Genomics driven discovery and engineering of fungal polycyclic polyketides

Subko, Karolina; Wolff, Peter Betz; Theobald, Sebastian; Frisvad, Jens Christian; Gotfredsen, Charlotte Held; Andersen, Mikael Rørdam; Mortensen, Uffe Hasbro; Larsen, Thomas Ostenfeld

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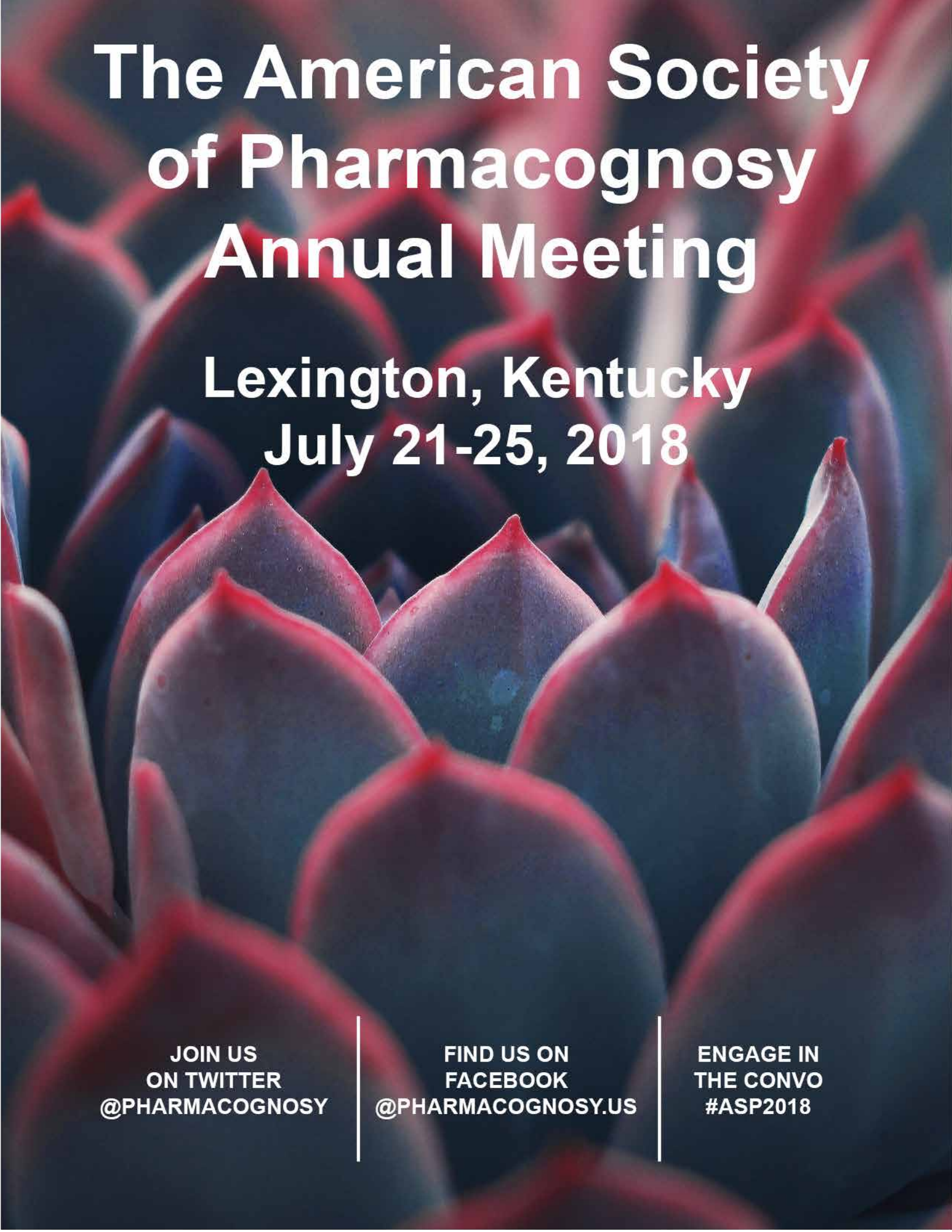
The American Society of Pharmacognosy Annual Meeting
Lexington, Kentucky
July 21-25, 2018
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ENGAGE IN THE CONVO #ASP2018

The American Society of Pharmacognosy Annual Meeting

July 21-25, 2018
Hilton Lexington Downtown
Lexington, Kentucky

ENGAGE IN THE CONVO #ASP2018

Welcome to 59th Annual Meeting of the American Society of Pharmacognosy (ASP) 2018 in beautiful downtown Lexington, Kentucky! The Local Organizing Committee would like to thank you for joining us for the ASP meeting at the Hilton Lexington Downtown. The scientific program includes podium presentations from an impressive and diverse group of speakers who will be discussing the latest advances in all areas of natural products discovery and development, a series of workshops supporting your technical and professional development, as well as two poster sessions to showcase work from around the globe. Additional activities will allow attendees to enjoy Kentucky to its fullest including an evening at the majestic Keeneland Racetrack, tours of Horse Farms and Distilleries, and an excursion to the Lloyd library and Winkler Center (repository for the founding ASP scientific and societal articles). For the Young Members, a fun filled evening event at Buffalo Trace Distillery is planned. Lexington and the Bluegrass have a lot to offer. We invite you to enjoy the many restaurants/bars and perhaps take some time to enjoy the natural beauty of the Red River Gorge, gain an appreciation for the majestry of the throughbred tradition at the internationally acclaimed Kentucky Horse Park, and many other attractions surrounding Lexington. We wish you a wonderful time in the Bluegrass.

The ASP 2018 Local Organizing Committee:
Jürgen Rohr, Chair
Joe Chappell
Sylvie Garneau-Tsodikova
Pete Spielmann
Jon Thorson
Oleg Tsodikov
Steven Van Lanen
and Laura Stoll, ASP Business Manager

ASP 2018

Lexington, Kentucky, July 21-25, 2018

ASP 2018 Organizers

Local Organizers

Jürgen Rohr, Chair (University of Kentucky)
Joe Chappell (University of Kentucky)
Sylvie Garneau-Tsodikova (University of Kentucky)
Pete Spielmann (University of Kentucky)
Jon Thorson (University of Kentucky)
Oleg Tsodikov (University of Kentucky)
Steven Van Lanen (University of Kentucky)

Scientific Committee

Jürgen Rohr, Chair (University of Kentucky)
John Beutler (National Cancer Institute)
Joe Chappell (University of Kentucky)
Sylvie Garneau-Tsodikova (University of Kentucky)
Mark O'Neil-Johnson (Sequoia Sciences)
Sheo Singh (SBS Pharma Consulting)
Pete Spielmann (University of Kentucky)
Jon Thorson (University of Kentucky)
Oleg Tsodikov (University of Kentucky)
Steven Van Lanen (University of Kentucky)
Chang-Guo Zhan (University of Kentucky)

Meeting Planning and Registration

Laura Stoll (The American Society of Pharmacognosy)

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ASP Award Winners 2018

Norman R. Farnsworth Research Achievement Award
James Gloer, Ph.D., University of lowa

Varro E. Tyler Prize
Guido Pauli, Ph.D., University of Illinois Chicago

Matt Suffness Young Investigator Award
Marcy Balunas, Ph.D., University of Connecticut

ASP Kilmer Prize

David Gallegos, Oregon State University

Undergraduate Research Award

Mario Augustinovic, University of North Carolina Greensboro
Manead Khin, University of North Carolina Greensboro
Malia L. Moore, University of California Berkley
Madison Patrick, Auburn University
Sabrina Ton, University of Oklahoma

Research Starter Grant

Ethan Van Arnam, Ph.D., Claremont McKenna College
Lukasz Ciesla, University of Alabama

Lynn Brady Student Travel Award

Brian Guo, University of Illinois at Chicago
Paige Mandelare, Oregon State University
Carla Meningatti, University of Sao Paolo
Sara Puckett, University of Connecticut
Robert Tokarski, Ohio State University

Jerry McLaughlin Student Travel Award

Oluwatofunmilayo Divaolu, University of Aberdeen (United Kingdom)
Anupama Tuladhar, Florida International University

David Carew Student Travel Award

Yihue Zhang, Auburn University

Waqar Bhatti Student Travel Award

Samantha Gromek, University of Connecticut

Student Travel Award

Kelsey Alexander, University of California-San Diego
Anina Buchman, Eberhard Karls University (Germany)
Maria S. Costa, University of Iceland
Camila M. Crnkovic, University of Illinois, Chicago
Pradeep Dewapriya, University of Queensland (Australia)
Taise T.H. Fukuda, University Sao Paolo/Harvard
Gabrielle M. Grandchamp, University of North Carolina-Chapel Hill
Riley Kirk, University of Rhode Island
Shamsunnahar Kushi, University of Queensland (Australia)
Sylvia Kunakom, University of Illinois at Chicago
Preston Manwil, Ohio State University
Laizuman Nahar, University Queensland (Australia)
George Neuhaus, Oregon State University
Paul Scesa, Florida Atlantic University
Abu Bakar Siddique, University of Louisiana Monroe
Hannah Whitmore, University Surrey (United Kingdom)
Mario Wibowo, Griffith University (Australia)

Travel Grant for Active Members

Asmaa Boufridi, Griffith University (Australia)
Jana Braesel, University of Illinois at Chicago
Narayan Chaurisiya, University of Mississippi
Anne-Clare Limon, University of South Florida
Pencheng Wang, University of Pittsburgh
Chen Zhang, University of California-San Diego

2018 Arthur E. Schwarting Award

Sang Kook Lee, Ph.D., SNU Korea
Hwa-Jin Chung, Won Kyung Kim, Jedo Oh, Me-riong Kim, Joon-Shik Shin, Jinho Lee, In-Hyuk Ha, and Sang Kook Lee.* Anti-Osteoporotic Activity of Harpagoside by Upregulation of the BMP2 and Wnt Signaling Pathways in Osteoblasts and Suppression of Differentiation in Osteoclasts. J. Nat. Prod. 2017, 80(2), 434-442. (DOI: 10.1021/acs.jnatprod.6b00964).

2018 Jack L. Beal Award

Chambers C. Hughes, Ph.D., University California, San Diego
Daniela Reimer and Chambers C. Hughes.* Thiol-Based Probe for Electrophilic Natural Products Reveals That Most of the Ammosamides Are Artifacts. J. Nat. Prod. 2017, 80(1), 126–133. (DOI: 10.1021/acs.jnatprod.6b00773).

The American Society of Pharmacognosy Annual Meeting

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Program Schedule

Saturday July 21, 2018

8:00 AM – 7:30 PM	Registration – ***Grand Kentucky Ballroom Foyer***
9:00 AM – 4:00 PM	Executive Committee Meeting (Invitation Only) – ***Grand Kentucky Ballroom Salon B***
9:00 AM – 1:00 PM	AMWS1 - ***Grand Kentucky Ballroom Salon C*** "Introduction to Global Natural Product Social (GNPS) Molecular Networking and 3D Visualization of Natural Product Data" with Dr. Pieter Dorrestein (UCSD)
9:00 AM – 11:00 AM	AMWS2 - ***Grand Kentucky Ballroom Salon A*** "Grant Writing Strategies" with Dr. D. Craig Hopp (NCCIH, National Institutes of Health)
2:00 PM – 4:00 PM	PMWS1 - ***Grand Kentucky Ballroom Salon A*** "New Approaches for the Biological Evaluation of Natural Products with Anticancer Potential" with Dr. April Risinger (University of Texas, Health Sciences Center, San Antonio)
2:00 PM – 5:00 PM	PMWS2 - ***Grand Kentucky Ballroom Salon C*** "How Can Countercurrent Separation help You with Your Natural Product Research?" with CENAPT team, in collaboration with Gregoire Audo (Armen/Gilson)
2:00 PM – 4:00 PM	PMWS3 – ***Grand Kentucky Ballroom Salon D*** "One Carbon Based Unit's / Computer's Approach to Structure Elucidation" with Mark O'Neil-Johnson, Sequoia Sciences, Inc and Arvin Moser, ACD Labs

12:00 PM – 5:00 PM Exhibitor Set Up – ***Grand Kentucky Ballroom Foyer***

5:00 PM – 10:00 PM Exhibition – ***Grand Kentucky Ballroom Foyer***

7:00 PM – 10:00 PM **President's Opening Reception** - Supported in part by the American Society of Pharmacognosy Foundation through a generous donation from the Estate of Gerry and Lynn Brady
Grand Kentucky Ballroom – (Ticketed Event)

Sunday July 22, 2018

6:45 AM – 6:00 PM Registration – ***Grand Kentucky Ballroom Foyer***

6:45 AM – 6:00 PM Exhibition – ***Grand Kentucky Ballroom Foyer***

6:45 AM – 7:45 AM Continental Breakfast – ***Grand Kentucky Ballroom Foyer***

7:45AM – 8:00 AM Welcoming Remarks and Announcements – ***Grand Kentucky Ballroom***

Grand Kentucky Ballroom
Symposium I – Structural Biology
Chair: Oleg Tsodikov

8:00 AM – 8:40 AM S-01
Hazel M. Holden (University of Wisconsin – Madison)

THE BIOSYNTHESIS OF N-FORMYLATED SUGARS IN PATHOGENIC BACTERIA

8:40 AM – 9:20 AM S-02
George N. Phillips, Jr. (Rice University)

STRUCTURAL BIOLOGY OF NATURAL PRODUCT METABOLISM: SOME EXAMPLES

9:20 AM – 10:00 AM S-03
Catherine L. Drennan (Massachusetts Institute of Technology)

STRUCTURAL ELUCIDATION OF ENZYMES INVOLVED IN OXETANOCIN-A BIOSYNTHESIS

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

Grand Kentucky Ballroom Salon A&B
Session S-AM1 – Carbohydrates
Chair: Jon Thorson

10:30 AM – 11:10 AM S-04
Hung-wen Liu (University of Texas at Austin)

RADICAL SAM ENZYMES IN THE BIOSYNTHESIS OF SUGAR-CONTAINING NATURAL PRODUCTS

11:10 AM – 11:50 AM S-05
George A. O'Doherty (Northeastern University)

DE NOVO SYNTHESIS OF NATURAL PRODUCTS FOR MEDICINAL CHEMISTRY

11:50 AM – 12:10 PM O-01
Chia-Chuan Chang, PhD. (National Taiwan University)

CHEMICAL INVESTIGATION OF SACCHARIDES OF DENDROBIUM CASSIOPE

12:10 PM – 12:30 PM O-02
Qingyao Shou (Unigen, Inc.)

TRITERPENOID SAPONINS FROM THE ROOTS OF GLYCYRRHIZA GLABRA

Grand Kentucky Ballroom Salon C&D
Session S-AM2 – Biosynthesis/Discovery
Chair: Steven Van Lanen

10:30 AM – 11:10 AM S-06
Jianhua Ju (South China Sea Institute of Oceanology, Chinese Academy of Sciences)

ANTI-INFECTIVE NATURAL PRODUCTS DISCOVERY AND BIOSYNTHESIS FROM MARINE MICROORGANISMS

11:10 AM – 11:30 AM O-03
Stephanie Grond (Eberhard Karls Universität Tübingen Institute of Organic Chemistry)

LUGDUNIN - A NEW ANTIBIOTIC FROM OUR NOSE: STRUCTURE, CHEMICAL SYNTHESIS AND BIOACTIVITY

11:30 AM – 11:50 AM O-04
Hope Ada Igboeli (University of Prince Edward Island)

DISCOVERY OF A NEW POLYKETIDE VIA CO-TREATMENT WITH AN EPIGENETIC MODIFIER AND OSMOTIC STRESS

11:50 AM – 12:10 PM O-05
Gina Grammbitter (Goethe-Universität Frankfurt am Main)

UNCOMMON MECHANISM OF A TYPE II PKS DERIVED ARYL POLYENE PIGMENT PRODUCED BY XENORHABDUS DOUCETIAE

12:10 PM – 12:30 PM O-06
Jana Braesel (University of Illinois at Chicago)

DIAZAQUINOMYCIN BIOSYNTHESIS IN MARINE AND FRESHWATER ACTINOMYCETES

12:30 PM – 2:00 PM Lunch (on your own)

12:30 PM – 2:00 PM Journal of Natural Products Editorial Board Meeting (Invitation Only) ***Triple Crown***

Grand Kentucky Ballroom Salon A&B
Session – S-PM1 - Discovery
Chair: Jürgen Rohr

2:00 PM – 2:40 PM S-07
Chirlei Glienke (Federal University of Parana, Brazil)

BIODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF ENDOPHYTES OF THE MEDICINAL PLANTS FROM THE BRAZILIAN BIOMES PANTANAL AND CERRADO

2:40 PM – 3:00 PM O-07
Jaclyn M. Winter (University of Utah)

EXPLORING THE CHEMICAL POTENTIAL OF GREAT SALT LAKE MICROORGANISMS

3:00 PM – 3:20 PM O-08
Andrés Mauricio Caraballo Rodríguez (University of California - San Diego, Skaggs School of Pharmacy and Pharmaceutical Sciences)

(University of California San Diego)

MOLECULAR CARTOGRAPHY OF FUNGUS-GROWING ANTS GARDENS REVEALS THE PRESENCE OF BIOACTIVE METABOLITES IN SITU

3:20 PM – 3:40 PM O-09
Serge Fotso
(Corteva Agriscience™, Agriculture Division of DowDuPont™)

ANTIFUNGAL PEPTIDES FROM MICROASCUS ALVEOLARIS ACTIVE AGAINST PHYTOPATHOGENIC FUNGI

3:40 PM – 4:00 PM O-10
David Cole Stevens (University of Mississippi)

MYXOBACTERIA DEGRADE ACYLHOMOSERINE LACTONE QUORUM SIGNALS AND PREVENT PSEUDOMONAS PUTIDA BIOFILM FORMATION

Grand Kentucky Ballroom Salon C&D
Session – S-PM2 – Plant Genetics
Chair: Joe Chappell

2:00 PM – 2:40 PM S-08
Cathie Martin (John Innes Centre**)**

SYNTHESIS OF BIOACTIVE 4' DEOXYFLAVONES IN SCUTELLARIA BAICALENSIS, THE GOLDEN HERB OF THE CHINESE MEDICINAL GARDEN

2:40 PM – 3:00 PM O-11
Debasis Bagchi, PhD, MACN, CNS, MAIChE
(Cepham Inc., University of Houston)

A RANDOMIZED, PLACEBO-CONTROLLED, CLINICAL TRIAL TO EVALUATE THE EFFICACY OF A NOVEL FENUGREEK SEED EXTRACT ON PHYSICAL FITNESS AND SPORTS NUTRITION

3:00 PM – 3:20 PM O-12
Hannah Whitmore (University of Surrey)

NOVEL HOMOISOFLAVONOIDS AS INHIBITORS OF OCULAR ANGIOGENESIS

3:20 PM – 3:40 PM O-13
Nga Yi Tsang (Hong Kong Baptist University)

DISCOVERY OF ANTI-AVIAN INFLUENZA COMPOUNDS FROM EURYA NITIDA AND MAESA PERLARIUS

3:40 PM – 4:00 PM O-14
Anina Buchmann (University of Tuebingen, Germany)

THE IMMUNOSUPPRESSANT BRASILICARDIN: HETEROLOGOUS EXPRESSION, REVISION OF THE GENE CLUSTER AND MUTASYNTHESIS STUDIES

4:00 PM – 7:00 PM **Poster Session I** – *Magnolia, Bluegrass, Crimson Clover, Lilly of the Valley, Black Berry Lilly, Lobby and Pre-Function*

(Poster #'s P-01 – P-140)

(All posters need to be picked up by Monday 6:00 AM, ASP is not responsible for lost or damaged posters)

4:00 PM – 7:00 PM Dinner (on your own)

Grand Kentucky Ballroom
Symposium II – Synthesis
Chair: Sylvie Garneau-Tsodikova

7:00 PM – 7:40 PM S-09
Peter Wipf (University of Pittsburgh)

ADVENTURES IN THE SYNTHESIS OF POLYCYCLIC ALKALOIDS

7:40 PM – 8:20 PM S-10
Shahriar Mobashery (University of Notre Dame)

COMPLEX BIOLOGICAL MACHINERIES: BACTERIAL CELL WALL, ITS TURNOVER AND LINK TO ANTIBIOTIC RESISTANCE

8:20 PM – 9:00 PM S-11
Paul J. Hergenrother (University of Illinois, Urbana-Champaign)

SYSTEMATIC CONVERSION OF GRAM-POSITIVE-ONLY COMPOUNDS INTO BROAD-SPECTRUM ANTIBIOTICS

9:00 PM – 9:40 PM S-12
Jeroen S. Dickschat (University of Bonn)

TRACING TERRESTRIAL TERPENES WITH ISOTOPES

Monday July 23, 2018

7:00 AM – 4:30 PM Registration – ***Grand Ballroom Foyer***

7:00 AM – 4:30 PM Exhibition **–** ***Grand Ballroom Foyer***

7:00 AM – 8:00 AM Continental Breakfast – ***Grand Ballroom Foyer***

Grand Kentucky Ballroom
Symposium III – Discovery Techniques
Chairs: Sylvie Garneau-Tsodikova

8:00 AM – 8:40 AM S-13
Nathan Magarvey (McMaster University)

DENDRAL TO DEEP LEARNING IN NATURAL PRODUCT CHEMISTRY

8:40 AM – 9:20 AM S-14
Rebecca Goss (University of St Andrews)

EXPLORING AND EXPLOITING BIOSYNTHESIS TO ACCESS NOVEL NATURAL PRODUCTS

9:20 AM – 10:00 AM S-15
Rebecca A. Butcher (University of Florida)

CHEMICAL SIGNALING IN WORMS

10:00 AM – 10:30 AM Break

Grand Kentucky Ballroom Salon A&B
Session M-AM1 - Biosynthesis
Chair: Jürgen Rohr

10:30 AM– 11:10 AM S-16
Yi Tang (University of California, Los Angeles)

DISCOVERING NEW ENZYMES FROM FUNGAL NATURAL PRODUCT BIOSYNTHETIC PATHWAYS

11:10 AM – 11:50 AM S-17
Ben Shen, Ph.D. (The Scripps Research Institute)

GENOME MINING OF ENEDIYNE NATURAL PRODUCTS PROVIDING NEW OPPORTUNITIES FOR ENEDIYNE BIOSYNTHESIS, ENGINEERING, AND DRUG DISCOVERY

11:50 AM – 12:10 PM O-15
Michael J. Smanski (University of Minnesota)

DESIGNED BIOSYNTHESIS OF A NATURAL PRODUCT OF UNKNOWN ORIGIN

12:10 PM – 12:30 PM O-16
Timothy A. Wencewicz
(Washington University in St. Louis, Department of Chemistry)

CROSSROADS OF ANTIBIOTIC BIOSYNTHESIS AND RESISTANCE

Grand Kentucky Ballroom Salon C&D
Session M-AM2 - Discovery
Chair: Oleg Tsodikov

10:30 AM– 11:10 AM S-18
Robert H. Cichewicz (The University of Oklahoma)

MAKE NATURAL PRODUCTS GREAT AGAIN: NO COLLUSION; PLENTY OF COLLABORATION

11:10 AM – 11:30 AM O-17
Rob Capon (The University of Queensland)

METABOLITES FROM A FISH GUT FUNGUS CHALLENGE THE DEFINITION OF WHAT IT IS TO BE A NATURAL PRODUCT?

11:30 AM – 11:50 AM O-18
Jason M. Crawford (Yale University)

BIOACTIVE POLYKETIDE TRANSFORMATIONS IN BACTERIAL PATHOGENS AND IN HUMANS

11:50 AM – 12:10 PM O-19
Danielle H. Demers (AnalytiCon Discovery)

NATLIFE 2020: DISCOVERING TASTE MODULATING NATURAL PRODUCTS FOR HUMAN HEALTH APPLICATIONS

12:10 PM – 12:30 PM O-20
Patricia M. Van Skaik (Lloyd Library and Museum)

WHAT'S OLD AND NEW AT THE LLOYD LIBRARY: AMERICA'S INDEPENDENT PHARMACOGNOSY LIBRARY

12:30 PM – 2:00 PM Lunch (on your own)

12:30 PM – 2:00 PM Fellows Meeting (Invitation Only)
Triple Crown Room

Grand Kentucky Ballroom Salon A&B
Session M-PM1 - Marine
Chair: Jon Thorson

2:00 PM – 2:40 PM S-19
Kerry McPhail (Oregon State University)

TARGETING INHIBITORS OF PROTEIN SECRETION FROM MARINE MICROBIAL COMMUNITIES

2:40 PM – 3:00 PM O-21
Glenroy (Dean) Martin (Fisk University)

INVESTIGATION OF BIOACTIVE CHEMICAL ENTITIES FROM MARINE MICROORGANISMS

3:00 PM – 3:20 PM O-22
Sandra Loesgen (Oregon State University)

MENSACARCIN AFFECTS MITOCHONDRIAL FUNCTION SELECTIVELY IN MELANOMA CELLS

3:20 PM – 3:40 PM O-23
Jeremy G. Owen (Victoria University of Wellington)

METAGENOME SEQUENCING OF THE NEW ZEALAND MARINE SPONGE MYCALE HENTSCHELI UNCOVERS THE BIOSYNTHETIC PATHWAYS FOR THE CYTOTOXIC POLYKETIDES MYCALAMIDE AND PATEAMINE

3:40 PM – 4:00 PM O-24
Shugeng Cao (Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo)

NEW NATURAL PRODUCTS FROM HAWAIIAN MARINE AND ENDOPHYTIC FUNGI

Grand Kentucky Ballroom Salon C&D
Session M-PM2 – Plant Metabolites
Chair: Steven Van Lanen

2:00 PM – 2:40 PM S-20
Christopher J. Schofield (University of Oxford)

ENZYMES THAT MAKE AND BREAK β-LACTAMS

2:40 PM – 3:00 PM O-25
Paul Scesa (Florida Atlantic University)

A KINETIC DEAROMATIZATION STRATEGY FOR AN EXPEDIENT BIOMIMETIC ROUTE TO THE BIELSCHOWSKYSIN SKELETON

3:00 PM – 3:20 PM O-26
Eduardo J. Caro-Diaz
(School of Pharmacy, University of Puerto Rico)

HIGHLY CONVERGENT TOTAL SYNTHESIS AND ASSIGNMENT OF ABSOLUTE CONFIGURATION OF MAJUSCULAMIDE D, A POTENT CYTOTOXIC METABOLITE FROM MOOREA SP.

3:20 PM – 3:40 PM O-27
Camila Manoel Crnkovic (University of Illinois at Chicago)

USING METABOLOMICS TO DISCOVER NATURAL PRODUCTS FROM CULTURED CYANOBACTERIA

3:40 PM – 4:00 PM O-28
Barry R. O'Keefe (National Cancer Institute)

THE NCI PROGRAM FOR NATURAL PRODUCT DISCOVERY

4:00 PM – 6:00 PM **Poster Session II –** *Magnolia, Bluegrass, Crimson Clover, Lilly of the Valley, Black Berry Lilly, Lobby and Pre-Function*

*(*Poster #'s *P-141 - P-275)*

(All posters need to be picked up by Tuesday 6:00 AM
ASP is not responsible for lost or damaged posters)

6:00 PM Bus Loading for Keenleand
Hilton Lexington Downtown Lobby

7:00 PM – 10:00 PM **An Evening at Keeneland** (Dinner - Ticketed Event)
4201 Versailles Rd.
Lexington, KY 40510
Directions to Keeneland

Tuesday July 24, 2018

7:00 AM – 12:30 PM Registration – ***Grand Ballroom Foyer***

7:00 AM – 12:30 PM Exhibition – ***Grand Ballroom Foyer***
7:00 AM – 8:00 AM Continental Breakfast – ***Grand Ballroom Foyer***

Grand Kentucky Ballroom
Symposium IV- Biosynthesis
Chair: Joe Chappell

8:00 AM – 8:40 AM S-21
Tim S. Bugni (University of Wisconsin)
Waters Award Recipient

SMALLMOLECULEMEDIATEDBACTERIALINTERACTIONSTRANSCRIPTIONALLY ACTIVATE BIOSYNTHETIC GENE CLUSTERS IN A MICROMONOSPORA SP.

8:40 AM – 9:20 AM S-22
Michael D. Burkart (University of California, San Diego)

PROTEIN-PROTEIN AND PROTEIN-SUBSTRATE INTERACTIONS IN FATTY ACID AND POLYKETIDE SYNTHASES

9:20 AM – 10:00 AM S-23
Christian Hertweck (Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute)

CHEMICAL INNOVATION IN MICROBIAL INTERACTIONS

10:00 AM–10:30 AM Break

Grand Kentucky Ballroom Salon A&B
Session T-AM1 - Discovery
Chair: Steven Van Lanen

10:30 AM – 11:10 AM S-24
Amy L. Lane (University of North Florida)

HARNESSING BIOSYNTHETIC PATHWAYS TO EXPAND DIKETOPIPERAZINE CHEMICAL SPACE

11:10 AM – 11:50 AM S-25
Sean F. Brady (The Rockefeller University)

NATURAL PRODUCTS FROM UNCULTURED BACTERIA

11:50 AM – 12:10 PM O-29
Andrew Osborn (Oregon State University)

CHARACTERIZATION OF CONSERVED ACTINOBACTERIAL MYCOSPORINE-LIKE AMINO ACID GENES THROUGH HETEROLOGOUS EXPRESSION

12:10 PM – 12:30 PM O-30
April L. Risinger, PhD (The University of Texas Health Science Center at San Antonio - UT Health)

OLD DRUGS, NEW TRICKS: NON-MITOTIC EFFECTS OF MICROTUBULE TARGETING AGENTS IN CANCER CELLS

Grand Kentucky Ballroom Salon C&D
Session T-AM2 - Methods
Chair: Jon Thorson

10:30 AM – 11:10 AM S-26
Roger Linington (Simon Fraser University)

THE NATURAL PRODUCTS ATLAS; AN OPEN ACCESS DATABASE PLATFORM FOR NATURAL PRODUCTS DISCOVERY

11:10 AM – 11:30 AM O-31
Brian Killday (Bruker Biospin)

TWO-DIMENSIONAL QUANTITATIVE NUCLEAR MAGNETIC RESONANCE FOR DETERMINATION OF COMPONENT CONCENTRATIONS IN COMPLEX MIXTURES

11:30 AM – 11:50 AM O-32
Kirk R. Gustafson (Molecular Targets Program, Center for Cancer Research, NCI)

NMR CHARACTERIZATION OF COMPLEX NATURAL PRODUCTS: NEW TOOLS FOR ASSIGNING NOVEL MOLECULAR SCAFFOLDS

11:50 AM – 12:10 PM O-33
Maria Sofia Ramos da Costa (University of Iceland)

IDBAC: A MALDI-TOF MS PLATFORM TO CREATE DIVERSE MICROBIAL LIBRARIES

12:10 PM – 12:30 PM O-34
James Hudson Tryon (Northwestern University)

LEVERAGING METABOLOMICS ACROSS HUNDREDS OF ACTINOMYCETE STRAINS TO CALIBRATE EMERGING BIOINFORMATICS PLATFORMS

1:30 PM Bus Loading for Optional Tours to Horse Farm and Distillery
Hilton Lexington Downtown Lobby

2:00 PM – 5:00 PM **Horse Farm and Distillery Tours**

2: 00 PM Bus Loading Starting for the Young Members Event
Hilton Lexington Downtown Lobby

4:00 PM – 8:00 PM **Young Members Event** (Ticketed Event)
Buffalo Trace Distillery
113 Great Buffalo Trace
Frankfort, KY 40601
Directions to Buffalo Trace Distillery

12:30 PM - Free Afternoon and Evening for others not attending Young Members Event or Optional Tours

Wednesday July 25, 2018

7:00 AM – 3:30 PM Registration – ***Grand Kentucky Ballroom Foyer***

7:00 AM – 11:50 AM Exhibition – ***Grand Kentucky Ballroom Foyer***

7:00 AM – 8:00 AM Continental Breakfast – ***Grand Kentucky Ballroom Foyer***

11:50 AM – 3:00 PM Exhibitor Dismantling **– *Grand Kentucky Ballroom Foyer***

Grand Kentucky Ballroom
Symposium V- Discovery
Chair: Jürgen Rohr

8:00 AM – 8:40 AM S-27
Jon Clardy (Harvard Medical School)

TARGETED-INTERACTION SCREENS AND ANTIBIOTIC DISCOVERY

8:40 AM – 9:20 AM S-28
Taiwo Olayemi Elufioye (University of Ibadan)

PHYTOCHEMICALS AS NEUROPROTECTIVE AGENTS: THE MULTI-TARGET APPROACH

9:20 AM – 10:00 AM S-29
Rolf Müller (Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS)

MYXOBACTERIA: BIODIVERSITY, SYNTHETIC BIOTECHNOLOGY AND SECONDARY METABOLOMICS FOR NATURAL PRODUCT DISCOVERY

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

Grand Kentucky Ballroom Salon A&B
Session W-AM1- Enzymes
Chair: Sylvie Garneau-Tsodikova

10:30 AM – 11:10 AM S-30
Andrew M. Gulick, Ph.D (University at Buffalo)

THE STRUCTURAL CYCLE OF NONRIBOSOMAL PEPTIDE SYNTHETASE ENZYMES

11:10 AM – 11:50 AM S-31
Sylvie Garneau-Tsodikova (University of Kentucky)

UNDERSTANDING AND ENGINEERING MULTIFUNCTIONAL ENZYMES FOR NONRIBOSOMAL PEPTIDE SYNTHESIS

Grand Kentucky Ballroom Salon C&D
Session W-AM2- Symbiosis
Chair: Oleg Tsodikov

10:30 AM – 11:10 AM S-32
Eric W. Schmidt (University of Utah)

STRUCTURAL AND BIOCHEMICAL DIVERSITY IN MARINE SYMBIOTIC NATURAL PRODUCTS

11:10 AM – 11:30 AM O-35
Paige Elizabeth Mandelare (Oregon State University)

CO-CULTURE OF TWO FORMS OF A MARINE-DERIVED ASPERGILLUS ALLIACEUS RESULTS IN THE PRODUCTION OF ALLIANTHRONES A-F

11:30AM – 11:50 AM O-36
Gabrielle Grandchamp (University of North Carolina at Chapel Hill)

COCULTURE STIMULATES ANTIBIOTIC BIOSYNTHESIS IN ACTINOMYCETES

11:50 AM – 1:30 PM Lunch (on your own)

Grand Kentucky Ballroom
Award Symposium

1:30 PM – 2:20 PM S-33
James B. Gloer (Department of Chemistry, University of Iowa)
Norman R. Farnsworth Research Achievement Award Lecture

ADVENTURES IN FUNGAL NATURAL PRODUCTS CHEMISTRY--FUNGAL ECOLOGY, BIODIVERSITY, AND THE SEARCH FOR NEW BIOACTIVE METABOLITES

2:20 PM – 3:00 PM S-35
Marcy J. Balunas (University of Connecticut)
Matt Suffness Young Investigator Award Lecture

INTERACTION-DRIVEN MOLECULE DISCOVERY FROM HOST-MICROE SYMBIOSES

3:00 PM – 5:00 PM	ASP Business Meeting – ***Magnolia Room***
6:30 PM – 7:30 PM	**Closing Reception** – ***Grand Kentucky Ballroom Foyer***
7:30 PM – 10:30 PM (Ticketed Event)	**Closing Ceremony and Banquet** – ***Grand Kentucky Ballroom***

Thursday July 26, 2018

9:00 AM	**Optional Excursion to Lloyd Library**

Thank you for your participation in the 2018 ASP Meeting!
See you in Madison, WI in 2019

Symposium Presentations

S-01

THE BIOSYNTHESIS OF *N*-FORMYLATED SUGARS IN PATHOGENIC BACTERIA

Hazel M. Holden and James B. Thoden
Department of Biochemistry, University of Wisconsin-Madison

Bacteria produce an astonishingly diverse array of carbohydrate-based macromolecules that serve important physiological roles. Approximately 11 years ago, my laboratory turned its research attention to those enzymes that are involved in the biosynthesis of novel sugars found attached to antibiotics, antifungals, anthelmintics, and antitumor agents. In addition to being found on natural products, however, unusual sugars have also been observed on the lipopolysaccharides or LPS of Gram-negative bacteria. There is growing evidence that the O-antigens of the LPS play important physiological roles including effective colonization of host tissues, protection from phagocytosis and serum-mediated killing, and resistance to antimicrobial peptides.

The occurrence of deoxysugars on the bacterial LPS has been known for more than 30 years. Due to the increased sensitivities of such techniques as NMR, however, it is becoming apparent that the O-antigens are far more complicated than originally thought. Recent research has demonstrated that some pathogenic Gram-negative bacteria contain quite remarkable *N*-formylated dideoxysugars. Indeed, my laboratory has shown that *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has all the necessary enzymes to produce *N*-formylated sugars. Our recent investigations on the enzymes involved in the biosynthesis of these sugars will be presented.

S-02

STRUCTURAL BIOLOGY OF NATURAL PRODUCT METABOLISM: SOME EXAMPLES

George N. Phillips, Jr.
Rice University, MS 140, Houston, Tx 77006

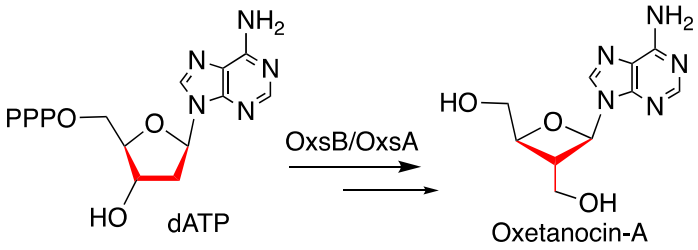
Complex natural products are often biosynthesized by modular polyketide synthase (PKS) systems, which extend and modify the nascent compound through sequential steps. We have characterized a set of PKS's that do not contain the usual customized *in cis* acyl transferase modules but can use more generic ones. This class of PKS have an interesting "variation on a theme" and suggest ways for horizontal gene transfer to play a role in new natural product pathways. The set of enzymes that modify the basic scaffold produced by the PKS systems, including cyclization, aromatization, methylation and glycosylation also exhibit interesting enzymatic activities that can be studied at the atomic level by structural biology to reveal mechanisms of action. Some of these systems will be reviewed with an eye toward the pathways that are involved for enediynes and other natural products. Finally, new crystallographic methods also allow time-resolved kinetics of natural product degradation to be revealed with near atomic detail Some results on beta-lactamase will be presented.

Supported by NIH grants GM094585, GM098248, CA106150 and the Department of Energy

S-03

STRUCTURAL ELUCIDATION OF ENZYMES INVOLVED IN OXETANOCIN-A BIOSYNTHESIS

Jennifer Bridwell-Rabb[1-3], Aoshu Zhong[4,5], Gyunghoon Kang[2], Hung-wen Liu[4,5], and Catherine L. Drennan[1-3]
[1]Howard Hughes Medical Institute, [2]Department of Chemistry, and [3]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, [4]Division of Chemical Biology and Medicinal Chemistry, College of Pharmacy, and [5]Department of Chemistry, University of Texas, Austin, TX 78712



The natural product oxetanocin-A is a potent antiviral compound produced by *Bacillus megaterium* NK84-0128. The biosynthesis of oxetanocin-A has been linked to a plasmid-borne gene cluster that contains four genes involved in oxetanocin-A production (*oxsA* and *oxsB*) and oxetanocin-A resistance (*oxrA* and *oxrB*). In terms of the biosynthetic enzymes, OxsB is a cobalamin (Cbl)-dependent *S*adenosylmethionine (SAM) radical enzyme, and OxsA is an HD-domain phosphohydrolase enzyme. We have determined crystal structures of both OxsA and OxsB, and in this talk, we will present our current thinking about how these enzymes work together to transform dATP into oxetanocin-A, a process that involves a chemically challenging carbon skeletal rearrangement.

S-04

RADICAL SAM ENZYMES IN THE BIOSYNTHESIS OF SUGAR-CONTAINING NATURAL PRODUCTS

Hung-wen Liu
Division of Chemical Biology and Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Texas at Austin, Austin, TX 78712, USA

Carbohydrates play a key role in the biological activity of numerous natural products. In many instances, their biosynthesis requires radical mediated chemical transformations, some of which are catalyzed by radical SAM enzymes. DesII is one such enzyme involved in the deamination reaction necessary for net C4 deoxygenation of a glucose derivative en route to desosamine formation. AprD4 is functionally related to DesII and catalyzes the C3 deoxygenation of paromamine, a key step in the biosynthesis of serval aminoglycosides. SpeY has been shown to catalyze dioxane ring formation in a disaccharide. GenK is a cobalamin-dependent radical SAM enzyme that catalyzes the methylation of an unactivated sp^3 carbon of $GenX_2$ to produce the intermediate G418 during the biosynthesis of gentamicin. OxsB is another cobalamin-dependent radical SAM enzyme responsible for the ring contraction of 2'-deoxyadenosine 5'-monophosphate to yield the four-membered heterocycle in oxetanocin. These enzymes are representative radical SAM enzymes that are involved in carbohydrate biosynthesis. The biosynthetic roles of some of these enzymes, their mechanisms of catalysis, the questions that have arisen during their study, and the insights they can offer for furthering our understanding of radical SAM enzymology will be discussed in this presentation.

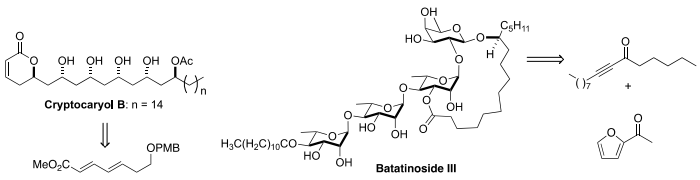
S-05

DE NOVO SYNTHESIS OF NATURAL PRODUCTS FOR MEDICINAL CHEMISTRY

George A. O'Doherty
Department of Chemistry Northeastern University, Boston, MA 02115

Over the years, the O'Doherty group has been working in two related areas of organic synthesis: carbohydrates and natural products. The unifying theme that connects our research in these two areas is our method of synthesis (asymmetric catalysis) and target selection (stereochemically complexity and biological activity). A recurring theme in the group's synthetic approaches to any target is the reliance on asymmetric catalysis and synthetic design for the control of asymmetry. The application of these "atom economical" approaches should allow simple conversion of readily available bulk chemicals (*e.g.*, polyenes, furans) to advanced chiral intermediates (*e.g.*, polyketides and carbohydrates). Fundamental to our approach is the development of highly efficient routes that transform, via catalysis, inexpensive achiral starting materials into enantiopure products, which are poised for the conversion into complex molecules with biologically relevant properties (*i.e.,* enantioselective synthesis of a new "chiral pool" via asymmetric catalysis). Recently, we have found that these approaches have matured to the point where we have developed enantioselective routes to these complex molecules in sufficient quantities that are amenable for biomedical investigations.

Scheme 1: *Sample target molecules*



S-06

ANTI-INFECTIVE NATURAL PRODUCTS DISCOVERY AND BIOSYNTHESIS FROM MARINE MICROORGANISMS

Jianhua Ju
CAS Key Laboratory of Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

Natural products play a pivotal role in drug discovery and development initiatives, especially in infectious disease arenas. However, the emergence of drug-resistance among human pathogenic bacteria has proven to be an increasingly important problem, necessitating the discovery of new drug candidates with novel structures to circumvent bacterial mechanisms of drug resistance. Marine microorganisms have emerged as an exciting resource for novel bioactive natural products discovery. Deciphering and engineering the natural product biosynthetic pathway provide an alternative strategy to modify the structures of complex natural products. In this talk, selected examples will be discussed on: i) isolation and identification of marine microorganisms from marine habitats; ii) Screening and discovery of novel anti-infective natural products as promising drug leads from marine microorganisms; and iii) genetic engineering of the biosynthetic pathways of the anti-infective polypeptide, polyketide and nucleoside natural products to make new drugable analogues and elucidating novel enzymes capable of catalyzing new chemistry.

S-07

BIODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF ENDOPHYTES OF THE MEDICINAL PLANTS FROM THE BRAZILIAN BIOMES PANTANAL AND CERRADO

Chirlei Glienke[1], Daiani C. Savi[1], Khaled A. Shaaban[2,3], Jon S. Thorson[2,3], Yvelise M. Possiede[4], Jürgen Rohr[2]
[1]Department of Genetics, Federal University of Paraná, Curitiba, Brazil; [2]Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY, United States; [3]Center for Pharmaceutical Research and Innovation, University of Kentucky, Lexington, KY, United States; [4]Department of Biology, Federal University of Mato Grosso do Sul, Campo Grande, Brazil

Multidrug resistant pathogens have been observed with increasing frequency in recent decades, driving the search for new drugs and stimulating the interest in Natural Products (NP) sources. Endophytes represent an important source of novel and useful bioactive compounds. In recent years, we have explored endophytes of medicinal plants from two Brazilian biomes, the Pantanal (wetland) and the Cerrado (savannah), known as a biodiversity hotspot. We isolated more than 3,000 endophytic strains from *Vochysia divergens* (Pantanal) and *Stryphnodendron adstrigens* (Cerrado) and the molecular phylogenetic analyzes revealed high diversity of actinomycetes and fungi. The strains belong to more than 30 genera, and about 30% of all isolates correspond to new species and even to new genera. Several strains are producers of secondary metabolites with high biological activity against human pathogens such as *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli, Micrococcus luteus, Klebssiella pneumoniae* (KPC), *Candida albicans* and *Saccharomyces cerevisiae*, and against plant pathogens, such as *Colletotrichum abscissum* and *Phyllosticta citricarpa*. These compounds belong to several classes of known compounds (indoles, b-carbolines, brevianamide, diaporthin, orthosporin, cercosporin, isocercosporin and diketopiperazines) and novel compounds, such as 3-(sec-butyl)-6-ethyl-4,5-dihydroxy-2-methoxy-6-methylcyclohex-2-enone. Our results indicate that the endophytes of Brazilian medicinal plants are producers of bioactive compounds and that their biodiversity and biotechnological potential remain underestimated.

S-08

SYNTHESIS OF BIOACTIVE 4' DEOXYFLAVONES IN SCUTELLARIA BAICALENSIS, THE GOLDEN HERB OF THE CHINESE MEDICINAL GARDEN.

Cathie Martin[1], Meng-Ying Cui[2], Dongfeng Yang[1,3], Jie Liu[2], Jie Li[2], Lionel Hill[1], Lei Yang[2], Yonghong Hu[2], Xiao-Ya Chen[2,4] and Qing Zhao[1,2]
[1]Department of Metabolic Biology, John Innes Centre, Norwich, NR4 7UH UK; [2]Shanghai Key Laboratory of Plant Functional Genomics and Resources, Shanghai Chenshan Botanical Garden, Shanghai Chenshan Plant Science Research Center, Chinese Academy of Sciences, Shanghai, China; [3]College of Life Sciences, Zhejiang Sci-Tech University, Key Laboratory of Plant Secondary Metabolism and Regulation of Zhejiang Province, Hangzhou 310018, China; [4]State Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China

Scutellaria baicalensis Georgi, known as Huang-Qin or Chinese skullcap, is one of the most widely used medicinal plants in traditional Eastern medicine. It has been applied in the treatment of inflammation, respiratory tract infections, diarrhoea, dysentery, liver disorders, hypertension, haemorrhaging, and insomnia. The major bioactive compounds isolated from *S. baicalensis* are 4'deoxyflavones such as baicalin, baicalein, wogonin and wogonoside. These flavones have been reported to have several pharmacological activities, which include anti-cancer, hepatoprotection, antibacterial and antiviral, antioxidant, anticonvulsant and neuroprotective effects.

I will describe the elucidation of the complete biosynthetic pathways for baicalein and wogonin in *S. baicalensis*, where new pathways have evolved

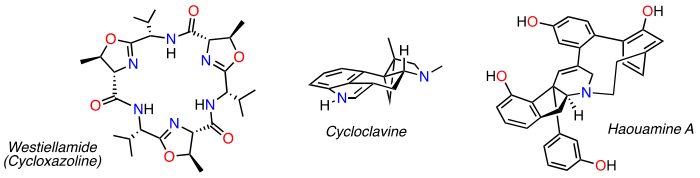
from the classic flavone biosynthetic pathway found in many angiosperms. A recently completed genome sequence for *S. baicalensis* sheds light on how this pathway evolved, principally by tandem duplications and neofunctionalizations. Metabolite profiling shows that different 4'deoxyflavones accumulate in different species of Scutellaria, particularly as a resulting in varying activity of P450 decorating enzymes. Screening of Scutellaria accessions also identified species where 4' deoxyflavones accumulate to high levels in leaves, so offering more sustainable natural sources of these bioactive compounds.

S-09

ADVENTURES IN THE SYNTHESIS OF POLYCYCLIC ALKALOIDS

Peter Wipf, Stephanie McCabe, and Liming Cao
Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA

A significant part of our work over the past two decades has been influenced by explorations of natural products, in particular alkaloids, with novel architectures. Small ring strain and transannular interactions in larger ring scaffolds have traditionally been a rich source of serendipity-driven discoveries in Chemistry. For example, we have studied the effects of configuration and hybridization of five-membered heterocycles embedded in the macrocyclic ring system of *Lissoclinum* peptides, giving raise to unique metal chelation selectivities. In the total synthesis of both enantiomers of the *Clavine* alkaloid cycloclavine, we used the strain of a methylenecyclopropene to assemble three consecutive stereocenters in an intramolecular Diels-Alder cycloaddition. In the last steps of a synthesis of the 3-aza-[7]-paracyclophane moiety of the ascidian *Aplidium haouarianum* metabolite haouamine A, an unexpected fragmentation generated a rearranged heterocyclic product.



S-10

COMPLEX BIOLOGICAL MACHINERIES: BACTERIAL CELL WALL, ITS TURNOVER AND LINK TO ANTIBIOTIC RESISTANCE

Shahriar Mobashery
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 USA

Members of *Enterobacteriaceae* and *Pseudomonas aeruginosa* have the ability to sense damage inflicted to their cell wall by β-lactam antibiotics. The process involves chemical signaling, which will be a subject of my presentation. A primary mechanism for this sensing and signalling involves the events of cell-wall recycling. The cell wall is degraded for recycling and then the cell wall is synthesized *de novo* for the repair function. The recycling events get initiated by the functions of a family of 11 lytic transglycosylases in *P. aeruginosa*, which generate the signalling factors that influence transcriptional events in the cytoplasm. The structures and mechanisms of these enzymes and those of the early cytoplasmic steps of recycling have been the subject of study in my lab, which I will disclose in my presentation.

S-11

SYSTEMATIC CONVERSION OF GRAM-POSITIVE-ONLY COMPOUNDS INTO BROAD-SPECTRUM ANTIBIOTICS

Professor Paul J. Hergenrother
Department of Chemistry, 600 S. Mathews, University of Illinois, Urbana-Champaign, Urbana, IL 61801

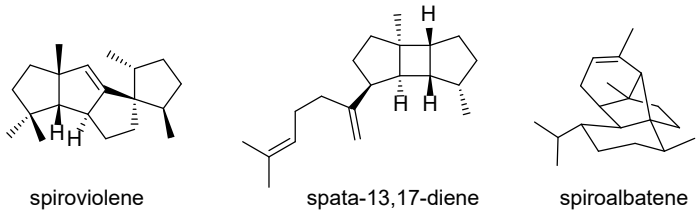
The incidence of multi-drug resistant Gram-negative infections has risen sharply in the last decade and is a growing health concern. This problem is exacerbated by the fact that it has been 50 years since the clinical introduction of a new antibacterial class for these pathogens. Central to the problem of Gram-negative antibiotic discovery are the challenges small-molecules face in traversing the Gram-negative outer membrane. This lecture will describe our guidelines, the "eNTRy rules", for predicting compound accumulation in Gram-negative bacteria. The successful application of the eNTRy rules to the conversion of several different classes of Gram-positive-only compounds into versions that have broad-spectrum antibiotic activity will be discussed.

S-12

TRACING TERRESTRIAL TERPENES WITH ISOTOPES

Jeroen S. Dickschat
Kekulé-Institute of Organic Chemistry and Biochemistry, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany (dickschat@uni-bonn.de).

Recent advances in genome sequencing have revealed a large number of terpene synthases in microorgansisms. During the past years my group has synthesised various isotopically labelled terpene precursors that can be used to efficiently unravel the cyclisation mechanisms of these astonishing enzymes. Their application in mechanistic investigations on the most interesting newly discovered terpene synthases, making the compounds shown below, will be discussed.



S-13

DENDRAL TO DEEP LEARNING IN NATURAL PRODUCT CHEMISTRY

Nathan Magarvey, PhD.
McMaster University, Biochemistry and Biomedical Sciences & Chemistry and Chemical Biology, 1280 Main Street West, Hamilton Canada L8S4L8

Bioactive natural products from microbes discovered from an era defined through bioactivity guided fractionation lead to many revolutionary products. Much history exists in these searches and like in other pursuits there is a need to create increased efficiency in mining microbes for new bioactive agents. The increase in, and capacities to, sequence microbes and microbiomes provides information that now is suggesting the unknown chemical dark matter awaiting interrogation in relevant assays/applications. Translating genetic information into accurate suggestion of chemistry demands computational tools and artificial intelligence. Among the first artificial intelligent systems, Dendral, focused on the unknown chemical dark matter from other planets. A similar level of ambition will be required to define the unknown chemistry encoded within microbial genomes. In this talk an attempt will be made to define the history, current progress and prospective future of deep learning and artificial intelligence in microbial natural product chemical discovery.

S-14

EXPLORING AND EXPLOITING BIOSYNTHESIS TO ACCESS NOVEL NATURAL PRODUCTS

Rebecca J. M. Goss[1,2*]
[1]Biomedicinal Ressearch Complex, University of St Andrews, KY16 9ST, UK; [2]School of Chemistry, University of St Andrews, KY16 9ST, UK

Though natural products represent a treasure trove of medicinally relevant compounds, they are commonly misperceived to be unsuitable for medicinal chemistry. We have an interest in the discovery of novel bioactive natural products and elucidating the biosynthesis of structurally unusual natural products. We are developing new approaches to natural product analogue synthesis by blending together synthetic biology and synthetic chemistry. By complementing the biosynthetic machinery encoding an existing natural product with foreign genes we are able to introduce chemically orthogonal, reactive and selectable functionalisable handles into natural products. We have been developing mild chemical methodologies to enable the chemical derivitisation of these handles.

S-15

CHEMICAL SIGNALING IN WORMS

Rebecca A. Butcher
Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

Our goal is to develop a comprehensive understanding of the chemical structures, biosynthesis, and mechanism of secondary metabolites in nematodes. This talk will describe our work on two important classes of natural products from *C. elegans*, the ascarosides and the nemamides. *C. elegans* secretes ascarosides, derivatives of the 3,6-dideoxysugar ascarylose, as pheromones to control its development and behavior. We are using a multidisciplinary approach, including RNAi-based screens, metabolomics, *in vitro* enzyme assays, organic synthesis of biosynthetic intermediates, and X-ray crystallography, to study the ascaroside biosynthetic pathway. Our work has shown how peroxisomal β-oxidation is used to control the production of different ascaroside pheromones and how environmental conditions influence this process. In a second area of research, we have used comparative metabolomics and NMR spectroscopy to discover the first hybrid polyketide-nonribosomal peptides from an animal, the nemamides from *C. elegans*. We are using CRISPR-Cas9 and comparative metabolomics to explore the role of specific enzymatic domains in nemamide biosynthesis. Our work is providing insights into the biosynthesis of natural products in the context of a complex animal system.

S-16

DISCOVERING NEW ENZYMES FROM FUNGAL NATURAL PRODUCT BIOSYNTHETIC PATHWAYS

Yi Tang
Department of Chemistry and Biochemistry, Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, 420 Westwood Plaza, Los Angeles, CA 90095

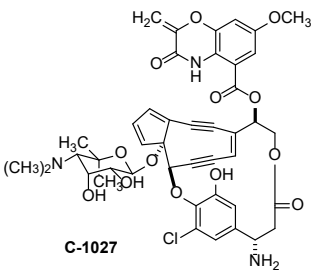
Fungal natural products are structurally diverse compounds with pharmaceutically relevant bioactivities. In this talk I will present our work in understanding the biosynthesis of hybrid polyketide-amino acid compounds synthesized by polyketide synthase-nonribosomal peptide synthetases (PKS-NRPS). We will focus on how assembly-line programming rules from the PKS-NRPS and novel enzyme activities from tailoring enzymes are precisely coordinated to build chemical complexity in a relatively few biosynthetic steps. For example, we discovered pericyclases that catalyze Diels-Alder and hetero-Diels Alder reactions are found in these pathways to install decalin and pyran rings, respectively. Other examples that will be presented include oxaleimides, a new family of succinimide and maleimide containing natural products discovered from genome mining of a cryptic PKS-NRPS gene cluster.

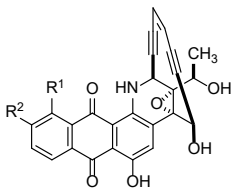
S-17

GENOME MINING OF ENEDIYNE NATURAL PRODUCTS PROVIDING NEW OPPORTUNITIES FOR ENEDIYNE BIOSYNTHESIS, ENGINEERING, AND DRUG DISCOVERY

Ben Shen
Departments of Chemistry and Molecular Medicine and Natural Products Library Initiative at The Scripps Research institute, The Scripps Research Institute, Jupiter, Florida, USA

The enediyne family of natural products has had profound impact on modern chemistry, biology, and medicine. Since the neocarzinostatin chromophore structure was first unveiled in 1985, the enediyne family has grown steadily but remains very small. Recent advances in microbial genomics, however, clearly revealed that the biosynthetic potential of soil actinomycetes to produce enediynes is underappreciated. Our current efforts on genome mining of enediyne natural products will be discussed to highlight the new opportunities for enediyne biosynthesis, engineering, and drug discovery.





Uncialamycin ($R^1 = R^2 = H$)
Yangpumicin ($R^1 = OH$, $R^2 = H$)
Tiancimycin ($R^1 = OH$, $R^2 = OCH_3$)

S-18

MAKE NATURAL PRODUCTS GREAT AGAIN: NO COLLUSION; PLENTY OF COLLABORATION

Robert H. Cichewicz[1]
[1]*Natural Product Discovery Group, Department of Chemistry and Biochemistry, Institute for Natural Product Applications and Research Technologies, University of Oklahoma, Norman, OK 73019*

Nature has long served as a source for bioactive molecules with many of these compounds ranking among the world's most renowned and widely used organic substances. Numerous examples exist including well-known cancer chemotherapeutic agents, insecticides, and antibiotics. The influence

that natural products have had on humankind and the chemical sciences is undeniable, yet these landmark molecules are the products of past discovery efforts, which has raised the question, what do natural products have to offer as a resource for bioactive compound discovery in today's world? An approach that has been implemented at the University of Oklahoma to transform fungal natural products drug discovery is the creation of a novel citizen-science-driven discovery effort called the Citizen Science Soil Collection Program (https://whatsinyourbackyard.org/). This program is aimed at recruiting citizen scientist from all walks of life to join our research team and explore the immense biological and natural-product-chemical diversity of fungi from across much of the North American continent. In addition to enabling the rapid development of one of the world's largest fungal isolate and natural product chemistry collections, the Citizen Science Soil Collection Program provides a grassroots mechanism for promoting STEM education. Several unique and promising compounds have already emerged through these endeavors, which have reached families and schools across America.

S-19

TARGETING INHIBITORS OF PROTEIN SECRETION FROM MARINE MICROBIAL COMMUNITIES

Kerry L. McPhail[1], David A. Gallegos[1], Xinhui Yu[1], Xuemei Wan[1], Patricia M. Flatt[2], Eric Isemonger[3], Jarmo C.J. Kalinski[3], Xavier Siwe Noundou[3], Samantha Waterworth[4], Rosemary A. Dorrington[2], Jason Kwan[4], Jane E. Ishmael[1]

[1]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, USA, [2]Department of Chemistry, Western Oregon University, Monmouth, OR 97361, USA, [3]Department of Biochemistry and Microbiology, Rhodes University, Grahamstown South Africa, [4]School of Pharmacy, University of Wisconsin, Madison, WI 53705.

The dominant role of "privileged" natural product (NP) structures in modern medicine is well established, especially for microbial NPs in the treatment of infectious diseases and cancers. Many microbial NP-based drugs have arisen from serendipitous discoveries involving relatively limited chemical and biological screening, followed by lengthy development paths for unanticipated indications. The discovery of extensive NP biosynthetic potential in sequenced microbial genomes, has prompted efforts to target biosynthetic genes for known NPs in short supply, or that potentially represent new chemical entities with new biological mechanisms but remain "cryptic". We have taken advantage of collaborations in metagenomics and pharmacology to facilitate our investigations of macrocyclic NPs from marine tunicate/microbial symbiont consortia, and microbial communities dominated by cyanobacteria, in some cases, linking genomics, transcriptomics and secondary metabolomics. Whole-cell functional assays are being used for the discovery of substrate-selective inhibitors of cellular protein secretion via the Sec61 translocon. In particular, we are targeting nonpolar cyclic depsipeptide structures that are consistent with the handful of known Sec61 translocon inhibitors, as well as secreted protease inhibitors. The protein secretory pathway is an emerging therapeutic target due to its central involvement in regulating cellular proteostasis and protein folding, and its dysregulation in human disease.

S-20

ENZYMES THAT MAKE AND BREAK B-LACTAMS

Christopher J. Schofield

Department of Chemistry, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK

The lecture will describe mechanistic and inhibition studies on enzymes involved in β-lactam biosynthesis and hydrolysis. It will aim to show how a desire to understand how these enzymes work led to the identification of unanticipated roles for homologues of the bacterial roles in humans, including in the regulation of protein biosynthesis and nucleic acid repair. Recently, this work has led to the identification of new mechanisms for antibiotic resistance. Work on the inhibition of metallo β-lactamases and other enzymes involved in antibiotic resistance will be described.

S-21

SMALL MOLECULE MEDIATED BACTERIAL INTERACTIONS TRANSCRIPTIONALLY ACTIVATE BIOSYNTHETIC GENE CLUSTERS IN A MICROMONOSPORA SP.

