The American Society of Pharmacognosy Annual Meeting

July 29 – August 2, 2017
Hilton Portland Downtown
Portland, Oregon

Welcome to ASP 2017 in beautiful downtown Portland, Oregon! The Local Organizing Committee for the American Society of Pharmacognosy would like to thank you for joining us for the 58th Annual Meeting of the American Society of Pharmacognosy at the Hilton Portland Downtown. The program has an impressive group of speakers discussing the latest advances in the broad areas of natural products chemistry and biology, and two poster sessions that will allow attendees to share their work in a collegial setting. In addition to the scientific program, we will share a relaxing evening at the Oregon Museum of Science and Industry on the Portland riverfront. Also, Portland offers a wide array of restaurants and bars to allow you to enjoy the excellent food and beverages for which this region is known. We hope that you all have a wonderful time at the meeting.

The ASP 2017 Local Organizing Committee
Phil Proteau, Chair
Taifo Mahmud, Scientific Committee Chair
Sandra Loesgen
Kerry McPhail
Dale Nagle
Benjamin Philmus
J. Fred Stevens
Laura Stoll, ASP Business Manager

ASP 2017

Portland, Oregon. July 29-August 2, 2017

Theme: Natural product corps of discovery: Venturing into the unknown

ASP 2017 Organizers

Local Organizers

Phil Proteau (Oregon State University, Chair)
Taifo Mahmud (Oregon State University)
Kerry McPhail (Oregon State University)
Fred Stevens (Oregon State University)
Benjamin Philmus (Oregon State University)
Sandra Loesgen (Oregon State University)
Dale Nagle (University of Mississippi)

Scientific Committee

Taifo Mahmud (Oregon State University, Chair)
Nadja Cech (UNC Greensboro)
Patricia Flatt (Western Oregon University)
Sandra Loesgen (Oregon State University)
Kerry McPhail (Oregon State University)
Dale Nagle (University of Mississippi)
Benjamin Philmus (Oregon State University)
Kevin Reynolds (Portland State University)
Amala Soumyanath (OHSU)
Fred Stevens (Oregon State University)
Mark Zabriskie (Oregon State University)

Registration and Logistical Support

Laura Stoll (The American Society of Pharmacognosy)

2017 ASP MEETING SPONSORS

Diamond Sponsor

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Gold Sponsor

BRUKER

Silver Sponsor

PALL ForteBio

Bronze Sponsors

ALKEMIST LABS
Your Natural Products Partners
EXTRASYNTHESE

GILSON®

Bronze Sponsors - *continued*

SABINSA

TELEDYNE ISCO

Invited Speaker Sponsor (Dr. Nagarkatti)

BRAIN Institute OHSU

Session Sponsor (Microbial and Fungal Natural Products)

NPDI™

General Meeting Sponsors

ACD/Labs

Aveda

BMS

Oregon's Wild Harvest, Inc.

OSU – Linus Pauling Institute

OSU – College of Pharmacy

U.S. Pharmacopeial Convention

2017 ASP MEETING EXHIBITORS

Advion, Inc.

Alkemist Labs/Extrasynthese

AnalytiCon Discovery

BioChromato

Biotage

Bruker

CRC Press, Taylor & Francis Group

Gilson

Interchim, Inc.

JEOL USA

MilliporeSigma

Pall ForteBio

PhytoLab GmbH & Co. KG

Sabinsa

Teledyne Isco

Waters Corporation

ASP Award Winners 2017

Norman R. Farnsworth Research Achievement Award

Guy Carter, Biosortia Pharmaceuticals

Varro E. Tyler Prize

Richard van Breemen, University of Illinois at Chicago

Matt Suffness Young Investigator Award

Katherine Ryan, University of British Columbia

D. John Faulkner Travel Award

Tripathi Siddharth, University of Mississippi

Travel Grant for Active Members

Jana Braesel, University of Illinois at Chicago
Skylar Carlson, University of Wisconsin Madison
Jie Li, Scripps Institute of Oceanography
Erin McCauley, University of California Santa Cruz
Charles Naman, Scripps Institute of Oceanography

Jerry McLaughlin Student Travel Award

Laura Bocanegra, National Auto. University Mexico

Lynn Brady Student Travel Award

Emily Britton, University of North Carolina Greensboro
Zhibin Liang, University of Hawaii, Manoa
Logan MacIntyre, University of Prince Edward Island
Nathan Moss, Scripps Institute of Oceanography

David Carew Student Travel Award

Manuel Rangel-Grimaldo, National Auto. University Mexico

ASP Kilmer Prize

Fatma Alawadhi, University of Florida

Student Research Award

Samantha Gromek, University of Connecticut

Research Starter Grant

Matthew Bertin, University of Rhode Island
Laura Sanchez, University of Illinois at Chicago
Katharine Watts, Cal Poly, San Luis Obispo
Jaclyn Winter, University of Utah
Tyler Johnson, Dominican University Of California (2016 Recipient)
Suthananda Sunassee, University of Cape Town (2016 Recipient)

Undergraduate Research Award

Abo Aoun Mohamed, University of Winnipeg
Haylee Padget, University of Oklahoma
Andrew Whiteley, Cal Poly, San Luis Obispo

Waqar Bhatti Student Travel Award

Anam Shaikh, UT Southwestern Medical Center

Student Travel Award

Lindsay Caesar, University of North Carolina Greensboro
Chase Clark, University of Illinois at Chicago
Camila Crnkovic, University of Illinois at Chicago
Maryam Elfeki, The University of Illinois at Chicago
Jake Haeckl, Simon Fraser University
Christopher Leber, Scripps Institute of Oceanography
Catherine McCaughey, Simon Fraser University
Corena Shaffer, UT Health Science, San Antonio
Mariam Salib, University of California, San Diego
Hannah Whitmore, University of Surrey
Fabien Schultz, Technical University of Berlin
Javier Ruiz Vargas, Centro de Investigacion Cientifica de Yucatan (2016 URG Recipient)

2017 Arthur E. Schwarting Award

Per Juel Hansen & Thomas Ostenfeld Larsen's manuscript, "Silas Anselm Rasmussen, Sebastian Meier, Nikolaj Gedsted Andersen, Hannah Eva Blossom, Jens Øllgaard Duus, Kristian Fog Nielsen, Per Juel Hansen,* and Thomas Ostenfeld Larsen.* Chemodiversity of Ladder-Frame Prymnesin Polyethers in *Prymnesium parvum*. *J. Nat. Prod.* **2016**, *79*, 2250-2256"

2017 Jack L. Beal Award

Adam S. Duerfeldt's manuscript, "Nathan P. Lavey, Jesse A. Coker, Eliza A. Ruben, and Adam S. Duerfeldt.* Sclerotiamide: The First Non-Peptide-Based Natural Product Activator of Bacterial Caseinolytic Protease P. *J. Nat. Prod.* **2016**, *79*, 1193-1197"

Stephen Chamberland's manuscript, "Stephanie J. Conn, Shannon M. Vreeland, Alexandra N. Wexler, Rebecca H. Pouwer, Ronald J. Quinn, and **Stephen Chamberland**.* Total Synthesis of Clavatadine A. *J. Nat. Prod.* **2015**, *78*, 120-124." – (2016 Recipient)

The American Society of Pharmacognosy Annual Meeting

July 29 – August 2, 2017

Hilton Portland Downtown

Portland, Oregon

Program Schedule

Saturday July 29, 2017

7:30 AM – 7:30 PM	**Registration** – ***Grand Ballroom Foyer***
9:00 AM – 4:00 PM	***Council Suite*** - Executive Committee Meeting (Invitation Only)
9:00 AM – 11:00 AM	***Galleria South*** "Updated Guide to Scientific Writing and Publishing" with Drs. A. Douglas Kinghorn (The Ohio State University), Daneel Ferreira (University of Mississippi), and Ms. Leslie Walker (ACS)
9:00 AM – 4:00 PM	***Grand Ballroom II*** (Lunch included at 12:00 PM) "Applications of NMR Beyond Structure Elucidation" with Drs. Charlotte Simmler and Guido Pauli (University of Illinois, Chicago)
11:30 AM – 1:00 PM	***Galleria South*** "Determining the mechanism of action of potential anticancer agents: beyond apoptosis" with Ms. Corena Schaffer, and Drs. April Risinger, Andrew Robles and Susan Mooberry (University of Texas, Health Sciences Center, San Antonio)
1:00 PM – 4:00 PM	***Forum Suite*** "Grant Writing Strategies" with Dr. D. Craig Hopp (NCCIH, National Institutes of Health)
1:00 PM – 5:00 PM	***Galleria North*** "Following the Path of a Natural Product Molecule (Extraction to Elucidation)" with Drs. Mark O'Neil-Johnson (Sequoia Biosciences) and Jimmy Yuk (Waters, Inc.)

12:00 PM – 5:00 PM	Exhibitor Set Up – ***Grand Ballroom Foyer***
7:00 PM – 10:00 PM	Welcome Reception – ***Grand Ballroom I – (Ticketed Event)***

Sunday July 30, 2017

7:30 AM – 6:00 PM	Registration – ***Grand Ballroom Foyer***
7:30 AM – 6:00 PM	Exhibition – ***Grand Ballroom Foyer***
7:15 AM – 8:15 AM	Continental Breakfast – ***Grand Ballroom Foyer***
8:15 AM – 8:30 AM	Welcoming remarks and announcements – ***Grand Ballroom I and II***

Grand Ballroom I and II

Symposium I - Natural Products Biosynthesis and Synthetic Biology

Chairs: Taifo Mahmud and Benjamin Philmus

8:30 AM – 9:15 AM	S-01 **Chaitan Khosla** (Stanford University) ASSEMBLY LINE BIOSYNTHESIS OF POLYKETIDE ANTIBIOTICS
9:15 AM – 10:00 AM	S-02 **Ikuro Abe** (The University of Tokyo) ENGINEERED BIOSYNTHESIS OF FUNGAL MEROTERPENOIDS
10:00 AM – 10:30 AM	Break – ***Grand Ballroom Foyer***
10:30 AM – 11:15 AM	S-03 **Huimin Zhao** (University of Illinois, Urbana-Champaign) BREAKING THE SILENCE: NEW STRATEGIES FOR DISCOVERING NOVEL NATURAL PRODUCTS
11:15 AM – 12:00 PM	S-04 **Jürgen Rohr** (University of Kentucky) POST-PKS ENZYME COMPLEXES
12:00 PM – 1:30 PM	Lunch (on your own)
12:00 PM – 1:30 PM	*Journal of Natural Products* Editorial Board Meeting (Invitation Only)

Grand Ballroom I

Session – S-PM1 - Natural Products Biosynthesis

Chair: Benjamin Philmus

1:30 PM – 1:50 PM

O-01

Steven Van Lanen (University of Kentucky)

BIOSYNTHESIS OF HIGHLY MODIFIED PEPTIDYL-NUCLEOSIDE ANTIBIOTICS INHIBITING BACTERIAL TRANSLOCASE I

1:50 PM – 2:10 PM

O-02

Hyun Bong Park (Yale University)

FUNCTIONAL CHARACTERIZATION OF A HYBRID NONRIBOSOMAL PEPTIDE SYNTHETASE-LIKE-PTERIDINE SYNTHASE BIOSYNTHETIC GENE CLUSTER

2:10 PM – 2:30 PM

O-03

Guojun Wang (Florida Atlantic University)

DEEP-SEA SPONGE NATURAL PRODUCTS: BIOSYNTHETIC GENES AND ENZYMES

2:30 PM – 2:50 PM

O-04

Jaclyn M. Winter (University of Utah)

IDENTIFICATION OF THE MANGICOL BIOSYNTHETIC GENE CLUSTER IN FUSARIUM EQUISETI CNC-477 AND CHARACTERIZATION OF ITS SESTERTERPENE SYNTHASE

Grand Ballroom II

Session S-PM2 Herbal and Plant Natural Products

Chair: Joe Chappell

1:30 PM – 1:50 PM

O-05

Joshua Kellogg (University of North Carolina at Greensboro)

STUDIES IN SIMILARITY: METABOLOMICS-BASED MULTIVARIATE CORRELATION FOR COMPARISON OF NATURAL PRODUCTS

1:50 PM – 2:10 PM

O-06

Gonzalo Rodolfo Malca-Garcia (University of Illinois at Chicago)

INVESTIGATION OF THE RESIDUAL COMPLEXITY OF RED CLOVER ISOFLAVONOIDS BY OFF-LINE CCS-qNMR

2:10 PM – 2:30 PM

O-07

Pei Chen (USDA)

FLAVONQ: AN "EXPERT SYSTEM" AUTOMATED DATA PROCESSING TOOL FOR PROFILING FLAVONOIDS USING ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY DIODE ARRAY DETECTION HIGH-RESOLUTION ACCURATE-MASS MASS SPECTROMETRY (UHPLC HRAM-MS)

2:30 PM – 2:50 PM

O-08

Fabien Schultz (Technical University of Berlin)

INVESTIGATION OF BIOACTIVE COMPOUNDS FROM MEDICINAL PLANTS COLLECTED IN THE TROPICAL RAINFORESTS OF EAST AFRICA

2:50 PM – 3:20 PM

Break – ***Grand Ballroom Foyer***

Grand Ballroom I

Session S-PM3 - Synthetic and Chemical Biology

Chair: Alessandra Eustaquio

3:20 PM – 3:40 PM

O-09

Rob J. Capon (The University of Queensland)

CANE TOAD CHALLENGE: EXPLOITING CHEMICAL ECOLOGY TO CONTROL A TOXIC INVASIVE PEST SPECIES

3:40 PM – 4:00 PM

O-10

Coran Watanabe (Texas A&M University)

CYCLORETINAL IN AGE-RELATED MACULAR DEGENERATION: ITS BIOSYNTHESIS BY THE MILK PROTEIN BETA-LACTOGLOBULIN AND ITS CATABOLISM AS A TREATMENT STRATEGY

4:00 PM – 4:20 PM

O-11

Mostafa E. Abugrain (Oregon State University)

CHEMOENZYMATIC SYNTHESIS OF NEW PACTAMYCIN ANALOGS

4:20 PM – 4:40 PM

O-12

Gilbert Gorr (Phyton Biotech GmbH)

BIOSYNTHESIS AND PRODUCTION OF ACTIVES BY PLANT CELL CULTURE

Grand Ballroom II

Session S-PM4 - Medicinal Chemistry and Drug Discovery

Chair: Tadeusz Molinski

3:20 PM – 3:40 PM

O-13

James Fuchs (Ohio State University)

LEAD OPTIMIZATION AND BIOLOGICAL EVALUATION OF CYTOTOXIC ARYLNAPHTHALENE LIGNAN LACTONE NATURAL PRODUCTS

3:40 PM – 4:00 PM

O-14

Lena Keller (University of California – San Diego)

A NATURAL PRODUCT 'MAKEOVER' – DEVELOPMENT OF PLASMODIUM-SELECTIVE PROTEASOME INHIBITORS AS ANTIMALARIAL AGENTS BASED ON CARMAPHYCIN B

4:00 PM – 4:20 PM O-15

Russell Williams (Sequoia Sciences, Inc.)

DISCOVERY AND DEVELOPMENT OF BIFIDENONE AS AN ANTICANCER DRUG LEAD

4:20 PM – 4:40 PM O-16

Paul Graupner (Dow AgroSciences)

INATREQ ™ ACTIVE – A NOVEL NATURAL PRODUCT BASED AGROCHEMICAL TO CONTROL FUNGAL INFECTIONS IN CEREALS

4:40 PM – 7:00 PM **Poster Session I – *Pavilion Ballroom and Foyer***

(P-001 – P-174)

Chair: Sandra Loesgen

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

Monday July 31, 2017

7:30 AM – 5:00 PM Registration – ***Grand Ballroom Foyer***

7:30 AM – 5:00 PM Exhibition **– *Grand Ballroom Foyer***

7:30 AM – 8:30 AM Continental Breakfast – ***Grand Ballroom Foyer***

Grand Ballroom I and II

Symposium II - Molecular Pharmacology of Natural Products and Complementary Medicine

Chairs: Fred Stevens and Sandra Loesgen

8:30 AM – 9:15 AM S-05

Prakash Nagarkatti (University of South Carolina)

UNDERSTANDING THE EPIGENETIC PATHWAYS OF REGULATION BY CANNABINOIDS AND THEIR USE AS ANTI-INFLAMMATORY AGENTS

9:15 AM – 10:00 AM S-06

Luc Pieters (University of Antwerp – 2017 Waters Award for Excellence in Natural Products Innovation)

NATURAL PRODUCTS AS PRODRUGS: SOME CASE STUDIES

10:00 AM – 10:30 AM Break

10:30 AM– 11:15 AM S-07

Amala Soumyanath (Oregon Health & Science University)

BOTANICALS AND ALZHEIMER'S DISEASE – CHOLINESTERASE INHIBITORS, ANTI-AMYLOID STRATEGIES AND BEYOND

11:15 AM – 12:00 PM S-08

Dale Nagle (University of Mississippi)

NATURAL PRODUCTS AS MOLECULAR PROBES OF BREAST TUMOR CELL METABOLISM AND METASTATIC ORGANOTROPISM

12:00 PM – 1:30 PM Lunch (on your own)

12:00 PM – 1:30 PM ASP Fellows Meeting – ***Council Suite (Invitation Only)***

Grand Ballroom I

Session M-PM1 - Plants and Neurology/Medical Marijuana

Chair: Amala Soumyanath

1:30 PM – 1:50 PM O-17

Arun Rajgopal (Amway)

GLABRIDIN, EVODIAMINE AND BOTANICAL EXTRACTS ACTIVATE AMP-ACTIVATED PROTEIN KINASE TO AUTOPHAGY POSSIBLY THROUGH mTOT, A PARKINSON'S AND ALZHEIMER'S DISEASE TARGET

1:50 PM – 2:10 PM O-18

Nam-Cheol Kim (United States Pharmacopeia)

USP'S EFFORTS FOR DEVELOPING CANNABIS PUBLIC STANDARDS

2:10 PM – 2:30 PM O-19

Chris Witowski (Alternative Medical Enterprises, LLC)

FORMULATION OF MEDICINAL CANNABIS DELIVERY DEVICES

2:30 PM – 2:50 PM O-20

Esperanza Carcache de Blanco (Ohio State University)

IDENTIFICATION OF PLANT SECONDARY METABOLITES AS NF-KB LEADS

Grand Ballroom II

Session M-PM2 - Microbial Natural Products

Chair: Sheo Singh

1:30 PM – 1:50 PM O-21

Hiroyasu Onaka (The University of Tokyo)

COMBINED-CULTURE: ACTINOMYCETES POTENTIAL SECONDARY METABOLISM IS AWAKENED BY CO-CULTURE WITH MYCOLIC ACID CONTAINING BACTERIA

1:50 PM – 2:10 PM O-22

Emily Mevers (Harvard University)

INDUCTION OF A POTENT AND SELECTIVE STAPHYLOCOCCUS AUREUS ANTIBIOTIC

2:10 PM – 2:30 PM O-23

Dong-Chan Oh (Seoul National University)

DISCOVERY OF NEW BIOACTIVE NATURAL PRODUCTS FROM INSECT SYMBIONTS

2:30 PM – 2:50 PM O-24

Jie Li (University of California - San Diego)

PRODUCTION OF NEW THIOLACTOMYCIN ANTIBIOTICS WITH POTENTIATED BIOACTIVITY BY ENZYMATIC TANDEM CARBOXYLATION-AMIDATION

Grand Ballroom I

Session M-PM3 - Dietary Supplements

Chair: Craig Hopp

2:50 PM – 3:10 PM O-25

Joseph Betz (NIH)

OVERVIEW OF THE 2017-2021 STRATEGIC PLAN FOR THE NIH OFFICE OF DIETARY SUPPLEMENTS

3:10 PM – 3:30 PM O-26

Mitzi Nagarkatti (University of South Carolina)

INDOLE-3-CARBINOL ATTENUATES COLITIS THROUGH ALTERATIONS IN GUT MICROBIOTA AND METABOLIC PATHWAYS

3:30 PM – 3:50 PM O-27

Teresa Horm (Unigen)

AMLEXIN, A BOTANICAL COMPOSITION, DEMONSTRATES EFFICACY IN MULTIPLE MODELS OF ARTHRITIS DISEASE PROGRESSION AND IN A HUMAN CLINICAL TRIAL

