring halogenated vinyl 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 topoisomeras es (LTopIB). 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 LTopIB 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.

METABOLOMIC PROFILING OF COFFEE PULP BY-PRODUCTS WITH ANTI-INFLAMMATORY PROPERTIES USING HIGH RESOLUTION MASS SPECTROMETRY

Darren Dumlao1, Scott Harder1, Randy Arnold2, Baljit Ubhi1, Mariialace Maldini1
1SCIEX, Redwood City, CA, 2SCIEX, 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 carded as waste, with severe environmental and economic consequences. 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.

IDENTIFYING NATURAL PRODUCT QUORUM SENSING INHIBITORS USING CASTANEA SPP. AS A MODEL SYSTEM FOR COMPOUND ACTIVITY MAPPING

James T. Lyles1, Sandeep Voleti2, Micah Dettweller3, Akram Salami4, and Cassandra L. Quave5,6
1Center for the Study of Human Health, Emory University, Atlanta, GA 30322, 2Department of Biology, Emory University, Atlanta, GA 30322, 3Department of Dermatology, Emory School of Medicine, Atlanta, GA 30322, 4Molecular 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 Castanea 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

Psyrax 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 Zhou1, Yvette Alania2, Marianna C. dos Reis3, Rasika S. Phansalkar1, Joo-Won Nam1, James McAlpine4, Shao-Nong Chen5, Ana K. Bedran-Russo3, and Guido F. Paoli2

1Dept Med Chem & Pharmacognosy, Coll Pharmacy and 2Dept Restorative Dentistry, Coll Dentistry, U of IL at Chicago, IL 60612, USA; 3College 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-(2→8)-→O→7,4β-catechin (PA1, 2), epicatechin-(2→8)-→O→7,4β-epicatechin (PA2, 4), epicatechin-(2→8)-→O→7,4β-ent-epicatechin (PA4, 5), and epicatechin-(2→8)-→O→7,4β-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 “C-γ-gauche” effect, differential chemical shifts (Δδ) relative to four stereo-chemically defined dimers (terminal unit II), and phospho-gluconolysis 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. massoniana 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 Vo1, Yuh Min, Chook2, John B. MacMillan2

1Department of Chemistry and Biochemistry, University of California, Santa Cruz, Santa Cruz, CA, USA; 3Department 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. Winter1, Laura Flores Bocanegra1, William J. Crandall1, Fridah Rotich1, Madeline Tillmann, Cedric J. Pearce2, Nicholas H. Oberlies1, Nadja B. Cech3

1Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412, 2Davidson College, Davidson, North Carolina, 28035, 3Mycosynthetic, 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 discovered.
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,1 and April L. Rissing1
1Department of Chemistry and Biochemistry, Institute for Natural Products Applications and Research Technologies, The University of Oklahoma, Norman, OK. 2Department 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.

P-215
BLOOD PRESSURE EFFECTS AND CHEMISTRY OF BLACK AND WHITE TEA FROM VIETNAM
Lucy Noon1, Ceri L. Davies1, and Laura M. Bystrom1
1School 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 evaluate 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
Tiiti Roy1, Sergette Banang-Mbeumi2, Pratik Basnet2, Mario Sechi2, Siva Murra2, Jean Christopher Chamcheu1,2
1College of Pharmacy and 2College of Art, Education & Science, University of Louisiana at Monroe, LA, USA and 1Department 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>cucurmin>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.

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ANTIMALARIAL XANTHONE FROM LEAF EXTRACT OF ANTHOCLEISTA VOGELII PLANCH (LOGANIACEAE)
Alaribe C.S.1,2, Adesegun S.1,2, Bamiro J.3, Shode. O.F.1 and Coker H.A.1,2
1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria, 2Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria, 3Dept of Obstetrics & Gynaecology Research Laboratory,College of Medicine,UTH,Nigeria, 4Dept of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, Durban. South Africa

Anthocheila 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 xanthenes 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-dimethoxyxanthone). 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 Anthooleista vogelli Planck (Loganiaceae)

REFERENCES

**P-218**

**RESOLUTION OF OPTICALLY ACTIVE α-HYDROXY KETONES IN PSEUDO-SOLID-PHASE BY MECHANOCATALYSIS**

Shuvendu Das1, Changan Li2, Shih Yang3, Leonard MacGillivray4, Mark Arnold2

1,2Center for Biocatalysis & Bioprocessing, Coralville, IA, USA, 3The 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 furonins 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 furonin 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 mechanobiocatalytic 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 Siddique1, Nehad Ayeoub2, Afsana Tajmim3, Sharon A. Meyer3, Khalid El Sayed4

1Department 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 recurrent 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.