Deepa Acharya[1], Navid Adnani[1], Marc G. Chevrette[2], Ian J. Miller[1], Shaurya Chanana[1], Yusi Cui[1], Cameron R. Currie[2], Lingjun Li[1], Jason C. Kwan[1], Tim S. Bugni[1]

[1]Pharmaceutical Sciences Division and [2]Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53705

Whole genome sequencing has shown that the biosynthetic potential of actinomycetes far exceeds what is observed using standard fermentation conditions. As such, products for many biosynthetic gene clusters are not observed under standard laboratory conditions. In particular, we found that members of the Micromonosporaceae, a group of actinomycetes common in marine environments, rarely produced extracts with antibiotic activity, despite evidence for rich biosynthesis capability. We developed microscale co-culture platforms to evaluate mixed cultures for induction of antibiotic production. One pair, a *Micromonospora* sp. and a *Rhodococcus* sp. led the production of an otherwise unattainable bis-nitroglycosylated antibiotic that we named keyicin. Our preliminary studies led to the hypothesis that a small molecule mediated the interaction between these organisms that activated production of keyicin. We sequenced the genomes of both organisms using PacBio and identified the *Micromonospora* sp. as the producer. Within the biosynthetic gene cluster, we identified a LuxR with homology to those found in *Pseudomonas* sp. Combined with evidence that the *Rhodococcus* sp. produced a molecule responsible for activating production, we established a working hypothesis that a small molecule led to transcriptional activation of the keyicin pathway. This presentation will discuss the data supporting our current hypothesis and a proof of concept for extending these studies to other similarly regulated clusters.

S-22

PROTEIN-PROTEIN AND PROTEIN-SUBSTRATE INTERACTIONS IN FATTY ACID AND POLYKETIDE SYNTHASES

Michael D. Burkart

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Metabolites from fatty acid synthase (FAS) and polyketide synthase (PKS) pathways differ broadly in their identities and functional roles. The former are considered primary metabolites that are linear hydrocarbon acids, while the latter are complex aromatic, partially reduced, or polyunsaturated secondary metabolites. Though the study of bacterial FAS has benefitted from decades of biochemical and structural investigations, PKSs have remained less understood, primarily due to challenges in protein expression and structural biology. Here we will discuss approaches to the study of FAS that have highlighted the critical role of the acyl carrier protein (ACP) with regard to how it stabilizes intermediates through sequestration and selectively delivers cargo to successive enzymes within these iterative pathways, utilizing the specificity conferred by protein-protein interactions to guide and organize enzymatic timing and specificity. Analogs of the tools that have shown promise in FAS elucidation have found new approaches to both type I and type II PKS systems, which will be further elaborated with applications to structural biology approaches.

S-23

CHEMICAL INNOVATION IN MICROBIAL INTERACTIONS

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The vast structural diversity of secondary metabolites has evolved over millions of years to address specific needs of the producing microorganisms in their niches. Thus, microbial natural products are not only highly specific mediators of microbial interactions, but also a valuable a source of molecular tools and therapeutics that have been pre-optimized for particular biological targets. Recent research has led to a massive body of knowledge on biosynthetic mechanisms, structures and functions. Yet, we know only very little about how structural diversity evolved. We are also perplexed by the hugely underestimated biosynthetic potential that remains invisible outside of the specific ecological context. This talk will present some compounds and pathways from various less explored bacteria, highlight the importance of considering ecological aspects in the search for biologically active molecules, and shed light on mechanisms and some evolutionary aspects of natural product biosynthesis.

S-24

HARNESSING BIOSYNTHETIC PATHWAYS TO EXPAND DIKETOPIPERAZINE CHEMICAL SPACE

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Natural products with 2,5-diketopiperazine (DKP) scaffolds offer a broad range of bioactivities and chemical structures. The functional and structural diversity of these cyclodipeptides arises via enzyme-catalyzed construction from a variety of amino acids as well as tailoring of DKP cores. For many years, nonribosomal peptide synthetases (NRPSs) were the only enzymes recognized as catalysts for DKP assembly. Cyclodipeptide synthases (CDPSs), employing two aminoacyl-tRNAs as substrates for DKP assembly, were first reported in 2009. Aminoacyl-tRNAs are uncommon players in natural product assembly, making CDPSs intriguing members of Nature's biosynthetic repertoire. Biochemical and bioinformatics analyses support that CDPSs are found from at least six bacterial phyla and some animals, yet biosynthetic pathways with CDPSs remain understudied relative to pathways featuring NRPSs or other common biosynthetic enzymes. Through studies of bacterial pathways that include CDPSs, my group has unveiled novel biosynthetic capabilities and developed tools for the engineered biosynthesis of DKPs. This presentation will highlight our strategies for the characterization of these pathways to discover uniquely functionalized DKPs and our development of a platform for the CDPS-catalyzed assembly of novel DKPs from unnatural aminoacyl-tRNA precursors. Our results establish the catalytic promise of CDPSs beyond natural cellular aminoacyl-tRNAs, and showcase the utility of biosynthetic strategies for expanding the breadth of chemical space provided natural products.

S-25

NATURAL PRODUCTS FROM UNCULTURED BACTERIA

Sean F. Brady
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One of the key revelations to arise from the large-scale sequencing of bacterial (meta)genomic DNA is that traditional approaches used for the discovery of bioactive small molecules have only gained functional access to a small fraction of the bacterial biosynthetic gene clusters present in nature. Uncultivated environmental bacteria no doubt produce additional undiscovered secondary metabolites that could serve as molecular probes of biological processes as well as new therapeutic agents. Although there appears to be no easy way to culture this collection of unstudied microorganisms, it is possible to isolate large fragments of bacterial DNA directly from environmental samples and clone this DNA into model cultured bacteria where it can be amplified and studied. We are using both functional and sequence-based screening strategies to identify bioactive natural products encoded by large DNA libraries constructed using DNA extracted from both soil or human microbiomes. Bioactive small molecules identified in these studies will be discussed.

S-26

THE NATURAL PRODUCTS ATLAS; AN OPEN ACCESS DATABASE PLATFORM FOR NATURAL PRODUCTS DISCOVERY

Roger G. Linington
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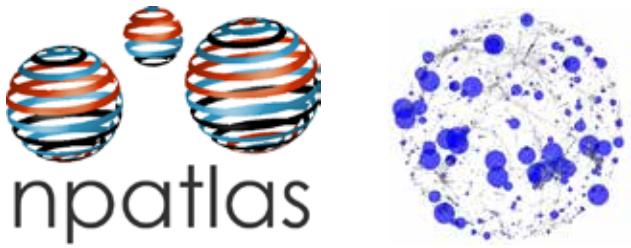
Data-driven methods are playing an increasing role in many areas of modern natural products science. However, despite the recognition that these tools can greatly accelerate discovery programs, the field still lacks a central open access repository containing all known natural products structures. Our laboratory has been developing such a platform for all microbially-derived natural products. This database, termed the Natural Products Atlas, aims to provide a community resource for data pertaining to microbial natural products, including structures, names, origins, physicochemical data, structural reassignments and corrections, total syntheses and other information. It is expected that future versions of the platform will include spectral data and information about biological activities. The 2018 ASP meeting will be the official launch of this new resource. Attributes, features and applications of this new tool will be presented.

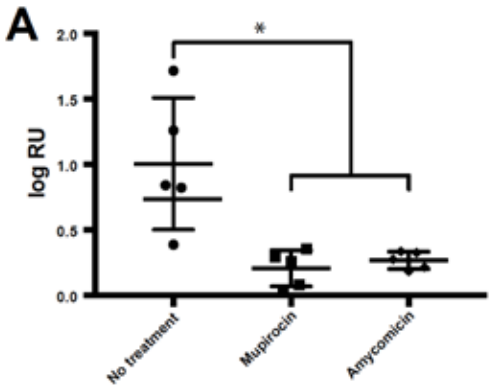


S-27

TARGETED-INTERACTION SCREENS AND ANTIBIOTIC DISCOVERY

Jon Clardy[1], Gleb Pishchany[2], Emily Mevers[1], Roberto Kolter[2]
[1]Department of Biological Chemistry & Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA, [2]Department of Microbiology and Immunology, Harvard Medical School, Boston, MA 02115.

The rapid and pervasive appearance of antibiotic resistance has invigorated efforts to discover new small molecule antibiotics that could become useful therapeutics. Many of these efforts have involved revisiting traditional sources – particularly environmental bacteria – with new methods. This lecture reports on one such method, which we have named targeted-interaction screens. The method capitalizes on the ability of complex bacterial communities to produce small molecules that are not observed when any of the single community members is cultured with the same. The discovery of amycomicin, a potent and specific antibiotic for *Staphylococcus aureus*, an important human pathogen, from a community of nine Actinomycetes will illustrate the method. Amycomicin is active against MRSA (methicillin resistant *S. aureus*) and active in an *in vivo* mouse skin infection model (see Figure).



S-28

PHYTOCHEMICALS AS NEUROPROTECTIVE AGENTS: THE MULTI-TARGET APPROACH

Taiwo Olayemi Elufioye
Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Neurodegenerative diseases (NDs) such as Alzheimer's, Parkinson's, and Huntington's diseases as well as amyotropic lateral sclerosis affect millions of people around the world with the main risk factor being advancing age. Each of these diseases involves characteristic pathological and molecular features resulting from their effects on specific neurons and/or regions in the brain. Neuroprotection refers to the preservation of the structure and function of these neurons from insults arising from cellular injuries induced by a variety of agents and/or NDs.

Several *in vitro* and *in vivo* study models specific to each ND have been employed with the aim of understanding their underlying mechanisms and identifying new therapeutic strategies. Of the most prevalent drug development efforts employed in the past few decades, mechanisms implicated in the accumulation of protein-based deposits, oxidative stress, neuro-inflammation, and certain neurotransmitter deficits such as acetylcholine and dopamine have been scrutinized in great detail.

This presentation will be discussing classical examples of plant-derived neuroprotective agents by highlighting their structural class and specific mechanisms of action.

S-29

MYXOBACTERIA: BIODIVERSITY, SYNTHETIC BIOTECHNOLOGY AND SECONDARY METABOLOMICS FOR NATURAL PRODUCT DISCOVERY

Rolf Müller
Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Department Microbial Natural Products, Coordinator Research Unit Novel Antibiotics, German Center for Infection Research, University Campus E8 1, 66123 Saarbrücken, Germany

Amongst the well-established bacterial producers myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promsing bioactivities. This is at least in part due to the fact that they have been much less studied in the past in comparison to other traditional sources such as actinomycetes and bacilli. Nevertheless, the issue of rediscovery is a major hurdle for myxobacterial extracts as well. I will disucss recent results from our efforts to culture previously uncultured myxobacteria and to connect phylogentically distant clades to novel metabolites by metabolome and genome mining. In addition, I will show examples of heterologous expression of myxobacterial compounds yielding producer strains making production of lead compounds for pharmaceutical development feasible.

S-30

THE STRUCTURAL CYCLE OF NONRIBOSOMAL PEPTIDE SYNTHETASE ENZYMES

Andrew M. Gulick
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The Nonribosomal Peptide Synthetases (NRPSs) are a family of large modular enzymes that are responsible for the production of many pharmaceutically important peptide natural products. During biosynthesis, the NRPSs employ an assembly line strategy in which the amino acid and peptide intermediates are covalently bound to a peptidyl carrier protein (PCP) domain. This loaded PCP transports the substrates to neighboring catalytic domains for substrate activation, modification, polymerization, and release. The core NRPS domains include the adenylation domain that activates and loads the amino acid substrate, the condensation domain that catalyzes peptide bond formation, and the thioesterase domain that catalyzes product release. Additional auxiliary domains may catalyze further modifications. Recently, insight into the structural mechanisms that govern NRPS choreography has been provided through the determination of structures of multidomain enzymes that capture the protein in distinct states of the catalytic cycle. In particular, a large conformational change that is employed by the NRPS adenylation domain is required for the domain to adopt the catalytic conformations necessary for both the adenylation and thioester-forming partial reactions. This conformational change also transports the PCP domain between the two core domains. We will present our studies with full NRPS modules that demonstrate how the adenylation and condensation domains simultaneously adopt active conformations, leading to an efficient catalytic cycle. Further, the structures illustrate limited interactions between the core of the NRPS and the downstream thioesterase domain suggesting limited structural constraints between modules.

S-31

UNDERSTANDING AND ENGINEERING MULTIFUNCTIONAL ENZYMES FOR NONRIBOSOMAL PEPTIDE SYNTHESIS

*Sylvie Garneau-Tsodikova**
** Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY, 40536-0596, USA.*

Nonribosomal peptides are natural products biosynthesized by multi-modular enzymatic assembly-lines comprised of domains performing various activities. Adenylating enzymes play a critical role in dictating the identity of building blocks to be incorporated in growing peptides during nonribosomal peptide biosynthesis. To increase the structural diversity of the products it generates, Nature has evolved unique interrupted adenylating enzymes capable of performing both adenylation and methylation reactions. We will present our efforts towards understanding the mechanism by which these unique enzymes function and our biochemical and structural work towards engineering novel interrupted enzymes with adenylating and methylating activities. We will also discuss introduction of diversity into natural products via halogenation.

S-32

STRUCTURAL AND BIOCHEMICAL DIVERSITY IN MARINE SYMBIOTIC NATURAL PRODUCTS

Eric W. Schmidt
Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84108

Marine animals contain unique compounds with unique scaffolds and chemical modifications. Technical advances have improved the direct

access to biosynthetic machinery and to active compounds for drug discovery. While many chemicals are produced by the symbiotic microbiota, others are made by the animals themselves. Recent advances in characterizing novel compounds and biochemical reactions from marine animals and their symbionts will be described.

S-33

ADVENTURES IN FUNGAL NATURAL PRODUCTS CHEMISTRY--FUNGAL ECOLOGY, BIODIVERSITY, AND THE SEARCH FOR NEW BIOACTIVE METABOLITES

James B. Gloer.
Department of Chemistry, University of Iowa, Iowa City, IA 52242, USA

My professional association with fungi and their metabolites began 35 years ago with a fortuitous opportunity to study the chemistry of an unusual host-selective toxin produced by a plant pathogen. The structural and biological novelty of that system ultimately inspired the development of a research program in fungal chemistry that has continued ever since. At the time, it was clear that many ecological groups of fungi were underexplored from a chemical standpoint, despite evidence that certain types might be fruitful sources of bioactive natural products. We initiated a program targeting fungi from biodiverse, undersampled communities on the basis of several criteria, including hypotheses about roles of chemistry in antagonistic or defensive interactions, as well as the distinctiveness of taxa prevalent in those communities. We proposed that this focus on organism selection could reduce redundancy inherent in random screening approaches, while shedding light on some fundamental questions about targeted species and providing direction for future prioritization strategies. Many different efforts along these lines were undertaken over the years, including studies of marine, freshwater, mycoparasitic, fungicolous, endophytic, coprophilous, and sclerotium-producing fungi, all of which proved to be productive sources of bioactive metabolites and other interesting findings to varying degrees. All of these projects have involved close collaborations with outstanding mycologists whose unique expertise and valuable insights made this kind of targeted approach possible. This presentation will highlight some results and observations arising from these collaborative studies over the long term, and offer some personal perspective on past and present developments in this rapidly-evolving field.

S-35

INTERACTION-DRIVEN MOLECULE DISCOVERY FROM HOST-MICROBE SYMBIOSES

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

Microbial symbioses are increasingly recognized to play an integral role in host structure and function. Co-evolution of these benign and/or beneficial relationships has been the focus of numerous microbiology studies, although the role of secondary metabolite interactions has received considerably less attention. Small molecule interactions in these host-microbe symbioses are likely to contribute to the complex molecular conversations occurring between bacterial symbionts, eukaryotic hosts, and their pathogens/prey. Given that these host-microbe associations have naturally evolved to select for biologically active bacteria, they provide a source of secondary metabolites more likely to have potent medicinal activity and thus be poised for future preclinical drug development. We utilize several unique host-microbe symbioses to explore interactions between eukaryotic hosts and their associated bacteria, integrating natural products and analytical chemistry, medicinal and synthetic chemistry, microbial chemical ecology, and biological screening with advanced molecular biology to provide a comprehensive understanding of secondary metabolite production in these symbioses. Our long-term goal is to use insights gained from these host-microbe symbioses to discover and develop new druggable lead compounds for a wide range of human diseases. Recent developments from these studies will be presented including experiments to allow for competitive interactions to enhance metabolite production and further our understanding of microbial interactions.

Oral Presentations

O-01

CHEMICAL INVESTIGATION OF SACCHARIDES OF DENDROBIUM CASSIOPE

Jhih-Jhong Wang[1], Mei-Kuang Lu[2] and Chia-Chuan Chang[1]
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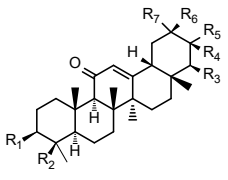
Dendrobium Cassiope (DC), a hybrid of *Dendrobium nobile* (♀) and *D. moniliforme* (♂), is a medicinal herb cultivated in Taiwan. *Dendrobium* shows several bioactivities, such as anti-hyperglycemic, beneficial for cardiovascular diseases, and immunity modulation. The present study utilized centrifugal filtration for rapid purification of the polysaccharides (PSs) of DC. The PSs were purified by DEAE-cellulose column chromatography and characterized by nuclear magnetic resonance spectroscopy (NMR) and monosaccharides composition analysis. The major PS (**DC-PS1**) is an glucomannan with a molecular weight over 3 kDa, and the ratio of Man-Glc is 100 : 12.5, and with partial 2-*O*- and 3-*O*-acetyl groups. The backbone of **DC-PS1** is β-(1→4)-mannose and β-(1→4)-glucose, with 1,6-α-mannosyl branches and β-mannosyl terminals. The molecular weight of **DC-PS1** was estimated to be 3.308 kDa, and the degree of polymerization (DP) is 20, by high performance size exclusion chromatography (HPSEC) and diffusion-ordered NMR (DOSY).

O-02

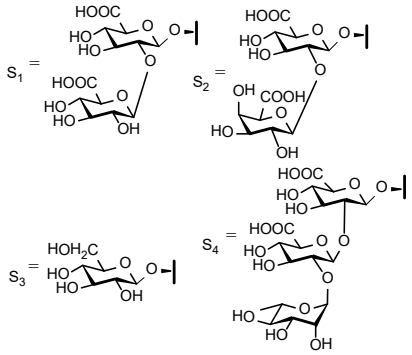
TRITERPENOID SAPONINS FROM THE ROOTS OF GLYCYRRHIZA GLABRA

Qingyao Shou[1], Ping Jiao[1], Mei Hong[1], Qi Jia[1], Indra Prakash[2], Sangphyo Hong[2], Bin Wang[2], Gil Ma[2], Allison Bechman[2]*
[1]Unigen USA, 2121 South State St., Suite 400, Tacoma, WA 98405[2] The Coca-Cola Company, One Coca-Cola Plaza North West, Atlanta, GA 30313

With a rising consumer demand for reduced-sugar in food and beverages, the discovery of natural low-/zero- calorie sugar alternatives brings more attention to global food industries and researchers. Licorice, the root of *Glycyrrhiza* species is one of the oldest and well-known sweet materials in the world. Its extract is widely used as a flavor in foods, beverages, and dietary supplements. Glycyrrhizin as one of the major metabolites obtained from Licorice, is up to 50 times as sweet as sucrose, and is approved as a natural sweetener and/or flavor in many countries. Except for glycyrrhizin, several other triterpene saponins from Licorice plants were identified sweet. This drove us to look deep into glycyrrhizin analogs from *G. glabra*. With the strategy of the LC/MS guided mining, ten new oleanane-type triterpenoid saponins (Glabasaponin A-J) were isolated from the roots of *G. glabra* together with a known compound Macedonoside A. The structures of the compounds **1**–**10 (**Figure 1) were determined based on 1D and 2D NMR, as well as the accurate molecular weight from QTOF-MS data analyses, and the sugar residues were identified by gas chromatography after hydrolysis.



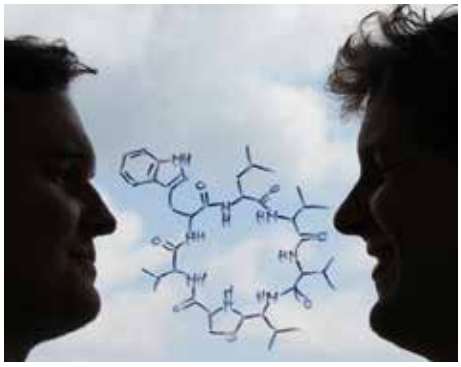
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	S_1	CH_2OH	H	OH	H	CH_3	COOH
2	S_1	CH_2OH	H	H	OH	CH_3	COOH
3	S_2	CH_2OH	H	H	OH	CH_3	COOH
4	S_2	CH_3	H	H	OH	CH_3	COOH
5	S_1	CH_3	H	S_3	H	CH_3	CH_2OH
6	S_1	CH_3	H	S_3	H	CH_2OH	CH_3
7	S_2	CH_3	H	S_3	H	CH_3	CH_2OH
8	S_4	CH_3	H	S_3	H	CH_3	CH_2OH
9	S_4	CH_3	S_3	H	H	CH_3	CH_2OH
10	S_4	CH_3	H	S_3	H	CH_2OH	CH_3
11	S_1	CH_3	H	H	OH	CH_3	COOH



O-03

LUGDUNIN – A NEW ANTIBIOTIC FROM OUR NOSE: STRUCTURE, CHEMICAL SYNTHESIS AND BIOACTIVITY

Nadine A. Schilling[1], Martin C. Konnerth[1], Alexander Zipperer[2], Hubert Kalbacher[3], Anne Berscheid[2], Heike Brötz-Oesterhelt[2], Andreas Peschel[2], Bernhard Krismer[2], Stephanie Grond[1]
[1]Institute of Organic Chemistry, [2]Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), [3]Interfaculty Institute of Biochemistry, Eberhard Karls Universität Tübingen, Germany



A *Staphylococcus lugdunensis* isolate from the human nose produces a compound with strong bactericidal activity against multi-resistant *Staphylococcus aureus*. Structure elucidation by NMR, high resolution mass spectrometry and chemical reactions revealed the hydrophobic cyclic heptapeptide, named lugdunin. Peptide synthesis was especially customized to form the thiazolidine heterocycle. The thiazolidine ring – for the first time in a cyclopeptide – and a tryptophan are crucial for full biological activity. With manifold products from our synthesis, SAR (structure activity relationship)-studies revealed nature's architectural concept of the antibacterial agent. We discuss that we have not observed resistance development yet, and present our current hypotheses of mechanisms underlying the structure-activity relationships.

O-04

DISCOVERY OF A NEW POLYKETIDE VIA CO-TREATMENT WITH AN EPIGENETIC MODIFIER AND OSMOTIC STRESS

Hope Ada Igboeli, Douglas Marchbank, Hebelin Correa, Russell Kerr. Department of Chemistry, University of Prince Edward Island

Genomic analysis of several filamentous fungi has revealed the presence of "silent" biosynthetic pathways which encode for unknown natural products. The number of these silent biosynthetic gene clusters often greatly outnumbers the number of commonly expressed natural products. This insight has fuelled the development of new strategies for natural products discovery in fungi to optimize their biosynthetic potential. Herein we de-

scribe a new method of natural product biosynthetic gene activation in fungi involving the initiation of both stress and epigenetic pathways to simultaneously up-regulate silent or trace natural products. This method has led to the discovery of a new polyketide which was isolated from the fermentation extract of *Asteromyces cruciatus* treated with suberoylanilide hydroxamic acid and salt. The polyketide was purified by reversed-phase flash column chromatography followed by preparative high performance liquid chromatography. The structure of this compound was established using one-and-two dimensional NMR experiments.

O-05

UNCOMMON MECHANISM OF A TYPE II PKS DERIVED ARYL POLYENE PIGMENT PRODUCED BY *XENORHABDUS DOUCETIAE*

Gina L.C. Grammbitter[1], Kudratullah Karimi[2], Nina Morgner[2] and Helge B. Bode[1,3]
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Aryl polyene (APE) pigments are a widely distributed class of bacterial polyketides, also present in γ-proteobacteria such as Xenorhabdus doucetiae. Nevertheless, little is known about the biosynthesis of these compounds, which are produced by an uncommon type II polyketide synthase (PKS) mechanism. We started by identifying the major yellow APE from X. doucetiae via HPLC-UV/MS. Co-purification experiments with APE enzymes revealed four stable protein complexes, three of them being new for PKS II systems. The focus of our work was to investigate the complex biosynthetic mechanism of APEs. Therefore, we isolated all enzymes involved in biosynthesis and characterised them in vitro. As a result, we reconstituted the whole APE biosynthesis by MS analyses of the ACP-bound intermediates of each reaction step. These findings not only shed light on the biosynthetic mechanism of this special PKS II system, but also on protein-protein interactions of the multi-enzyme complex.

O-06

DIAZAQUINOMYCIN BIOSYNTHESIS IN MARINE AND FRESHWATER ACTINOMYCETES

Jana Braesel[1], Brian T. Murphy[1], and Alessandra S. Eustáquio[1]
[1]University of Illinois at Chicago, College of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, Chicago, IL 60607

Tuberculosis (TB) remains a global health problem, accounting for 1.7 million deaths in 2016 and 6.3 million new cases. The most significant threat is multidrug- and extensively drug-resistant strains of *Mycobacterium tuberculosis*, which are resistant to first- and second-line drug regimes. It was recently reported that diazaquinomycins show potent and selective inhibitory activity against *M. tuberculosis*, including a panel of drug-resistant TB strains. Understanding of the genetic and molecular basis of diazaquinomycin biosynthesis can help generate structural analogs for structure-activity relationship studies.

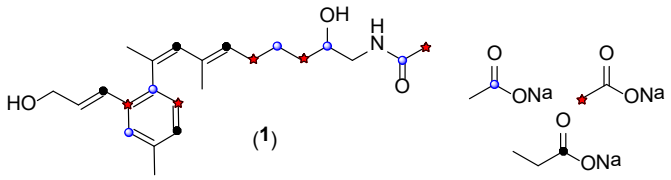
To identify the diazaquinomycin biosynthetic gene cluster, the genomes of two known diazaquinomycin producers, *Streptomyces* sp. F001 and *Micromonospora* sp. B006, were sequenced. Since automated genome mining techniques, such as antiSMASH 4.0, failed to identify a putative diazaquinomycin biosynthetic gene cluster, both genomes were compared using progressive MAUVE alignment. A 22.3 kb continuous region shared between the strains F001 and B006 was identified. This presentation will highlight our latest results towards confirming the diazaquinomycin biosynthetic gene cluster by deleting the identified region using CRISPR/Cas9. Our goal is to further study diazaquinomycin biosynthesis by a combination of gene inactivation and biochemical *in vitro* characterization of pathway enzymes. We are especially interested in the molecular basis for the different diazaquinomycin analogs produced by each strain.

O-07

EXPLORING THE CHEMICAL POTENTIAL OF GREAT SALT LAKE MICROORGANISMS

Emilio Cortes-Sanchez, Guangwei Wu and Jaclyn M. Winter.
Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT 84112

Biological pressures can influence the chemical diversity of secondary metabolites and microorganisms isolated from extreme environments have proven to ideal resources for drug discovery efforts and for characterizing novel biosynthetic pathways. The Great Salt Lake, also recognized as America's Dead Sea, is an endorheic hypersaline lake in Utah. Recently, we started a natural product drug discovery campaign aimed at interrogating halophilic bacteria isolated from this unique environment. One of our strains, *Streptomyces* sp. GSL-15, was shown to produce BE-5221 D (**1**) a new beta-hydroxy acetamide belonging to the rare class of trialkyl-substituted aromatic acids. Following de novo genome sequencing and assembly, genome mining was used to identify the biosynthetic cluster responsible for the synthesis of this unique metabolite. A 57 kb type I polyketide cluster was identified and confirmed to be responsible for the synthesis of **1** through an engineered CRISPR-Cas9 approach. Feeding experiments with various ^{13}C-labeled precursors revealed how the backbone of **1** is biosynthesized and resulted in the identification of a new offloading mechanism in polyketide biosynthesis. Investigation into this mechanism, as well as the genes responsible for the unusual carbon-carbon bond formation, is ongoing.



(**1**)

O-08

MOLECULAR CARTOGRAPHY OF FUNGUS-GROWING ANTS GARDENS REVEALS THE PRESENCE OF BIOACTIVE METABOLITES IN SITU

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

Fungus-growing ants are useful models to study the role of molecules in intraspecies interactions. We applied mass spectrometry approaches for detecting, identifying and mapping molecules from *Trachymyrmex septentrionalis* and *Atta texana* fungus gardens. Molecular mapping of *Trachymyrmex septentrionalis* fungus gardens obtained from the Eastern coast of USA enabled to visualize geographical distribution of molecules with a wide range of biological activities, from antibiosis to anticancer properties. These included fungal peptides from *Trichoderma* species; fungal sterols; plant and microbial saponins and bacterial peptides from *Pseudomonas* species. Molecular mapping of lab reared *Atta texana* fungus garden

enabled us to visualize 3D spatial distribution of flavonoid-related plant metabolites, which suggests biotransformation processes occurring *in situ*. Thus, molecular cartography of fungus gardens provided insights into the chemical diversity of natural products *in situ*, a necessary step for revealing the biological role molecules play in natural environments.

O-09

ANTIFUNGAL PEPTIDES FROM MICROASCUS ALVEOLARIS ACTIVE AGAINST PHYTOPATHOGENIC FUNGI.

Serge Fotso[1], Paul Graupner[1], Quanbo Xiong[1], Jeffrey R. Gilbert[1], Don Hahn[1], Avila Adame Cruz[1], George Davis[1] and Kengo Sumiyoshi[2]
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Agricultural crops face tremendous yield and quality losses due to pests, pathogenic diseases and weed damages. In addition, the emergence and spread of disease resistance to chemical agents used in modern agricultural to control pathogens and pests is a growing concern for the farmers and scientists. Added to those challenges, the stringent regulatory pressure on some synthetic pesticides (withdraw or usage restriction), the modern agricultural practice is facing a global food challenge to meet the growing need of food production to feed 9 billion people by 2050. Natural products can play a crucial role in the discovery of new Ag active molecules. Nature produces many compounds with biological activity such as fungicide, insecticide and herbicide which may present high acceptable environmental and fate profile. The Corteva Natural product (NP) program intent to utilize that advantage of NP to solve these challenges that are facing modern agriculture. Through our process, the investigation of the crude extract from the fungi *Microascus alveolaris* strain PF1466 led to the isolation of the known scopularide and three new cyclodepsipeptides named alveolarides A, B and C. Alveolaride A provided strong *in vitro* activity against the plant pathogens *Pyricularia oryzae*, *Zymoseptoria tritici* and *Ustilago maydis*. The structures and biological activities of these compounds will be presented.

O-10

MYXOBACTERIA DEGRADE ACYLHOMOSERINE LACTONE QUORUM SIGNALS AND PREVENT PSEUDOMONAS PUTIDA BIOFILM FORMATION

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Quorum quenching enzymes such as acylhomoserine lactonases (AHLases) are typically associated with Gram-negative bacteria that participate in quorum signaling and facilitate maintenance of acylhomoserine lactone (AHL) metabolic flux. We propose that myxobacteria utilize these AHLases, likely acquired via horizontal gene transfer, to disrupt the quorum signaling networks of neighboring prey. Investigated AHLases from *Archangium* sp. strain Cb G35 and *Cystobacter ferrugineus* strain Cfe23 were chosen due to their sequence similarity to AHLases associated with the genus *Pseudomonas*. Both AHLases were capable of degrading a variety of AHLs including 3-oxo-C12-AHL the regulatory quorum signal involved in *Pseudomonas putida* biofilm formation. Addition of purified AHLase from *C. ferrugineus* to *P. putida* cultures not only prevented biofilm formation but also provided denser cultures when compared to AHLase-unexposed controls. We propose that predatory myxobacteria utilize quorum quenching enzymes to prevent organized, multicellular prey behaviors that are recalcitrant to predation such as biofilm formation.

O-11

A RANDOMIZED, PLACEBO-CONTROLLED, CLINICAL TRIAL TO EVALUATE THE EFFICACY OF A NOVEL FENUGREEK SEED EXTRACT ON PHYSICAL FITNESS AND SPORTS NUTRITION

Anand Swaroop,[1] Sreejayan Nair,[2] Derek T. Smith,[3] Scarlet L. Barnes,[3] Boyi Dai,[3] Timothy J. Robinson,[4] Rama P. Nair[5] and Debasis Bagchi[1,6]
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Background: Our previous studies demonstrated that a saponin rich isolate from the spice fenugreek (*Trigonella foenum-graecum*) improved glucose tolerance, insulin sensitivity and cardiometabolic parameters in diabetic mice. The current study was aimed at evaluating the effect of a novel fenugreek seed extract enriched in 20% protodioscin (Furosap®, FS) supplementation on body mass, serum testosterone levels, cardiorespiratory endurance and muscle strength in healthy human subjects. **Methods:** A prospective, double blind, placebo-controlled, randomized trial was performed in healthy male human subjects (n= 40, 24.02±3.9 years), who received FS or placebo capsules (250 mg, twice daily) for 12 weeks. Prior to and following intervention, hand-grip, body fat mass/lean mass/fat distribution, upper and lower body strength and maximal graded exercise stress testing were determined using a digital hand dynamometer, dual-energy X-ray absorptiometer (DEXA), force plate, and treadmill with open-circuit spirometry, respectively. Testosterone levels and C-reactive proteins were determined in the serum samples. **Results:** Mean lean mass, fat-free mass and total serum testosterone levels were significantly elevated in the group receiving FS. Furthermore, a tendency towards lowering blood pressure was observed in the FS-treatment group. No changes were observed in grip-strength, jump height, peak jump force and push-up force between the treatment or placebo groups. Similarly, measures of cardiorespiratory endurance (heart rate, VO_2 max, peak and submaximal respiratory exchange ratio), systolic and diastolic blood pressures, and fat-mass remained unchanged in both groups following intervention. **Conclusions:** FS supplementation over a period of 12 weeks increased lean body mass and total testosterone levels. No adverse events were reported. Further long-term studies in larger population are necessary to validate these findings.

O-12

NOVEL HOMOISOFLAVONOIDS AS INHIBITORS OF OCULAR ANGIOGENESIS

Hannah Whitmore[a], Dulcie Mulholland[a], Sianne Schwikkard[b], Timothy Corson[c], Kamakshi Sishtla[c], Walter Knirsch[d], Wolfgang Wetschnig[d]
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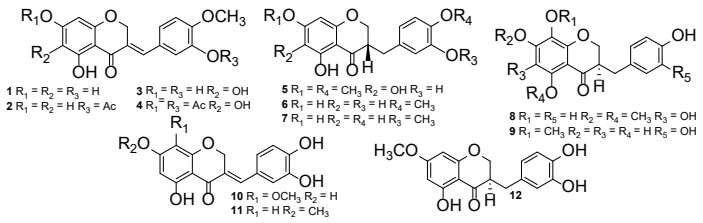
Novel homoisoflavonoids were extracted from *Rhodocodon intermedius* (**1**,**3**,**5**), *R. cryptopodus* (**6**), *R. rotundus* (**7**), *Eucomis autumnalis* (**8**), *E. comosa* (**9**,**10**) and *Massonia bifolia* (**11**,**12**). These, along with acetate derivatives, **2** and **4**, have been tested in cell proliferation assays to determine their anti-angiogenic abilities, producing excellent results. Anti-angiogenic compounds are of interest in the treatment of retinopathy, a major cause of blindness in patients with diabetes and 'wet' age-related macular degeneration. The absolute configuration of compounds **5-7** was found to be *S* at C-3, unusual for 3-benzyl-4-chromanones from the Hyacinthaceae. The structures and biological activity of novel natural-source and synthetic homoisoflavonoids will be presented.



O-13

DISCOVERY OF ANTI-AVIAN INFLUENZA COMPOUNDS FROM *EURYA NITIDA* AND *MAESA PERLARIUS*

Nga Yi Tsang[1], Wan-Fei Li[1], Yang Zhao[1], Eric Yip[2], Yi Guan[2], and Hong-Jie Zhang[1,]*

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Viral infections cause various human diseases that threaten public health. Avian influenza (AIV) refers to influenza caused by viruses adapted to birds. The largest and most deadly pandemic in recorded history occurred in 1918 with an estimated death toll of more than 40 million people worldwide, which was caused by an H1N1 subtype influenza A virus. H5N1 is another deadly pathogenic avian influenza subtypes that cause severe disease and death both in human and poultry. Herbal medicines provide vast resources for the development of novel antiviral drugs. We have recently identified two anti-bird flu entry active plant leads (*Eurya nitida*, Theaceae; *Maesa perlarius*, Myrsinaceae) from evaluation of >2,000 plant extracts. Bioassay-guided separation of the MeOH extracts of the stems of the two plant has led to the identification of a number of anti-avian flu compounds. The isolated compounds have been evaluated against the influenza viruses of H1N1, H3N2, H5N1 and H9N2. *Acknowledgements: The work described in this paper was supported* by *the Health and Medical Research Fund (12132161) of the Food and Health Bureau, Hong Kong SAR, and the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/15-16/02).*

O-14

THE IMMUNOSUPPRESSANT BRASILICARDIN: HETEROLOGOUS EXPRESSION, REVISION OF THE GENE CLUSTER AND MUTASYNTHESIS STUDIES

Anina Buchmann[1], Michael Eitel[2], Pierre Koch[2], Paul N. Schwarz[3], Evi Stegmann[3], Wolfgang Wohlleben[3], Marcin Wolański[4], Michał Krawiec[4], Jolanta Zakrzewska-Czerwińska[4], Carmen Méndez[5], Alma Botas[5], Francisco Morís[6], Jesus Cortés[6], and Harald Gross[1]

[1]Department of Pharmaceutical Biology, University of Tübingen, Tübingen, Germany, [2]Department of Pharmaceutical Chemistry, University of Tübingen, Tübingen, Germany, [3]Department of Microbiology and Biotechnology, University of Tübingen, Tübingen, Germany, [4]Department of Molecular Microbiology, University of Wroclaw, Wroclaw, Poland, [5]Department of Functional Biology, University of Oviedo, Oviedo, Spain, [6]EntreChem SL, Oviedo, Spain.

Brasilicardin A (BcA) is a novel and potent immunosuppressant consisting of a unique diterpene *anti/syn/anti*-perhydrophenanthrene skeleton with two sugar residues, an amino acid-like portion, and a hydroxybenzoate moiety, which is produced in low quantity by the human pathogen *Nocardia terpenica*. BcA is comparable in potency with standard immunosuppressants such as cyclosporine or tacrolimus, but is less toxic due to a novel mode of action. In order to develop a safe biotechnological platform for the production of brasilicardin analogs in high yields, a fosmid containing the putative brasilicardin gene cluster was introduced into more than 70 bacterial strains belonging to the risk group 1. Heterologous expression of the putative brasilicardin gene cluster led to the production of non-glycosylated or rhamnosylated brasilicardins in most of the strains tested, with one strain producing ~300 mg/L of rhamnosylated brasilicardins. Subsequent genetic engineering measures led to gram-scale production of brasilicardins. In order to gain insight into the biosynthesis of brasilicardin, the genome of the natural producer was sequenced and a systematic gene deletion study was conducted. This led to the revision of the so far proposed brasilicardin biosynthetic pathway and opened up the possibility for mutasynthetic studies. Application of the latter strategy, yielded a broad spectrum of 17 new brasilicardin derivatives.

O-15

DESIGNED BIOSYNTHESIS OF A NATURAL PRODUCT OF UNKNOWN ORIGIN

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Serofendic acid (SA) is a natural product isolated from fetal calf serum with a chemical structure that suggests plant or microbial origin. Initially isolated due to its nanomolar neuroprotective activity, SA shows potent activity preventing damage from myocardial and cerebral ischemia. It is a preclinical lead for the development of drugs to treat victims of stroke or heart attack. Here we report the design and validation of a synthetic metabolic pathway that affords a late stage intermediate, *ent*-atiserenoic acid in a *Streptomyces* host, at titers exceeding 500 mg/L titers. We demonstrated the utility of this platform by synthesizing SA and analogs that are more potent than clinically approved anti-apoptotic compound, ursodeoxycholic acid.

O-16

CROSSROADS OF ANTIBIOTIC BIOSYNTHESIS AND RESISTANCE

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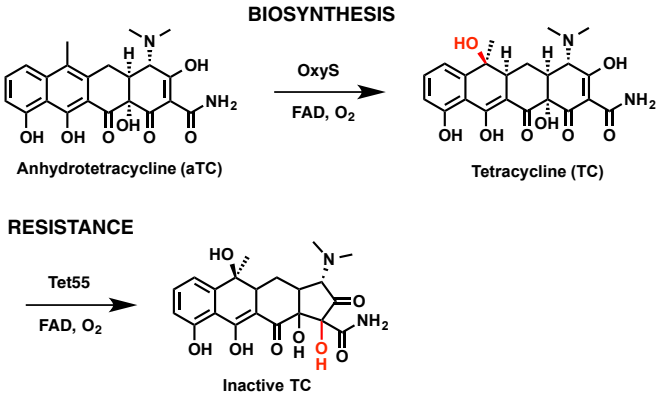
Antibiotic biosynthetic gene clusters (BGCs) often contain genes encoding for self-protection mechanisms employed during antibiotic production. These self-protection genes can become antibiotic resistance genes (ARGs) in human pathogens following horizontal gene transfer. We recently discovered an emerging family of tetracycline ARGs encoding for tetracycline inactivating enzymes ("tetracycline destructases") using functional screens of metagenomic samples. The tetracycline destructases are flavin monooxygenases (FMOs) that covalently inactivate tetracycline antibiotics via scaffold oxidation. We determined the structural and mechanistic basis for tetracycline oxidation by several tetracycline destructases, which suggests an evolutionary origin for these resistance enzymes in tailoring FMOs from type II polyketide BGCs of polycyclic natural product scaffolds with a high degree of *C*-hydroxylation. The story of the tetracycline destructases will be presented to highlight how the delicate balance between enzymatic construction (biosynthesis) and destruction (resistance) of antibiotics can drive microbial selection and inspire clinical solutions for antibiotic resistance.



O-17

METABOLITES FROM A FISH GUT FUNGUS CHALLENGE THE DEFINITION OF WHAT IT IS TO BE A NATURAL PRODUCT?

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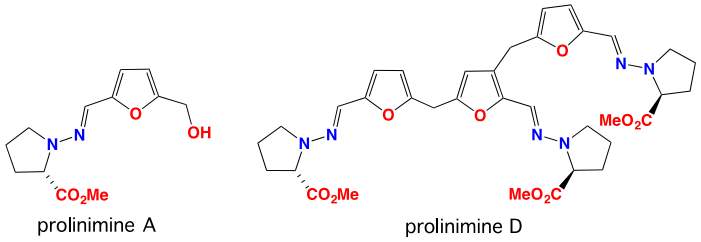
Cultivation trials on a fungus, *Trichoderma* sp. CMB-F563, isolated from the gastrointestinal tract of a mullet purchased from a local market, revealed a remarkable level of chemical diversity. The prolinimines A-D, observed exclusively on rice media, were identified as an unprecedented set of *N*-amino-L-proline methyl ester (hydrazine) Schiff bases. Structures, inclusive of absolute configuration, were assigned by detailed spectroscopic analysis, chemical degradation and total synthesis, with dimeric and trimeric prolinimines C-D identified as isolation artefacts of monomeric prolinimines A-B. Intrigued by this structure class, we developed chemical probes to detect the putative biosynthetic precursor, *N*-amino-L-proline methyl ester. These studies revealed an unexpected level of chemical reactivity and biosynthetic complexity, challenging our perceptions on the relationship between microbes and culture media, and prompting a re-evaluation of the very definition of *what is to be a natural product*.



O-18

BIOACTIVE POLYKETIDE TRANSFORMATIONS IN BACTERIAL PATHOGENS AND IN HUMANS

Hyun Bong Park[1,2], Tyler Goddard[1,2], Joonseok Oh[1,2], Corey E. Perez[1,2], Brandon Mercado[1], and Jason M. Crawford[1,2,3]*
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Gammaproteobacterial pathogens belonging to the *Photorhabdus* genus participate in a multilateral symbiosis with nematodes, insects, and in some cases, humans. *P. luminescens* establishes an infection in the circulatory fluid of insect larvae, whereas *P. asymbiotica* can also cause systemic infections in humans, such as bloodstream infections (bacteremia and sepsis). Under cellular stress cultivation conditions mimicking host pathogenesis, we observed the stimulation of a novel polyketide harboring a complex 6,6,5,3,6 pentacyclic core, which we named the carbocyclinone core. We also identified a conserved *Photorhabdus* oxidase that initiates an oxidative cyclization sequence – an electrocyclization followed by a Diels Alder cyclization – utilizing two achiral aromatic polyketide substrates *en route* to product formation. The resulting product contains 6-stereocenters and includes a strained cyclopropane in conjugation with quinone and pseudo-quinone moieties. Humans contain a distant homolog of the oxidase, and whole blood could similarly catalyze carbocyclinone conversion. Other related polyketides, including ones found in dietary plants and are known to access the bloodstream, also served as suitable substrates. Model chemical studies demonstrated that the cyclopropane serves as an electrophilic site, likely underscoring its mode of action. Through structural and biological considerations, we speculated that the carbocyclinones might inhibit blood coagulation. Coagulation participates in innate immunity and pathogen defense in both insects and humans. In a *Mycobacterial* model of coagulation, the product was more potent than the anticoagulant drug warfarin, yet it did not display eukaryotic cytotoxicity in our assays. We discuss the structure, biosynthesis, genetics, and biomedical implications of the carbocyclinones from insects to humans.

O-19

NATLIFE 2020: DISCOVERING TASTE MODULATING NATURAL PRODUCTS FOR HUMAN HEALTH APPLICATIONS

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Contemporary dietary patterns, including the overconsumption of salt, sugar, and fats, have paved a new landscape of nutrition and health science. Numerous salt, sugar, and fat replacing ingredients have been discovered, however a vast majority are curtailed by undesirable off-tastes or untenable production pathways. As part of the NatLifE 2020 strategic alliance, funded in part by the German Federal Ministry for Education and Research (BMBF), AnalytiCon Discovery is looking to the natural products of edible plants in order to discover and develop a new generation of sustainably produced taste modulating ingredients. This case study describes the high throughput screening of natural compounds for bitter masking properties, and the discovery of a lead compound which blocks the bitter off-notes of important sugar replacing ingredients such as saccharin and acesulfam K.

O-20

WHAT'S OLD AND NEW AT THE LLOYD LIBRARY: AMERICA'S INDEPENDENT PHARMACOGNOSY LIBRARY

Patricia Van Skaik
Executive Director of the Lloyd Library and Museum

Lloyd Brothers Start a Pharmacognosy Library (1870s)

- Pharmacognosy in the 19th Century (Eclectic Medicine)
- Lloyd Collections Off the Beaten Path
- Research, Development & Manufacture of Natural Pharmaceuticals
- Research Publications

Fast Forward 140 Years-Grow, Connect, Sustain and Impact

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- Advancing Scholarship-Fellows
- Educating through Lectures & Exhibits
- Forming Partnerships & Celebrating

O-21

INVESTIGATION OF BIOACTIVE CHEMICAL ENTITIES FROM MARINE MICROORGANISMS

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The marine environs consist of a rich untapped biodiversity that has shown to produce a wide range of natural products with novel structural features and interesting biological activities. The marine microorganisms, for example, have yielded compounds with new structural classes and bioactivities. A few of these include FDA approved drugs with antibiotic, antiviral and antitumor activities. In our previous work, we have isolated 12 marine microorganisms that have exhibited antimicrobial activities. Of the twelve, the extracts of two (GM03 and GM06) displayed activities against triple negative breast cancer (TNBC) cell lines. Additionally, coculture experiments were carried out with microorganisms GM01 and GMN6 that exhibited antimicrobial activities in mixed cultures. The purification and identification of the bioactive chemical entities from the above extracts are currently under investigation.

O-22

MENSACARCIN AFFECTS MITOCHONDRIAL FUNCTION SELECTIVELY IN MELANOMA CELLS

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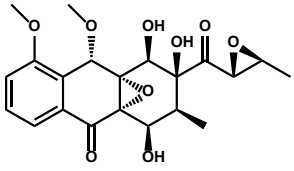
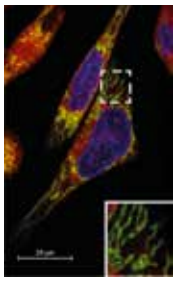
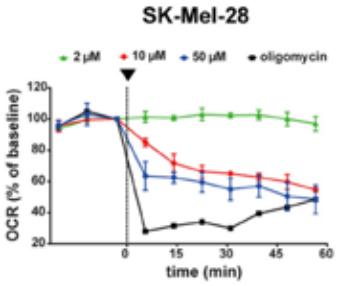
Department of Chemistry, Oregon State University, Corvallis, OR 97331, USA.

Mensacarcin is a highly produced bacterial natural product with unique cytotoxic and cytostatic effects. A combination of molecular and cell-based assays was used to provide insight into mensacarcin cytotoxicity and selective induction of cell death in melanoma. First, different cell proliferation and cytotoxicity assays as well as western blot analysis show that mensacarcin induces caspase depending apoptosis in melanoma cells but not in the non-sensitive colon carcinoma cells. Secondly, subcellular compartmentalization studies using confocal microscopy was used to determine mensacarcin's site of action. Fluorescently-labeled mensacarcin retains activity and co-localizes in mitochondria within minutes where it affects energy metabolism. Finally, live cell bioenergetic flux experiments were used to determine how mensacarcin induces mitochondrial dysfunction.







O-23

METAGENOME SEQUENCING OF THE NEW ZEALAND MARINE SPONGE MYCALE HENTSCHELI UNCOVERS THE BIOSYNTHETIC PATHWAYS FOR THE CYTOTOXIC POLYKETIDES MYCALAMIDE AND PATEAMINE

*Matthew A. Storey[1], David F. Ackerley[1], Robert A. Keyzers[2], Peter T. Northcote[3] and Jeremy G. Owen[1]**
[1] School of Biological Sciences, Victoria University of Wellington, New Zealand; [2] School of Chemical and Physical Sciences, Victoria University of Wellington, New Zealand; [3] Ferrier Research Institute, Victoria University of Wellington, New Zealand.

The New Zealand marine sponge *Mycale hentscheli* is the source of the cytotoxic polyketides pateamine, peloruside and mycalamide. By using a combination of Illumina and PacBio sequence data, coupled to metagenome binning and hybrid assembly, we have resolved complete genomes for the major sponge symbiotic bacteria living in association with a *M. hentscheli* specimen. Here we describe the primary and secondary metabolic characteristics of the microbiome of *M. hentscheli*, and present complete biosynthetic pathways for mycalamide and pateamine.

O-24

NEW NATURAL PRODUCTS FROM HAWAIIAN MARINE AND ENDOPHYTIC FUNGI

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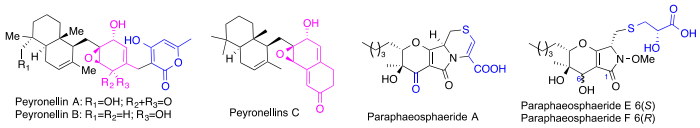
Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, HI, USA

The Hawaiian Islands are the most remote dry land on earth. They are located almost 2,400 miles from California, 3,800 miles from Japan, and 2,400 miles from the Marquesas Islands, from which the first settlers arrived in Hawaii around 300-400 AD. The natural resources of Hawai`i are unique due to its mid-oceanic environment with ecologically rich habitats, which present a wide variety of terrestrial ecosystems including tropical rain forests, coastlines, and marine life. From literature and unpublished sources, approximately 21,383 species have been recorded from the Hawaiian Islands and surrounding waters, of which 8,759 are endemic to the Hawaiian Islands, and 4,532 are nonindigenous species. Of these, approximately 15,000 species are terrestrial, 300 are found in freshwater, and 5,500 are marine-inhabiting. Literature search also revealed that Hawaiian microorganisms, especially marine and endophytic fungi are under-explored. Investigation of Hawaiian fungi isolated from marine and plants led to the discovery of many new and diverse secondary metabolites, for examples peyronellins and paraphaeosphaerides.



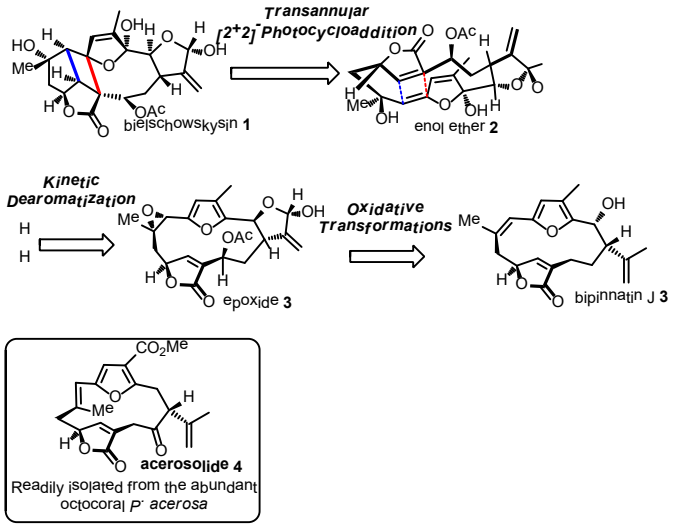
O-25

A KINETIC DEAROMATIZATION STRATEGY FOR AN EXPEDIENT BIOMIMETIC ROUTE TO THE BIELSCHOWSKYSIN SKELETON

Paul Scesa[1], Medhi Wangpaichitr[2], Niramol Savaraj[2], Lyndon West[1], and Stephane Roche[1]

[1]Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, Florida 33431, USA, [2]University of Miami School of Medicine, 1475 NW 12 Ave, Miami, FL 33136 USA

Bielschowskysin (**1**), the flagship of the furanocembranoid diterpene family, has attracted attention from chemists owing to its intriguing and daunting polycyclic architecture and medicinal potential against lung cancer. The high level of functionalization of **1** poses a considerable challenge to synthesis. Herein, a stereoselective furan dearomatization strategy of furanocembranoids was achieved via the intermediacy of chlorohydrins. The stereochemical course of the kinetic dearomatization was established, and the C3 configuration of the resulting *exo*- enol ether intermediates proved to be essential to complete the late- stage transannular [2+2] photocycloaddition. Overall, this biomimetic strategy starting from the natural product acerosolide (**4**) featured an unprecedented regio- and highly stereoselective furan dearomatization, which provided rapid access to the pivotal *exo*- enol ethers en route to the intricate bielschowskyane skeleton.



O-26

HIGHLY CONVERGENT TOTAL SYNTHESIS AND ASSIGNMENT OF ABSOLUTE CONFIGURATION OF MAJUSCULAMIDE D, A POTENT CYTOTOXIC METABOLITE FROM *MOOREA SP.*

Eduardo J.E. Caro-Diaz[1], Fred Valeriote[2], and William H. Gerwick[1,3]

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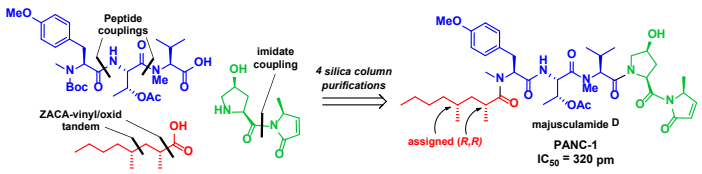
We describe the first total synthesis of majusculamide D, a lipopentapeptide isolated from *Moorea sp.* that has shown potent anti-cancer activity. Our strategy was to produce large amounts of compound *via* a scalable synthesis taking advantage of a convergent route and minimal number of purifications by making use of simple work-ups for the production of large amounts of synthetic intermediates. We have also determined the absolute configuration of the 1,3-dimethyloctanamide motif and achieved by an elegant synthesis of this buliding block *via* ZACA chemistry.



O-27

USING METABOLOMICS TO DISCOVER NATURAL PRODUCTS FROM CULTURED CYANOBACTERIA

Camila M. Crnkovic[1], Aleksej Krunic[1], Daniel S. May[1], Diana Kao[2], Tyler A. Wilson[3], James R. Fuchs[3], Joanna E. Burdette[1], Nicholas H. Oberlies[2], Jimmy Orjala[1]

[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612, [2]Department of Chemistry & Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27402, [3]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210

The discovery of natural products has been traditionally based on bioassay-guided approaches. Recently, *omics* techniques have added new tools to the field of natural product discovery. Cyanobacteria are chemically rich organisms shown to produce valuable metabolites. In this study, we applied two strategies using mass spectrometry metabolomics to explore the chemical diversity of cultured cyanobacteria. In the first strategy, *in situ* metabolomics using a droplet–liquid microjunction–surface sampling probe (droplet probe) coupled with UPLC-UV-HRMS/MS led to the discovery of calothrixamides A and B from strain *Calothrix* sp. UIC 10520. In the second approach, comparative metabolomics using UPLC-HRMS was applied to analyze three strains growing in four liquid media that contained different levels of phosphate and nitrate. Among many relevant observations, these experiments revealed a new linear peptide, named scytoamide, produced in increased amounts by strain *Scytonema* sp. UIC 10036 under low nitrate and high phosphate conditions. Calothrixamides A-B and scytoamide were elucidated by HRMS, MS/MS, and 1D and 2D NMR, along with chemical degradation and derivatization reactions.

O-28

THE NCI PROGRAM FOR NATURAL PRODUCT DISCOVERY

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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. Importantly, this national resource is available to the research community for the screening of extracts and the isolation of bioactive natural products. However, despite the success of natural products in drug discovery, compatibility issues that make crude natural product extracts challenging have reduced enthusiasm for the high-throughput screening (HTS) of crude natural product extract libraries in targeted assay systems. To address

these limitations and make the NCI's Natural Products Repository more amenable to HTS, we have initiated the prefractionation of extracts using an automated, high-throughput robotics platform capable of generating a library of 1,000,000 partially purified extracts. The talk will discuss this and other efforts to increase the utility of the NCI Natural Products Repository.

O-29

CHARACTERIZATION OF CONSERVED ACTINOBACTERIAL MYCOSPORINE-LIKE AMINO ACID GENES THROUGH HETEROLOGOUS EXPRESSION

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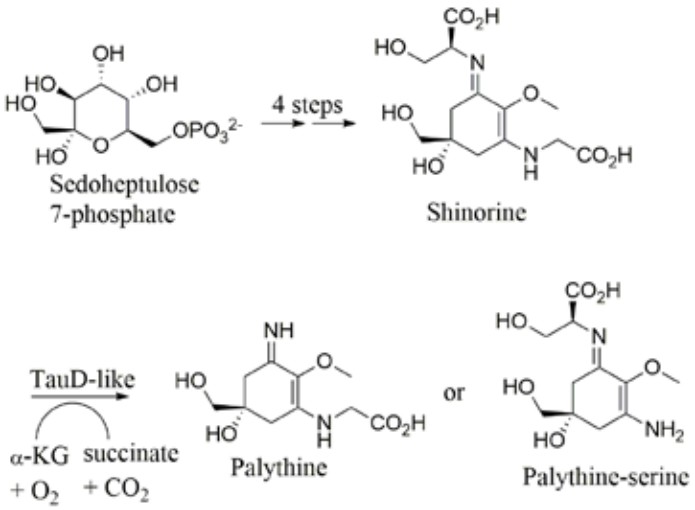
Mycosporine-like amino acids (MAAs) are sunscreen compounds identified in algae, bacteria, and marine invertebrates. MAAs are formed by a four-step pathway that uses sedoheptulose 7-phosphate as substrate where it undergoes cyclization, methylation, and two amino acid condensations. Over 30 MAAs have been identified, yet the known pathway cannot explain all this diversity. Genome mining identified three putative genes encoding a haloacid dehalogenase, a taurine dioxygenase (TauD-like), and a membrane protein conserved within actinobacterial MAA operons. The *Rhodococcus fascians* D188 MAA operon was cloned into fragments with and without the putative genes to determine their function by heterologous expression in *Streptomyces coelicolor* M1152. The MAA shinorine was found in all cultures, but expressions that contained the *tauD*-like gene also formed the MAAs palythine and palythine-serine. Thus, the TauD-like enzyme is needed for cleaving the amino acid C-N bond to form palythines, whose biosynthesis was previously unknown.



O-30

OLD DRUGS, NEW TRICKS: NON-MITOTIC EFFECTS OF MICROTUBULE TARGETING AGENTS IN CANCER CELLS

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The discovery and development of microtubule targeting agents (MTAs), including the taxanes, vinca alkaloids, ixabepilone, and eribulin, represent some of the most notable success stories for natural products drug discovery. The clinical anticancer efficacy of these drugs has long been attributed strictly to their antimitotic activities and they are commonly referred to as "antimitotics." However, increasing evidence of the non-mitotic effects of MTAs on oncogenic signaling has prompted a reevaluation of their mechanisms of action. The finding that individual patients can respond differently to the 4 classes of MTAs used to treat breast cancer prompted evaluations into the distinct effects of these drugs on oncogenic signaling pathways. We evaluated early events that link MTA-induced microtubule disruption to the signaling pathways involved in the epithelial-mesenchymal transition (EMT), an important event in oncogenesis. Striking differences were identified among MTAs in their ability to disrupt Src-dependent E-cadherin internalization and TGF-β signaling. Within 2 h of treatment, eribulin has unique effects on the localization of E-cadherin. Additionally, eribulin and vinorelbine inhibit expression of key transcription factors that drive EMT as compared to a taxane. These findings demonstrate the ability of diverse MTAs to rapidly and differentially alter non-mitotic oncogenic signaling. Identification of differences among MTAs might facilitate the rational selection of specific drugs depending on tumor characteristics. These studies highlight the importance of continually revisiting the mechanisms of action of compounds, particularly in light of clinical observations. Studies were funded by Eisai Inc.

O-31

TWO-DIMENSIONAL QUANTITATIVE NUCLEAR MAGNETIC RESONANCE FOR DETERMINATION OF COMPONENT CONCENTRATIONS IN COMPLEX MIXTURES

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Numerous applications of Nuclear Magnetic Resonance Spectroscopy (NMR) for quantitative analyses of chemical mixtures have been utilized. 1D ^{1}H-NMR typically provides the highest sensitivity analyses with excellent linear response to component concentrations which can be converted to absolute concentrations by reference to an internal standard (including the solvent) or an external reference via the PULCON method. Analyses of complex mixtures via 1D-NMR however are often hindered by resonance overlap. Utilization of ^{1}H-^{13}C heteronuclear correlation (HSQC) provides excellent dispersion along the ^{13}C chemical shift dimension, thereby greatly reducing peak overlap. The added dispersion along with the high reproducibility of NMR enables molecular identification with high confidence even with closely related molecules. The HSQC cross-peak intensity responses are influenced by resonance-specific signal attenuation and therefore the volumes for different cross peaks from the same molecule can vary. Nevertheless, by utilizing the same experimental parameters for any set of standards and unknowns, the areas for the corresponding resonances scales linearly with molar concentrations. This study investigates the quantitative accuracy of HSQC on synthetic model mixtures. In addition, it will be demonstrated that Quantitation via Heteronuclear Multiple Bond Correlation (Q-HMBC) experiments can be performed utilizing the PULCON method. Applications to natural product extracts and formulations and implementation into automated analysis methods will be presented.