3:50 PM – 4:10 PM O-28

Debasis Bagchi (University of Houston)

CLINICAL EVALUATION OF A STANDARDIZED PRUNUS DOMESTICA EXTRACT ON BENIGN PROSTRATE HYPERPLASIA (BPH) IN MALE VOLUNTEERS

Grand Ballroom II

Session M-PM4 - Fungal Natural Products

Chair: James Gloer

2:50 PM – 3:10 PM

O-29

Andrea Stierle (University of Montana)

THE BERKELEYLACTONES, ANTIBIOTIC MACROLIDES FROM FUNGAL COCULTURE

3:10 PM – 3:30 PM

O-30

Christine Salomon (University of Minnesota)

ANTIFUNGAL DISCOVERY FOR THE TREATMENT OF WHITE NOSE SYNDROME (WNS) IN BATS

3:30 PM – 3:50 PM

O-31

Sandra Loesgen (Oregon State University)

BIOTRANSFORMATION OF THE HDAC INHIBITOR VORINOSTAT YIELDS NEW ANILINE –CONTAINING FUNGAL METABOLITES

3:50 PM – 4:10 PM

O-32

Andrew Robles (UT Health San Antonio)

IDENTIFICATION AND MECHANISTIC STUDIES OF A FUNGAL METABOLITE WITH SELECTIVE CYTOTOXIC ACTIVITY AGAINST EWING SARCOMA CELLS

4:10 PM – 6:30 PM

Poster Session II – Pavilion Ballroom and Foyer

(P-175 – P-346)

Chair: Nadja Cech

(All posters still up by 6:30 PM will be moved to Broadway Rooms and need to be picked up by Tuesday 9:00 AM)

6:30 PM

Bus Loading for OMSI

7:00 PM – 10:00 PM

Oregon Museum of Science and Industry (Dinner - Ticketed Event)

1945 SE Water Avenue

Portland, OR 97214

Directions to OMSI

Tuesday August 1, 2017

8:00 AM – 5:00 PM	Registration – ***Grand Ballroom Foyer***
7:30 AM – 10:30 AM	Exhibition – ***Grand Ballroom Foyer***
7:30 AM – 8:30 AM	Continental Breakfast – ***Grand Ballroom Foyer***
10:30 AM – 3:00 PM	Exhibition Dismantling – ***Grand Ballroom Foyer***

Grand Ballroom I and II

Symposium III - Natural Products from Unique Ecosystems

Chairs: Kerry McPhail and Taifo Mahmud

8:30 AM – 9:15 AM	S-09 **Bill Baker** (University of South Florida) CHEMISTRY AND BIOACTIVITY OF ANTARCTIC MARINE INVERTEBRATES
9:15 AM – 10:00 AM	S-10 **Julia Kubanek** (Georgia Institute of Technology) METABOLOMIC APPROACHES FOR UNDERSTANDING THE ECOLOGICAL IMPACTS OF NATURAL PRODUCTS IN MARINE SYSTEMS
10:00 AM–10:30 AM	Break
10:30 AM – 11:15 AM	S-11 **Pei-Yuan Qian** (Hong Kong University of Science and Technology) GENOME-MINING COUPLED WITH MANIPULATION OF BIOSYNTHESIS PATHWAYS AS A POWERFUL TOOL FOR BIOACTIVE COMPOUND DISCOVERY
11:15 AM – 12:00 PM	S-12 **Ryuichi Sakai** (Hokkaido University) BIOACTIVE METABOLITES FROM WATER-SOLUBLE MARINE EXTRACTS
12:00 PM – 1:30 PM	Lunch (on your own)

Grand Ballroom I

Session T-PM1 - Molecular Pharmacology of Natural Products

Chair: Dale Nagle

1:30 PM – 2:00 PM

O-33

David Kingston (Virginia Tech University)

GOLD NANOPARTICLES FOR DRUG DELIVERY OF PACLITAXEL AND DOXORUBICIN

2:00 PM – 2:20 PM

O-34

Stephen Polyak (University of Washington)

NETWORK PERTURBATIONS THAT UNDERLIE ANTI-INFLAMMATORY BIOACTIVITY OF NATURAL PRODUCT COMBINATIONS

2:20 PM –2:40 PM

O-35

Cassandra Quave (Emory University)

SENSITIZATION OF BETA-LACTAM ANTIBIOTICS WITH RESISTANCE MODIFYING AGENTS FROM A MEDICINAL PLANT

2:40 PM – 3:00 PM

O-36

Charlotte Simmler (University of Illinois at Chicago)

IDENTITY AND PURITY VERIFICATION OF COMMERCIAL COMPOUNDS IS ESSENTIAL FOR THE ACCURACY OF BIOLOGICAL RESULTS

Grand Ballroom II

Session T-PM2 - Advanced Technologies in Natural Products Research

Chairs: Roger Linington and John MacMillan

1:30 PM – 2:00 PM

O-37

Marnix Medema (Wageningen University)

NEW COMPUTATIONAL APPROACHES TO NATURAL PRODUCT DISCOVERY IN PLANTS AND MICROBES

2:00 PM – 2:20 PM

O-38

Giorgis Isaac (Waters Corporation)

CHEMICAL ANALYSIS AND ADULTERANT CHARACTERIZATION OF ELEUTHEROCOCCUS SENTICOSUS AND CI-WU-JIA TEA BY UHPLC UV-MS USING NOVEL INFORMATICS PLATFORM

2:20 PM – 2:40 PM

O-39

Kenji Kurita (Genentech Research and Early Development)

BRIDGING THE GAP BETWEEN MOUNTAINS OF DATA

2:40 PM – 3:00 PM

O-40

Brian Murphy (University of Illinois at Chicago)

MALDI-TOF MS-BASED TAXONOMIC AND METABOLOMIC PROFILING FOR INFORMED MICROBIAL LIBRARY GENERATION

3:00 PM– 3:20 PM Break – ***Grand Ballroom Foyer***

Grand Ballroom I

Session T-PM3 - Plant Natural Products

Chair: Angela Hoffman

3:20 PM – 3:40 PM O-41

K. Brian Killday (Bruker)

NMR CHEMOMETRICS: DEVELOPMENT OF AUTOMATED MULTICLASS CLASSIFICATION METHODS FOR BOTANICALS

3:40 PM – 4:00 PM O-42

Anna Berim (Washington State University)

ELUCIDATION OF METHOXYLATED FLAVONE BIOSYNTHESIS IN SWEET BASIL AND USE OF UNDERLYING ENZYMES TO FOR FLAVONOID BIODIVERSIFICATION

4:00 PM – 4:20 PM O-43

Angela Calderon (Auburn University)

LC-MS-BASED STRATEGY FOR SCREENING OF PASSIVELY ABSORBED AÇAÍ AND MACA EXTRACT CONSTITUENTS FOR CYP3A4 INHIBITION

4:20 PM – 4:40 PM O-44

Xavier Siwe Noundou (Rhodes University)

A TRIP TO THE AFRICA RAINFOREST: SCIENTIFIC RATIONALE OF SOME MEDICINAL PLANTS

Grand Ballroom II

Session T-PM4 - Natural Products from Unique Sources

Chair: Tim Bugni

3:20 PM – 3:40 PM O-45

Lilibeth Salvador-Reyes (University of the Philippines)

ANTIMALARIAL COMPOUNDS FROM PHILIPPINE SPONGE-ASSOCIATED MICROORGANISMS

3:40 PM – 4:00 PM O-46

Shugeng Cao (University of Hawai'i at Hilo)

BACTERIAL METABOLITES AND LARVAL RECRUITMENT FOR BENTHIC MARINE COMMUNITIES

4:00 PM – 4:20 PM O-47

Matthew Bertin (University of Rhode Island)

CHEMICAL PROFILING OF A TRICHODESMIUM BLOOM FROM THE GULF OF MEXICO REVEALS A BIOSYNTHETICALLY INTRIGUING METABOLOME

4:20 PM – 4:40 PM	O-48 **Laura Sanchez** (University of Illinois at Chicago) BACTERIAL COMMUNICATION IN SITU: A TALE OF TWO VIBRIOS
4:40 PM –	Free evening (Except for Younger Members)
6:30 PM	**Walk to Younger Members Event at Punch Bowl Social** 340 SW Morrison Street Portland, OR 97204 Directions to Punch Bowl Social
7:00 PM – 10:00 PM	**Younger Members Event – *Punch Bowl Social*** (Ticketed Event)

Wednesday August 2, 2017

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

Grand Ballroom I and II
Award Symposium

8:10 AM – 9:00 AM	S-13 **Guy Carter** (Biosortia Pharmaceuticals – Norman R. Farnsworth Achievement Award Lecture) NATURAL PRODUCTS IN PHARMA 1980-2010: A PERSONAL PERSPECTIVE
9:00 AM – 9:50 AM	S-14 **Richard Van Breemen** (University of Illinois at Chicago – Varro Tyler Prize Award Lecture) FROM BOTANICAL AUTHENTICATION THROUGH CLINICAL EVALUATION, SAFETY AND EFFICACY OF BOTANICAL DIETARY SUPPLEMENTS
9:50 AM – 10:20 AM	Break – ***Grand Ballroom Foyer***
10:20 AM – 11:00 AM	S-15 **Katherine S. Ryan** (University of British Columbia – Matt Suffness Young Investigator's Award Lecture) USING COFACTORS IN NEW WAYS IN NATURAL PRODUCTS BIOSYNTHESIS
11:00 AM – 11:20 AM	S-16 **Fatma Alawadhi** (University of Florida – Kilmer Prize Award) GRASSYSTATINS D-F, POTENT ASPARTIC PROTEASE INHIBITORS FROM MARINE CYANOBACTERIA AS POTENTIAL ANTIMETASTATIC AGENTS TARGETING INVASIVE BREAST CANCER

11:20 AM – 11:40 AM S-17

Samantha Gromek (University of Connecticut – Student Research Award)

ANTIFUNGAL LEAD DISCOVERY FROM BACTERIA ASSOCIATED WITH THE HAWAIIAN BOBTAIL SQUID TO TREAT FUSARIAL INFECTIONS

11:40 AM – 12:00 PM S-18

Siddharth Tripathi – (University of Mississippi – John Faulkner Travel Award)

A SMALL MOLECULE SCREEN IDENTIFIES AN Hsp90 INHIBITOR THAT DISRUPTS THE FUNGAL CELL WALL INTEGRITY PATHWAY

12:00 PM – 1:30 PM Lunch (on your own)

Grand Ballroom I

Session W-PM1 – The Future of ASP I

Chair: Chris Thornburg

1:30 PM – 1:45 PM O-49

Tiago Leao (University of California – San Diego)

OMICS APPROACH FOR ACCESSING THE NATURAL PRODUCT UNIVERSE OF MARINE CYANOBACTERIA

1:45 PM – 2:00 PM O-50

Andrew Osborn (Oregon State University)

EVOLUTION OF C_7-CYCLITOL SYNTHASES AND THEIR DISTRIBUTION THROUGHOUT PROKARYA AND EUKARYA

2:00 PM – 2:15 PM O-51

Erin P. McCauley (University of California Santa Cruz)

BIOACTIVE NATURAL PRODUCTS FROM INDO-PACIFIC MARINE SPONGES

2:15 PM – 2:30 PM O-52

Danielle Demers (University of South Florida)

UNCOVERING THE CHEMICAL DIVERSITY OF A LIBRARY OF EPIGENETICALLY MODIFIED FUNGAL EXTRACTS

2:30 PM – 2:45 PM O-53

Matt McErlean (University of Kentucky)

INDUCIBLE ACTINOMYCIN HETEROCYCLE CAUSES CHANGES IN ACTIVITY AND SHOWN IN MULTIPLE ACTINOMYCIN FAMILIES

2:45 PM – 3:00 PM O-54

Mary Choules (University of Illinois at Chicago)

ANTI-M.TB RUFOMYCIN IN COMBINATION WITH CLPC1 TARGETING COMPOUNDS

3:00 PM – 3:15 PM

O-55

Jarmo-Charles Kalinski (Rhodes University, South Africa)

BIOLOGICAL ACTIVITY OF PYRROLOIMINOQUINONE ALKALOIDS FROM SOUTH AFRICAN LATRUNCULID SPONGES

3:15 PM – 3:30 PM

O-56

Seoung Rak Lee (Sungkyunkwan University, Republic of Korea)

ISOFLAVONOID GLYCOSIDES FROM THE TERMITE-ASSOCIATED *STREPTOMYCES* SP. RB1

Grand Ballroom II

Session W-PM2 - The Future of ASP II

Chair: Angela Calderon

1:30 PM – 1:45 PM

O-57

Emily Rue (University of Illinois at Chicago)

PROCYANIDINS: IDENTIFICATION AND ANALYSIS USING ION MOBILITY MASS SPECTROMETRY

1:45 PM – 2:00 PM

O-58

Vaclav Vetvicka (University of Louisville)

ANTI-STRESS ACTION OF AN ORALLY-GIVEN COMBINATION OF RESVERATROL, BETA-GLUCAN, AND VITAMIN C

2:00 PM – 2:15 PM

O-59

Zhibin Liang (University of Hawaii at Manoa)

COMPUTER-AIDED DRUG DISCOVERY OF SELECTIVE GSK3B INHIBITORS INSPIRED BY NATURAL PRODUCTS FOR ALZHEIMER'S DISEASE

2:15 PM – 2:30 PM

O-60

Stephen Teo (University College London)

ANTIBACTERIALS FROM THE TROPICAL RAIN FORESTS OF BORNEO

2:30 PM – 2:45 PM

O-61

Ean-Jeong Seo (Johannes Gutenberg University)

BOTH PHENOLIC AND NON-PHENOLIC GREEN TEA FRACTIONS INHIBIT MIGRATION OF CANCER CELLS

2:45 PM – 3:00 PM

O-62

Tehane Ali (The Ohio State University)

IDENTIFICATION OF NEW AND BIOACTIVE COMPOUNDS FROM FERMENTATION OF THE ENDOPHYTIC FUNGUS PENICILLIUM CONCENTRICUM

3:00 PM – 3:15 PM

O-63

Richard Tehan (Oregon State University)

EVOLUTIONARY METABOLOMICS IN TOLYPOCLADIUM FUNGI TO GUIDE NATURAL PRODUCTS DISCOVERY

3:15 PM – 3:30 PM	O-64 **Yixuan Xia** (Hong Kong Baptist University) ANTICANCER EFFECTS OF MILIUSANES ON COLORECTAL CANCER CELLS
3:45 PM – 5:30 PM	ASP General Meeting – ***Grand Ballroom II***
6:00 PM – 7:00 PM	Closing Reception – ***Grand Ballroom Foyer***
7:00 PM – 10:00 PM	Closing Ceremony and Banquet – ***Grand Ballroom I and II*** (Ticketed Event)

Symposium Presentations

S-01

ASSEMBLY LINE BIOSYNTHESIS OF POLYKETIDE ANTIBIOTICS

Chaitan Khosla
Stanford University, Stanford CA 94305

Many complex natural products, such as polyketide antibiotics, are synthesized by multi-enzyme systems that operate as assembly lines. Their apparently modular architecture has opened the door to engineering of new antibiotics by rationally manipulating the DNA encoding these megasynthases. Understanding the structures and mechanisms of these assembly lines represents a challenging and exciting frontier. Recent progress toward this goal will be discussed.

S-02

ENGINEERED BIOSYNTHESIS OF FUNGAL MEROTERPENOIDS

Ikuro Abe
Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113-0033, Japan

Meroterpenoids are hybrid natural products that are partially derived from terpenoid origin, and those from fungi exhibit an extremely wide range of structural diversity and biological activities. Recent advances in genome sequencing technologies and development of tools for biosynthetic studies have allowed the discovery of many biosynthetic gene clusters for fungal meroterpenoids and intensive researches at genetic and enzymatic level. We have been working on the meroterpenoids derived from a simple aromatic precursor, 3,5-dimethylorsellinic acid (DMOA), and discovered several fascinating enzymes that catalyze drastic structural rearrangement which dramatically increase structural complexity of the molecules. For example, multifunctional, nonheme iron-dependent dioxygenases are the key components in the austinol and the anditomin pathway, in which the enzymes are responsible for the construction of the spiro-lactone and the bicyclo[2.2.2]octane core, respectively. On the other hand, the terretonin biosynthesis involves a cytochrome P450 and an isomerase, which work collaboratively to perform the unprecedented ring expansion reaction to afford the terretonin scaffold. This presentation will focus on our recent advances in engineered biosynthesis of complex fungal meroterpenoids.

S-03

BREAKING THE SILENCE: NEW STRATEGIES FOR DISCOVERING NOVEL NATURAL PRODUCTS

Huimin Zhao
Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Microorganisms are a major source of new therapeutic agents. My group has been developing new genomics-driven, synthetic biology-enabled strategies to discover and produce novel natural products from sequenced genomes and metagenomes. One strategy is to refactor target cryptic gene clusters in heterologous hosts. As proof of concept, we used this strategy to awaken the silent polyketide spectinabilin pathway from *Streptomyces spectabilis* in *Streptomyces lividans* and activate a cryptic pathway containing a polyketide synthase-non-ribosomal peptide synthetases from *Streptomyces grieseus* in *Streptomyces lividans*, which led to the discovery of two novel tetramic acid natural products that have never been reported in literature. To increase the throughput, we are establishing a fully integrated robotic system to automate all the steps in gene cluster refactoring and product detection. A second strategy is to activate the target cryptic gene clusters in their native hosts by knocking-in strong promoters upstream of the target cryptic gene clusters using a CRISPR/Cas9 system. We successfully activated more than 10 cryptic gene clusters from five different Streptomyces and uncovered a number of novel natural products. A third distinct yet complementary strategy is to express the uncharacterized biosynthetic gene clusters in heterologous hosts using a direct cloning method based on artificial restriction enzymes.

S-04

POST-PKS ENZYME COMPLEXES

Jürgen Rohr
Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506, USA

PKS complexes are well known and thoroughly studied. However, the post-PKS tailoring steps are still often viewed a linear sequence of events catalyzed by single, independent enzymes. This picture is rapidly changing, since more and more multiple-function enzymes and/or co-dependent enzymes were discovered in context with post-PKS tailoring steps. For example, multiple function enzymes as well as post-PKS enzyme complexes of were observed in the tailoring steps of mithramycin and gilvocarcin biosyntheses. To achieve the unique benzo [*d*]naphtho[1,2-*b*] pyran-6-one chromophore of the gilvocarcins, polyketide biosynthesis is carried out by a type II polyketide synthase (PKS), and a post-PKS enzyme complex is proposed to convert an angucyclinone intermediate (dehydrorabelomycin) into the defuco-gilvocarcin chromophore. Its components, consisting of GilOII, GilM and GilR, established a complicated and intriguing collaboration to share an FAD co-factor necessary for all three enzymes. While the basic functions of these enzymes have been determined, a more complete biochemical characterization is still needed to understand their interplay and substrate channeling. Likewise, the two crucial saccharide chains as well as the highly functionalized pentyl side chain of mithramycin, all hallmarks of the pharmacophore of the aureolic acids anticancer drugs, are assembled using post-PKS enzyme complexes, in which its key components also play multiple roles.

S-05

UNDERSTANDING THE EPIGENETIC PATHWAYS OF REGULATION BY CANNABINOIDS AND THEIR USE AS ANTI-INFLAMMATORY AGENTS

Prakash Nagarkatti[1]
Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29223

Studies from our lab have shown that cannabinoids, including Δ(9)-tetrahydrocannabinol (THC) and cannabidiol (CBD), exert potent anti-inflammatory properties and attenuate autoimmune diseases through multiple pathways: 1) Switch from Th1 to Th2, 2) Induction of Tregs, 3) Induction of apoptosis and 4) Promotion of myeloid-derived suppressor cells (MDSCs). Recently we noted that epigenetic regulation of the immune response genes, by cannabinoids, plays a critical role in mediating anti-inflammatory effects. THC treatment caused histone activation related to Th2 cytokine genes and repressive modification signals to Th1 cytokine genes, thereby inducing a switch from Th1 to Th2. We also noted that the induction of MDSCs by THC was regulated by epigenetic changes including induction of unique microRNA. THC caused hypermethylation at the promoter region of DNMT3a and DNMT3b in MDSCs, which correlated with reduced expression of DNMT3a and DNMT3b. Furthermore, promoter region methylation was decreased at Arg1 and STAT3 in THC-induced MDSCs. THC also suppressed miRNA-17/92 cluster, which targeted Pten, an inhibitor of the PI3K/Akt signaling pathway, thereby inducing T-regulatory cells. Altogether, our study demonstrates that cannabinoids may modulate immune response through multiple epigenetic pathways involving

histone modifications, DNA methylation as well as microRNA induction (Supported by NIH grants P01AT003961, R01AT006888, R01AI123947, R01AI129788, R01ES019313, R01MH094755, P20GM103641).