**P-220**

**QUANTIFICATION OF ANTIMICROBIAL GINKGOLIC ACIDS BY MASS SPECTROMETRY BASED ON EXTRACTION METHOD**

William L. Crandall1, Heather L. Winter1, Nadja B. Cech1

1Department 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,
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ISOLATION OF T. CRISPA AND T. SINENSIS TO ADDRESS QUALITY CONCERNS FOR TINOSPORA DIETARY SUPPLEMENTS
Abidah Parveen1,2, Omer Fantoukh1, Zulfiqar Ali1, Yan Hong Wang1, Vijayasankaran Raman1, Ikbas A. Khan1,2
1Department of Biomolecular Sciences, Division of Pharmacognosy, 2National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, 38677, USA, 3Abbottabad 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. Adaikpoh1, D. Cole Stevens1
1Department 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, 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, 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, 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, the gliding Gram-negative bacteria. 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MULTIFUNCTIONAL REAGENTS FOR IDENTIFICATION AND ISOLATION OF NATURAL PRODUCTS FROM CRUDE EXTRACTS
Gabriel Castro-Falcón, Grant S. Seller, 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-moieties for UV/Vis detection, Br/Cl atoms and ionizing and fragmenting units for MS and MS’ detection, chromatographically enrich-able components like perfluorinated alkyl chains, and crystallizing units for X-ray analysis. We have found that thiol, nitroso alyl and tetrazine reagents react chemoselectively with natural products containing electrophoric 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 zebrasfish 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-
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TETRAHYDROQUINOLINES FROM ALLOMYRINA DICHOTOMA AND THEIR INHIBITORY EFFECTS ON LPS-MEDIATED HUMAN ENDOTHELIAL CELLS VIA NF-KB PATHWAY

InWha Park¹, Wonhwa Lee², Joosook Oh³, and MinKyun Na²*¹
¹College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea, ²Aging Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea, ³Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States.

The most commonly consumed insects are beetles (Coleoptera), Allomyrina dichotoma L. (family Scabelidae, order Coleoptera), a rhinoceros beetle, is mainly distributed in Korea, Japan, China, and Taiwan. The biological activities 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 1H-1H coupling constants and the NOE cross-peaks, as well as the computational chemical shifts calculation followed by D4 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-κB pathway.

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 Desrosiers¹, Alexis Mitteleman², and Pamela J. Weathers¹
¹Dept. Biology and Biotechnology; ²Dept. 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 isozymes (CYPs) responsible for AN metabolism. We showed that methanolic DLA extracts had an IC₅₀ 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 CYCLODIPETIDES ISOLATED FROM PSEUDOMONAS FLUORESCENS IB-MR-66E

Mariana Lozano-González¹, Berenice Ovalle-Magallanes¹, Manuel Rangel-Grimaldo¹, Susana de la Torre-Zavala¹, Lilia Noriega¹, Claudia Tovar-Palacio¹, Armando Tovar-Palacio¹, and Rachel Mata²
¹Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico. ²Instituto de Biotecnología, Universidad Autónoma de Nuevo León, Monterrey, Mexico. ³Instituto 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 Fosch1, Verena Schönig1, Greta Marie Assmann2, Christin Moser1, Beate Sievert1, Veronica Butterweck1, Jürgen Drewe1

1Max Zeller Söhne AG, CH-8590 Romanshorn, Switzerland, 2University of Konstanz, D-78464 Konstanz, Germany

Ze 339, a CO3 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 9 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 investigated for the observed 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 Kim1,2, Eun Hee Park1,2, Won-Kyung Cho1, and Jin Yeal Ma1

1Korean Medicine (KM) Application Center, Korea Institute of Oriental Medicine (KIOM), Daegu, Republic of Korea, 41062, 2Institute of New Drug Research, Myungmoon Bio, Daegu, Republic of Korea, 41059, 3Korean 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-2015E and KIOM-2015EE 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-2015E 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-xB 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.

**P-231**

**EMBRACING MODES FOR QUALITY AND SAFETY ANALYSIS FOR SAFER BOTANICAL PRODUCTS AND AUTHENTIC PLANT SAMPLES**

Abidah Parveen1,2, Omer Fantoukh1,2, Jane Manfron Budel1, Mir Tahir Maqbool1, Zulfijar Ali3, Vijayasankar Raman2, Ganesh Babu NM4, Jianping Zhao5, M. Khalid Ashfaq2, Yan-Hong Wang2, Ikhtias A. Khan2

1Department of Biomolecular Sciences, Division of Pharmacognosy, 2National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA, 3Department of Pharmaceutical Sciences, Abbottabad University of Science and Technology, Havelian, Abbottabad District, KP, Pakistan, 4Department of Pharmaceutical Sciences, State University of Ponta Grossa, UEPG, PR 84030-900, Brazil, 5The 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 investigations were 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 *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.

**P-232**

**BIOASSAY-GUIDED ISOLATION OF CYTOTOXIC COMPONENTS FROM THE STEMS OF STREPTOCAULON JUVENTAS**

Ermias Mekuria Addo1, Joshua M. Henkin2, Gerardo D. Anaya-Eugenio3, Tran Ngoc Ninh4, Yulin Ren4, Harihantenableinai L. Rakotondraibe1, Esperanza J. Carcache de Blanco1, Dja D. Soeijarto1, A. Douglas Kinghorn5

1Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, 2Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, 3Vietnam Academy of Science and Technology, Hanoi, Vietnam, 4Science 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-154 cells, for which all of them showed potent activity [e.g., periplogen digiotoxioside (2) with IC50 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.
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JAUNDICE: SURVEY OF TRADITIONAL REMEDIES AND IN-VITRO LIPID PEROXIDATION IN THE PROGRESSION OF LIVER INJURY AT THE CELLULAR LEVEL