O-32

NMR CHARACTERIZATION OF COMPLEX NATURAL PRODUCTS: NEW TOOLS FOR ASSIGNING NOVEL MOLECULAR SCAFFOLDS

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NMR provides powerful structural elucidation tools that are particularly well suited for natural products studies. Comprehensive spectroscopic characterization of a native metabolite may be sufficient to fully assign a planar structure. However, assignment of the relative and absolute configuration of a molecule when there are multiple stereogenic centers often requires the development and application of additional experimental strategies. Many successful approaches in this regard rely on the formation of appropriate derivatives for more detailed NMR study. Since natural products are often obtained in very limited quantities, micro-scale chemical manipulations and the ability to analyze the structure of the resulting products is often key to the complete structural and configurational assignment of complex metabolites. Anisotropic NMR parameters such as residual dipolar coupling (RDC) and residual chemical shift anisotropy (RCSA) provide a powerful and complementary means to help assign and verify structures deduced from conventional NMR analyses. Application of a wide spectrum of NMR techniques and methodologies will be described in the structural elucidation of two novel natural product scaffolds, each of which contain multiple stereogenic centers.

O-33

IDBAC: A MALDI-TOF MS PLATFORM TO CREATE DIVERSE MICROBIAL LIBRARIES

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In the course of a nearly century-long global effort to discover new bacterial-derived antibiotics from the environment, there have been few innovations to the way that researchers have collected samples and subsequently created microbial libraries sourced for therapeutic discovery. As a result, it is difficult to discover novel antibiotic scaffolds due to the degree of taxonomic and chemical redundancy that exists in these strain libraries. To address the need for isolating novel taxa from environmental samples, we developed a high-throughput matrix assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) technique that allows us to readily group bacterial colonies by putative taxonomic identity and further discriminate them based on *in situ* natural product production. In May 2017, we embarked on a collection expedition to Iceland, which has a unique geology and geographical position in the North Atlantic Ocean. Using SCUBA and sampling off of vessels, we collected eighty-six samples from thirty sites. After purifying 1,607 strains from the samples that we collected, we acquired consecutive protein and specialized metabolite MS spectra from single colonies of these strains. In approximately four hours we are able to prepare, acquire data for, and visualize 384 colonies using our semi-automated, freely available IDBac bioinformatics pipeline. This pipeline will be presented.

O-34

LEVERAGING METABOLOMICS ACROSS HUNDREDS OF ACTINOMYCETE STRAINS TO CALIBRATE EMERGING BIOINFORMATICS PLATFORMS

James H. Tryon[1], Jorge C. Navarro-Muñoz[2], Elizabeth Parkinson[3], Michael Mullowney[1], Nelly Selem-Mojica[4], Emmanuel L.C. De Los Santos[5], Marnix Medema[2], Francisco Barona-Gomez[4], Regan Thompson[1], William Metcalf[3], Neil L. Kelleher[1].
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Bioinformatic analyses of microbial genome sequences have unveiled large numbers of uncharacterized natural product biosynthetic gene clusters (BGCs). Despite growing interest in studying natural products across large datasets, no publicly available tool exists that provides in-depth BGC comparisons across massive genome libraries. During the development of BiG-SCAPE, a tool to provide the field with these analyses, we became interested in applying a large library of untargeted LC-MS/MS metabolomics data collected from hundreds of actinomycete strains to calibrate BiG-SCAPE BGC comparison metrics. A benchmarking strategy was developed using a subset of known natural products and their characterized BGCs within our dataset. Analysis of these benchmark compounds as well as correlation score distributions for thousands of unknown compounds within our dataset has been used to demonstrate the potential of BiG-SCAPE to enable large-scale genome mining efforts.

O-35

CO-CULTURE OF TWO FORMS OF A MARINE-DERIVED *ASPERGILLUS ALLIACEUS* RESULTS IN THE PRODUCTION OF ALLIANTHRONES A-F

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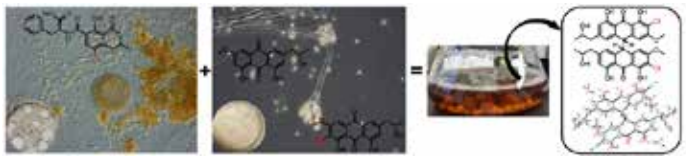
Two developmental forms of marine alga-derived *Aspergillus alliaceus* were explored as sources of new metabolites. The asexual (conidial) and the sclerotial form of this endophytic fungus can be separated and each produce their own, distinct secondary metabolite profile. After combining both fungal phenotypes in a co-culture experiment, nalgiolaxin, a chlorinated anthraquinone pigment was more abundant and bianthrones were newly produced. The structures of allianthrones A-F were determined by extensive NMR spectroscopic analysis, and supported by chiroptical properties, and X-ray crystallography. Allianthrone A and exhibited moderate cytotoxic activity against colorectal carcinoma (HCT-116) and melanoma (SK-Mel-5) and allianthrones A and D were subjected to the NCI-60 cell line panel.



O-36

COCULTURE STIMULATES ANTIBIOTIC BIOSYNTHESIS IN ACTINOMYCETES

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Actinomycetes are one of the largest producers of specialized metabolites, including many commonly used antibiotics. Under laboratory conditions, most actinomycetes only produce a fraction of the metabolites they are predicted to synthesize. The signals that activate expression of these cryptic biosynthetic gene clusters are unknown, although other bacteria may provide these signals. Based on this hypothesis, we are using coculture to mimic interactions that occur in native environments to elicit specialized metabolite production and identify novel antibiotics. We isolated actinomycetes from individual soil particles to capture interactions that could feasibly occur in the environment. We tested 11 isolates in all 55 possible pairwise combinations for antibiotic activity against *Staphylococcus aureus* using an antibiotic overlay assay. We identified multiple actinomycete pairs that displayed coculture-specific antibiotic activity, some of which were also active against a beta-lactam resistant strain of *S. aureus*. Using bioassay-guided fractionation we have obtained a semi-pure active fraction from one of the active cocultures. We are now using liquid-chromatography-mass spectrometry and NMR to identify the active compound. Our results indicate that, in microbial coculture, secreted metabolites can serve as signals that alter the expression of antibiotic biosynthetic genes. Exploring the metabolic consequences of microbial coculture may therefore allow us to identify novel antibiotics with potential therapeutic applications as well as reveal how bacteria communicate in native settings.

Poster Presentations

P-001

CO-CULTURING OF TROPICAL MARINE CYANOBACTERIA OF *MOOREA* AND *LEPTOLYNGBYA* SPECIES

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Antagonistic interaction between co-cultured organisms has been a productive method by which to activate normally silent secondary metabolite biosynthetic gene clusters. To this end, we performed a four week long co-culture experiment using four isolated, non-axenic, tropical filamentous cyanobacteria cultures. As identified by 16S rRNA sequencing, two of the cultures were *Leptolynby*a species while other two were *Moorea producens*. All four cultures were isolated from original field collections made in shallow waters near Jamaica, Palmyra atoll, American Samoa and Sulawesi. These cultures have been established as producers of potent natural products such as hectochlorin, jamaicamides, palmyramide A, leptochaelin, fagaaluamides and phormidolide. The biomass and media of each sample were harvested, dried and extracted to produce crude extracts with yields of 5-40%. We tracked the progress of the co-culture process using light microscopy, and recorded that *Moorea* filaments in *Moorea* and *Leptolynby*a co-cultures were dying, thus indicating an impact of the one species on the other. The addition of diluted crude extracts of *Leptolyngbya* or its coculture with *Moorea* did not kill *Moorea* filaments, suggesting a non-chemical reason for this phenomenon. The crude extracts of each of the experiments will be analyzed by mass spectrometry, MS^2 molecular networking, UV absorbance and biological activity to cancer cells.

P-002

NEW ALCYOPTEROSINS AND STEROIDS ISOLATED FROM AN UNDESCRIBED ANTARCTIC CORAL

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Marine invertebrates from Antarctica have been investigated for their potential natural product chemistry. Often sessile, these organisms must develop chemical protective mechanisms to survive and defend themselves against predators. The biodiversity of these organisms is of particular interest due to the extremely low temperatures and the circumpolar current around the Antarctic continent serving as an ecological isolating shield. The chemodiversity that emanates from these organisms can be a significant source of novel chemistry to be further developed into new drugs. The chemical investigation of an undescribed *Gersemia sp*. Antarctic coral has led to the isolation of two different kinds of bioactive compounds. After lyophilization of the organism, two different extractions were performed: the first one used a methylene chloride: methanol (1:1) solvent mixture, and the second one used methylene chloride only as solvent in a Soxhlet extraction. After a partition followed by normal phase Medium Pressure Liquid Chromatography, stages of normal phase and reverse phase High Performance Liquid Chromatography purifications were performed and revealed two kinds of new bioactive compounds: new acetylated sesquiterpenoids with alcyopterosin scaffolds from the first process and new acetylated steroids from the second. One and two-dimensional nuclear magnetic resonance, mass spectrometry, X-ray crystallography, and circular dichroism were the methods performed to elucidate and confirm the structures. Furthermore, biological testing against *Leishmania* sp. and ESKAPE pathogens, Zika virus, *Clostridium difficile*, and HeLa cancer cells were performed to extend the scope of drug discovery potential.

P-003

INVESTIGATING THE CYTOTOXIC SECONDARY METABOLITES OF AN AMERICAN SAMOAN MARINE CYANOBACTERIA *LEPTOLYNGBYA* SP.

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Marine cyanobacteria have emerged as one of the most prolific producers of structurally unique and biologically active marine natural products. In this regard, living collections of the cyanobacterium *Leptolyngbya* sp. ASX22JUL14-1 were made in American Samoa and maintained in laboratory culture. The secondary metabolites of this culture were determined to be of interest due to in vitro cytotoxicity of the organic extract against NCI-H460 lung cancer cells. After extended culture efforts to produce adequate biomass, LC-MS/MS based molecular networking, various forms of NMR, and cancer cell bioassay were used to guide the isolation of a novel natural product. Accelerated NMR techniques were used for faster data collection, and its structure was determined using the full assortment of spectroscopic methods.

P-004

NEW BIOACTIVE COMPOUNDS FROM SINGAPORE'S MARINE CYANOBACTERIA AND SPONGE-ASSOCIATED ACTINOMYCETES

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Filamentous marine cyanobacteria and bacteria, such as actinomycetes, are well known to be prolific sources of novel bioactive natural products for drug discovery and development. As part of a drug discovery research program in Singapore, a new cyclic depsipeptide, benderamide A, was isolated from a marine cyanobacterial bloom collected from St. John's Island. Its planar structure was deduced based on extensive 1D and 2D NMR spectroscopy while the stereochemistry was determined using Marfey's analysis. In addition to five amino acid-derived residues, benderamide A contained a polyketide unit, 2-2-dimethyl-3-hydroxyoctynoic acid (Dhoya). Preliminary biological result revealed benderamide A to exhibit 100% toxicity in the brine shrimp toxicity assay when tested at 10ppm. Cytotoxicity data of the new cyclic depsipeptide will also be presented in this study. We have recently embarked research on drug discovery from marine bacteria associated with a number of deep water marine sponge species collected at the Singapore Straits. Using culture-dependent method, a total of 187 marine actinomycetes were isolated and identified based on 16S rRNA gene sequences. About 3% of the bacterial extracts showed anti-quorum sensing activity in a dose dependent manner. Furthermore, the bacterial extracts were subjected to MS-based molecular networking analysis for dereplication and their bacterial genome analysed for the presence of novel biosyn-

thetic gene clusters. Both the metabolomic and genomic data of a marine bacterial strain #52 are presented here to illustrate the use of innovative techniques for the discovery of novel bioactive compounds.

Acknowledgments: This project is supported by the NRF MSRDP P-15 grant.

P-005

MINING NOVEL LINEAR AND CYCLIC PEPTIDES FROM AN AUSTRALIAN MARINE TUNICATE-DERIVED FUNGUS, *TALAROMYCES* SP. CMB-TU011.

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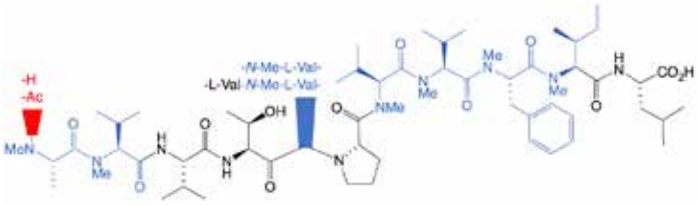
An Australian marine tunicate-derived fungus, *Talaromyces* sp. CMB-TU011, was subjected to analytical (MATRIX) cultivations to identify optimal conditions for the production of four new extensively *N*-methylated linear peptides, talaropeptides A-D (shown), and an unprecedented cyclic peptide hydroxamate, talarolide A. Identification of these peptides was achieved by detailed spectroscopic analysis, and chemical methods (C_3 and 2D C_3 Marfey's). Genomic analysis identified the talaropeptide NPRS, the 2nd largest described to date, as similar to cyclosporine.



P-006

MARINE DERIVED PROTEINS WITH POTENT BIOACTIVTY

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Natural product extracts represent a substantial repository of bioactive compounds that have resulted in treatments currently used in numerous therapeutic regimens. These drugs have largely been discovered by natural product chemists investigating the small organic solvent-soluble components of these extracts. However, clinically approved biologics from natural sources provide evidence that the discovery of novel bioactive proteins from nature can lead to viable, clinically relevant therapeutics. We examined the proteinaceous components of aqueous marine extracts from the Natural Products Branch repository for proteins with potent anticancer or antiviral activities using the NCI-60 cancer-cell panel and an in-house anti-HIV-1 assay. Extracts with activities concentrated in the proteinaceous fraction were further characterized to identify the proteins of interest. Here we report the isolation, characterization, and initial sequencing efforts of two lectins from marine aqueous extracts. Proteins isolated from both the sponge *Vagocia* sp. and the sea cucumber *Isostichopus badionotus* that displayed nanomolar bioactivities against HIV-1 and NCI-60 cancer cell lines, respectively, will be discussed.

P-007

DISCOVERY OF ANTILEISHMANIAL NATURAL PRODUCTS FROM ACTINOBACTERIA ASSOCIATED TO BRAZILIAN FUNGUS-GROWING ANTS

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In the quadripartite symbiosis in the fungus-growing ant ecosystem between three mutualist (Attine ant, fungal garden and symbiotic actinomycetes) and one parasite (specialized pathogenic fungus *Escovopsis* sp) some interspecies interactions are mediated by small molecules. The actinobacteria associated to the ant host produce secondary metabolites to inhibit this pathogen but not the crop fungus. This specific ecological function can guide the discovery of natural products potentially active against human pathogens. Indeed, Interesting bacterial-derived natural products have been reported with a wide spectrum of biological activities. In an ongoing ICBG project, we have isolated several actinobacteria strains from the exoskeleton of fungus-growing ants to prospect for active compounds against protozoan parasites such as *Leishmania donovani*. The bioassay guided fractionation of active extracts led to the isolation of antibiotics Mer-A2026B (**1**), Piericidin-A_1 (**2**) and Nigericin (**3**), from cultures of *Candidatus Streptomyces philanthi bv. triangulum* ICBG292, which showed IC_{50} of 37 μM, 42 μM and 334 nM against *L. donovani,* respectively. The compounds were also active against *Escovopsis*. Dimeric dinactin (**4**), produced by *Streptomyces puniceus* AB10, showed potent anti-*L. donovani* activity with an IC_{50} of 55 nM. Compounds **3** and **4** were more active than positive control, miltefosine (IC_{50} of 7.26 μM). Structures of additional leishmanicidal compounds are currently being established. Therefore, the ecological function of natural products from bacterial symbionts of attine ants can be aligned pharmacological activities.

P-008

CACOLIDES A-L, UNPRECEDENTED SESTERTERPENE BUTENOLIDES FROM A SOUTHERN AUSTRALIAN MARINE SPONGE, CACOSPONGIA SP.

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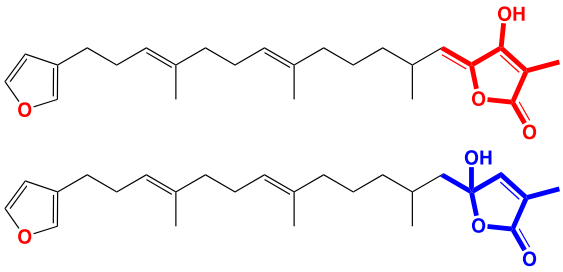
Preliminary HPLC-DAD-MS and ^{1}H NMR analysis of triturated fractions prepared from an extract of a southern Australian marine sponge, *Cacospongia* sp. (CMB-03404), suggested the presence of secondary metabolites belonging to the variabilin-*like* class of sesterterpene tetronic acid (highlighted in red). As this sponge-specific structure class is well represented in the scientific literature, initial thoughts were that this extract had little to offer in the way of structure novelty. However, a closer examination using a molecular networking (GNPS) approach, followed by careful fractionation and structure elucidation using detailed 1D and 2D NMR spectroscopic analysis, identified an array of fourteen new metabolites, spanning cacolides A-L and cacolic acids A-B. Of note, none of the cacolides were tetronic acids, but many possessed an isomeric, and very rare γ-substituted γ-hydroxybutenolide moiety (highlighted in blue).



cacolide A

P-009

DEEP-SEA ACTINOBACTERIA AS AN ATTRACTIVE SOURCE FOR NATURAL PRODUCTS

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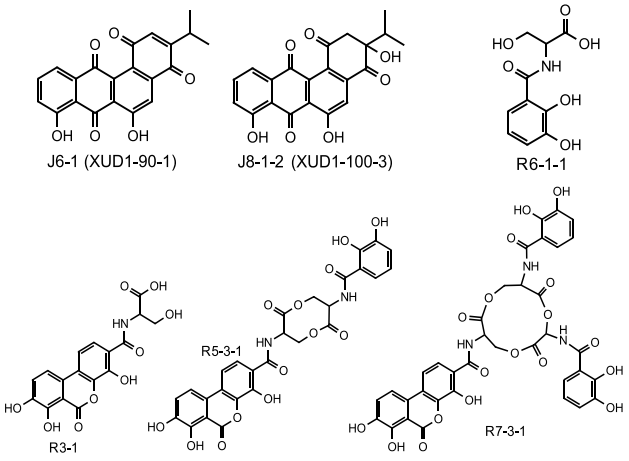
Though natural products (NPs) derived from terrestrial actinobacteria are the major source of current antibiotics, the less-exploited deep-sea Actinobacteria may serve as an unprecedented source of novel NPs. In a preliminary study of 50 Actinobacteria isolated from diverse deep-sea sponges or unique environmental niches, we found more than half of the tested strains (27) were identified as active in at least one anti-microbial assay. We also proved the rare earth salt lanthanum chloride ($LaCl_3$) is an effective elicitor. Of the 27 strains, the anti-microbial activity of 15 (>50%) were induced or enhanced by the addition of $LaCl_3$. Here, we report the identification of new metabolites (except R6-1-1) isolated from R818, a deep-sea *Streptomyces* sp. (the R series of compounds) and J378, a deep-sea *Nocardiopsis* sp. (the J series of angucyclines). A brominated derivative (structure not shown) of J6-1 showed an anti-MRSA activity at least 160-fold higher than that of J6-1. The genome of both strains were determined. Genome- and structure-guided biosynthesis of these metabolites are now being studied.



P-010

FACILE SYNTHESIS AND MICROBIAL METABOLISM OF PRENYLQUERCETINS

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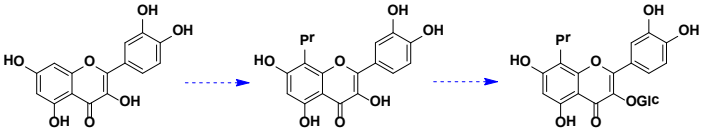
Quercetin, one of the major constituents of propolis, was found to show various biological activities. Enhancement of bioactivity of flavonoids has often been closely associated with nuclear prenylation, and thus three prenylated derivatives of quercetin were synthesized through Claisen rearrangement in the presence of boron trifluoride etherate. Microbial metabolism studies were carried out in order to identify microbial metabolites of prenylquercetins with increased water solubility and improved bioavailability. During the screening process, it was revealed that the fungus *M. hiemalis* is capable of metabolizing prenylquercetins into more polar metabolites. Antioxidant activity of the prenylated quercetin derivatives and microbial metabolites were evaluated by DPPH assay. Synthesis, isolation, structure elucidation and bioassay of the prenylquercetins and their metabolites will be presented.



P-011

BIOGENESIS OF HALOGENATED AMINO ACID BUILDING BLOCKS IN THE BIOSYNTHESIS OF THE LIPOPEPTIDE ANTIBIOTIC TAROMYCIN

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An orphan biosynthetic gene cluster from the marine actinomycete *Saccharomonospora* sp. CNQ-490 was successfully captured, engineered, and heterologously expressed, which resulted in the isolation of two novel lipopeptide antibiotics, taromycin A and B. Both molecules are highly similar to the clinically used antibiotic daptomycin, but have several structural differences. The two aromatic amino acid residues in taromycins, tryptophan (Trp) and kynurenine (Kyn), are chlorinated. Although 6-Cl-Trp has been reported in other peptides, 4-Cl-Kyn has not been previously observed in nature. The work to be presented describes *in vivo* and *in vitro* studies that elucidated the unique enzymology underlying the formation of these residues. Tar14, a new member of flavin-dependent tryptophan halogenases, was biochemically characterized and its crystal structure was solved. Tar13 (tryptophan 2,3-dioxygenase, TDO) and Tar16 (kynurenine formamidase, KF), have been identified as the enzymes that perform the unprecedented transformation of 6-Cl-Trp to 4-Cl-Kyn. Tar13 and Tar16 fall into a separate new subgroup in the phylogenetic tree of known TDO and KF homologues. The characterization of these enzymes enriches the functional diversity of TDO and KF families and provides a new strategy for discovering novel halogenated natural products by genome mining.

P-013

CHEMICAL CHARACTERIZATION, POTENTIAL ANTIOXIDANT AND NEUROPROTECTIVE ACTIVITIES OF POLYSACCHARIDES OF SOME SEAWEEDS

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This study sought to characterize and determine the antioxidant and neuroprotective potentials of polysaccharides from *Ecklonia maxima*, *Ulva rigida* and *Gelidium Pristoides*. The antioxidant activity of the polysaccharides was determined via their ability to scavenge 2,2-diphenyl -1-picrylhydrazyl (DPPH), 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS) and hydroxyl (OH) radicals. The effect of the polysaccharides was

also tested on acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and beta-amyloid ($A\beta_{1-42}$) protein. Sugars such as D-xylose, D-ribose, D-galactose, D-arabinose and L-rhamnose and L-fucose were identified in the polysaccharides via Gas Chromatography-Mass Spectrometry. The polysaccharides exhibited ABTS, DPPH and OH radical scavenging activities. The polysaccharides also reduced AChE and BChE activities *in vitro*. Electron micrographs from the transmission electron microscope revealed that polysaccharides from the seaweeds incubated with $A\beta_{1-42}$ at different intervals (0-96 h) were devoid of protein fibrils or showed very low levels of fibrils compared to the control. The observed antioxidant activity and inhibitory effects of the polysaccharides on AChE, BChE and beta-amyloid aggregation suggest that they are potential neuroprotective agents and could be explored for the treatment and management of Alzheimer's disease.

P-014

ANTIMICROBIAL SECONDARY METABOLITES FROM MARINE-INVERTEBRATE ASSOCIATED BACTERIA.

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C. difficile infections (CDIs) and methicillin-resistant *S. aureus* (MRSA) infections constitute a major health threat worldwide emphasizing the need for new drug entities. Within this scope, ecteinamycin (**1**) was isolated from a marine *Actinomadura* sp. Ecteinamycin demonstrated potent activity against *C. difficile* NAP1/B1/027 (MIC = 59 ng/μL), as well as other toxigenic and non-toxigenic strains. Chemical genomics using *E. coli* barcoded deletion mutants revealed particularly sensitive mutants such as those deficient in *trkA* and *kdpD* (potassium cation transport-related genes) suggesting that **1** likely exerts antibacterial activity via its ionophoric activity. Also, a potent bioactive spiro-napthoquinone (**2**) was isolated from a marine *Micromonospora* sp. Compound **2** demonstrated potent activity against MSSA and MRSA strains, with MICs of 0.25 μg/mL. Further *in vivo* testing and MOA studies are being pursued.

P-015

CHARACELLA PACHASTRELLOIDES; TREASURE OF THE IRISH DEEP-SEA

Sam Afoullouss[1,2], Kevin Calabro[1], Christine Morrow[2], Rob Nesbitt[1,2], Claire Laguionie-Marchais[2], Ryan Young[1,2], Bill Baker [3], Louise Allcock[2], Olivier P. Thomas[1]

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We are investigating the chemical diversity of deep-sea sponges collected from the Irish continental shelf. Sampled from between 1000 – 3000 m, these sponges inhabit a rarely explored *cornucopia* of biodiverse fauna. Due to the extreme physical conditions, these organisms have developed unique secondary metabolites with previously unseen chemical structures and characteristics. We aim to isolate these new compounds and determine their potential to be developed into new marine drugs. Our search for new compounds begins with a chemical screening process. This technique gives an overall view of the chemical diversity present in each sponge and increases our probability of finding novel compounds.

We report herein the isolation and structure elucidation of two novel epimer peptides from the sponge *Characella pachastrelloides* (Carter, 1876). Both of them (867 Da) consist of three separate moieties, a tripeptide (O-Me-Tyr, Asp, Thr), a ten-member alkyl chain capped with dimethyl substituted cyclic tetrahydropyran bonded to the α-carbon of the threonine and 2-α-aminopyranuronamide connected to the tripeptide *via* a *O*-glycosidic linkage. Both compounds are epimers which only differ with the sugar moieties, where the 2-α-glucuronamide replaces a 2-α-galacturonamide.

P-016

A NEW CYCLOPENTAPEPTIDE ISOLATED FROM THE MARINE-FACULTATIVE *ASPERGILLUS* SP. (MEXU 27854)

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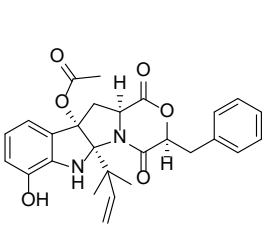
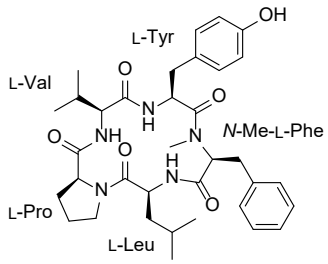
Reinvestigation of the marine-facultative *Aspergillus* sp. (MEXU 27854) yielded the known dioxomorpholine PF 1233A, along with a new cyclic *N*-methyl amino acid-containing cyclopentapeptide (**1**). Their structures were established by 1D and 2D NMR and spectrometric analysis, and the absolute configuration of **1** was elucidated via the Marfey's method on a UPLC-PDA-HRESIMS-MS/MS system.





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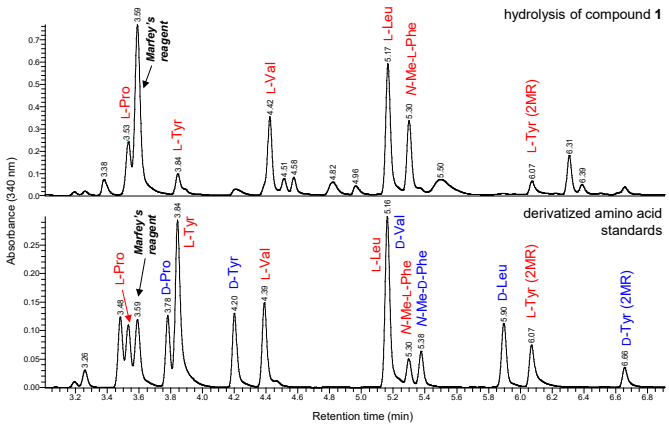

P-017

BIOACTIVE COMPOUNDS PRODUCED BY ACTINOBACTERIA ASSOCIATED WITH *ACROMYRMEX* ANTS

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Microbial symbionts are sources of novel and 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. This association can be harmed by pathogenic fungi of the genus *Escovopsis*. Based on this ecological evidence, this work aims to study natural products biosynthesized by symbiotic microorganisms associated with *Acromyrmex* leaf-cutter ants collected in remaining areas of Atlantic Forest in Brazil. We have screened bacterial strains against the specific fungal pathogen *Escovopsis* and the parasites *Trypanosoma cruzi* and *Leishmania donovani*. Two actinobacteria identified by 16S rRNA gene sequencing as *Streptomyces luridiscabiei* ICBG 328 and *Streptomyces nodosus* ICBG 197 showed antifungal activity in antagonism assay and their extracts displayed antiprotozoal activity against *T. cruzi* and *L. donovani*. Bioguided fractionation of the extracts by SPE and HPLC led to the identification of six analogous polyketides and a polyene macrolide produced by *S. luridiscabiei* ICBG 328 and *S. nodosus* ICBG 197, respectively. Analyses of HR-ESI-MS data, 1D and 2D NMR data, and searches on databases, allowed the structure determination of compounds known as chromomycins A2-3G, A3, AP, A2, 02-3D, 4B-A3 and Nystatin, the polyene macrolide. Nystatin showed antifungal activity against two *Escovopsis* strains whereas chromomycins displayed significant antiprotozoal activity against *L. donovani*.

P-018

DISCOVERY OF MARINE NATURAL PRODUCT INHIBITORS OF NEW DELHI METALLO BETA-LACTAMASE 1

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Carbapenem Resistant *Enterobacteriaceae* (CRE) is a growing health concern in desperate need of new treatment options. New Delhi metallo beta lactamase 1 (NDM-1) is the newest member of the metallo beta lactamase (MBL) family of carbapenemase enzymes that contribute to CRE antibiotic resistance to beta lactam antibiotics. NDM-1 is the most prevalent MBL CRE infection in the United States, and at present, there are no clinically relevant inhibitors for NDM-1. Our group sought to identify new inhibitors of NDM-1 from our fractionated libraries of marine natural product extracts. Expression of the NDM-1 gene on a pBAD33 plasmid in BW27749 *E. coli* cells was tightly regulated through arabinose incorporation in the media. A cell-based screen was developed from these cells to screen for inhibitors of NDM-1. Promising fractions were assessed via an LC/MS-based screen. Pure compounds were analyzed in pure protein inhibition assays with nitrocefin. Assay-guided fractionation of active fractions coupled with HRMS and NMR has led us to the discovery of new natural product inhibitors of NDM-1.

P-019

ACID-CATALYZED RING CONTRACTION OF THE MACROLACTONE RHIZOXIN

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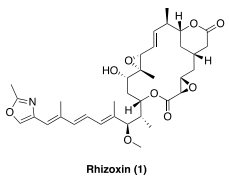
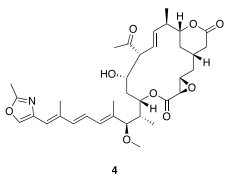
The macrolactone rhizoxin (**1**) and several analogues (**2**-**6**) were isolated from a *Rhizopus microsporus* isolate, which contained an endosymbiotic bacterium, *Burkholderia rhizoxinica*. Compound **4** contains an unprecedented 15-membered macrolactone rather than the 16-membered macrolactone present in all previously reported metabolites in this family. Investigation into the formation of **4** led to the hypothesis that the formation of the new rhizoxin analogues is dependent on the mildly acidic nature of the culture medium. Specifically, **4** is proposed to arise through a Meinwald-like rearrangement of the allylic epoxide to yield the contracted macrolactone. This report details the formation of these rhizoxin analogues and discusses their biological activities.


Rhizoxin (1)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P-020

SCREENING IRISH DEEP-SEA INVERTEBRATES FOR BIOACTIVE COMPOUNDS AGAINST THE APOPTOSOME.

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Although 95% of the sea floor is deeper than 1000 m, marine metabolites of deep-sea origin only account for 2% of described marine metabolites. With the development of new technologies we can now access and collect in these biodiverse and species-rich habitats. Ireland's territorial waters are among the largest in mainland Europe (*c.a.* 880 000 km^2), and its continental margin has a high prevalence of submarine canyons and highly diverse corals mounds, dominated by Porifera and Cnidaria. We have sampled these environments using the ROV Holland aboard the R/V Celtic Explorer in support of our biodiscovery program.

Among our screening priorities is a targeted approach focused on programmed cell death (PCD). PDC occurs in many pathological situations from inflammation to ischaemia. It is also the mechanism through which many anti-cancer therapies act. Screening the extracts of our deep-sea organisms in a primary caspase-3 screen identified three extracts of interest. The activity of these extracts was confirmed utilizing a novel split-luciferase apoptosome secondary screen designed to identify both activators and inhibitors of this protein complex. Bioguided fractionation of the most active extract originating from a deep-sea zoanthid yielded a bromotyrosine metabolite. The structure was elucidated using contemporary 1D and 2D NMR spectroscopy and HRMS. Further work is ongoing into the mode of action as well as isolation and elucidation of additional bioactive metabolites from this zoanthid.

P-021

GENOME MINING OF FRESHWATER CYANOBACTERIA FOR RARE METABOLITES

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Freshwater cyanobacteria are producers of metabolites of biomedical and ecological significance. Among the natural products that cyanobacteria produce are polyketides biosynthesized by type III polyketide synthases (T3PKSs). For example, a T3PKS is involved in the biosynthesis of the cytotoxic molecules known as cyclophanes. The Orjala lab has built a large collection freshwater cyanobacteria strain collection at UIC. We have sequenced the genome of *Nostoc* sp. strain UIC 10110, a merocyclophane producer. We used the gene sequence coding for the merocyclophane T3PKS to find homologs using BLAST, and designed a degenerate primer pair to probe the strain library for T3PKSs by PCR. After screening 449 strains, 11 strains were identified to contain type III PKSs. Based on phylogenetic analysis, three hits appear to encode novel polyketides, whereas eight are probable cyclophane producers. This work will ultimately expand the current knowledge of cyanobacterial chemistry and has the potential to discover new molecules that may be of biomedical relevance.

P-022

LANTIPEPTIDE VITILEVUAMIDE AND CO-METABOLITES FROM SOUTH AFRICAN DIDEMNID TUNICATES

David Gallegos[1], Xinhui Yu[1], Xuemei Wan[1], Jeffrey Serrill[1], Shirley Parker-Nance[2], Rosemary Dorrington[3], Eric Schmidt[4], Jane Ishmael[1], Kerry McPhail[1]
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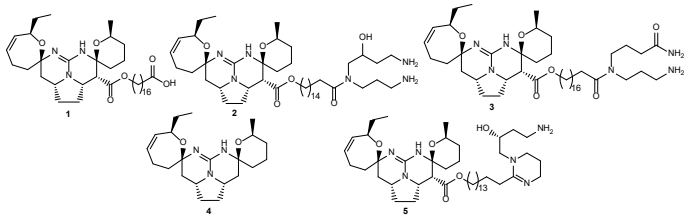
Unique populations of endemic, biologically diverse sessile marine organisms that contain may house specific microbial consortia represent critical opportunities to discover new chemistry. We have targeted "gelatinous" didemnid tunicates found in Algoa Bay, South Africa, as a potential source of diverse suites of metabolites with potent biological activities. A bioassay-guided isolation approach yielded the known natural product vitilevuamide from several new species of didemnid tunicates. Vitilevuamide (1602 Da) was previously isolated from two tunicates collected from Papua New Guinea,[ref1] and was described as an anti-tubulin agent with an unknown specific mechanism,[ref1,2] although its molecular structure could not be characterized fully. With assistance from biosynthetic pathway predictions, we have assigned the absolute configuration of this nonribosomal peptide. Remarkably, we have also characterized low molecular weight polyketide co-metabolites that appear to associate closely with vitilevuamide, and which may enhance the cytotoxicity of vitilevuamide, as well as additional large peptide congeners. Our further bBiological investigation to date, including submission to the NCI60 cell line panel, has confirmed the inhibition of tubulin polymerization by vitilevuamide, and cytotoxicity of the associated polyketides.

P-023

GUANIDINE ALKALOIDS FROM TWO MARINE SPONGES OF GENUS *MONANCHORA* WITH ANTIFUNGAL ACTIVITY AGAINST *CRYPTOCOCCUS* SP.

Mohamed M. Radwan[1], Ali E. Raslan[1,2], Amira S. Wanas[1], Safwat A. Ahmed[3], Alaa M. Nafady[2], Siddharth K. Tripathi[1], Melissa R. Jacob[1], Hashim A. Hassanean[3], Mahmoud A. ElSohly[1,4]
[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA, [2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt, [3]Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt, [4]Department of Pharmaceuitcs and Drug Delivery, School of Pharmacy, University of Mississippi, MS 38677, USA

Crambescidic acid-671 (**1**), a new guanidine alkaloid along with the known alkaloids: crambescidin 800 (**2**), crambescidin 826 (**3**), crambescidin 359 (**4**), and formiamycalin (**5**) were isolated from the marine sponges *Monanchora clathrata* and *Monanchora unguiculata*. Compounds **1**-**4** isolated from *M. clathrata* and Compounds **2**-**5** isolated from *M. unguiculata*. Compounds **1**-**3** and **5** showed potent antifungal activity against three *Cryptococcus* strains with IC_{50} values ranging from 0.04 μM to 1.20 μM, MIC values ranging from 0.24 μM to 1.27 μM, and MFC values ranging from 0.24 μM to 2.55 μM, comparable to the positive control Amphotericin B.



P-024

BUTUANIMIDES: NOVEL HALOGENATED NRPS-PKS COMPOUNDS FROM A PHILIPPINE SHIPWORM ASSOCIATED BACTERIUM

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The marine wood-boring bivalve mollusks of the family *Teredinidae,* also know as shipworms, play host to cellulolytic symbionts, primarily of the genus *Teredinibacter*. A novel species of *Teredinibacter* was isolated from the gill homogenate of *Bactronophorus sp.* collected in a mangrove forest in the southern Philippines. A series of compounds bearing structural resemblance to andrimid and moiramide, herein named the butuanimides, were isolated from the organic extract of this strain. Similar to the known compounds, butuanimides have a methyl succinidmide moiety conjugated to valine. However, this series lacks the B-phenylalanine subunit found in the known compounds, and the PKS-derived tail portion is halogenated and includes a highly unstable, epoxyalcohol-containing six-membered ring. Preliminary bioassay data shows activity against both a strain of MRSA and *P. aeruginosa*, without cytotoxicity to the mammalian cell lines MDCK or HCT-116. Furthermore, the genome of this symbiont was obtained and a biosynthetic pathway will be proposed.

P-025

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

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

The Antarctic sponge *Dendrilla membranosa* was found to contain diterpenoid secondary metabolites with activity in a screening campaign focused on *Leishmania donovani*, *Naegleria fowlerii* and the ESKAPE pathogens. In total, 12 natural products and 12 semi-synthetic derivatives were isolated or derived from this cold-water poriferan resulting in a library of 21 compounds, with 3 compounds being both isolated directly from the sponge as well as alternatively obtained via semi-synthetic routes. Seven of the 21 compounds within the library are previously unreported structures.

P-026

ANTIMICROBIAL NATURAL PRODUCTS FROM SOUTH AFRICAN MARINE ALGAE

Jameel Fakee[1], Edmund Mabande[2], Omolola Afolayan[2], Marilize le Roes-Hill[3], John J. Bolton[4] and Denzil R. Beukes[2]
[1]Faculty of Pharmacy, Rhodes University, Grahamstown, 6140, South Africa, [2]School of Pharmacy, University of the Western Cape, Bellville, 6535, South Africa, [3]Biocatalysis and Technical Biology Research Group, Cape Peninsula University of Technology, Bellville, 7535, South Africa, 4Department of Biological Sciences, University of Cape Town, Rondebosch, 7700, South Africa

Natural products remain the leading source of new antibiotics against pathogenic microorganisms. However, the rapid development of resistance against common antibiotics combined with the slow pace of new antimicrobial drug discovery present a significant risk to human health. We have therefore initiated a programme to explore the antimicrobial potential of South African marine organisms. In the current study, we screened a library of more than 200 marine algal extracts for activity against *Acinetobacter baumannii*, *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus subsp. aureus* and *Candida albicans*. The natural products with the most promising antimicrobial activities were isolated and their structures determined using standard spectroscopic methods.

In this paper we will present the structures and antimicrobial activities of representative examples of more than 50 natural products.

P-027

PHYTOCHEMICAL COMPARISM AND GENOTOXICITY STUDIES OF TWO SELECTED GASTROPODS SHELL SPECIES

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Nature continues to be an attractive source of new therapeutic agent since a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms. However, their safety is of paramount important both at the cellular and molecular level. In order to stress how important lower animals are which can be sources of pharmacological substances, as well as the level at which they can be safe for use motivates the drive of this research more so the fact that they are extincting species. The objectives of study are to investigate the genotoxic, cytotoxic and phytochemical properties of Land and Marine snail, *Achatina achatina* and *Thais clavigera,* respectively.

The n-hexane extracts from each powdered shells *Achatina achatina* (AaH) and *Thais clavigera* (TcH) were subjected to GC-Mass spectrometry, Diphenylamine Assay and Gel Electrophoresis Assay for Phytochemical and Genotoxic assays respectively.

The GC-MS results revealed the presence of two toxins in n hexane extracts of *Thais clavigera*; dibutyl phthalate, an endocrine disruptor and Bis (2-ethylhexyl) phthalate, a carcinogen and a teratogen while the n hexane extracts of the *Achatina achatina* showed aromatic hydrocarbons ; *p*-cymene; 1,3,8-*p*-menthatriene and dimethoxyethybenzene.

The genotoxic properties of the extracts, AaH and TcH evaluated on liver and testis tissues of mice, using Diphenylamine Assay and Gel Electrophoresis assay showed that the DNA fragmentation in the was insignificant with AaH and TcH in the liver (0 %) and testis (36 %). This finding suggests that the two extracts may play protective role in the genomic DNA of the liver and testis cells. The Cytotoxic properties of two extracts were evaluated using brine shrimp lethality. Findings revealed that n hexane extract of the *Thais clavigera* was the most toxic.

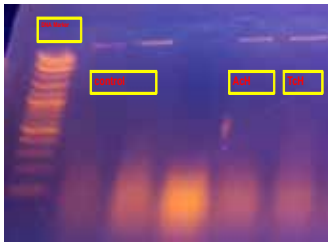

Figure 1: Gel Electrophoresis assay of AaH and TcH

P-028

ABSOLUTE STEREOSTRUCTURES OF ALKALOIDS AND AMINO ALCOHOLS FROM TWO MARINE INVERTEBRATES

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The structures of indole alkaloids and sphingoid bases from marine organisms often deviate from conventional patterns of functionalization found in the plant Kingdom, and present challenges to assignment of their absolute stereochemistry. We describe, here, the absolute stereostructure of the new dioxindole **1**, from a sub-Arctic specimen of *Geodia baretti*, and re-evaluation of barettin (**2**) and dihydrobarettin (**3**) using ECD and the new Marfey's-type reagent, L-N_a-(1-fluoro-2,4-dinitrophenyl)tryptophanamide (L-FDPT, **4**). Finally, (*S*)-distaminolyne A (**5**), from the New Zealand ascidian, *Pseudodistoma opacum*, is re-evaluated against **6** and **7** obtained by asymmetric synthesis.

P-029

DISCOVERY OF NOVEL NATURAL PRODUCTS FROM A CONSORTIUM OF PANAMANIAN CYANOBACTERIAL STRAINS

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Marine microorganisms are rich sources of structurally intriguing natural products and a number of marine microbial secondary metabolites have been reported with potent anti-cancer activities. As a part of on-going research to discover new bioactive natural products from marine microorganisms, chemical investigation on an extract of environmental collection of Panamanian cyanobacteria *Symploca* sp. with *Scytolyngbya* sp. were performed. Based-on MS-based dereplication and MS2-based molecular networking study on the extract showed the presence of a series of novel secondary metabolites. Four new natural products were isolated by normal-phase vacuum liquid chromatography (NP-VLC) followed by reversed-phase high performance liquid chromatography (RP-HPLC). Their structures were determined by spectroscopic data analyses and absolute configurations were proposed from the biosynthetic speculation. Among four compounds, a compound with 4-*O*-methyl β-ribofuranose showed most potent cytotoxicity against H-460 human lung cancer cells (23% cell survival at 7.5 μM).

P-030

ISOLATION OF METABOLITES FROM ANTARCTIC SPONGE *ARTEMISINA PLUMOSA* FOR POTENTIAL DNA TOPOISOMERASE I INHIBITION BIOACTIVITY

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Studies of the plant alkaloid camptothecin isolated from *Camptotheca acuminata* have shown the antitumoral potential of compounds that inhibit mammalian DNA Topoisomerase I activity. Topoisomerases are necessary for cellular functions such as DNA replication, transcription, and recombination, hence disruption of this activity poses a viable mechanism for drugs that target the need for innovative anticancer agents. It is well established that marine sponges represent a rich source for novel secondary metabolites, with approximately 30% of all marine natural products isolated to date being derived from sponges. This vast diversity of secondary metabolites combined with the historical success of natural products as anticancer drugs makes marine sponges ideal candidates for new compounds capable of Topoisomerase I inhibition.

Freeze-dried samples of the Antarctic sponge *Artemisina plumosa* were extracted using solutions of 1:1 dichloromethane:methanol, and subsequent extraction with 1:1 methanol:water. These extracts were fractionated using MPLC. A bioassay was conducted on the MPLC fractions to determine DNA relaxation activity, with 9 positive results, which was used in parallel with Nuclear Magnetic Spectroscopy (NMR) to guide separation of the samples using HPLC. Through this sequence of isolation and separation on sponge extracts preliminary results indicate a potential for DNA Topoisomerase I inhibition bioactivity.

P-031

AN OVERVIEW OF DELITPYRONES: A-PYRONE DERIVATIVES FROM A FRESHWATER *DELITSCHIA* SP.

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Of the approximately 120,000 identified species of fungi (3-8%) within the 2.2-3.8 million that is estimated within the kingdom, limited research has been conducted on freshwater ascomycetes with only 675 species described. Interestingly, less than 250 metabolites have been reported from freshwater ascomycetes. Our group's research on these fungi has established that further investigation into their chemical space is paramount. Using an eight step procedure, a peak library was constructed and screened against the African American prostate cancer cell line E006AA-hT as this population encounters higher rates of incidence and mortality. Cytotoxity and dereplication results of a *Delitschia* sp. designated as G858, lead to the isolation of 8 new α-pyrone derivatives, 1 new dihydronaphthalenone, and 8 known fungal compounds. Of these 17 isolated compounds, 14 were tested against the prostate cancer cell line E006AA-hT, with one compound showing a promising lead.

P-032

NEW SECONDARY METABOLITES FROM AN ANTARCTIC TUNICATE

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Marine organisms have attracted the interest of the scientific community in the past few decades, followed by the discovery of new and novel compounds. An area of great interest has been marine organisms native to Antarctica, which due to the circumpolar current have created a unique ecosystem. In this project, we worked with a *Synoicum* sp. from which new compounds were isolated. The new compounds belong to the family of indole alkaloids and have been subjected to various bioassays in order to determine their activity.

P-033

STRUCTURAL INTERROGATION OF MICROBIAL DYNEMICIN BIOSYNTHETIC ENZYMES

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Dynemicin is a 10-membered enediyne isolated from a soil microbe, *Micromonospora chersin*, employed for bacterial defense. The enediynes, including dynemicin, have also distinguished themselves as promising candidates for antibacterial or cancer therapeutics due to their unparalleled cytotoxicity via chromosomal double-stranded DNA cleavage. Dynemicin possesses an unusual and complex molecular architecture; the enediyne comprises the characteristic bicyclo "warhead" group and a distinctive anthraquinone peripheral moiety that acts as the anchor governing the bicyclo group's positioning for hydrogen abstraction. The dynemicin anthraquinone group is synthesized and assembled by an extensive host of enzymes. DynU16, a putative cyclase, DynA1 and DynA2, putative oxidases were selected for pioneering structural efforts to facilitate decoding of the elaborate anthraquinone molecular assembly. Structural determination and analysis via X-ray crystallography will provide high-resolution structures exposing the biocatalytic features that enable regio-, chemo- and stereo-selectivity of the enzymes. Moreover, synergistic biochemical and structural efforts have the promising potential to reveal paradigm-shifting biological chemistry. If the proposed objectives are achieved, structural characterization of the dynemicin biosynthetic machinery will afford valuable perspectives into the intricate enediyne molecularassembly.

Supported by a training fellowship from the Gulf Coast Consortia, on the Houston Area Molecular Biophysics Program (Grant No. T32GM008280) and National Cancer Institute, R01-CA217255.

P-034

MODEL ACTINOCIN PROVIDES INFORMATION FOR ACTINOMYCIN SUBSTRUCTURE FORMATION AND PROMOTION OF ANTIBIOTIC ACTIVITY

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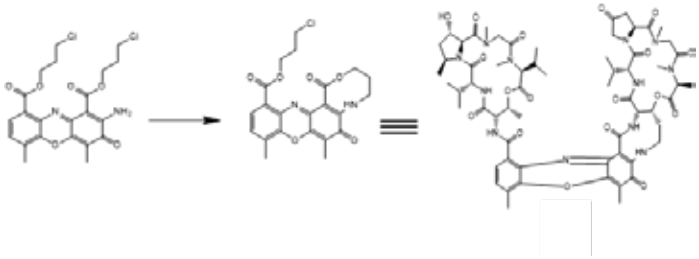
The actinomycin scaffold remains the gold standard to measure DNA binding. However, since its discovery in 1949, several other families of actinomycin have been discovered, some containing an unusual β-ring heterocycle. Though there is evidence the formation of this substructure drastically changes the biological activity of the molecule, little is known how these β-ring heterocycles form. Furthermore, it is unclear how the mechanism of activity changes in these actinomycins to produce this unique activity. Herein, we synthesized a model actinocin to (1) probe the mechanism of β-ring heterocycle formation and (2) probe for a change in activity. The information gleaned from our model was applied to actinomycins we believe could undergo this structural transformation.



P-035

ENGINEERING MULTIFUNCTIONAL ENZYMES CAPABLE OF ADENYLATING AND SELECTIVELY METHYLATING THE SIDE CHAIN OR CORE OF AMINO ACIDS

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Nonribosomal peptides (NRPs) are a large class of natural products with therapeutic relevance. These NRPs are synthesized by mega-enzymes called nonribosomal peptide synthetases (NRPSs). NRPSs are subdivided into catalytic domains (adenylation (A), condensation (C), and thiolation (T)) and auxiliary domains (*e.g.*, methyltransferase (M)). NRPSs string together amino acids to yield a final NRP product. These NRPs can then be decorated with additional chemistry by auxiliary domains. Nature has added further diversity to NRPSs by embedding auxiliary domains into A domains, most commonly an M domain, to yield an interrupted A domain. A domains are key targets for NRPS engineering as they dictate the amino acids incorporated into NRPs. We aim to emulate and expand what Nature has created. To investigate this, we generated two fully functional 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. Additionally, 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 methylate the amino acid in two different locations. This provides an exciting proof-of-concept for generating interrupted A domains as future tools to modify NRPSs and increase their NP diversity.

P-036

HOW ARE NONRIBOSOMAL PEPTIDES METHYLATED? A TALE OF INTERRUPTED ADENYLATION DOMAINS

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Natural products (NPs) are one of the most important sources of drugs. They are produced by various enzymes including nonribosomal peptide synthetases (NRPSs) that assemble amino acids into small peptide NPs. NRPSs are modular enzymes that contain multiple catalytic domains in each module. Each domain normally has a specific function, whose orchestrated actions synthesize complex peptide NPs. However, the adenylation (A) domains sometimes embed 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 the bisintercalator NP, thiocoraline. Thiocoraline and its analogous NP, thiochondrilline A, contain unique *N*,*S*-dimethyl-L-cysteine 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 out of 12 possible pathways by a series of radiometric assays. We also solved the structure of one interrupted A domain involved in thiocoraline biosynthesis and doing so gained insight into the mechanism by which interrupted bifunctional A domains work. We are now using the information gathered by these studies to guide our effort towards combinatorial biosynthesis of methylated NRPs.

P-037

PRENYLATED FLAVONE DERIVATIVES FROM THE TWIGS OF *ARTOCARPUS HETEROPHYLLUS*

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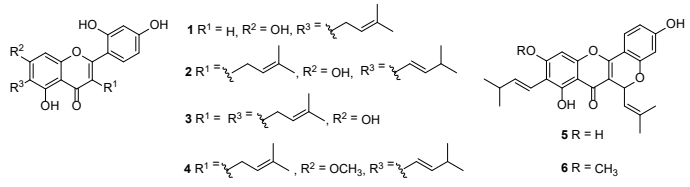
The chromatographic separation of the methanolic crude extract from the twigs of *Artocarpus heterophyllu*s led to the isolation of six known prenylated flavones named artocarpesin (**1**), noratocarpin (**2**), cudraflavone C (**3**), artocarpin (**4**) brosimone I (**5**) and cycloartocarpin (**6**). Their structures were identified on the basis of spectroscopic data, mainly 1D and 2D NMR data. Compounds **2**, **4** and **5** exhibited cytotoxicity against oral human carcinoma (KB), human breast carcinoma (MCF-7), small lung carcinoma (NCI-H187) and noncancerous cell lines with the IC_{50} values in the range of 10 - 37.9 μM. In addition, compound **6** showed cytotoxicity against NCI-H187 with an IC_{50} value of 44.2 μM.



P-038

TOWARD AN ENZYME-COUPLED, BIOORTHOGONAL PLATFORM FOR METHYLTRANSFERASES

Tyler D. Huber[1,2], Brooke R. Johnson[1,2], Yang Liu[1,2], Jonathan A. Clinger,[3] Fengbin Wang,[3] Shanteri Singh[1,2,4], Jianjun Zhang[1,2,5], Jürgen Rohr[2], Steven G. Van Lanen[2], Andrew J. Morris[6], George N. Phillips, Jr.,[3,7] and Jon S. Thorson[1,2]
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Methyl group transfer from *S*-adenosyl-L-methionine (AdoMet) to various substrates including DNA, proteins, and natural products (NPs), is accomplished by methyltransferases (MTs). Analogs of AdoMet, bearing an alternative *S*-alkyl group can be exploited, in the context of an array of wild-type MT-catalyzed reactions, to differentially alkylate DNA, proteins, and NPs. This technology provides a means to elucidate MT targets by the MT-mediated installation of chemoselective handles from AdoMet analogs to biologically relevant molecules and affords researchers a fresh route to diversify NP scaffolds by permitting the differential alkylation of chemical sites vulnerable to NP MTs that are unreactive to traditional, synthetic organic chemistry alkylation protocols. We present novel chemoenzymatic strategies that employ methionine adenosyltransferases (MATs) and methionine (Met) analogs to synthesize AdoMet analogs *in vitro*, which are utilized in a one-pot reaction by MTs for the alkylrandomization of NP scaffolds. We will also present the development, use, and results of a high-throughput screen to identify mutant-MAT/Met-analog pairs suitable for postliminary bioorthogonal applications.

P-040

UTILIZATION OF AROMATIC PRENYLTRANSFERASES FOR ALKYL-DIVERSIFICATION OF NATURAL PRODUCTS

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Institute for Natural Products Applications and Research Technologies, Department of Chemistry and Biochemistry, University of Oklahoma, 101 Stephenson Parkway Norman Oklahoma 73019, USA

The structural diversification of natural products (NPs) presents an important means for generating novel biologically active molecules. While the systematic diversification of structurally complex NPs using conventional chemical synthesis can be challenging, a chemoenzymatic method of diversification provides a promising alternative for regio- and stereo- selective NP-diversification. To this end, NP tailoring enzymes are known to exhibit relaxed substrate specificity towards their substrates and some of these tailoring enzymes such as glycosyltransferases, acyltransferases etc. have been successfully used for selective late stage modification of NPs. A class of highly promiscuous NP late stage modification enzymes are soluble aromatic prenyltransferases (PTs) that catalyze the transfer of prenyl groups from their corresponding activated donors onto aromatic moieties. While several promiscuous PTs have been characterized to date, their utility towards alkyl-diversification of NPs largely remains unexplored. Herein, we present representative examples of utility of PTs for alkyl-diversification of NPs using synthetic unnatural alkyl-donors. In addition, we demonstrate the substrate specificity of PTs can be modulated via rational-engineering of the PTs. Thus, this study provides a basis for further investigation of chemoenzymatic utility of PTs for alkyl-diversification of therapeutically important molecules towards the generation of alkyl-derivatized molecules with altered activity.

P-041

CINNAMIC ACID DERIVATIVES FROM *BAILLONELLA TOXISPERMA* EXTRACT DISLAYING ANTIHYPERALGESIC AND ANTIINFLAMMATORY ACTIVITIES *IN VIVO*

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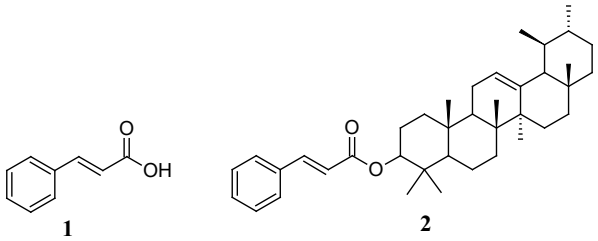
Decoctions of the aerial plant parts of *Baillonella toxisperma* in Cameroon, has long been used in traditional medicine for pain-relief, as well as for their anti-inflammatory properties. Corroborating the ethnomedicinal uses, the stem bark methanol extract of *B. toxisperma* showed significant antihyperalgesic and antiinflammatory properties in *in vivo* rat models of acute and chronic pain. To identify the compounds responsible for the observed activities, the extract was subjected to a series of partitioning and chromatographic techniques which led to the discovery of cinnamic acid (**1**) and its α-amyrin hybrid, **2**, a new chemical entity. The isolation and characterization of **2** from this bioactive fraction will be discussed.



1 **2**

P-042

CHARACTERIZATION OF THE CALICHEAMICIN ORSELLATE FLAVIN-DEPENDENT HALOGENASE CALO3

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While a number of flavin-dependent halogenases involved in the bromination or chlorination of microbial secondary metabolites have been characterized, corresponding flavin-dependent iodinases remain largely unstudied. We report on the preliminary in vitro characterization of the putative flavin-dependent halogenase CalO3 responsible for iodination or bromination of the calicheamicin orselinate moiety. Studies to be discussed include heterologous CalO3 overproduction in *E. coli*, CalO3 purification and SAR studies [using a series of synthetic orselinate SNAC esters in the absence or presence of the other key calicheamicin orselinate modifying enzymes (oxidase CalO2, methytransferase CalO6 and CalO1)]. These studies will help clarify the timing and fundamental details of the uniquely-substitued calicheamicin orselinate moiety and may also pave the way to understanding the molecular features that define halogen selectivity in flavin-dependent halogenases.

P-043

NEW AQUEOUS-SOLUBLE PEPTIDIC ALKALOIDS AND SIDEROPHORES FROM *PENICILLIUM SOLITUM* IS1-A FROM THE ANTARCTIC CONTINENT

Julie P.G. Rodriguez[1], Raquel P. Morais-Urano[1], Jairo I. Bulla[1], Antonio G. Ferreira[2], Raquel A. Santos[3], Lara D. Sette[4], Roberto G. S. Berlinck[1].

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Water-soluble secondary metabolites represent a smallest fraction of investigated natural products, allegedly due to the difficulty of handling water-soluble extracts. Since the investigation of very polar metabolites is rather neglected, we developed a new strategy for the investigation of water-soluble extracts. The hydrophilic fraction from the growth media of *Penicillium solitum* IS1-A isolated from a marine isopod collected at the Antarctic Continent displayed cytotoxic activity and was investigated using several separation approaches. A series of novel water-soluble peptidic alkaloids and siderophores have been isolated, whose structures and bioactivities will be presented and discussed.

Funding FAPESP, CAPES and CNPq.

P-045

BACTERIAL-DERIVED EXTRACELLULAR ELECTRON SHUTTLE

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Shewanella oneidensis MR-1 is a facultative anaerobic γ-proteobacterium that has the ability to utilize a diverse suite of terminal electron acceptors, including insoluble solid metal oxides. The mechanisms underlying how MR-1 indirectly shuttles electrons to these solid substrates are poorly understood. In 2000 chemical analyses of MR-1's spent supernatant revealed that MR-1 excretes a small labile molecule that has the ability to recover anaerobic respiration of mutants on solid substrates, but the active metabolite was never identified. Revisiting this lack of identity with specialized resin, HR-LCMS analysis, and total synthesis, led to its identification as 2-amino-3-carboxy-naphthoquinone (ACNQ). ACNQ potently recovers anaerobic respiration in a mutant strain (Δ*menC*: menaquinone mutant) on solid substrates (EC_{50} of 25 nM) and can significantly increase current generation in Mtr-expressing *E. coli* strains. ACNQ is derived non-enzymatically from a primary metabolite, 1,4-dihydroxy-2-naphthoic acid (DHNA) and is produced by all other anaerobic bacteria (facultative and obligate) investigated. In summary, the discovery of ACNQ provides a better understanding as to how MR-1 shuttles electrons to insoluble terminal electron acceptors. This discovery has several potential applications, including medical uses like treatment of mitochondrial disease and bioenergy.

P-046

NEW ANTI-*CRYPTOCOCCUS* WORTMANNIN DERIVATIVES FROM A *NIESSLIA* SP. PREVIOUSLY KNOWN ONLY FROM THE METAGENOME

Nicole M. Krausert,[1] Lijian Xu,[2] Yan Li,[2] Gerald F. Bills,[2] and James B. Gloer.

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In the course of our studies of coprophilous (dung-colonizing) fungi as sources of antifungal agents, strain TTI-426 was obtained from horse dung collected at Lake Houston in Montgomery County, Texas. The crude extract of a fermentation of this strain grown in YES medium showed activity against *Cryptococcus neoformans* and *Candida albicans* and was therefore selected for chemical investigation. rDNA sequences of TTI-426 were identical to sequences of an undescribed *Niesslia* sp. from soil metagenomes in northern Texas. The strain was later identified as a new species of the genus *Niesslia*, based on morphology and sequence analysis. Silica gel column chromatography and reversed phase HPLC afforded two new anti-*Cryptococcus* wortmannin derivatives, as well as additional new related compounds. The structures of these metabolites were established mainly by analysis of HRESIMS and 2D NMR data. Relative configurations were assigned using NOESY data, and the structure assignments were supported by NMR comparison with similar compounds.

P-047

CHALLENGES IN ABSOLUTE CONFIGURATION – NEW POLYKETIDES FROM MARINE-ASSOCIATED *ASPERGILLUS POROSUS*

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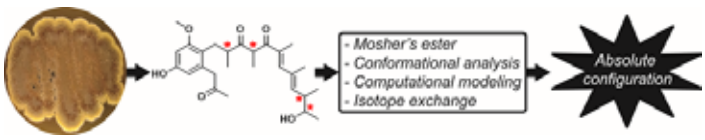
Fungal natural products have inspired and enabled countless modern therapeutics. We found *Aspergillus porosus* to produce novel polyketides with interesting structural features alongside known cytotoxins. Chemical screening revealed the presence of novel, dynamic secondary metabolites of polyketide origin. Structural elucidation was performed primarily with 1D and 2D NMR techniques. Mosher's ester analysis along with a *J*-based conformational analysis, utilizing C-H coupling constants measured via HETLOC, established the absolute configuration of two asymmetric carbons. Further comparative analysis of experimental NMR and electronic circular dichroism spectra with computed spectra, along with isotope exchange studies support the assignment of the remaining asymmetric centers.