S-06

NATURAL PRODUCTS AS PRODRUGS: SOME CASE STUDIES

Luc Pieters
Natural Products and Food Research & Analysis (NatuRA), Department of Pharmaceutical Sciences, University of Antwerp, Belgium

Natural products are often prodrugs, e.g. glycosides, which must undergo *in vivo* metabolic conversion (activation). A *Gloriosa superba* seed extract containing colchicine, a well-known cytotoxic compound, 3-*O*-demethylcolchicine and its glycoside colchicoside, was found to be active in a murine pancreatic tumor model. Also a colchicoside-rich / colchicine-poor extract with the same total level of colchicine and derivatives was active in the same model, indicating that colchicoside can be considered as a prodrug. The activity of gemcitabine, a drug widely used against pancreatic cancer, could be improved by combining it with a *Gloriosa superba* seed extract.

Extracts of the herb *Herniaria hirsuta* are traditionally used in Morocco against kidney and gall stones. Prolonged use of a *H. hirsuta* extract resulted in a cholesterol-lowering effect in the bile of dogs, a pharmacological effect that can prevent the formation of gallstones and can contribute to dissolving existing gallstones. Saponins (medicagenic acid glycosides) have been hypothesized as active principles, but before absorption they need to be deglycosylated. The aglycones (or metabolites thereof) can be absorbed, and may further be metabolized to the ultimate active molecules.

Therefore, an *in vitro* gastro-intestinal dialysis model (GIDM) was developed, including microbial fermentation in the colon, to mimic human biotransformation processes. Cyclopeptide alkaloids will be discussed as an example.

S-07

BOTANICALS AND ALZHEIMER'S DISEASE – CHOLINESTERASE INHIBITORS, ANTI-AMYLOID STRATEGIES AND BEYOND.

Amala Soumyanath
Department of Neurology, Oregon Health & Science University, Portland, OR 97239, USA.

Alzheimer's Disease (AD), the most common cause of dementia, currently affects 5.3 million adults in the USA. Recent increases in federal funding for AD research recognize the urgent need for better treatments for this disease. Current FDA approved drugs for AD are either cholinesterase inhibitors or glutamate receptor antagonists. Botanicals have already provided molecular templates (physostigmine) and ligands (galantamine, huperzine A) for these targets. However, while these approaches improve neurotransmission in AD, they do not limit disease progression. Natural products have therefore been explored as means of reducing brain β-amyloid plaques, a hallmark of AD. However, in recent trials, anti-amyloid immunological agents have failed to show clinical cognitive benefits. The toxic sequelae of β-amyloid deposition have now been proposed as more relevant targets to limit AD progression. The targets include other known features of AD such as hyperphosphorylated tau protein, mitochondrial dysfunction, oxidative stress, and loss of synapses in affected brain regions. Using multiple *in vivo* and *vitro* approaches, we have found that *Centella asiatica* (CA), a traditional herbal memory enhancer, not only improves cognition, but can improve mitochondrial activity, antioxidant response and dendritic arborization, and decrease tau phosphorylation. Active compounds in CA include triterpenes and caffeoylquinic acids. These studies will be described both to illustrate models applicable to the study of anti-AD botanicals, and to showcase CA's potential as a disease-modifying, botanical agent for AD.

S-08

NATURAL PRODUCTS AS MOLECULAR PROBES OF BREAST TUMOR CELL METABOLISM AND METASTATIC ORGANOTROPISM.

Yu-Dong Zhou[1,2,3] and Dale G. Nagle[1,2,4]
[1]Institute of Interdisciplinary Integrative Biomedical Research, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China, [2]Marine Biopharmaceutical Research Center, School of Marine Sciences, Ningbo University, Ningbo 315211, China, [3]Department of Chemistry and Biochemistry and [4]Department of Biomolecular Sciences and RIPS, School of Pharmacy, University of Mississippi, University, MS 38677-1848

Breast tumors reprogram their cellular energy metabolism, nutrient uptake and utilization-associated biochemical processes. These processes become further transformed as genetically predisposed metastatic breast tumor cells colonize specific organs. Breast tumor cells often specifically metastasize to the brain, bone, lung and liver. Massagué and collogues isolated organotropic subclones and established organ-specific gene signatures associated with lung-, bone-, and brain-specific metastatic triple-negative breast cancer (TNBC) MDA-MB-231 cells. Using these genetically characterized metastatic subclones specific to lung (LM4175), bone (BoM1833), and brain (BrM-2a), we evaluated natural products for the ability to selectively suppress metastatic breast cancer cells in a target organ-dependent manner. Natural product-based metabolic regulators were used to demonstrate that metastatic subclone organotropism is accompanied by a corresponding bioenergetic and metabolic adaptation to specific organs. Similarly, through the evaluation of marine natural products, histone deacetylase (HDAC) inhibitors were found to disrupt organotropic metastatic TNBC subclone growth and differentiation. These breast tumor metabolism and epigenetic dysregulation studies are yielding potential new directions for anti-metastatic breast tumor research and drug discovery.

S-09

CHEMISTRY AND BIOACTIVITY OF ANTARCTIC MARINE ORGANISMS

Bill J. Baker,[1,2] Charles D. Amsler,[3] James B. McClintock,[3] Nerida G. Wilson,[4] Dennis E. Kyle[2,5] and Lindsey N. Shaw.[2,6]
Departments of [1]Chemistry, [5]Global Health and [6]Cell Biology, Microbiology and Molecular Biology, and [2]Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL; [3]Department of Biological Sciences, University of Alabama at Birmingham, Birmingham, AL; Western Australian Museum, Perth, Australia.

Antarctica is a continent of enigmas. Stunning geographic beauty belies its inhospitable climate. Covered a mile thick in ice, it is the world's largest desert. Fossil ferns found in its mountains speak of its prehistory as a tropical rainforest, but now is largely devoid of life. Its most famous inhabitant, the penguin, is thought of as a flightless bird, but soars underwater much as a falcon glides the sky. Perhaps one of the greatest enigmas is the contrast between the terrestrial and marine environments. On land, monochromatic snow and ice support little life, yet the sea teams with life, life that expresses itself with the full rainbow of colors.

Color is but one manifestation of chemical ecology. The Antarctic benthos supports an extensive community of predators and prey, competitors and facilitators. A harsh geographic history has contributed to marine diversification and enhanced what we now recognize as a rich flora and fauna, commensurate in some instances with temperate kelp forests and even approaching the richness of tropical marine environments. Not surprisingly, Antarctic benthic ecology

is highly dependent on chemical mediation of interspecific interactions, interweaving chemodiversity with biodiversity in a classical yin and yang feedback loop. The evolution of selective chemical defenses facilitates drug discovery research, producing suites of metabolites that inform structure-activity studies and add breadth to bioactivity profiles. This presentation will focus on recent and contextual research from our lab which has demonstrated the potential for new biomedical leads and scaffolds from these difficult to access biological resources.

S-10

METABOLOMIC APPROACHES FOR UNDERSTANDING THE ECOLOGICAL IMPACTS OF NATURAL PRODUCTS IN MARINE SYSTEMS

Julia Kubanek[1,2], Remington X. Poulin[2], Kelsey Poulson-Ellestad[1], Emily Schwartz[1], Facundo Fernandez[2], Marc Weissburg[1]
[1]School of Biological Sciences and [2]School of Chemistry & Biochemistry, Aquatic Chemical Ecology Center, Georgia Institute of Technology, Atlanta, GA 30332,

Among the many pressures that marine organisms face, intense competition and predation have contributed to the evolution of chemical defenses and the ability to sense chemical cues. Chemical ecologists and natural product chemists have long sought to understand the identities, functions, and consequences of these compounds in the marine environment. However, traditional approaches including bioassay-guided fractionation have often led to unsatisfying outcomes, especially for cases in which chemical cues and signaling molecules are waterborne and unstable; yields are low or variable; multiple compounds act synergistically or additively; and bioassays are labor-intensive or consume considerable amounts of compound. We have developed a metabolomics-based strategy to take advantage of the natural variation in production of chemical cues across different environmental conditions towards identifying ecologically important waterborne molecules. We have also applied metabolomics to decipher mechanisms of action of allelopathic compounds in marine systems. As expected, marine organisms respond to a diversity of chemical species in their watery worlds, exhibiting dramatic behavioral and physiological changes when exposed to predators and competitors.

S-11

GENOME-MINING COUPLED WITH MANIPULATION OF BIOSYNTHESIS PATHWAYS AS A POWERFUL TOOL FOR BIOACTIVE COMPOUND DISCOVERY

Pei-Yuan Qian
Division of Life Science and Environmental Science Programs, Hong Kong University of Science and Technology, Hong Kong SAR

Genome-mining based bioactive compound discovery coupled with biosynthesis pathways manipulation allows us to reveal novel compounds and unusual biosynthetic pipeline at an unprecedented rate. Our genome-mining based compound discovery was triggered by the discovery of a didemnin-producing marine bacterium *Tistrella mobilis* and then didemnins biosynthesis pathways. Analysis of genomes of the bacteria related to the genus *Tistreila* led to discovery a group of calpain inhibitors (thalassospiramides) and relevant biosynthesis pathways but more importantly, the remarkable plasticity of the thalassospiramide NRPS-PKS assembly line that generates amazing chemical diversity. Using the same approach and techniques, we also successfully discovered and elucidated the chemical structure of several precolibactins of *Escherichia coli*, including the largest and most complete precolibactin-886 of colibactin family isolated and characterized to date. The individual gene inactivation study indicated that the introduction of the aminomalonate unit to precolibactin-886 is catalyzed by the modular polyketide synthase ClbKpks and a divergent colibactin biosynthesis pathway generates diverse colibactins. More recently, we analyzed 5585 complete bacterial genomes spanning the entire Domain of bacteria, using a network associated genome mining platform that links the chemical building blocks to components associated with biosynthetic gene clusters. We revealed the hidden biosynthetic logic of D-amino acids containing nonribosomal peptides (DNRPs). Through further chemical and enzymatic analysis, we demonstrated the D-amino acid specific resistance mechanism of DNRP antibiotics and reveal both the widespread distribution and broad-spectrum potential of D-stereospecific resistant peptidases, which provides an early warning of antibiotic resistance when developing peptide antibiotics.

S-12

BIOACTIVE METABOLITES FROM WATER-SOLUBLE MARINE EXTRACTS

Ryuichi Sakai
Faculty of Fisheries Sciences Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041-8611, Japan

Exploration of aqueous extracts of marine benthic organisms resulted in discovery of highly unusual molecules ranging from small aromatics to proteins. Here, I overview our recent progress in isolation of small acetylcholinesterase inhibitors from solitary tunicate *Cnemidocarpa irene*,[1] putative serotonin receptor ligands mellpaladines from the Palauan Didemnidae tunicate,[2] and KB343, an unusual guanidine alkaloid from a *Epizoanthus* sp. I also introduce a discovery of an intriguing protein, soritesidine (SOR), from the Okinawan marine sponge *Spongosorites* sp. SOR is a 120kD protein with very potent cytotoxicity. We determined its whole amino acid sequence and found it has AB-toxin like motif. AB-toxins are widely known as bacterial virulent factors such as diphtheria toxin, but have never been reported from marine organisms. Isolation, biological activities and structural feature will be discussed.

1. Tadokoro, Y. *et.al. ACS Omega* **2017,** *2* (3), 1074-1080. 2. Uchimasu, H. *et.al. Tetrahedron* **2016,** *72* (45), 7185-7193.

S-13

NATURAL PRODUCTS IN PHARMA 1980-2010: A PERSONAL PERSPECTIVE

Guy T. Carter
Biosortia Pharmaceuticals, 350 Phillips Hill Rd, New City, NY 10956

In 1980, Pharma NP programs were already well beyond the "Golden Age of Antibiotic Discovery". As antibiotics discovery was de-emphasized, new applications for NP were explored. Lederle Laboratories (American Cyanamid) focused its human health efforts on anti-cancer agents, while devoting half of its resources to new chemistry for agricultural products. Cyanamid's agricultural division was exceptionally receptive, resulting in: nemadectin (Moxidectin®), dioxapyrrolomycin (chlorfenapyr), and ganefromycin. Lederle in the 1980's remained focused on NP discovery with the conviction that these would continue to provide a viable platform for new products. In this context we began to explore marine microorganisms (Valerie Bernan) as a source for novel NP. This work was facilitated by the NCDDG (Ireland/Andersen) and ICBG (Barrows) programs. After Wyeth acquired Cyanamid in 1994, a retrospective program was launched to re-evaluate Lederle's antibiotic assets. The chemistry and biology of several antibiotics were explored: mannopeptimycin (Haiyin He), muraymycin (Leonard McDonald) and saccharomicin (Fangming Kong). Rapamycin is an immunosuppressant drug, however there is a strong belief that it has greater potential in human medicine. In 2005, Wyeth engaged Biotica, a UK-based biotech, to pursue biosynthetic approaches to new rapalogs. Fascinating new structures were unraveled (McDonald /Gerhard Schlingmann). Taking another approach, Edmund Graziani led a team that synthesized ILS-920, a non-immunosuppressive rapalog with neuroprotective activity and novel MOA. In 2010, Wyeth's NP function

was incorporated into Pfizer's Worldwide Medicinal Chemistry, where it's assets continue to play a role in drug discovery.

S-14

FROM BOTANICAL AUTHENTICATION THROUGH CLINICAL EVALUATION, SAFETY AND EFFICACY OF BOTANICAL DIETARY SUPPLEMENTS

Richard B. van Breemen
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, Univ. of Illinois College of Pharmacy, Chicago, IL 60612

The development of standardized botanical dietary supplements followed by preclinical and clinical assessments of their safety and efficacy require a multidisciplinary effort. Since the founding of our botanical center under the leadership of Norman Farnsworth, our team has pursued a stepwise approach to the development, evaluation and production of safe, reproducible and effective botanical dietary supplements. This approach includes acquisition and authentication of botanical materials, studies of mechanisms of action, identification of active compounds, chemical and biological standardization, investigation of the metabolism and bioavailability of active compounds, GMP production of a standardized formulation, and concludes with clinical studies of safety and efficacy. For 30 years, all of our natural product studies have relied upon LC-MS/MS and high resolution MS. We utilize high resolution MS to characterize new natural products, active compounds and human metabolites, and we rely on UHPLC-MS/MS for quantitative analysis during chemical standardization, studies of pharmacokinetics and drug-botanical interactions, and measuring biomarkers of efficacy. A noteworthy innovation for identifying pharmacologically active natural products has been the invention of the MS-based screening approach, pulsed ultrafiltration LC-MS. A faster alternative to bioassay-guided fractionation, PUF-MS enables rapid identification of ligands to therapeutically important receptors in complex botanical extracts. Highlights of our research on botanical dietary supplements will be discussed including studies of botanical alternatives to hormone therapy for menopausal women and effects of lycopene in a tomato oleoresin in the prevention of prostate cancer in men.

S-15

USING COFACTORS IN NEW WAYS IN NATURAL PRODUCTS BIOSYNTHESIS

Katherine S. Ryan
Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, V6T 1Z1, Canada.

Organic and metal-based cofactors are widely employed in natural products biosynthesis to construct new molecules. Through our studies on the biosynthesis of natural products, my research group recently identified new roles for two well-known cofactors. First, through our studies of the biosynthesis of the antibiotic indolmycin, we discovered an enzyme that uses the organic cofactor pyridoxal phosphate (PLP) together with molecular oxygen to form a double bond across an unactivated carbon-carbon bond in L-arginine, ultimately leading to production of the indolmycin precursor D-dehydroarginine. This result on Ind4 suggests that PLP has the previously unexpected ability to use oxygen to catalyze challenging oxidation reactions. Second, we investigated the biosynthetic pathway to piperazate, which is a cyclic amino acid containing a nitrogen-nitrogen bond and a precursor to diverse non-ribosomal peptide molecules. We discovered that a widely distributed piperazate synthase enzyme employs a heme cofactor to activate a hydroxylamine precursor for nitrogen-nitrogen bond formation, giving piperazate. This result is a significant step forward in understanding mechanisms for heme-dependent nitrogen-nitrogen bond formation in nature. In this talk, I will describe the studies that led to identification of these enzymes, the *in vitro* work that allowed us to elucidate their functions, the implications for our understanding of enzyme catalysis, and the potential applications of our work in development of biocatalysts and the identification of new natural product drugs.

S-16

GRASSYSTATINS D–F, POTENT ASPARTIC PROTEASE INHIBITORS FROM MARINE CYANOBACTERIA AS POTENTIAL ANTIMETASTATIC AGENTS TARGETING INVASIVE BREAST CANCER

Fatma H. Al-Awadhi[1,2], Brian K. Law[3], Valerie J. Paul[4] and Hendrik Luesch[1,2]
[1]Department of Medicinal Chemistry, [2]Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville. [3]Department of Pharmacology and Therapeutics, University of Florida, Gainesville. [4]Smithsonian Marine Station, Fort Pierce, Florida, USA.

Three novel modified peptides named grassystatins D–F (**1**–**3**) were discovered from a marine cyanobacterium from Guam. Their structures were elucidated using NMR spectroscopy and mass spectrometry (MS). The hallmark structural feature in those peptides is the statine unit which contributes to its aspartic protease inhibitory activity preferentially targeting cathepsins D and E. The antiproteolytic activity was assessed using purified enzymes, lysates, and intact live invasive breast cancer MDA-MB-231 cells. Grassystatin F (**3**) was the most potent analogue with IC_{50} values of 50 and 0.5 nM against cathepsins D and E, respectively. Molecular docking was carried out to rationalize the differences in potency between **1** and **3**. The acidic tumor microenvironment is known to increase the activation of some of the lysosomal proteases associated with tumor metastasis such as cathepsins. Since cathepsin D is a biomarker in aggressive forms of breast cancer and linked to poor prognosis, the effect of cathepsin D inhibition by **1** and **3** on the downstream cellular substrates, cystatin C and PAI-1, was investigated. Furthermore, the functional relevance of targeting cathepsin D substrates was evaluated by examining the effect of **1** and **3** on the migration of MDA-MD-231 cells. Grassystatin F (**3**) inhibited the cleavage of cystatin C and PAI-1, the activity of their downstream targets cysteine cathepsins and tPA, as well as the migration of the highly aggressive triple negative breast cancer cells.

S-17

ANTIFUNGAL LEAD DISCOVERY FROM BACTERIA ASSOCIATED WITH THE HAWAIIAN BOBTAIL SQUID TO TREAT FUSARIAL INFECTIONS

Samantha M. Gromek[1], Allison H. Kerwin[2], Andrea M. Suria[2], Spencer V. Nyholm[2], and Marcy J. Balunas[1,]*
[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269, USA, [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA

Within the *Fusarium* genus, the *Fusarium solani* species complex (FSSC) is the dominant clade, known to contain many highly drug resistant fungi. FSSC fungi are generally resistant to a majority of azole antifungal drugs (e.g., fluconazole and itraconazole) and thus amphotericin B is most often used either alone or in combination with rifampin or 5-flucytosine (5-FC). However, for many patients that acquire fusarial infections, especially those who are immunocompromised, these treatments incur a high mortality rate. In addition to their impact on human disease, FSSC fungi have emerged as marine pathogens, threatening extinction of endangered species through their association with developing egg clutches. We selected the Hawaiian bobtail squid, *Euprymna scolopes*, as a model system for the discovery and development of new antifungal drug leads, exploring biologically active secondary metabolites produced by bacteria found in the

accessory nidamental gland (ANG) of sexually mature female cephalopods. Our ongoing research supports the hypothesis that bacteria from the ANG are deposited into the egg jelly coat (JC) where they produce antimicrobial compounds to defend eggs throughout their physically unprotected embryonic period. When treated with antibiotics, squid eggs developed a biofilm primarily composed of the FSSC fungi, *F. keratoplasticum*, that infiltrated the JC resulting in severely reduced hatch rates. Using three *F. keratoplasticum* strains, we have found differential biological activity among ANG and JC bacterial extracts and have performed extensive secondary metabolite profiling of active strains using a suite of spectroscopic techniques. Bioactive compound isolation and identification will be presented and future studies will involve investigation of the mechanism(s) of action of these potent antifungal isolates.