Adeyoyin Adeniyi
Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, College of Medicine Campus, PMB 12003, Sarsu-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), Caillandra portoricensis (Mimosidae), Celastrus paniculatus (Celastraceae), Cochlospermum tinctorum (Cochlospermaceae), Curcuma longa (Zingiberaeae), Curculigo pilosa (Hyloxalidaceae), Cymbopogon citratus (Poaceae), Enantia chlorantha Oliv. (Anonaceae), Gossypium barbadense (Malvaceae), Kigelia africana (Bignoniaceae), Lawsonia inermis (Lythraceae), Lophira alata (Ochnaceae), Mangifera indica (Anacardiaceae), Morinda lucida (Rubiacceae), Phyllanthus amarus (Euphorbiaceae), P. muelleriatus (Euphorbiaceae), Rauwolfia vomitoria (Apocynaceae), and Sarcocephalus latifolius (Rubiacceae). 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 (Cichlidae) and Scomber japonicus (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 1.0384 x10-5 (mg/tissue) and 8.2304 x10-5 to 5.4100 x10-5 (mg/tissue) in C. gariepinus and S. japonicus fish models, respectively. This indicated the mapping 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 PERVERDIS

Garima Agarwal,1 Steven Kurnia,1 Gerardo Anaya Eugenio,1 Tran Ngoc Ninh,1 Joanna E. Burdette,1 Djaya D. Soejarto,1 Esperanza J. Carcache de Blanco,1 L. Harinantenaina Rakotondraibe,1 and A. Douglas Kinghorn1
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[β]benzofurans, specific to the Aglaia spp., have garnered much interest as potential anticancer, anti-infective, and antiviral agents owing to their apoptotic and translation factor inhibition activity. As part of our continuing efforts to discover potential anticancer agents, the aqueous extract of Aglaia perverdis Hiern roots were found to contain four new flavaglins 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 roagogue derivatives.

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NEW MEGASTIGMANES FROM OPUNTIA HUMIFUSA CLADODES

Min Seok Jo, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Su Cheol Baek, Kwang Ho Lee, Yong Hoon Lee, Joon Min Cha, Kyong Jin Park, Tae Hyun Lee, Dong Hyun Kim, Youn Sung Choi, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Siwon 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 Snatke’s method. All the isolated compounds were evaluated for their inhibitory effects on nitric oxide (NO) production in LPS-activated RAW 264.7 macrophages.

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NEW 1,3-DIPHENYL PENTANE NORLIGNANS FROM PUERARIA LOBATA ROOTS

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

Glycosmis ovoidea 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-κB 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′,4′-trihydroxy-3,6,7,8,4′-pentamethoxyflavone (1), a coumarin with a previously unassigned absolute configuration, six known coumarins inclusive of kinucoumarin (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:
P-241  
**INHIBITION OF CHOLINESTERASE BY ALKALOIDS FROM THE RHIZOMES OF COPTIS CHINENSIS**  
Thao Quyen Cao, Jeong Ah Kim, Jae Sae, Byung Sun Min  
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 with comparison with those reported in the literature. Compounds 4, 5, and 7 showed potent inhibition against acetylcholinesterase (AChE) with IC\textsubscript{50} values of 1.1, 5.6, and 12.9 Î¼M, respectively. Compounds 2 and 4 showed inhibition of butyrylcholinesterase (BChE) with IC\textsubscript{50} values of 11.5 and 27.8 Î¼M, 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, Jeong Ah Kim, Byung Sun Min  
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\textsubscript{50} values ranging from 4.25 to 6.78 Î¼M, which was significantly higher than that of the positive control, ursolic acid (IC\textsubscript{50} = 11.34 Î¼M). 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 Îµ-viniferin (8) and mixed for piceatannol (7) and Ï–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 ACTIVITIES**  
Dong Hyoung Kim, Kyung Jin Park, Joon Min Cha, Tae Hyun Lee, Youn Sung Choi, Kang Ro Lee  
1 Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea.

NIRAM was obtained by fermentation of the aerial parts from Polygonum tinctoria. P. tinctoria has been traditionally used as natural blue dye in Korea. NIRAM was reported to have anti-inflammatory 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 EiOH extract of NIRAM. The investigation of EiOH 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\textsuperscript{H} and 13\textsuperscript{C} NMR, 1H–1H 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\textsubscript{50} values of 22.87, 6.65, and 14.17 Î¼M.

P-244  
**ANTIPLASMODIAL ACTIVITY OF PULCHERRIN A, I AND J FROM CAESALPINIA PULCHERRIMA**  
Osahan K. Ogbeide, Vincent O. Inmieje, Osayemwere Erharuyi, Irene Osoghabe, Joseph B. Owolabi, Sammer Youssaf, Ibdl M. Choudhary, Abiodun Faladure  
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 investigations 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\textsubscript{50} ranging from 5.36 - 11.27 Î¼M. 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**  
Freyen M Abdelmalek, Fazila Zulficar, Mahmoud A Ramadan, Faten M Darwish, Mahmoud H Assaf, Samir A Ross  
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 distrib-
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 anti-diabetic 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 Zulfiqar1, Samir A. Ross1,2, Zulfiqar Ali1, Ikhas A. Khan1,2

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 ijeju 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 Elwaa1,2, Fazila Zulfiqar1, Abdel-Rahim Ibrahim1, Amal Kabbash1, Mona El-Aaer1,2, Samir A. Ross1,2

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 Department 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 *Limonum* 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-ferulool-3-methoxytyramine, N-trans-cafeoyltyramine, N-trans-feruloyltyramine and gallates. Their structures were identified by spectroscopic methods including 1H NMR, 13C NMR, 2D NMR and HRESIMS.