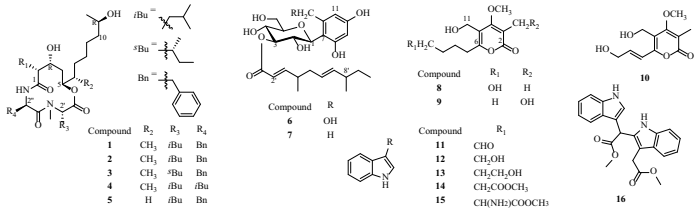
P-048

FOUR CLASSES OF SECONDARY METABOLITES FROM AN ENDOPHYTIC FUNGUS *COLLETOTRICHUM GLOEOSPORIOIDES* JS0417

Changyeol Lee[1], Soonok Kim [2], and Sang Hee Shim [1,*]

[1]College of Pharmacy, Duksung Women's University, Seoul 01369, South Korea [2]National Institute of Biological Resources, Incheon 22689, South Korea

Endophytic fungus *Colletotrichum gloeosporioides* JS0417 was isolated from roots of *Suaeda japonica* Makino and cultivated on rice media, and then extracted with ethyl acetate (EtOAc). Chemical investigation of the EtOAc extracts led to the isolation of four classes of secondary metabolites including five cyclic lipodepsipeptides (**1**-**5**), two chaetiacandins (**6**-**7**), three α-pyrones (**8**-**10**) and six indoles (**11**-**16**). Their chemical structures were elucidated based on chemical methods including Mosher's method, advanced Marfey's method as well as spectroscopic data. Nine metabolites (**1**-**9**) were first reported from this fungal strain. Details of isolation and structure determination are presented.



P-049

A NEW GLYCOSYLATED CYCLIC LIPODEPSIPEPTIDE FROM A HALOPHYTE-ASSOCIATED FUNGUS, *COLLETOTRICHUM GLOEOSPORIOIDES* JS419

Sunghee Bang[1], Changyeol Lee[1], Soonok Kim[2], Ki Sung Kang[3] and Sang Hee Shim[1,*]

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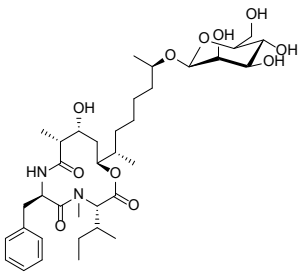
A novel glycosylated cyclic lipodepsipeptide was isolated from cultures of a halophyte *Sueda japonica*-associated fungus, *Colletotrichum gloeosporioides* JS419. Spectroscopic analysis revealed that its planar structure is a glycosylated cyclic lipodepsipeptide, in which 3,5-dihydroxy-2,6-dimethyl-11-*O*-mannosyl dodecanoic acid was linked to a phenylalanine and an *N*-methyl isoleucine. Its relative and absolute stereochemistry were established by ROESY, *J*-based configuration analysis and chemical reaction such as modified Mosher's method, advanced Marfey's method, and sugar derivatization. Despite of the precedent of the polyketide-derived lipid moiety with 14 carbons in two cases, its relative as well as absolute stereochemistry was elucidated in this study for the first time. It exhibited mild anti-proliferative effect in MCF-7 cells by blocking nuclear translocation of estrogen receptor-α.



P-050

ISOLATION OF ANTIFUNGAL ACTIVE SUBSTANCES FROM *BOESENBERGIA PULCHERRIMA* AGAINST FUSARIUM WILT (*FUSARIUM OXYSPORUM*)

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With the aim of developing environment-friendly agricultural products with antifungal activity against *Fusarium oxysporum f.* sp. *lycopersici,* a causative agent of Fusarium wilt, active substances from *Boesenbergia pulcherrima* roots were isolated. The hexane fraction from *B. pulcherrima* root extract was analyzed by GC/MS. The main peaks were estimated and identified to be methyl eugenol, methyl isoeugenol, elemicin, α-asarone, and 1,2-dimethoxy-4-(2-methoxyethenyl)benzene based on the Wiley library and by comparing retention times and mass spectra with their corresponding standards using GC/MS. For the identification of the compound in peak D that was estimated to be 1,2,4-trimethoxy-5-vinylbenzene, for which no reference standard was available, the hexane fraction was processed by column chromatography before NMR analysis. The result confirmed the compound to be 1,2,4-trimethoxy-5-vinylbenzene. Almost all compounds showed antifungal activity against *F. oxysporum* based on bioassays, and α-asarone had the highest activity.

P-051

COMBATING MICROBIAL DRUG RESISTANCE: IDENTIFICATION AND SAR STUDY OF INDOLE-FUNCTIONALISED POLYAMINES AS ANTIBIOTIC ENHANCERS.

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Antibiotics have been the cornerstone of modern medicine saving lives by virtue of being able to cure infectious diseases. The combination of increased incidence of drug-resistant strains of bacteria, with a lack of novel drugs in development, creates an urgency for antibiotics with new mechanisms of action. An attractive strategy for overcoming bacterial resistance is to identify compounds that can enhance the activity of antibiotics that are becoming ineffective. Initial screening of compounds from our in-house library identified a lead indole-functionalised polyamine that restored activity of the antibiotic doxycycline towards the drug-resistant Gram-negative bacterium, *P. aeruginosa* while also exhibiting intrinsic antibacterial activity. SAR studies were conducted and a number of novel analogues were identified as potent doxycycline enhancers towards *P. aeruginosa*, *E. coli* and *K. pneumoniae*. The results of a comprehensive SAR on the observed doxycycline enhancing activity along with the mechanism of action of the more potent compounds and their ability to enhance the activity of other classes of antibiotics towards drug-resistant bacteria will be presented.

P-052

ANTIMALARIAL AND ANTIOXIDANT PROPERTIES OF THE LEAVES OF *ALAFIA BARTERI*

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High resistance and cost to available antimalarials and free radicals involvement in the development of complication caused by malaria has arouse the need to explore plant materials that have antioxidant activity and contain new chemical compounds that can treat this deadly disease. Thus we investigated a popularly used plant for its antioxidant activity and its effectiveness in the treatment of malaria. Dried and powdered leaves of *Alafia barteri* was extracted in hexane, dichloromethane, ethyl acetate and methanol. Fractionation of dichloromethane extract yielded Compound 1; methanol extract yielded compounds 2 and 3. The in-vivo antimalarial activity of the compounds aganist *P. berghei* (NK-65) were evaluated. The antioxidant activity investigation included various radicals and oxidation systems (FRAP, $ABTS^{\bullet+}$ and ORAC). Methanol extract of the leaves of *A. barteri,* possessed significant chemo suppressive activity and exhibited good clearance against chloroquine resistant *Plasmodium Berghei* (NK-65). Compound 1 showed significant antimalarial activity compared to compounds 2 and 3. The leaves of *A. barteri* presented a good antioxidant activity and can be explored as therapeutic agents in the attenuation of free radical as well as in the treatment of malaria disease.

P-053

GRAM SCALE PRODUCTION OF THE ANTIFUNGAL POLYENE SELVAMICIN

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Fungal infections are associated with high mortality rates. Recent outbreaks of pan resistant strains of *Candida auris* demonstrate the dire need for new antifungal agents. Selvamicin was discovered as a new polyene antifungal agent that does not bind ergosterol like amphotericin suggesting a different mechanism of action. Additionally, selvamicin shows better solubility and activity towards *Candida auris*. The promising characteristics of selvamicin combined with a putative new mechanism of action have led us to pursue further evaluation of this promising antifungal agent. As a prerequisite to preclinical evaluation, we pursued production of gram-scale quantities of the antifungal polyene selvamicin. We tested a range of media conditions aiming for minimal production of molecules aside from selvamicin in parallel with optimizing isolation and purification. Following just two iterations of HPLC on the crude product yields ~0.5-1 g of selvamicin, at a purity >95%. This poster will highlight aspects of how gram scale production was achieved.

P-054

ANNONACEAE FAMILY AND THEIR ACTIVITY TOWARDS NRF-2/ARE PATHWAY

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Annonaceae family or custard apple family has over a hundred genera and thousands of species. This family is distributed worldwide and some species have been used traditionally for medicinal effects. The purpose of this investigation is to study the anti-cell proliferative effects of a collection of Annonaceae plant extracts in colon cancer cells (HCT-116 and HT-29) and to determine their effects against inflammation via Nrf-2 activation pathway. A collection of 85 Annonaceae plant extracts (16 genera, 32 species) from the repository of the NCNPR was screened for anti-cell proliferative activity and their effects on the activation of Nrf-2/ARE pathway. The cell proliferation was determined by a colorimetric method based on tetrazolium salts WST-8 in which viable cells are able to reduce WST-8 salts to water-soluble dye. Reporter gene assay was employed to determine Nrf-2 activation. The results indicated that of the 85 extracts, 25 extracts inhibited cell proliferation of colon cancer cells at the concentration of 50 µg/ml or less; five extracts showed Nrf-2 activation of greater than two fold at 100 µg/mL in HCT-116 cells.

P-055

MACROLACTONIZATION OF MULTIPLE MICROVIRIDIN CORE PEPTIDES WITHIN A SINGLE POLYPEPTIDE SUBSTRATE BY AN ATP-GRASP LIGASE

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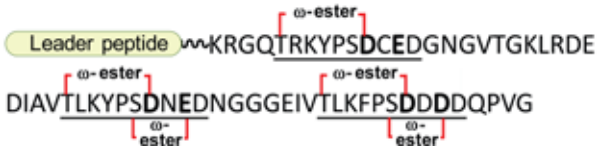
Ribosomally synthesized and post-translationally modified peptides (RiPPs) are an important family of natural products. Their biosynthesis follows a common scheme in which the leader peptide of a precursor peptide guides the modifications of a single core peptide. Here we describe biochemical studies of the processing of multiple core peptides within a precursor peptide, rare in RiPP biosynthesis. In a cyanobacterial microviridin pathway, an ATP grasp ligase, AMdnC, installs up to two macrolactones on each of the three core peptides within AMdnA. The enzyme catalysis occurs in a distributive fashion and follows an unstrict *N*-to-*C* overall directionality. Furthermore, AMdnC is catalytically versatile to process unnatural substrates carrying one to four core peptides, and kinetic studies provide insights into its catalytic properties. Collectively, our results reveal a distinct biosynthetic logic of RiPPs, opening up the possibility of modular production via synthetic biology approaches.



P-056

ENEDIYNE NATURAL PRODUCTS BIOSYNTHESIS

Minakshi Bhardwaj, Steven Van lanen, Jon Thorson
University of Kentucky

Enediyne natural products are secondary metabolites produced by soil and marine microorganisms (Actinomycetes). Enediyne natural products are the most cytotoxic molecules and use as anticancer drugs. Various antibody–drug conjugates (ADCs) have shown great clinical success in anticancer therapy.

Enediynes have complex structures that are unusual among naturally occurring natural products. The structure is characterized by an unsaturated core with two acetylenic groups conjugated to a double bond with a 9- or 10-membered ring. The enediyne part of the structure leads to interesting questions about their biosynthesis in Actinomyces.

We are working on synthesizing potential precursor for the enediyne natural product. It has been observed that knockout of polyketide synthase resulted in hampering in the production of enediyne compounds. The potential precursors will be fed to polyketide synthase mutant and production of enediyne will be monitored. This will help us to decipher the precursors for the enediyne natural product.

P-057

DELINEATING THE BIOSYNTHESIS OF CAPURAMYCIN-TYPE ANTIBIOTICS

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Department of Pharmaceutical Science, College of Pharmacy, University of Kentucky

Mycobacterium tuberculosis (TB) has been significant global concern for centuries despite the advances in modern medicine. The standard therapeutic regimen against TB remains a long process involving an extensive drug cocktail, and the prominence of various drug resistant strains further complicates the treatment of TB. Thus, it is imperative to discover and develop new antibiotic compounds that will improve upon the therapeutic regimen and circumvent resistance mechanisms. An underexplored class of nucleoside natural products—the capuramycins—have excellent anti-TB properties and have been shown to potently inhibit MraY, a ubiquitous and essential enzyme that functions in bacterial cell wall biosynthesis. However, the capuramycins' mechanism of action against MraY is not yet understood, and are key for improving that activity. The biosynthetic pathway of capuramycin has been proposed, but many steps remain uncharacterized. We have chosen to investigate the enzymatic activities of the putative methyltransferase, CapK, and the putative glycosyltransferase, CapG, found in the gene cluster of capuramycin-type compound A-503083 in order to clarify the biosynthetic pathway further. Based on our findings, we aim to exploit the activities of CapK and CapG in order to diversify the capuramycin scaffold and assemble a library of novel capuramycins with favorable anti-TB properties: moderate potency, maintained target specificity, and no resistance.

P-058

OPTIMIZATION OF THE BIOSYNTHESIS OF VERTICILLINS AND USE OF EXPANSILE NANOPARTICLES FOR *IN VITRO* STUDIES.

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Verticillins are members of the epipolythiodioxopiperazine (ETP) alkaloid class of fungal metabolites and are known as potent cytotoxic agents with IC_{50} values lower than 10 nM. Studies showed that verticillin A has activity as a selective histone methyl transferases inhibitor with important anticancer properties. However, two major challenges slow down moving this class forward. Initially, the reliable supply was the first challenge to provide amounts required for preclinical advancement of these leads. Thus, having a rapid way to probe a suite of fermentation conditions was desired, so as to optimize the biosynthesis of the ETPs resulting in the selection of the best fungal strain, the best medium for growth and the optimum growth time. The other challenge, like many natural products, these analogues are poorly soluble, which makes their administration a challenging process. Encapsulating verticillin A inside expansile nanoparticles (eNPs) enhanced greatly their solubility in physiologic conditions, thereby facilitating *in vivo* studies. eNPs were prepared in a 5% loading, within a range of 70-80% of the initial amount encapsulated. The verticillin A loaded eNPs showed promising results *in vitro* against triple negative MDA-MB-231 breast cancer cells and is currently being tested *in vivo* against ovarian cancer in murine models.

P-059

DECRYPTING ORPHAN BIOSYNTHETIC GENE CLUSTERS IN BACTERIAL CO-CULTURES THROUGH CHEMICAL AND BIOINFORMATIC ANALYSES

Deepa Acharya[1], Ian Miller[1], Doug Braun[1], Yusi Cui[1], Marc Chevrette[2], Mark Berres[3], Cameron Currie[2] Lingjun Li[1], Jason Kwan[1], Tim Bugni[1]
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We have developed methods of co-cultivation as a means to unlock the potential of orphan gene clusters in marine Actinobacteria. We discovered that a *Micromonospora* sp. (WMMB235) produced a new antibiotic, keyicin, when co-cultured with a *Rhodococcus* sp. (WMMA185)[1]"ISSN" : "1554-8929", "abstract" : "Advances in genomics and metabolomics have made clear in recent years that microbial biosynthetic capacities on Earth far exceed previous expectations. This is attributable, in part, to the realization that most microbial natural product (NP. We performed global transcriptomics and quantitative proteomics on the producing organism WMMB235 in co-culture and mono-culture to delineate the scope of interspecies interactions for gene activation. We found significant increases in gene expression not only for the *kyc* gene cluster responsible for keyicin, but also a number of other biosynthetic gene clusters (BGCs) encoding putative novel molecules. Moreover, we observed that semi-crude extracts of *Rhodococcus* sp. are sufficient to activate the production of Keyicin. This suggests that the two species communicate via small molecule chemical signaling akin to quorum sensing. Very little is known about the quorum sensing mechanisms in Actinobacteria, and lesser still about the mechanisms regulating cryptic BGCs. Taken together, these studies show co-culture is an effective approach to access orphan BGCs and shed light on new quorum sensing signaling pathways in Actinobacteria.

P-060

ELUCIDATION OF THE COMPLETE NARGENICIN BIOSYNTHETIC GENE CLUSTER FROM A HUMAN PATHOGENIC NOCARDIA ISOLATE

Sacha J. Pidot[1], Marion Herisse[1], Jessica L. Porter[1], Torsten Seemann[1,2], Benjamin P. Howden[1,2,3], Timothy P. Stinear[1,3]
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As part of our antibiotic discovery program, we isolated a human pathogenic *Nocardia arthritidis* isolate that produced an antibiotic highly active against Gram positive bacteria, including methicillin resistant *Staphylococcus aureus* and vancomycin resistant Enterococci. Upon further investigation, the isolated compound was identified as nargenicin, a 28-membered macrolide containing a unique ether bridge, which is also known to have antineoplastic activity. Shortly after its discovery in the 1980s, nargenicin was found to be of polyketide origin. However, the biosynthetic gene cluster responsible for nargenicin production and the tailoring enzymes involved have remained unidentified. We performed Pacbio SMRT sequencing of our *N. arthritidis* isolate, which allowed rapid and complete assembly of a putative nargenicin biosynthetic gene cluster (*nar*), whose polyketide synthase architecture matched the known nargenicin structure. To show conclusively that *nar* is responsible for nargenicin production, we inactivated the *nar* gene cluster in the producing strain, abolishing nargenicin production. Furthermore, we investigated the biosynthetic tailoring enzymes that form part of the *nar* gene cluster to determine their precise roles in nargenicin biosynthesis. The identification of the *nar* gene cluster paves the way for heterologous expression and biosynthetic engineering studies to derive future bioactive compounds based on the nargenicin scaffold.

P-061

ISOLATION OF MURAYMYCIN CONGENERS AND SAR STUDY

Zheng Cui,[1] Xiachang Wang,[2,3] Stefan Koppermann,[4] Patrick D. Fischer,[4] Jannine Ludwig,[4] Jon S. Thorson,[2] Christian Ducho,[4] and Steven G. Van Lanen.[,1]*
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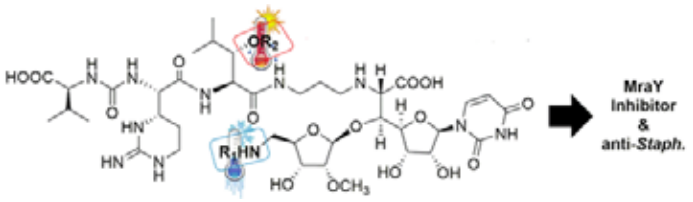
Three new muraymycin congeners, named B8, B9, and C6, as well as six known muraymycin congeners were isolated from three mutant muraymycin producer strains using new media conditions. Structures of these compounds were elucidated by HRMS and NMR. The entirety of the NMR data is reported in the public for the first time. Muraymycin B8 and B9 with an elongated terminally branched fatty acid side chain, had picomolar IC_{50} values against *Staphylococcus aureus* and showed good antibacterial activity against *S. aureus*. Muraymycin C6, with an N-acetyl modification of the primary amine of the disaccharide core showed greatly reduced antibacterial activity. SAR investigation on four representative muraymycin subgroups A-D was studied.



P-062

TESTOSTERONE BOOSTING EFFICACY OF A NOVEL *CURCULIGO ORCHIOIDES* EXTRACT IN MALE RATS

Kanwaljit Chopra[1], Anand Swaroop[2], Pawan Kumar[3], Manashi Bagchi[2] and Debasis Bagchi[2,4]

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Curculigo orchioides Gaertn (family Hypoxidaceae, also known as black or kali musli), is an endangered medicinal plant used for diverse medicinal purposes including impotency, aphrodisiac, diuretic, tonic, jaundice, and skin ailments. Phytochemical investigations of rhizomes revealed the presence of a novel phenolic glycoside, curculigoside, triterpenoid, saponins, flavones, cellulose, hemicellulose, and calcium oxalate. We developed a novel extract of *Curculigo orchioides* (Blamus™, standardized to 30% curculigosides) and assessed its dose- and time-dependent efficacy (0, 10, 25 and 50 mg/kg body weight p.o.) on body weight, serum free and total testosterone levels in male rats (200-230 grams; n = 6) over a period of 28 days. Blamus didn't cause any marked elevation in serum free testosterone levels at either 10 or 25 mg/kg body weight doses, however, a 50 mg/kg body weight dose of showed a significant increase in serum free testosterone level (*$p < 0.0001$). However, no significant increases were observed in serum total testosterone levels at 0, 10, 25 or 50 mg/kg body weight doses of Blamus. Extensive testicular histopathological analyses including investigations on the seminiferous tubules, spermatogenesis, sperm cell morphology, Leydig cells and Sertoli cells following treatment with either 0, 10, 25 or 50 mg/kg body weight doses of Blamus demonstrated dose-dependent improvement in structural integrity. Furthermore, no significant changes were observed in serum SGOT, SGPT, BUN and creatinine levels following treatment with any of the given doses, which demonstrated its safety profiles. Thus our data ascertained that Blamus may serve as a safe and novel, natural testosterone booster and provide broad spectrum application is sports nutrition, muscle building and exercise pathophysiology.

P-063

IDENTIFICATION OF POTENT AHR LIGANDS FROM HUMAN-ASSOCIATED BACTERIA BY STRUCTURE-GUIDED GENOME MINING

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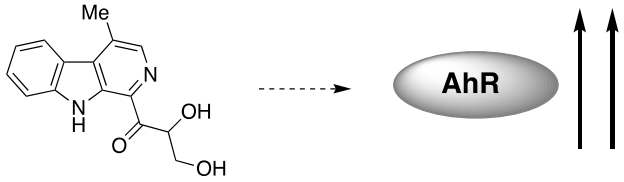
Aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor that regulates inflammation and cell proliferation in response to aromatic hydrocarbons. Recent studies reported that human microbiota are capable of metabolizing tryptophan to indole-derived compounds that activate AhR signaling pathway; however, whether microbiota can *de novo* biosynthesize AhR ligands remains poorly understood. In this study we investigated AhR ligands from human microbiota by genome mining targeted on biosynthetic genes that produce polyaromatic secondary metabolites. By searching for genes responsible for β-carboline biosynthesis, we isolated oxopropaline, streptonigrin, and a new derivative from a gut-associated *Streptomyces* strain. An assay for AhR signaling activation revealed that oxopropaline has stronger AhR signaling induction than tryptophan metabolites such as diindolylmethane. These results will aid the discovery and design of drugs targeting AhR signaling pathway.



P-064

INSIGHT INTO THE BIOSYNTHETIC PATHWAY OF A NOVEL NUCLEOSIDE ANTIBIOTIC

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Antibiotic-resistance has become a widespread problem in the United States and across the globe. Meanwhile, new antibiotics are entering the clinic at an alarmingly low rate. Highly-modified nucleosides, a class of natural products often produced by actinobacteria, target MraY bacterial translocase I. MraY is a clinically unexploited enzyme target that is ubiquitous and essential to peptidoglycan cell wall biosynthesis. The nucleoside antibiotics known vary in efficacy and the functionalities contributing to improved activity is poorly understood. Sphaerimicin, a newly discovered modified nucleoside, has potent inhibitory activity with an IC_{50} of 13.65 nM against MraY. In general, sphaerimicin is primarily effective against gram-positive bacteria (MIC ranges from 2-16 μg/mL against *Enterococcus faecium* and *Staphylococcus aureus*), but little is known about the biosynthesis and mechanism of action. Sphaerimicin has highly unusual structural features, including a heavily modified ribosylated glycl-uridine disaccharide core that is appended to a dihydroxylated piperidine ring. The novel biosynthesis of these features was investigated, leading to the functional characterization of six enzymes critical for the disaccharide core formation from uridine monophosphate. Recently, a unique *S*-adenosylmethionine- and PLP-dependent alpha-aminobutyric acid transferase and a nonheme Fe(II)- and alpha-ketogluterate-dependent hydroxylase from the sphaerimicin biosynthetic pathway were characterized. This part of the pathway extends the carbon scaffold, which will be crucial for formation of the piperidine ring. Not only does this study provide new chemical entities to help better understand MraY as a target, it could potentially reveal new enzymatic chemistries that will power innovative chemoenzymatic synthesis and genome mining to uncover new natural products.

P-065

1,2,4-TRIAZINE CONTAINING NATURAL PRODUCTS: INVESTIGATIONS INTO THE BIOSYNTHESIS, SELF-RESISTANCE AND GENOME MINING.

Khaled H. Almabruk[1], Michael K. Fenwick[2], Brenda T. Shaffer[3], Qing Yang[3], Joyce E. Loper[3], Steven E. Ealick[2], Benjamin Philmus[1]
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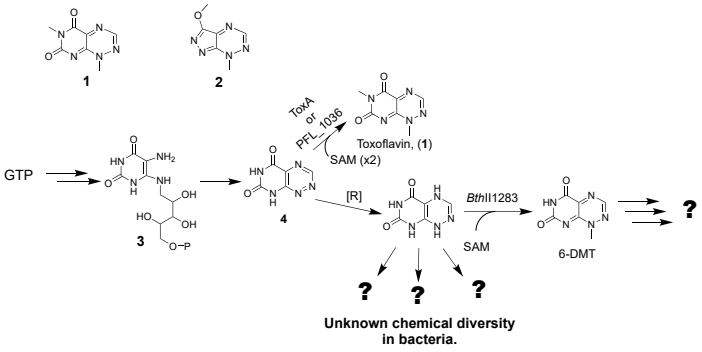
Triazine containing natural products constitute a small set of known metabolites including toxoflavin (**1**), a virulence factor produced by the plant pathogenic bacteria *Burkholderia glumae* and nostocine A (**2**), an alleochemical produced by the cyanobacterium *Nostoc spongiaeforme*. Here we present our lab's findings involving the biosynthetic route used by *Pseudomonas protegens* Pf-5 to form the 1,2,4-triazine ring which goes through 5-amino-6-(5-phospho-D-ribitylamino)uracil (**3**), an intermediate shared by the riboflavin biosynthetic pathway. In addition, we have looked at the self-resistance that *P. protegens* Pf-5 utilizes to prevent self-toxicity. By screening microbial genomic sequences available publically, we have also identified multiple biosynthetic gene clusters suggesting that they have a common core of enzymes that we have postulated synthesize a common intermediate (**4**) that is then diversified into a larger chemical space then previously appreciated.



P-066

ELUCIDATING THE BIOSYNTHESIS OF PENILUMAMIDE AND IDENTIFYING NEW THERAPEUTIC TARGETS FOR ANTIBIOTIC DEVELOPMENT

Stephanie C. Heard and Jaclyn M. Winter

While resistance mechanisms continue to rise among disease-causing bacteria, the number of effective antibiotics is quickly being depleted. One particular pathway we are targeting for developing new antibiotic agents is folate metabolism. We searched the literature for natural products containing structural components similar to intermediates in the folate pathway 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 lumazine functional group is extremely rare and has only been reported in three natural products, to date. Penilumamide therefore is an excellent candidate for biosynthetic interrogation and for revealing not only how this unprecedented monomer is synthesized, but if the pterin-like core possesses antimicrobial activity. The 33 Mbp genome of the penilumamide-producing fungal strain *A. 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. Independent inactivation of all four NRPS genes within close proximity of this putative lumazine-producing operon confirmed that only a single module-containing enzyme is required for penilumamide production. The biosynthesis of the lumazine building block will be presented, as well as gene inactivation studies and reconstitution of the nonribosomal peptide synthetase.

P-067

IDENTIFICATION OF THE BIOSYNTHETIC GENE CLUSTER OF TRIACSINS

Wenlong Cai[1], Fredrick Twigg[1], Wei Huang[1], Joyce Liu[1], and Wenjun Zhang[1,2]
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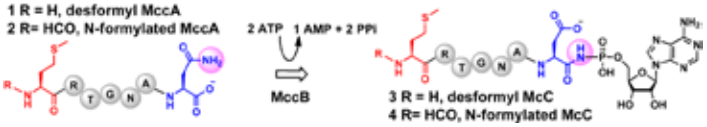
Triacsins, having been discovered for over three decades from *Streptmyces aureofaciens*, are widely used today as potent acyl-CoA synthetase inhibitors. Triacsins are notable for their unique a unique N-hydroxytriazene moiety at the terminus which is not found in any other natural products. Simpler conjugated nitrogen-nitrogen bonds are found in a variety of natural products while only a handful of biosynthetic pathways have been characterized. We have recently identified the biosynthetic gene cluster for triacsins in *Streptomyces aureofaciens* via genome mining and gene inactivation. Further genome mining effort led to the discovery of an additional Streptomyces producer of triacsins. Our *in vivo* and *in vitro* characterization of candidate biosynthetic genes and possible intermediates revealed new biosynthetic insights regarding triacsin biosynthesis in *Streptomyces*.

P-068

BIOSYNTHESIS OF THE RIPP TROJAN HORSE ANTIBIOTIC MICROCIN C IS DIRECTED BY THE *N*-FORMYL PEPTIDE SUBSTRATE

Shi-Hui Dong[‡,§], Alexey Kulikovsky[‡,†], Svetlana Dubiley[†], Konstantin Severinov[†], and Satish K. Nair[‡,§,ᶜ,]*
[‡]Department of Biochemistry, [§]Institute for Genomic Biology, and [ᶜ]Center for Biophysics and Quantitative Biology, University of Illinois at Urbana-Champaign, Illinois, USA. [†]Institute of Gene Biology, Russian Academy of Science, 34/5 Vavilo str. 11934 Moscow, Russia

Microcin C7 (McC) is a ribosomally produced heptapeptide that is subsequently modified by the linkage of the terminal Asp α-carboxylate to AMP via a phosphoramidate bond. Post-translational modification on the heptapeptide precursor is carried out by MccB, which consumes two equivalents of ATP to generate the P-N linkage. Here, we demonstrate that MccB only efficiently processes the precursor heptapeptide that retains the *N*-formylated initiator Met (fMet). Binding studies and kinetic measurements evidence the role of the *N*-formyl moiety, and structural studies reveal substrate bound in a productive mode. Structural data show that the *N*-formyl peptide binding results in an ordering of residues in the "crossover loop", which dictates specificity in homologous ubiquitin activating enzymes. Importantly, the *N*-formyl peptide exhibits substrate inhibition, and cannot be displaced from MccB by the desformyl counterpart. Such substrate inhibition may be a strategy to avert the unwanted buildup of McC and avoid toxicity in the cytoplasm of producing organisms.



P-069

DESIGN AND SYNTHESIS OF PHYLLANTHUSMIN ANALOGUES TO IMPROVE PHARMACOLOGICAL PROPERTIES

Andrew C. Huntsman[1], Alexandria Young[2], John L. Woodard[1], Hee-Byung Chai[1], Yulin Ren[1], Mitch A. Phelps[3], A. Douglas Kinghorn[1], Joanna E. Burdette[2], James R. Fuchs[1]
[1]Medicinal Chemistry and Pharmacognosy, The Ohio State University
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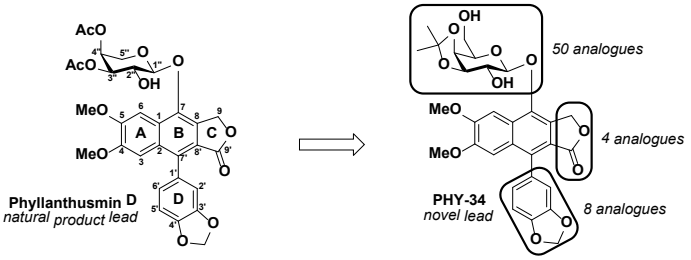
[3]Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University.

Structure activity relationship studies on the phyllanthusmin class of natural products have led to a novel lead compound (**PHY-34**) with potent antiproliferative activity. Its design has facilitated additional functionalization to further explore pharmacological properties, such as selectivity, solubility, and potency. In brief, this work has provided insight into key structural motifs and chemical transformations that may be able to be exploited to overcome additional challenges associated with the development of this class, including toxicity. Our current focus is on identification of the molecular target, addressing metabolic liabilities, and mediating potential off-target effects.

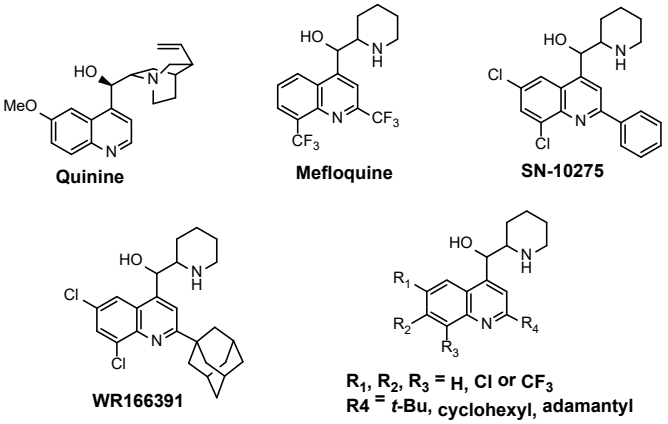


P-070

ANTIMALARIAL AND ANTIMICROBIAL ACTIVITIES OF A-(2-PIPERIDYL)-2-ALKYL-4-QUINOLINEMETHANOL ANALOGS

H. M. T. Bandara Herath, H. Ranjith W. Dharmaratne, Melissa Jacob, N. P. Dhammika Nanayakkara
National Center for Natural Product Research, School of Pharmacy, University of Mississippi, MS 38677

Mefloquine, a currently used antimalarial drug, is an analogs of the highly effective antimalarial alkaloid, quinine. A related analog, SN-10275, had better prophylactic and therapeutic activities than quinine against *Plasmodium vivax* malaria in humans, potent *in vitro* activity against *Staphylococcus aureus* and *Cryptococcus neoformans*, and strong *in vivo* antischistosomal activity in a mouse model. However, drug-induced phototoxicity remains the major drawback in development of this compound. The observed photosensitivity of SN-10275 has been attributed to the increased resonance conjugation from the 2-aryl group. A subsequent study has shown that WR166391 also has potent antimicrobial activity. In our study, we prepared a number of WR166391 analogs with bulky alkyl groups (*t*-butyl, cyclohexyl, adamantyl) at the 2-position and different substitutions on the quinoline ring. Some of them showed potent *in vitro* activity against *C. neoformans*, methicillin-resistant *S. aureus* (MRSA) and *Mycobacterium intercellulare*.

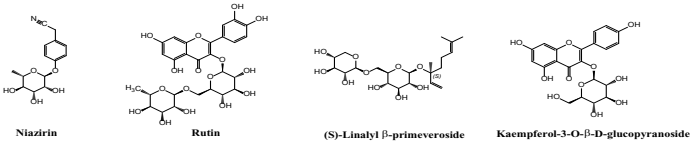


P-071

ISOLATION, CHARACTERIZATION AND SYNTHESIS OF SECONDARY METABOLITES DERIVED FROM THE MIRACLE TREE (*MORINGA OLEIFERA*) LEAVES

Omer I. Fantoukh[1,2,3], Mohamed Albadry[1,2], Mohammed F. Hawwal[1,2,3], Abidah Parveen[1,2], Taghreed Majrashi[1,2], Zulfiqar Ali[1], Amar G. Chittiboyina[1,2], Ikhlas A. Khan[1,2]
[1]National Center for Natural Products Research, [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA, [3]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Moringa oleifera Lam. is known as the miracle vegetable because all parts of *Moringa* have been exploited to treat a variety of ailments. It shows antihypertensive, antimicrobial, antidiabetic, anticancer, and anti-inflammatory activities. In this study, phytochemical investigation of *M.oleifera* leaves resulted in the isolation and characterization of one new compound in addition to fifteen known secondary metabolites from several chemical classes including flavonoids, terpenoids, lignans and phenyl propanoids. Because of the low yields and limited supply, the new compound and niazirin were successively synthesized for anticancer activity screening.



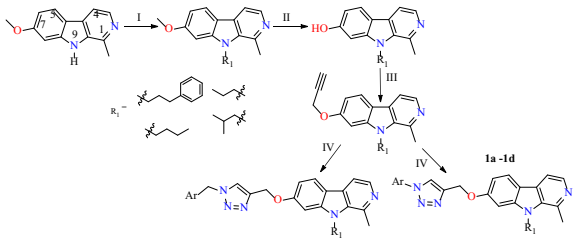
P-072

ISOFORM SELECTIVITY OF HARMINE CONJUGATED 1,2,3-TRIAZOLES AGAINST HUMAN MONOAMINE OXIDASE

Saqlain Haider[1], Manal Alhusban[1], Narayan Chaurasiya[1], Babu L. Tekwani[1,2], Amar G. Chittiboyina[1], and Ikhlas A Khan[1,2]
[1]National Center for Natural Products Research, [2]Division of Pharmacognosy, Department of BioMolecular Sciences; School of Pharmacy, The University of Mississippi, University, MS 38677, USA.

There is little information available on Monoamine oxidase isoform selectivity of N-alkyl harmine analogs which exhibit a myriad of activities including MAO-A, tyrosine-phosphorylation-regulated- kinase (DYRK1A) and cytotoxicity to several select cancer cell lines. Keeping in mind the selective inhibitory activity of harmine on MAO-A over MAO-B (>10,000-fold selectivity) we have designed the synthesis of single and double methylene bridged 1,2,3- triazole derivatives through simple 'Click' chemistry and

screened them for their MAO inhibitory potentials. The compounds **3e** and **4c** exhibited an IC50 of 0.83 ± 0.03 and 0.43 ± 0.002 μM against MAO-A and an IC50 of 0.26 ± 0.04 and 0.36 ± 0.001 μM against MAO-B respectively. The details on chemistry, computational results and biological assay will be disclosed.



P-073

FIVE NEW STILBENES FROM THE STEM BARK OF *ARTOCARPUS COMMUNIS*

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Artocarpus communis J.R.Forst. & G.Forst. (Moraceae), synonymous with *Artocarpus altilis*, is a flowering tree that is native to Indonesia, Papua New Guinea, and Tropical Asia and the Pacific. It has been used in traditional medicine in the Pacific Islands for a variety of ailments including liver cirrhosis, hypertension, diabetes, skin ailments, and tapeworm infection. Previous phytochemical investigations of this plant have yielded over 130 compounds, of which flavonoids, aryl benzofurans, stilbenoids, and lectins are largely responsible for the pharmacological effects of the plant. In our investigation, five new prenylated stilbenes, along with the known compounds cudraflavone C, *trans*-4-isopentenyl-3,5,2′,4′-tetrahydroxystilbene, *trans*-4-(3-methyl-*E*-but-1-enyl)-3,5,2′,4′-tetrahydroxystilbene, pannokin G, morusin, cycloartobiloxanthone, artonin P, artocarpin, artonin E, kuwanon C, artobiloxanthone, and artoindonesianin C were isolated from the stem bark of *A. communis*. The structures were established by NMR spectroscopic analysis, MS studies, and comparison of experimental data with spectral data reported in the literature. Although the organic extract of *Artocarpus communis* modulated the activity of the oncogenic transcription factor HIF-2α in preliminary testing, none of the isolated pure compounds had a significant inhibitory effect on the transcriptional activity of HIF-2α.

P-074

BIOMIMETIC TOTAL SYNTHESIS OF THE CYCLIC IMINE NATURAL PRODUCT SCYTONEMIDE A USING WEINREB AM SOLID PHASE RESIN

Tyler A. Wilson[1], Robert J. Tokarski[1], Jimmy E. Orjala[2], L. Harinantenaina Rakotondraibe[1], James R. Fuchs[1]

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As part of a continued effort to identify novel compounds with anticancer properties, scytonemide A was isolated from the cyanobacterium *Scytonema hofmannii* in 2010. This macrocyclic peptide contains a rare imine linkage previously seen in only a handful of other natural products, including the nostocyclopeptides and koranimine. Scytonemide A displayed promising inhibition against human 20S proteasome chymotrypsin catalytic activity, with an IC_{50} of 96 nM. However, further exploration and development of this unique natural product as a proteasome inhibitor was hindered due to the small quantities obtained from the cyanobacterium, necessitating an efficient synthetic route for sufficient quantities of the compound. Therefore, inspired by the biosynthetic construction of cyclic peptide imines using non-ribosomal peptide synthetases (NRPSs), a biomimetic solid-phase peptide synthesis (SPPS) approach was developed and executed using the Weinreb AM resin. Thorough spectroscopic analysis was performed to confirm the structure of the desired product, shedding light on the differences observed between the synthetic and natural samples.

P-075

NATURE PRODUCT BASED ANTI-CANCER PROBE/LEAD DEVELOPMENT VIA SYNTHETIC DIVERSIFICATION STRATEGIES

Yang Liu[1], Yinan Zhang[1], Qing Ye[2,3], Joseph Eckenrode[1], Xiachang Wang[1], Yubin Guo[2,3], Yanan Cao[2,3], Weijia Cai[2,3], Jamie Horn[1], Larissa V. Ponomereva[1], Khaled A. Shaaban[1], Markos Leggas[1], Qing-Bai She[2,3], and Jon S. Thorson[1].

[1]Center for Pharmaceutical Research and Innovation, College of Pharmacy, University of Kentucky; [2]Center for Applied Energy Research, University of Kentucky; [3]Kentucky Geological Survey, University of Kentucky

Natural products are a productive source of leads for drug development. As part of our ongoing translational research and drug discovery and development program at the Center for Pharmaceutical Research and Innovation (CPRI) at the University of Kentucky (UK), we carried out chemical probe synthesis, corresponding target identification, structure-activity relationship (SAR) studies and in vivo evaluation of novel semi-synthetic analogs deriving pyranonaphthoquinones and mithramycin. We will report on key synthetic modification strategies, the discovery of the pyranonaphthoquinone molecular target, semi-synthetic mithramycin analogs with selectivity toward Ewing's sarcoma and preliminary in vivo studies of optimized early potential lead candidates.

P-076

INSPIRED BY NATURAL PRODUCTS, RATIONAL DESIGN AND SYNTHESIS OF NOVEL STEROIDAL LXR AGONISTS AS WELL AS THEIR BIOLOGICAL EVALUATION

Derong Ding[1,2], Rupinder Kaur[1,2], Yaxia Yuan[1,2], Gregory A. Graf[1,2], Chang-Guo Zhan[1,2], David S. Watt[1,3], Jon S. Thorson[1,2]

[1]Center for Pharmaceutical Research and Innovation, College of Pharmacy, University of Kentucky, Lexington, KY, USA; [2]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, USA ; [3]College of Medicine, University of Kentucky, KY, USA,40508

Live X receptors (LXRs) are the ligand-activated transcription factors and have LXRα and LXRβ isoforms. They play an important role in lipid metabolism and have been the useful target for treatment of cardiovascular diseases, Alzheimer`s disease, dermatological ailments and cancers. LXRβ is ubiquitously expressed and is a more attractive drug target owing to the hepatic lipogenesis caused by the agonism of LXRα. Naturally occurring oxysterols such as 24(*S*), 25-epoxycholesterol were identified as LXR ligands. To develop more potent LXRβ agonists for the treatment of cardiovascular disease, we embarked on a rational ligand design strategy. Specifically, using the crystal structure of LXRβ in complex with 24(*S*),25-epoxycholesterol, computational modeling predicted a series of novel structurally-realted ligands with putative high affinity for LXRβ. We report on the development of a 12-step synthetic strategy from lithocholic acid to enable the synthesis of the top computational target. In addition, preliminary biological evaluation of the corresponding analog will be presented as the first step in validating and refining our current LXRβ computational model.

P-077

CHARACTERIZATION OF IMMUNOMODULATORY POLYSACCHARIDES ISOLATED FROM *PANAX QUINQUEFOLIUS* (NORTH AMERICAN GINSENG) CALLUS CULTURES

Rajarshi Ghosh[1,2], Shannon Smith[1], Evidence Nwangwa[2], Paul Kline[2] and Anthony Farone[1]
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Botanical polysaccharides have been identified as attractive candidates for the development of novel therapeutics in recent years, mainly due to their ability to modulate the host immune system. However, there has been several challenges in the commercialization of such products because of high purification costs, erratic yields, and variable chemical characteristics. This study proposes the application of a plant tissue culture-based system for the production of pathogen-free natural immunomodulatory polysaccharides with consistent chemical characteristics. Four polysaccharide fractions were isolated from callus cultures of Panax quinquefolius, a well-known medicinal herb with immunomodulatory properties. The heterogeneous fractions ranged in molecular weight between 5 kDa and 50 kDa. The fractions displayed characteristics of arabinogalactan type II polysaccharide based on composition and linkage analysis data. The compounds were found to stimulate a range of pro-inflammatory markers (IL-6, TNF-α, MCP-1, GM-CSF and nitric oxide) in vitro (RAW 264.7 murine macrophage cells) and ex vivo (mice splenocytes). The potency of the callus polysaccharides as immunostimulatory agents was comparable to mature Panax quinquefolius root polysaccharides. The results demonstrate the potential applicability of callus culture as a novel source of bioactive polysaccharides.

P-078

SMALL MOLECULE CHEOENZYMATIC GLYCOSYLATION PLATFORM DEVELOPMENT AND APPLICATIONS

Ryan R. Hughes, Khaled A. Shaaban, Larissa Ponomareva, Jamie Horn, Markos Leggas, Jon S. Thorson*
** Center for Pharmaceutical Research and Innovation, Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536; Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536, United States*

Glycosylation is a key process through which a glycosyltransferase (GT) attaches sugars to a wide variety of potential acceptors that include nucleic acids, polysaccharides, proteins, lipids, carbohydrates and medicinally relevant secondary metabolites. While GT-catalyzed formation of *O*-glycosides is most common, corresponding GTs involved in the biosynthesis of *C*-, *S*-, and *N*-glycosides have also been characterized. OleD is an *O*- catalyzing GT from *Streptomyces antibioticus* that has been successfully engineered by the Thorson lab to give mutants that are more proficient and permissive in the context of both sugar donors and glycosyl substrates. The corresponding engineered GTs serve as enabling catalysts for glycorandomization (a robust platform for small molecule differential glycosylation) and set the stage to assess the impact of glycosylation on basic physical properties, mechanism and *in vivo* pharmacology of natural products, drugs, drug leads and/or bioactive probes. As part of this broad effort, the extension of a 2-chloro-4-nitrophenol glycoside-based high throughput screen to identify additional new GT substrates (including tertiary amines and hydroxamates) and new GT inhibitor pharmacophores will be presented.

P-079

EVALUATION OF ANTIOXIDANT AND ANTI-CANCER ACTIVITY OF FUCOSE-CONTAINING SULFATED POLYSACCHARIDE FROM MARINE ALGAE

Priscilla Lin,[1] Tamara Kondratyuk, [2] San Teh,[1] Leng Kar Chang,[1] Leng Chee Chang[2]
SKA Academy of Art and Design, 2645 North Berkeley Lake Road, Suite F142, Duluth, GA, 30096, USA. [2]Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, 96720, USA.

Fucoidan, a sulfated polysaccharide purified from brown algae, possesses a variety of pharmacological effects, including anti-inflammatory, antioxidant, and anticancer properties; however, different extraction method affect the biological activity and type of extracted fucoidan. The objectives of this study were to extract crude fucoidan and evaluate the medicinal properties found in *Undaria pinnatifida* and dried kelp with the aim to ascertain whether those have these activities, as well as which functional groups confer them. *U. pinnatifida* and dried kelp are common brown algae species eaten in soups. Both are easily accessible to consumers and are believed to have health benefits. Both specimens were pre-treated and extracted using two methods - the conventional method and a microwave-assisted extraction. Following extraction, samples were evaluated for antioxidant activity using the ferric ion-reducing antioxidant power (FRAP) assay. The structure of fucoidan was analyzed using NMR, and Fourier Transform Infrared Spectroscopy. Extracted fucose was tested for cytotoxicity using an *in vitro* sulforhodamine B (SRB) assay against human prostate (LNCaP, PC-3), breast (MCF-7), colon (Caco-2), and lung (Lu-1) cancer cell lines. Analysis of data revealed activities with inhibitory concentrations greater than 50% against several cancer cell lines. These results demonstrated fucoidan isolated from *U. pinnatifida* and dried Kelp does possess antioxidant and anticancer properties.

P-080

GENOME MINING-GUIDED SCREENING FOR PSEUDOSUGAR-CONTAINING NATURAL PRODUCTS

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Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331-3507, U.S.A.

Sugar-containing compounds are ubiquitous in nature, and many of them have significant biological activities. The attachment of sugars to natural products is catalyzed by members of the glycosyltransferase (GT) superfamily. Similarly, pseudosugar-containing natural products have been recognized to be important secondary metabolites with significant potential. The addition of pseudosugars to natural products is catalyzed by the variants of GTs known as pseudo-glycosyltransferases (PsGTs). Using bioinformatic and genome mining approaches we discovered a number of promising gene clusters in different actinomycetes that are expected to produce pseudosugar-containing natural compounds. Inactivation of these gene clusters resulted in mutant strains that showed distinct secondary metabolite profiles. Isolation, characterization, and biological activities of these natural products will be presented.

P-081

SILYMARIN PREVENTS MEMORY IMPAIRMENTS, ANXIETY, AND DEPRESSIVE-LIKE SYMPTOMS IN A RAT MODEL OF POSTTRAUMATIC STRESS DISORDER

Tamam El-Elimat[1], Zeinab Y. Al Subeh[2], Mahmoud Abu AlSamen[1], Karem H. Alzoubi[1], Tyler N. Graf[2], and Nicholas H. Oberlies[2]
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Post-traumatic stress disorder (PTSD) is a debilitating psychopathological disease that is triggered by exposure or witnessing a traumatic event, and usually associated with substantial co-morbidities, such as cognitive impairment, anxiety, and depression. Silymarin, from *Silybum marianum* (L.) Gaertn. (Asteraceae), has been reported to exhibit a neuroprotective activity against various neurodegenerative diseases. Herein, the beneficial effects of silymarin in ameliorating PTSD symptoms such as memory impairments, anxiety- and depression-like behaviors were evaluated on a single prolonged stress (SPS) rat model of PTSD. Male Wistar rats were randomly assigned into four groups: control, silymarin, PTSD, and PTSD/silymarin groups. Rats were administered silymarin, 100 mg/kg i.p. for three weeks. Animals in all groups were tested for short- and long-term memory in the radial arm water maze, for anxiety behavior using open field and elevated plus maze tests, and for depressive symptoms using tail suspension test. Results showed that rats in PTSD group suffer from long-term memory impairment and depressive symptoms compared to the control group, and silymarin treatment prevents the development of memory impairments, anxiety, and depressive-like symptoms in PTSD rats.

P-082

APOPTOTIC INDUCTION BY (+)-BETULIN FROM *SYZYGIUM CORTICOSUM* ON MDA-MB-321 BREAST CANCER CELLS

Gerardo D. Anaya-Eugenio[1,2], Yulin Ren[1], A. Douglas Kinghorn[1], Esperanza J. Carcache de Blanco[1,2]
[1]Division of Pharmacy Practice and Science, and [2]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA.

Betulin, one of the main secondary metabolites from *Syzygium corticosum* (Lour.) Merr. & Perry (Myrtaceae), has shown cytotoxic activity against MDA-MB-231 (triple negative) breast cancer cells[1]. However, its effect and mechanism of action remains unclear. The present study focuses on the study of the biological effects of betulin on MDA-MB-231 breast cancer cells, as well as its possible mechanisms underlying these effects. After 72 hours of treatment with betulin, the growth of MDA-MB-231 cells were significantly inhibited (IC_{50}= 1.5 μM) in the SRB assay. Betulin also reduced protein concentrations of NF-κB (p65 and p50/105), both IκB kinase (IKK) α and β in western blot analysis. However, betulin does not seem to modify the expression of PARP-1. These preliminary data suggest that betulin induces apoptosis in MDA-MB-231 cancer cells by inhibition of the NF-κB complex.

Reference: Król SK, Kiełbus M, Rivero-Müller A, Stepulak A (2015). Comprehensive review on betulin as a potent anticancer agent. Biomed Res Int. 2015: 584189.

P-083

DE-REPLICATION COUPLED WITH AMOEBIC BIOASSAY GUIDED FRACTIONATION IDENTIFIES BIOACTIVE SECONDARY METABOLITES IN EPIGENETIC MODULATED FUNGAL EXTRACTS

Santana Thomas,[1] Christopher A. Rice,[2] Dennis Kyle,[2] Bill J. Baker[1]
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Fungal endophytes are suggested to be ubiquitous among plants to essentially provide a symbiotic relationship. The fungal bioactive natural products have profound effects on its host with provision of growth enhancement and competitiveness. As a good source of bioactive secondary metabolites fungal endophytes were collected and isolated from mangrove tissues. To encourage the production of these bioactive compounds, epigenetic modulators were introduced to the cultures. After a screen of over 8000 extracts against amoebic infections biologically active extracts were grown in a large scale for isolation and purification of the active components. Using anti-amoebic activity and de-replication strategies in tandem several bioactive secondary metabolites were isolated from these fungal cultures.

P-084

INHIBITION OF THE 20S PROTEASOME: STRUCTURE-ACTIVITY RELATIONSHIP OF SCYTONEMIDE A AND RELATED ANALOGUES

Robert J. Tokarski[1], Tyler A. Wilson[1], Jimmy E. Orjala[2], Joanna E. Burdette[2], L. Harinantenaina Rakotondraibe[1], James R. Fuchs[1].
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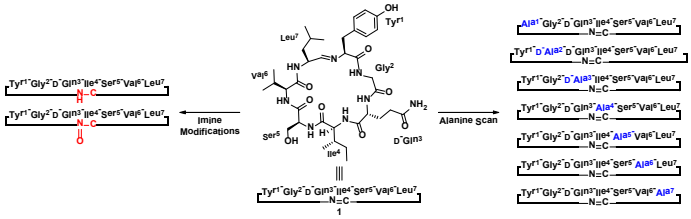
Scytonemide A (**1**), a unique cyclic peptide imine discovered from the cyanobacterium *Scytonema hofmannii* in 2010, shows promising 20S proteasome inhibition, with an IC_{50} = 96 nM. Recently, the total synthesis of **1** was reported utilizing solid-phase peptide synthesis (SPPS) on Weinreb AM resin. To facilitate further development as a potential therapeutic, this synthetic route has been modified to access structural analogues and establish structure-activity relationships. The syntheses and biological activities of a series of analogues of **1**, including an alanine scan and imine modifications, will be presented.



P-085

NEW ^{1}H-NMR BASED TECHNIQUE FOR THE DETECTION OF NOVEL NATURAL PRODUCTS

Asmaa Boufridi[1], Dominique Gorse[2], RuAngelie Edrada-Ebel[3], Ronald J. Quinn[1]
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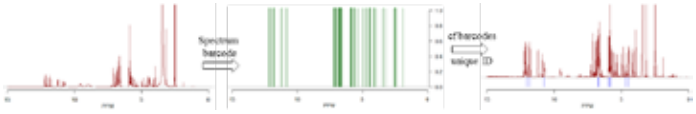
We have developed an untargeted ^{1}H-NMR method in the R language. We have acquired and digitised ^{1}H-NMR spectra of more than 4000 fractions

from plants and marine animals. The digitised ^{1}H-NMR data were imported into the software R and processed (smoothing the intensity, removing the baseline and binning). Each detected peak was set to 1 to create a barcode related to each fraction. Each barcode is compared to the whole dataset in order to detect unique peaks and the corresponding spectrum selected. Compounds are isolated by ^{1}H-NMR guided isolation.



P-086

NATIVE MASS SPECTROMETRY AS A DRUG DISCOVERY TOOL

Angela Di Capua[1], Miaomiao Liu[1], Asmaa Boufridi,[1] Ali Elnaas,[1] Tin Mak,[1] Yang Yang,[1] Carl Nathan,[2] Ruslana Bryk,[2] Peter Myler[3], and Ronald J. Quinn[1]

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We have reported the use of native mass spectrometry to investigate 62 potential protein targets for malaria using a natural product-based fragment library. 97 low molecular natural products were identified as binding partners of 32 of the putative malarial targets. Seventy-nine (79) fragments have direct growth inhibition on *Plasmodium falciparum* at concentrations that are promising for development of fragment hits. This adds a fragment library to the published HTS active libraries in the public domain.

As far as we are aware, the use of natural products as a fragment library has not been previously reported. The work should have broad impact as it can be applied to any therapeutic target. The use of a mass-spec based approach is novel as the vast majority of FBDD campaigns use either DSF or NMR as the primary screen. This work likely represents the largest single dataset disclosed for a FBDD campaign with data for 62 targets against 643 unique fragments, providing a wealth of information on both ligand and target promiscuity.

We will present an extension of this work applied to TB. We conducted a phenotypic HTS campaign against *M. tuberculosis* H37Rv and the TB target Lipoamide Dehydrogenase (Lpd). The phenotypic active fractions have been used for target identification using a panel of cloned and expressed *Mycobacterium* proteins.

P-087

A MS/MS MOLECULAR NETWORKING BASED APPROACH TOWARD THE CHARACTERIZATION OF THE TRICHODESMIUM METABOLOME

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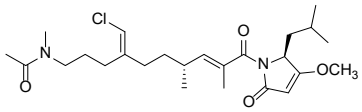
Trichodesmium species are highly adapted marine cyanobacteria, known for their beneficial role as nitrogen fixers in tropical and subtropical waters. Currently, there is little knowledge available with respect to the diversity and abundance of secondary metabolites. Using a MS/MS based molecular networking approach that clusters ions based on their similar MS^2 fragmentation patterns, we aimed to characterize the *Trichodesmium* metabolome. The approach led to the isolation and characterization of new smenamide analogs (**1**-**3**), which are structurally similar to the previously elucidated smenamides A and B, adding to a class of biosythetically interesting hybrid polyketide-peptide compounds. Among the newly described smenamides, **1** and **3** showed potent cytotoxicity against neuro-2A cells. The isolation, characterization and cytotoxicity of these new smenamide molecules (**1**-**3**) will be presented.



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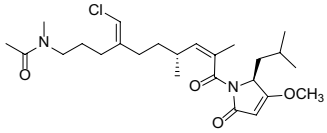

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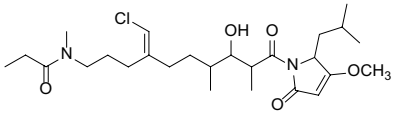

3

P-088

EXPLORATION OF XANTHINE OXIDASE INHIBITORS FROM *HYPTIS SUAVEOLENS*

Fu-Chun Hsu, Yi-Li Lin, and Shoei-Sheng Lee.*

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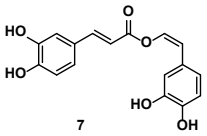
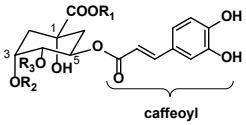
Bioassay-guided fractionation of the EtOH extract of the *Hyptis suaveolens* stems and seeds indicated both EtOAc- and *n*-BuOH- soluble fractions to be active against xanthine oxidase which catalyzes the formation of uric acid, a main cause of gout. Separation of these fractions via Sephadex LH-20, centrifugal partition chromatography, and reversed-phase chromatography led to the isolation of 17 compounds from the stems and four from the seeds. Additional ten minors in the seeds were characterized via the assistance of HPLC-SPE-NMR. Of these isolates, nepetoidin B (**7**), 4,5-*O*-dicaffeoylquinic acid (**25**), and methyl 3,5-*O*-dicaffeoylquinate (**27**) have been reported to display good inhibitory activity against xanthine oxidase.



7



caffeoyl

	R_1	R_2	R_3
25	H	H	caffeoyl
27	Me	caffeoyl	H

P-089

NEW NMR APPLICATIONS FOR BOTANICAL MIXTURES: THE USE OF HSQC TO DETERMINE LIGNAN CONTENT IN *SAMBUCUS WILLIAMSII HANCE*

Raymond Cooper[1], Juan Lu[2], Hui-Hui Xiao[1,3], Daniel Mok[1,3], Xin-Sheng Yao[4] and Man-Sau Wong[1,3]
c

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Lignans found in the botanical extract of the Traditional Chinese Medicine, *Sambucus williamsii* Hance exhibit protective effects on trabecular bone mass and mechanical strength of cortical bone of ovariectomized (OVX) rats. A novel approach was adapted to standardize and determine quantities of the lignan content in the aqueous alcoholic extract of *Sambucus williamsii* using HSQC NMR methods and provides data to meet the registration requirements for a Class 5 botanical drug in China. The assigned oxy-benzyl substituted carbon signal in the 13C NMR spectrum of a known lignan was compared to the equivalent carbon signal in the lignan mixture, observed in the botanical extract, under the same solvent and concentration conditions. A linear response curve was obtained for the peak height of the assigned oxy-benzyl carbon at ~86ppm for the standard lignan at various dilutions. Results were obtained by comparing the correlation peak (oxy-benzyl substituted carbon signal in the HSQC for the 13C signal ~ 86ppm, and 1H signal at ~4.6ppm) of pinoresinol in the HSQC spectrum with the equivalent correlation peaks of lignans in the botanical extract. Using these data, the lignan amount in the extract was calculated to be approximately 60% of the total extract. The application of this simple and reliable method can be used to estimate other compound families in complex mixtures or botanical extracts.

P-090

ENHANCED LC–MS/MS MOLECULAR NETWORKING WORKFLOW FOR ACCELERATED AND MASSIVE DEREPLICATION OF NATURAL PRODUCTS

Kyo Bin Kang[1,2], Justin J.J. van der Hooft[1,3], Madeleine Ernst[1], Ricardo da Silva[1], Marnix H. Medema[3], Sang Hyun Sung[2], and Pieter C. Dorrestein[1].
[1]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA, [2]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Republic of Korea, [3]Bioinformatics Group, Department of Plant Sciences, Wageningen University, Wageningen 6708PB, The Netherlands.

MS/MS molecular networking, which groups and visualizes molecules based on their MS/MS spectral similarity suggesting structural similarity, was previously presented by our group as a data analysis technique for accelerating dereplication processes. Here, we introduce an enhanced workflow that facilitates mapping and identifying massive specialized metabolite spaces, based on enhancement of MS/MS molecular networking by two computational MS/MS data processing methods: network annotation propagation (NAP) and MS2LDA substructure topic modeling. By combining these two processing tools, information about chemical scaffolds and substructures could be annotated automatically, even for spectral clusters without any spectral library matches. We demonstrate accuracy and efficiency of our integrative workflow in large-scale dereplication of natural products with a case of extracts from 71 Rhamnaceae plants. More than 1,000 compounds could be putatively annotated by NAP, and their substructures, for example, different phenolic moieties (e.g. vanilloyl or coumaroyl) of triterpenoid esters or aglycone types of flavonoid glycosides were easily recognized by MS2LDA.

P-091

CELLULAR MEMBRANE AFFINITY CHROMATOGRAPHY AND CELL MEMBRANE COATED NANOPARTICLES IN DRUG DISCOVERY FROM NATURAL MATRICES

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Identification of pharmacologically active compounds targeting transmembrane proteins (TMPs) is a challenging task that demands high resource commitment. Innovative approaches: cell membrane affinity chromatography (CMAC) columns and cell membrane coated nanopartciles (nanoghosts) are proposed to screen complex natural matrices for biologically active, secondary metabolites. CMAC and ligand fishing with nanoghosts are robust approaches allowing for the immobilization of fully functional TMPs on the surface of either immobilized artificial membrane (IAM) stationary phase HPLC particles or magnetic nanoparticles. Both techniques allow for the identification of phytochemicals specifically interacting with TMPs, without the need to isolate any of the compounds present in the screened extract. These innovative techniques speed up the process of the identification of potential new drug leads and are ideally suited for building libraries of natural compounds, interacting with the specific protein targets. The preparation steps and application of both approaches in most recent drug discovery projects will be presented.