S-18

A SMALL MOLECULE SCREEN IDENTIFIES AN HSP90 INHIBITOR THAT DISRUPTS THE FUNGAL CELL WALL INTEGRITY PATHWAY

Siddharth K. Tripathi, Qin Feng, Melissa R. Jacob, Xing-Cong Li, Alice M. Clark and Ameeta K. Agarwal
National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677

New therapies are greatly needed to overcome resistance to current antifungal drugs such as caspofungin (CAS). The goal of this project is to discover natural products that potentiate CAS activity by interfering with the cell wall integrity pathway (CWIP) which is required for adaptation to the cell wall stress exerted by CAS. Using a new promoter-reporter-based high-throughput assay, we have screened ~5000 natural products from our in-house collection and identified compounds that improve CAS potency in fungal pathogens. A sesquiterpene quinone compound named NP75451 was identified that enhanced CAS activity in CAS-resistant clinical isolates of *Candida albicans* and *Candida glabrata* and, also in the inherently CAS-insensitive pathogen *Cryptococcus neoformans*. To investigate its CAS-potentiating mechanism, we conducted RNA-seq analysis in *S. cerevisiae*. Our studies revealed that CWIP-related genes that were strongly induced by CAS alone were not induced by CAS + NP75451; thus, NP75451 synergizes with CAS by preventing cell wall repair through the CWIP. Further studies revealed that NP75451 targets Hsp90. The transcript profile of NP75451 was similar to that of the Hsp90 inhibitor celastrol. To confirm NP75451's effect on Hsp90, a well-established promoter-reporter assay system was used that monitors the rat glucocorticoid receptor (GR), an Hsp90 client protein. We observed that NP75451 inhibited GR induction in a concentration-dependent manner. We also showed that NP75451 inhibits the activation of the yeast protein Mpk1, another Hsp90 client protein. Because Mpk1 is a critical kinase in the CWIP pathway, this result demonstrates that NP75451 inhibits the CWIP by disrupting Mpk1 activity. In summary, using a new high-throughput assay, we have identified a new compound that improves CAS potency in fungal pathogens. Further evaluation in mammalian models of fungal infection will be required for its development into an effective antifungal combination therapy.

Oral Presentations

O-01

BIOSYNTHESIS OF HIGHLY MODIFIED PEPTIDYL-NUCLEOSIDE ANTIBIOTICS INHIBITING BACTERIAL TRANSLOCASE I

Zheng Cui, Ying Huang, Xiaodong Liu, Ashley Arlinghaus, Jonathan Overbay, and Steven G Van Lanen
Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

Several new natural products have been discovered the past decade by using an activity-based screen to identify inhibitors of bacterial translocase I (UDP-*N*-acetylmuramic acid-pentapeptide:undecaprenyl phosphate transferase), an essential enzyme involved in the biosynthesis of peptidoglycan cell wall. An interesting feature of most of these potential antibiotics is that they structurally consist of unusually modified nucleoside cores that are likewise appended with unusual sugars, peptides, and polyketides/fatty acids. We have identified the biosynthetic gene clusters for several of these natural products including several capuramycin-type antibiotics, which are structurally characterized as peptidyl-sugar-nucleoside hybrids, and caprazamycin-type antibiotics, which are structurally characterized as hybrids of all the aforememtioned components. We will present progress toward delineating the biosynthesis of these two groups of nucleoside antibiotics, highlighting the novel enzymatic strategies that have been discovered for diverting the canonical nucleoside into secondary metabolism, which includes reactions catalyzed by a non-heme, Fe(II)-dependent a-ketoglutarate:UMP dioxygenase and a pyridoxal-5-phosphate-dependent L-Thr:uridine-5-aldehyde transaldolase. The discovery of these enzymes has enabled a genomics-guided approach to identify distinct yet structurally related nucleoside antibiotics.

O-02

FUNCTIONAL CHARACTERIZATION OF A HYBRID NONRIBOSOMAL PEPTIDE SYNTHETASE-LIKE-PTERIDINE SYNTHASE BIOSYNTHETIC GENE CLUSTER

Hyun Bong Park[1,2], Corey E. Perez[1,2], Karl W. Barber[3,4], Jesse Rinehart[3,4], and Jason M. Crawford[1,2,5]
[1]Department of Chemistry, Yale University, New Haven, CT 06520, USA, [2]Chemical Biology Institute, Yale University, West Haven, CT 06516, USA, [3]Department of Cellular & Molecular Physiology, Yale School of Medicine, New Haven, CT 06520, USA, [4]Systems Biology Institute, Yale University, West Haven, CT 06516, USA, [5]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06510, USA

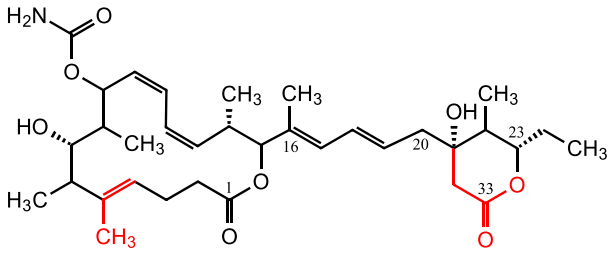
Nonribosomal peptides are a large class of small molecules that possess a wide range of biological activities. Pteridines are heterocyclic compounds that often serve as redox-active cofactors. The biosynthetic machineries for the construction of these distinct classes of secondary metabolites operate independently in the cell. Using genome synteny analysis, we identified a mixed nonribosomal peptide synthetase-like-pteridine synthase biosynthetic gene cluster in a Gammaproteobacterium *Photorhabdus luminescens*. Through comprehensive gene deletion, pathway-targeted molecular networking, *in vitro* reconstitution, quantitative proteomic analysis, and NMR, we show that the genetic locus affects the regulation of quorum sensing and secondary metabolic enzymes and encodes new pteridine metabolites functionalized with *cis*-amide acyl-side chains, named pepteridine A (**1**) and B (**2**). *P. luminescens* undergoes phenotypic variation and can serve both pathogenic and mutualistic roles in two different invertebrate hosts. The pepteridines are only produced in the pathogenic phenotypic variant and represent the first reported secondary metabolites to be synthesized by an unprecedented hybrid NRPS-pteridine pathway.

O-03

DEEP-SEA SPONGE NATURAL PRODUCTS: BIOSYNTHETIC GENES AND ENZYMES

Guojun Wang, Esther Guzmán, and Amy Wright
Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 US Highway 1 North, Fort Pierce, FL 34946

Sponges are the prolific producers of marine natural products (MNPs) and an attractive source for drug discovery. One probable reason is the extremely rich reservoir of natural product biosynthetic genes present in their diverse symbiotic micro-organisms. The supply of MNPs is often the major hurdle banning them from further clinical studies. The Wang laboratory focuses on the biosynthesis of deep-sea sponge MNPs to identify the biosynthetic origin and pathways and ultimately to develop sustainable supplies for them. Leiodermatolide (LDM), isolated from the sponge *Leiodermatium* sp., is a potent mitosis-targeting type I polyketide with *in vivo* activity against pancreatic tumors. Its mode of action differs fundamentally from anti-mitotic drugs including taxanes and vinca alkaloids. LDM does not bind to tubulin, nor does it induce or inhibit in vitro polymerization of purified tubulin. Live cell imaging revealed that LDM causes an immediate block of microtubule elongation. LDM is notably active (GI_{50}=1.0 nM) against the HEL92.1.7 cell line which contains the Pgp efflux transporter, a major cause of leukemia drug-resistance. Biosynthesis of LDM is being studied including the construction of a high-capacity fosmid library and the targeted screening for the highly conserved keto-synthase (KS) domains of *trans*-AT (acyl transferase) type I polyketide synthase. Preliminary sequencing revealed a 25-kb DNA loci showing a high probability of LDM biosynthesis. Domains such as KS, ACP (acyl carrier protein), KR (ketoreductase) and DH (dehydrogenase) were identified, but no AT domains. Our results to date will be presented.



Leiodermatolide

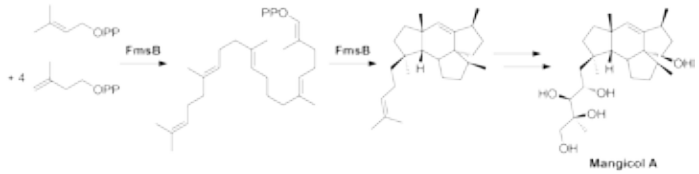
O-04

IDENTIFICATION OF THE MANGICOL BIOSYNTHETIC GENE CLUSTER IN FUSARIUM EQUISETI CNC-477 AND CHARACTERIZATION OF ITS SESTERTERPENE SYNTHASE

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

The marine-derived fungal strain *Fusarium equiseti* CNC-477 was characterized by the Fenical laboratory and shown to be a prolific producer of rare sesterterpene (C25) polyols. Mangicols A–G contain an unprecedented spirotricyclic core and possess remarkable anti-inflammatory activity, whereas neomangicols A–C contain a tetracyclic skeleton and exhibit antimicrobial activities. Both classes of molecules contain multiple hydroxylations on the uncyclized prenyl chain and neomangicols A and B contain an additional chlorine or bromine atom. Following de novo genome sequencing and assembly, genome mining was used to identify the biosynthetic cluster responsible for the synthesis of these sesterterpene polyols. Heterologous expression of the corresponding sesterterpene synthase allowed for the characterization of the cyclopent[*e*]-s-indacene ring formation observed in the

mangicols. The biosynthetic cluster, development of an alternative platform for the heterologous production of terpenes, and characterization of the mangicol sesterterpene synthase will be presented.



O-05

STUDIES IN SIMILARITY: METABOLOMICS-BASED MULTIVARIATE CORRELATION FOR COMPARISON OF NATURAL PRODUCTS

Joshua J. Kellogg,[1] Olav M. Kvalheim[2], Nicholas H. Oberlies,[1] and Nadja B. Cech[1]

[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC 27412, Department of Chemistry, University of Bergen, Bergen 5020, Norway

Natural products and botanical supplements are inherently complex mixtures of secondary metabolites, with significant variability in structural characteristics and concentration present. Efforts to tackle the complex problem of identifying bioactive metabolites from natural products have increasingly favored metabolomics-based approaches, using multivariate data analysis to evaluate large numbers of metabolites and ascertain their respective contributions to an observed biological activity. To facilitate these efforts, a new statistical metric was developed, the reproduced correlation coefficient (RCC), a univariate statistic that incorporates the entire principal component analysis (PCA) statistical model to enable sample comparison. As noise and irrelevant factors remain in the residuals after PCA modeling, this approach captures the essential features of the data better than correlations calculated from raw data. By incorporating the scores and loadings components of the model, the RCC provides a useful measure of similarity, enabling more quantitative comparisons than are possible with visual inspection of a PCA plot or hierarchical cluster analysis (HCA). Using green tea (*Camellia sinensis* (L.) Kuntze (Theaceae)) as a case study, RCC values for commercial tea samples were tabulated to yield a correlation matrix that quantitatively compared sample similarity based upon the whole metabolome, as opposed to individual metabolites. This chemometric approach has the potential to provide quantitative measures of data for comparisons of multiple, complex data sets independent of the analytical platform (IR, MS, NMR).

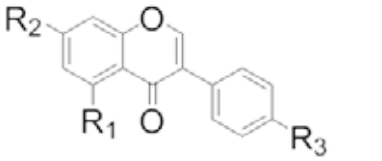
O-06

INVESTIGATION OF THE RESIDUAL COMPLEXITY OF RED CLOVER ISOFLAVONOIDS BY OFF-LINE CCS-QNMR

Gonzalo R. Malca-Garcia, David C. Lankin, James McAlpine, Shao-Nong Chen, and Guido F. Pauli

UIC/NIH Center for Botanical Dietary Supplements Research, Dept. of Med. Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA

The present objective was to determine the purity and residual complexity of isoflavonoid knock-out fractions from Red Clover Extract (RCE) using an off-line combination of countercurrent separation (CCS) and qNMR. Each centrifugal partition chromatography (CPC) fraction of RCE was analyzed by qNMR to determine the purity of the major components and the residual complexity of fractions enriched in the target compounds biochanin A (**1**) and formononetin (**2**). CPC using *n*-hexane-EtOAc-MeOH-H_2O (5.5/4.5/5/5) was used to separate the RCE. The *K* values of the target compounds, **1** and **2**, were determined to be 1.25 and 1.18, respectively, using liquid-liquid partition experiments. A total of 83 fractions was obtained containing 11 major isoflavonoids, including the target compounds, exhibiting a range of purities. ^{1}H NMR fingerprints of isoflavonoid reference standards were employed for dereplication. A one-step CPC separation afforded highly pure **1**, in fraction 65 (93.8% purity) still retaining 0.6% of prunetin as a residual component. Similarly, fraction 24 consisted of **2** (78.4% purity) as a major component in addition to 0.75% of genistein (**3**). A major challenge is to elucidate the structural nature of the residual complexity. Residual complexity is evident as chromatographic fractions retain varying degrees of the original metabolomic diversity and has to be considered for compound isolation and generation of knock-out extracts.



Isoflavone	R_1	R_2	R_3
Biochanin A(1)	OH	OH	OMe
Formononetin(2)	H	OH	OMe
Genistein (3)	OH	OH	OMe
Daidzein (4)	H	OH	OH
Prunetin (5)	OH	OMe	OH

O-07

FLAVONQ: AN "EXPERT SYSTEM" AUTOMATED DATA PROCESSING TOOL FOR PROFILING FLAVONOIDS USING ULTRA HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY DIODE ARRAY DETECTION HIGH-RESOLUTION ACCURATE-MASS MASS SPECTROMETRY (UHPLC HRAM-MS)

Mengliang Zhang, Jianghao Sun, and Pei Chen

Food Composition and Methods Development Lab, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705-2350, USA

Liquid Chromatography and mass spectrometry methods, especially ultra-high performance liquid chromatography coupled with diode array detection and high resolution accurate-mass multi-stage mass spectrometry (UHPLC-DAD-HRAM/MSn), have become the tool-of-the-trade for profiling flavonoids in foods. However, manually processing acquired UHPLC-DAD-HRAM/MSn data for flavonoid analysis is very challenging and highly expertise-dependent due to the complexities of the chemical structures of the flavonoids and the food matrices. A computational expert data analysis program, FlavonQ, has been developed to facilitate this process. The program uses an UV-Vis spectral library for an initial step-wise division of flavonoids into sub-classes. Then individual flavonoids in each class is identified based on their mass spectra with the assistance of in-house LC/MS database that contains 5686 flavonoids. Quantitation is based on the UV-Vis spectra. The step-wise classification strategy to identify classes significantly improved the performance of the program and resulted in more accurate and reliable classification results. The program was validated by analyzing data from a variety of samples, including mixed flavonoid standards, blueberry, mizuna, purple mustard, red cabbage, and red mustard green. Accuracies of identification for all samples were above 88%. FlavonQ-2.0v greatly facilitates the identification and quantitation of flavonoids from UHPLC-HRAM-MSn data. It saves time and resources and allows less experienced people to analyze the data.

O-08

INVESTIGATION OF BIOACTIVE COMPOUNDS FROM MEDICINAL PLANTS COLLECTED IN THE TROPICAL RAINFORESTS OF EAST AFRICA

F. Schultz[1,2], G. Anywar[3], L.-A. Garbe[1,2]
[1]Institute of Bioanalytics, Technical University of Berlin, Germany, [2]Applied Chemistry, School of Agriculture and Food Sciences, Neubrandenburg University of Applied Sciences, Germany, [3]Department of Plant Sciences, Microbiology and Biotechnology, Makerere University, Uganda

The majority of plant and insect species of the tropical rainforests in western Uganda and eastern DRC have not yet been discovered; 90% have not yet been screened for bioactivity. Approx. 60% of the world's population relies almost entirely on plants for medication and knowledge of East African plants and their traditional uses is mainly transferred orally from one generation to the next by traditional healers. Our study provides documentation of 16 different African medicinal plants, which are claimed to possess anti-malarial, anti-cancer and anti-biotic properties among others. One possible methodology for the discovery of novel bioactive compounds is screening selected plant extracts for a broad array of pharmacological activities. We present results of diverse bioassays performed with 61 different plant extracts: 1. Anti-biotic resazurin assay (e.g. *Mycobacterium smegmatis, Listeria monocytogenes*, *Legionella pneumophila*); 2. Anti-malarial heme biocrystallization assay as a pre-screen for upcoming *in vitro* and *in vivo* evaluation; 3. GC-MS-assisted Ames test with human S9 liver fractions for investigation of mutagenic / potential carcinogenic effects. Bioassay-guided fractionations combined with GC/LC-MS techniques enabled identification of bioactive compounds in medicinal plants. For instance, extracts of *Zanthozylum chalybeum* contained 8% of antimalarial lupeol and one compound was isolated from *Harungana madagascariensis,* exhibiting a MIC of 7.8 µg/ml against *L. monocytogenes*. Traditional use could be scientifically validated in many cases.

O-09

CANE TOAD CHALLENGE: EXPLOITING CHEMICAL ECOLOGY TO CONTROL A TOXIC INVASIVE PEST SPECIES

Angela Salim[1], Venkatanambi Kamalakkannan[1] and Robert J Capon[1]

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

The cane toad *Rhinella marina* indigenous to south/middle America was introduced to major sugar growing regions last century as a biological control for cane beetles. A biocontrol failure, the cane toad became a devastating invasive pest species. For example, with an invasion front extending across northern Australia from Queensland to Western Australia, and south to New South Wales, cane toads are poisoning and killing iconic native predator species, including lizards, snakes, crocodiles and marsupials, as well as domestic pets. Despite the public and authorities expending considerable effort over many decades, lasting cane toad control has proved elusive – perhaps until now. We report on a chemical ecology inspired investigation into cane toad toxins - spanning storage, structure, biology and ecology, as well as bacterial and enzymatic biotransformation – that has informed innovative solutions for cane toad control. For example, knowledge of toad toxin has enabled the production of a natural attractant that can be used in cheap, environmentally sustainable traps, to selectively eradicate cane toad tadpoles from the environment. We provide an account of the science behind this patented trapping technology, and demonstrate how it is being trialled under the Cane Toad Challenge, a community engagement and citizen science initiative.

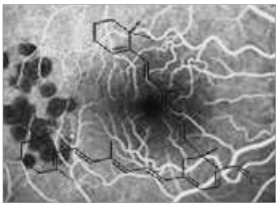
O-10

CYCLORETINAL IN AGE-RELATED MACULAR DEGENERATION: ITS BIOSYNTHESIS BY THE MILK PROTEIN BETA-LACTOGLOBULIN AND ITS CATABOLISM AS A TREATMENT STRATEGY

Vishruth Gowda, Irum Perveen, Brendan Foley, Jasmine Du, Jennifer Foulke-Abel, Hillary Agbo, Coran M. H. Watanabe

Department of Chemistry, Texas A&M University, College Station, TX 77845.

The cycloterpenals are a family of natural products whose name reflects their terpenoid biosynthetic origin and cyclohexadienal motif. Cycloretinal is a representative member of this natural product class, which has been isolated from the human eye and is one of several compounds associated with causing age related macular degeneration (AMD). We have demonstrated that betalactoglobulin (BLG), the principle whey protein found in milk promotes the synthesis of cycloretinal, including that of pasteurized milk. The protein has been identified within drusen pigments (extracellular deposits) that accumulate in the retina of AMD patients. Experiments to address the mechanism of cycloretinal formation will be discussed in addition to experiments that have been undertaken to catabolize the compound enzymatically as a treatment strategy.



Liposfuscins (yellow particles) contain singlet oxygen generating chromophores such as cyclorefinal

Section of the macula

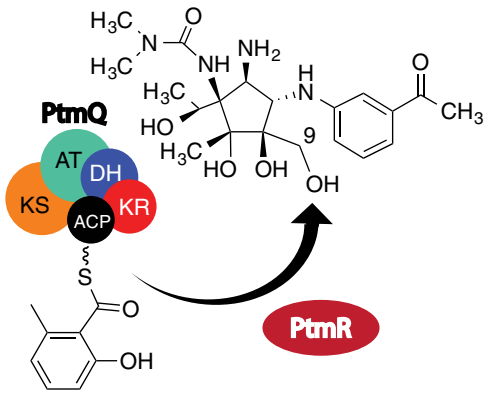
O-11

CHEMOENZYMATIC SYNTHESIS OF NEW PACTAMYCIN ANALOGS

Mostafa E. Abugrain[1], Corey J. Brumsted[2], Andrew R. Osborn[1], Benjamin Philmus[1], and Taifo Mahmud[*1,2]*
[1]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97333, [2]Department of Chemistry, Oregon State University, Corvallis, OR 97333, USA.