### P-248

**ARRABIDAEEA CHICA VELROT: CHALLENGES ENCOUNTERED FROM CROP TO HERBAL MEDICINE**

Núbia C. A. Queiroz,1,2 Michelle P. Jorge,1 Iza M. O. Sousa,1,2 Carmen S. P Lima,2 Maria Christina de Miranda Mattias,1 Ana Cristina Dal Rio,2 Eduardo Baldon Pereira,1 Victória Hahn Kakas Galassi,1 João Ernesto de Carvalho,1 Tais Freire Galvão,1 Mary Ann Foglio1

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 (RENNISUS) in Brazil. The species is rich in anthocyanins with previous reports describing healing and antilercrogenic activity in preclinical studies. The crude standardized extract dried by atomization favored calcaneous tendons healing in an animal model, as well as the reduction of cutaneous ulcerations, evaluated in experimental in vivo healing experimental model with semisolad basis incorporated extract1,2. 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 cancer1.  

**P-249**

**ARTEMISININ-BASED COMBINATION THERAPY BY TRANSDERMAL ROUTE AS THE POTENTIAL FOR IMPROVED OPTIONS IN MALARIA TREATMENT**

Fabiana Volpe Zamuto 1,2, Fonseca-Santos, Bruno 1, Marlus Chorilli 1, Mary Ann Foglio 1,2

1Faculty of Pharmaceutical Sciences, State University of Campinas, São Paulo, Brazil. 2Graduate School of Bioscience and Technology of Bioactive Products, Institute of Biology, University of Campinas, 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 1. 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 1. 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/cm² ART and 94 ± 13 µg/cm² LUM of drug permeation. The results presented here show the first transdermal delivery system containing the ART-LUM association.

**P-250**

**NCI PROGRAM FOR NATURAL PRODUCTS DISCOVERY: AUTOMATING THE PRODUCTION OF AN HTS FRIENDLY NATURAL PRODUCTS LIBRARY**

Jason R. Evans, Spencer K. Trinh, Matthew R. Harris, John R. Britt, Tanja Grkovic, and Barry R. O’Keefe 1,2,3

1Natural Products Support Group, Leidos Biomedical Research Inc., Frederick, MD 21702. 2Data Management Services Inc., Frederick, MD 21702. 3Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, 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-Hyun Kim 1, Dong-Seon Kim 2

1Dept of Horticulture, Chungnam National University, Daejeon 34134, Korea. 2Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Korea. 3Institute 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 adiponecic 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 1,2, Huaqiao Tang 1,2, James T. Lyles 1, John Bacsa 1, Cassandra L. Quave 1,3

1Center for the Study of Human Health, Emory University. 2Department of Pharmacy, Sichuan Agricultural University, Chengdu, China. 3X-ray Crystallography Center, Emory University. 4Department of Dermatology, Emory University School of Medicine, Atlanta, GA, USA.

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-7,24Z-dien-26-oic acid (2) and 3α-hydroxytirucalla-7,24Z-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 (IC50, 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. Pardi
UIC/NIC 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, Hexane/Ethyl acetate/Methanol/Water (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.

P-254

TRAIL-SENSITIZING ACTIVITY OF C-27-CARBOXYLATED TRIERPENOID ISOLATED FROM ASTILBE RIVULARIS

Sujung Im¹, InWha Park², Hee Sun Byun³, Gang Min Hur³, and MinKyun Na³
¹College of Pharmacy, Chonnam National University, 99 Daehak-ro, Yusong, Daejeon 34134, Republic of Korea, ²Department of Pharmacology and Physiology, College of Medicine, Chonnam National University, 266 Munhwir-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-coumaroyloxyolean-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. Oseghale¹, Vincent O. Imije¹, Osayemwenre Erharuyi¹, Chidimma Iheanacho¹, Abiodun Falodun¹
¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. ²Department 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 anti-diabetic 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 (IC50 = 29.65 μg/mL). The extracts and fractions of ECS significantly reduced the average blood glucose 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.

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.
P-257  NEW ANTICANCER METABOLITES FROM STAHLIANTHUS THORELLII
Nhuan-Linh Nguyen1, Hoa Thanh Vo1,2, Wen-Chi Wei3, Yu-Chi Lin1, Zhi-Hu Lin1, Mei-Chuan Chen1, and Yao-Haur Liu1,3
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 thorellii has been used to treat the diseases relating to inflammation, ulcer, and cancer etc. We present here that three new phenolic compounds, stilbithoroides 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. thorellii. 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-259  ANTI-LISTERIA DITERPENOIDS FROM MADIA ELEGANS FROM NORTH AMERICA
Jason A. Clement1, Sung Ryed Park1, Matthew Todd2, Fangyuan Zhang2, and Zaiyi Huang3, Yanhong Liu1
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 dichloromethan-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-258  COMPETITIVE A-GLUCOSIDASE INHIBITORS FROM ARTOCARPUS ELASTICUS
Janar Jenis1, Aizhalan Baiseitova2, Ki Hun Park2
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 IC50s 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 potentials (IC50). 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-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 phytoprogesterins 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 phytoprogesterins, which modulated PR activity.
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 Sang 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.