P-092

NEW METHODS FOR THE DETECTION OF ADULTERANTS IN GINKGO BILOBA LEAF EXTRACT

Elizabeth Corwin, Meide Pan, PhD, and Michael Harvey
NSF International

A new reverse-phase HPTLC method combined with a dual-wavelength HPLC-UV fingerprint method (detection at 370 and 260 nm) has been developed in our laboratory to evaluate the quality of ginkgo products and to identify adulterants in ginkgo biloba leaf raw materials, leaf extract, and finished products. More than 20 commercial ginkgo supplements from North American and European markets were evaluated. Of the ginkgo dietary supplements tested between 2015 and 2018, only three of the products contained authentic ginkgo leaf extracts. The adulterations included uncharacteristically high levels of rutin, quercetin, keampferol, or extracts from Japanese Sophora or green tea. The methods used will be presented, as well a systematic approach for determination of quality ginkgo biloba products.

P-093

AN APPLICATION OF ^{19}F NMR SPECTROSCOPY IN NATURAL PRODUCT CHEMISTRY TO INVESTIGATING THE EFFECT OF CHEMICAL ENVIRONMENT ON PARTITION COEFFICIENT

Laura Tyler, David C. Lankin, Shao-Nong Chen, Guido F. Pauli
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, IL.

In the context of pharmacokinetics, the absorption potential and membrane permeability of natural products (NPs) can be reflected in the partition coefficient (K_p) between the lipid bilayer of phosphatidylcholine (PC) vesicles and a physiological buffer. However, measurement of K_p values in complex NP matrices is a major challenge. To fill this gap, a quantitative ^{19}F NMR (qFNMR) method was developed to determine the K_p of a semi-synthetic, fluorinated NP (F-mimic) produced from a chalcone enriched fraction of *Glycyrrhiza inflata* (GI) rhizome extract. The qFNMR spectra of F-mimic in the presence of a PC unilamellar vesicle suspension showed a

single PC concentration dependent signal of varying shift and line broadening related to fast exchange between the PC vesicles and buffer. GI extracts of variable preparation and polarity were applied to this methodology to investigate the effects of co-administered phytochemicals on the K_p of F-mimic. The results are supported by Caco-2 biological assay and a second derivative spectrophotometric method. The proposed method leverages the highly selective nature of ^{19}F NMR to exclude the signal interference of biological systems and extracts to investigate the effects of complex chemical environments on a target metabolite.

P-094

THE AXOLOTL AS A NEW MODEL FOR THE DISCOVERY AND VALIDATION OF CHEMICAL GENETICS TOOLS FOR REGENERATIVE BIOLOGY

Larissa V. Ponomareva,[1] Qingchao Qiu,[2] Varun B Dwaraka,[2] Khaled A. Shaaban,[1] Jon S. Thorson,[1] S. Randal Voss[2]
[1]Center for Pharmaceutical Research and Innovation, College of Pharmacy, University of Kentucky; [2]College of Medicine, Spinal Cord and Brain Injury Research Center, University of Kentucky

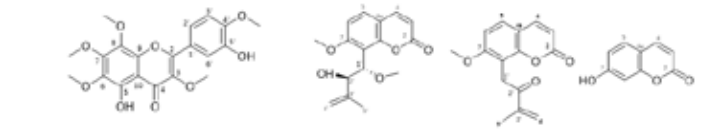
Amphibian vertebrates are important models in regenerative biology because they present exceptional regenerative capabilities throughout life. However, it takes considerable effort to rear amphibians to adulthood for regeneration studies and the relatively large sizes that frogs and salamanders achieve during development make them difficult to use in chemical screens. Here we develop a new tail regeneration model using early hatchling stage Mexican axolotl larvae. We show that axolotl hatchlings completely regenerate amputated tails in 7 days. Further, we show that axolotl hatchlings can be efficiently reared in microtiter plates to achieve moderate throughput screening of chemicals to investigate toxicity and identify molecules that alter regenerative outcome. Importantly, the use of pre-feeding salamanders circumvents the need for an IACU protocol and the screen has also been adapted as an undergraduate student teaching laboratory exercise. Here we report results from screening of four compound collections: the Tocriscreen Stem Cell Toolbox (80 compounds), Selleckchem Epigenetics library (151 compounds), the representative sets from the MicroSource Discovery Systems Spectrum Collection (2650 compounds) and the Center for Pharmaceutical Research and Innovation (326 natural products). Several tail regeneration modulators were identified where subsequent dose response, expression-profiling for select agents have been pursued. Our study establishes the axolotl hatchling as a chemical screening model to investigate signaling pathways associated with tissue regeneration and implicates utility for toxicology screening.

P-095

INVESTIGATION OF THE CYTOTOXIC CONSTITUENTS OF *GLYCOSMIS OVOIDEA* COLLECTED IN VIETNAM

Peter J. Blanco Carcache[1], Choon Y. Tan[1], Austin A. Czarnecki[3], Tran Ngoc Ninh[2], Nelson Freitas Fernandes[1], Gerardo D. Anaya-Eugenio[1], Yulin Ren[1], Djaja D. Soejarto[3,4], H. Liva Rakotondraibe[1], Joanna E. Burdette[3], A. Douglas Kinghorn[1].
[1]College of Pharmacy, The Ohio State University, Columbus, OH, 43210, [2]Institute of Ecology and Biological Resources, Vietnam Academy of Science of Technology, Hanoi, Vietnam, [3]College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612, [4]Science and Education, Field Museum of Natural History, Chicago, IL, 60605

As part of a continuing effort to discover new anticancer agents, *Glycosmis ovoidea* Pierre (Rutaceae) was selected for further studies due to the cytotoxicity of the chloroform partition of its stems, fruits, and leaves against the HT-29 and MCF-7 cancer cell lines. No previous phytochemical studies have been reported on *G. ovoidea*. The chloroform extract has afforded thus far a potent flavonoid cytotoxic agent (**1**), a coumarin with a previously unassigned absolute configuration (**2**), and two known coumarins (**3**, **4**). Compound **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 the MDA-MB-435, MDA-MB-231, OVCAR-3, and HT-29 human cancer cells.

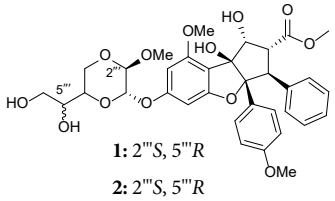


P-096

HPLC-MS METHOD VALIDATION FOR SILVESTROL AND 5'''-EPISILVESTROL AND ITS APPLICATION IN DETECTING THEIR ANALOGS

Garima Agarwal[1], Andrew C. Huntsman[1], James R. Fuchs[1], Esperanza Carcache de Blanco[1], and A. Douglas Kinghorn[1].
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

Silvestrol (**1**) and its analog 5'''-episilvestrol (**2**), first isolated from the twigs and barks of *Aglaia foveolata* Pannell,[1] have garnered much interest due to their inhibitory activity against an array of cancer cell lines both *in vitro* and *in vivo* along with their ability to inhibit protein synthesis via binding to eukaryotic initiation factor (eIF) 4A.[2] As part of our continuous effort to discover potential anticancer agents, the roots of *Aglaia perviridis* Hiern. were fractionated and the chloroform partition was found to contain both **1** and **2**. To aid in accurate identification and quantification of **1**, **2**, and their derivatives in a fraction, a LC-MS dereplication method has been developed and validated.



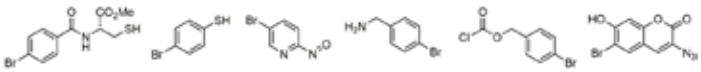
1: 2'''*S*, 5'''*R*
2: 2'''*S*, 5'''*R*

P-097

LABELING NATURAL PRODUCTS IN COMPLEX EXTRACTS

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

An enormous number of biologically-active natural products that could lead to new drugs have not been discovered because there is a shortage of innovative methods for their discovery. Reactivity-guided isolation is a novel approach to access natural product chemical space. It identifies biologically-active metabolites based on their reactivity and has the potential to transform the way in which natural products chemists find new natural products. Chemical probes consisting of a prominent UV-MS (or fluorogenic) tag and a chemoselective reagent are designed to label natural products with a specific pharmacophore or functional group in an unprocessed extract. The resulting derivatives are then readily detected by LC/MS, isolated, and characterized. The unlabeled metabolites are easily targeted for isolation from the original extract on the basis of their calculated masses. We have developed an assortment of probes over the past five years for various purposes, which we will present in some detail.



P-098

RAW NMR DATA: RESEARCH INTEGRITY AND NO MORE MULTIPLETS

James B. McAlpine,[*] Shao-Nong Chen,[*] Jonathan Bisson,[*] Charlotte Simmler,[*] David C. Lankin,[*] Matthias Niemitz,[†] Mark S. Butler,[‡] Michael T. Crimmins,[#] Harald Gross,[&] Guido F. Pauli[*]
[*]Center for Natural Product Technologies (CENAPT), PCRPS & MCP, Coll. of Pharmacy, Univ. of IL at Chicago, Chicago, USA; [†]NMR Solutions, Kuopio, Finland; [‡]UQ Centre Clin. Res.,Royal Brisbane& Women's Hosp. Herston, 4029, Australia; [#]Kenan & Caudill Laboratories of Chemistry, Univ. of North Carolina, Chapel Hill, USA; [&]Dept. of Pharm, Biology, Eberhard Karls Univ., Tübingen, Germany

Whereas the role of NMR in natural product research is undisputed, what is still not appreciated is the fact that, surprisingly, it is underutilized. Almost invariably the first step in characterizing a new isolate is a simple ^{1}H NMR spectrum, collected as a free induction decay (FID) from a broad band pulse. The ^{1}H NMR incorporates a wealth of data on the electronic environment of each hydrogen and its relationship to nearby hydrogens and ^{13}C atoms. Unfortunately, FIDs are not human-readable and is submitted to a Fourier Transformation (FT) to give us the NMR spectrum that we can to some extent analyze. The result is commonly listed in tables, including frequent designation of signal patterns as multiplets, i.e., a loss of data and an invitation to typographical errors and misassignments. There are now programs which will analyze FIDs to reproduce the exact FT'ed spectrum and provide accurate chemical shifts, coupling constants, line widths, and peak areas. This and other developments make FIDs, the original raw data, invaluable for the integrity of science. They provide an avenue to structural correctness, purity analysis, build of databases, which can be used for dereplication, and enable reviewers to verify structures in submitted manuscripts. Examples of the use of these advanced FID analysis, deconvolution of "multiplets", and of compounds whose published structures were shown to be erroneous by re-examination of their NMR spectra will be presented.

P-099

NATURAL PRODUCT DISCOVERY USING AUTOMATED HIERARCHICAL CLUSTERING AND PRINCIPAL COMPONENT ANALYSIS (HC-PCA) IN R

Shaurya Chanana[1], Chris S. Thomas[1], Tim S. Bugni[1].
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Rediscovery of known natural products hinders discovery efforts aimed at identifying new and interesting structural scaffolds for drug discovery purposes. Liquid chromatography / mass spectrometry in combination with principal component analysis (LC/MS-PCA) based metabolomics has been successfully used to address this challenge. However, the inherent complexities of metabolomics data necessitate the development of a pre-processing step to determine appropriate group membership and manageable group sizes for computing informative PCA models. Datasets with large inherent diversity fare poorly in PCA since inter- and intra-group variance tends to be similar. Hierarchical clustering analysis (HCA) is able to group similar bacterial strains based on their LC/MS chromatograms after which PCA can be successfully employed on these smaller sub-groups for the discovery of putative novel compounds. We have developed a script that allows for a more robust automated analysis of large collections of bacterial strains. The script is written in R, and supports integration with other existing metabolomics workflows and can be linked with automated mass matching using existing databases.

P-100

TARGETED ISOLATION OF NATURAL PRODUCTS USING *IN SILICO* MOLECULAR NETWORK ANNOTATION PROPAGATION: A CASE OF *SAGERETIA THEEZANS*

Kyo Bin Kang[1,2], Eun Jin Park[2], Ricardo R. da Silva[1], Hyun Woo Kim[2], Sang Hyun Sung[2], and Pieter C. Dorrestein[1].
[1]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA, [2]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 08826, Korea.

Network Annotation Propagation (NAP) is a recently developed *in silico*-based MS/MS spectra annotation tool, which takes an advantage of network topology from MS/MS molecular networking. NAP improves accuracy of *in silico* fragmentation predictions by re-ranking candidate structures based on the network consensus of structural similarity, even when there is no match to a MS/MS spectrum in spectral libraries. In the present study, NAP was applied to annotate molecular families in the MS/MS spectral network of the *Sageratia theezans* (Rhamnaceae) twig extract for dereplication. Based on the *in silico* dereplication results, molecules expected to be unknown were prioritized and isolated. In total, six dicoumaroyl 8-*O*-4' neolignans (**1**–**6**) and three dicoumaroyl lignans (**7**–**9**) were isolated and structurally characterized by spectroscopic analyses. We also confirmed the feedback effect of expanding structural library to the improvement of NAP annotation; this highlights the importance of community contributions of structures to databases in addition to providing reference spectra. Among the isolates, compounds **7**–**9** showed potent protective effect against glutamate-induced oxidative stress in mouse HT22 cells.

P-101

GENOMICS DRIVEN DISCOVERY AND ENGINEERING OF FUNGAL POLYCYCLIC POLYKETIDES

Karolina Subko[1], Peter P. Wolff[1], Sebastian Theobald[1], Jens C. Frisvad[1], Charlotte H. Gotfredsen[2], Mikael R. Andersen[1], Uffe H. Mortensen[1], and Thomas O. Larsen[1].
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Polycyclic aromatic polyketides represent an important group of Natural Products (NPs) exhibiting a broad variety of antimicrobial and cytotoxic activities. In filamentous fungi their backbone structures are synthesised by iterative non-reducing polyketide synthases (NR-PKSs) that undergo regioselective cyclizations and are further altered by additional enzymatic modifications. In this study genome sequences of more than 300 species in genus *Aspergillus* were genome mined against selected polycyclic NR-PKS gene sequences to identify a hit list of species with homologous gene clusters. The resulting hit strains were then grown under a number of growth media conditions and their crude extracts were dereplicated using HPLC-DAD-HRMS, to separate the strains with expressed and silent polyketide pathways. The selected silent hit NR-PKSs, in association with the modification enzymes encoded by their gene clusters, were overexpressed in their native strain, leading to isolation and structural elucidation of the resulting products and characterization of their biosynthetic pathways. The case study in *Aspergillus sydowii* will be presented.

P-102

EVALUATING A NEW TOOL TO AID IN STRUCTURE ELUCIDATION BY DOUBLE BOND PLACEMENT USING OZONE-INDUCED DISSOCIATION MASS SPECTROMETRY

Sonja L. Knowles[1], Ngoc Vu[1], Huzefa A. Raja[1], Matthew E. Mead[3], Jacob L. Steenwyk[3], Antonis Rokas[3], Qibin Zhang[1,2], and Nicholas H. Oberlies[1].
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The location of double bonds can play a vital role in the activity of secondary metabolites. Most often, organic structures are solved by using NMR, but it can be challenging to locate double bond locations, particularly for fully substituted double bonds and compounds with multiple double bonds in similar chemical environments. Ozone-Induced Dissociation Mass Spectrometry (OzID-MS) serves as an orthogonal structure elucidative tool due to initiation of ozonolysis across a carbon-carbon double bond. Ozonolysis breaks a bond into an aldehyde/ketone and a Criegee ion, which produces unique fragments that can be detected. Using a Synappt G2 Mass Spectrometer, fungal secondary metabolites were analyzed to confirm the placement of double bonds, including, the structure elucidation of a new compound. To confirm that the unique fragment ions being observed were due to OzID-MS and can be used as indicative ions for characterizing double bond placement, the compounds were also examined on the same instrument without the presence of ozone. In addition to serving as a complement to structure elucidation via NMR, we hypothesize that this technique could be valuable when analyzing the structures of secondary metabolites in situ.

P-103

STABLE ISOTOPE LABELING AND COMPARATIVE METABOLOMICS FACILITATE GENOME MINING IN CYANOBACTERIA

Daniel S. May[1], Camila M. Crnkovic[1,2], Aleksej Krunic[1], Jimmy Orjala[1].
[1]Department of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, USA; [2]CAPES Foundation, Ministry of Education of Brazil, Brasília, DF 70040-020, Brazil

Improvements in both sequencing and bioinformatic technologies have made genome mining a powerful approach for the identification of bacterial natural products. Bioinformatic tools, such as AntiSMASH, can be used to identify biosynthetic gene clusters from genomic data and provide predictions on the structures of the produced compounds. However, the predicted structures are often not complete, making identification of the produced compounds in a cell extract through mass spectrometry challenging. Taking advantage of the unique growth conditions for cyanobacteria, ^{15}N stable isotope labeling was used to match biosynthetic gene clusters to their produced compounds in a cyanobacterial cell extract. Bioinformatic analysis with AntiSMASH was able to identify six biosynthetic gene clusters from the sequenced genome of the cyanobacterium UIC 10630 *Nostoc* sp. Three of the six biosynthetic gene clusters had high similarity to reported biosynthetic gene clusters, while three appeared to be orphan gene clusters. Stable isotope labeling with ^{15}N labeled nitrate, and subsequent comparative metabolomic analysis, matched four of the six biosynthetic gene clusters with compounds in the cell extract. Two of the identified compounds were new natural products and their structures were elucidated by NMR and mass spectrometry.

P-104

USING MALDI-TOF MS TO CREATE LOW-REDUNDANCY LIBRARIES FROM THE CULTIVABLE FRESHWATER SPONGE MICROBIOME

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[1]University of Illinois at Chicago, Chicago, IL, USA, [2]Northwestern University, Evanston, IL, USA

Our lab recently developed a mass spectrometry (MS) and bioinformatics pipeline, IDBac, that allows MS fingerprinting of up to 384 microbial strains in four hours by a single user. We demonstrated IDBac's capabilities by simultaneously extracting protein and specialized metabolite MS profiles from unidentified species of bacteria cultivated on an agar plate. With unsupervised machine learning and visualizations, IDBac created hierarchical groupings of protein MS fingerprints that accurately mirrored phylogenetic groupings and further distinguished isolates based on inter- and intra-species differences in specialized metabolite production. While created for a broad range of applications, we are interested in IDBac's application in creating bacterial strain libraries for drug discovery, a process that has seen little innovation in nearly 80 years of biomedical research. Current practice relies on colony morphology and/or limited gene sequencing analyses to prioritize strains, which has led to libraries plagued with a high degree of taxonomic and chemical redundancy and a general divestment in natural products by pharmaceutical companies and other agencies. To address this problem, we designed a detailed roadmap for creating a low-redundancy bacterial library, using the cultivable microbiomes of the understudied freshwater sponge *Eunapius fragilis* var. minuta as an example. Our pipeline is grounded in shareable data, and suitable for easily evaluating sample collection strategies. Built with collaborative efforts in mind, IDBac has potential in bringing a new paradigm to the front-end of microbial drug discovery.

P-105

IN VIVO AND *IN VITRO* ACTIVITY OF PERFOLIATIN A, AN *A-GLUCOSIDASE* INHIBITOR ISOLATED FROM *MELAMPODIUM PERFOLIATUM*

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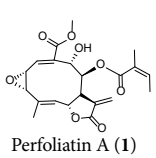
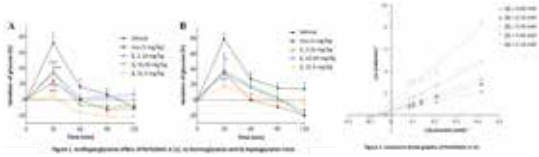
The known melampolide, perfoliatin A (**1**) isolated from the aerial parts of *Melampodium perfoliatum* (Asteraceae) possessed antihyperglycemic activity in normal and hyperglycemic (NA/STZ, 50/130 mg/kg) mice during an oral sucrose tolerant test (3.1-31.6 mg/kg, Figure 1). Compound **1** exhibited *in vitro* inhibitory activity against rat small intestinal and *Ruminococcus obeum* α-glucosidases (IC_{50} = 6.5 ± 0.63 mM and 0.47 ± 0.01 mM, respectively). Kinetic assays using the *R. obeum* enzyme showed that compound **1** behaved as noncompetitive inhibitor, according with the Lineweaver Burk graphic (Figure 2).



Perfoliatin A (**1**)



P-106

MORE THAN THE SUM OF ITS PARTS: MONITORING ANTIVIRULENCE WITH DROPLET-LIQUID MICROJUNCTION-SURFACE SAMPLING PROBE

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is often the face of "superbugs," bacterial infections that develop antibiotic resistance. To combat this, we turn our attention to alternative methods, such as antivirulence, to disrupt the quorum sensing pathway in MRSA and hinder its pathogenesis. One known quorum sensing inhibitor is ω-hydroxyemodin, which was isolated from the fungal endophyte, *Penicillium restrictum*. The antivirulence effect of ω-hydroxyemodin was observed *in vitro* and *in vivo*. We have incorporated droplet-liquid microjunction-surface sampling probe (droplet probe), which applies an innocuous droplet to the surface of the bacteria and performs a microextraction of the metabolites. We can couple this technique to UPLC-HRMS to broaden the scope of our analysis. Previously this technique was used to sample fungi and some plants; we demonstrate its use to measure the virulence of pathogenic bacteria by monitoring the inhibition of the virulent factor, δ-toxin, with the fungal metabolite, ω-hydroxyemodin, *in situ*.

P-107

SMALL MOLECULE ACCURATE RECOGNITION TECHNOLOGY (SMART) TO ENHANCE NATURAL PRODUCTS RESEARCH

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We present the Small Molecule Accurate Recognition Technology (SMART), a system that integrates the benefits of Fast 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 as well as the automatic identification of a new compound as belonging to a particular compound family. To effectively accomplish this goal, we first developed a state of the art protocol for the rapid accumulation of this particular type of NMR data, and this involved an innovation in the data processing methodology. Next, a deep Convolutional Neural Network (CNN) with contrastive loss was trained on a database containing over 2,000 HSQC spectra as the training set. This resulted in a remarkable and highly accurate 3D clustering of different classes of molecules based on their respective HSQC spectra. To demonstrate the utility of SMART, several newly isolated compounds were automatically located with their known analogues in the embedded clustering space, thereby streamlining the discovery pipeline for new natural products. In addition, because white Gaussian 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 significant noise or artifacts.

P-108

DEVELOPMENT OF A BACTERIAL HOST FOR ANTIBIOTIC DISCOVERY AND PRODUCTION

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Microbial metabolites are remarkable starting points for antibiotic discovery and development. Bacterial genomes encode a large and mostly untapped capacity for antibiotic biosynthesis. In order to realize the full extent of genome mining for antibiotic discovery is heterologous expression in a robust host. *Burkholderia* sp. FERM BP-3421, a β-Proteobacterium, is a non-pathogenic, industrial strain that offers the desired characteristics of a heterologous host. FERM BP-3421 has a doubling time of ~1 hour, has genetic tractability, has been optimized to produce a drug lead in high yields, and has potential to produce complex metabolites of various biosynthetic classes based on the >20 biosynthetic gene clusters (BGCs) encoded in its genome. We aim to test FERM BP-3421 as a heterologous host for the discovery and production of antibiotics identified by genome mining. We have established an electroporation protocol and have constructed suitable vectors for FERM BP-3421. This presentation will cover our latest results towards testing the host using model BGCs and selected orphan BGCs.

P-109

NEW APPROACHES FOR TARGET IDENTIFICATION OF NATURAL PRODUCTS

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Our mechanism-blind screening program aimed at identifying compounds targeting triple negative breast cancer (TNBC) subtypes from extracts and fractions of diverse source organisms has been successful and multiple compounds targeting different subtypes have been identified. A major benefit of mechanism-blind drug discovery is the opportunity to identify new and unanticipated drug targets. This is critical for TNBCs because there are few defined molecular targets for this heterogeneous disease. A major challenge with this approach is identification of the biological targets. A CRISPR-Cas9 mediated genome-wide knockout screen is being used to identify the mechanisms of action of a compound with selective activity against the mesenchymal stem-like (MSL) subtype of TNBC. One gene per cell is knocked out by viral transduction of guide RNA (sgRNA). The cell population is treated with the compound of interest as a selection pressure and sgRNAs are isolated and sequenced from surviving cells to identify gene disruptions that confer a survival advantage. A cytotoxin with no selectivity for the subtypes of TNBC is used as a control to discriminate genes that confer non-specific drug resistance, for example genes involved in apoptosis. The approach is expected to identify gene products critical for the sensitivity of MSL cells to the selective compound. This will inform on the compound's mechanism of action and identify potential molecular targets that will be validated. Ultimately, these new targets will facilitate drug discovery for this challenging type of cancer. Funded by U01CA182740

P-110

ACCURATE COMPOUND IDENTIFICATION OF COMPLEX TRADITIONAL HERBAL MEDICINE USING A NOVEL MASS SPECTROMETRY ACQUISITION METHOD

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Yu Ping Feng San (YPFS) is a three herbs TCM formulation which has been traditionally used for treatment of immune system related diseases. The LC-MS data generated from such multiple herbs contain fragments from co-eluting multiple precursor ions. This makes the fragment data more complex and hence difficult to make correct compound identification. Here we describe the application of a novel data independent acquisition (DIA) approach called SONAR™ for improved spectral clarity and confident compound identification from a complex samples such as TCM. SONAR™ utilizes a low resolution quadrupole mass filter, which is scanned continuously and both precursor and MS/MS data are acquired. Data was also collected for comparison purposes using a traditional DIA method such as MS^e which provides both precursor and fragment ion information but without a resolving quadrupole. From the results, the specificity of SONAR™ provides cleaner precursor and fragment ion spectra compared to the traditional DIA acquisition method. As an example, the identification of prim-O-glucosylcimifugin acquired using a traditional DIA and SONAR™ was compared. When using the traditional DIA there are multiple co-eluting compounds which could confound the structural analysis. The presence of these co-eluting precursor ions with prim-O-glucosylcimifugin provided a high complexity with 97 high energy fragment ions, making compound identification very complex and challenging. On the other hand when the data is acquired using SONAR™, cleaner precursor and fragment ion spectra were generated. The selected narrow precursor mass window from SONAR™ provided specific fragment ions and contains only the parent ions prim-O-glucosylcimifugin $[M+H]^+$ and $[M+Na]^+$. Eight clean relevant fragment ions generated only from the parent ion prim-O-glucosylcimifugin which leads to correct and confident compound identification. In summary, compared to the traditional DIA method, the specificity of SONAR™ provides cleaner precursor and high energy fragment ion spectra, which results in confident compound identification.

P-111

AUTHENTICATION OF AYURVEDIC HERBAL PRODUCTS AVAILABLE IN EUROPEAN MARKET USING DNA METABARCODING RAISES QUALITY CONCERNS

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Ayurveda is one of the oldest systems of medicine in the world, widely recognized as part of the complementary and alternative system of medicine. The growing commercial interest in Ayurvedic herbal products increases the incentive for adulteration and substitution in the medicinal plants market. Such adulteration threatens the efficacy, safety and may seriously alter the consumer's health. Most likely, fraudulent practices as the use of fillers and plant materials of inferior quality is driven by the increasing level of consumption of herbal products which exceed the supply capacity for some plant species. Accidental substitutions may often occur, in addition to the deliberate fraudulent adulteration leading to an improper utilization of the plant material. The quality of herbal products is directly reflected in their safety and efficacy, thereby proposing novel strategies to exhaustively assess and monitor both the quality of the raw material and of the final marketed herbal product, is now a challenge in the herbal pharmacovigilance. In this study we used DNA metabarcoding to authenticate the Ayurvedic products available on the European market, and to evaluate its feasibility to check the authenticity of the product. Seventy-nine Ayurvedic herbal products sold as tablets, capsules, powders and extracts were randomly purchased via e-commerce and pharmacies in Europe, and analyzed using DNA metabarcoding approach. Our analysis reveals that only two out of 12 single ingredient products contained only one species, whereas six products contained the species listed on the label along with several other species, and four products did not contained any species listed on the label but contained several other species. Eight out of 27 multiple ingredient products, contained no species listed on the label, whereas the remaining 19 products contained between of 1 to 5 of the species listed on the label along with many other species not specified on the label. The fidelity for single ingredient products was 67%, and the overall ingredient fidelity for multi ingredient products was 20% and for all products 23%. Also, detection of threatened species requiring conservation strategies raises concerns about illegal trade. The study highlights the necessity for quality control of the marketed herbal products, and shows that DNA metabarcoding is an effective analytical approach to authenticate complex multi ingredients herbal products. However, effort needs to be done to standardize the protocols for DNA metabarcoding for plant identification is necessary before this approach can be implemented as routine analytical approaches and approved by the competent authorities for use in regulated procedures.

P-112

PROGRESS TOWARD A BIOORTHOGONAL ALKYLATION PLATFORM: MODEL METHYLTRANSFERASE STUDIES

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Methyltransferases (MTs) utilize *S*-adenosyl-L-methionine (AdoMet) as a cosubstrate and catalyze the methylation of nucleic acids, proteins, small molecules, macromolecules and natural products. These methylation events are crucial for a number of important regulatory and metabolic reactions. These events are difficult to study, however, as MTs are highly prevalent within a cell, the AdoMet domains of which are highly conserved. Therefore, the development of bioorthogonal tools and strategies, which would allow us to study or exploit specific methylation events, would aid in our understanding of key biochemical reactions and further enable chemoenzymatic diversification of complex natural products.

P-113

CYTOTOXIC BISBENZYLISOQUINOLINE ALKALOIDS ISOLATED FROM THE LEAVES AND STEMS OF *PACHYGONE ODORIFERA*

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Bisbenzylisoquinoline alkaloids have been reported extensively for their promising antitumor potential. In a continuing search for anticancer agents from higher plants, a chloroform extract of the leaves and stems of *Pachygone odorifera* Miers (Menispermaceae), collected in Vietnam, was found to be cytotoxic against the HT-29 colon, the MDA-MB-231 breast, the MDA-MB-435 melanoma, and the OVCAR3 ovarian human cancer cell lines. Fractionation of this extract guided by bioassay toward these cell lines yielded several new and known bisbenzylisoquinoline alkaloids. All these dimeric alkaloids contain a *N,N'*-dimethyl group and a methoxy group at the C-6 position, with two benzylisoquinoline alkaloid monomers connected through two oxygen bridges between the isoquinoline units and a single C-C linkage between the benzyl moieties. When tested toward the small panel of human cancer cell lines selected, all isolates obtained showed moderate activity, with IC_{50} values in the range 3–10 μM. Investigation of these compounds and their activity showed that the configuration of the C-1 or C-1' positions is not crucial for these bisbenzylisoquinoline alkaloids to mediate their cytotoxicity.

P-114

TOWARDS QUANTITATIVE SPATIAL PROFILING OF NATURAL PRODUCTS GINSENOSIDES FROM PANAX GINSENG ROOTS USING MASS SPECTROMETRY IMAGING

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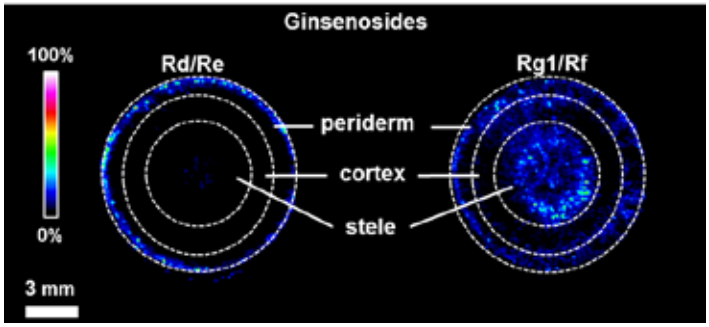
Panax ginseng C.A. Mey roots have been widely used as traditional herbal medicine in East Asia for several millennia. P. ginseng root contains diverse bioactive natural products, such as ginsenosides, that have been reported to show pharmacological effects on the tumor, immune system, diabetes. Therapeutic utility of ginseng primarily depends on its ginsenosides contents. The effective use of ginsenosides requires profiling the types of ginsenosides and their quantitative distribution in ginseng roots. Mass spectrometry (MS) imaging is gaining momentum for profiling the spatial distribution of natural products in plant tissues. Here, we utilize desorption electrospray ionization (DESI) MS on a quadrupole Tof mass spectrometer (Xevo G2-XS, Waters Corporation, MA, USA) to obtain the spatial distribution of ginsenosides within the ginseng root sections. The frozen transverse cross sections of the ginseng root at 20 μm thickness were prepared for MS imaging. The MS image was plotted and analyzed by High Definition Imaging (HDI) software (Waters Corporation, MA, USA) at 100 μm pixel size. MS images were overlaid with microscopic images to define regions of interest (ROI) for three sections in the root cross-section, periderm, cortex, and stele.

Fold-change analysis of ion counts in ROI of that section provided quantitative spatial distribution of the ginsenosides. Preliminary analysis shows ginsenosides Rg1/Rf were mostly found within the outer bark (periderm) and inner core (stele) of the root. While, ginsenosides Rd/Re Rs1/Rs2, Ra1/Ra2, and pseudoginsenoside Rc1 were more abundant in periderm. Ginsenoside Ra3 exhibited a diffuse distribution within the cross-section and a high concentration around the periderm. To distinguish between isomers, such as Rf/Rg1, tandem mass spectrometry (MS/MS) with DESI was employed. The characteristic separate fragmentation pattern showing monosaccharide or disaccharide group help discern between the spatial distributions of two ginsenosides. The spatial profile of ginsenosides may differ depending on the age, cultivar, year, geographic origin and quantitative mass spectrometry imaging of ginsenosides from roots can be a helpful tool to study these variations.



Acknowledgement: Authors acknowledge the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences in Beijing, China for generously proving the root samples.

P-115

AN IMPROVED DEREPLICATION STRATEGY FOR NOVEL ACTIVE MARINE NATURAL PRODUCTS DISCOVERY

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

P-116

DEVELOPMENT AND APPLICATION OF MAGNETIC MICROBEAD AFFINITY SELECTION SCREENING FOR NOVEL RETINOID X RECEPTOR-A ANTI-INFLAMMATORY AGENTS

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With the aim of discovering new drugs, different methods have been developed to facilitate the screening of complex mixtures from natural products,

including for example, pulsed ultrafiltration mass spectrometry (PUF-MS) that was conceived in our lab. Though PUF-MS is superior to bioassay-guided fractionation and the classical non MS-based bioassays that test compounds singly, its throughput is challenged by incompatibility with microtiter well plates and automation. Therefore, MS-based magnetic microbead affinity selection screening (MagMASS) was designed and developed to overcome these challenges. The retinoid X receptor-α (RXR-α), a potential target for cancer treatment and prevention, and anti-inflammation was chosen to demonstrate the application of MagMASS. The protein-bound compounds from the extracts, after isolation using MagMASS, were analyzed by LCMS on a Shimadzu ion trap time-of-flight (IT-ToF) accurate mass tandem spectrometer. The MagMASS assay for RXR-α was developed with a throughput of at least 3 96-well plates per day which was an enhancement of over 100-fold compared to the previous PUF-MS RXR-α assay developed in our lab. Additionally, MagMASS was applied to screen complex mixtures of botanical extracts as well as synthetically derived compounds. New ligands of RXR-α were identified and characterized. The experimental details and results from this study will be presented.

P-117

PREPARATIVE METABOLOMICS TO PROBE STRUCTURAL DIVERSITY OF *RHODIOLA ROSEA* BY COMBINED CPC, UHPLC, LC-MS, AND NMR

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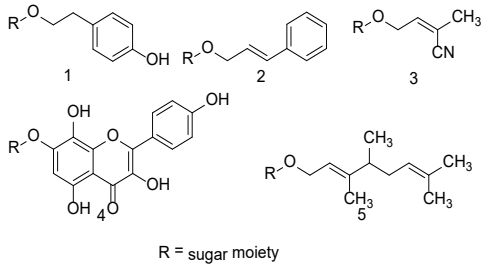

We are developing a combined analytical and preparative method using centrifugal partition chromatography (CPC), UHPLC, LC-MS, and NMR to analyze the metabolomic diversity of *Rhodiola rosea* phytoconstituents. This approach involves a proanthocyanidin (PAC) knock-out CPC procedure, developed to remove the large amounts of PACs, which interfere with the analytical methods by causing UHPLC baseline issues and broad resonances in ^{1}H NMR spectra. Subsequently, 1D/2D NMR and qTOF experiments were applied to investigate the PACs-free portion of the metabolome consisting of five types of compounds: phenylethanoid, phenylpropanoids, nitriles, flavonoids, and monoterpenes. Certain monoterpene glycosides were found to be sensitive to silica gel and their decomposition products were investigated. Collectively, this supports the use of liquid-liquid techniques in preparative metabolomic analysis.

P-118

APPLICATION OF A PHYLOGENETIC-MOLECULAR NETWORKING STRATEGY TO IDENTIFY TRICHORMAMIDE-LIKE SECONDARY METABOLITES

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Cyanobacteria have emerged as a prominent source of biomedically relevant natural products. The accumulation of metabolites isolated from the phylum over the last few decades has resulted in the need to assess the novelty of a compound at the onset of the drug discovery process to prevent rediscovery of known chemistry. Components extracted from UIC cyanobacterial strain 10484, found to be active against three cancer cell lines, were dereplicated by first identifying the taxonomic position of the strain using the 16S rRNA sequence. The strain was found to clade with trichormamide-producer UIC 10045. Comparison of the MS/MS fragmentation profiles of the 10484 bioactive compounds to trichormamides using the Global Natural Products Social Molecular Networking (GNPS) platform confirmed the presence of novel trichormamide analogs. Using 2D-NMR, MS/MS, and an advanced Marfey's analysis, we were able to elucidate the structure of compounds found in the active HPLC subfractions.

P-119

PURIFICATION OF PHENOLIC COMPOUNDS USING A COMBINATION OF FLASH AND PREPARATIVE CHROMATOGRAPHY

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Phenolic flavonoids are common plant secondary metabolites. They occur as flavonoids, anthocyanidins, and anthocyanins. The interest in flavonoids is due to their anti-oxidant activity; they are also studied as potential natural food colorants that may also possess nutraceutical benefits. As the compounds are chemically similar to each other, obtaining pure material can be difficult. A simple three-step purification procedure utilizing ion exchange chromatography for the initial separation, followed by purification on polyamide resin, and final separation with C18 reverse phase chromatography is shown. The methanolic extract from *Camellia sinensis*, a rich source of phenolic flavonoids, is used as a model system for purification of these compounds.

P-120

A VALIDATED UNTARGETED METABOLOMICS METHOD FOR DIVERSITY SCREENING OF NATURAL PRODUCTS FROM ASTERACEAE

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In natural products research, despite currently available state-of-the-art analytical techniques for the separation and detection of compounds, a bottleneck is to locate the desired or new compounds. The analysis of biological matrices remains a challenge while compound re-isolation is still a significant problem. LC-MS-based metabolomics combined with chemoinformatics tools represent a rapid and efficient holistic approach for the large-scale analysis of plant material. In this work, we propose the development and validation of an analytical methodology for diversity screening. Twelve compounds from a pure compound library were used to verify the efficiency and performance of reversed-phase UHPLC gradients. A full factorial design, using extracts from 41 Asteraceae species, was used to select

chromatographic conditions. The resulting UHPLC-DAD-(ESI)-HRMS (Orbitrap) method was validated according to international analytical guides. In total, 244 extracts were evaluated using this approach. As a result, a robust and efficient analytical method, which is capable of analyzing a broad range of secondary metabolites, was developed and their compound diversity could be verified by checking the similarity of known structures. This approach is also useful for finding novel compounds.

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P-121

Chiral/achiral analysis of naturally occurring cannabinoids using a new sub-2 µm chiral stationary phase with ultra high performance SFC-MS

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The interest in the medical use of *Cannabis Sativa L.* is steadily increasing because of its therapeutic efficacy towards a wide variety of ailments, and its unique chemistry, characterized by the presence of cannabinoids that are concentrated in the female inflorescence. The fiber type of *Cannabis Sativa L.* has a low concentration of psychoactive (-)-Δ9-*trans*-tetrahydrocannabinol ((-)-Δ9-THC) that is typically less than 0.2%. The main cannabinoid in the fiber type of *Cannabis Sativa L.* is (-)-cannabidiol ((-)-CBD) but there is also (-)-cannabidivarin ((-)-CBDV), cannabigerol (CBG), cannabinol (CBN) and the racemic cannabichromene (*rac*-CBC), each having various therapeutic actions. The analysis of the original composition of plant material is necessary for phenotype determination and quality control of medicinal cannabis used in therapeutic treatments.

The presence of natural racemic compounds (*rac*-CBC) in plant extract was investigated using Chiral Stationary Phases (CSPs) with Ultra High Performance Supercritical Fluid Chromatography (UHPSFC). The Chiral Stationary Phase (CSP) allowed resolution of the racemic compound cannabichromene (*rac*-CBC) in plant extract and the synthetic racemic (+/-)-Δ9-*trans*-tetrahydrocannabinol. Good separation, in terms of chemio- and enantio- selectivity, was obtained with high resolution for all cannabinoids, and their acid forms, under isocratic conditions.

P-122

Chiral chromatographic resolution of racemic terpenoids using SFC with a tris-(3,5-dimethylphenyl) carbamoyl amylose coated stationary phase

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Regis Technologies, Inc., 8210 Austin Avenue, Morton Grove, IL 60053, USA

Terpenes and terpenoids are secondary metabolites produced by a variety of plants and consist of two or more five-carbon isoprene units. They are major constituents of essential oils, which are often used in fragrances and aromatherapies, and both natural and synthetic derivatives have been used as flavors in food additives. Additionally, many terpenoids have shown a diverse range of biological activities, including anti-bacterial, anti-fungal, anti-viral, anti-imflammatory, anti-tumoral, and immunomodulatory functions.

Both enantiomeric forms of a particular terpenoid are often naturally occurring, with each eliciting distinct biological responses. For example, (*R*)-(-)-linalool, known as licareol, has a woody, lavender-like scent, while (*S*)-(+)-linalool, or coriandrol, has a sweet, floral aroma. As such, enantiomeric profiling of terpenes in natural products is potentially important for both product formulation as well as cultivar determination.

Herein, the chromatographic resolution of two different naturally occurring enantiomeric terpenoids, linalool and terpinen-4-ol, is shown on an analytical scale using supercritical fluid chromatography (SFC) and a polysaccharide coated chiral column [tris-(3,5-dimethylphenyl) carbamoyl amylase]. A small-scale purification of 250 mg of racemic linalool is also demonstrated.

P-123

Application of certified reference material (CRM) in natural products

Shibiao Wu, Nick Hauser, Jenna Milliken, Andy Ommen, Christopher Rucinski
MilliporeSigma, 2931 Soldier Springs Rd, Laramie, WY 82070

MilliporeSigma Pharmaceutical Secondary Standards are Certified Reference Materials (CRM) that are considered to provide the highest level accuracy, uncertainty, and traceability to an SI unit as well as direct traceability to a Compendial Standard or other Primary Standard when available. In this presentation, we describe the best practices followed to accurately qualify these Reference standards including mass balance approach and assay calculation by direct comparison techniques in these reference standards. The full characterization of the material for use as a Reference Standard gives confidence to the measured results for its intended purpose. Also, Snap-N-Shoot® reference solutions and mixes for common botanicals and other dietary supplements are available for improving laboratory efficiency. These CRMs are prepared and certified to the highest industry standards including ISO Guide 34 and ISO/IEC 17025 and are accompanied by a comprehensive Certificate of Analysis which includes, as appropriate, a traceable assay and a mass balance assignment including water determination, residue analysis, and residual solvents. Additionally, the COA provides qualitative data for identity confirmation such as LC-QTOF-MS, optical rotation, FTIR and NMR. This combination of a comprehensive COA and user-friendly CRMs are highly applicable to the analytical requirements in phytochemical identification, minor component quantification, dietary supplement and food analyses, positive/negative control, and in *vivo/vitro* bioactivity tests.

P-124

Absolute stereostructures of cyclopenta[*g*] indoles from the west australian sponge *Trikentrion flabelliforme* by NMR and ECD

Mariam N. Salib[1], and Tadeusz F. Molinski[1,2]
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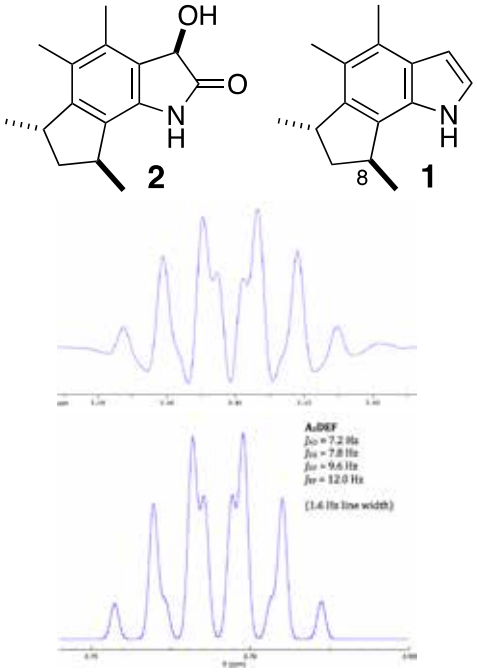
Six new cyclopenta[*g*]indoles, *trans*-herbindole A (**1**), trikentramides E-H and I (**2**), were isolated from the sponge *Trikentrion flabelliforme* collected in Exmouth Gulf, and their structures were elucidated by integrated spectroscopic analysis. Accurate scalar couplings (*J*) of the deceptively simple H-8 methine multiplet of **1** were secured through analysis of the resolution-enhanced ^{1}H NMR signal and simulations. The absolute configurations of the new compounds were established through interpretation of $[a]_D$, ECD and comparison with known analogues. Chemical interconversion of five of the natural products reveal their stereochemical uniformity. A hypothetical non-tryptophan biosynthesis is proposed based on a polyketide synthase and pyrrole-2-carboxylic thioester as the starter unit.



P-125

COMPREHENSIVE SPECTROSCOPIC CHARACTERIZATION OF A NEW FUSICOCCANE DITERPENE: APPLICATION OF NEW NMR STRUCTURE ELUCIDATION TOOLS

Dongdong Wang[1], Emily Smith[1], David Durrant[2], Deborah Morrison[2], Robert H. Cichewicz[3], Gary E. Martin[4], Curtis J. Henrich[1], Kirk R. Gustafson[1], and John A. Beutler[1]

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A high-throughput screening assay for RAS/RAF pathway inhibitors identified a cultured fungal extract as a hit for further chemical investigation. Bioassay-guided isolation led to several different compound classes, including new fusicoccane-type diterpenoids. In addition to conventional 2D NMR analyses, new NMR pulse sequences and experimental approaches were employed to assign and confirm the structure of compound **1**. These included newly described pulse sequences such as LR-HSQMBC, which effectively extends the range of heteronuclear correlations to $^{4}J_{CH}$, and 1,1-HD-ADEQUATE, which provides the functional equivalent of unambiguous $^{2}J_{CH}$ correlations. Anisotropic NMR parameters such as residual chemical shift anisotropy (RCSA) and residual dipolar coupling (RDC) provide a powerful and complementary means to assign new small molecule structures. Anisotropy measurements are possible when compounds partially align within a gel or liquid crystaline medium. We obtained RCSA data for **1** in PBLG, a new liquid crystal that enhances molecular alignment and thus increases the RCSA values. Large RCSAs resulting from stronger molecular alignment improve structural analysis, especially in compounds rich in sp^3-hybridized carbons. In addition to confirming the proposed structure of **1**, RCSA data was particularly effective for assigning the relative configuration of a hemiketal carbon that was problematic to assign by other means.

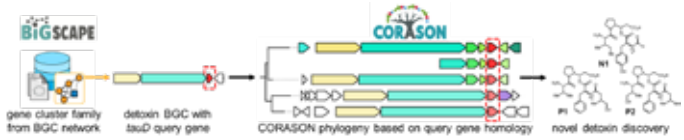
P-126

BIG-SCAPE/CORASON GENOME DATBASE MINING REVEALS NOVEL DETOXINS

Michael W. Mullowney[1], Jorge C. Navarro-Muñoz[2], Nelly Selem-Mojica[3], Luciana Teresa Dias Cappelini[1], James H. Tryon[1], Satria Kautsar[2], Elizabeth Parkinson[4], Emmanuel L.C. De Los Santos[5], William W. Metcalf[4], Francisco Barona-Gomez[3], Marnix H. Medema[2], Regan J. Thomson[1], and Neil L. Kelleher[1]

[1]Dept. of Chemistry, Northwestern University, Evanston, IL, [2]Bioinformatics Group, Wageningen University, Wageningen, The Netherlands, [3]Langebio, Guanajuato, Mexico, [4]Institute of Genomic Biology, University of Illinois, Urbana, IL, [5]Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry, United Kingdom

Using BGC identification tools in a new workflow with BiG-SCAPE and CORASON for gene cluster relatedness and phylogenomic analysis, respectively, we mined a large actinomycete genome library to identify two new detoxin biosynthetic gene cluster subfamilies and discover three novel detoxin analogs. Detoxin N1 was purified, then characterized using metabolic labeling, tandem MS, and NMR, while detoxins P1 and P2 were identified using metabolic labeling and tandem MS.



P-127

SAR OF PENTACYCLIC TRITERPENES AND THEIR CYTOTOXICITY AND NF-KB AND MITOCHONDRIAL TRANSMEMBRANE POTENTIAL (MTP) INHIBITORY EFFECTS

Yulin Ren[1], Gerardo D. Anaya-Eugenio[1], Austin A. Czarnecki[2], Chunhua Yuan[3], Hee-Byung Chai[1], Djaja D. Soejarto[2,4], Joanna E. Burdette[2], Esperanza J. Carcache de Blanco[1], A. Douglas Kinghorn[1]

[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA. [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, USA. [3]Campus Chemical Instrument Center, The Ohio State University, Columbus, Ohio, USA. [4]Science and Education, Field Museum of Natural History, Chicago, Illinois, USA.

Pentacyclic triterpenes of plant origin are attracting increasing interest, owing to their promising bioactivities. In a continuing search for anticancer agents from higher plants, (+)-ursolic acid was characterized as the main active compound from *Syzygium corticosum* (Lour.) Merr. & Perry (Myrtaceae), which showed potent NF-κB inhibitory activity, with an IC_{50} value of 31 nM. Several (+)-ursolic acid analogues have been prepared in the present study, and their bioactivities were evaluated. A preliminary structure-activity relationship (SAR) study indicated that the substitution of the 19,20-dimethyl group plays a key role for (+)-ursolic acid to mediate its cytotoxicity and NF-κB and mitochondrial transmembrane potential (MTP) inhibitory effects, and these activities were decreased greatly by esterification of the C-3 hydroxy group with 4-chlorobenzoic acid or reduction of the C-28 carboxy group to a primary alcohol. In addition, this same reduction increased the cytotoxicity of (+)-oleanolic acid and (+)-betulinic acid but decreased considerably the NF-κB and MTP inhibition of both compounds. Interestingly, esterification of the C-3 hydroxy group with a ferulic acid unit increased greatly the MTP inhibition of (+)-betulinic acid.

P-128

PROBING DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF BOTANICALS USING IN VITRO TECHNIQUES

Karen M. VanderMolen[1], Jorge Naciff[2], Yuching Shan[2], George P. Daston[2], Timothy R. Baker[3], Catherine Mahony[4]
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As complex mixtures, botanicals present unique challenges when assessing safe use, particularly when endpoint gaps exist that cannot be fully resolved by existing toxicological literature. Obtaining data for developmental and reproductive toxicity can be particularly difficult, and so a weight of evidence strategy for these endpoints is illustrated here which utilizes new in vitro approaches. Both receptor binding assays and gene expression studies have been explored as tools to inform on modes of action. Several extracts of both botanicals suspected to have reproductive effects and herbs with a significant history of use were tested against a suite of receptors and enzyme activity assays at biologically relevant doses to probe developmental and reproductive activity at a molecular level. Additionally, gene expression changes in different cell types were analyzed using the connectivity mapping approach to identify major modes of action through a functional read-across approach. Together, these two data streams have been shown to increase confidence in predictions of botanical biological mode of action, and allow for assessment of relative potency in the decision-making process.

P-129

QUANTITATIVE ANALYSIS OF *SALVIA CIRCINATA* AQUEOUS EXTRACT

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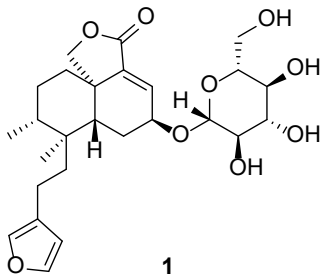
Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

Salvia circinata Cavanillesii (Lamiaceae) is a medicinal Mexican plant widely commercialized for the treatment of diabetes, ulcers and parasites. Recently the antidiabetic potential was confirmed using *in vivo* and *in vitro* experiments. Herein, a suitable validated UPLC–ESI/MS method to quantify the most relevant active principles, amarisolide (**1**) and pedalitin (**2**), is described. UPLC analyses were carried out using a C_{18} column, a gradient elution [(H_2O + 0.1% formic acid) and CH_3CN] and a flow rate of 0.3 mL min^{-1}. Detection wavelength was set at 270 and 330 nm and with an electrospray source in negative mode. The method was successfully validated in terms of linearity, accuracy and precision.



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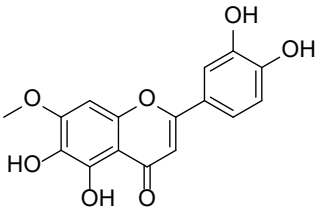


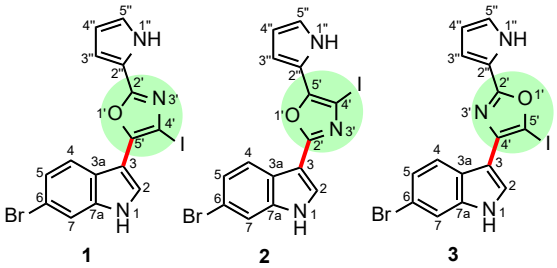
2

P-130

UNEQUIVOCAL DETERMINATION OF A BREITFUSSIN ANALOG BY NMR ANISOTROPIC MEASUREMENTS

Ikenna E. Ndukwe,[1] Yizhou Liu,[2] Ryan D. Cohen,[1] Colin Lam,[1] Kirill A. Blinov,[3] Edward R. Sherer,[1] R. Thomas Williamson,[1] Johan Isaksson[1] and Gary E. Martin[1]
[1] Process and Analytical Research and Development, Merck & Co., Inc., Rahway, NJ 07065. [2]Analytical Research and Development, Pfizer, CT 06340. [3]MestReLab Research S. L., Santiago de Compostela, A Coruna, 15706 Spain

Structural characterization of breitfussin analogs and compounds with similar molecular features – *vis-a-vis* flat, sp^2-hybridized, proton-deficient heterocycles – has been accomplished in the past by a combination of analytical/computational techniques including NMR spectroscopy, mass spectrometry (MS), Computer-Assisted Structure Elucidation (CASE) program utilization, DFT calculations and atomic force microscopy (AFM). The challenge of unequivocally determining the structure of the breitfussins by traditional NMR methodologies is exacerbated by the iodo-substituted oxazole ring. The molecules lack the requisite long-range heteronuclear correlations to enable unambiguous determination of its constitution while DFT calculation of carbon chemical shifts is adversely impacted by the relativistic effect of the iodine. Herein, we show the utility of NMR anisotropic measurements – Residual Dipolar Coupling (RDC) and Residual Chemical Shift Anisotropy (RCSA) – for the unambiguous constitutional definition of the breitfussin analogs.



1 **2** **3**

P-131

ADVANCES IN HIGH-THROUGHPUT AFFINITY EXTRACTION MASS SPECTROMETRY FOR CHARACTERIZING ACTIVE COMPOUNDS IN NATURAL PRODUCT MIXTURES

Richard B. van Breemen
Linus Pauling Institute, Oregon State University, 305 Linus Pauling Science Center, Corvallis, OR 97331

As faster alternatives to bioassay-guided fractionation, various affinity extraction mass spectrometry methods such as pulsed ultrafiltration (van Breemen 1987), gel permeation chromatography (Kaur 1987) and magnetic microbead affinity selection (Choi and van Breemen, 2008) have been used for years for the isolation, characterization and identification of pharmacologically active compounds in complex natural product mixtures such as botanical and microbial extracts. Often requiring manual sample handling and data analysis, we enhanced the productivity of these MS-based methods by incorporating multichannel well plate format, automated sample preparation, substituting UHPLC for HPLC, and automating data analysis using metabolomics software. Although all MS-based affinity selection screening approaches can benefit from the application of metabolomics software for data analysis and fast UHPLC, magnetic microbead affinity selection screening (MagMASS) was particularly compatible with automated multi-well plate technology. Together, the application of these improvements enhanced the throughput of MagMASS mass spectrometry for natural products screening by over 100-fold in applications using the estrogen receptors ER-alpha and ER-beta, RXR, lipoxygenase, and fructose-1,6-bisphosphatase.

P-132

ASSESSING THE EFFICIENCY OF CULTIVATION TECHNIQUES TO RECOVER NATURAL PRODUCT BIOSYNTHETIC GENE POPULATIONS FROM SEDIMENT.

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[1]Dept. of Medicinal Chemistry and Pharmacognosy, Center for Biomolecular Sciences, College of Pharmacy, [3]DNA Services Facility, University of Illinois at Chicago, Chicago, IL 60607; [3]Dept. Microbiology and Biotechnology, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Despite decades of cultivating microorganisms for use in drug discovery, few attempts have been made to measure the extent to which common cultivation techniques have accessed existing chemical space. Metagenomic studies have shown that cultivable bacteria represent a fraction of those that exist in the environment, and that uncultivated populations in sediment have genes that encode for a high diversity of novel natural product (NP) biosynthetic enzymes. Quantifying these genes in both sediment and cultivatable bacterial populations allows us to assess how much diversity is present on nutrient agar and is critical to guiding the trajectory of future NP discovery platforms. Herein we employed next generation amplicon sequencing to assess the NP biosynthetic gene populations present in two Lake Huron sediment samples, and compared these with populations from their corresponding cultivatable bacteria. We highlight three findings from our study: 1) after cultivation, we recovered between 7.7% and 23% of three common types of NP biosynthetic genes from the original sediment population; 2) between 76.3% and 91.5% of measured NP biosynthetic genes from nutrient agar have yet to be characterized in known biosynthetic gene cluster databases, indicating that readily cultivatable bacteria harbor potential to produce new NPs; 3) even though the predominant taxa present on nutrient media represented some of the major producers of bacterial NPs, the sediment harbored a significantly greater pool of NP biosynthetic genes that could be mined for structural novelty, and these likely belong to taxa that typically have not been represented in microbial drug discovery libraries.

P-133

ISOLATION OF NEW CASTANOPSININS FROM THE LEAVES OF CASTANOPSIS SIEBOLDII

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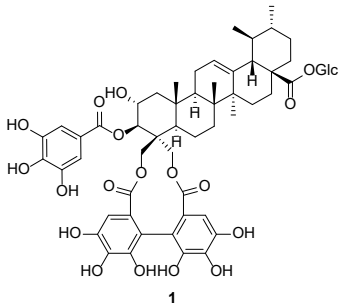
College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Gwanak-gu, Seoul 08826, Republic of Korea.

Castanopsis sieboldii (Fagaceae) is a species of evergreen tree that is widely distributed in East Asia. *Castanopsis* species has characteristic secondary metabolites, castanopsinins, which are the novel triterpene glycosides containing a hexahydroxydiphenic acid (HHDP) group. They have only been isolated from *castanopsis* species, so these compounds are considered as chemotaxonomic makers for *castanopsis* species. In a search for this type of compounds from *C. sieboldii*, two new compounds, castanopsinins J (**1**) and K (**2**) were isolated. The structures of **1** and **2** were elucidated by interpreting their NMR, HRESIMS, and ECD spectra.



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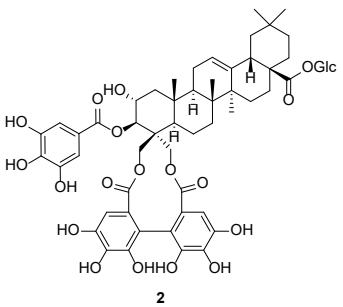


2

P-134

NEUROPROTECTIVE EFFECT OF METHANOL EXTRACT OF *ANNONA MURICATA* IN HT22 CELLS

Ye Lim Son[1], Lee Hyeon Woo[1], Ma Choong Je[1,2]
[1]Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Chuncheon 24341, Republic of Korea; [2]Institute of Bioscience & Biotechnology, Kangwon National University, Chuncheon 24341, Republic of Korea

Annona muricata lives in tropics. It is known to be effective in anticancer, antioxidant properties, and strengthens immunity. Glutamate is an excitatory neurotransmitter, but excessive amounts of glutamate cause oxidative stress. And if oxidative stress causes damage to brain cells. The objective of this study was to establish the neuroprotective effect of 80% methanol extracts of *Annona muricata*. In this experiment, extracts of *Annona muricata* reduce the Ca^{2+}, ROS levels and keep up the level of mitochondrial membrane potential. These results led to decrease the glutamate induced cell death. *Annona muricata* significantly subsist at the cell viability test, as a result *Annona muricata* seems to have a neuroprotective effect in HT22 cell.

P-135

PROTECTIVE EFFECT OF METHANOL EXTRACT OF *ORIGANUM VULGARE* IN HT22 CELL

Ye Lim Son[1], Lee Hyeon Woo[1], Ma Choong Je[1,2]
[1]Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Chuncheon 24341, Republic of Korea; [2]Institute of Bioscience & Biotechnology, Kangwon National University, Chuncheon 24341, Republic of Korea

Origanum vulgare is a herb used as spice in Italian, Mexican and Spanish cuisine. *Origanum vulgare* contains a phenolic component, phenols have antioxidant, antibacterial, blood pressure reduction effect. Glutamate, an excitatory neurotransmitter, causes oxidative stress when released in large amounts. It leads to brain cell damage. This experiments showed *Origanum vulgare* rebating the level of intracellular ROS, Ca^{2+} and maintaining the mitochondrial membrane potential level. As a result, the glutamate induced cell death is decreased in HT22 cell. The purpose of this study was to identify the neuroprotective effect of 80% methanol extract of *Origanum vulgare.* It was effective at the cell viability test. Consequently, We confirm that *Origanum vulgare* has neuroprotective effect in HT22 cell.