Pactamycin is a potent antitumor antibiotic produced by the soil bacterium *Streptomyces pactum*. This structurally unique natural product consists of a highly functionalized cyclopentitol core unit, two aromatic rings [3-aminoacetophenone (3AAP) and 6-methylsalicylic acid (6MSA)], and a 1,1-dimethylurea moiety. Despite its strong biological activity, its development was hampered by its high-toxicity profile. Our previous studies revealed that the type I iterative PKS (PtmQ) is a 6MSA synthase that supplies 6MSA for pactamycin biosynthesis. However, the enzyme that is responsible for the attachment of 6MSA to the aminocyclitol unit was unknown. Through genetic and biochemical characterization, we discovered that PtmR, a β-ketoacyl-ACP synthase (KAS) III-like protein, is responsible for the direct transfer of the 6-methylsalicylyl moiety from PtmQ to the aminocyclopentitol unit. The enzyme also recognizes a wide array of synthetically prepared acyl-*N*-acetylcysteamines (acyl-NACs) as substrates. Using a che-

moenzymatic approach, we generated a suit of new pactamycin derivatives with diverse alkyl and aromatic functionalities.



O-12

BIOSYNTHESIS AND PRODUCTION OF ACTIVES BY PLANT CELL CULTURE

David A. Ullisch[1], Yantree D. Sankar-Thomas[1], Stefan Wilke[1], Thomas Selge[1], Matthias Pump[1], Thomas Leibold[1], Harald Heckenmüller[1], Kai Schütte[1] and Gilbert Gorr[1]
[1]Phyton Biotech GmbH, Alter Postweg 1, 22926 Ahrensburg, Germany

Plants have been considered as a source of pharmaceutically active substances for ages. A promising approach for the sustainable production of natural products can be plant cell fermentation (PCF®) i.e. cultivating plant cells in a controlled bioreactor environment.

To demonstrate the power of PCF® we present three case studies:

1. For more than 17 years Phyton Biotech has been producing paclitaxel at an industrial scale of up to 75,000 L. This fully controlled, and cGMP-compliant process results in yields of up to 60 g/kg biomass (dw).
2. Thapsigargin is a secondary plant metabolite which is currently isolated from seeds of *Thapsia garganica*. Thapsigargin is a powerful cytotoxin – a SERCA inhibitor – and the precursor for the derivative ADT, the key ingredient of the investigational prodrug Mipsagargin (G-202) which is in several clinical trials. Phyton Biotech successfully generated plant cell lines capable of expressing this compound. Here we present data about the screening for high producing cell lines.[(a)]
3. The third case study covers ingenol-3-mebutate. This compound is found in the milky sap of intact plants of the *Euphorbiaceae* family at very low concentrations. Ingenol-3-mebutate is used in Picato® which is approved against actinic keratosis. Generation of cell lines expressing significant amounts of ingenol-3-mebutate is another example underlining the strength of plant cell culture.
4. The authors gratefully acknowledge Inspyr Therapeutics for funding.

O-13

LEAD OPTIMIZATION AND BIOLOGICAL EVALUATION OF CYTOTOXIC ARYLNAPHTHALENE LIGNAN LACTONE NATURAL PRODUCTS

James R. Fuchs[1], Andrew C. Huntsman[1], Alexandria N. Young[2], Bernadette K. Latimer[1], Hee-Byung Chai[1], Yulin Ren[1], Mitch A. Phelps[1], A. Douglas Kinghorn[1], and Joanna E. Burdette[2]
[1]College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA, [2]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA

A series of natural products was recently identified from the plants *Phyllanthus poilanei* and *Phyllanthus songboiensis* by the Kinghorn lab as a part of a collaborative program project grant (P01 CA125066) in an effort to discover novel anticancer agents. Several of the isolated diphyllin-type arylnaphthalene lignans, including phyllanthusmin D and acutissimalignan A, showed potent *in vitro* activity against HT-29 cancer cells. In an effort to improve both their potencies and drug properties, structure-activity relationship (SAR) studies have subsequently been carried out on these compounds. To date, more than fifty structural analogues have been prepared, exploring the roles of the aromatic substituents, the lactone ring, and the sugar moiety. The synthesis of analogues of the glycone portion in particular has resulted in increases of in vitro antiproliferative activity of between 100- and 1000-fold as compared to the natural product phyllanthusmin D. One of the most potent of these compounds has also proven effective in both a hollow fiber assay and a xenograft study. In addition to observed increases in potency, efforts have also been directed at addressing potential metabolic liabilities and the synthesis of probe compounds designed to explore the mechanism through which these compounds exert their cytotoxic effects.

O-14

A NATURAL PRODUCT 'MAKEOVER' – DEVELOPMENT OF PLASMODIUM-SELECTIVE PROTEASOME INHIBITORS AS ANTIMALARIAL AGENTS BASED ON CARMAPHYCIN B

Lena Keller[L], Gregory M. LaMonte[2*], Jehad Almaliti[3*], Betsaida Bibo-Verdugo[4*], Bing Yu Zou[2], Jennifer Wang[2], Yevgeniya Antonova-Koch[2], Pamela Orjuela-Sanchez[2], Colleen A. Boyle[2], Edgar Vigil[2], Lawrence Wang[2], Greg Goldgof[2], Lena Gerwick[1], Anthony J. O'Donoghue[4§], Elizabeth A. Winzeler[2§], William H. Gerwick[1,4§] and Sabine Ottilie[2§]*
** Equal author contributions*
[1]Scripps Institution of Oceanography, University of California, San Diego, CA 92093, USA [2]Department of Pediatrics, School of Medicine, University of California, San Diego, CA 92093, USA [3]Department of Pharmaceutical Sciences, University of Jordan, Amman 11912, Jordan [4]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA 92093, USA

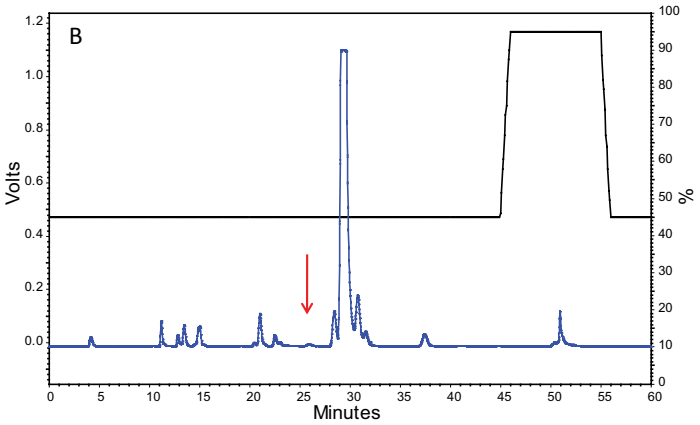
The potent proteasome inhibitors carmaphycin A and B were isolated from a marine cyanobacterium in 2012, and were found to potently inhibit all stages of the human malaria lifecycle. However, significant toxicity towards host cells necessitated the development of more selective inhibitors. Employing total chemical synthesis, we produced a library of 20 carmaphycin analogues that were assayed for their biological activity against *P. falciparum* and HepG2 cells. The most promising candidate retained the high antimalarial potency of carmaphycin B but exhibited a 100-fold reduced toxicity towards liver cells. The results were confirmed through *in vitro* activity assays. Using molecular modeling approaches, we identified key residues that affect the efficient ligand binding of the synthetic natural product-derivative to the human proteasome and therefore provides a structural basis for the increased selectivity. Together, these studies conclusively demonstrate that toxicity of proteasome inhibitors to human cells can be dramatically reduced while maintaining potent anti-microbial activity.

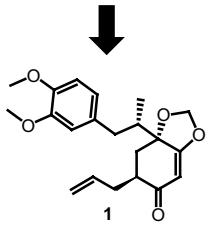
O-15

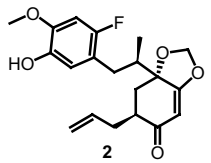
DISCOVERY AND DEVELOPMENT OF BIFIDENONE AS AN ANTICANCER DRUG LEAD

Russell B. Williams[1], Zhongping Huang[2], Steve M. Martin[1], Julie A. Lawrence[1], Vanessa L. Norman[1], Mark O'Neil-Johnson[1], John E. Mangette[2], Courtney M. Starks[1], Gary R. Eldridge[1]
[1]Sequoia Sciences, Inc. 1912 Innerbelt Business Center Dr. St. Louis, MO 63114, USA, [2]Albany Molecular Research Inc., 1001 Main Street, Buffalo, NY 14203, USA.

Bifidenone (**1**) was isolated in microgram quantities from material collected from a small tree in Gabon. The resulting synthesis project to obtain enough material for further investigation was ultimately successful, but there were many bumps along the way. From the synthesis we were able to confirm the observed activity, determine the absolute configuration, and establish a mode of action. Based on those results, a medicinal chemistry study was conducted to improve potency and bioavailability. A preclinical candidate (**2**) was selected for efficacy and toxicity studies. This project, from initial discovery to current status, will be presented.







O-16

INATREQ ™ ACTIVE – A NOVEL NATURAL PRODUCT BASED AGROCHEMICAL TO CONTROL FUNGAL INFECTIONS IN CEREALS.

Paul Graupner, Kevin Meyer, John Owen, Chenglin Yao, and David Young AgChem Discovery, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268.

Natural Products have made an impact historically to control various agriculturally relevant pests and diseases. Examples include the pyrethroids which have persisted in many forms since their introduction over 70 years ago. More recently such products such as the spinosyns have been used as insecticides both as pure natural products and semi-synthetic variants, and a family of products inspired by the naturally occurring strobilurins have proven very successful as broad spectrum fungicides.

One of the more important cereal diseases is *Septoria* leaf blotch caused by the ascomycete fungus *Zymoseptoria tritici* which may reduce wheat yields by up to 50 %. Strobilurin and azole based chemistries have been used for many years to control *Septoria* leaf blotch, but are increasingly becoming less effective due to a buildup of resistant strains affecting large areas of the European grain belt. With resistance issues and raised regulatory hurdles for some current chemistries, there is always a need for new solutions in the plant protection arena.

Through our natural product program we have identified a class of picolinamide natural products that give excellent control of *Z. tritici,* including resistant strains. In this talk we will present fenpicoxamid (Inatreq™) which is a protected derivative of the natural product published as UK-2A and represents our first foray in this disease area. Fenpicoxamid offers a new target-of-action for S*eptoria* control and we expect it to be launched in 2018. Aspects about the discovery, chemistry, and its site-of-action will be shared.

O-17

GLABRIDIN, EVODIAMINE AND BOTANICAL EXTRACTS ACTIVATE AMP-ACTIVATED PROTEIN KINASE TO AUTOPHAGY POSSIBLY THROUGH MTOT, A PARKINSON'S AND ALZHEIMER'S DISEASE TARGET.

Molly Hood[1], Brian J. Doyle[2], Jeffrey D. Scholten[1], Arun Rajgopal[1], and John F. Rebhun[1]
[1]Amway R&D, Ada, Michigan, USA, [2]Alma College, Alma, Michigan, USA

Recently, MSDC-0160, a compound that inhibits mTOT, mitochondrial **T**arget **o**f **T**hiazolidinedione (pyruvate carrier complex (Mpc1 and Mpc2)), emerged as a drug candidate for the treatment of Parkinson's disease in specific animal models. It blocks pyruvate transport, activating AMPK, (an ATP (energy) sensor) and activating autophagy. Previous work demonstrated that like control compounds, thiazolidinediones, (troglitazone, etc.), phytochemicals, glabridin and evodiamine, activate an isolated PPARγ ligand binding domain (LBD) but, behave differently in gene expression tests. Others have shown that glabridin activates AMPK indirectly by impairing cellular respiration. Do glabridin and evodiamine affect mTOT? Here we show that MSDC-0160, activates PPARγ LBD assay and other compounds glabridin, evodiamine, troglitazone modulate AMPK, S6 kinase and autophagy. Further, rutaecarpine (similar compound to evodiamine) does not activate PPARγ LBD, and does not modulate these other targets. Methyl pyruvate, a cell permeable pyruvate analog interrupts glabridin, evodiamine, troglitazone and MSDC-0160's activation of AMPK. We present an alignment between the results of these assays using botanical extracts from the Alma College Botanical Extract Library. The alignment suggests that the PPARγ LBD assay can be used to initially identify botanicals that may bind to mTOT modulate AMPK activity and may be important for possible health benefits. Authors thank Dr. Mark L. Proefke and Mr. Mark Gammage and the Biology Department of Alma College.

O-18

USP'S EFFORTS FOR DEVELOPING CANNABIS PUBLIC STANDARDS

Nam-Cheol Kim, Nandakumara D. Sarma, and Gabriel I. Giancaspro
Dietary Supplements and Herbal Medicines, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852, USA

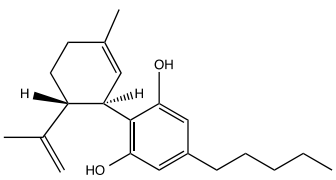
Following legalization of the medical use of cannabis in several U.S. States and internationally, United States Pharmacopeia (USP) responded to the requests from stakeholders to investigate advisability and feasibility of developing quality standards for cannabis used for medical purposes. Development of quality standards for cannabis used for medical purposes requires consideration of a wide range of scientific, legal, and policy issues that reach far beyond its' classification as a botanical drug or herbal medicine. USP published a Stimuli Article in the USP *Pharmacopeial Forum* [*PF* 42(1)] which reviewed the regulatory and scientific landscape regarding medical cannabis, identified issues related to the lack of quality standards, and explored the potential options for developing quality standards. USP also organized a stakeholder Roundtable and received stakeholder comments to consider the development of quality standard for cannabis. As the use of cannabis for medical purposes is growing, the need for a USP public quality standard to help ensure avoidance of adulteration, accurate identification, control of contaminants, and considerations regarding constituent composition and strength has been identified. To initiate the task of standard-development process, USP Botanical Dietary Supplements and Herbal Medicines Expert Committee formed Cannabis Expert Panel. The Panel is working on appropriate methods for the Identification, Composition and Limits for Contaminants. The proposed standards will be open for public comment before adoption by the USP Expert Committee.

O-19

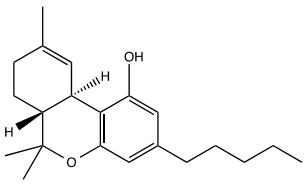
FORMULATION OF MEDICINAL CANNABIS DELIVERY DEVICES

Christopher G. Witowski[1]
[1]*Alternative Medical Enterprises, LLC, Sarasota, FL 34240*

Cannabis use dates back 5,000 years in Traditional Chinese Medicine; cannabis is now widely accepted and a majority of U.S. states have adopted laws to allow medical cannabis use. Typical combustion methods of cannabis do not constitute a proper medical delivery method. AltMed's goal was to adopt pharmaceutical principles to develop a product pipeline utilizing cannabis extracts as the active ingredient. Under the MüV brand, AltMed has launched transdermal patches, metered-dose inhalers, topicals, and vaporizer pens with varying ratios of cannabidiol (CBD, **1**) and tetrahydrocannabinol (THC, **2**), the major cannabinoid constituents of various cannabis cultivars. Product development was undertaken by selecting only FDA approved inactive ingredients to ensure safe and effective drug delivery systems. The work presented herein will outline the formulation development of the cannabinoid transdermal patch and metered-dose inhaler.



Cannabidiol (**1**)



Tetrahydrocannabinol (**2**)

O-20

IDENTIFICATION OF PLANT SECONDARY METABOLITES AS NF-KB LEADS

Ulyana Munoz Acuna[1,2], *Nelson Freitas Fernandes*[1,2], *Nicole A. Eggers-Woodard*[2], *Angela A. Salim*[2], *Li Pan*[2], *Yulin Ren*[2], *A. Douglas Kinghorn*[2], *and Esperanza Carcache de Blanco*[1,2]
[1]*Division of Pharmacy Practice and Administration, and* [2]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University.*

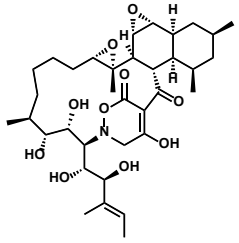
As part of our efforts toward natural products drug discovery, we have screened hundreds of tropical plant extracts and compounds that have exhibited significant activity in the NF-κB assay. This assay has been used in our lab to evaluate the ability of the natural product samples to act as inhibitors of nuclear factor translocation from the cytoplasm to the nucleus in human cells. Compounds found active in the nanomolar range can be classified into pseudolignans, benzoic acid glycosides, benzofurans, and flavonoids. Compounds found active in the micromolar range belonged to the following classes of compounds: pseudolignans, phenylpropanoids, benzofurans, and sesquiterpene lactones. In summary, a total of seven compounds have been identified as being active in the nanomolar range and sixteen compounds in the micromolar range from the groups of compounds to be presented. The biodiversity observed in the biologically active compounds that will be presented go to show that plant secondary metabolites can serve as good leads in the development of new NF-κB inhibitors as potential new drugs.

O-21

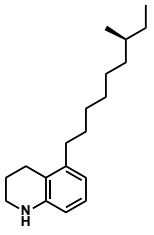
COMBINED-CULTURE: ACINOMYCETES POTENTIAL SECONDARY METABOLISM IS AWAKENED BY CO-CULTURE WITH MYCOLIC ACID CONTAINING BACTERIA

Shumpei Asamizu, and Hiroyasu Onaka
Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo, Japan 1138657

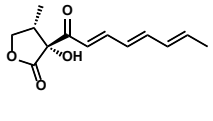
Streptomyces contains 30–40 secondary metabolite biosynthetic gene clusters, however the expression of most metabolite biosynthetic gene clusters is cryptic or silent. To activate such a silent secondary metabolite fermentation, co-culture is one of attractive methods. In our developed Combined-culture[1], the activator strain is a mycolic acid-containing bacterium (MACB), and about 90% of *Streptomyces* species show changes in secondary metabolism in combined-culture compared with pure culture. We developed this combined-culture method for antibiotic screening, and 5 novel type of antibiotics, alchivemycins **1**, 5-alkyl THQs **2**, arcyriaflavin E **3**, chojalactones **4**, niizalactams **5** were isolated. 1, Onaka H.et al., *Appl Environ Microbiol.* 77(2): 400-406 (2011)



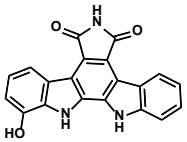
1



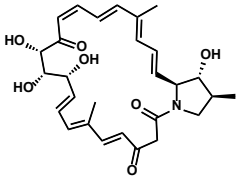
2



3



4



5

O-22

INDUCTION OF A POTENT AND SELECTIVE *STAPHYLOCOCCUS AUREUS* ANTIBIOTIC

Emily Mevers[1], Gleb Pishchany[2], Roberto Kolter[2], Jon Clardy[1]
[1] Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, [2] Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115

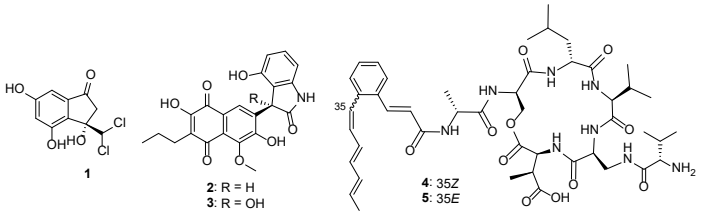
The emergence of highly resistant bacterial pathogens, such as carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*, stresses the importance of the need for the discovery of new selective and potent antibiotics. Silent bacterial biosynthetic gene clusters (BGCs), if upregulated, represent an untapped resource to meet this need, though finding the right laboratory culture conditions to 'turn on' these BGCs has proven challenging. In the natural environment, a single soil bacterium chemically interacts with hundreds of different microbes, all of which are competing with one another for limited nutrients. It is these microbial interactions that likely regulate the production of silent BGCs and thus improving our understanding of these interactions will help lead to the discovery of new antibiotics. In this study, we successfully upregulated the production of novel antibiotics by reconstituting an ecologically-relevant bacterial community. This led us to one specific bipartite system, involving the interaction of *Amycolatopsis* sp. AA4 and *Streptomyces coelicolor* M145, to kill *S. aureus*. When AA4 is grown in the presence of M145 or its spent supernatant, the production of a highly potent and selective *S. aureus* antibiotic is significantly upregulated. Bioassay-guided isolation led to the isolation of a common cell well component as the inducing agent and amycomycin A, a novel and chemically-intriguing natural product that exhibits single digit nanomolar potency against *S. aureus*. Interestingly, AA4 also produces large quantities of palmitoleic acid, which acts as an antidote by inhibiting the toxicity amycomycin A has on *S. aureus* and presumably serves as a resistant mechanism for AA4.