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 Sang 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 tested for their biological properties.

EVALUATING THE EFFECT OF OREGANO ESSENTIAL OIL AND CARVACROL ON SESTRIN 2 EXPRESSION IN HCT116 COLON CELLS

Jacob Veenstra, Bhaskar Venmi, Mirielle Nauman, Brian Guo, CT Che, Jeremy Johnson

1Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607, 2Department 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 HCT116 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 HCT116 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 HCT116 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.

TARGETED ISOLATION OF PHOSPHODIESTERASE-4 INHIBITORY SELAGINELLIN ANALOGUES USING MS/MS-BASED DEREPLICATION ENHANCED BY IN SILICO ANNOTATION STRATEGY

Sunmin Woo, Jinwoong Kim, Sang Hyun Sung, and Kyo Bin Kang

1College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Korea, 2College 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 1H,3H-dibenzo[de,h]isochromene analogues named selariscins A (1) and B (2). Some isolates showed PDE4 inhibitory activity with IC50 values in the range of 2.8–33.8 μM, and their binding modes were suggested by a molecular docking study.
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 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.

QUASSINOID GLYCOSIDES MAY CONTRIBUTE TO THE ANTICANCER PROPERTIES OF FRUCTUS BRUCEAE IN VIVO
Yuan Xu1, Mingming Xu1, Lin Zhang1, Zhenhua Zhu1, Sheng Guo1, Shulan Su1, Jianming Guo1, Chun-Tao Che1, Zhi-Xiu Lin1, Ming Zhao1, Jin-Ao Duan1
1Jiangsu 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, 2Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, United States, 3School 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 brucocin. 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.

DEFINING THE ROLE OF XANTHONES FROM THE MANGOSTEEN FRUIT IN PROMOTING ANDROGEN RECEPTOR DEGRADATION
Mireille Nauman1,2, Blaskar Vern1,2, and Jeremy Johnson1,2
1Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago College of Pharmacy, Chicago, IL 60612, 2Department 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.

CHEMICAL CONSTITUENTS AND IN-VITRO ANTI-CANCER ACTIVITY OF LEPTADENIA PYROTECHNICA
Khasawneh, Mohammad A.; Elwey, Hanan M.; Hamza, Alaueddin A.
1United Arab Emirates University, Al-Ain, United Arab Emirates, 2National Organization for Drug Control & Research, Cairo, Egypt

The aim of this study is to isolate the chemical constituents of the Leptadenia pyrotechnica (LP) (Forsk.) 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 lupeol 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; lupeol
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DERIVATIZATION OF CASSANE DITERPENOIDS FROM CAESALPINIA PULCHERRIMA (L.) SW.
AND EVALUATION OF THEIR CYTOTOXIC AND LEISHMANICIDAL ACTIVITIES
Osayemwenre Erharsuyi1, Achyuat Adhikari2, Abiodun Falodun1, Rehan Imad1, Iqbal M. Choudhary1,2,3
1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, 2H.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, 3Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21412, Saudi Arabia.

Oxidation of the cassane diterpenoids 6β-cinnamoyl-7α-hydroxyvouacapan-5β-ol (1) and pulcherrimin A (2), which were isolated from the roots of Cassapelia 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.

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TWO NEW LIGNANS AND A NEW STILBENE FROM SECUINEGA SUFFRUTICOSA
Kyoung Jin Park1, Joon Min Cha1, Tae Hyun Lee1, Dong Hyun Kim1, Youn Sung Choi1, Kang Ro Lee1
1Natural Products Laboratory, School of Pharmacy, Sungkyunkwan 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, CHCl3, EtOAc, and n-BuOH soluble fractions and repeated column chromatographic purification of the n-hexane-, CHCl3-, 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 (1H and 13C NMR, COSY, HSQC, and HMBC). The isolated compounds (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.

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LABDANE DITERPENE GLYCOSIDES OF PINUS KORAIENSIS TWIGS AND THEIR BIOLOGICAL ACTIVITIES
Kyoung Jin Park1, Joon Min Cha1, Tae Hyun Lee1, Dong Hyun Kim1, Youn Sung Choi1, Kang Ro Lee1
1Natural Products Laboratory, School of Pharmacy, Sungkyunkwan 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 (1H and 13C 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 Olga Tsiftsoglou,1 Erjon Mamoci,1 Sokol Abazi,1 Dimitra Hadjiapavou-Litina,1 Diamanto Lazari,1 Jon Clardy1

1Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115. 2Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania, 3Canadian Institute of Technology, Tirana, Albania. 4School 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 vladichorum Hartvig, collected in 2011 from Mt. Kunora of Lura in Albania. Phytochemical investigations into the aerial parts and seeds of C. vladichorum led to the isolation of eleven compounds, including five sesquiterpene lactones, two flavonoids, two indole alkaloids and two dibenzylbutyro lactone 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.