P-136

MEMORY IMPROVEMENT EFFECT OF METHANOL EXTRACT OF *MAGNOLIA DENUDATA* IN THE SCOPOLAMINE-INDUCED AMNESIA MOUSE MODEL

Ye Lim Son[1], Hyeon Woo Lee[1], Choong Je Ma[1,2]
[1]Department of Medical Bio Engineering, College of Biomedical science, Kangwon National University. Chuncheon 24341, Republic of Korea,[2]Institute of Bioscience & Biotechnology, Kangwon National University, Chuncheon 24341, Republic of Korea.

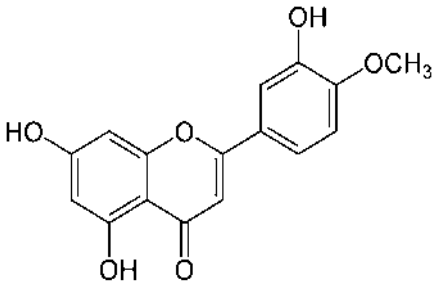
Magnolia denudata is known to have anti-oxidant, anti-inflammatory, anticancer effect. But it has not been reported that it has memory improvement effect. We experimented whether the methanol extract of *Magnolia denudata* (50, 100 or 200 mg/kg) could improve memory improvement effect in scopolamine induced amnesia mouse model using the Morris water maze test and passive avoidance test. In the Morris water maze test, the extract of *Magnolia denudata* improved the impairment of memory ability by scopolamine. As well as it made cognitive dysfunction in mice better. These results show that methanol extract of *Magnolia denudata* decrease the cognitive dysfunction caused by scopolamine. Consequently, We expect that methanol extract of M*agnolia denudata* can be useful for improving memory impairment.

P-137

INFLUENCE OF AÇAÍ BERRY CONSTITUENTS ON CELLULAR PROTECTION AND APOPTOSIS IN HEPG2 CELLS: TO DEFEND OR SURRENDER.

Thomas Siff[1], Bryan Berguson[1], Noelle Addair[1] Suneel Kandagatla[2], José Rivera-Chávez[2], Tyler N. Graf[2], Nicholas H. Oberlies[2], Gregory M. Raner[1]
[1]Department of Biology and Chemistry, Liberty University, Lynchburg, VA 24515, USA, [2]Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, Greensboro, NC 27402

The Brazilian açaí berry (*Euterpe oleracea*) is one of the most highly marketed and consumed natural products world-wide, with a projected market value of over $2 billion by 2025. A variety of health benefits have been attributed to this superfruit, however, studies linking these proposed effects to specific biochemical pathways are lacking. Using an activity guided fractionation approach, we have identified several compounds with distinct effects on biochemical pathways related to anti-oxidant activity, xenobiotic metabolism and apoptosis in human hepatoma cells. One of these compounds, a flavonoid called diosmetin, is active in multiple biochemical assays, which suggests a potential coordinated influence on cell death and protective pathways in the cell. This compound is an attenuator of the Nrf2 antioxidant pathway in human liver cells and has modest pro-apoptotic activity at low micromolar concentrations. It is also a nanomolar inhibitor of several different human cytochrome P450 enzymes and appears to reduce induction of CYP enzymes following treatment with β-naphthoflavone. Combined, these findings present a coordinated biochemical model that may provide a missing element in our understanding of the true health effects of this popular product.



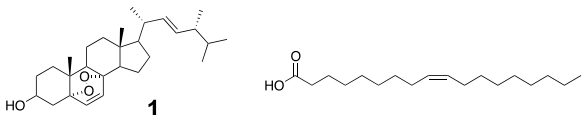
diosmetin

P-138

BIACTIVE COMPOUNDS ISOLATED FROM *EPICOCCUM NIGRUM* NFW7, AN ENDOPHYTIC FUNGUS FROM *TAXUS FUANA*.

Gerardo D. Anaya-Eugenio[1], Hira Mehboob Mirza[1], Safia Ahmed[2], Masoom Yasinzai[3], Esperanza J. Carcache de Blanco[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA. [2]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan. [3]SA-CIRBS, International –Islamic University, Islamabad, Pakistan.

Endophytic fungi represent an important source of natural products with medicinal properties. In the present work, the endophytic fungus *Epicoccum nigrum* was isolated from the plant *Taxus fuana*. Seven compounds were isolated from the ethyl acetate extract that showed growth inhibition against PC-3 prostate cancer cells. Structure identification and elucidation was completed using spectroscopy techniques (1D-NMR ^{1}H and ^{13}C, and 2D-NMR such as HMBC, HSQC, COSY, and DEPT). Two compounds have been fully identified and tested, including ergosterol-5α, 8α-peroxide (**1**) and oleic acid (**2**), which have previously been reported with potential anticancer properties[1-2]. The anti-proliferative activity of all compounds isolated will be evaluated against a panel of cancer cell lines. They will also be tested against target-based assays such as NF-κB and mitochondrial trans-membrane potential (MTP).

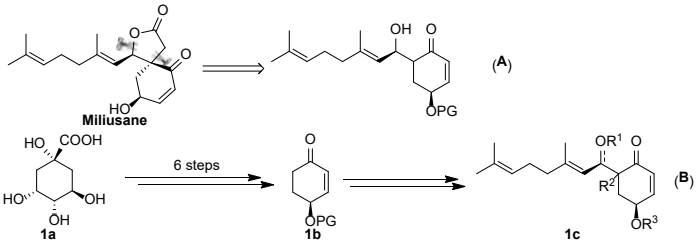


P-139

SYNTHESIS AND BIOACTIVITY EVALUATION OF MILIUSANE ANALOGUES

*Yu Zhu, Yi-Fu Guan, Ming-Yu Ye, Ni Shi, Siu Wai Tsang, and Hong-Jie Zhang**
School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, P.R. China.

Miliusanes are a cluster of compounds with a skeleton containing 18 carbons. They were identified from the *Miliusa* plants (Annonaceae), and showed cytotoxic activities against a panel of cancer cell lines (Zhang et al., *J. Med. Chem.* 2006, 49:693-708). To elucidate the structure-activity relationship (SAR), we have synthesized miliusane analogues by modifying the functional groups of the miliusane structures. Due to the bioactivity potency and structural novelty of this type of compounds, we have further carried out total synthesis of miliusane-like compounds (**Scheme 1**). The biological activities of the synthetic compounds have been evaluated against different disease targets. *Acknowledgements: The work described in this paper was supported by the Research Grant Council of the Hong Kong Special Administrative Region, China (Project No. HKBU 12103014), the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/15-16/02) and the Hong Kong Baptist University, Knowledge Transfer Office, Matching Proof-of-Concept Fund (MPCF-003-2017/18).*



Scheme 1. *A)* Synthetic design of the miliusane-like compounds. *B)* D-(-)-quinic acid (**1a**) was used as the starting compound toward the total synthesis. Through the key intermediate **1b,** a series of miliusane-like compounds (**1c**) were synthesized.

P-140

CHEMICAL COMPOSITION OF ESSENTIAL OILS, BIOLOGICAL ACTIVITY AND SECRETORY STRUCTURES OF SPECIES OF *BACCHARIS* FROM BRAZIL

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Chemical composition and biological activities of essential oils, and anatomy of leaves and stems of *Baccharis microdonta*, *B. pauciflosculosa*, *B. punctulata*, *B. reticularioides*, and *B. sphenophylla* growing in Brazil were studied. The essential oils extracted by hydrodistillation were analyzed by GC/MS and evaluated in vitro for their antimalarial and insecticidal activities. Anatomical analysis was performed by the usual light and scanning

microtechniques. Analyses of the essential oils from the five species showed significant differences in their chemical compositions. Some compounds are unique to some species hence can be used as chemical markers for species identification. The major compounds of the essential oils were spathulenol (23.27%) and kongol (22.43%) in *B. microdonta*, β-pinene (18.31%) and limonene (18.75%) in *B. pauciflosculosa*, α-bisabolol (23.63%) in *B. punctulata*, α-pinene (24.47%) in *B. reticularioides*, and β-pinene (15.20%), limonene (14.50%) and spathulenol (13.11%) in *B. sphenophylla*. *Baccharis pauciflosculosa*, *B. reticularioides* and *B. sphenophylla* exhibited antimalarial activities. *Baccharis microdonta* and *B. punctulata* showed cytotoxicity. None of the oils showed insecticidal toxicity to bed bug assessed in using three different bioassays except *B. sphenophylla* with 66.67% and 83.33% mortality in insecticide resistant strain 'Bayonne' and susceptible strain'Ft. Dix', respectively, only in fumigation bioassay at 250µg/125 mL of air. The vegetative aerial parts of all the five species showed glandular trichomes and ducts as secretory structures. This study was supported by Capes and Araucária Foundation (Brazil).

P-141

NATURAL PRODUCTS FROM AUSTRALIAN CELASTRACEAE PLANTS AND THEIR LEUCINE TRANSPORT INHIBITION IN PROSTATE CANCER CELLS

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Australian endemic plants have been superficially explored for both their chemistry and pharmacology. Thus, the use of this unique bioresource for drug discovery and chemical biology research holds great promise for the identification of biologically active natural products. The Celastraceae family is widely found in tropical and subtropical parts of the world, with some of the species endemic to Australia. These plants are a source of diverse chemical compounds with various bioactivities, one of which is inhibition of L-type amino acid transporters (LATs). This interesting biological activity has been recently identified during our research. LATs uptake neutral amino acids such as leucine into cells and are vital for protein synthesis as well as stimulating the mammalian target of rapamycin complex 1 (mTORC1) signalling pathway. The mTORC1 pathway is a central regulator of cell proliferation, growth, and metabolism. The LAT family is expressed in both normal and cancer cells, however the expression levels of LAT family members are upregulated in various human cancers, including prostate cancer. Leucine plays an important role as a rate-limiting signalling molecule in the mTORC1 pathway; therefore, targeting LATs by inhibiting leucine uptake affects cancer. Accordingly, the inhibition of leucine transporters may be a novel therapeutic approach for treating a variety of cancers. This presentation will cover the chemical and pharmacological aspects of dihydro-β-agarofurans from several Australian endemic Celastraceae plants.

P-142

CYTOTOXIC ANTHRACENONE *C*-GLYCOSIDES ISOLATED FROM THE LEAVES AND TWIGS OF *ALVARADOA AMORPHOIDES*

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Previously, several anthracenone *C*-glycosides were isolated from the leaves of *Alvaradoa haitiensis* Urb. (Simaroubaceae), and they were found to be potently cytotoxic against KB human oral epidermoid cancer cells. In a continuing search for anticancer agents from higher plants, a chloroform extract of the leaves and twigs of *Alvaradoa amorphoides* Liebm., collected in Guatemala (voucher specimen *Soejarto et al. 7236* in deposit at the Herbarium of the Field Museum under accession number: F-2057539), was found to be cytotoxic against the HT-29 human colon cancer cell line. Fractionation of this extract guided by bioassay against HT-29 cells yielded several pairs of new and known isomeric anthracenone *C*-glycosides, as well their aglycone. The structures of these isolates were determined by analysis of their spectroscopic data, and their cytotoxicity was evaluated toward HT-29 cells. All the anthracenone *C*-glycosides were found to be active, with IC_{50} values in the range 2–6 µM, but their aglycone was inactive, indicating that the *C*-glycose unit plays an important role for these anthracenones to mediate their cytotoxicity toward HT-29 cells. This is the first report of identification of anthracenone *C*-glycosides as the major cytotoxic components of *A. amorphoides*.

P-143

MOLLIC ACID, A CYCLOARTANE TRITERPENOID ISOLATED FROM *MARKHAMIA TOMENTOSA* LEAVES EXHIBITED ANTIPROLIFERATIVE ACTIVITY ON HELA CELLS

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Previous pharmacological investigations have reported the antiproliferative activity of *Markhamia tomentosa* (Benth.) K. Schum Ex Engl. (Bignoniaceae) leaf extract on HeLa cells [1]. In this study, the antiproliferative phytochemicals present in the plant were isolated and characterized using bioactivity-guided approach and spectroscopic techniques respectively. Bioassay-guided fractionation of the crude leaf extract of *M. tomentosa* revealed that the dichloromethane (Mdf) and ethyl-acetate (Mef) fractions showed potent cytotoxicity activity against HeLa cells with IC_{50} values of 83.26 and 104.5 µg/ml respectively in the MTT assay. From the isolation and purification of Mdf and Mef fractions by repeated column chromatography, followed by characterization by 1D and 2D NMR spectroscopy, sitosterol, phytol, oleanolic acid and mollic acid were isolated for the first time from *M. tomentosa*. Mollic acid showed more potent antiproliferative activity. These results suggest that mollic acid isolated from Mef fraction of the crude extract may be responsible for the earlier reported antiproliferative activity of *M. tomentosa* on HeLa cells.

P-144

CARDIOPROTECTIVE AND HYPOCHOLESTEROLEMIC EFFECT OF ETHANOLIC EXTRACT OF *MORMODICAL CHARANTIAL* IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN ADULT WISTAR RATS.

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The ethanolic extract of the plant of *Mormodical charantial and* standard drug, metoprolol were prepared in normal saline and then administered orally to rats at the doses of 250 and 100mg/kg body weight (b.wt) respectively for a period of thirty days .ISO was freshly prepared in normal saline and was then used to induce MI by intraperitoneal injection at the dose of 100mg/kg to Wistar rats on the 30th day. Serum lipid profile and cardiac marker enzymes such as creatine phosphokinase (CK-MB) Isoenzyme , lactate dehydrogenase (LDH), Alanine transaminase (ALT)and Aspartate (AST) were obtained in the serum and in the heart homogenate of the experimental rats and then measured calorimetrically.The results show that isoproterenol-induced myocardiac infarction were associated with significant ($p<0.05$) increase in the activities of cardiac marker enzymes such as AST, ALT, CK-MB and LDH in the serum with concomitant decrease in the activities of these enzymes in the myocardial tissue as compared to control group. There were also significant ($p<0.05$) increase in serum level of total cholesterol (TC) ,trigyceride(TG), low density lipoprotein(LDL) and very low density lipoprotein(VLDL) in the group injected with isoproterenol (group ii)as compared with control group . Pretreatment with leaf extract of *Mormodical charantial* at a dose of 250mg\ kg b.wt and also by Metoprolol at dose 100 mg/kg body weight significantly ($p<0.05$) prevented this alteration of the lipid profile and also of the activities of these cardiac marker enzymes both in the serum and myocardial tissue as compared to isopreterenol-induced control group. The histological examinations of the heart further confirmed the cardioprotective effect *Mormodical charantial* as there were absent of swollen myocardium whereas ISO induced groups were characterized with these toxicological features . *Mormodical charantial* possesses cardioprotective and hypocholesterolemic effects.

P-145

CHEMISTRY AND BIOAVAILABILITY OF THE FLAVONOIDS AND SAPONINS ISOLATED FROM *GYNOSTEMMA PENTAPHYLLUM* (THUNB.) MAKINO

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Gynostemma pentaphyllum (Thunb.) Makino has been the focus of extensive research over the past decade. The work presented here encompasses the characterisation of total flavonoids and saponins (the principal active constituents) in *G. pentaphyllum*. Two new flavonoids and five new dammarane saponins, together with sixteen known compounds were isolated, purified and characterised from *G. pentaphyllum*. The two new flavonoids, namely, kaempferol-3-O-α-rhamnopyranosyl-(1→2)-β-galactopyranoside and quercetin-3-O-α-rhamnopyranosyl-(1→2)-β-galactopyranoside and two new dammarane saponins, gypenoside LVI-acetate and gypenoside XLVI-acetate were isolated from the methanol extract of *G. pentaphyllum* leaves and the other three new saponins, namely, Damulin E, F and yixinoside BI were isolated from the heat treated ethanol extract of Active-AMP (a commercial preparation of *G. pentaphyllum* powder). A cell permeability assay, using Caco-2 cell model was performed on the isolated compounds resulting in compounds (both flavonoids and saponins) demonstrating moderate to good permeability across Caco-2 cell monolayer which can be considered for potential bioavailable drugs. To identify the potential bioactivities of the isolated quercetin flavonoids, we did an enzyme inhibitor assay against PARP14, a key enzyme for inflammatory diseases and various cancer types. Two quercetin derivatives, quercetin-3-glucoside and quercetin-dirhamnoglucoside showed excellent inhibition of PARP14.

P-147

CYTOTOXICITY OF EUPHOL, A TETRACYCLIC TRITERPENE

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Euphol, a tetracyclic triterpene alcohol, is one of the major compounds present in Euphorbia species, and cytotoxic activity has been attributed to this compound. The MTT reduction assay was used to evaluate the cytotoxicity of euphol (7.30 to 234.30 μM) against cancer cell lines. The selectivity index was determinate by the ratio of IC_{50} of lymphocytes or monocytes and K-562 cells. To evaluate the death mechanisms, morphological and cell cycle assays in K-562 were done by fluorescence optical microscopy (acridine orange and ethidium bromide staining) and flow cytometry (propidium iodide), respectively. K-562 cells were more sensitive to the euphol with IC_{50} values of and 34.56±2.12 μM. Whereas, Jurkat, HL-60, HRT-18 and B16F10 showed an IC_{50} of 84.33±11.76 μM, 49.36±2.76 μM, 53.63±10.16 μM and 69.80±7.31 μM. The selectivity index was 2.08 for lymphocytes and 2.35 for monocytes. The morphological analysis showed a reduction in the total cell number and was possible to observe apoptotic cells. In the analysis of the cell cycle it was observed that euphol promoted progression into S-phase and reduction of population cellular in G2/M-phase. This study demonstrated that euphol exhibited cytotoxic effects against a variety of cancer cells lines and suggest that the mechanism involved could be related with apoptosis.

P-148

DITERPENE ACIDS FROM *GUTIERREZIA SAROTHREA* AND *G. MICROCEPHALA*: CHEMICAL DIVERSITY, CHEMOTYPES AND IMPLICATIONS FOR ABORTIFACIENT ACTIVITY IN CATTLE.

Dale R. Gardner

USDA-ARS Poisonous Plant Research Laboratory, 1150 E. 1400 N., Logan, Utah, USA

Broom snakeweed (*Gutierrezia sarothare*) and threadleaf snakeweed (*G. microcephala*) are perennial plants found on western US rangelands. If eaten, the plants can be toxic to cattle, sheep and goats. In cattle they may cause premature parturition (abortions) in late term animals. The toxic components are not known, but some propose that the diterpene acids may be both toxic and abortifacient. A number (133) of plants were collected from Colorado, Oklahoma, New Mexico, Texas, Arizona and Utah. Plants were identified by classical taxonomy and then analyzed by a general GC-MS procedure to determine individual chemotypes. The GC-MS fingerprints were diverse showing a possible 15 different chemotypes. There were four major chemotypes accounting for 73% of the samples from which the major diterpenes acids were determined by extraction, preparative chromatography and characterization by NMR, MS, IR and UV spectroscopy. The diterpene acids were found to be a mix of furano, lactone, di-acid and

labdane type acids. Several of the diterpene acids appear to be new compounds and most have not been previously described from *G. sarothare* or *G. microcephala*. Chemotype BSW-2 is of particular interest because it was found to contain a compound identified as labd-7-en15,18-dioic acid, which is structurally similar to isocupressic acid and agathic acids which are known to cause abortions in cattle.

P-149

CYTOTOXIC CONSTITUENTS WITH NF-KB AND MITOCHONDRIAL TRANSMEMBRANE POTENTIAL (MTP) INHIBITORY EFFECTS FROM *SYZYGIUM CORTICOSUM*

Yulin Ren[1], Gerardo D. Anaya-Eugenio[1], Austin A. Czarnecki[2], Tran Ngoc Ninh[3], Chunhua Yuan[4], Hee-Byung Chai[1], Djaja D. Soejarto[2,5], Joanna E. Burdette[2], Esperanza J. Carcache de Blanco[1], A. Douglas Kinghorn[1]

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In a continuing search for anticancer agents from higher plants, a chloroform extract of the leaves and twigs of *Syzygium corticosum* (Lour.) Merr. & Perry (Myrtaceae), collected in Vietnam, was found to be cytotoxic against the HT-29 human colon cancer cell line. Fractionation of this extract guided by bioassay against HT-29 cells yielded a new hydroxylated lupane derivative and 18 known natural products. All isolates obtained were evaluated for their cytotoxicity toward the HT-29 colon, the MDA-MB-231 breast, the MDA-MB-435 melanoma, and the OVCAR3 ovarian human cancer cell lines. The pentacyclic triterpenoids, methylated 2,3-dihydroflavonoids, and ellagic acid derivatives purified were found to be the main active components, but the megastigmanes did not contribute to this type of activity. The most abundant and cytotoxic compound, (+)-ursolic acid, exhibited also potent NF-κB (IC_{50} 31 nM) and mitochondrial transmembrane potential (MTP) (IC_{50} 3.5 μM) inhibitory activities.

P-150

CHARACTERIZATION OF PHYTOPROGESTIN COMPOUNDS IN HERBAL SUPPLEMENTS

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Progestins are used to treat gynecological diseases like uterine fibroids, endometriosis, endometrial cancer, and ovarian cancer that result from abnormal levels of progesterone. Detrimental side effects like breast cancer, cardiovascular disease, and stroke are associated with progestin therapy. To prevent these side effects, alternative progestins that are selective for the progesterone receptor are necessary. Herbal supplement sales have increased in recent years, although it is unclear what they contain since they are not tightly regulated. Previous studies have shown that some herbal supplements contain compounds that modify steroid signaling and specifically our lab has identified molecules in herbal supplements that act similarly to progesterone, termed phytoprogestins. Phytoprogestin compounds were identified by bioassay guided fractionation using a luciferase reporter assay. The compounds were tested to see if they had agonist or antagonist effects. Apigenin, a flavonoid that is present in many herbal supplements and teas, was found to be a mixed agonist and had *in vivo* progestin-like effects by inhibiting genistein induced uterine proliferation. Surprisingly, irilone and cornuside, compounds isolated from red clover and dogwood respectively, 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.

P-151

UNVEILING THE IMPACT OF CRUDE OIL POLLUTION ON THE PHYTOCHEMICAL PROFILE OF *VERNONIA AMYGDALINA*

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Vernonia amygdalina (VA) is used by traditional medical healers to treat pregnant indigenous women for the prevention of miscarriages. The present study was aimed at investigating the effect of crude oil pollution on the phytochemical profile and safety of the use of VA during pregnancy. VA was collected from Kokori (affected by an oil-spill) and Abraka (oil-free environment). Both samples were extracted with methanol followed by liquid–liquid separation. After repeated chromatography, the butanol fraction gave two compounds which were characterized based on their NMR spectral data. In addition, ICPMS was carried out on the crude extracts of VA to assess the levels of heavy metal accumulation. The elemanolide-type sesquiterpene lactone, Vernodalol (**1**) was found in VA from both communities. However, the related Lasiopulide (**2**) which has never been reported from VA was exclusively found in VA collected from the oil-polluted community. Furthermore, ICP-MS revealed crude oil polluted VA to display elevated levels of lead, arsenic and cadmium. Our results thus show a marked difference in the phytochemical content of VA from Abraka (oil-free) and Kokori (oil-spill) communities, which may lend support to the respondents' claims that the medicinal plants in the crude oil spill community may lose their potency upon sustained oil exploration. Work is on-going to investigate the toxicity of the isolated compounds as well as plants extracts of both communities.

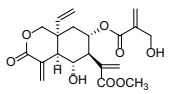

Figure (**1**): Vernodalol

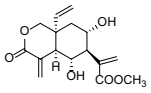

Figure (**2**): Lasiopulide

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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL STUDIES OF AN AUSTRALIAN NATIVE PLANT

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This study aimed to evaluate the bactericidal efficacy of leaves obtained from an Australian native plant (denoted as 8479) against 19 bacteria. The antibacterial activity of 8479 was determined using the well diffusion assay, whilst broth microdilution assay was used to measure the minimum inhibitory concentration (MIC). Several chromatographic (*i.e.,* normal phase flash column, reversed phase high performance liquid chromatography) and spectroscopic (*i.e.* nuclear magnetic resonance, mass spectroscopy) techniques were used to isolate and identify bioactive compounds derived from the plant. The crude methanolic extract at a concentration of 100 mg/mL was bactericidal against numerous Gram-positive bacteria (*B. cere-*

us, MSSA, MRSA isolates, *S. pyogenes, B. subtilis* and *S. epidermidis*) and vancomycin-resistant enterococci *E. gallinarum, E casseliflavus, E. faecalis* and *E. faecium*. To date, phenolic compounds have been purified from the methanolic extract of 8479. The MICs of the compounds ranged between 8-64 μg/mL. Further, current results demonstrate that crude methanolic extracts, as well as purified compounds have antibacterial activity against several common-wound colonising bacteria.

P-154

THREE NEW DITERPENOID ALKALOIDS FROM *ACONITUM JAPONICUM*

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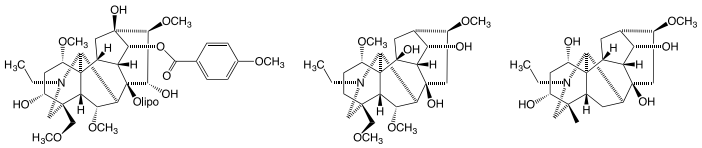
Three new C_{19}-diterpenoid alkaloids, lipojesaconitine (**1**), 10-hydroxy-chasmanine (**2**), and 8-demethylcrispulidine (**3**), have been isolated from *Aconitum japonicum* THUNB. subsp. *subcuneatum* (NAKAI) KADOTA together with two known C_{19}-diterpenoid alkaloids, foresticine and neo-linine, and four known C_{20}-diterpenoid alkaloids, acomicarchamine A, 9-hydroxynominine, kobusine and torokonine. The structures of these alkaloids were determined by their ms, 1D and 2D-nmr data. Two of the new C_{19}-diterpenoid alkaloids (**1**, **3**) and five of the known diterpenoid alkaloids were evaluated for cytotoxic activity against five human tumor cell lines (A549, MDA-MB-231, MCF-7, KB, and KB-VIN).



P-155

PHYTOCHEMICALS DERIVED FROM THE POPULAR SOUTH AFRICAN ROOIBOS TEA (*ASPALATHUS LINEARIS*) SPECIES AND THEIR DRUG INTERACTION POTENTIAL

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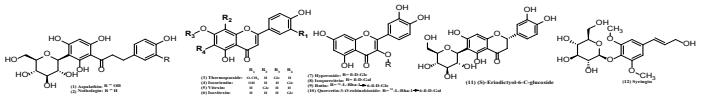
Aspalathus linearis is a leguminous shrub endemic in South Africa, and used for production of the quaffable herbal rooibos tea. Although it is enjoyed in 37 countries, rooibos extracts were reported to inhibit CYP2C9, CYP2C8 and CYP3A4 isozymes. In our study, phytochemical investigation of unfermented rooibos tea leaves and seeds resulted in the isolation and characterization of eleven unique flavonoid C- and O-glycosides **(1-11)** in addition to syringin **(12)**. This is the first study aims mainly to investigate the toxicity profile for the unfermented methanol extract of rooibos tea and its isolated unique secondary metabolites focusing on drug metabolizing enzymes and efflux transporters.



P-156

INVESTIGATION OF THE CHEMICAL COMPOSITION AND ANTICANCER POTENTIAL OF ANDROCYMBIUM PALAESTINUM BAKER (COLCHICACEAE) LEAVES

Tamam El-Elimat[1], Maram B. Alhawarri[1], José Rivera-Chávez[2], Joanna E. Burdette[3], Austin Czarnecki[3], Feras Alali[4], and Nicholas H. Oberlies[2]
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Thirteen compounds were isolated from the methanolic extract of *Androcymbium palaestinum* Baker (Colchicaceae) leaves. Of these, three were new, two were new natural products, and eight were known. The new isolated compounds were 1-demethyl-(+)-androcine (**5**), (-)-androcnizine (**8**), and 2-demethyl-*β*-lumicolchicone (**10**), while the new natural products were (+)-*O*-methylkreysigine-*N*-oxide (**3**) and 5′-methoxy-(+)-homolaudanosine (**9**). Moreover, (-)-colchicine (**11**), (-)-demecolcine (**12**), and 3-demethyl-(-)-demecolcine (**13**) were reported for the first time from this species. The structures of the isolated compounds were elucidated using a series of spectroscopic and spectrometric techniques, principally HRESIMS, 1D-NMR (^{1}H and ^{13}C-NMR) and 2D-NMR (COSY, edited-HSQC, and HMBC). The cytotoxic activities of the isolated compounds were evaluated using the MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovary) cancer cell lines. Compound **11** was the most potent against all tested cell lines, with IC_{50} values of 12, 95, and 23 nM, respectively.

P-157

REALTIME PCR ANALYSIS OF FLAVORNOID BIOSYNTHESIS GENES IN TIBETAN MEDICINAL PLANT *SWERTIA MUSSOTII*

Yue Liu[1,2§], Yi Wang[3§], Jiaqing Sun[1], Fengxian Guo[1], Lin Zhan[1], Toni Mohr[3], Prisca Cheng[3], Naxin Huo[3,4], Danning Pei[1], Li Tang[1], Chunlin Long[1], Luqi Huang[2*], Yong Q. Gu[3]*
[1]College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China, [2]National Resource Center for Chinese Materia Medica, China Academy of Traditional Chinese Medicine, Beijing 100700, China, [3]USDA-ARS, Western Regional Research Center, Albany, CA 94710, U.S.A., [4]Department of Plant Science, University of California Davis, Davis, CA 95616, U.S.A.

Swertia mussotii Franch. is an important traditional Tibetan medicinal plant with pharmacological properties effective in the treatment of hepatitis. Flavonoid are the major bioactive compounds in this plant. Transcriptome sequences of *S. mussotii* was used to mine the genes involved in flavonoid biosynthesis pathway. The expression profiles of 17 candidate transcripts encoding the key enzymes for flavonoid biosynthesis were examined in different *S. mussotii* tissues, root, stem, leaf, and flowers, validated by qRT-PCR. We found that some allelic genes had different expression pattern. Maybe the allelic genes had the different role in the plant. Acknowledgment: financial support by project of NSFC-81274185, 81373765, NCET-13-0624, 111-B08044, and Yong Talent project 2016-3-1, sponsored by the State Ethnic Affairs Commission, China.

P-158

THREE NEW TRITERPENOIDS FROM EUONYMUS ALATUS FORMA CILIATODENTATUS

Yamashita H[1], Matsuzaki M[1], Kurokawa Y[1], Nakane T[2], Shibata T[3], Bando H[1] and Wada K[1]

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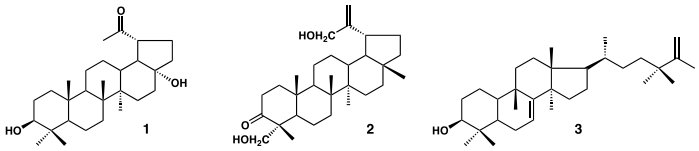
The bark of *Euonymus alatus* forma *ciliato-dentatus* (Celastraceae) has been used as an analgesic for toothache by the Ainu tribe (AINU-people), an indigenous people of Japan. In the course of our studies on natural drug resources used by the AINU-people, we became interested in chemical constituents and morphological differences among *Euonymus* species. Three new triterpenoids, 3β,17α–dihydroxy-28,30-bisnorlupan-20-one (1), 23,30-dihydroxylup-20(29)-en-3-one (2) and 24,24-dimethylcucurbita-7,26-dien-3β-ol (3) were isolated along with eight known lupane derivatives, 30-norlupane-3,20-dione (4), 28,30-dihydroxylup-20(29)-en-3-one (5), 23,30-dihydroxylup-20(29)-en-3-one (6), (20*S*)-30-norlupane-3,20-diol (7), 3,28-dihydroxy-30-norlupan-20-one (8), 3,28-dihydroxylup-20(29)-en-30-al (9), 3,23-dihydroxylup-20(29)-en-28-al (10) and lup-20(29)-ene-3,23,28-triol (11) from an ethanol extract of the dried bark of *E. alatus* forma *ciliato-dentatus.* These known compounds were found in this plant for the first time.



P-159

STRUCTURAL ELUCIDATION OF Ω-PHENYLPENTAENE FATTY ACID GLYCOSIDES FROM FRUITS OF TWO RHAMNACEAE PLANTS

Kyo Bin Kang[1,2], Ming Gao[3], Eun Jin Park[1], Geum Jin Kim[3], Jinwoong Kim[1], Hyukjae Choi[3], and Sang Hyun Sung[1]

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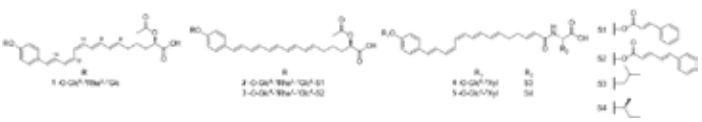
Three 2-acetoxy-ω-phenylpentaene fatty acid triglycosides berchemiosides A–C (**1**–**3**) and two ω-phenylpentaene α,β-unsaturated fatty acid amide diglycosides rhamnellosides A (**4**) and B (**5**) were isolated and characterized from fruits of two Rhamnaceae plants *Berchemia berchemiifolia* and *Rhamnella franguloides*, respectively. Their structures were determined by spectroscopic analysis in combination with chemical derivatization; especially, 2D *J*-resolved and ROESY NMR analyses were applied to reveal the absolute configurations of pentaene groups. Compounds **1**, **4**, and **5** were found to have (6*E*,8*E*,10*Z*,12*Z*,14*E*)-geometry, whereas **2** and **3** exhibited all-*E* geometries.



P-160

NEW PREGNANE GLYCOSIDES ISOLATED FROM STAPELIA GIGANETEA

Hyeon Seok Jang[1], Seong Yeon Choi[1], Birang Jeong[1], Jiho Lee[1], Kiwon Ko[1], Heejung Yang[1]

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Stapelia gigantea (Asclepiadaceae) is a succulent plant native to dry lands of South Africa and a popular garden in Korea. We isolated four new pregnane glycosides (**1**-**4**) from *S.gigantea*. The structures were determined by 1D and 2D NMR spectral analysis. The absolute configuration of compounds (**1**-**4**) were determined by NOESY and coupling constants analyses.

P-161

CYTOTOXIC COMPOUNDS FROM SEDUM MIDDENDORFFIANUM

Jiho Lee[1], Seong Yeon Choi[1], Hyeon Seok Jang[1], Birang Jeong[1], Kiwon Ko[1], Heejung Yang[1]

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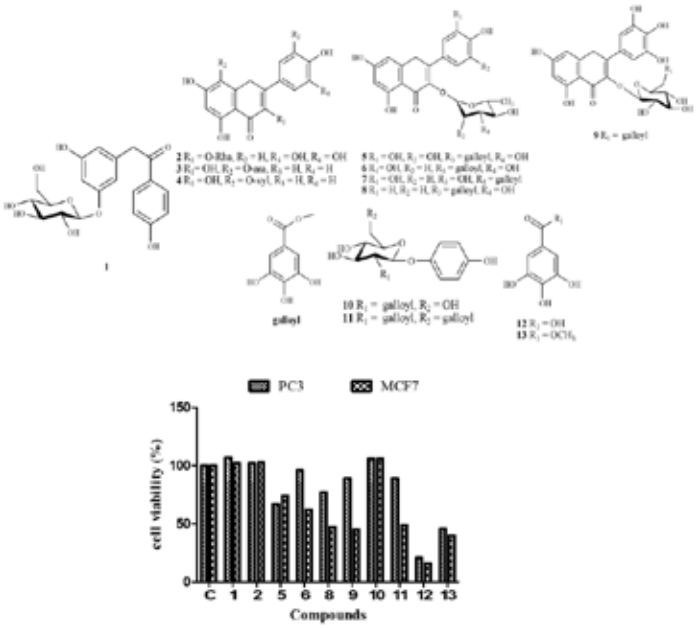
A new phenolic compound, belamphenone-5-*O*-β-D-glucoside (**1**), was isolated from *S. middendorffianum* along with eight flavonoids (**2**-**9**), two phenolic compounds (**10** and **11**) and two benzoic acid (**12** and **13**). Compounds **8**, **9**, **11**, **12** and **13** showed anti-proliferative activities against MCF-7 than PC-3 cell line.



P-162

ACAI EXHIBITS ANTI-DIABETIC AND OSTEOGENIC ACTIVITY IN FISH MODELS

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Acai berries from the *Euterpe oleracea* Martius (Arecaceae) palm tree, used in South American cultural medicine, have made a resurgence in popular culture for treating osteoarthritis, high cholesterol, weight loss, and obesity. However, many of these claims have not yet been supported by scientific evidence, so potential osteogenic and anti-diabetic effects of acai extract (AE) are being investigated. A methanol extract of dried organic acai berries was found active in several *in vitro* assays for anti-inflammatory (NFκB), anti-diabetic (PPAR-γ), and osteogenic effects. AE increased osteoblast proliferation and reduced apoptosis in cultured human osteoblasts (hFOB). The AE was further tested in an osteogenesis medaka model and a diabetic zebrafish model. Double transgenic *osterix/Sp7*:mCherry medaka larvae treated with AE exhibited osteoblast proliferation and increased osterix expression. In wildtype zebrafish with chemically-induced diabetes, HbA_{1C} levels decreased upon treatment with AE, reversing hyperglycemia. In addition, PPAR-γ expression was increased at a level greater than positive control rosiglitazone, an FDA-approved anti-diabetes drug. Hence, acai is a promising lead.

P-163

VARIATION OF THE PRODUCTION OF 4-NEROLIDYLCATECHOL BY *PIPER UMBELLATUM*

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Piper umbellatum, previously known by the popular name Pariparoba, has a high medicinal potential. The major compound is 4-nerolidylcatechol (4-NC), responsible for several biological activities such as antioxidant, antimicrobial, anti-edema and anti-malaria. The aim of the present study was to evaluate the yield of 4-NC produced by *P. umbellatum* collected at different times. Three isolations of 4-NC were performed, the first extraction being carried out at the beginning of August 2016, the second in January 2017 and the third in October 2017. A dichloromethane extract was prepared for each collect, using fresh leaves of *P. umbellatum*, obtained from the Garden of Medicinal Plants of the School of Pharmaceutical Sciences - Unesp, Araraquara, SP. After total evaporation of the solvent the extract was partitioned three times using hexane and acetonitrile. The acetonitrile phase was then fractionated on octadecylsilane column with isocratic elution of acetonitrile:water (50:50) and 20 fractions were collected. The fractions were applied in thin layer chromatography eluting with hexane:ethyl acetate:acetic acid (60:28:2) and developing with sulfuric anisaldehyde and NMR (^{1}H and ^{13}C) analysis for confirmation of purity. The data obtained showed that in the first extraction the yield was 0.015%, followed by the second extraction with 0.010% and the third extraction with 0.006%, indicating variation of the metabolite production in different collections performed in the same place. The results suggest that climatic and environmental factors may influence the increase or decrease of 4-NC production needing more attention to achieve higher levels.

P-165

A SINGLE ENZYME INTRODUCES BOTH C–O BONDS OF THE ETHER BRIDGE IN LOLINE ALKALOIDS. REGIO- AND STEREOCHEMICAL COURSE OF THE OXIDATIONS.

Juan Pan,[1,2] Minakshi Bhardwaj,[1] Bo Zhang,[2] Wei-chen Chang,[2] Christopher L. Schardl,[1] Carsten Krebs,[2] Robert B. Grossman,[1] and J. Martin Bollinger, Jr.[2]

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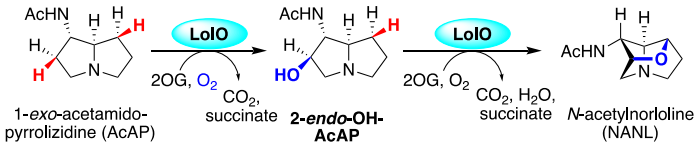
The loline alkaloids, insecticidal natural products produced by fungal endophytes of cool-season grasses, contain an ether bridge connecting two bridgehead C atoms, an unusual context for an ether in natural products. We show that a single nonheme Fe oxygenase, LolO, oxidizes two unactivated C–H bonds of 1-*exo*-acetamidopyrrolizidine (AcAP) to give *N*-acetylnorloline (NANL). A stop-flow kinetics study and isolation and characterization of a hydroxylated intermediate show that LolO oxidizes the C2 position of AcAP first, and feeding of stereospecifically labeled precursors to live fungi shows that LolO selectively abstracts the endo H atoms from C2 and C7. These studies lay the groundwork for understanding the mechanism of the very unusual ether-forming event that leads to NANL.



P-166

COMPARISON OF ANTIOXIDANT ACTIVITIES BETWEEN *CRINUM ASIATICUM* AND *RICINUS COMMUNIS* LEAVES EXTRACTS

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[1]Department of Applied Thai Traditional Medicine, Faculty of medicine, Thammasat university, Pathumthani, 12120, Thailand.

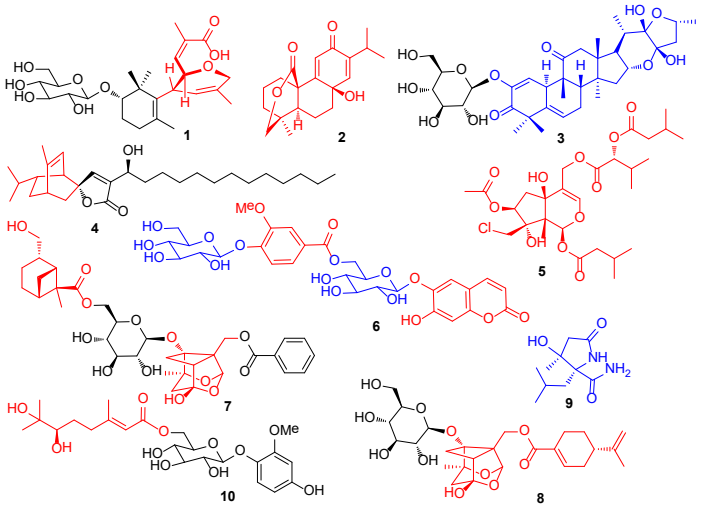
Hot Salt-pot Compression is a method of postpartum care that has been widely used in Thai traditional medicine. The method is used for treating several symptoms of women's postpartum. *Crinum asiaticum* (CA) leaf is the main component of this method. *Ricinus communis* (RC) leaf is also commonly used in the method instead of CA leaf. Therefore, in this work, we focuses on the comparison of antioxidant activities of the water and ethanolic extracts of CA and RC leaves. Antioxidant activities were assessed by DPPH and FRAP methods. The results demonstrated that the ethanolic extract of RC leaf showed the strongest antioxidant activity by using DPPH and FRAP assays (EC_{50} value of 17.37 µg/mL and FRAP value of 412.66 mg Fe(II)/g, respectively) while the lowest antioxidant activity was reported for the ethanolic extract of CA (EC_{50} value of > 100 µg/mL and FRAP value of 74.60 mg Fe(II)/g, respectively). From the findings, extracts from RC having better antioxidant activity than extracts from CA. However, these methods are fundamental assays which do not clearly indicate antioxidant effect. Thus, these extracts should further investigate for another antioxidant methods.

P-167

STRUCTURES AND BIOLOGICAL EVALUATION OF NEW COMPOUNDS FROM TRADITIONAL CHINESE MEDICINES (TCMS)

Jing-Fang Zhang, Gui-Yang Xia, Rui Li, Yu-Zhuo Wu, Ling-Yan Wang, Bo-Lin Qiu, Huan-Xia, Sheng Lin and Jian-Gong Shi**
State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Guided by bioactivity-directed fractionation has resulted in discovery of new compounds (**1**-**10**) with chemical and/or biological diversity from six Traditional Chinese Medicines (TCMs), *Paeonia lactiflora*, *Valeriana jatamansi*, *Litsea cubeba*, *Fraxinus sieboldiana*, *Machilus yaoshansis,* and red yeast rice [1-11]. Their structures were assigned via spectroscopic techniques, and the absolute configurations were verified via chemical methods, specific rotation, electronic circular dichroism (ECD) data, and X-ray crystallographic analysis. Compound **3** has an unusual 16,23:22,25-diepoxy unit. Compound **4** was a novel tricyclic spirolactones bearing long linear alkyl chains formed by Diels_Alder [4+2] cycloaddition of a molecule of each butenolide with β-phellandrene, and it showed cytotoxicity against the A549 cell lines with IC_{50} values of 5.1 μM. Compounds **7** and **8** are rare cage-like paeoniflorin derivatives comprising two monoterpenoidal moieties. Compound **9** was an unusual γ-lactam with inhibitory activity against histone deacetylase 1 (HDAC1) with an IC_{50} of 3.7 μM.



P-168

CRYSTAL STRUCTURE IN COMPLEX WITH CARRIER PROTEIN CHARACTERIZES PYRROLE BIOSYNTHESIS BY A TYPE II NRPS OXIDASE

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Sequential enzymatic reactions on payloads tethered to carrier proteins (CPs) generates non-proteogenic amino acids that are then delivered to non-ribosomal peptide synthetases (NRPSs) to generate peptidic natural products. The underlying diversity of non-proteogenic amino acid building blocks is the principal driver of the chemical diversity of NRPS-derived natural products. Structural insights into recognition of CPs by tailoring enzymes that generate these non-proteogenic amino acids is non-existent. Here, we present the crystal structure of FAD-dependent L-prolyl oxidase in complex with its cognate CP in the holo- and product bound states. The thiotemplated pyrrolyl product CP is a universal player in natural product biosynthetic pathways and our results delineate the interactions between the CP and the oxidase, while also providing insights into the stereospecificity of the enzymatic reaction. Our results demonstrate that NRPSs recognize and bind to their CPs using interactions quite different to that from fatty acid and polyketide biosynthetic enzymes and that structural diversity in natural product biosynthesis can be, and is, derived from subtle modifications of primary metabolic enzymes.

P-169

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *PILIOSTIGMA THONNINGII*.

Chijoke Ezennaka[1,2], Michael Afolayan[1-4], Radhakrishnan Srivedavyasasri[1], Olayinka Asekun[2], Oluwole Familoni[2], Margaret Sofidiya[3], Abayomi Orishadipe[4], Mohamed Ibrahim[1], Samir Ross[1,5,]*
[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Department of Chemistry, [3]Department of Pharmacognosy, University of Lagos, Lagos, Nigeria. [4]Chemistry Advanced Research Center, Sheda Science and Technology Complex, PMB 186, Garki-Abuja, Nigeria. [5]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Piliostigma thonningii, (Milne-Redhead, Fabaceae) is used for various medicinal purposes in African countries. The decoction of the leaves and bark is used for the treatment of ulcers, wounds, heart pain, arthritis, malaria, pyrexia, leprosy, sore throat, diarrhea, toothache, gingivitis, cough and bronchitis. Phytochemical investigation of *P. thonningii* yielded two new compounds: 2*β*-methoxyclovan-9*α*-ol (**1**), methyl-*ent*-3*β*-hydroxylabd-8(17)-en-15-oate (**2**), and twenty known compounds which were identified by their NMR, MS and GC-MS spectral analyses as: clovane-2*β*,9*α*-diol (**3**), alepterolic acid (**4**), anticopalic acid (**5**), (3*S*,5*R*,6*S*)-trihydroxy-7*E* -megastigmen-9-one (**6**), *β*-amyrin (**7**), vitamin E (**8**), piliostigmin (**9**), (+)-epicatechin (**10**), quercetin (**11**), quercitrin (**12**), afzelin (**13**), 3-hexenyl-1-O-*β*-D-glucopyranoside (**14**), garcinielliptone Q (**15**), epoxydammarane-3*α*,25-diol (**16**), palmitic acid, oleic acid, linoleic acid, stigmasterol, sitosterol and *β*-sitosterol glucoside. Compounds **1**, and **4** showed potential selectivity towards *Trypanosoma brucei* with IC_{50} 7.89 and 3.42 μM, respectively. Compound **2** showed moderate activity towards *T. brucei and Leishmania donovani* Amastigote with IC_{50} 3.84 and 7.82 μM, respectively. The structure activity relationship of the isolated metabolites suggested that hydroxylation at C-2 enhances the activity towards *T. brucei* in sesquiterpenes **1** and **3**. Similarly hydroxylation at C-3 in labdane diterpenes elevates the activity towards *T. brucei*.

Acknowledgements: Supported by Association African Universities; SHESTCO, NG; NCNPR, USA.

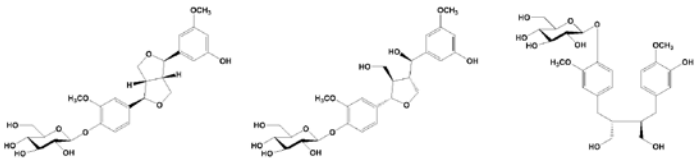
P-170

CONSITUTENTS OF *FORSYTHIA KOREANA* FLOWERS (OLEACEAE) AND THEIR FUNCTIONALITY AS PHARMACOLOGICAL AGENTS

Yeong-Geun Lee, Jung Su Ko, Jung Eun Gwag, Tong Ho Kang, Se Chan Kang, Hyoung-Geun Kim, Nam-In Baek
Graduate School of Biotechnology and Department of Oriental Medicine Biotechnology, Kyung Hee University, Yongin 17104, Korea.

Forsythia koreana (Oleaceae), a perennial shrub, is widely distributed in east Asia. From the *F. koreana* flowers, twenty phenolic compounds including four new ones were isolated through repeated column chromatographies and identified based on spectroscopic data such as 1D and 2D NMR, IR, FAB/MS, and CD. As well the examination of extracts, fractions, and isolated compounds was carried out for the functionality such as anti-diabetes, anti-oxidantion, and anti-inflammation. Some compounds showed significant anti-oxidant, anti-inflammatory, anti-diabetes, and whitening

activity without visible toxic effect. Quantitative analysis of active materials in *F. koreana* flowers was also conducted using LC/MS experiments.



koreanaside A koreanaside B koreanaside C

P-171

A POLYKETIDE SYNTHASE TRIO LEADS TO *ORTHO*-HYDROQUINONE BIOSYNTHESIS TO FACILITATE IRON ASSIMILATION BY ENDOPHYTIC FUNGI

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[1]Department of Chemical and Biomolecular Engineering, [2]Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, United States

Collaboration between iterative polyketide synthases (iPKS) represents an effective strategy to construct molecules of diverse functions. Through genome mining, we identified and characterized *Tln* cluster in biocontrol agent *T. harzianum* t-22 that synthesizes phenylpropanoid-like *ortho*-hydroquinone molecules, termed tricholignan, via a PKS trio followed by tailoring enzymes. Tricholignan, resembling coumarins in plant, can reduce Fe(III) to assimilate iron. The synthesis was initiated by sequential actions of a high-reduced polyketide synthase (HRPKS) and a non-reducing polyketide synthase (NRPKS). An unusual pseudo acyl-carrier protein (ψACP) fused with methyltransferase (MT) *in trans* is essential to execute proper programming rules of the partner NRPKS, via inhibiting the KS domain in an ACP-specific manner.

P-172

ADDITIONAL α-GLUCOSIDASE INHIBITORS FROM *MALBRANCHEA FLAVOROSEA* (LEOTIOMYCETES, ASCOMYCOTA)

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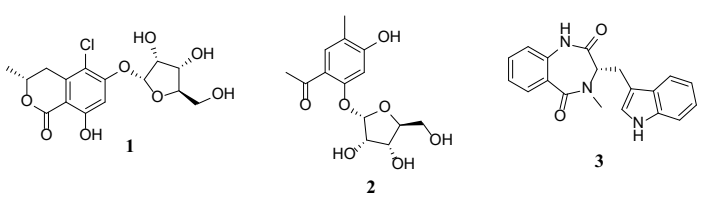
Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

From the rice-based culture of *Malbranchea flavorosea* three new compounds, namely flavoroseoside B (**1**), 4-hydroxy-2-*O*-α-ribofuranosyl-5-methylacetophenone (**2**), and (*S*)-3,4-dihydro-3-(1*H*-indol-3-ylmethyl)-4-methyl-1*H*-1,4-benzodiazepine-2,5-dione (**3**); along with three known compounds, rosigenin, massarilactone B, and riboxylarinol B were obtained. The structures were determined by spectroscopic methods. Compound **3** and its synthetic analog *(R)*-3,4-dihydro-3-(1*H*-indol-3-ylmethyl)-1-methyl-1*H*-1,4-benzodiazepine-2,5-dione inhibited the activity of *Ruminococus obeum* α-glucosidase enzyme.



1 2 3

P-173

GENOME MINING GUIDED DISCOVERY OF BIOACTIVE NATURAL PRODUCTS FROM ENDOPHYTIC FUNGI

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State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

Filamentous fungi are prolific producers of bioactive natural products. Recent genome sequencing efforts for many fungal species have revealed significant biosynthetic potential, as represented by a large number of cryptic and diverse biosynthetic pathways. Endophytic fungi are increasingly recognized as significant underachievers in natural product biosynthesis. The high biosynthetic potential is a reflection of the complex natural ecological environment, in which small molecules play important roles in host-fungus interactions. These complex ecological interactions, which are difficult to replicate in the laboratory, also result in most gene clusters being silent in axenic cultures.

Epigenetic modification, overexpression of transcription factor, heterologous expression of gene cluster in *Aspergillus nidulans* as well as combinational strategies were used to active and overexpress the cryptic biosynthetic genes, which provide a rapid approach to mine the chemical diversity of endophytic fungi.

P-174

PHENOLIC CONSTITUENTS FROM THE TWIGS OF *BETULA SCHMIDTII* REGEL AND THEIR BIOLOGICAL ACTIVITY

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As a part of our ongoing search for bioactive constituents from Korean medicinal sources, *Betula schmidtii* Regel (Betulaceae) twigs were studied. MeOH extract of *B. schmidtii* twigs was subjected to solvent-partitioning to yield *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH soluble fractions and repeated column chromatographic purification of the EtOAc-soluble and *n*-BuOH-soluble fractions afforded four new phenolic glycosides (**1**-**4**), a new lignan glycoside (**19**), a new isoflavonoid glycoside (**27**), along with thirty-two known ones. The chemical structures of the new compounds (**1**-**4, 19,** and **27**) were elucidated by spectroscopic data analysis including 1D and 2D NMR (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC, and NOESY) and chemical methods. All the isolated compounds (**1**-**38**) were evaluated for their anti-inflammatory activity through the measurement of nitric oxide (NO) production levels in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cell line, for their neuroprotective effects via induction of nerve growth factor (NGF) in C6 glioma cells, and for their cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) were evaluated.

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SECURINEGA ALKALOIDS OF *SECURINEGA SUFFRUTICOSA* AND THEIR ANTIPROLIFERATIVE ACTIVITIES

Kyoung Jin Park[1], Joon Min Cha[1], Tae Hyun Lee[1], Dong Hyun Kim[1], Sang Un Choi[2], Kang Ro Lee[1]
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Securinega suffruticosa (Pall.) Rehder (Euphorbiaceae) is widely distributed in Korea, China, and Japan. The twigs of this plant have been used in Korean traditional medicine to treat infantile paralysis, rheumatic disease, and blood circulation disorder and its roots have been used to treat quadriplegia and acute ear infection. Previous phytochemical research on *S. suffruticosa* repored alkaloid, flavonoid, tannin, and diterpenoid. As continuing search for bioactive secondary metabolites from Korean medicinal plants, the MeOH extract from the twigs of *S. suffruticosa* successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Each fraction was assessed for their cytotoxic activities against four cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the SRB bioassay and the active fraction was shown to be the $CHCl_3$ extract. The $CHCl_3$-soluble fraction was separated using repeated column chromatography to afford four new alkaloids (**1**-**4**) and five known compounds (**5**-**9**). The chemical structures of the new compounds (**1**-**4**) were established using diverse NMR techniques (^1H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY) and HRMS data analysis. Cytotoxic study for the all isolates (**1–9**) is in progress.

P-176

CYTOTOXIC CONSTITUENTS FROM *EUPHORBIA HUMIFUSA*

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Euphorbia humifusa Willd. (Euphorbiaceae), an annual plant, is distributed widely in Korea, Japan and China. *E. humifusa* has been used to treat jaundice, dysentery, enteritis, poisonous snake bites, and hepatitis caused by virus. A previous phytochemical investigation of *E. humifusa* reported pyrrolidinonoids, sesquiterpenoids, triterpenoids, and phenolic compounds. As a part of our continuing search for bioactive constituents from Korean medicinal plants, we investigated the MeOH extract of the whole plants of *E. humifusa*. The MeOH extract of *E. humifusa* were subjected to repeated column chromatography and semi-preparative HPLC to give a new megastigmane glycoside (**11**), along with twenty one known compounds (**1** – **10**, and **12** – **22**). The structures of isolated compounds were determined by spectroscopic methods, including 1D and 2D NMR analyses and chemical reaction.The cytotoxicities of the isolated compounds (**1** – **22**) against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines were evaluated using the SRB assay *in vitro*. Compounds **3**, **7**, and **10** showed cytotoxic activity against the four human tumor cell lines with IC_{50} values in the range of 1.21 – 27.19 μM.

P-177

A NEW PHENOLIC GLYCOSIDE FROM THE TWIGS OF *QUERCUS ACUTISSIMA* CARUTH

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In the course of our continuing search for biologically active compounds from Korean medicinal plants, we investigated the twigs of *Q. acutissima*. *Quercus acutissima* Caruth (Fagaceae), known as Saw tooth oak, is widely distributed in Korea, Japan, and China. The edible fruits of *Q. acutissima* have been used in Korean traditional medicine to treat atopic dermatitis. Previous investigation of *Q.acutissima* reported several triterpenoids, flavonoids, lignans, tannins, and phenolic compounds with antioxidant, and antiviral activities. The MeOH extract of the twigs of *Q. acutissima* was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc and *n*-BuOH-soluble fractions led to the isolation of a new diarylheptanoid glycoside, named acuttissioside A (**1**), together with thirteen known phenolic glycoside derivatives (**2**-**14**). The structure of new compound (**1**) was elucidated by spectroscopic methods including 1D and 2D NMR (^1H and ^{13}C NMR, COSY, HSQC, and HMBC). The isolated compounds (**1**-**14**) were tested for their anti-inflammatory activity measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine microbial cell line.

P-178

PHENOLIC COMPOUNDS FROM THE TWIGS OF ALEURITES FORDII AND THEIR CYTOTOXIC ACTIVITY

Joon Min Cha, Kyoung Jin Park, Tae Hyun Lee, Dong Hyun Kim, Seung Rak Lee, Jae Sik Yu, Seulah Lee, Hae Min So, Sil Kim, Kang Ro Lee
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Aleurites fordii Hemsl. (=*Vernicia fordii* Hemsl., Euphorbiaceae), known for tung oil tree, is widely distributed throughout northeast asia. The fruits, leaves and roots of this tree have been used in Korean traditional medicine for treating sore throat, respiratory illness, constipation and dieresis. In the course of our continuing search for biologically active compounds from Korean medicinal sources, we investigated the MeOH extract of the twigs of *A. fordii*. From the MeOH extract, three new phenolic compounds (**1**, **10**, and **11**), including fourteen known phenolic compounds (**2**-**9**, and **12**-**17**) were isolated. The chemical structures of new compounds (**1**, **10**, and **11**) were determined through NMR (^1H and ^{13}C NMR, ^1H-^1H COSY, HMQC, HMBC, and NOESY), HRMS, and CD data. Cytotoxic activities of the isolated compounds (**1**-**17**) against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines were evaluated using the SRB assay. Compound **11** exhibited weak cytotoxic activity against A549 cell line (IC_{50} value = 13.76 μM).

P-179

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

Ermias Mekuria Addo,[1] Joshua M. Henkin,[2] Austin A. Czarnecki,[2] Tran Ngoc Ninh,[3] Yulin Ren,[1] Joanna E. Burdette,[2] Djaja D. Soejarto,[2,4] A. Douglas Kinghorn[1]

[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210. [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612. [3]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. [4]Science and Education, Field Museum of Natural History, Chicago, IL 60605.

The cardenolides periplogenin (**1**) and alloperiplogenin (**2**) and their glycosides periplocymarin (**3**), periplogen *β*-D-glucoside (**4**), periplocin (**5**), and corchorusoside C (**6**) were isolated as cytotoxic constituents of the stems of *Streptocaulon griffithii* Hook. f. (Apocynaceae), collected in Vietnam. Preliminary SAR observations based on the activity of **1**, **3**, **5**, and **6** against four cancer cell lines (MDA-MB-435, OVCAR3, HT-29, and MDA-MB-231) can be summarized as: (1) the glycosides were ~20–30 more potent than the aglycones (cardenolides), (2) selectivity may be reflected as to the extent of glycosylation for most of the glycosides, and (3) glycosides with a methoxylated 2,6-dideoxy sugar unit were ~2–3 times more potent than their non-methoxylated congeners.

P-180

NEUROTHERAPEUTIC EFFECTS OF *GINKGO BILOBA* EXTRACT AND GINKGOLIDE B

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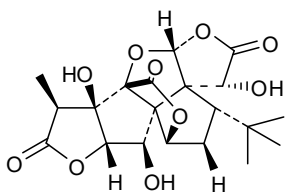
In addition to its documented use in traditional Chinese medicine, *Ginkgo biloba* is used in modern herbal medicine. Its extract (GBE) is indicated in the treatment of many diseases such as Alzheimer, age-associated dementia, and intermittent claudication. GBE, terpene trilactones-enriched extract (TTEE) and ginkgolide B (GB) were prepared *via* several chromatographic processes and the identity of GB was confirmed based on its spectral data. The potential of GBE, TTEE and GB to recover sciatic nerve crush injury in rat models was investigated. Six groups of Wistar male rats; naïve, sham, crush, crush + GBE (50 mg/kg), crush + TTEE (50 mg/kg) and crush + GB (10 mg/kg) were prepared. The test extracts/compound were diluted with normal saline and administered intraperitoneally, one hour following the injury and daily for 14 days. Functional and sensory neurobehavioral tests, morphological, histological and immunohistochemistry analyses were performed at weeks 3 and 6. Results revealed that GBE, TTEE and GB enhance functional and sensory behavioral parameters and protected the histological and the ultrastructural elements in the sciatic nerve. Additionally, all treatments prevented spinal cord neurons from further deterioration following sciatic nerve injury. Results demonstrated that GB has the most significant potential effects among other treatments.



Ginkgolide B

P-181

ETHNO-MEDICINAL AND PHARMACOGNOSICAL STUDY OF THE CHAKMA COMMUNITY OF JURACHARI UPAZILLA OF RANGAMATI DISTRICT, BANGLADESH

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Investigation and documentation of the status of medicinal plants and associated knowledge was conducted of the Chakma community in Juraicchari upzilla of Rangamati district, Bangladesh. The information has been documented by interviewing traditional herbalists, various elderly man and women following different recommended ethnobotanical methods. A total of 144 vascular plants have been recorded which have been used to treat 80 diseases/illness. These species belonging to 127 genera under 62 families. The most used family in terms of number of species is Asteraceae (6.25%). Leaves (35.57%) have been found to be used frequently in herbal formularies and the most frequently treated disease/illness is cough, dysentery, dermal disease, diarrohea, jaundice and fever.