O-23

DISCOVERY OF NEW BIOACTIVE NATURAL PRODUCTS FROM INSECT SYMBIONTS

Dong-Chan Oh[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea

Symbiotic bacteria in insects have recently drawn a significant attention as an untapped source of new bioactive compounds, which may play protective roles for insect hosts. Chemical studies of symbiotic bacteria in phylogenetically diverse insects such as the dung beetle *Copris tripartitus*, the burying beetle *Nicrophorus concolor*, the carpenter ant *Componotus japonicus*, and the silkworm *Bombyx mori* resulted in the discovery of bioacitve natural products with novel structures. For example, a new dichlorinated indanone (**1**), naphthoquinone-oxinoles (**2**-**3**), and branched-chain cyclic peptides (**4**-**5**) were discovered from dung beetle symbionts and antibiotic macrocyclic lactams were isolated from a silkworm-gut bacterium. Their structures, biological activities, and potential functions in insect symbiotic systems will be presented.



O-24

PRODUCTION OF NEW THIOLACTOMYCIN ANTIBIOTICS WITH POTENTIATED BIOACTIVITY BY ENZYMATIC TANDEM CARBOXYLATION-AMIDATION

Jie Li[1], Xiaoyu Tang[1], Shaun Mckinnie[1], Takayoshi Awakawa[1,2] and Bradley S. Moore[1,3]
[1]Scripps Institution of Oceanography, University of California (UC), San Diego, La Jolla, CA 92093, [2]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, UC, San Diego, La Jolla, CA 92093

The selective activation of unreactive hydrocarbons by biosynthetic enzymes has inspired new synthetic methods in C–H bond activation to form chiral molecules. The work to be presented reports the unprecedented C–H amide functionalization of an unreactive ethyl group in the biosynthetic conversion of thiotetromycin to thiotetroamide that results in a ten-fold increase in antibiotic potency. We detail the genetic and biochemical basis for the terminal amidation in thiotetroamide biosynthesis, which involves a uniquely adapted cytochrome P450-amidotransferase enzyme pair and highlights the first carboxylation-amidation enzymatic cascade reaction leading to the selective formation of a terminal amide group from a chemically inert alkyl group. The second part of the work shows the application of this unusual enzyme pair as a biotransformation tool to introduce a terminal amide group, a functionality that is frequently incorporated into the chemical structures of lead molecules to achieve improved bioactivity and bioavailability, as reflected by a fair number of marketed drugs that terminate in a primary amide group (e.g., Briviact® and Adlyxin® among the total 12 non-antibody drugs approved by U.S. FDA in 2016). Motivated by the enhanced bioactivity of thiotetroamide ascribed to the terminal amidation process as well as the pharmaceutical relevance of this process, structurally diverse thiolactone and lactone derivatives were isolated or synthesized, and chemoenzymatically interrogated as potential substrates of the cytochrome P450-amidotransferase enzyme pair, through which unnatural thiotetroamide analogues with potentiated bioactivity were prepared.

O-25

OVERVIEW OF THE 2017-2021 STRATEGIC PLAN FOR THE NIH OFFICE OF DIETARY SUPPLEMENTS

Joseph M. Betz, Paul M. Coates, Paul R. Thomas
Office of Dietary Supplements, National Institutes of Health, 6100 Executive Blvd., 3B01, Bethesda, MD 20892 USA

The Office of Dietary Supplements (ODS) at the National Institutes of Health (NIH) has released its strategic plan for 2017-2021, *Strengthening Knowledge and Understanding of Dietary Supplements*. The document presents a refreshed set of goals, strategies, and activities that ODS plans for the next 5 years. It also provides a review of ODS activities between 2010 and 2016 and includes examples of ODS collaborations, programs, and summaries of its extramural investments. The plan was shaped by input received from ODS stakeholder communities throughout the federal government, academia, industry, consumer advocacy and education groups, and interested consumers.

The ODS mission is to support, conduct, and coordinate scientific research and provide intellectual leadership for the purpose of strengthening knowledge and understanding of dietary supplements (DS) to foster an enhanced quality of life and health for the U.S. population. Our vision is that researchers, health professionals, government officials, other policymakers, and consumers will have ready access to scientific information of the highest quality on the health effects of DS. The mission is achieved by setting out several strategic goals. They are to: 1) Expand scientific knowledge of DS by stimulating and supporting biomedical research and by developing and contributing to collaborative initiatives, workshops, meetings, and conferences; 2) Enhance the DS research workforce through training and career development; 3) Foster development and dissemination of research

resources and tools to enhance the quality of DS research; 4) Translate DS research findings into useful information for consumers, health professionals, researchers, and policymakers.

This presentation will provide an overview of ODS activities of the past 5 years and goals for the next 5 years.

O-26

INDOLE-3-CARBINOL ATTENUATES COLITIS THROUGH ALTERATIONS IN GUT MICROBIOTA AND METABOLIC PATHWAYS

Mitzi Nagarkatti and Philip B. Busbee
Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29208

Colitis is an inflammatory disease of the large intestine, which is difficult to treat. In the current study, we demonstrate that indole-3-carbinol (I3C), found in cruciferous vegetables, attenuates clinical symptoms in Trinitrobenzenesulfonic acid (TNBS)-induced murine colitis. In particular, I3C prevented weight loss, reversed colon shortening, and reduced infiltrating immune cells and serum amyloid A. We performed 16S rRNA metagenomic sequencing to investigate alterations in the gut microbiome after induction of colitis by TNBS and following treatment with I3C. 16S rRNA analysis of cecal flushes and validation by RT-PCR revealed that TNBS-induced colitis triggered significant increase in the species Bacteroides acidifaciens, whereas colitis mice treated with I3C had decreased levels of this species, compared to controls. In addition, I3C was able to modulate gut microbial metabolites by way of altering short chain fatty acid (SFCA) production during disease induction. Specifically, I3C treatment was able to increase production of SCFA butyric acid, and treatment of colitis mice with sodium butyrate (NaB) was able to alleviate deleterious effects attributed to colitis. Collectively, these data suggest that I3C attenuates colitis by preventing pathogenic gut microbial dysbiosis and restoring gut microbiome composition to a more homeostatic state (Supported by NIH grants P01AT003961, R01AT006888, R01MH094755, P20GM103641, R01AI129788 and R01AI123947).

O-27

AMLEXIN, A BOTANICAL COMPOSITION, DEMONSTRATES EFFICACY IN MULTIPLE MODELS OF ARTHRITIS DISEASE PROGRESSION AND IN A HUMAN CLINICAL TRIAL

Mesfin Yimam, Teresa Horm, Laura Wright, Ping Jiao, Mei Hong, Lidia Brownell, Qi Jia.
Unigen, Inc. 3005 1st Ave, Seattle, WA 98121, USA.

Arthritis is a chronic and complicated disease characterized by inflammation of the joints, leading to pain, stiffness, and degradation of joint structure. Current treatments focus on symptom relief by inhibiting the immune system to reduce inflammation. These treatments may treat pain and swelling, but often do not stop joint deterioration. Here, we describe AmLexin, a proprietary blend of the bioflavonoid extracts from heartwood of *Acacia catechu* and the root bark of *Morus alba*. We demonstrate that Amlexin reduces arthritic phenotypes *in vitro*, *ex vivo*, and *in vivo*, using various models of arthritis. These include: anti-oxidation; COX/LOX inhibition; excised rabbit knee cartilage treated with Interleukin-1β (IL-1β), a cytokine that is increased in arthritis patients and contributes to inflammation of the joints; the monoiodoacetate (MIA) rat model of osteoarthritis; and the carrageenan-induced rat paw edema model. In a human clinical trial wherein arthritis patients were treated with AmLexin, a Glucosamine/Chondroitin combination, and a placebo, patients treated with AmLexin exhibited an 8.9% decrease in C-telopeptide of type II collagen (CTX-II) in their urine, as compared to a 0.5% increase in the Glucosamine/Chondroiting group and a 25% increase in the placebo group. uCTX-II is an important and established biomarker for osteoarthritis, as its increase in urine is a direct result of cartilage breakdown in the joints. This human clinical data in combination with all other pre-clinical data demonstrate that AmLexin can be used for maintaining joint health and protecting joint cartilage.

O-28

CLINICAL EVALUATION OF A STANDARDIZED *PRUNUS DOMESTICA* EXTRACT ON BENIGN PROSTRATE HYPERPLASIA (BPH) IN MALE VOLUNTEERS

D. Bagchi[1,2], S.N. Sankhwar[3], N. Verma[3], N. Patel[3], A. Swaroop[2], P. Kumar[4], and M. Bagchi[2]
[1]Dept of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX 77204, USA, [2]Cepham Research Center, Piscataway, NJ, [3]King Georges' Medical University, Lucknow, India, [4]Chemical Resources, Panchkula, India

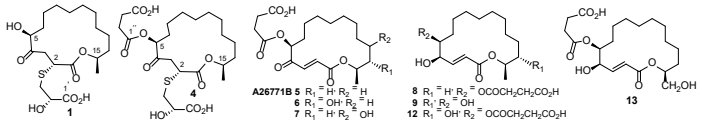
We previously demonstrated the efficacy of a novel *Prunus domestica* stem extract (Prosprune™) in testosterone propionate-induced BPH in male Wistar rats. This IRB approved clinical study assessed the efficacy of Prosprune™ (100 mg capsule b.d.) over a period of 12 weeks in 140 male subjects (age: 40-65 years; International Prostate Symptom Score (IPSS) ≥ 8; Prostate volume ≥20 mL and ≤ 70 mL) suffering from moderate BPH for 6 months. Subjects were examined at baseline, 4-, 8- and 12 weeks of treatment for IPPS, prostate volume, urinary flow, blood pressure, serum testosterone level and sonographic evaluation. Significant reductions in IPSS were observed at 4- (35.11%, p = 0.000**), 8- (61.50%, p = 0.000**) and 12-weeks (79.83%, p = 0.000**) of treatment, while prostate volume was reduced by 11.52% (p = 0.000**), 20.73% (p = 0.000**) and 29.46% (p = 0.000**) at the completion of 4-, 8- and 12-weeks of treatment, respectively. Approximately 56% reduction in PSA was observed following 12 weeks of treatment (p = 0.041**), and 76% of subjects showed a decrease in serum PSA levels at the end of treatment. Sonographic evaluation exhibited a time-dependent reduction in prostate volume. Serum testosterone level increased by 17.28% (p = 0.000**). This study demonstrates that Prosprune™ is safe and effective in ameliorating the symptoms of BPH in male volunteers.

O-29

THE BERKELEYLACTONES, ANTIBIOTIC MACROLIDES FROM FUNGAL COCULTURE

Andrea Stierle[1], Donald Stierle[1], Daniel Decato[2], Nigel Priestley[2], Jeremy Alverson[2], John Hoody[2], Kelly McGrath[1], Dorota Klepacki[3]
[1]Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT, 59812, USA, [2]Department of Chemistry and Biochemistry, University of Montana, Missoula, MT, 59812, USA, [3]Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60607, USA

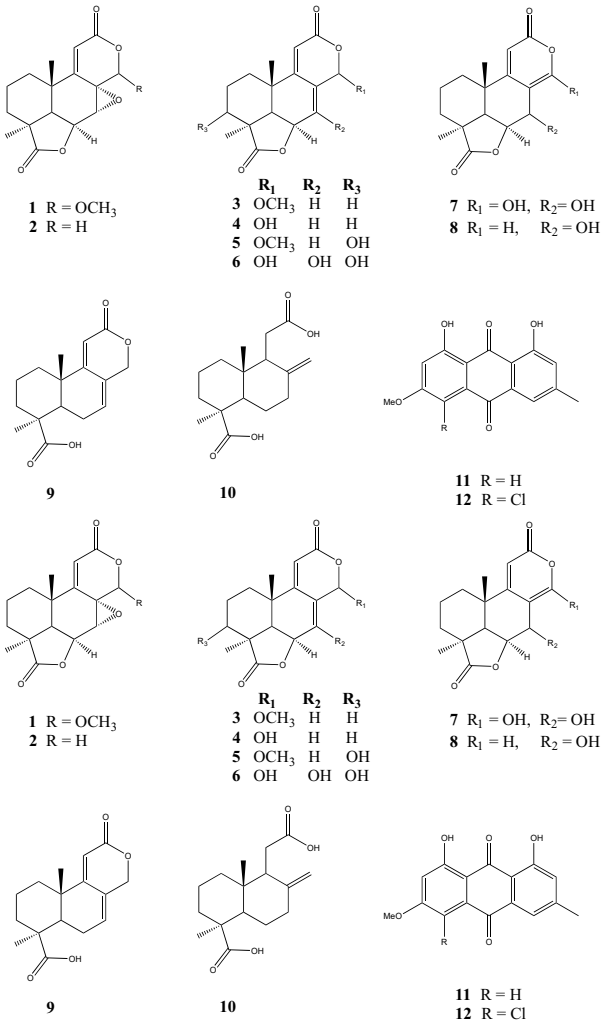
Our ongoing studies of the extremophilic fungi isolated from the Berkeley Pit have shown little evidence of antibiotic production. However, coculture fermentation of two of these fungi has yielded eight new macrolides, berkeleylactones A-H (**1**, **4**, **6-9**, **12**, **13**), as well as the known antibiotic A26771B (**5**). The structures were deduced from analyses of spectral data, and the absolute configurations of **1** and **9** were determined by single crystal X-ray crystallography. Compound **1** exhibited low μM activity against four MRSA strains, including erythromycin-resistant strains. Unlike other macrolide antibiotics, **1** does not inhibit protein synthesis nor target the ribosome, which suggests a novel mode of action for its antibiotic activity.



O-30

ANTIFUNGAL DISCOVERY FOR THE TREATMENT OF WHITE NOSE SYNDROME (WNS) IN BATS

Yudi Rusman, Michael B. Wilson and Christine E. Salomon
Center for Drug Design, University of Minnesota, Minneapolis, MN 55455, USA



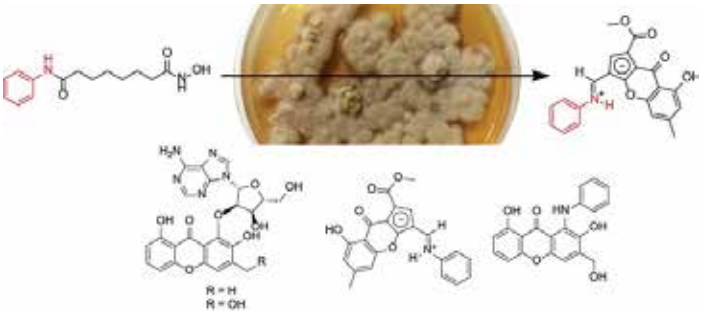
White nose syndrome (WNS) is a recently discovered fungal disease spreading across North America and decimating entire bat colonies. Million of bats have been killed since WNS was first discovered in 2006, and the disease has been confirmed in 31 states and 5 Canadian Provinces. WNS is caused by the fungus *Pseudogymnoascus destructans*, a psychrophilic dermatophyte that infects hibernating bats and leads to mortality as high as 95-100% for some species. There are currently no viable methods for preventing or treating infection; however, one potential approach using microbial biological control agents to provide direct and sustained inhibition of *P. destructans* on hibernaculum substrates, surfaces, and bats. We have identified 32 fungal and 59 bacterial isolates from subterranean habitats that inhibit the growth of *P. destructans*. One of the most potent isolates is *Oidodendron* sp., an ascomycete fungus isolated from wood collected in the Soudan Iron Mine. Bioassay guided fractionation of a rice culture extract of this fungus led to the identification of six new norditerpene lactones together with several known natural products. Compounds 1-4 exhibit moderate antifungal activity against *P. destructans* (MIC < 10 μg/mL) and the human fungal pathogens *Candida albicans* and *Cryptococcus neoformans*.

O-31

BIOTRANSFORMATION OF THE HDAC INHIBITOR VORINOSTAT YIELDS NEW ANILINE –CONTAINING FUNGAL METABOLITES

Donovon A. Adpressa[1], Kayla Stalheim[1], Philip J. Proteau[2], Sandra Loesgen[1]
[1]Department of Chemistry, [2]College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA.

The diversity of genetically encoded small molecules produced by filamentous fungi remains largely unexplored. Epigenetic perturbation of the endophytic ascomycete *Chalara* sp. 6661 with the HDAC inhibitor vorinostat resulted in the isolation of four new modified xanthones. Incorporation studies with deuterium-labeled vorinostat indicate that the aniline moiety in chalalanine A is derived from vorinostat itself. This is the first report of fungal biotransformation of the popular epigenetic modifier and subsequent incorporation of the released aniline into a polyketide metabolite. All structures were determined by extensive NMR spectroscopic analyses and the compounds tested in cytotoxicity and antimicrobial assays.



O-32

IDENTIFICATION AND MECHANISTIC STUDIES OF A FUNGAL METABOLITE WITH SELECTIVE CYTOTOXIC ACTIVITY AGAINST EWING SARCOMA CELLS

Andrew J. Robles[1], Saikat Haldar[4,5], Wentao Dai[4,5], April L. Risinger[1,2], Robert H. Cichewicz[4,5], Peter J. Houghton[2,3], and Susan L. Mooberry[1,2]
[1]Department of Pharmacology, [2]Cancer Therapy & Research Center, and [3]Greehey Children's Cancer Research Institute, The University of Texas Health Science Center at San Antonio. [4]Natural Product Discovery Group, Institute for Natural Products Applications and Research Technologies, and [5]Department of Chemistry & Biochemistry, University of Oklahoma.

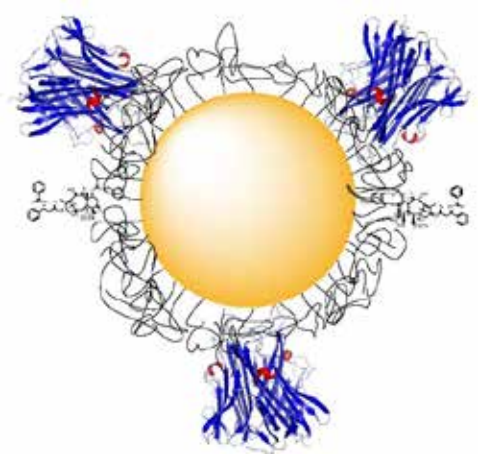
Ewing sarcomas (EWS) are bone and soft tissue malignancies that affect children and adolescents. Targeted therapies for EWS could be identified because EWS result from chromosomal translocations that lead to expression of the abnormal EWS-FLI1 fusion protein, a transcriptional activator and modulator of RNA splicing. A screening program to identify compounds with selective actions against pediatric solid tumor types identified that the fungi-derived compound altertoxin II (ATXII) has highly selective activities against 5 cell lines that express the EWS-FLI1 fusion protein, compared to 7 other pediatric and adult cancer cell lines. The concentrations that cause 50% growth inhibition (GI_{50}) were determined for each cell line and indicated that ATXII has an average of 33-fold selectivity for EWS-expressing cell lines. To further evaluate the selectivity for EWS and determine if this activity is specific to sarcomas, we confirmed these results in a secondary panel of EWS and rhabdomyosarcoma (RMS) cell lines. On average, the IC_{50} of ATXII was 94-fold higher (range 16- to 400-fold) in RMS cells than EWS cells, indicating high selectivity for EWS. Mechanistic studies with a luciferase reporter and immunoblotting indicated that 100 nM ATX inhibits the promoter activity of the EWS-FLI1 target NR0B1 and reduces NR0B1 protein levels. This concentration also induced cell cycle accumulation in EWS cells and phosphorylation of p53 and checkpoint ki-

nases 1 and 2. These experiments suggest ATXII inhibits EWS-FLI1-driven transcription, induces DNA damage and cell cycle accumulation, leading to apoptosis of EWS cells. Ongoing studies will evaluate the antitumor effects of ATXII in xenograft models of EWS. Funded by R01 GM107490, CA165995.