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**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, Kyong 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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**TWO NEW ECDYSTEROIDAL GLYCOSIDES FROM SPHENOCENTRUM JOLLYANUM**

TemtayO. Ajayi1,2, Radhakrishnan Srivedayasarasi,1 Emmanuel E. Nyong3,4, Michael A. Odeniyi3, Jones O. Moody5, Samir A Ross1,5,6

1National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. 2Department of Pharmacognosy Faculty of Pharmacy, University of Ibadan, Nigeria. 3Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State, Nigeria. 4Department of Pharmacaceutics, Faculty of Pharmacy, University of Ibadan, Nigeria. 5BioMolecular 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 Menispermacaeace. It is a perennial plant that grows naturally along the west coast sub region of Africa with expatiate from Cameroon across Nigeria to Sierra Leone. It has found use as chewing sticks, relief for conjugation, cough medicine, for sickle cell disease, rheumatism and other inflammatory conditions. Pharmacological activities include the use as antimarial, antiviral, anti-angiogenic, analgesic, aphrodisiac and vermifugue. It has also been used in tradition to treat 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 IC50 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 IC50 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 ecdysoirids: polyphoamurein, polyphoamurine B, ecysterone, and 20, 26-dihydroxyecystone.

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**CHEMISTRY AND BIOLOGY OF SILANE RUBELLA GROWING IN EGYPT**

Ismail A. Hussein1,2, Atef A. El-Helei3, Abd-elsalam I. Mohammad3, Radhakrishnan Srivedayasarasi,1 Emmanuel E. Nyong3,4, Samir A Ross1,5,6

1National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. 2Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo 11371, Egypt. 3Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University MS 38677, USA.

The genus Silene (Caryophyllaceae) is one of the largest genera of flowering plants in the world consisting of about 700 species. In Egypt, 29 species of Silene are distributed in Suez area, Aqaba Gulf, 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), Bacillus subtilis and Salmonella typhimurium and antiviral activity against HAV-10. It also showed moderate activity towards colon carcinoma cell line HCT-116 with IC50 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), queretin (6), isovitexin (7), rutin (8), vicinin 2 (9) (R)-naringin (10), (S)-naringin (11), chlorogenic acid (12), betulinic acid (13), oleancolic acid (14), ursolic acid (15), spinasterol (16), ecystosterone (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.
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SCREENING ENDOLICHENIC FUNGI FOR ANTIMICROBIAL METABOLITES
Sabina S. Hossain, Joshua J. Kellogg, Emily D. Wallace, Nadja B. Cech
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC 27402

Endolichecnic fungi are a diverse group of fungi that live asymptptomatically 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 endolichecnic 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 endolichecnic 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 endolichecnic 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 μg/mL. Efforts to identify the active antimicrobial compounds in this extract are ongoing.

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ISOLATION AND ELUCIDATION OF A NEW DITERPENE FROM THE BARKS OF CINNAMOMUM CASSIA
Suk Woo Chang¹, Joon-pyo Hong¹, Du Sik Jang¹
¹Department 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 elucidation of compound 1 is described herein.

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ISOLATION AND STRUCTURE ELUCIDATION OF ISOQUINOLINE ALKALOIDS FROM ASIMINA TRILOBA AND THEIR CYTOTOXICITY AND NEUROTOXICITY EVALUATIONS
Taghreed A. Mairashi¹,², Shabana Khan¹,², Nicole Ashpole³, Fazila Zulfiquar³, Amar G. Chittiboyina¹, Zulfiquar Ali⁴, and Ikhlas A. Khan¹,²
¹National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. ²Department of BioMolecular Sciences, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA. ³College of Pharmacy, King Khalid University, Abha, Saudi Arabia.

Annonaceous 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 Lee¹, Kyoung Jin Park¹, Joon Min Cha¹, Dong Hyun Kim¹, Youn Sung Choi¹, and Kang Ro Lee¹
¹Natural 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 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, CHCl₃, EtOAc, and BuOH. The repeated column chromatography and purification of CHCl₃ 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 (¹H and ¹³C NMR, ¹H-¹H 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 Pham¹, and Won Keun Oh¹
¹Korea 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 Ayurvedic 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.

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**SCALE-UP AND INCREASED PRODUCTIVITY FOR PURIFICATION OF SECO-IRIDOIDS FROM CENTAURIUM ERYTHRAEA RAFN. USING CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)**

Tvstelina Mandova¹, Frédéric Dijoux¹
¹Gilion 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 column volumes, 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-iridoïds were separated from the common centaury plant (Centauriurn erythraea Rafn., Gentianaceae). Separation was monitored using DAD and MS analysis, and for one of the most enriched fractions of the experiment, swertiamarin accounted for 231mg/g, compared to the crude extract where swertiamarin was quantified as 8.72mg/g.

**P-283**

**DIHYDROANTHAQUINONES FROM RUBIA PHILIPPINENSIS**

Quan Trong Khang¹, Joonseok Oh¹, InWha Park¹, Nam Giang Pham¹, and MinKyun Na¹
¹College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea, ²Department 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 spectrscopic 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 I.C. analyses.