To ascertain the level of agreement among the informants of the Chakma community regarding the use of plants to treat certain disease categories, informant consensus factors (FIC) values were determined. In the treatment of neurological disorder, the highest FIC value (0.67) was observed, with 17 use-reports for 3 plant species. The fidelity level of *Trevesia palmata* (Roxb. *ex* Lindl.)Vis. among the three plants is 54.17%. On the basis of FIC value and Fidelity level it is quite clear that the plant *Trevesia palmata* has a good medicinal background and selected for ethnopharmacognosical study.

In the present study, alkaloids were assessed qualitatively, indicates the presence of alkaloid in the fresh sample of *Trevesia palmata* leaf. The *in vitro* thrombolytic activity study revealed that the percentage of weight loss of clot after application of *Trevesia palmata* leaf extract solution was taken as the functional indication of thrombolytic activity. The herbal preparation may be incorporated as a thrombolytic agent for the improvement of the patient suffering from a therothrombotic diseases. Fresh leaves is also used to investigate the sedative/anxiolytic effect using some recommended behavioral models, such as Tail suspension test (TST), Forced swimming test (FST), Elevated plus maze test (EPM), Hole cross test (HCT) and Open field test (OF). Observation of all implied methods and protocols help us to be enthusiastic enough to explain and anticipate that *Trevesia palmata* possesses significant level of neurological properties. Therefore, after analyzing the resultant findings it can be confidently claim that, *Trevesia palmata* has satisfactory levels of anxiolytic and sedative capacity along with depressive domination. However the causative active ingredients isolation and modification probably can help with eradication of side effect like depression.

P-182

TRITERPENOIDAL SAPONINS ISOLATED FROM *CAMELLIA JAPONICA* ROOTS WITH NRF2-MEDIATED ANTIOXIDANT ACTIVATION

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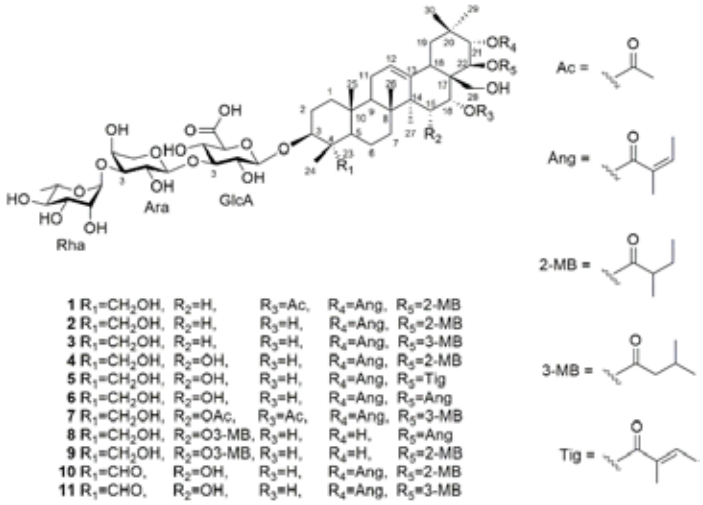
We isolated new triterpenoidal saponins (**1**-**11**) from the *Camellia japonica* roots and investigated antioxidant response element (ARE) luciferase activities of compound **1**-**11** against human keratinocyte (HaCaT-ARE cells). Compound **1**-**11** increased ARE luciferase activities via nuclear factor erythroid 2-related factor 2 (Nrf2) accumulation in the nucleus. The ARE-luciferase activities of compounds **4**, **5**, **6**, **8** and **11** significantly increased more than two folds at 25 μM.



1 R_1=CH_2OH, R_2=H, R_3=Ac, R_4=Ang, R_5=2-MB
2 R_1=CH_2OH, R_2=H, R_3=H, R_4=Ang, R_5=2-MB
3 R_1=CH_2OH, R_2=H, R_3=H, R_4=Ang, R_5=3-MB
4 R_1=CH_2OH, R_2=OH, R_3=H, R_4=Ang, R_5=2-MB
5 R_1=CH_2OH, R_2=OH, R_3=H, R_4=Ang, R_5=Tig
6 R_1=CH_2OH, R_2=OH, R_3=H, R_4=Ang, R_5=Ang
7 R_1=CH_2OH, R_2=OAc, R_3=Ac, R_4=Ang, R_5=3-MB
8 R_1=CH_2OH, R_2=O3-MB, R_3=H, R_4=H, R_5=Ang
9 R_1=CH_2OH, R_2=O3-MB, R_3=H, R_4=H, R_5=2-MB
10 R_1=CHO, R_2=OH, R_3=H, R_4=Ang, R_5=2-MB
11 R_1=CHO, R_2=OH, R_3=H, R_4=Ang, R_5=3-MB

P-183

DRUG DISCOVERY FROM NATURAL AND STRUCTURALLY MODIFIED BUFADIENOLIDES

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Cardiotonic steroids (CTS), e.g. digoxin and ouabain, are clinically important drugs for the treatment of heart failure owing to their potent inhibition of cardiac Na^+,K^+-ATPase (NKA). Bufadienolides constitute one of the two major classes of CTS, but no drug was discovered from pure bufadienolides so far. We have performed systematic studies on bufadienolides. A number of novel bufadienolides were isolated from plants and animals, some of which displayed potent stereo-selective inhibition against NKA (*J. Nat. Prod*. **2017**, 80, 1182-1186; *Fitoterapia* **2015**; 105: 7-15 and *J. Nat. Prod.* **2013**, 76, 1842–1847). Structural modifications using both chemical and biosynthetic methods were performed to further improve the activities (*Org Lett*. **2018**, 20(3):534-537 and *RSC Adv.*, **2018**, 8, 5071–5078). Finally, the inhibition mechanism was investigated by molecular docking and solid state NMR (*Sci. Rep*. **2016**; 6:29155).

P-184

NEW SECONDARY METABOLITES ISOLATED FROM EPIGENETICALLY MODIFIED FUNGI FOR ANTI-INFECTIVE DRUG DISCOVERY

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Whether from tropical or lower latitude ecosystems, the chemical survival mechanisms used by fungi offer a potential axis for research to find new drugs. Moreover, epigenetic regulation is a key mechanism to orchestrate the expression or suppression of gene activity; 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. In our lab, two kinds of epigenetic modifiers are used: sodium butyrate as a Histone Deacytalase (HDAC) inhibitor, and 5-azacytidine as a DNA Methyl Transferase (DNMT) inhibitor. Screening studies in the lab have shown that fungi from Floridian mangroves and from the coastal portions of the Tapachula region of Mexico, cultured on rice in the presence of DNMT epigenetic regulators, exhibited activity against microbial agents such as ESKAPE, leishmaniasis and *Naegleria fowleri* pathogens. Through a bioassay-mass spectroscopy-guided sequence compiling extractions, partitions, and chromatographic methods, the separation of a crude extract material has shown potential new chemistry. Two different DNMT treated fungi were respectively active in leishmaniasis and ESKAPE screenings. After an ethyl acetate:water partition, the crude extracts were fractionated on normal phase MPLC. Then, stages of normal phase and reverse phase HPLC purifications have already brought forward known and new bioactive compounds and the work is still ongoing and fruitful. With the pool of isolated compounds, one and two-dimensional nuclear magnetic resonance, mass spectroscopy, and X-ray crystallography are providing the data necessary to elucidate the structures and characterize the stereochemistry involved. Further biological testing is being performed to extend the scope of drug discovery potential.

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SECONDARY METABOLITE CAPACITY OF *PSEUDONOCARDIA* SYMBIONTS OF THE FUNGUS-GROWING ANT *TRACHYMYRMEX SEPTONTRIONALIS*

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Trachymyrmex septentrionalis is a fungus-growing ant found primarily along the eastern coast of the United States. Like other fungus-growing ants, *T. septentrionalis* has adaptively incorporated *Pseudonocardia* bacteria onto its propleural plate, creating a symbiotic network in which bacterial secondary metabolites protect the fungus garden from pathogens, while the fungus garden provides a food source for the ants. In the current study, comparative metabolomic analyses of extracts of *Pseudonocardia* collected from geographically distinct colonies from New York to Florida were performed to investigate evolutionary relationships along this geographic gradient. Colonies from North Carolina, Georgia, and Florida had the largest diversity of secondary metabolites when comparing mass spectrometric (MS) data. Alongside this MS networking, genomic analysis of biosynthetic capacity for secondary metabolite production revealed that *Pseudonocardia* from Florida and North Carolina have the highest degree of similarity in their capacity for and production of secondary metabolites. These studies will be combined with antimicrobial and antiproliferative assays to direct isolation and identification of bioactive metabolites from this unique host-microbe system.

P-186

UNCOVERING NEW FUNGAL NATURAL PRODUCTS AND THEIR BIOSYNTHETIC PATHWAYS USING FAC-MS TECHNOLOGY

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[1]Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, [2]Intact Genomics, Inc., St Louis, MO 63132, [3]Department of Medical Microbiology and Immunology and Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, [4]Proteomics Center of Excellence, Northwestern University, Evanston, IL 60208, [5]Department of Chemistry, Northwestern University, Evanston, IL 60208.

Filamentous fungi are prolific producers of secondary metabolites with drug-like properties, and their genome sequences have revealed an untapped wealth of potential therapeutic leads. To better access these secondary metabolites and characterize their biosynthetic gene clusters, we have developed a new platform for large-scale screening and heterologous expression of intact gene clusters that uses fungal artificial chromosomes and metabo-

lomic scoring (FAC-MS). We have utilized the FAC-MS platform to identify the biosynthetic machinery responsible for production of acu-dioxomorpholine, a metabolite produced by the fungus, *Aspergilllus aculeatus*. Using stable isotope labeling, MS, and bioinformatics analyses, we determine that the diketomorpholine scaffold is assembled from a tryptophan and phenyllactate by a highly unusual condensation domain. Acu-dioxomorpholine is highly related to orphan inhibitors of P-glycoprotein targets in multidrug-resistant cancers and identification of the biosynthetic pathway for this compound class enables genome mining for additional derivatives.

P-187

IDENTIFICATION OF A NOVEL DEPSIPEPTIDE FROM SHEEP FECES-DERIVED FUNGUS TALAROMYCES SP. CMB-NF091.

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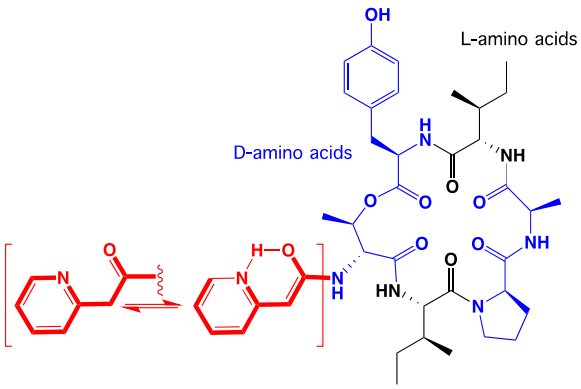
[1]*Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, QLD 4072, Australia.*

To fully explore the secondary metabolite potential of a sheep feces-derived fungus, *Talaromyces* sp. CMB-NF091, the strain was cultivated in a microbioreactor (MATRIX) array of ×33 different conditions, and solvent extracts subjected to chemical analysis. Optimised conditions were used to produce a hexa-depsipeptide, talaropyrine (shown) bearing a mixture of L (black) and D (blue) amino acid residues as determined by C_3 Marfey's analysis, and an unprecedented pyridinyl-acetamide moiety (red). 2D NMR and QTOF-MS/MS analysis supported the assignment of the amino acids sequences. Curiously talaropyrine appear to exist as different, equilibrating tautomers about the pyridinyl-acetamide moiety, prompts the synthesis of model compounds to better understand the phenomena.



P-188

FREE RADICAL SCAVENGING ABILITY OF THE SECONDARY METABOLITES PRODUCED IN THE SCLEROTIA AND MYCELIUM OF *INONOTUS OBLIQUUS*

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The medicinal fungus *Inonotus obliquus*, commonly called Chaga, has been used in traditional Chinese medicine and Siberian folk medicine for centuries. In the current study, Chaga specimens were collected on three *Betulacae* tree species, to evaluate the substrate effect on secondary metabolite production. This perennial fungus contains an outer protective layer, called the sclerotia which is rich in polysaccharides as well as an inner portion which is abundant in triterpenoid molecules. The water soluble, polysaccharide portion has shown antioxidative activity in DPPH models, which is consistent with the common medicinal tea ingestion. However, certain triterpenoid molecules have shown to be good free radical scavengers and could augment the scavenging ability from oxidative damage. This would require extraction of Chaga biomass in a more lipophilic solvent such as alcohol, and ingestion in tincture form. Herein a combinatorial approach using the various molecules of Chaga will be investigated for maximum antioxidant activity. Furthermore, the permeability of these molecules will be explored using the parallel artificial membrane permeability assay (PAMPA) which uses membrane simulators in the human biological system such as the gut, the blood brain barrier, and the skin for uses in pharmaceuticals, nutraceuticals, and skincare.

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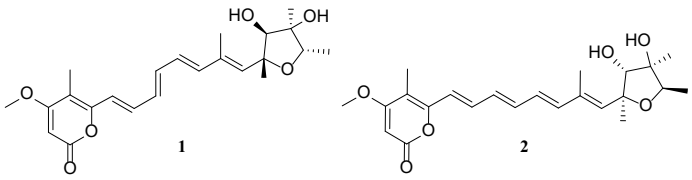
BIOASSAY GUIDED ISOLATION OF SECONDARY METABOLITES FROM *PENICILLIUM AURANTIACOBRUNNEUM*, A FUNGAL ASSOCIATE OF THE LICHEN *NIEBLA HOMALEA*

Choon Yong Tan[1], *Fengrui Wang*[1], *Kristie D. Goughenour*[2], *Chad Rappleye*[2], *Harinantenaina L. Rakotondraibe*[1]
[1]*Division of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210,* [2]*Department of Microbiology, Center for Microbial Interface Biology, The Ohio State University, Columbus, OH 43210*

Lichens are groups of organisms comprising photobionts (microalgae and cyanobacteria) and mycobionts (fungi) living symbiotically and have shown a myriad of biological activities attributable to their secondary metabolites. As such, a preliminary cytotoxic screening on an extract of *Penicillium aurantiacobrunneum*, a fungal associate of an endemic lichen of the U.S., *Niebla homalea* (Ach.) Rundel & Bowler, has elicited potent cytotoxicities against human breast cancer (MCF-7, IC_{50} = 1.8 mg/mL) and human ovarian cancer (A2780, IC_{50} = 2.2 mg/mL) cells. Bioassay-guided isolation on this extract resulted in an identification of **1** with structure closely related to the mycotoxin, citreoviridin (**2**). The structure elucidation including the stereochemistry of **1** and the isolation of other cytotoxic constituents produced by the fungus in various media, such as rice and barley, will be presented.



P-190

PERSEPHACIN, A NOVEL FUNGUS-DERIVED DEPSIPEPTIDE EXHIBITING BROAD-SPECTRUM ANTIFUNGAL ACTIVITY

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[1]*Natural Products Discovery Group, Institute for Natural Products Applications and Research Technologies, University of Oklahoma.*[2]*Department of Chemistry and Biochemistry, University of Oklahoma.*

Persephacin, a unique cyclic depsipeptide bearing a novel hydrophobic amino acid residue, was discovered from an endophytic fungal isolate, Elsinoe sp. The bond-line structure of persephacin was ascertained through an analysis of its NMR and MS data, whereas its absolute configuration was determined by a combination of the Marfey's analysis, acidic hydrolysis with amino acid modifications, and comparisons of experimental versus computational spectroscopic data (^{13}C NMR chemical shifts, ECD spectra, and specific rotation values). Persephacin exhibited broad-spectrum antifungal and fungicidal activities against a wide range of clinically important and drug-resistant fungal pathogens (yeasts and filamentous fungi). Details

pertaining to the structure analysis and bioactivity of persephacin are presented.

P-191

THE FRAGRANCE OF FUNGI: SPME-ASSISTED GC/MS HEADSPACE ANALYSIS OF VOLATILE METABOLITES

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Fungi produce a plethora of secondary metabolites, some of which have become drugs while others are toxins. They also produce volatiles, which evaporate easily under certain pressures and temperatures and are often lost through conventional extraction methods. The use of an alternative extraction method would allow for the elucidation of the missing volatile metabolites, and more importantly, further insight into the chemical ecology of a fungus. Environmental pressures can influence the development of conventional secondary metabolites as well as volatile compounds. For headspace analysis, a solid phase microextraction is coupled to a GC/MS system, thereby allowing for real time collection of metabolites produced by a fungus. *Penicillium restrictum* unambiguously produced over 25 volatiles over the course of extraction. Some of these are known to cause harm to humans, while others are used in perfumes. Further analysis of the *P. restrictum* will allow for complete description of its volatile metabolome, while the volatile metabolites of other fungi can subsequently be elucidated.

P-192

ISOLATION AND STRUCTURE DETERMINATION OF ANTIVIRAL NATURAL PRODUCTS FROM FUNGI

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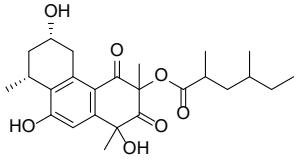
We have focused on metabolites of fungi isolated from sand, seaweed, mosses, and plants to isolate novel antiviral natural products. In this study, we report the isolation and structural elucidation of anti-hepatitis B virus (HBV) compound, vanitaracin A, and anti-hepatitis C virus (HCV) compound, peniciherquamide C. The chemical structures of these compounds were determined from spectroscopic data (1D/2D NMR, MS and IR).



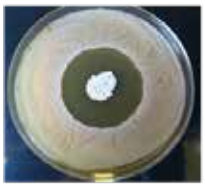
Vanitaracin A

P-193

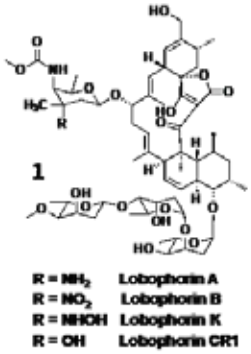
CHEMICAL-ECOLOGICAL INVESTIGATION OF ACTINOBACTERIA ASSOCIATED WITH STINGLESS BEE *MELIPONA SCUTELLARIS*

Diego Rodríguez-Hernández[1] and Mônica T. Pupo[1]
[1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil.

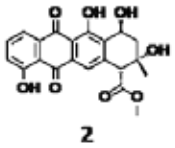
Social insects are frequently associated with symbiotic microorganisms that produce antimicrobial compounds to protect their colonies against pathogens. As part of an ongoing ICBG-Brazil project, we have studied the ability of bacterial symbionts associated with stingless bees and fungus growing ants to suppress pathogenic microbes. Six actinobacteria strains associated with the stingless bee *Melipona scutellaris* were isolated from nurse and foraging bees. The strains ICBG1323 and ICBG1321 were identified as *Streptomyces pactum* and *Micromonospora tulbaghiae*, respectively, using 16S rRNA sequencing. Both exhibited pronounced inhibitory activity against *Paenibacillus larvae*, the causative agent of American foulbrood. Strains were cultured in ISP-2 liquid medium and bioassay-guided HPLC fractionation, led to the isolation of some compounds. NMR and MS data allowed the identification of spirotetronate (**1**) and anthracycline (**2**) families of metabolites, known as potent antibiotics. The ecological role for this class of metabolites is being investigated.



S. pactum against *P. larvae*



M. tulbaghiae against *P. larvae*



P-194

BIOLOGICALLY ACTIVE COMPOUNDS FROM THE ENDOPHYTIC FUNGUS *PLECTANIA MILLERI* NFL1, ISOLATED FROM *TAXUS FUANA*

Hira Mehboob Mirza[1,2], Ulyana Muñoz Acuña[1], Karl A. Werbovetz[2], Safia Ahmed[3], Masoom Yasinzai[4], Liva Rakotondraibe[2], Esperanza J. Carcache de Blanco[1,2]
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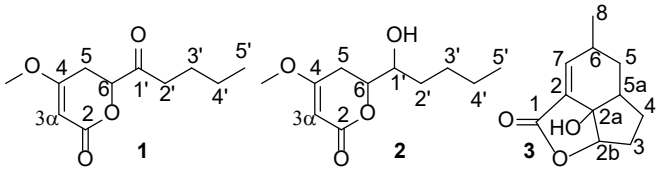
Microbial natural products, particularly endophytes, have recently become primary targets in drug screening programs. Northern areas of Pakistan are considered biodiversity hotspots of medicinal plants with ethno-pharmacological importance (e.g *Taxus fuana*). In the present study, crude extracts obtained after solid state fermentation of endophytic fungus *Plectania milleri* NFL1 from *Taxus fuana* have been screened in a panel of bioassays.

Pestalotin (**1**), its analogue (**2**) and galiellalactone (**3**) were isolated from the ethyl acetate extract. Galiellalactone showed growth inhibition against the PC-3 prostate cancer cell line and promastigotes of *Leishmania* sp. Structure identification and elucidation was completed using spectroscopy techniques including 1D-NMR ^{1}H, ^{13}C, and DEPT, and 2D-NMR such as HMBC, HSQC, and COSY. *In-silico* studies will also be performed to optimize the activity of the active compound (s) and will serve as a foundation in the search for effective drugs against cancer and leishmaniasis.



P-195

WIDESPREAD DISTRIBUTION OF A COMMON ANTIFUNGAL AGENT AMONG BRAZILIAN FUNGUS-GROWING ANTS' BACTERIAL SYMBIONT

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Attine ants cultivate a fungus with which they maintain a permanent and obligatory mutualistic association. To help prevent nest infection by parasites, especially by the specialized fungal pathogen *Escovopsis* sp., the ants have an association with symbiotic actinobacteria of the genus *Pseudonocardia* that produce protective secondary metabolites. Investigations of ants in a previously unstudied region, colonies from around Brazil including the Amazon Forest, Atlantic Forest and Brazilian Cerrado (Savanna), led to an interesting ecological finding. Metabolomic analysis [HR-LCMS, MS/MS and Global Natural Products Social Molecular Networking (GNPS)] on over 100 *Pseudonocardia* strains representing five distinct collection sites revealed that a significant number of strains produced cahuitamycins and/or oxachelins, which are structurally related. Interestingly, in preliminary assays, many of these metabolites selectively inhibited the parasite over the mutualistic fungus. Based on genomic analysis, it appears that the BGC was likely acquired by horizontal gene transfer as other cahuitamycin producers are distantly related strains. The geographically widespread production of oxachelins/cahuitamycins by distantly related *Pseudonocardia* sp. and the selective inhibition of the pathogenic fungus suggest that these metabolites have an important ecological role in protecting the nest from infection.

P-196

UNUSUAL 19-NOR-PIMARANES FROM *ICACINA TRICHANTHA*

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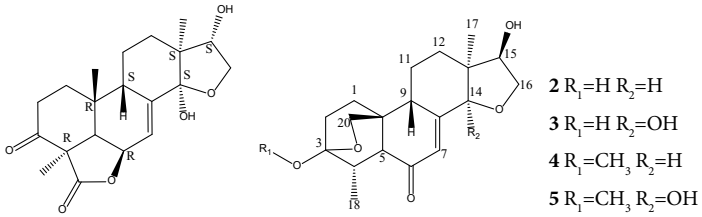
The Niger Delta region of West Africa possesses a rich biodiversity of plants with various ethnobotanical uses, including *Icacina trichantha* Oliv. (Icacinaceae), a flowering shrub indigenous to Nigeria with a long history of traditional medicinal use that continues to this day. Our group has conducted the first phytochemical analysis of this species, leading to the identification of over 20 novel compounds. The current project presented here has resulted in the isolation of five new pimarane derivatives from the EtOAc-soluble fraction (**1**-**5**), including four rare 19-*nor*-pimaranes (**2**-**5**).



P-197

LIGNANS FROM *FORSYTHIA VIRIDISSIMA* ROOTS AND THEIR CYTOTOXIC EFFECTS IN HCT-116 COLON CANCER CELLS

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Sixteen compounds, including eight dilignans (**1**–**8**) and eight lignan glycosides (**9**–**16**), were isolated from the roots of *Forsythia viridissima*. Compounds **1**-**6** and **16**, which were newly isolated in nature, were unambiguously determined by 1D and 2D NMR analyses and chemical methods. Compounds **1**–**8** were dimers of dibenzylbutyrolactone lignan analogues. The cytotoxic activities of the isolates were examined against human colon cancer HCT-116 cells. Compounds **8** and **10** showed cytotoxic activities with IC_{50} values of 9.65 and 5.67 μM, respectively.

P-198

ANTIMICROBIAL ACTIVITIES OF TREGAYSORNMAS FORMULA

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Tregaysornmas formula, including *Jatropha multifida* L., *Nelumbo nucifera* Gaertn. and *Aegle marmelos* L., was discovered in Thai Pharmacy scripture. This research aimed to investigate antimicrobial activities of the 95% ethanol and water extracts of Tregaysornmas formula using Disc diffusion method, Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal/Fungicidal Concentration (MBC/MFC) assay. The results showed that 95%EtOH and water extracts of Tregaysornmas formula exhibited antibacterial activity against *Staphylococcus aureus* with inhibition zone of 10 and 6.5 mm., respectively. The 95%EtOH and water extracts showed MIC value of 0.625 and 1.25 mg/ml, respectively against *S. aureus* and MIC value of 5 and 1.25 mg/ml, respectively against *Escherichia coli*. However, both were not show antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albicans*. We concluded that Tregaysornmas formula showed antibacterial activities and it was related with the ethnomedical use as maintaining body balance of patients in Thai traditional medicine.

P-199

DISCOVERY OF NOVEL ANTIMALARIAL AGENTS FROM PLANTS

David G. I. Kingston[1], Yongle Du[1], Rolly Fuentes[1], Namki Cho[1], Ana Lisa Valenciano[2], Maria B. Cassera[2], Jason Clement[3], and Michael Goetz[3]
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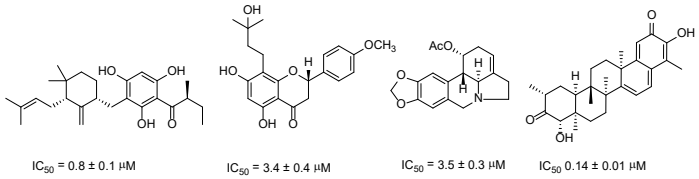
Malaria is one of the world's most devastating diseases, with over 3.2 billion people at risk for contracting it, and an estimated 216 million malaria cases and over 440,000 deaths in 2016. The potent antimalarial drugs quinine and artemisinin are natural products, and natural products continue to make key contributions of new antimalarial agents. Recent work from our joint groups will be presented, leading to the isolation and structure elucidation of new antiplasmodial compounds as well as known compounds with previously unreported antiplasmodial activity.



P-200

ETHNOBOTANICAL AND PHYTOCHEMICAL STUDIES ON MAIANTHEMUM ATROPURPUREUM, A TIBETAN FOOD PLANT IN NORTHWEST YUNNAN

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Maianthemum atropurpureum (Asparagaceae), *nibai* in Tibetan or *zhu-ye-cai* in local Chinese, is a wild vegetable commonly consumed by the Tibetan people in Northwest Yunnan, China. An ethnobotanical investigation had been carried out to understand its significance in traditional Tibetan diet. The phytochemical analysis involved multiple chromatographic and spectral methods including LC-TOF-MS analysis while the nutrient content for *nibai* was determined by the China National Standards (GB) methods. The phytochemical content of *nibai* was examined by conventional isolation strategies, as well as HR-ESI-TOF-MS to detect and identify 16 compounds including nine steroid saponins and seven flavonoids. Dried *nibai* is a rich source of protein (24.6%), with 18 of the 20 common amino acids. The amino acid content of *nibai* can reach up to 17.9 g/100 g, with the essential amino acids as major contributors, corresponding to 42.3 % of the total amino acids. *Nibai* contains mineral elements, dietary fiber, vitamins, β-carotene, carbohydrates, and lipids. These results help to confirm that the local Tibetan practice of consuming *Maianthemum atropurpureum* is warranted due to its high levels of vitamins, minerals, essential amino-acids, and other phytochemicals. *Nibai* may be further developed in Tibet and beyond as a health food and/or dietary supplement product.

P-201

EFFECTS OF ASSOCIATION OF *PTERODON PUBESCENS* AND *CORDIA VERBENACEA* ON CELL MIGRATION AND ANTI-INFLAMMATORY ACTIVITY IN HACAT CELLS

Rosanna Tarkany Basting[1,2], Ilza Maria de Oliveira Sousa[1], Veronika Butterweck[3], Mary Ann Foglio[1]
[1]Faculty of Pharmaceutical Sciences – FCF/ [2]Faculty of Medical Science – FCM/ State University of Campinas – Unicamp, 13083-887, Campinas – SP, Brazil, [3]Institute for Pharma Technology, School of Life Sciences, University of Applied Sciences Northwestern Switzerland - FHNW, Muttenz, 4132, Switzerland.

Pterodon pubescens (Pp) and *Cordia verbenacea* (Cv) are Brazilian folk medicinal plants, known for anti-inflammatory effects. The extracts' association demonstrated a synergistic effect on *in vivo* inflammatory models. Herein the effects on cell migration is reported for the first evaluating samples [Association (A): Pp + Cv – ratio ½ : ½] effects in TNFαstimulated cells. Time-lapse microscopy detected a decrease in cell migration of A at 3.125 and 12.5 μg/mL tested concentrations (at t= 24 h: 28.6 ± 1.3% for 3.125 μg/mL and 46.3 **± 10.7% for 12.5** μg/mL) in comparison to the untreated control (at t=24 h 43.8 ± 3.5%). For 6.25 μg/mL, the cell migration was significantly higher, at t=24 h, a gap closure of 56.2 ± 11.0% was reached. In the TNFαstimulated cells, only A (12.5 μg/mL) increased the levels of vascular endothelial growth factor (VEGF). All concentrations significantly reduced Interleukin 8 levels, demonstrating a potential anti-inflammatory effect and exerted inhibitory effects on cell migration.

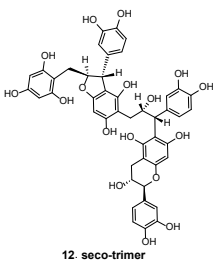
Acknowledgment: FAPESP grant #2016/23816-4; #2015/08600-2

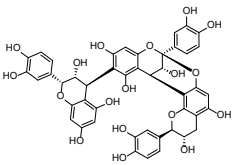
P-202

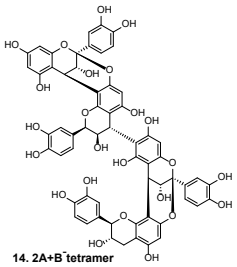
INVESTIGATION ON THE STRUCTURAL DIVERSE PROCYANIDINS FROM *PINUS MASSONIANA*

Bin Zhou[1], Joo-Won Nam[1,3], Rasika S. Phansalkar[1], Yvette Alania[2], Mariana C. dos Reis[2], James McAlpine[1], Shao-Nong Chen[1], Ana K. Bedran-Russo[2] and Guido F. Pauli[1]
[1]Department of Medicinal Chemistry & Pharmacognosy, College of Pharmacy; [2]Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, Illinois 60612, USA. [3]College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea

Our previous studies of proanthocyanidin (PAC) action on dentin showed that the affinity of PACs to type-I collagen is directly correlated with their exact structural features and degree of polymerization. In order to explore the SARs of PACs, scaled-up phytochemical isolation from the bark of *Pinus massoniana* was carried out. So far, 14 PACs have been isolated from enriched active fractions by means of previously developed countercurrent separation (CCS) protocols and identified by NMR and LC-MS: two residual monomers, *epi*-catechin and catechin (**1** and **2**); nine dimers including three seco-dimers (**3**–**5**); five A-type (**6**–**10**) and one 4,6-linked B-type dimer (**11**); two trimers (**12** and **13**); one tetramer (**14**). By including biosynthetic considerations, the new seco-trimer, **12**, is likely formed from EC(C) by oxidative ring opening, nucleophilic addition, and intramolecular esterification reactions. NOE evidence for its relative configuration will be presented. Two major dentin active components (**13** and **14**, purity ≥90% by qHNMR) were successfully enriched to near-gram levels by CCS, LH-20, and C-18 columns, ensuring sufficient quantities for biological studies.


12. seco-trimer


13. A+B trimer


14. 2A+B tetramer

P-203

EFFECTS OF TOTAL FLAVONOIDS OF ASTRAGALUS SEEDS ON TG TTRANSPORT PATHWAY IN OVARIECTOMIZED HYPERLIPIDEMIA RATS

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Semen Astragali Complanati is a traditional Chinese medicine which could warm kidney-yang, commonly used for hyperlipermia. Previous studies indicate that the Total Flavonoids of Semen Astragali Complanati (TFA) could reduce TG synthesis of ovariectomized rats fed high-fat diet. This study aimed to explore the effects of TFA on TG transport pathways. Female SD rats were randomly divided into blank group, model group, positive control group, and TFA 28.5, 57, 114mg/kg groups. Furthermore, total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in serum and liver were detected. Tests of very low density lipoprotein (VLDL) and apolipoprotein B100 (ApoB100) in serum, and protein expression of MTP in liver were applied. Results showed that TFA could significantly reduce lipids in blood and liver, increase the levels of VLDL and ApoB100 in serum and upregulate protein expression of MTP. Thereby, TG transport from liver to blood was increased while TG deposition in liver was reduced. However, blood lipid was not up but down. It might be related to fat hydrolysis metabolism, which is needed to further research.

P-204

PREPARATION OF RED CLOVER (*TRIFOLIUM PRATENSE*) ISOFLAVONE KNOCKOUT AND ENRICHED DESIGNER EXTRACTS FOR *IN VIVO* STUDIES

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[1]UIC/NIH Center for Botanical Dietary Supplements Research, Dept. of Med. Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA. [2]Dominican University, Physical Science Dept. River Forest, IL 60305, USA.

Focusing on isoflavone-rich red clover extract (RCE), this study expands on the DESIGNER approach to Deplete and Enrich Select Ingredients to Generate Normalized Extract Resources using countercurrent separation (CCS). Knockout and highly-enriched RCEs for biochanin A (**1**) and formononetin (**2**) were prepared by centrifugal partition chromatography (CPC). The biphasic solvent system *n*-hexane-ethyl acetate-methanol-water (HEMWat 5.5/4.5/5/5) yielded the target *K* values of 1.25 and 1.18 for **1** and **2**, respectively. The qHNMR characterization and quantitation of 81 CPC fractions identified 11 major isoflavonoids, including the target compounds with sufficient resolution for the preparation of knockout and highly-enriched extracts. To prepare sufficient quantities for animal studies, it was necessary to improve RCE solubility and loading capacity to 1.0 g per run by addition of DMSO. This did not disrupt the separation profile. The preparation of knockout extracts was performed with two sequential CPC separations. The first CPC separation afforded 93.8% pure **1** with 0.6% of prunetin as a residual component. The second CPC step used HEMWat 4/6/4/6 and produced high purity **2**. The isoflavone metabolome can resolved preparatively with high resolution by CPC technology large-scale DESIGNER extract preparation. Gravimetric investigations demonstrated the high efficiency of CPC technology for full and unbiased sample recovery (99.8%).

P-205

DISCOVERY OF PHYTOPROGESTINS WITH MIXED AGONIST AND SYNERGISTIC ACTIVITY FROM TRIFOLIUM PRATENSE AND CORNUS OFFICINALIS

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Department of Medicinal Chemistry and Pharmacognosy, Center for Pharmaceutical Biotechnology, College of Pharmacy, 833 S. Wood St., University of Illinois at Chicago, Chicago, IL 60612, United States

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 seven phytoprogestins present in red clover (*Trifolium pratense*) and dogwood (*Cornus officinails*) extracts. While looking for compounds in both plants, irilone (**1**), cornuside (**5**), (*7α*)-*7-O*-methylmorroniside (**6**), and a new derivative, demethoxycornuside (**7**) exhibited a synergistic interaction with progesterone. Prunetin (**2**), formononetin (**3**) and biochanin A (**4**) exhibited mixed agonist activity. Collectively these results suggest that the effects of red clover and dogwood extracts repeatedly observed in cultured cells, and the inverse correlation between risk of various cancers and flavonoid intake may be due, in part, to altered progesterone signaling.

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INDOLE DITERPENES IN *IPOMOEA ASARIFOLIA* AND *IPOMOEA MUELLERI*

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Ipomoea asarifolia and *Ipomoea muelleri* have been associated with a tremorgenic syndrome in livestock in Brazil and Australia, respectively. *Ipomoea asarifolia* and *I. muelleri* were investigated by high-performance liquid chromatography–high-resolution mass spectrometry (HPLC–HRMS) and high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) for indole diterpene composition. The high-resolution mass spectrometric data in combination with MS/MS fragmentation mass spectral data provided valuable information for indole diterpene characterization. Several indole diterpenes were detected in each species. Three new indole diterpenes were isolated and their structures determined by 1D and 2D NMR spectroscopy and given the names 11-hydroxy-12,13-epoxyterpendole K, 6,7-dehydroterpendole A, and 6,7-Dehydro 11-hydroxy-12,13-epoxyterpendole A. The tremorgenic potential of the new indole diterpenes were evaluated in a mouse model.

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PHYTOCHEMICAL AND ANTIPROLIFERATIVE ACTIVITY INVESTIGATIONS OF THE LICHEN *NIEBLA HOMALEA*

Yan Zhang[1], Choon Yong Tan[1], Richard Spjut[2], L. Harinantenaina Rakotondraibe[1]
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, [2]World Botanical Associates, P.O. Box 81145, Bakersfield, CA 93380

Lichens are symbiosis organisms composed of fungi (mycobionts) and one or more algae and/or cyanobacteria (photobionts). Triterpenes, phenolic compounds (e.g. depsides, depsidones, quinones, xanthones, diphenyl ethers), and sesquiterpenes were reported from lichens and their mycobionts. Lichen metabolites have been shown to display biological activities such as cytotoxicity, antiviral, and antimicrobial activities. During our systematic search for antiproliferative (against A2780, ovarian and MCF-7, breast cancer cell lines) compounds from lichens and their mycobionts, we selected a lichen identified *Niebla homalea* (Ach.) Rundel & Bowler (Ramalinaceae) for further studies on cytotoxicity. *N. homalea* is a species of fruticose lichen endemic to the coastal fog regions California and Baja California. To date, no bioactive chemical investigation has been reported on the lichen genus *Niebla*. To determine the unknown chemotaxonomy of the genus and to identify secondary metabolites that can display antiproliferative activity, we carried out the phytochemical investigation of the present *N. homalea*. We discovered eight stictane triterpenoids (including five new, **1**-**5**) and isolated four known phenolic compounds. The structure elucidation, antiproliferative evaluation of the secondary metabolites as well as the chemotaxonomy of the genus *Niebla* will be presented.

P-208

UP480P, AS A NATURAL PRESERVATIVE

Ping Jiao[1], Mei Hong[1], Misun Oh[2], Qi Jia[1]
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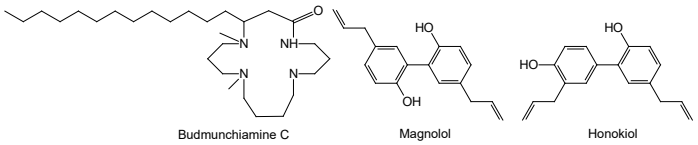
There is a need for natural compositions with broad antimicrobial activity to be utilized as safer and more environment-friendly natural preservatives for cosmetic and personal care products. UP480P, a unique combination of two plant extracts of *Albizia amara* seeds and *Magnolia officinalis* barks, has been developed as a natural preservative with proven anti-microbial activities. *Magnolia officinalis* barks contains two major lignans - Magnolol and Honokiol, which are responsible for the antibacterial and antifungal activities. The *Albizia amara* seed extract showed potent inhibition against both Gram-positive and Gram-negative bacteria with macrocyclic alkaloids - budmunchiamines identified as active components. UP480P showed potent antimicrobial activities with unexpected synergistic effect by combining Magnolia and Albizia extracts with MIC values determined against five microorganisms including *E. coli*, *P. aeruginosa*, *S. aureus*, *A. brasiliensis*, and *C. albicans*. UP480P were also evaluated following USP <51> guideline at two concentrations of 0.5% and 0.1% in a non-ionic Oil/Water emulsion system. The safety of UP480P has been demonstrated by both *in vitro* and human safety tests.



Budmunchiamine C Magnolol Honokiol

P-209

PHYTOTOXIC NEW FURANOCOUMARIN AND ANALOGS FROM LEAVES OF *AMYRIS ELEMIFERA*

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Plants produce secondary metabolites to compete with other fungi, plants, and insects. Thus, these secondary metabolites can have various biological activities such as antifungal, insecticidal, and phytotoxic activities. The plants in the Rutaceae family are especially enriched in these compounds. We investigated *Amyris elemifera*, a plant in the Rutaceae family in search of such compounds. Chromatographic fractionation of the ethyl acetate extract of the leaves of *A. elemifera* afforded a novel furanocoumarin, 2-(9-((3-methylbut-2-en-1-yl)oxy)-7-oxo-2,3-dihydro-7H-furo[3,2-g] chromen-2-yl)propan-2-yl acetate with phytotoxic and antifungal activities. In the seedling development bioassay for evaluating phytotoxic activity, this compound was more active against the monocot *Agrostis stolonifera* than the dicot *Lactuca sativa*. This novel compound is the major constituent in the ethyl acetate extract of the leaves. Isolation of the active metabolite, elucidation of structure and biological activities, and synthesis of analogs will be discussed.

P-210

EVALUATION OF MACA AND AÇAÍ FOR CYP3A4 INHIBITION AND INDUCTION AND THEIR POTENTIAL TO PRODUCE BOTANICAL-DRUG INTERACTIONS

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The consumption of botanical dietary supplements (BDS) is a common practice among the US population, but potential of metabolic botanical-drug interactions exists and its mechanism has not been well studied. CYP3A4 is an important enzyme that contributes to the metabolism of 60% clinically used drugs, including most anticancer agents. This study focused on *Lepidium meyenii* Walpers (maca) root and *Euterpe oleracea* Mart. (açaí) berries since they are commonly used BDS that may be co-administered with CYP3A4-metabolized drugs. Parallel artificial membrane permeability assay (PAMPA) was conducted to filter intestinal passively diffused compounds of maca and açaí extracts that were subsequently screened for liver CYP3A4 inhibition and induction. Passively diffused constituents of methanol açaí extract with IC_{50} of 28.03 μg/μL and non-passively diffused constituents of acidic methanol açaí extract with IC_{50} of 0.49 μg/μL demonstrated the highest inhibition potential. Moreover, both passively diffused and non-passively diffused compounds in methanol açaí extract at 1.5 μg/μL displayed the most significant hepatic CYP3A4 induction. This study suggests more potential of açaí to produce metabolic CYP3A4 interactions than maca extracts.

P-211

TWO NEW SERRATANE-TYPE TRITERPENES FROM *HUPERZIA SERRATA*

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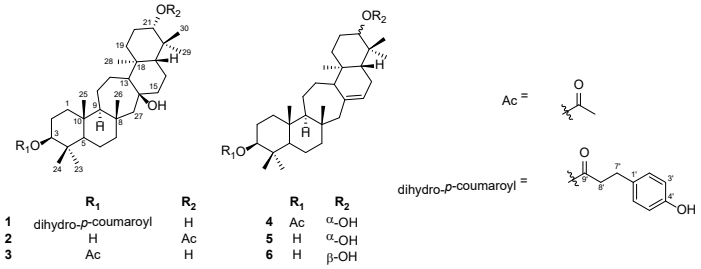
Huperzia serrata (Thunb.) Trev. (Huperziaceae, also named *Lycopodium serratum*) is a traditional Chinese herbal medicine used for the treatment of contusion, strain, swelling, and schizophrenia. To search for structurally interesting and bioactive serratane-type triterpenes, we have focused on nonalkaloidal fraction and two new compounds (**1** and **2**) along with four known compounds (**3 - 6**) were isolated. The chemical structures of isolates were determined by spectroscopic data interpretation, particularly by 1D- and 2D-NMR studies. Thereafter all the isolated compounds (**1 - 6**) were evaluated for cytotoxicity against hepatic stellate cells (HSC-T6).



	R_1	R_2
1	dihydro-*p*-coumaroyl	H
2	H	Ac
3	Ac	H

	R_1	R_2
4	Ac	α-OH
5	H	α-OH
6	H	β-OH

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NEW LIGNANS AND SECOIRIDOID GLYCOSIDES FROM *JASMINUM SINENSE*

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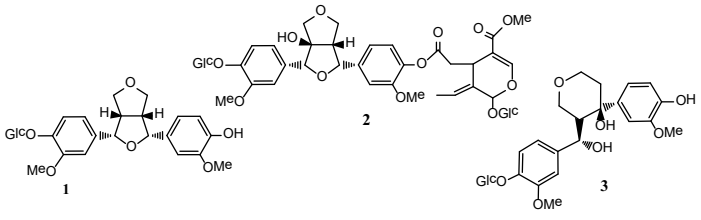
Three new lignans and secoiridoid glycosides were isolated by chromatographic fractionation of alcoholic extract of *Jasminum sinense* and named jasminolignans A-C (**1**-**3**). They were identified as 2α-(4β-D-glucosyloxy-3-methoxy-phenyl)-4α-(3-methoxy-4-hydroxy-phenyl)-3,7-dioxabicyclo [3.3.0]-octane (**1**), 2α-(4β-D-glucosyloxy-3-methoxy-phenyl)-4α-[3-methoxy-4-*O*- (5-ethylidene- 6β-D-glucosyloxy-5,6-dihydro-2*H*-pyran-3-carboxylic acid methyl ester-7-oxoyloxy)- 2-acetyl- phenyl]-1β-hydroxy-3,7-dioxabicyclo[3.3.0]-octane (**2**), and 3β-[(4β-D-glucosyloxy-3-methoxy-phenyl)-α-hydroxy]-methyl-4β-hydroxy-4α-(3-methoxy-4-hydroxy-phenyl)-tetrahydro-4*H*-pyran (**3**). The structures and the relative configurations of the new metabolites were elucidated through extensive spectroscopic analyses.



P-213

NEW TRITERPENOIDS FROM STEMS OF *SCHISANDRA ARISANENSIS*

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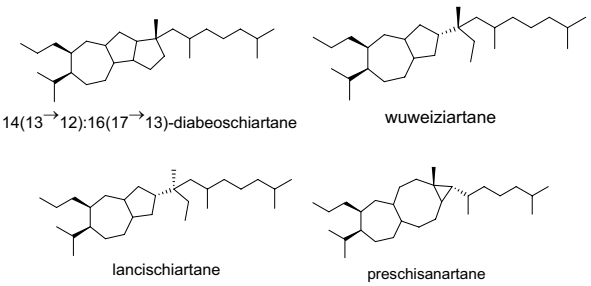
Schisandra arisanensis is an endemic medicinal plant grown in mountainous area of Taiwan. Previously, many new triterpenoids have been isolated from the fruits of this species. Chemical investigation of triterpenoids from the stems of *S. arisanensis* revealed 22 triterpenoids including eight new compounds. The new compounds are classified into four types according to their carbon skeleton patterns, 14(13→12)16(17→13)–diabeoschiartane–type, wuweiziartane–type, lancischiartane–type and preschisanartane–type. All the isolated triterpenoids will be discussed in the meeting.



P-214

BLACK COHOSH (ACTAEA RACEMOSA) EXTRACTS AND COMPOUNDS ENHANCE OSTEOBLASTOGENESIS IN HFOB OSTEOBLASTS BY ACTING AS HDAC AGONISTS

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Extracts of the roots and rhizomes of *Actaea racemosa,* commonly known as black cohosh are widely used for the management of menopausal symptoms and bone health. In mouse myoblasts, extracts of black cohosh (EBC) enhanced osteoblast differentiation and increased trabecular bone structure and BMD in mice, however the mechanisms have not been entirely elucidated. In this work, we investigate the effects of EBC, actein (Ac) and deoxyactein (Dc) in hFOB cultured human osteoblasts. The extract, Ac and Dc all enhanced osteoblast proliferation and reduced apoptosis. EBC, Ac and Dc increased osteoblast proliferation and reduced apoptosis in cultured human osteoblasts (hFOB) by reducing the Bax/Bcl-2 ratio, and acting as HDCA agonists by concentration dependently increasing SIRT1, HDAC1, and HDAC3. Ac and Dc (5-20 ng/ml) also induce mitochondrial biogenesis in hFOB osteoblasts by increasing the expression of PGC1α mRNA. These data support the hypothesis that black cohosh enhances bone formation by increasing osteoblastogenesis through epigenetic regulation of HDACs and mitochondrial biogenesis.

P-215

BLACKCURRANT (RIBES NIGRUM L., GROSSULARIACEAE) EXTRACTS ENHANCE OSTEOBLASTOGENESIS IN HFOB OSTEOBLASTS AND TELEOST FISH

Nishikant Raut[1], Zhitao Ren[2], Temitope O. Lawal[3], Sheila M. Wicks[4], Shitalben Patel[5], Gail B. Mahady[5]
[1]Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India, [2]Univerity of Macau, Macau, China, [3]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria, [4]Department of Cellular and Molecular Medicine, Rush University, Chicago, IL 60607. [4]Department of Pharmacy Practice, PAHO/WHO Collaborating Centre, University of Illinois at Chicago, Chicago, IL 60607, USA.

Blackcurrants (*Ribes nigrum*) are native to Central and Eastern Europe and Northern Asia and are used in traditional medicine for the treatment of cardiovascular disease, diabetes, viral infections and inflammation. The berries contain four major anthocyanins: delphinidin-3-O-glucoside, delphinidin-3-O-rutinoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside. We have previously shown that blackcurrant extracts (BCE) increase myoblastogenesis, and in this work, we investigate its effects in cultured hFOB human osteoblasts and osterix/Sp7:mCherry transgenic medaka. BCE and delphinidin-3-glucose increased osteoblast proliferation and reduced apoptosis in cultured human osteoblasts (hFOB) by reducing Bax and p53 expression and increasing HDAC1, HDAC3 and PGC1α mRNA expression. In *osterix/Sp7*:mCherry medaka, BCE treatment (10 μg/ml) increased osteoblast proliferation by increasing osterix/Sp7 expression. These data suggest that BCE and D3G increase osteoblastogenesis through epigenetic regulation of HDACs and mitochondrial biogenesis.

P-216

CHEMICAL INVESTIGATION OF *BOEHMERIA FORMOSANA* ASSISTED BY HPLC-SPE-TT-NMR/MS

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Chemical investigation of the constituents from the EtOAc-soluble fraction of *Boehmeria formosana* root via Sephadex LH-20, reversed phase HPLC, and centrifugal partition chromatography led to the isolation of nine compounds (**1**—**9**). As the hyphenated HPLC-SPE-TT-NMR/MS technique can provide much more information for structural elucidation of the minor compounds than the general approaches, this hyphenation was applied in this study to characterize the minor natural constituents. This approach led to characterization of four additional compounds (**10**—**13**) from a partially purified mixture, about 2 mg at most. Among these isolates, compound **13** is a novel tetrameric phenylpropanoid. Its structural elucidation, based on extensive spectroscopic analysis, was presented.

P-217

MASS SPECTROMETRY DETECTION OF BOTANICAL SECONDARY METABOLITE MODIFICATIONS BY THE GUT MICROBIOME

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Secondary metabolites derived from botanical sources have historically been utilized in the medical community due to their extensive exhibition of beneficial effects on human health. The potential role of the gut microbiome in dictating or altering biological activity of such secondary metabolites has recently become a topic of great interest. Modifications to already active components of botanicals may serve as a source of new drug candidates, or may explain the beneficial health effects that result from consumption of plant based foods or medicines. We have conducted experiments to explore the influence of gut microbes on the compound parthenolide, isolated from the botanical medicine *Tanacetum parthenium*. Mass spectrometry was used as a tool to detect structural modifications to parthenolide after *in vitro* exposure to gut microbes. Several unique compounds derived from parthenolide, including methylated and methoxylated forms, were detected by mass spectrometric analysis in these studies.

P-218

ENERGY METABOLISM AND IMMUNOMODULATORY MECHANISM OF MACA BASED ON SPLEEN-DEFICIENCY SYNDROME MICE

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Maca (*Lepidium meyenii* Walp.), native to Peru, is quite popular used with a variety of traditional Chinese medicinal materials in China. However, its usage is still lack of theoretical basis on the issue of Traditional Chinese Medicine(TCM). This study put forward TCM properties of Maca and aimed to verify it by investigating the mechanism of energy metabolism and immunomodulatory on spleen-deficiency mice. Ginseng, a major strengthen spleen herb, as control. Maca significantly increased the numbers of peripheral blood cells and reversed the atrophy of thymus and spleen. In addition, Maca increased lymphocyte proliferation inhibition rate and lymphocytes cycle were detected by flow cytometry. The proportion of cells in G_2/M phase and S phase increased, but the proportion of cells in G_0/G_1 phase decreased. The levels of IL-2, IL-4, TNF-β, IFN-γ in serum increased, measured by RIA. Then study of hot and cold property indexes, the differences in the thermotropism behaviors of mice after administration showed that Maca has the warm property. Furthermore, the ratio of cAMP/cGMP in serum and the level of mitochondrial energy metabolism enzymes increased. This study revealed the mechanism of improving the immune system and promoting energy metabolism by Maca. Our results suggest that Maca has the function of strengthen spleen and the TCM property of warm.

P-219

ABSOLUTE CONFIGURATION AND CONFORMATION OF TWO ISOPRENYLATED FLAVONOIDS FROM *DODONAEA VISCOSA* BY NMR AND ECD SPECTROSCOPIC ANALYSIS

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Two isoprenylated flavonoids from *Dodonaea viscosa* have been isolated, previously, without determination of their configuration. The present study shows that a combination of NMR and electronic circular dichroism (ECD) techniques are a relatively straightforward method to determine the absolute configuration of isomer compounds. HREMS values, ^{1}H-NMR and ^{13}C-NMR chemical shifts, are the same for the two isomers, except their stereocenters and their adjacent atom. we complement our study by carrying out (ECD) spectral simulations for the two models and the related diastereomers. The calculated ECD spectra was consistent with the experimental results for both isomers.

P-220

DETERMINATION OF ANTHRAQUINONES FROM *BULBINE NATALENSIS* (*BULBINE LATIFOLIA*) AND DIETARY SUPPLEMENTS USING UHPLC-PDA-MS

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[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA, [2]Department of Pharmaceutical Sciences and [3]SAMRC Herbal Drugs Research Unit, Tshwane University of Technology, Pretoria, South Africa, [4]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Bulbine natalensis Baker (synonym *Bulbine latifolia* (L.f.) Spreng.), family Xanthorrhoeaceae, is widely distributed in South Africa and traditionally used as an aphrodisiac and skin remedies. A validated ultra-high performance liquid chromatography-photodiode-array method was developed for the quantification of seven anthraquinone type of compounds. The separation was achieved using a reversed phase (C-18) column, photodiode array detection, and a gradient of water/acetonitrile as the mobile phase. The seven compounds could be separated within 15 minutes using the developed UHPLC method with detection limits of 25 ng/mL with 2 µL injection volume. The analytical method was validated for linearity, repeatability, accuracy, limits of detection (LOD) and limits of quantification (LOQ). The Relative Standard Deviations (RSD) for intra- and inter-day experiments were less than 5%, and the recovery efficiency was 98-101%. Nine supplements labeled as containing *B. natalensis* were available for sale, five products showed for the profile of *B. natalensis* with total content ranging from 11.3 to 90.4 mg per recommended daily dose. Compounds of *B. natalensis* were not detected in four of nine supplements. The developed method is simple, economic, rapid and especially suitable for quality control analysis of *B. natalensis*. LC-mass spectrometry coupled with electrospray ionization (ESI) method is described for the identification and confirmation of compounds in plant samples and dietary products.

P-221

EXTRA-VIRGIN OLIVE OIL C-MET INHIBITOR OLEOCANTHAL-LAPATINIB: A NOVEL SYNERGISTIC COMBINATION FOR THE CONTROL OF HER2+ BREAST MALIGNANCIES

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[1]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana 71201,[2]Department of Pathology and Public Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan.

Dysregulation of EGFR/HER2 family is a hallmark of aggressive breast cancer (BC). Small-molecule tyrosine kinase inhibitors (TKIs) are among the most effective cancer targeted treatments. (-)-Oleocanthal (OC) is a naturally occurring phenolic secoiridoid lead from extra-virgin olive oil (EVOO) with documented anti-cancer activities via targeting c-Met. Dysregulation of c-Met promotes resistance to BC targeted therapies. Lapatinib (LP) is a dual EGFR/HER2 inhibitor for HER2-amplified BC. Combined OC-LP treatment was hypothesized to be mechanistically synergistic against HER2-overexpressing BC. Combined sub-effective concentrations of OC-LP resulted in synergistic anti-proliferative effects against the HER2-positive BT-474 and SK-BR-3 BC cell lines. Antibody Array and Western blot analysis showed that combined OC-LP treatment inhibited EGFR, HER2, and c-Met receptor activation as well as multiple downstream signaling proteins. OC-LP combination significantly inhibited BC cell invasion, migration, and reduced activation of FAK, and paxillin. Combined treatment of OC (5 or 10 mg/kg) with LP (50 or 12.5 mg/kg) suppressed BT-474 tumor growth by more than 90% in a nude mice xenograft model. c-Met, EGFR, HER2 were significantly suppressed in treated mice tumors. This study reveals OC translational potential for use to sensitize HER2-overexpressing BC and synergize with chemotherapeutics targeting HER family.

P-222

INTER-KINGDOM BEACH WARFARE: A TALE OF TWO MICROBES.

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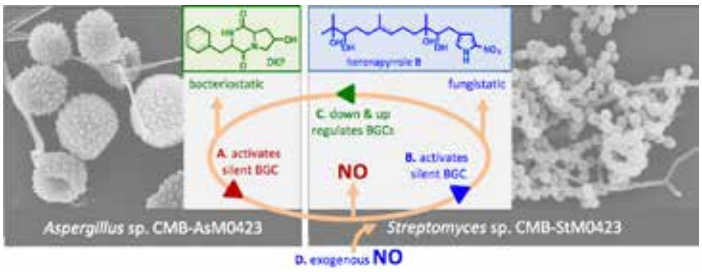
[1]Institute for Molecular Bioscience and [2]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Queensland 4072, Australia.

Inter-kingdom beach warfare between *Streptomyces* sp. CMB-StM0423 and *Aspergillus* sp. CMB-AsM0423, co-isolated from beach sand collected on Heron Island, Australia, sees the *Aspergillus* metabolite *cyclo*-(L-Phe-*trans*-4-hydroxy-L-Pro) (DKP) stimulate the *Streptomyces* to produce nitric oxide (NO), which mediates transcriptional activation of silent biosynthetic gene clusters (BGCs) for heronapyrrole B, and in turn prompts the *Aspergillus* to upregulate DKP. Structure activity relationship studies, coupled with the use of NO synthase inhibitors, NO donors and scavengers, and chemical, biochemical, microscopic, genomic and transcriptomic analyses, confirmed the specificity of DKP as an NO activator. These findings highlight the importance of inter-kingdom chemical cues (particularly NO) in regulating silent BGCs. We have since determined NO mediated transcriptional activation (NOMETA) has a broader ecological footprint, and has potential application in microbial biodiscovery.



P-223

IN-VITRO AND IN-VIVO ANALYSIS OF FORMULATED COLA NITIDA TABLET AND CREAM IN THE TREATMENT OF DERMATOPHYTE INFECTION

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[1] Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan.

This study assessed the formulation and evaluation of anti-dermatophytic activities of tablets and creams prepared from ethanol extract of *Cola nitida* stem bark and Avicel by direct compression with simple ointment B.P.C. The tablet in synergy with the simple ointment as a combination therapy for the treatment of dermatophyte infection by *Trichophyton rubrum* and *Trichophyton tonsurans in-vitro* was evaluated. The variables used in the formulation of the cream and tablet elicited synergistic potency of *Cola nitida* for the treatment of dermatophyte infection with varied zones of inhibition. The statistical ratio obtained from this study indicated that H (2.5% extract + 10% glycerine + 87.5% base) and F (5% extract + 5% propyleneglycol + 90% base) in combination with 200 mg of CNSB (*Cola nitida* stem bark) tablet elicited the greatest efficacy with zones of inhibition of 25±0.2 and 20± 0.2. While for the non-combination treatment, zones of inhibition were 14±0.1 and 10± 0.1. The in-vivo analysis revealed histologically and hematologically that though the control drug, ketoconazole and 5-fluorouracil were active against the tested dermatophytes, there is rupturing of cells at the site of infection after treatment. In treatment with formulated *Cola nitida* stem bark; there were no obvious rupturing of the cells of the wistar rats after treatment. This affirmed the fear of medical practitioners in prescribing oral drugs when patients present with dermatophyte infection because they are eukaryotic in nature.

P-224

NONTUBERCULOUS MYCOBACTERIA SPECIES ARE INHIBITED BY METHANOL EXTRACTS OF *COLA NITIDA* (VENT.) SCHOTT

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Cola nitida (Vent.) Schott. used in traditional medicine to treat asthma, whooping cough and other respiratory tract diseases including tuberculosis was investigated for antimycobacterial activities in five nontuberculous mycobacteria species viz: *Mycobacterium fortuitum* ATCC 684, *Mycobacterium smegmatis* ATCC 19420, *Mycobacterium phlei* ATCC 19240, *Mycobacterium abscessus* and *Mycobacterium smegmatis*. Preliminary antimycobacterial screening was done by the agar diffusion method. The minimum inhibitory concentration (MIC) of the methanol extract was determined by the agar dilution method while the bactericidal activity was studied by the viable counting technique. Phytochemical screening for the detection of secondary metabolites revealed the presence of saponins, tannins, reducing sugar, phenol and glycoside in the bark while alkaloids, tannins, reducing sugar, phenolics, glycoside, resin and terpernoids were detected in the leaf sample. Alkaloids, tannins, reducing sugar and resin were the secondary metabolites detected in the seed. The methanol extracts of the bark at 100 mg/mL demonstrated highest activity with diameter of zone of inhibition between 14±0.2 mm and 24±0.4 mm. The MIC for the bioactive extracts was in the range of 12.5 – 50 mg/mL while the minimum bactericidal concentration (MBC) ranged from 75 mg/mLto 200 mg/mL. Time-kill assay revealed a drastic decline (> 50%) in population after 6 hours of exposure to methanol extracts of seed and bark at concentrations equivalent to 2 x MIC and 4 x MIC accompanied with a total kill (100%) of the population at 24 hour

P-225

COMBINATION THERAPY WITH β-GLUCAN AND COENZYME Q10 IN MURINE EXPERIMENTAL AUTOIMMUNE DISEASE AND CANCER

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Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone or ubidecarenone, is an essential substance for electron transport in oxidative phosphorylation and also serves as an important anti-oxidant. CoQ_{10} is recently gaining attention as a substance with significant anti-inflammatory properties. In addition to the effects on various aspects of immune system, CoQ_{10} was also found to positively affect fatigue, have positive effects on type 1 and type 2 diabetes mellitus and serve as a preventive agent against toxicity acting via modulation of oxidative stress.