O-33

GOLD NANOPARTICLES FOR DRUG DELIVERY OF PACLITAXEL AND DOXORUBICIN

David G. I. Kingston[1], Long Xia[1], Jielu Zhao[1], Shugeng Cao[1], Giulio F. Paciotti[2], Lawrence Tamarkin[2], and Marion F. Ehrich[3]
[1]Department of Chemistry and Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061, USA, [2]CytImmune Sciences Inc., 15010 Broschart Road, Rockville, Maryland 20850, USA, [3]Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA,



The natural products paclitaxel (Taxol®) and doxorubicin are widely used and effective anticancer agents, but both suffer from significant side effects that could be alleviated by targeted drug delivery. The synthesis of a series of thiolated paclitaxel and doxorubicin analogs is described as part of a novel nanomedicine program aimed at developing formulations of these natural anticancer drugs that will bind to gold nanoparticles for tumor-targeted drug delivery and tumor-specific drug release. Preliminary evaluations of the new nanomedicines composed of gold nanoparticles, thiolated polyethylene glycol (PEG-thiol), and one of several thiolated paclitaxel or doxorubicin analogs is presented. The addition of tumor necrosis factor alpha (TNFα) targets the nanoparticles to the tumor and reduces the drug-eliminating interstitial fluid pressure in the tumor.

O-34

NETWORK PERTURBATIONS THAT UNDERLIE ANTI-INFLAMMATORY BIOACTIVITY OF NATURAL PRODUCT COMBINATIONS

Stephen J. Polyak[1], Jessica Wagoner[1], Nicholas Oberlies[2], Avi Ma'ayan[3], Nicholas Lyons[4], Benjamin Siranos[4], Aravind Subramanian[4], Nadja Cech[2] Departments of Laboratory Medicine[1], University of Washington, Seattle, WA, USA; Department of Chemistry and Biochemistry[2], University of North Carolina at Greensboro, Greensboro NC, USA; Icahn School of Medicine at Mount Sinai[3], New York, NY, USA; Broad Institute[4], Cambridge, MA, USA.

The mechanisms that underlie the biological effects of natural product combinations are rarely known. Using case studies with the herbal extract silymarin (from *Silybum marianum*), and purified lactones from feverfew (parthenolide from *Tanacetum parthenium*) and ashwaganda (withaferin A from *Withania sominfera*), we i) identify combinations of natural products that additively suppress nuclear factor kappa B (NF-κB) activity, ii) define the cellular networks that are perturbed when NF-κB is inhibited by complex extracts and combinations of natural products, and iii) validate natural product-induced network and pathway alterations that feed into NF-κB. Parthenolide and withaferin A individually inhibit NF-κB activity with nanomolar potency, and when combined show additive suppression of NF-κB. Transcriptional profiling of a combination matrix of parthenolide and withaferin A using the L1000 assay, and Connectivity Map (CMAP) analyses strongly predict significant regulation of the proteasome, which is central to regulation of NF-κB, and NF-κB itself. Silymarin also inhibits NF-κB with micromolar potency, and causes activation of adenosine monophosphate kinase (AMPK). In AMPK knock out mouse cells, silymarin does not inhibit NF-κB as effectively, indicating that silymarin activation of AMPK feeds directly into suppression of NF-κB. Collectively, the data demonstrate our ability to identify how complex mixtures, single compounds, and combinations of natural products modify cellular inflammatory status, some of which are active at doses that are relevant and achievable *in vivo*.

O-35

SENSITIZATION OF BETA-LACTAM ANTIBIOTICS WITH RESISTANCE MODIFYING AGENTS FROM A MEDICINAL PLANT

Cassandra L. Quave[1,3], Roberta Melander[2], James T. Lyles[3], Kate Nelson[1], and Christian Melander[2]

[1]Department of Dermatology, Emory University School of Medicine, Atlanta, GA 30322, USA, [2]Department of Chemistry, NC State University, Raleigh, NC 27695, USA, [3]Center for the Study of Human Health, Emory University, Atlanta, GA 30322, USA.

Antimicrobial resistance is currently responsible for an estimated 700,000 deaths annually, and projected to reach 10 million deaths per year by 2050. Alternative approaches to restore the activity of existing antibiotics are urgently needed. Medicinal plants have a long and complex history in the traditional treatment of bacterial infections; however the mechanism of action of many botanical therapies remains poorly understood. To identify novel resistance modifying agents (RMAs) that act to sensitize, or restore, the therapeutic efficacy of β-lactam antibiotics, we evaluated >900 extracts of a unique natural products library composed of >400 medicinal plant and fungal extracts, constructed based on evidence of traditional medical use for infectious and inflammatory diseases. We identified extract 649 as a potent RMA, dropping the minimum inhibitory concentration (MIC) of oxacillin (Ox) in a MRSA strain in a synergistic fashion, with a fractional inhibitory concentration (FIC) index of 0.06. The extract is derived from the leaves of a shrub used by Native Americans as an indigenous medicine. Further refinement of the extract yielded an active partition (649C), which was determined to be composed of 42 compounds with relative abundance >1% based on LC-FTMS analysis. This extract exhibited a FIC Index of 0.016 and dropped the MIC of Ox against MRSA from 64 to 2 μg/mL (the CLSI breakpoint) at a concentration of just 2 μg/mL of partially purified extract.

O-36

IDENTITY AND PURITY VERIFICATION OF COMMERCIAL COMPOUNDS IS ESSENTIAL FOR THE ACCURACY OF BIOLOGICAL RESULTS

Charlotte Simmler,[1,2] Obinna Mbachu,[1] Dejan C. Nikolic,[1] Richard B. van Breemen,[1,2] Judy L. Bolton,[1] Guido F. Pauli[1,2]

[1]UIC/NIH Center for Botanical Dietary Supplements Research, [2]Center for Natural Product Technologies (CENAPT), Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Research Street, Chicago, Illinois

A total of 15 flavonoids and isoflavonoids were sourced from various providers in order to investigate the structure activity relationships (SARs) related to their estrogenic potency in vitro. As part of good research practices, both NMR and MS analyses were performed in order to confirm the identity, and determine the purity of each compound. Surprisingly, 3 out of 15 compounds were adulterated. Moreover, chiral analysis of the claimed enantio-pure flavonoids (*e.g.*, 8-prenyl-naringenin) revealed them to be racemic. The purity of the compounds with confirmed identity, determined by qHNMR using the 100% method,[1] was found to be ~95% w/w, except for one flavonoid (85% w/w). More importantly, some impurities were structurally related flavonoids with estrogenic potency, such as genistein present in dihydrogenistein, thus requiring further purification prior to biological studies. Any bioassays performed with uncontrolled samples would have led to wrong conclusions due to mislabeled structures and/or presence of bioactive impurities, thereby affecting not only the accuracy but also the reproducibility of the results. The presentation will show details of the identity/purity verification and illustrate the impact on selected biological outcomes of the SAR study.

(1) Pauli GF, et al. *J. Med. Chem. 57*, 9220 (2014)

O-37

NEW COMPUTATIONAL APPROACHES TO NATURAL PRODUCT DISCOVERY IN PLANTS AND MICROBES

Marnix H. Medema[1]
[1]Bioinformatics Group, Wageningen University, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

Plants and microorganisms produce a wealth of specialized metabolites, which are of great importance from both ecological and clinical perspectives. Due to the accelerated accumulation of genomic, transcriptomic and metabolomic data, computational methods have become more and more important to identify new molecules and to assess their biological activities. In this lecture, I will highlight several tools and algorithms recently developed in our research group. Specifically, I will discuss recent developments in the antiSMASH software framework for biosynthetic gene cluster analysis, as well as BiG-SCAPE, a new tool to automatically group biosynthetic gene clusters into families that recapitulate the chemical structures of their products. Also, I will introduce plantiSMASH, a new tool for the analysis of plant biosynthetic gene clusters. Putting this all together, I will conclude with a vision on computation-driven natural product discovery and engineering in both plants and microorganisms.

O-38

CHEMICAL ANALYSIS AND ADULTERANT CHARACTERIZATION OF *ELEUTHEROCOCCUS SENTICOSUS* AND CI-WU-JIA TEA BY UHPLC UV-MS USING NOVEL INFORMATICS PLATFORM

Giorgis Isaac[1], Yan-Hong Wang[2], Yonghai Meng[3], Chunmei Zhai[3], Bharathi Avula[2], Mei Wang[2], Jimmy Yuk[1], Kerri Smith[1], and Ikhlas Khan[2]
[1]Waters Corporation, MA 01757, USA, [2]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, MS 38677, USA, [3]School of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin, 150040,P.R.China,

Eleutherococcus senticosus (syn. *Acanthopanax senticosus*) species of the Araliaceae family is native to Northeastern Asia and commonly known as Siberian ginseng and ci-wu-jia or eleuthero. Ci-wu-jia tea is made from tender leaf of *E. senticosus* following the procedure of green tea preparation. Triterpenoid saponins, lignans, coumarins, organic acids, and flavonoids have been identified from different parts of *E. senticosus* by LC-UV and LC-MS. In this study, a novel LC-MS informatics platform will be used to screen for compounds in these complex natural product extracts. It provides an intuitive workflow encompassing data processing, characterization and identification of potential marker compounds, visualization, and reporting. A UHPLC-UV-MS method was developed to analyze 24 samples that included authentic *E. senticosus* leaf samples and ci-wu-jia tea products. A library containing 243 compounds was created from the genus of *Eleuthercoccus* and green tea. Out of 11 tea products, three tea samples were adulterated with green tea. The present work will explore the strategy of determining the marker compounds and adulterants from *E. senticosus* and ci-wu-jia tea products along with MS/MS characterization of key makers of *E. senticosus*.

O-39

BRIDGING THE GAP BETWEEN MOUNTAINS OF DATA

Kenji L. Kurita[1], Elizabeth A. MacMillan[2], Suzie Hight[2], Anam F. Shaikh[2], Scott La[2], Walter M. Bray[3], R. Scott Lokey[3], Michael A. White[2], John B. MacMillan[2], Roger G. Linington[4]
[1]Small Molecule Analytical Chemistry and QC, Genentech Research and Early Development, South San Francisco, CA 94080, USA, [2]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA, [3]Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, USA, [4]Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6

Natural products remain an indispensable source of new molecules that provide handles to better understand and modulate ecological systems and biological pathways important to human health. As improvements in analytical chemistry, biological screening, and genetic sequencing continue to generate mountains of data from natural products libraries and producing organisms, there is an urgent to need to develop technologies to the integrate these data that are specific to the field of natural products. Integration of secondary metabolomics and high-content biological screening data enabled the prediction of the identity and mechanism of action of individual molecules from natural product extract libraries. Leads were selected using a global view of the data from 720 marine bacterial fractions. Following their selection, analysis of network representations of high-content screening data yielded hypotheses of the mechanism of action of these compounds. Target molecules could then be isolated for further biological assessment and to confirm their identity. This generalized approach to hypothesis-driven natural products discovery will be presented.

O-40

MALDI-TOF MS-BASED TAXONOMIC AND METABOLOMIC PROFILING FOR INFORMED MICROBIAL LIBRARY GENERATION

*Chase Clark[1], Sofia Costa[1,2], Sesselja Omarsdottir[2], Laura M. Sanchez[1], Brian T. Murphy[1]**
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612; [2]University of Iceland, Reykjavik, Iceland

A high degree of taxonomic and chemical redundancy is a major limitation of microbial strain libraries that are sourced for drug discovery. Currently, the creation of these libraries relies on outdated and costly methods, namely visual inspection of morphological differences of colonies from agar plates, or ribosomal RNA gene sequencing methods that afford data that are not necessarily indicative of a microbe's natural product (NP) producing capacity. Despite the incredible potential of microorganisms to produce NPs – as revealed by exhaustive genome mining studies – this redundancy remains one of the major barriers to maximizing the use of chemical space toward

drug discovery efforts. In response to this, we have developed a rapid, facile mass spectrometry technique designed to maximize both the taxonomic and chemical diversity in microbial strain libraries. We employ matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to *single* colonies of bacteria from agar-based diversity plates in order to *a)* generate ribosomal protein fingerprints that are used to putatively identify the genus and species of the colony, and *b)* generate small molecule fingerprints of each colony to compare intra-species differences in NP production potential. Fewer than four hours are required to prepare, acquire data for, and process 384 colonies in a semi-automated bioinformatics pipeline. Details of this process will be discussed.

O-41

NMR CHEMOMETRICS: DEVELOPMENT OF AUTOMATED MULTICLASS CLASSIFICATION METHODS FOR BOTANICALS.

K. Brian Killday[1], Amy S. Freund[1], Christian Fischer[2] and Kimberly L. Colson[1]
[1]Bruker BioSpin, Billerica, MA, USA, [2]Bruker BioSpin GmbH Rheinstetten, Germany

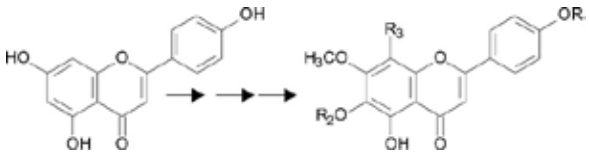
Adulteration of botanicals, both intentional and unintentional, has been proven to be widespread. Intentional adulteration is typically economically driven, such as diluting or substituting cheaper ingredients for more valuable ones. An example of this is Borage seed oil (*Borago officinalis*), which contains the fatty acid γ-linolenic acid (GLA). GLA has been studied for its anti-inflammatory and anti-thrombotic effects. As borage is costly to grow, an emerging problem in the market is the intentional mixing of cheaper oils such as safflower oil to increase the final product volume. Unintentional adulteration of botanicals can also occur from, for example, misidentification of species. Blueberry leaf extract (*Vaccinium angustifolium*) is known to have antidiabetic activity. It can however be adulterated with other *Vaccinium* species extracts which may reduce its efficacy.[1] Chemometrics utilizes statistical methods to understand chemical information and can be used to correlate product quality parameters or physical properties to analytical instrument data. Nuclear Magnetic Resonance (NMR) is well suited for chemometric analyses of complex mixtures in that it is information rich and highly reproducible. We have utilized multiclass classification methods with the Bruker AssureNMR software package for developing models, including PCA-ANOVA, PLS-DA, Multi-SIMCA, and k-Nearest Neighbors. The program generates a confusion matrix and recommends the best classification method for each particular botanical comparison. Once a model is built, samples can be submitted for fully automated NMR botanical identification analysis. Also, a quantification method using PLS to detect the level of safflower oil in borage oil to a level of 0.1% safflower oil was developed. Classification models for *Vaccinium sp.*, North American ginseng, black cohosh, and grape seed extracts will also be presented.

[1] Jonathan Ferrier et al, *Botany* 90 (2012) 401-406.

O-42

ELUCIDATION OF METHOXYLATED FLAVONE BIOSYNTHESIS IN SWEET BASIL AND USE OF UNDERLYING ENZYMES FOR FLAVONOID BIODIVERSIFICATION

Anna Berim and David R. Gang
Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA.



1 **2-6**

2: R_1=R_2=CH_3, R_3=H; **3**: R_1=R_3=H, R_2=CH_3

4: R_1= CH_3, R_2=R_3=H; **5**: R_1=R_2=CH_3, R_3=OH

6: R_1=R_2=CH_3, R_3= OCH_3

Microbial production and diversification of flavonoids are of interest for pharmaceutical and nutraceutical research and industry. Sweet basil (*Ocimum basilicum*) accumulates methoxylated flavones with characteristic hydroxyl residues at positions 8 and/or 6 and up to four *O*-methylations. In the past few years we combined multiple approaches to investigate the biosynthesis of these flavones in the plant. Our work resulted in complete pathway elucidation including several unprecedented reactions in flavonoid biosynthesis, such as a hydroxylation by a Rieske-type oxygenase and an *O*-demethylation. To test the performance of the isolated enzymes in vivo, we combined appropriate enzymes and constructed five baker's yeast strains producing salvigenin (**2**), cirsimaritin (**3**), ladanein (**4**), 8-hydroxysalvigenin (**5**), and gardenin B (**6**) using apigenin (**1**), the natural entry-point of the plant pathway, as fed substrate. First, we evaluated the conversion rates and flux using the salvigenin-producing strain under various culture conditions. We then probed the utility of the strains for biodiversification by supplying them with three further substrates representing different classes of flavonoids. Notably, we observed that the substrate specificity of *O*-methylating enzymes is limiting to several conversions, as are the stringent substrate preferences of the 6- and 8-hydroxylases. Overall, we created a promising first platform for the production of polymethoxylated flavonoids in baker's yeast, which can now be expanded for higher efficiency and versatility.

O-43

LC-MS-BASED STRATEGY FOR SCREENING OF PASSIVELY ABSORBED AÇAÍ AND MACA EXTRACT CONSTITUENTS FOR CYP3A4 INHIBITION

Angela I. Calderón[1], Thankhoe A. Rants'o[1], Da S. Jung[1], Lane McLendon[1]
[1]Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, 4306 Walker Building, Auburn, AL 36849, USA

CYP3A4 is the most abundant and main metabolic enzyme involved in biotransformation of antineoplastics. The aim of this research was to develop an LC-MS strategy to assess açaí and maca extracts for potential botanical-antineoplastic drug interaction through CYP3A4 inhibition. This study used LC-MS-based assays to assess transcellular passive absorption using parallel artificial membrane permeability assay (PAMPA) for methanol, acidic-methanol and dichloromethane açaí and maca constituents at 2.5 - 7.5 µg/µL and their standards followed by the evaluation of the Phase I and Phase II metabolism and CYP3A4 inhibition of the permeable constituents using human liver microsomes. Permeable constituents of methanol, acidic-methanol and dichloromethane açaí and maca extracts displayed moderate CYP3A4 inhibition. Additionally, Phase I and II metabolites of

maca extracts showed weak to moderate CYP3A4 inhibition whereas Phase I and II metabolites of açaí extracts showed weak CYP3A4 inhibition indicating sufficient detoxification. A total of sixteen metabolites of passively permeable maca chemical constituents were confirmed, and these consisted of thirteen hydrolysed or oxidized Phase I metabolites and three Phase II glucuronides. In the case of permeable açaí constituents, three Phase I and two Phase II metabolites were identified.

O-44

A TRIP TO AFRICA RAINFOREST: SCIENTIFIC RATIONALE OF SOME MEDICINAL PLANTS

Xavier Siwe-Noundou[1,2], Jean E. Mbosso-Teinkela[3], Bertha Chithambo[2], Louis P. Sandjo[4], and Rui W.M. Krause[2]
[1]Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa, [2]Department of Chemistry, Rhodes University, Grahamstown, 6140, South Africa, [3]Department of Biological Sciences, University of Douala, PO Box 2701 Douala, Cameroon, [4]Department of Pharmaceutical Sciences, Universidade Federal de Santa Catarina, Trindade, Brazil

The rainforest in Africa is wide, diverse and rich in important medicinal plants. The majority of the population in this region rely on medicinal plants for their primary health care due to high cost associated with Western medicine. Our study was designed to scientifically validate the traditional claims of the following plant species: *Ficus elastica* (Moraceae), *Morinda lucida* (Rubiaceae), *Echinops giganteus* (Asteraceae), *Alchornea cordifolia*, *A. laxiflora*, *A. floribunda* (Euphorbiaceae), *Erythrina sigmoidea* (Leguminoseae) and *Cylicodiscus gabunensis* (Mimosaceae). Fifty-one secondary metabolites (11 new derivatives and 40 known ones), including flavonoids, iridoids, polyphenols, fatty acids, anthraquinones, ceramides, thiophenes, terpenoids and steroids were identified. Chemical structures of isolated compounds were established by extensive NMR and MS analyses. Several of these natural products displayed noteworthy antimicrobial (2–39.1 μg/ml), antiproliferative (5–83 μM), antimalarial (17–25 μM) and anticancer (6–55 μM) activity. Our results provide a scientific rationale for the studied plants and suggest some of the isolated compounds as potential leads for further studies in drug discovery.