![Dihydroanthraquinones](image)

**P-284**

**ANTIBACTERIAL COMPOUNDS FROM THE AUSTRALIAN FRUIT OF CORDYLNE MANNERS-SUTTONIAE**

Trong D. Tran¹, Malin Olsson², Nahid Choudhury³, David J. McMillan³, Jason K. Cullen³, Peter G. Parsons³, Paul V. Bernhardt³, Craig M. Williams⁴, Paul W. Reddell⁵, and Steven M. Ogborne¹
¹GeneCology Research Centre, University of the Sunshine Coast, Queensland, Australia. ²School of Health and Sports Sciences, University of the Sunshine Coast, Queensland, Australia. ³QIMR Berghofer Medical Research Institute, Queensland, Australia. ⁴School of Chemistry and Molecular Biosciences, University of Queensland, Queensland, Australia. ⁵QBiotics 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 (EcoLogicSM) 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 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. Khan3, Babu Teykwan1, Shabana I. Khan1, Abiodun Falodun1

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, 200001, Nigeria, 2National Center for Natural Products Research, Institute of Pharmaceutical Sciences, University of Mississippi, 38677, USA, 3Department 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 IC₅₀ 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 IC₅₀ and IC₀ 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.

![Compounds 1-4](image)

**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 cytotoxic 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 IC₅₀ values of 14.65 ± 1.45 and 13.03 ± 0.33 μM, respectively. Compounds 4 (xanthopurpurin) and 5 (lucidin-α-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.

![Compounds 4 and 5](image)

**P-287**

**EFFECTS OF CYCLOCARYA PALIURUS LEAVES ON ENDOCRINE AND IMMUNE FUNCTION IN TYPE II DIABETES RATS WITH YIN DEFICIENCY SYNDROME**

**Jingxia Wang**1, Jinkai Yao1, Lu Fu1, Leilei Wang1, Lan Jia1, Liang Meng1, Jian Hua1, Wei Li1.

1Beijing University of Chinese Medicine, 11 Bei San Huan Dong Lu, Beijing 100029, China

Cyclocarya palius 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 thyrone (T4) 1 week (0.2 mg/kg), and single intraperitoneal injection of streptozotocin (30 mg/kg). Compared with the model group, the levels of thyroid hormone (T3), 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(CANAVAIIA GLADIATIA) POD EXTRACT**

**Pureum Im**, Jeong Yoon Im, Ae-Jin Choi and Jeong-Sook Choi

Division of Functional Food & Nutrition, Department of Agrofood Resources, National Institute of Agricultural Sciences, Wanju 53656, 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 bean 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 μg/ml(Aspirin; 30.7 sec). Also the results of mucous cell(Human conjunctival epithelial cells) recovery test were 140.46% in 100 μg/ml(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, Wenyun 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, (7S,8S,7R,8'R)-isooamericanol B (1) and amERICANOL B (2), and two dioneolignans, moricitin A (3) and B (4) were isolated from Noni (Morinda citrifolia) fruit powder. 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 Compositae 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 spectrometry 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 GERANIOLIODS, HERBACETINODS, AND ROSAVINS FROM A RHODIOLA ROSEA COUNTERCURRENT SEPARATION FRACTION
Yu Tang*, Dejan Nikolić, Daniel Zagal, J. Brent Friesen, James B. McAlpine, David C. Lankin, Guido F. Pauli, and Shao-Nong Chen
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 proanthocyanidins, flavonoids, phenylpropanoids, and phenylethanoids. In our study of R. rosea, a series of geranioliods, herbacetinoids, and rosavins were isolated and identified. To build standardization protocols, we developed a qHNMRI 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 qHNMRI (and HPLC-UV) integration, a countercurrent separation (CCS) method was developed to remove PAs and FAs prior to quantification. The content of geranioliods, 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 qHNMRI enables the poly-targeted chemical standardization of R. rosea for its putative bioactive compounds.

P-292

INDOLE AND B-CARBOLINE ALKALOIDS FROM THE FRUITS OF FLUEGGEA VIROSA
Zhen-Long Wu, Ming-Tao Xie, Qi-Fang He, Ying Wang, Wen-Cai Ye, and Chun-Tao Che
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 and a new spirooxindole alkaloid. 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 l-lysine and tyrosine.
**P-293**

**FADOGIA AGRESTIS: A PHYTOCHEMICAL STUDY AND SCREENING FOR ANTI-INFECTIVE ACTIVITIES OF AN AFRICAN TRADITIONAL HERB**

Ahmed Galal Osman¹, Zulfiquar Ali², Omer Fantoukh¹,², Vijayasankar Ramani², and Ikhlas Khan¹,²  
¹National Center for Natural Products Research, ²Division 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 benzoporphnone 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 IC₅₀ 9.4 µg/mL for 6 and IC₅₀ 8.3 µg/mL, IC₅₀ 9.2 µg/mL for 2.

**P-294**

**NMR CHARACTERIZATION OF NEW MERODITERPENOIDS FROM THE RED ALGA DELISEA SP.**

Dongdong Wang¹, Heidi R. Bokesch,¹,² Josep Sauri,¹ Kirk R. Gustafson,¹ and John A. Beutler¹  
¹Molecular Targets Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA; ²Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA; ³Structure 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 callophycolols 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 callophycolols A and B were on the basis of ¹³C chemical shift predictions and empirical data. The band-selective HSQC and CLIP-HSQMBC experiments, which can visualize the ³⁵Cl isotope effect on ¹³C nuclei, were applied to provide unequivocal support for the halogen assignments in compounds 1–8.