In our study, we decided to evaluate the possibility that Q_{10} effects will be potentiated by simultaneous addition of well-established immune modulator β-glucan. A Q_{10} and β-glucan were used both in vivo and in vitro and their effects were evaluated using phagocytosis, cytokine secretion, two types of experimentally-induced inflammatory problems and cancer. Our study confirmed strong anti-inflammatory effects of CoQ_{10} and showed that these effects were further potentiated by addition of β-glucan. The anti-cancer effects of CoQ_{10} were less pronounced, but again, stronger with added β-glucan. Based on our studies, we can conclude that there is significant synergy between CoQ_{10} and β-glucan, suggesting that this combination has a potential for further development in anti-inflammatory and anti-cancer treatment.

P-227

GASTROPROTECTIVE EFFECTS OF CARVONE ENANTIOMERIC PAIR.

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Mentha aquatica (Lamiaceae) essential oil contains almost 60% of carvone. This study evaluated the gastroprotective effects of both R-(-)-carvone (R-C) and S-(+)-carvone (S-C). The carvone enantiomeric pair was purchased from Merck. The Ethics Committee (CEUA/UNICAMP) approved the *in vivo* protocols using male Wistar rats. The anti-ulcer activity was evaluated in ethanol-induced gastric ulcer; the animals (n = 7) were treated (v.o.) with PBS (10 ml/kg, vehicle), Carbenoxolone (200 mg/kg, positive control), R-C or S-C (10, 30, 100 mg/kg) 1 h before ethanol administration (4 mL/Kg). After 1 h, the stomachs were removed, opened along the greater curvature, photographed at a fixed distance and the relative ulcerative areas (%) were analyzed using ImageJ®. The anti-secretory activity was evaluated in pylorus ligation model; the animals (n = 7) were treated (intraduodenal) with PBS (2.5 ml/kg, vehicle), Cymetidine (100 mg/kg, positive control), R-C or S-C (10, 30, 100 mg/kg) immediately after pylorus ligation. After 4 h, the gastric content was collect to volume, pH and hydrogenionic concentration evaluations. All results were submitted to statistic evaluation (ANOVA followed by Tuckey). Both R-C (ED_{50} = 25.6 mg/kg). and S-C (ED_{50} = 16.2 mg/kg) prevented ethanol-induced ulcers.

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TARGETED DEREPLICATION OF MICROBIAL NATURAL PRODUCTS BY HIGH-RESOLUTION MS AND PREDICTED LC RETENTION TIME

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A new strategy for identification of known compounds in *Streptomyces* was developed via combined screening of two natural product databases. **StrepDB** contains LCMS data for 5555 natural products, and was screened using a high-throughput processing algorithm that rapidly identifies known compounds using HRMS data and predicted LC retention times. **MbcDB** contains structures and analytical data for 665 natural products and was generated using the ACD/Spectrus DB Platform. StrepDB was used to screen a mutant *Streptomyces albus* extract, which led to the identification of two new compounds, legonmaleimides A and B. Their proposed structures were then confirmed by computer-assisted structure elucidation methods using ACD/Structure Elucidator Suite. This strategy suggests an extract dereplication pipeline approach that can be applied to natural product discovery.

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THE MIXTURE OF DAUCOSTEROL AND LUPEOL AMELIORATE DSS-INDUCED INFLAMMATORY BOWOL DISEASE BY ENHANCING IMMUNE RESPONSES

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Inflammatory bowel disease (IBD) is known to be associated with inflammation and ulceration in intestinal environment, but its pathogenesis is not clearly yet. *Canavalia gladiata* (CG) and *Arctium lappa* (AL) are widely used in traditional medicine and food, and have been demonstrated to have anti-inflammatory effects. Although CG and AL are popularly used in traditional medicine and food, the CGAL prevented the clinical signs of IBD and restored excessive production of ROS and IgA in DSS-induced IBD mice. The marker compounds of CG and AL mixture were identified as daucosterol (DA) and lupeol by HPLC. This study demonstrated that LPS-induced reactive oxygen species (ROS) and nitric oxide (NO) were measured after treatment with CG, AL, CGAL, lupeol, DA, and the mixture (lupeol+DA) of marker compounds such as DA and lupeol in Raw264.7 cell. And the enhancing immune responses including increased population and activation of immune cells, up-regulation of cell cycle and induction of IgA and IgG production were observed in WT mice after administration of lupeol+DA. Furthermore, the lupeol+DA also ameliorated the clinical signs of IBD and improved the population and activation of immune cells in DSS-induced IBD mice. Moreover, the functional defects of NK cells and amplified production of IgA were reversed by the mixture of lupeol+DA. In conclusion, the lupeol+DA ameliorate the progression of DSS-induced IBD by enhancing the immune responses and recovering the functional defects of immune cells.

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A BACTERIAL PRODUCT INDUCES MATING IN ANIMAL'S CLOSEST RELATIVE

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Choanoflagellates are the closest living relatives of animals, and thus serve as an excellent evolutionary model for the common ancestor of the animal kingdom. Notably, choanoflagellates share many signaling and cell-cell adhesion proteins with animals. Furthermore, many choanoflagellate species can exist in both single celled and multicellular forms. The choanoflagellate *Salpingoeca rosetta*'s unicellular-to-multicellular transition is regulated by specific lipids produced by its prey bacteria (*Algoriphagus machipongonensis* and other Bacteroidetes). In our search for new multicellularity-inducing lipids, we serendipitously discovered that choanoflagellates begin to mate when exposed to a secreted product from a different environmental and symbiotic bacterium (*Vibrio fischeri*). To our surprise, the secreted product was an enzyme. Bioassay-guided fractionation revealed the enzyme to be a chondroitinase. The chondroitinase digests chondroitin in the choanoflagellate proteoglycan coating, which is reminiscent of sperm cells releasing hyaluronidase to penetrate the egg coat in animal fertilization. Significantly, this discovery revealed that (a) chondroitin is present in choanoflagellates—suggesting for the first time that this glycosaminoglycan preceded the evolution of animals, and (b) bacteria can induce fertilization in choanoflagellates via a mechanism similar to animal fertilization—suggesting that bacteria may induce fertilization in primitive animals.

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METABONOMICS STUDY OF MECHANISM OF PAEONIFLORIN ON CHRONIC RESTRAINT STRESS AND RADIOTHERAPY-INDUCED MYELOSUPPRESSION RATS

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Paeoniflorin (PF) is considered as an effective ingredient of Paeoniae Radix Alba(PRA), in this study a novel approach using metabonomics was used to investigate the mechanism of PF on nourishing blood and anti-depress effect. The Chronic restraint stress and Radiotherapy-induced myelosuppression model rats were established by solitary and chronic restraint stress combined with radiation, a metabonomic approach based on LC-MS was used to profile metabolic pathway changed of the serum samples. The results showed that the changes in the levels of endogenous metabolites in the serum of model rats included higher levels of Phosphatidylcholine, L-Kynurenine, L-Glutamine and other 11 metabolites, and a lower concentration of Triglyceride, Butyrylcarnitine and other 9 metabolites, while PF group regulated insignificantly in the 11 metabolites. Five key metabolic pathways including ether lipid metabolism, glycerophospholipid metabolism, alanine, aspartate and glutamate metabolism, arginine and proline metabolism and tryptophan metabolism were the most relevant pathways involved in the model rats, the mechanism of the efficiency of PF on nourishing blood and anti-depress may be related to the regulation of these metabolic pathways.

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STUDY ON ANTIOXIDANT AND IMMUNE REGULATIONEFFECTS OF DENDROBIUM HUOSHANENSE ON DEFICIENCY OF KINDEY-YIN MICE

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Dendrobium huoshanense (DH) is a species of Dendrobium, which is not included in the Chinese Pharmacopoeia clearly but has a history of several thousand years of application. *Shennong Bencao Jing*, the earliest monograph of Chinese medicine, records that Dendrobium have the efficacies of replenishing the deficiency and reinforcing yin essence. In order to explore the antioxidant and immune regulation effects of DH, the kidney-yin deficiency mice were set up with thyroxine under the guidance of the TCM theory in this study, both Dendrobium candidum, a collected in Chinese Pharmacopoeia and commonly used Dendrobiumand in clinic, and Liuwei Dihuang pills were used as control drugs, and the changes of hair, food intake, autonomous activity in mice were observed, and the body weight, thymus index and spleen index were measured, and the level of IL-6 and IL-2 in serum, the content of SOD, GSH-Px, CAT and MDA in liver tissues were detected separately. The results showed that DH can obviously alleviate the yin deficiency symptoms of mice, such as yellowing dull hair, dry stool, yellow urine, restlessness, irritability, gain body weight of mice, increase the thymus index and spleen index, reduce the secretion of IL-6 and increase IL-2 in serum, enhance the content of SOD,GSH-Px,CAT and decrease MDA in liver tissues, which indicates that DH has the abilities to improve the antioxidant ability and enhance immunity, and its mechanism may be related to nourish kidney-yin deficiency. This study not only further clarifies the pharmacological effect of DH, but also provides experimental basis for the rational clinical application and the development of health products.

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HUNTING FOR NEW CLASSES OF PANCREATIC CANCER THERAPEUTIC LEADS FROM CYANOBACTERIA

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Pancreatic cancer is the twelfth most abundant form of cancer and yet has the third highest mortality rate in the United States.[1] The high mortality rate from pancreatic cancer may be attributed to difficulties in early diagnosis and the lack of effective therapeutic agents.[2] To address the latter issue, we aim to discover new classes of pancreatic cancer therapeutic leads from the secondary metabolites, including exo-metabolomes, produced by various strains of cyanobacteria. We also seek to generate preliminary hypotheses on mechanisms of action of these active natural products from the principal component analyses of the whole cell NMR spectra of pancreatic carcinoma cells treated with these compounds, as well as with drugs with known mechanisms of action.

Extracts of *Leptolyngbya* sp. and *Phormidium corium* exhibited significant inhibition of proliferation of Panc-1 cells. Bioactivity guided separation of the media extract of *Leptolyngbya* sp. resulted in the isolation of a few chlorinated metabolites. Structure elucidation and evaluation of biological activity of these compounds will be discussed

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BOTANICALS CONTAIN MITOCHONDRIALLY-ACTIVE COMPOUNDS

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Mitochondrial agonism and antagonism are both viable therapeutic strategies in different contexts. Botanical dietary supplements (BDS) were evaluated for mitochondria-targeting molecules. Human T47D breast cancer cells, and osteosarcoma cytoplasmic hybrids carrying a pathogenic mitochondrial mutation [complex I-driven ATP synthesis] were exposed to BDS crude extracts and O_2 consumption, ATP synthesis, and cell viability were measured. Guggul[2](*Commiphora wightii)* and 11 other extracts inhibited cellular respiration by ≥50%. Guggulsterol III and sesamin from guggul suppressed cellular respiration by inhibiting mitochondrial electron transport chain (ETC) complex I. Both compounds inhibited hypoxic activation of hypoxia-inducible factor-1 (HIF-1) and exhibited antiproliferative effects against T47D cells [IC_{50} values (95% CI) 3.4 (3.06–3.73) μM and 22.2 (19.78–24.81) μM, respectively]. Twelve BDS extracts stimulated rotenone-inhibited mitochondrial complex I-driven ATP synthesis. Three *Colchicum* spp. compounds from the NCI natural product library stimulated mitochondrial O_2 consumption. About 4% of BDS evaluated contained mitochondrial agonists or antagonists, with the antagonists being three-times more prevalent. Depending on their potency/concentration, consumption of BDS mitochondrial antagonists and agonists could either produce organ toxicity or act therapeutically.

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LOBARIC ACID AND PSEUDODEPSIDONES FROM THE LICHEN *STEREOCAULON PASCHALE* INHIBITS NF-KB SIGNALING PATHWAY

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Lichens produce a vast diversity of highly bioactive defence compounds in response to environmental stress to protect the symbiotic partners. Therefore, lichens of Northen Quebec represent a source of potential bioactive natural products due to the extreme growing conditions in the Nunavik region. Chemical investigation of the lichen *Stereocaulon paschale* has led to the isolation and identification of two new dibenzofurans and 11 knowns lichen metabolites.[2] Six pseudodepsidone-type metabolites were identified and derived from the cleavage of the depsidone linkage of lobaric acid, the major compound of the crude extract. Lobaric acid and the pseudodepsidones metabolites demonstrated significant *in vitro* inhibitory activity against major pro-inflammatory targets (NF-κB, TNF-α and IL-1β). Docking simulations were performed to investigate the mechanism involved. To further investigate their anti-inflammatory potency, we have developed a synthetic methodology to give access to a variety of lichens metabolites. Phytochemical investigation, inhibitory activity against pro-inflammatory targets and the synthetic methodology will be presented.

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SUPPLEMENTAL CAFFEIC ACID SUPPRESSES ROS ACCUMULATION AND HSC SENESCENCE IN THE BONE MARROW OF TBI-EXPOSED MICE

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Repetitive total body irradiation (TBI) irreversibly accumulated radiotoxic stress in bone marrow (BM) HSPCs, induced hematopoietic stem cell (HSC) senescence, and decreased hematopoietic function greater than single TBI did. Supplemental caffeic acid (CA) ameliorated TBI-mediated long-term BM injury by inhibiting ROS accumulation and HSC senescence. Supplementation with CA also exhibited a radioprotection effect on the survival of mice exposed only to single TBI. Collectively, our results indicate that supplemental CA ameliorates HSC senescence-accompanied long-term BM injury in TBI-exposed mice.

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CHEMOSELECTIVE DEHYDRATION OF GLUTAMINES TO EXPLORE STRUCTURE-ACTIVITY RELATIONSHIPS OF THE PEPTAIBOL ALAMETHACIN F50

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The reactions of primary amides to chemoselectively generate nitriles was accomplished via the Selectfluor modification of a palladium (II) catalyst. Illustration of the utility of this process was demonstrated via using this reaction with alamethicin F50, a peptaibol that has three different glutamine groups. Conditions were optimized to generate a mixture of all seven possible products in a single reaction. Separation protocols were used to purify them for examination of structure-activity relationships, and process rapidly determined which amides were important for the peptaibol's cytotoxic activity. Thus, this dehydration protocol can act similar to an alanine scan for the glutamines in a peptide.

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DEVELOPING NEW SOURCES OF ANTICANCER DRUGS: ENDEMIC COASTAL MACROLICHENS AND THEIR MICROBIAL ASSOCIATES

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Natural product compounds derived from plants and microbes have been frequently used as anticancer leads and chemical probes for validation of new pharmacological targets in anticancer drug discovery during the last decade. This is due to their biologically relevant chemical space that can be utilized not only to fight against recently discovered drug resistant cancers but also to contribute to the challenging drug discovery work of synthetic chemists by giving insights for the design of new lead molecules that better mimic the chemical space of natural products. As part of our effort to keep the discovery of new anticancer candidates that originate from natural products moving forward, we investigate the antiproliferative activity of unexplored and highly stressed endemic lichens (and their myco- and photobionts) growing on rocks of the west coast of the U.S. The results of bioassay-guided isolation and structure determination of antiproliferative compounds from an U.S. endemic lichen, *Niebla homalea* (Ach.), Ramalinaceae, the isolation of the fungal associates, and bioassay-guided isolation studies that led to the identification of new and bioactive compounds will be presented.

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CLINICAL EVALUATION OF INTERACTION OF A HOP BOTANICAL DIETARY SUPPLEMENT WITH DRUG METABOLISM IN WOMEN

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Extracts of hops (*Humulus lupulus* L.) containing prenylated flavanones are being used as botanical dietary supplements consumed by women for the management of menopausal symptoms. Our previous phase I clinical data (1) indicate that the half-lives of hop compounds exceed 20 hours. Additionally, our in vitro data suggest that hops might inhibit certain cytochrome P450 enzymes involved in drug metabolism (2). To evaluate this safety issue in the clinic, we tested a botanically authenticated and chemically and biologically standardized hop dietary supplement in 16 peri- and postmenopausal women for possible interactions with the pharmacokinetics of four FDA-approved drugs (caffeine, tolbutamide, dextromethorphan, and alprazolam) as probe substrates for cytochrome P450 enzymes using UHPLC-MS/MS. Data suggest no clinically relevant interactions of hop dietary supplement with drug metabolism.

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CHLOROPHYLL CLEAN-UP AND KNOCK-OUT MAY ENHANCE REPRODUCIBILITY OF BOTANICAL RESEARCH

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Chlorophylls, present in virtually all phytochemical extracts of aerial plant materials, can act as nuisance compounds, preventing reproducible and meaningful measurement of bioassay readouts via fluorescence interference and possible precipitation in aqueous media. To our knowledge, no detailed report comparing the specificity and reproducibility of chlorophyll clean-up methods exists. Therefore, a collection of scientific literature and web resources were mined systematically in order to evaluate existing chlorophyll removal methods, and thus, offer comprehensive protocols. Frequently reported are the use of (1) charcoal; (2) gel permeation, mainly using Sephadex LH-20; (3) adsorption resins, mainly Diaion HP-20; (4) the QuEChERS method; (5) liquid/liquid partitioning. Herein, we report a single step countercurrent separation (CCS) method that utilizes a biphasic solvent system for the targeted removal of chlorophyll from crude botanical extracts, thereby leading to the production of chlorophyll knock-out extracts (KOEs). The proposed CCS method was evaluated using two well-identified and chemically characterized plant materials, *Epimedium koreanum* and *Senna alexandrina*, both chosen for their mainstream usage as dietary supplements and for their substantial chlorophyll content. The extent of chlorophyll removal, as well as the preservation of the KOE chemical integrity were both evaluated by means of orthogonal ^{1}H NMR fingerprinting and UHPLC-UV/fluorescence.

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QUANTIFICATION OF IRIDOID AND PHENOLIC COMPOUNDS FROM ROOTS AND AERIAL PARTS OF *FADOGIA AGRESTIS* AND DIETARY SUPPLEMENTS

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An UHPLC-PDA-MS method was developed for the determination of one iridoid and ten phenolic compounds from roots and aerial parts of *Fadogia agrestis* Schweinf. ex Hiern (synonym *Vangueria agrestis* (Schweinf. ex Hiern) Lantz). The separation was achieved within 7 minutes by using C-18 column material, a water/acetonitrile mobile phase, both containing 0.1% formic acid gradient system. The method was validated for linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). The limits of detection of compounds was found to be in the range from 0.025-0.1 µg/mL. The wavelengths used for quantification with the diode array detector were 238, 254, 291 and 325 nm. Twelve of 17 dietary supplements contained phenolic compounds in the range from 0.3-2.5 mg/day. The phenolic compounds were not detected in five dietary supplements. LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification and confirmation of compounds from plant samples and dietary supplements claiming to contain *Fadogia*. This method involved the use of $[M+H]^+$ and $[M+Na]^+$ ions in the positive ion mode and $[M-H]^-$ ions in the negative ion mode with extractive ion monitoring (EIM). The developed method is simple, economic, rapid and especially suitable for quality control analysis of *Fadogia*.

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CHEMICAL PROFILING AND CHARACTERIZATION OF PRENYLFLAVONES FROM AERIAL PARTS OF *EPIMEDIUM* SPECIES USING UHPLC-QTOF-MS

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Epimedium, also known as horny goat weed, is a genus of flowering plants in the family Berberidaceae. The majority of the species are endemic to northeastern Asia. The dried aerial parts of *Epimedium brevicornu* Maxim., *Epimedium sagittatum* Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T.S. Ying, and *Epimedium koreanum* Nakai contain the highest concentrations of prenylflavonoids, which are the major compounds present in *Epimedium*, and have shown multiple beneficial therapeutic effects *in vitro.* UHPLC coupled with PDA is used for the quantitative determination of 15 components from different species of *Epimedium* (*Epimedium brevicornu* Maxim., *Epimedium sagittatum* Maxim., and *Epimedium grandiflorum* C.Morren) and dietary supplements claiming to contain *Epimedium*. These three species of *Epimedium* showed distinct chemical profile and icariin is found to be a major compound in *Epimedium grandiflorum.* LC-mass spectrometry coupled with electrospray ionization (ESI) interface method is described for the identification of compounds and involved the use of $[M+H]^+$, $[M+Na]^+$ and $[M-H]^+$ ions in the positive and negative ion mode with extracted ion chromatogram (XIC).

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BIOASSAY-GUIDED ISOLATION AND STRUCTURE ELUCIDATION OF FUNGICIDAL AND HERBICIDAL COMPOUNDS FROM *AMBROSIA SALSOLA* (ASTERACEAE).

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Plant species are a vast reservoir of biologically active compounds that have been used for agrochemical applications. The genus *Ambrosia* L. comprises more than 40 species and belongs to one of the most extensive botanical family, Asteraceae. Species of the genus or compounds isolated has been reported for its allelopathic, anti-inflammatory and antiprotozoal activities, among others. *Ambrosia salsola* (Torr. & A. Gray) Strother & B. G. Baldw (synonym *Hymenoclea salsola* Torr. & A. Gray), a species that thrives in the United States of America, was chosen for the discovery of phytotoxic and fungitoxic compounds. Dried and powdered leaves and twigs of *A. salsola* were extracted successively with hexane, ethyl acetate and methanol. The bioactive ethyl acetate extract was subjected to flash chromatography, and fractions eluted were screened for their phytotoxic and fungitoxic potential. Confertin and some methylated quercetin derivatives were identified, most of them described for the first time from this species. Bioassays for phytotoxic compounds showed that confertin inhibited the germination and growth of *Lactuva sativa* L. and *Agrostis stolonifera* L. Confertin was also active against the fungal plant pathogen *Colletotrichum fragariae* A.N. Crooks. with a thin layer chromatography bioautography assay.

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CHEMICAL DIVERSITY OF FUNGAL METABOLITES VIA EPIGENETIC MODIFICATION AND PRECURSOR DIRECTED BIOSYNTHESIS.

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Beauvericin is a well known mycotoxin that belongs to the ennitiatin antibiotic family and the cyclic depsipeptide class. It was first discovered from *Beauveria bassiana,* which is a commercial entomopathogenic mycoinsecticide fungus. The fungal strain MSX61576 from the Mycosynthetix library showed the production of beauvericin as the main secondary metabolite. A series of strategies was used to generate new chemical diversity, including OSMAC, epigenetic modifiers, and precursor directed biosynthesis. In this study we screened growth conditions, adding epigenetic modifiers and precursor units to the media to explore the new chemical diversity biosynthesized by the fungus. The experiments were analyzed *in situ* via droplet probe, and then a scale up of the selected culture conditions was prepared for isolation purposes.

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CHEMICAL PROFILING AND CHARACTERIZATION OF ANNONACEOUS ALKALOIDS AND ACETOGENINS FROM *ASIMINA* SPECIES USING UHPLC-QTOF-MS

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Alkaloids and acetogenins were reported to be the major physiologically active constituents in *Asimina* species. Chemical profiling of alkaloids and acetogenins from methanolic extracts of *Asimina* species (*A. triloba* and *A. parviflora*) have been analyzed using UHPLC-QToF in positive ion mode. These compounds were tentatively characterized based on the accurate mass of mass spectra and fragment ions. The fragments produced by collision induced dissociation (CID) revealed the characteristic cleavage and the fragmentation pattern provided structural information. The alkaloids of the aporphine, oxoaporphine and benzylisoqionolines type represent the predominant group found in *Asimina* species. Acetogenins are an important group of long-chain fatty acid derivatives containing one to three tetrahydrofuran (THF) rings and have a long aliphatic chain on one side (belonging to a series of C_{35}-C_{38} compounds) and aliphatic chain ending in an α, β-unsaturated γ-lactone on the other side. These compounds can be used to distinguish *Asimina* species. It also provides an excellent approach for rapid screening of chemical components from plant extracts. Magnoflorine was used as an example to discuss the fragmentation patterns. The fragment ions at *m/z* 297.1127 $[M+H-(CH_3)_2NH]^+$, 282.0886 $[M+H-(CH_3)_3NH]^+$, 265.0865 $[M+H-(CH_3)_2NH-CH_3OH]^+$, 237.0916 $[M+H-(CH_3)_2NH-CH_3OH-CO]^+$, and 222.0681 $[M+H-(CH_3)_2NH-CH_3OH-CO-CH_3]^+$ resulted from the protonated molecular ion. A total of 120 compounds were identified from the different parts of *A. triloba* and *A. parviflora* samples. However, for definite identification of these unknown components, further investigation is required. This may provide a model for the rapid screening and structural characterization of bioactive constituents from plant extracts.

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EXAMINATION OF CHEMICAL IONIZATION MASS SPECTRAL CALIBRATION METHODS FOR THE ANALYSIS OF ALKALOIDS IN POISON FROGS

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The quantitation of compounds in analytical samples typically relies on calibration curves of known analytes in order to provide reliable data. Unfortunately, for most published natural products, standards of analytically pure materials are not readily available and for novel compounds, no such standards could exist. Various modes of chromatography-mass spectrometry are commonly used for identifying natural products in extracts. However, mass spectrometry is generally viewed as being unreliable as an *a priori* quantitation method due to variation in ion yields and/or propensity for fragmentation or ion-molecule reactions. We have examined the feasibility of obtaining reliable quantitation based on the use of commercially available surrogate calibration compounds in structural classes (pyrrolizidines, indolizidines and quinolizidines) that are representative of the majority of poison frog alkaloids. Chemical ionization methods (ammonia chemical ionization in GC-MS and electrospray and atmospheric pressure chemical ionization in LC-MS) were evaluated. Our results to date will be presented, including a discussion of structural influences on response factors and the effects of post-column additives in LC-ESI-MS.

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CHLORELLA SP. MODULATES CHOLINESTERASE ACTIVITY AND B-AMYLOID AGGREGATION AND DIS-AGGREGATION IN VITRO

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The antioxidant, anti-cholinesterase and anti-amyloidogenic activities of extracts of *Chlorella sorokiniana* and *Chlorella minutissima* were investigated in this study. The extracts showed antioxidant activity through their radical and metal chelating abilities. Furthermore, the ethanol extract of *C. sorokiniana* exhibited highest acetylcholinesterase inhibitory activity while dichloromethane extract of *C. minutissima* showed the highest butyrylcholinesterase activity. Results of Thioflavin-T assay and electron microscopy study revealed that incubation of β-amyloid protein increased the aggregation of fibrils after 96 hours. However, ethanol extracts of *C. sorokiniana* induced disaggregation of β-amyloid fibrils. This study reveals the effective action of *C. sorokiniana* and *C. minutissima* extracts on some mediators of Alzheimer's disease and gives insights into their potential benefits as functional food or source of therapeutic agents relevant for the management of this disease.

P-248

CHEMICAL DEFENSE OF THE COMMON EUROPEAN GLOWWORM *LAMPYRIS NOCTILUCA*

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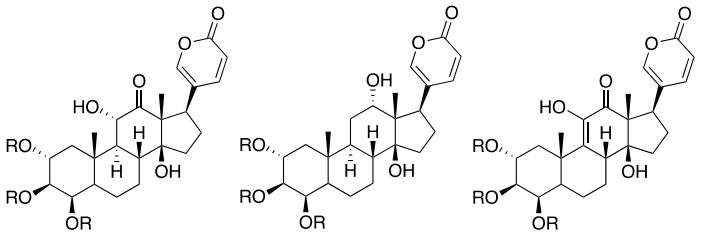
Chemical investigations of several genera of fireflies have demonstrated the presence of steroidal pyrones known as lucibufagins (LBGs, example structures shown below). These compounds have been shown to be the primary chemical defense mechanisms of these taxa, as they affect Na^+/K^+–ATPase, an enzyme essential to all animals. A prior HPLC-MS study of the Common European Glowworm *Lampyris noctiluca*, indicated that this species contains LBGs, but was unable to determine which of the many LBGs were present. Using specimens collected from Belgium and separated by life-stage and sex, we describe here HPLC-MS and NMR studies that are part of our ongoing efforts to determine the precise chemical structures of the LBG components of *L. noctiluca*. We also provide evidence that *L. noctiluca* larvae contain at least 11 different LBGs.



P-249

SELECTIVE DEGALLOYLATION AND GALLIC ACID KNOCK-OUT OF PROANTHOCYANIDIN-RICH PLANT EXTRACTS WITH DENTIN BIOMODIFYING PROPERTIES

*Bin Zhou[1], Yvette Alania[2], Mariana C. dos Reis[2], James McAlpine[1], Shao-Nong Chen[1], Ana K. Bedran-Russo[2], and Guido F. Pauli[1]**
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Long-term dental bioassays have shown that plant extracts containing gallated procyanidins (PACs) lose a significant amount of their bioactivity over time. Thus, the gallic acid (GA) moiety is thought to be a major contributors to the relative instability PACs dentin complexes, making galloyl degradation in PACs a critical factor in abating their activities in dental applications. In order to produce more stable and active dentin biomodifiers, an efficient and mild hydrolysis reaction was pursued. Preliminary data identified tannase as capable of hydrolyzing galloyl moiety of EGCG rapidly and under mild conditions. Applied to Green Tea Extract (GTE), the liberated GA was removed by countercurrent separation (CCS) using *n*-hexane-EtOAc-MeOH-H_2O (1/9/1/9, K = 0.697). Processing an aqueous solution (8 mL) of GTE (200 mg) and enzyme (20:1 w/w) at 37 °C for 20 hrs yielded 22.0 mg of GA (15% of GTE, w/w, purity ≥ 98% by HPLC at 280 nm), which was knocked-out from the extract by CCS. The same protocol was applied to witch hazel and grape seed extracts. Their phytochemical profiles and the dentin strengthen activities of all three GA knock-out extracts will be presented.

P-250

ALKALOIDS FROM *MALBRANCHEA ALBOLUTEA* SIGLER & CARMICHAEL (MYXOTRICHACEAE)

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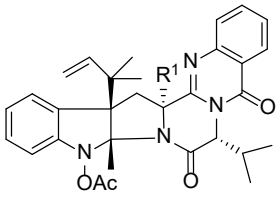
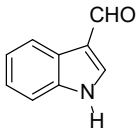
Extensive fractionation of an extract from the rice-based culture of the fungus *Malbranchea albolutea* Sigler & Carmichael (Myxotrichaceae) led to the isolation of 5-*N*-acetyl-8-β-isopropyl-ardeemin (**1**) and 5-*N*-acetyl-15-β-hydroxy-8-β-isopropyl-ardeemin (**2**), along with 1*H*-indole-3-carboxaldehyde (**3**). The structures of **1** and **2** were elucidated by spectroscopic and molecular modeling methods. The absolute configuration at the stereogenic centers of **1** and **2** was established by comparison of their experimental CD spectra with those calculated using DFT at the B3LYP/6-311G+(2d,p) level of theory.



1 R^1 = H
2 R^1 = OH



3

P-251

ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES OF BIOACTIVE CONSTITUENTS FROM *BOCCONIA FRUTESCENS*

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The number of infectious disease cases and the threat of drug-resistant bacteria is globally increasing. Some bacteria on the list of "biggest threats" compiled by the Centers for Disease Control and Prevention, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are increasingly viewed as community- acquired (CA). While the sources of CA-infections are not precisely known, evidence suggests that environmental exposures in rural areas puts these communities at greater risk: Hawai'i was identified as the state with the highest prevalence of MRSA as compared with the national average. Treatment for MRSA is challenging due to development of drug resistance thus requiring novel treatment approaches. During the course of research on new natural product-derived antimicrobials, *B. frutescens* extracts of roots and seeds collected in Hawai'i exhibited potent activity against methicillin-sensitive *S. aureus* Newman with MIC values of IC_{50} of 0.8, 3.25 μg/mL, respectively. *B. frutescens* has been used in traditional medicine to treat respiratory and skin infections in human. Following a chemically-guided approach, several alkaloids, including 8-hydroxydihydro sanguinarine, and chelerythrine were isolated. Their structures are determined using NMR spectroscopy and mass spectrometry. Compounds were tested evaluated against inflammation by following decreasing pro-inflammatory cytokines tumor necrosis factor (TNF)-α that are exacerbated by *S. aureus in vitro*. Indeed, extracts inhibited TNF-induced nuclear factor (NF-κB) activity by 95% without cytotoxicity. NF-κB pathways are known to play a vital role in regulating the activity of cyclooxygenase-2 and pro-inflammatory cytokines. The structure elucidation of alkaloids, their biological activity will be presented.

P-252

FORSYTHIA OVATA INHIBITS LIPOPOLYSACCHARIDE-INDUCED NITRIC OXIDE PRODUCTION IN RAW 264.7 CELLS VIA MODULATION OF NF-KB AND NRF2-MEDIATED HO-1 PATHWAYS

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Forsythia ovata Nakai is a deciduous shrub in the family Oleaceae, and distributed in the central area of Korea. Inflammation is an essential defensive response to harmful stimuli including pathogens, irritants, and inflammatory cytokines, which interact with other cells. Although the inflammatory process is a beneficial physiologic reaction, excessive or persistent inflammation causes serious inflammatory diseases including septic shock, rheumatoid arthritis, and autoimmune diabetes. Lipopolysaccharide (LPS), a bacterial endotoxin, induces the production of inflammatory mediators such as iNOS, COX-2, TNF-α, IL-1, and IL-6 in macrophages. In this study, we established anti-inflammatory effects of the 70% EtOH extract of *F. ovata* leaves in LPS-stimulated RAW 264.7 macrophage cells. *F. ovata* suppressed the LPS-induced expression of iNOS, COX-2, and proinflammatory cytokines in RAW 264.7 cells. *F. ovata* inhibited NO production in LPS-stimulated RAW 264.7 cells through suppression of their regulatory genes. The anti-inflammatory effects are associated with suppression of NF-κB and enhancement of Nrf2-mediated HO-1 activation. To characterize

the 70% EtOH extract of *F. ovata* leaves, we used reverse phase HPLC and UPLC-QTof MS. Major peaks were identified, and contents of major peaks were determined in the extract.

P-253

STRUCTURAL DIVERSITY AND ANTICANCER ACTIVITY OF MARINE-DERIVED ELASTASE INHIBITORS: KEY FEATURES AND MECHANISMS MEDIATING THE ANTIMETASTATIC EFFECTS IN INVASIVE BREAST CANCER

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Three new 3-amino-6-hydroxy-2-piperidone (Ahp)-containing cyclic depsipeptides named loggerpeptins A-C (**1**-**3**) along with molassamide (**4**) were discovered from a marine cyanobacterium, extending the structural diversity of this prevalent scaffold of cyanobacterial serine protease inhibitors. Molassamide (**4**), containing the 2-amino-butenoic (Abu) unit in the cyclic core, was the most potent and selective analogue against human neutrophil elastase (HNE). Given the growing evidence supporting the role of HNE in breast cancer progression and metastasis, we assessed the cellular effects of compounds **3** and **4** in the context of targeting invasive breast cancer. Both compounds inhibited the cleavage of the elastase substrate CD40 in biochemical assays; however, only **4** exhibited significant cellular activity. As CD40 and other receptor proteolytic processing culminates in NF_KB activation, we assessed the effects on the expression of target genes, including ICAM-1. ICAM-1 is also a direct target of elastase, and in our studies compound **4** attenuated both elastase-induced ICAM-1 gene expression and ICAM-1 proteolytic processing by elastase, revealing a potential dual effect on migration through modulation of gene expression and proteolytic processing. Molassamide (**4**) specifically inhibited the elastase-mediated migration of highly invasive triple negative breast cancer cells.

P-254

ACTIVATION OF SILENT BIOSYNTHETIC GENES OF RARE-ACTINOMYCETES BY COMBINED-CULTURE WITH MYCOLIC ACID-CONTAINING BACTERIUM

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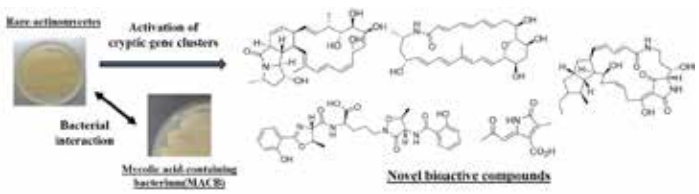
Mycolic acid-containing bacterium (MACB) could interact with broad-spectrum of actinobacteria and induce the gene expression responsible for natural products biosynthesis. The co-culture method using MACB was described as "combined-culture". Because rare-actinomycetes (=non-*Streptomyces*) are less-exploit resources for natural product discovery than *Streptomyces* species, we targeted the rare-actinomycetes for the combined-culture method. As a result, MACB induced the secondary metabolites production in 11 out of the 40-tested rare-actinomycetes by co-culture, and we finally obtained 11 novel compounds with unique and diverse structures and bioactivities.



P-255

MICROBIAL NATURAL PRODUCTS DISCOVERY FROM UNIQUE TERRESTRIAL ENVIRONMENTS

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Natural products remain a major inspiration and source for drug leads and bioactive probes. While the trends in microbial natural products discovery over the last decade have moved away from terrestrial microbes, we seek to explore the microbial diversity (and corresponding biosynthetic potential) of untapped terrestrial microbes from environments. As part of our ongoing natural product discovery program at the Center for Pharmaceutical Research and Innovation (CPRI) at the University of Kentucky (UK), we examined soil samples collected from different sites in Kentucky (including thermal vents from underground coal mine fires, coal and lead mine reclamation sites, active underground and surface coal mines, and deep subterranean drilling sites as unique access to the rich biodiversity of Appalachian Kentucky and throughout the Commonwealth) with a focus upon culturable actinomycetes capable of producing novel secondary metabolites. Cumulatively, this program has led to the deposition of >900 non-redundant bacterial strains and >360 pure bacterial metabolites (nearly half of which are new natural products exclusive to the CPRI collection). This CPRI natural product repository represents broad chemical diversity (terpenes, macrolides, coumarins, peptides, phenazines, glycosides, etc.). CPRI has enabled UK investigators with novel biochemical, cell-based and/or animal-model based assays access to the repository and this broad collaborative effort has led to discoveries of relevance to chemical probe in the areas of cancer, infectious disease, neurodegenerative diseases, regeneration and drug addiction.

P-256

RACEMIC X-RAY STRUCTURES OF JATROPHA'S ORBITIDES

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Orbitides are plant-derived cyclic peptides that have a range of therapeutically-relevant bioactivities. They are typically of low molecular weight, comprising 5 to 12 standard *L*-amino acids amino acid residues, ribosomally synthesized and have a head-tail cyclization as a post-translational modification[1]. Among the wide range of plant species that contain orbitides, *Jatropha* species (Euphorbiaceae) are one of the most promising sources of biologically active orbitides. Our objective was to elucidate the three-dimensional structures of these cyclic peptides to provide a basis for further functional or drug design studies. To determine their tertiary structures, we used racemic crystallography, an emerging structural technique that enables rapid crystallization of biomolecules by combining equal amounts of each stereo-isomer[2]. It has recently been successfully applied to determine structures of several cyclic disulfide-rich peptides that were previously recalcitrant to crystallization[3]. In this study, we synthesized both *L*- and *D*-enantiomeric forms of three *Jatropha's* orbitides: ribifolin, pohlianin C and jatrophidin, and determined their X-ray structures by direct methods. Overall, our results highlight the utility of racemic crystallography for obtaining high-resolution structures and also a useful approach to design new orbitides analogues.

P-257

INTERACTION-DRIVEN MOLECULE DISCOVERY LEADS TO ISOLATION OF A NEW LINCOMYCIN DERIVATIVE FROM *LABRENZIA* SP. ANG18, A BACTERIAL SYMBIONT

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Host-microbe symbioses are emerging as promising sources for drug discovery, with many bacterial symbionts producing biologically active compounds designed to interact with their host and/or protect against pathogens and prey. We utilize the Hawaiian bobtail squid, *Euprymna scolopes*, as a model system for the discovery and development of novel antimicrobial drug leads. Our previous research has shown that bacteria associated with the accessory nidamental gland (ANG) and the egg jelly coat (JC) of *E. scolopes* produce defensive metabolites that inhibit fungal fouling and/or bacterial growth. *Labrenzia* sp. ANG18 was isolated from an *E. scolopes* ANG and exhibited potent antifungal activity against three phylogenetically related *Fusarium keratoplasticum* strains. Using a suite of mass spectroscopic (MS) and nuclear magnetic resonance (NMR) techniques, we identified known lincomycins and isolated a new lincomycin derivative. Genomic sequencing confirmed the presence of key lincomycin biosynthesis genes in *Labrenzia* sp. ANG18. Lincomycin A, an FDA-approved antibiotic, was originally isolated from an actinomycete, *Streptomyces lincolnensis* var. *lincolnensis*. To our knowledge this is the first report of lincomycin derivatives produced by a bacterial isolate within *Alphaproteobacteria*.

P-258

MANUMYCIN-A IS A POTENT INHIBITOR OF MAMMALIAN CYTOPLASMIC THIOREDOXIN REDUCTASE AND AN ACTIVATOR OF MITOCHONDRIAL THIOREDOXIN REDUCTASE

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The thioredoxin system is the major cellular reductant system present in the cell, whose role is to maintain cellular redox homeostasis. It does this in part, by regulating the activity of many other enzymes including ribonucleotide reductase, which is essential for DNA synthesis. It also acts as an antioxidant, reducing destructive reactive oxygen species. The thioredoxin system is comprised of thioredoxin (Trx) which reduces target protein disulfide bridges by thiol-disulfide exchange and thioredoxin reductase (TrxR) which reduces Trx back to its active state. TrxR is a common target for many cancer drugs including cisplatin and auranofin. Recently we have shown that the Florida red tide toxin, brevetoxin can inhibit mammalian TrxR. Several compounds which are similar to brevetoxin in size and functionality have a similar effect on TrxR. These compounds include antitumor and antibiotics such as manumycin A (Man-A), geldanamycin and algal toxins such as nodularin and microcystin-LR. Man-A behaves as a typical TrxR1 (cytoplasmic) inhibitor. Other compounds screened activate the reduction of small disulfides such as DTNB (5,5'-dithiobis-(2-nitrobenzoic acid). Inhibition of TrxR at the C-terminal redox center produces a prooxidant known as SecTRAP (Selenium Compromised Thioredoxin Reductase-derived Apoptotic Proteins), which uses NADPH to produce superoxide radical anion. This might explain the observed burst of ROS in cells exposed to these compounds. We have also tried to characterize the molecular mechanism of action by using site-specific mutant enzymes which allow us to determine the specific site of interaction between enzyme and the compounds. The use of mutant enzymes has revealed that Man-A interacts in very different ways with mitochondrial TrxR by activating DTNB reduction similar to other compounds but still inhibiting the reduction of Trx. Our attempt to evaluate such activation observed in DTNB reduction due to these compounds will also be discussed. This study will thus identify a novel mechanism of action of these compounds.

P-259

THE STRUCTURE-ACTIVITY RELATIONSHIP OF NATURAL AND SEMI-SYNTHETIC CINNAMODIAL ANALOGUES AGAINST THE *AEDES AEGYPTI* MOSQUITO

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The *Aedes aegypti* mosquito serves as a major vector for viral diseases, such as dengue, chikungunya, and Zika, which continue to spread across the globe and threaten public health. In addition to increased vector transmission, the prevalence of insecticide-resistant mosquitoes is on the rise. Thus, new, safe and effective insecticides to control mosquito populations are needed. Cinnamodial, an unsaturated drimane sesquiterpene dialdehyde isolated from the Madagascan medicinal plant *Cinnamosma fragrans,* was recently shown to exhibit significant larval and adult toxicity to *Ae. aegypti* and outperformed DEET – the gold standard for insect repellents – at repelling adult female *Ae. aegypti* from feeding. In this study, a library of natural and semisynthetic analogues of cinnamodial were used to probe the structure-activity relationship for larvicidal, adulticidal and antifeedant activity against *Ae. aegypti*. Initial efforts were focused on modification of the unsaturated dialdehyde, aimed at probing the importance of the 1,4-dialdehyde and the α,ß-unsaturated carbonyl functionalities in the observed bioactivity of cinnamodial. Previous studies have highlighted the antifeedant and toxicant activity of drimane sesquiterpenes against agricultural pests, but this study represents the first comprehensive investigation into the SAR of cinnamodial against the medically important *Ae. aegypti* mosquito.

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BRAZILIAN GREEN PROPOLIS: *IN VITRO* SCHISTOSOMICIDAL PROPERTIES

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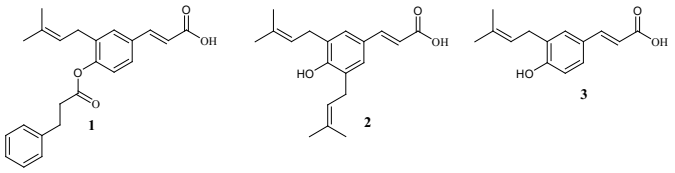
Brazilian green propolis produced by *Apis mellifera* has great economic importance due to their biological activities, including the activity against several parasites causing human diseases. Thus, the hydroalcoholic extract of Brazilian green propolis (**HEGP**), as well as their fractions obtained from partitions of the crude extract in aqueous methanol with hexanes (**GPH**), methylene chloride (**GPMC**) and *n*-butanol (**GPB**), in sequence, were tested *in vitro* against male and female worms of *Schistosoma mansoni*. The results revealed that both, **HEGP** and **GPMC**, showed potent schistosomicidal effect with LC_{50} values of 57.5 and 28.2 $\mu g.mL^{-1}$, respectively. Then, three metabolites isolated from **GPMC** (**compounds 1**-**3**) were also evaluated, and baccharin (**1**) was the most effective one with LC_{50} value of 52.7 µM. In addition, **1** at 12.5 and 25.0 µM abolished the *in vitro* egg production, thus denoting its capability of efficiently cease the *S. mansoni* life cycle. In this context, the results described here pointed out baccharin as a natural prototype for further medicinal chemical studies against *S. mansoni*.



P-261

NEW HYDROXYOLEOSIDE-TYPE *SECO*-IRIDOIDS WITH INSULIN MIMETIC ACTIVITY FROM *SYMPLOCOS COCHINCHINENSIS*

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To find active constituents with insulin mimetic activity from *Symplocos cochinchinensis* (Lour.) S. Moore, dereplication application using the EtOAc fraction of *S. cochinchinensis* resulted in ten new hydroxyoleoside-type compounds conjugated with a phenolic acid and monoterpene (**1**-**6** and **8**-**11**), as well as four known compounds (**7** and **12**-**14**). The absolute configuration of isolates were determined by ECD analysis of derivatives obtained after a series of reactions, such as those with dirhodium tetrakis and dimolybdenum tetraacetate. Compounds **3, 7** and **8** exhibited moderate 2-NBDG uptake increasing activity in differentiated 3T3-L1 adipocytes by GLUT4 translocation. Selected compounds also showed moderate inhibitory activity on PTP1B.

3. $R_1 = X_1$, $R_2 = X_2$, $R_3 = X_3$
7. $R_1 = X_4$, $R_2 = X_5$, $R_3 = X_6$
8. $R_1 = X_7$, $R_2 = X_8$, $R_3 = X_9$

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CYTOTOXIC VERAGUAMIDES SELECTIVELY INDUCE APOPTOSIS IN TRIPLE NEGATIVE BREAST CANCER CELLS

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One in eight women will be diagnosed with breast cancer in their lifetime. Of these women, 15%-20% will be diagnosed with triple negative breast cancer (TNBC), which is characterized by a lack estrogen receptors, progesterone receptors, and human epidermal growth factor 2 receptors. The treatment options for TNBC are limited to radiation, chemotherapy, and surgery, so there is dire need for small molecule drugs to treat this disease. One known and two new veraguamides were isolated from the extracts of an *Okeania* sp cyanobacteria collected in the Las Perlas Archipelago of Panama, and showed cytotoxicity for TNBC cells (MDA-MB-231). The novel linear analogues, veraguamides M and N showed minimal (>100 μM) cytotoxicity against ER positive cells (MCF-7) with low micromolar (12 and 3 μM) toxicity against TNBC cells. This suggests that the compounds may act through a mechanisms unique to TNBC cells, and for this reason could be used as molecular probes for the mechanisms of action.

P-263

DEGRADATION PRODUCTS OF ARTEPILLIN C AND *P*-COUMARIC ACID IN BRAZILIAN GREEN PROPOLIS

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In Brazilian green propolis, artepillin C (**1**) and *p*-coumaric acid (**2**) are responsible, along with other compounds, for several of its biological activities. Their stability as single compounds and in the crude propolis raw material were evaluated by exposure to heat, air oxygen and light. A full factorial experimental Design with two levels and eight experiments was selected, in which lower levels corresponded to absence or presence of light and oxygen and temperatures of -20ºC or 40ºC in the higher levels. To establish the best storage conditions, a Central Composite Design was selected. The two factors evaluated were temperature, from -20 to 50ºC, and time from zero to 30 days. Light is the main factor responsible for degradation of both **1** and **2**, and temperature also causes moderate loss of **1**. Combination of light and heat caused the degradation of 100% of **1** and 20% of **2**, in 30 days. Aiming to establish the best storage conditions, samples were protected from the ambient light. According to these experiments, when the compounds are isolated, by increasing the temperature the concentration decreases. The desirability of **1** states that a temperature of -2ºC is enough to reduce its degradation. Regarding **2**, between 15 and 25ºC, no significant degradation was observed. Both compounds, in the crude propolis, showed degradation of approximately 20% at 50ºC after 20 days. Therefore, to decrease degradation, the best storage and transport conditions include protection from the light, the use of dark packing and low temperatures.

P-264

(-)- EPICATECHIN MODULATES VASOREACTIVITY AND CELLULAR SIGNALING IN ENDOTHELIAL CELLS

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Vascular dysfunction heralds the onset of diabetic cardiovascular disease. The botanical compound (-)- epicatechin (EPICAT) is a known vasodilator. We hypothesized that EPICAT restores vasodilation in the diabetic vasculature by bolstering mitochondrial function via support of endothelial nitric oxide synthase (eNOS) activity and mitigation of reactive oxygen species (ROS). We examined the impact of EPICAT on vasoreactivity ex situ and endothelial cell function in vitro. EPICAT increased endothelium dependent vasodilation in rat aortic vessels (64.74%, p=0.002). HUVEC cells were incubated with 7 mM (NG) or 30 mM glucose (HG) with or without 0.1 or 1.0 μM EPICAT for 2 hours. Mitochondrial superoxide was detected via electron spin resonance spectroscopy, respiration was measured using Oroboros Oxygraph 2k, and cellular signaling was assessed by protein expression. Superoxide concentration was significantly elevated in HG cells but not in cells with HG and 1.0 μM EPICAT (p=0.008). EPICAT at 1.0 μM stimulated peNOS protein expression (p=0.006), and both concentrations stimulated protein expression of mitochondrial complexes I and II (p=0.02 for both). No changes in respiration were detected. We surmise that EPICAT potentiates vasodilation by activating eNOS and increasing mitochondrial complex expression, while attenuating excess ROS production.

P-265

SCREENING OF A NATURAL PRODUCT LIBRARY FOR ANTIMICROBIAL ACTIVITY TARGETING METAL HOMEOSTASIS

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Plants such as *Fouquieria splendens* and *Larrea tridentata* have been used by natives to treat several ailments such as fluid congestion, influenza, and bacterial infections. As antibiotic resistance in bacterial pathogens continues to become a more and more serious problem, new strategies for antibacterial control become more important to investigate. Zinc and iron are essential micronutrients for all living organisms due to their role in catalytic, regulatory, and structural proteins. We have explored the chemistry of Sonoran Desert plants for their ability to disrupt the metal homeostasis by targeting proteins in the zinc or iron physiological pathways. The leaves, bark, inner tissue, and flowers of *F. splendens* and *L. tridentata* were extracted and used to generate 88 enriched fractions for each tissue type. The crude extracts and enriched fractions were screened for activity testing using *lux, lacZ*, or *xylE* reporter systems expressed in *E. coli* and *Klebsiella* sp. Crude extracts of *L. tridentata* inner core, leaf, and flower and *F. splendens* flower extracts showed significant suppression of P*zntA-lux* and were confirmed by P*zntA-xylE* assay. Enriched fractions of these samples continued to show suppression of both reporter systems. Addition of crude and enriched extracts of *F. splendens* flowers also led to impaired P*znuA-lux* and P*fepA-lux* expression. Results were further confirmed with growth curves and microbial viability assays. These data strongly suggest the presence of small molecules impairing metal homeostasis.

P-267

AN ANTIPLASMODIAL COUMARIN AND A NORNEOLIGNAN FROM *ANIBA CITRIFOLIA*

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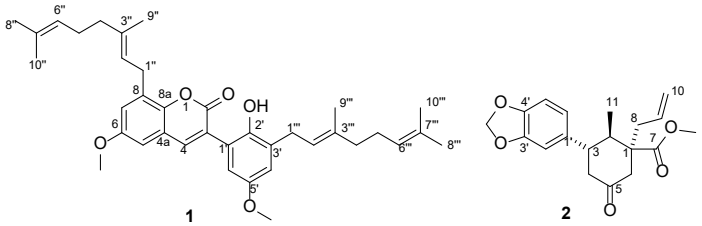
An extract of *Aniba citrifolia* from the Natural Products Discovery Institute was found to have good antimalarial activity, with an IC_{50} value of <1.25 μg/mL. After purification by liquid-liquid partition, chromatography on Sephadex LH-20, diol open column and C-18 reverse phase HPLC, the new coumarin **1**, the new neolignan **2** and six known neolignans were isolated. The structures of the new and known compounds were determined by NMR spectroscopy, MS and ECD. Among these compounds, the new coumarin **1** displayed the strongest antiplasmodial activity.



P-268

NCI PROGRAM FOR NATURAL PRODUCTS DISCOVERY: BUILDING AN INFORMATICS PLATFORM TO FACILITATE DISCOVERY OF BIOACTIVE NATURAL PRODUCTS

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The NCI Program for Natural Product Discovery (NPNPD) is a new, priority initiative for the NCI. The program is tasked with generating up to 1 million pre-fractionated samples from the existing repository of crude extracts at the NCI Natural Products Branch for the purposes of modern high-throughput targeted screening technologies. This effort will also encompass the development of integrated analytical resources for the isolation and structure elucidation of biologically active natural products. Here we present an informatics system for rapid identification of bioactive components that combines analytical, historical and source material information in a unified platform. Aspects of the system will eventually be publicly available, serving as a central repository of data on natural product samples derived from NCI collections.

P-269

NCI PROGRAM FOR NATURAL PRODUCTS DISCOVERY: RAPID ISOLATION AND IDENTIFICATION OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS FROM A PRE-FRACTIONATED LIBRARY

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The NCI Program for Natural Products Discovery (NPNPD) is a newly launched, priority program for the NCI. The new initiative aims to generate pre-fractionated extracts (up to 1,000,000) for modern high-throughput targeted screening technologies, and develop integrated analytical resources for the isolation and structure elucidation of biologically active natural products. 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 methodology is capable of processing 44 fractions in 12 hours to generate 968 sub-fractions in an assay ready-format. Examples of rapid isolation and identification of biologically active natural products from plant, marine and microbial biota will be presented.

P-270

EXPLORING SOUTH CHINA SEA CYANOBACTERIAL DIVERSITY FOR THE DISCOVERY OF NEW MARINE DRUG LEAD MOLECULES

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The structures of many cyanobacterial compounds differ significantly from marine natural products from other producers, and the discovery of new molecules from this source continues to be productively achieved especially when investigations focus on genetically and geographically diverse samples. The efficiency of this research has been demonstrated to be greatly improved by using targeted isolation methods after dereplication, for example guided by comparative metabolomics and cheminformatics, which is further supported here. It was observed during a dive trip near Hainan Province in the South China Sea, in the Tropic of Cancer, that this region maintains a biodiverse collection of marine filamentous cyanobacteria. Small- and medium-scale samples were there collected for chemical analysis, research prioritization, and preliminary isolation experiments. This has informed the decision to re-visit several nearby dive sites for larger-scale collection of cyanobacteria samples, where plausible and responsible to do so, as well as permitting the targeted isolation of a few prevalent secondary metabolites that, after MS, NMR, and chemical degradation structure elucidation studies, have been determined to be new bioactive marine natural products. The continued investigation of this underexplored natural resource is expected to provide further lead molecules for drug discovery and development.

P-271

ANTITUMOR AND ANTIMETASTATIC ACTIVITIES OF ESCULETIN BY TARGETING AXIN2/E-CADHERIN AXIS IN COLON CANCER

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Colorectal cancer (CRC) is the most common malignant disease worldwide due to its metastasis via the epithelial-mesenchymal transition (EMT) process. E-cadherin and Wnt signaling are emerging as potential targets for suppressing the EMT. In this context, Axin2 has been recognized as a negative regulator that inhibits glycogen synthase kinase 3β (GSK3β)-mediated degradation of Snail1, a transcriptional repressor of E-cadherin. However, Axin2 can also impede Wnt signaling via β-catenin degradation. Therefore, Axin2 may serve as either a promoter or suppressor of tumors, and the effects of its inhibition on the cell proliferation and metastasis of CRC require further elucidation. Here, esculetin (ES), a coumarin, was found to have the most potential effects on both β-catenin-responsive transcriptional and *E-cadherin* promoter activities. ES also showed anti-proliferative and anti-invasive activities in CRC cells. Mechanistically, Axin2 suppression by ES contributed to E-cadherin-mediated Wnt signaling inhibition. Moreover, the ability of ES to inhibit tumor growth and metastasis via Axin2 suppression was further supported in an HCT116-implanted orthotopic mouse model. Collectively, these findings suggest that targeting the Axin2/E-cadherin axis by ES may be an attractive therapeutic strategy for the treatment of metastatic CRC.

P-272

A STRUGGLE FOR FUNGAL SURVIVAL: ANALYSIS OF THE CO-CULTURE OF *ASPERGILLUS FISHERI* AND *XYLARIA CUBENSIS*

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Fungi naturally grow in competitive environments, and in order to prosper they have evolved the ability to biosynthesize a wide range of secondary metabolites. Co-culturing is one way to capitalize upon the fact that they have evolved to respond to a wide range of chemical stimuli. In turn, this could produce secondary metabolites that are not typically observed in mono-culture. To illustrate this, *Xylaria cubensis*, which is a known producer of griseofulvin, was grown in co-culture with *Aspergillus fischeri* in order to induce new secondary metabolite production. The co-culture grown on oatmeal agar was analyzed in situ via microextractions (droplet probe) on the surface of the fungi. The junction between the two fungi was observed to have a different chromatographic profile than that of the mono culture, suggesting the stimulation of cryptic biosynthesis. Isolation and structure elucidation was performed and other griseofulvin analogues as well as mycotoxins not previously see in the mono-culture were observed.

P-273

A NEW TETRANORLABDANE DITERPENE FROM *BOTRYOSPHAERIA PARVA* AN ENDOPHYTIC FUNGUS IN *EUGENIA JAMBOLANA* (LAM.)

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Tetranorlabdanes diterpenes are well-known secondary metabolites of both fungi and higher plants, and members of this class display several activities. As part of our ongoing screening for bioactive compounds from endophytic fungi, we have investigated the endophytic fungus *Botryosphaeria parva*, isolated from *Eugenia jambolana* (Lam.). *B. parva* was cultured in maize (90 g) for 21 days at 26°C in static mode. The culture was extracted with methanol (7 days) furnishing the crude CH_3OH extract, which was dissolved in CH_3CN and defatted with hexane by liquid partitioning. The CH_3CN fraction was evaporated to give 3.0 g of the crude extract. The CH_3CN extract was fractionated by CC using reversed-phase silica gel and eluted with H_2O:CH_3OH (70:30→100%) to afford eight fractions (A-H). The fraction B (168.3 mg) was subjected to $HPLC_{prep.}$ (40-63μm) using a gradient of H_2O:CH_3OH (63:37) yielding six known tetranorlabdane diterpene **1**, **3**-**7** and one new **2**. the structure of these compounds was established by extensive spectroscopic data analysis including NMR 1D and 2D and HRESIMS analysis, while the relative stereochemistry of **1**-**7** were determined by NOESY. To the best of our knowledge this is the first reporter of chemical studies of *B. parva.* The biological activities of these compounds are under investigation.

P-274

A BIOBRICKS SYNTHETIC BIOLOGY TOOLBOX FOR BIOSYNTHESIS OF ANTHRACYCLINONES

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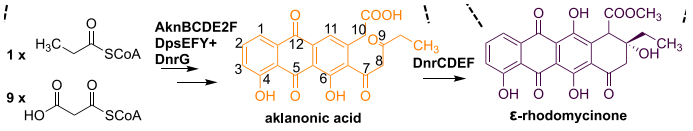
BioBricks® is a synthetic biology standard for interchangeable genetic parts including promoters, genes, noncoding sequences, and terminators. We have developed a BioBricks® toolbox of codon-optimized genes, strong promoters, and chromosomal integrating vectors for the metabolic engineering of anthracyclinone biosynthetic pathways in *Streptomyces coelicolor* M1146. Here, we report the initial assessment of a BioBricks® polyketide synthase pathway (module 1) and post-PKS tailoring genes (module 2) for production of aklanonic acid, aklanonic acid methyl ester, aklaviketone, aklavinone, and ε-rhodomycinone using HPLC-UVvis, HRMS-QTOF analysis, and ^{1}H- and ^{13}C-NMR spectroscopy.



P-275

MAMMOSPHERES AS MODELS FOR PREDICTING P450 1A1/1B1 METABOLISM

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Since the Women's Health Initiative reported that hormone replacement therapy directly correlated with increased risk of breast cancer and heart disease, many American women have turned to botanical supplements to seek relief from menopausal symptoms. Little is known about how these extracts modulate the chemical carcinogenic effects of estrogens. In the genotoxic pathway, P450 1B1 performs 4-hydroxylation of estrone/estradiol whereas P450 1A1 catalyzes detoxification of estrogen through 2-hydroxylation. These pathways are classically regulated by the aryl hydrocarbon receptor (AhR), and estrogen receptor alpha (ERα) regulates P450 1A1 epigenetically. Botanical supplements can affect both ERα and AhR, causing differential effects within the same supplement. The ethoxyresorufin-O-dealklase (EROD) assay measures activity of P450 1 family of enzymes. Unfortunately, in 2D MCF-7 cells, EROD signal was low and the AhR-mediated effect could not be separated from the ERα-mediated effect. 3D-Mammospheres are considered to be better models of humans than 2D monolayers. qPCR showed increased CYP1A1, but not CYP1B1 expression in 3D models, and with the P450 1B1 selective inhibitor, 2,3',4,5'-tetramethoxystilbene (TMS), ERα-mediated effects can be separated from those mediated by AhR. These results indicate that MCF-7 mammospheres, not monolayers, can be utilized to screen for modulation of estrogen chemical carcinogenesis and should be investigated in other assays as a way to achieve *in vitro* results more similar to humans. *Supported by NIH Grant* P50AT000155.

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