**P-295**

**EFFECT OF SAMPLE DEGASSING ON 'H NMR CHEMICAL SHIFTS OF AN N-CONTAINING NATURAL PRODUCT**

¹UIC/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 (T₁, T₂) 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 (∆δ) of 0.312 ppm. The signals of aromatic hydrogens exhibited the least ∆δ of 0.006 – 0.029 ppm. As expected, T₁ and T₂ 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 CO₂ 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**

Mohammed A. Albury¹,², Sateesh C. Ratte³, Pradeep B. Lasonkar³, Amar G. Chittiboyina³, and Ikhlas A. Khan¹,²  
¹Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt ²Departments of BioMolecular Sciences, Division of Pharmacognosy, and ³National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Mesembhrine is a naturally occurring benzylphenethylamine-type alkaloid which has been isolated from the South African plant *Sceletium tortuosum*, family Aizoaceae, known as Kanna. This plant has been used as a stimulant 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-arylactahydroindole 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-dimethoxy cinnamic acid, the desired stereogenic center at carbon 3a was created, afterward, intramolecular alkylidene C-H insertion and aldol
P-297
HIGHLIGHTS FROM A WORKSHOP ON ENHANCING NATURAL PRODUCT CLINICAL TRIALS
Guido Pauli1, Adam Kusza2, Naomi Fukagawa1, Gregory Bloss1, Mahtab Jafari1, Michael A Walters2, Bruce Barrett3, Frederic Bushman4, Steven Casper4, Floyd Chilton5, Christopher Coffee6, Mario Ferruzzi7, Craig Hopp8, Mairread Kiedy9, Daniel Lakens10, John MacMillan10, David Meltzer11, Jeffrey Paul12, Kathleen Pritchett-Corning13,14, Sara Quinney15, Sharon Ross16, Harold Seifried17, Kenneth Setchell18, Nisha Sipes19, Jacqueline Stephens20, D Lansung Taylor21, Herve Tiriac22, Dan Xf23, Freddie Ann Hoffman23, Barbara C Sorkin1
1University of Illinois at Chicago, Chicago, IL, USA; 2National Institutes of Health, Bethesda, MD, USA; 3United States Department of Agriculture (USDA), Agricultural Research Service, Beltsville, MD, USA; 4University of California, Irvine, Irvine, CA, USA; 5University of Minnesota, Minneapolis, MN, USA; 6University of Wisconsin, Madison, WI, USA; 7University of Pennsylvania, Philadelphia, PA, USA; 8U.S. Food and Drug Administration, College Park, MD, USA; 9University of Arizona, Tucson, AZ, USA; 10University of Iowa, Iowa City, IA, USA; 11North Carolina State University, Raleigh, NC, USA; 12University College Cork, Cork, Ireland; 13Eindhoven University of Technology, Eindhoven, Netherlands; 14University of California, Santa Cruz, Santa Cruz, CA, USA; 15University of Chicago, Chicago, IL, USA; 16Drexel Graduate College of Biomedical Sciences, College of Medicine, Evanston, IL, USA; 17University of Washington, Seattle, WA, USA; 18Harvard University, Cambridge, MA, USA; 19Indiana University, Indianapolis, IN, USA; 20Cincinnati Childrens Hospital Medical Center, Cincinnati, OH, USA; 21National Institute of Environmental Health Sciences, Durham, NC, USA; 22Louisiana State University, Baton Rouge, LA, USA; 23University of Pittsburgh, Pittsburgh, PA, USA; 24University of California, San Diego, La Jolla, CA, USA; 25Heterogeneity, 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 these 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 a caution against proceeding with large RCT of botanicals and other NP supported by NIH that have not shown evidence of benefit versus a placebo control. This lack of effect may be due to low sample size, high variability, or poor experimental design.

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.

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TRANSLATIONAL STUDIES ON CENTELLA ASIATICA – PRODUCT DEVELOPMENT AND REGULATORY APPROVALS FOR HUMAN CLINICAL STUDIES
Amala Soumyanath1, Kirsten Wright1, Armando Alcazar Magana1,4, Maya Caruso1, Nora Gray1, Donald Matthews1, Randall Wolter2, Ronald M. Laemmle3, Caroline L. Mozeley4, Troy T. Banks4, Claudia S. Maier3, Jan F. Stevens4, Joseph Quinn1
1Departments of 1Neurology and 5Pathology, Oregon Health and Science University, Portland, OR, 97239, USA; 2Departments of Chemistry and 4Pharmaceutical Sciences and 5Linus Pauling Institute, Oregon State University, Corvallis, OR, 97331, USA; 4BioVT, 2810 Meridian Parkway, Suite 100, Durham, NC, 27713, USA; 5Department 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 intervention 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 AMYLOLIQUEFACENS INVOLVES THE EXPRESSION OF RAPAMYCIN.
M. Chris James1, Xiaoyan Wang1, Theodore Leininger1, Amala Dass2 and Mark T. Hamann3
1BioFactura, 435 Progress Dr., Ste. Z, Frederick, MD 21701, USA; 2Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC 29425; 3US Forest Service, 1515 Dickey, Stoneville, MS 38776.

In an effort to better understand the mechanism by which the phytopathogenic bacterium called Xyella 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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