O-45

ANTIMALARIAL COMPOUNDS FROM PHILIPPINE SPONGE-ASSOCIATED MICROORGANISMS

Kevin Bossie Davis[1], Wesley Wu[2], Thatcher Pancho[1], Jannelle Casanova[1], Gisela P. Concepcion[1], Joseph DeRisi[2], Christine C. Hernandez[3], Lilibeth A. Salvador-Reyes[1]
[1]Marine Science Institute, University of the Philippines, Diliman 1101 Quezon City, Philippines, [2] Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA 94143, USA, [3]Institute of Chemistry, University of the Philippines, Diliman 1101 Quezon City, Philippines

Drug resistant *Plasmodium falciparum* parasites are an emerging concern and pose a major health security risk. There are currently no alternative treatment regimens to artemisin-based therapy for malaria. Therefore, there is an urgent need to identify new antimalarial compounds with novel mechanism of action. Here, we assess the antimalarial activity of extracts from sponge-associated microorganisms collected from the Philippines and identify the bioactive components from prioritized samples. A total of 2,044 Diaion fractions generated from 520 microbial isolates were tested against *Plasmodium falciparum* W2 strain. Significant antimalarial activity (% *P. falciparum* proliferation < 30%) were observed in 44 Diaion fractions. Two microorganisms were prioritized for further purification of the bioactive component based on the antimalarial activities, chemical profiles and taxonomic identification of the source microorganism. Bioactivity-guided purification of the microorganism Pm-0021 afforded five structurally-related compounds with IC_{50} of 0.5 – 4.0 μM against *P. falciparum* W2 strain. Critical structural features for antimalarial activity were identified. Resistance generation was commenced to identify the cellular target and to elucidate the mechanism of action of this class of antimalarial compounds from marine microorganisms.

O-46

BACTERIAL METABOLITES AND LARVAL RECRUITMENT FOR BENTHIC MARINE COMMUNITIES

You-Sheng Cai[1], Marnie Freckelton[2], Helen Turano[3], Brian T. Nedved[2], Michael Hadfield[2], Rosanna Alegado[3] and Shugeng Cao[1,]*
[1]Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, HI, USA; [2]Kewalo Marine Laboratory, Pacific Biosciences Research Center, University of Hawai'i at Mānoa, Honolulu, HI , USA; [3]Oceanography and Sea Grant College, Center for Microbial Oceanography: Research and Education University of Hawai'i, Mānoa, Honolulu, HI , USA.

For more than a century, marine biologists have been seeking an understanding of how the minute larvae of marine invertebrate animals, cast out into the broad ocean, find and settle in the right ecological settings for survival, growth and reproduction. While evidence that larvae are induced to settle and metamorphose by "factors" specific to certain muds or sands began to accumulate in the late 1940s, recognition that bacteria in surface biofilms are the 'factors' in most instances has grown significantly only in the last two decades. Publications on the topic cite nearly every major marine phylum, from sponges to cnidarians, polychaete worms, arthropods, echinoderms and chordates as requiring biofilms for larval settlement. This breadth of examples strongly suggests that the establishment and maintenance of most benthic marine populations, including those on rocky shores, coral reefs, mudflats, and sub-tidal regions, depend largely on bacterial stimulation for recruitment. Yet, we know very little of the diversity of bacteria that stimulate larvae to settle, and less of small molecules bacterial metabolites that might be important for larval recruitment for benthic marine communities. We have isolated marine bacteria that produce inductive mono-specific biofilms and we will identify small molecules from the OMVs (Outer Membrane Vehicles) of these bacteria that strongly induce larval settlement and metamorphosis.

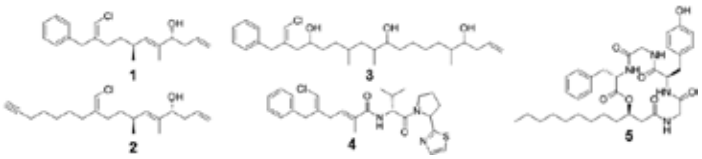
O-47

CHEMICAL PROFILING OF A *TRICHODESMIUM* BLOOM FROM THE GULF OF MEXICO REVEALS A BIOSYNTHETICALLY INTRIGUING METABOLOME

Matthew J. Bertin[1], Christopher W. Via[1], Alexandre F. Roduit[1], Miguel A. Gonzalez[1], Danielle G. Goldstein[1], Paul V. Zimba[2], and Peter D. R. Moeller.[3]
[1]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA, [2]Department of Life Sciences, Texas A&M Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412, USA, [3]Emerging Toxins Program, National Ocean Service/NOAA, Hollings Marine Lab, 331 Fort Johnson Road, Charleston, SC 29412, USA

Trichodesmium thiebautii, a marine filamentous cyanobacterium of the order Oscillatoriales, is globally significant both for its biogeochemical role in N_2 fixation and the biological community associated with its dense blooms. We are currently investigating the secondary metabolic composition of a *Trichodesmium thiebautii* bloom collected from Padre Island, TX in 2014 using multiple analytical approaches. Our results reveal a remarkably diverse metabolome with polyketides (**1**-**3**), hybrid PKS-NRPS molecules (**4**)

and peptides (**5**) posing intriguing questions with respect to biosynthesis of these metabolites and the chemical space represented in these blooms.



O-48

BACTERIAL COMMUNICATION IN SITU: A TALE OF TWO *VIBRIOS*

Alanna R Condren, Katherine E Zink, Chase Clark, Sofia Costa, Brian T Murphy, Laura M Sanchez
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612

Microbiome studies have laid the groundwork demonstrating that bacteria are major players in host health, however the exact mechanisms by which these bacteria influence or protect hosts represents a major gap in knowledge. There are strong correlative observations from microbiome surveys that suggest microbes play essential roles in host function, but very little work has been done to uncover the chemical mechanisms underlying host-microbe interactions. We have developed orthogonal approaches to study natural products in situ. First, we have developed a matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) pipeline to rapidly profile metabolic fingerprints directly from Petri dishes. We simultaneously applied this technique to probe two model host-microbe interactions; one being a symbiotic relationship leading to colonization of epithelial cells by one specific bacterium (*Vibrio fischeri* and the Hawaiian bobtail squid), and the other exploring how enteric pathogens can colonize and infect a host vertebrate (*V. cholerae* and zebrafish). Both of these systems are native to the macroorganism and microbe, allowing us to fully characterize the underlying chemistry involved in colonization, infection, and maintenance of the relationship. We use a combination of MALDI-TOF imaging MS techniques and orthogonal molecular networking approaches to probe these two distinct systems.

O-49

OMICS APPROACH FOR ACCESSING THE NATURAL PRODUCT UNIVERSE OF MARINE CYANOBACTERIA.

Tiago Leao[a], Guilherme Castelão[b], Lena Keller[a], Sheila Podell[a], Evgenia Glukhov[a], Eric Allen[a], William H. Gerwick[a]. Lena Gerwick[a]
[a]Center for Marine Biotechnology and Biomedicine, [b]Climate, Atmospheric Sciences, and Physical Oceanography, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, United States.

Historically, natural products have been discovered and isolated via chemical and/or bioactivity-guided approaches (referred to as "top-down approaches"). However, the emergence of the genomics era has enabled a combined approach of genome mining and metabolomics by which to discover novel natural products ("bottom-up"). A core challenge in bottom-up discovery is to efficiently group biosynthetic gene clusters (BGCs) into families, constructing a 'gene cluster network'. Therefore, we designed a solution for networking gene clusters based on synteny and homology between BGCs (http://biocompass.net). To test our pipeline, we evaluated one of the most prolific natural products producers, the cyanobacterial genus *Moorea*. By applying our pipeline to *Moorea*, we demonstrated that many of its BGCs are unique among bacterial genomes.[1] Our pipeline was able to dereplicate known NP pathways and by combining it with mass spectrometry, we could predict the most promising pathway candidates for drug discovery and the proper isolation methods for those candidates.

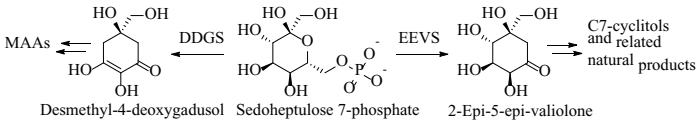
[1] Leao T, Castelão G, Korobeynikov A, Monroe EA, Podell S, Glukhov E, Allen E, Gerwick WH, Gerwick L. (2017) PNAS: 114, 3198–3203

O-50

EVOLUTION OF C_7-CYCLITOL SYNTHASES AND THEIR DISTRIBUTION THROUGHOUT PROKARYA AND EUKARYA

Andrew R. Osborn[1], Kelsey M. Kean[2], Khaled M. Alseud[1], Khaled H. Almabruk[1], Shumpei Asamizu[1], Janet A. Lee[1], P. Andrew Karplus[2], and Taifo Mahmud[1]
[1]Department of Pharmaceutical Sciences, [2]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

2-Epi-5-epi-valiolone synthase (EEVS), a C_7-sugar phosphate cyclase (SPC) was discovered during biosynthetic studies of the C_7N-aminocyclitol-containing natural products. Originally thought to be limited to certain actinomycetes, EEVS has since been identified in both prokaryotes and eukaryotes. Another SPC, desmethyl-4-deoxygadusol synthase (DDGS), was later found in the biosynthesis of mycosporine-like amino acid (MAAs) sunscreen compounds. We identified sequence features useful for distinguishing these enzymes, note active site differences, and demonstrate the importance of two active site residues for catalysis by point mutations. We examine the distribution of EEVS and DDGS in various prokaryotes and eukaryotes which suggests their broad potential biological roles in nature.

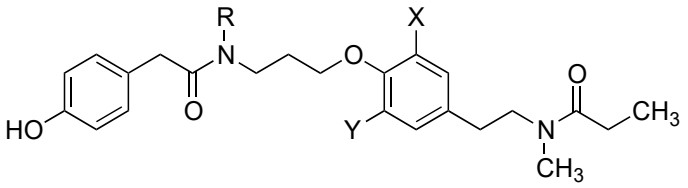


O-51

BIOACTIVE NATURAL PRODUCTS FROM INDO-PACIFIC MARINE SPONGES

Erin P. McCauley[1], Nicholas Lorig-Roach[1], Karen Tenney[1], Frederick A. Valeriote[2], and Phillip Crews[1]
[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064, United States, [2]Department of Internal Medicine, Division of Hematology and Oncology, Henry Ford Hospital, Detroit, MI 48202, United States

The Crews research group at UCSC has developed an extensive repository of sponges from coral reefs of the Indo-Pacific. This research focused on a sub set that was collected from the Coral Triangle in Indonesia. Extracts of these sponges were submitted to two bioassays, the first of which was performed through a collaboration with the Josephine Ford Cancer Center, where the extracts are screened for cytotoxicity against human solid tumor cell lines. This led to renewed interest in the makaluvamines, in particular makaluvamine J, which is very potent (IC_{50} = 54 nM) against the PANC-1 cell line (*Mar. Drugs* **2017**, *15*, 98). The second bioassay was performed with the Auerbuch-Stone research group at UCSC, where extracts were screened for metabolites that act as type three-secretion system (T3SS) inhibitors (*Antimicrob. Agents Chemother.* **2014**, *58*, 1118). This led to the identification of nine bromo- and iodotyrosine-derived metabolites, such as **1**-**7** that have putative T3SS inhibition activity.



(**1**) R = H X = Br Y = H (**5**) R = H X = Br Y = I
(**2**) R = H X = I Y = H (**6**) R = H X = I Y = I
(**3**) R = CH_3 X = Br Y = H (**7**) R = CH_3 X = Br Y = I
(**4**) R = H X = Br Y = Br

O-52

UNCOVERING THE CHEMICAL DIVERSITY OF A LIBRARY OF EPIGENETICALLY MODIFIED FUNGAL EXTRACTS

Danielle H. Demers,[1,2] Matthew A. Knestrick,[1,2] Renee Fleeman,[1,3] Ala Azhari,[1,4] Ashley Souza,[1,4] Brian Vesely,[1,4] Mandy Netherton,[5] Beatrice Colon,[1,4] Christopher A. Rice,[1,4] Kyle Rohde,[5] Dennis E. Kyle,[1,4] Lindsey N. Shaw,[1,3] Bill J. Baker.[1,2]

[1]Center for Drug Discovery and Innovation and Departments of [2]Chemistry, [3]Cell Biology, Microbiology and Molecular Biology, and [4]Global Health, University of South Florida, Tampa, FL, USA, [5]Burnett School of Biomedical Sciences, University of Central Florida, Orlando, FL, USA

A library of over 500 fungal isolates was cultured under conditions to modulate epigenetic expression, and extracts were screened against a range of infectious disease causing organisms. The resulting extract library was screened against *Leishmania donovani*, the ESAKPE pathogens, *Mycobacterium tuberculosis*, *Naegleria fowleri*, and J774 macrophages. Using stringent definitions of 'active' to moderate the hit rate, 19% of extracts were determined to be active. Strikingly, around 80% of the hits were hits in only one assay, illustrating the exquisite selectivity fungi have for targets of their chemical defenses. Additionally, the findings show that each of the culture treatments were equally successful in producing active extracts, while approximately 40% of active fungi only produced hits if they were epigenetically modified. We believe this to be the first example of an epigenetically controlled fungal extract library screened against such a comprehensive panel of infectious diseases, and find the results to be informative and supportive of this method of natural products screening efforts for drug discovery.

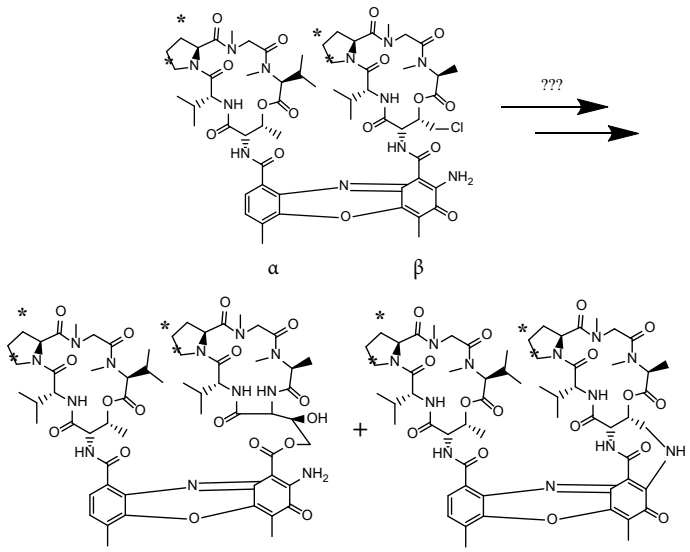
O-53

INDUCIBLE ACTINOMYCIN HETEROCYCLE CAUSES CHANGES IN ACTIVITY AND SHOWN IN MULTIPLE ACTINOMYCIN FAMILIES

Matt McErlean, Wenlong Cai, Khaled Shaaban, Aubree Zimmer, Jürgen Röhr, Jon Thorson, Steven Van Lanen

College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA

The actinomycin scaffold has been well studied for its ability to bind DNA and interfere with mRNA production. Since the discovery of the parent compound, Actinomycin D, in 1964, several other families of actinomycins have been discovered. Some of these contain β-ring heterocycles and other rearrangements to the β-ring that drastically change the biological activity of these compounds. However, it remains unknown how such rearrangements occur *in vivo*. By investigating two similar families of actinomycins—Z-type and Y-type—we have shown progress towards inducing these activity-changing rearrangements *in vitro* as well offer a hypothesis for how such a rearrangement would affect the activity of these compounds.



*indicates variable position between Y and Z families

O-54

ANTI-*M.TB* RUFOMYCIN IN COMBINATION WITH CLPC1 TARGETING COMPOUNDS

Mary P. Choules[1,2], Sang-Hyun Cho[1], Nina M. Wolf[1], Yang Yu[2], Wei Gao[1,2], Jeffrey R. Anderson[1], David C. Lankin[2], Hyun Lee[2,3], Bernard Santarsiero[2,3], Jinhua Cheng[4], Hanki Lee[4], Joo-Won Suh[4,5], Birgit U. Jaki[1,2], Scott G. Franzblau[1], and Guido F. Pauli[1,2]

[1]Institute for Tuberculosis Research, [2]Dept. of Med. Chem. & Pharmacognosy, and [3]Center for Biomolecular Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612, USA; [4]Center for Nutraceutical and Pharmaceutical Materials; [5]Div. of Bioscience and Bioinformatics, College of Natural Science, Myongji University, Cheoin-gu, Gyeonggi-do 17058, Korea, Republic of

ClpC1 is the chaperone portion of a protease complex responsible for protein degradation in *M. tuberculosis (M.tb)* and is currently not targeted in tuberculosis treatment. Several cyclic peptides have been identified that target ClpC1: rufomycin (RUF), ecumicin (ECU), and cyclomarin A (CymA). Studies were undertaken to better understand how these compounds bind to ClpC1. *M. tb* spontaneous resistance mutants to either RUF (rRUF) or ECU (rECU) have single nucleotide mutations within *clpc1*, but lack cross resistance. The same mutants demonstrated variable susceptibility to CymA. Surprisingly, when ECU, RUF, and CymA were tested in combination, the kinetics of growth inhibition suggests possible competitive effects. Preliminary surface plasmon resonance data and reported co-crystallization studies on CymA indicate that the three compounds have variable affinity to ClpC1. Research pertaining to characterization of RUF will be presented with the results from several combination studies.

Acknowledgments. T32AT007533 (OD, NCCIH); R21 AI093919-02 (NIAID), Next-Generation BioGreen 21 Program (No. PJ01133003; Rural Development Administration, Republic of Korea).

O-55

BIOLOGICAL ACTIVITY OF PYRROLOIMINOQUINONE ALKALOIDS FROM SOUTH AFRICAN LATRUNCULID SPONGES

Jarmo-Charles J. Kalinski[1], Rosemary A. Dorrington[1], Heinrich C. Hoppe[1], Meesbah Jiwaji[1], Xavier Siwe Noundou[1], Shirley Parker-Nance[2], Rui W. Krause[3] and Kerry L. McPhail[4]

[1] Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, South Africa. [2] Ellwandle Coastal Node, South African Environmental Observation Network, Port Elizabeth, South Africa. [3] Department of Chemistry, Rhodes University, Grahamstown, South Africa. [4] College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

Marine sponges of the family *Latrunculiidae* produce an array of bioactive pyrroloiminoquinones, a class of highly conjugated alkaloids known to exhibit anticancer, antibacterial, antiviral and antimalarial activity. The crude extracts of five South African latrunculid species of the genera *Tsitsikamma* and *Cyclacanthia* were investigated by LC-MS/MS analysis, revealing the presence of known and many novel pyrroloiminoquinones. Several compounds, including tsitsikammine B, 3-dihydrodiscorhabdin C and a novel makaluvamine, were isolated employing standard chromatographic techniques and characterised using NMR and MS analysis. Bioassays revealed differential biological activity, including cytotoxicity towards human (HeLa and HEK293) cells (0.29-3.1 μM and 0.55-14 μM respectively) and *P. falciparum* (0.56-8.9 μM). All pyrroloiminoquinone isolates exhibited anti-retroviral activity in HIV-1 pseudovirus assays. *In vitro* assays demonstrated tsitsikammamine B and the novel makaluvamine to inhibit human topoisomerase I, the latter exhibiting particularly potent activity, while 3-dihydrodiscorhabdin C was inactive.

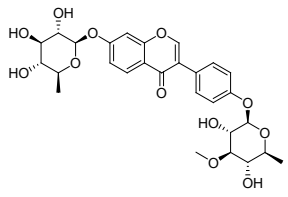
O-56

ISOFLAVONOID GLYCOSIDES FROM THE TERMITE-ASSOCIATED *STREPTOMYCES* SP. RB1

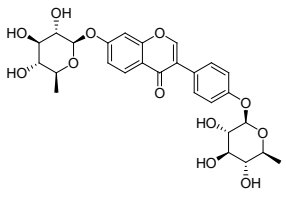
Seoung Rak Lee[1], Jae Sik Yu[1], Seulah Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Hae Min So[1], Sil Kim[1], Dahae Lee[1], Won Se Suh[1], Kyoung Jin Park[1] and Ki Hyun Kim[1]

[1] School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

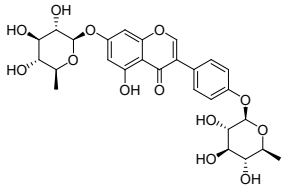
As part of our continuing study to explore structurally novel and bioactive secondary metabolites from insect-associated bacteria, we investigated the metabolites of the termite-associated *Streptomyces* sp. RB1. One new isoflavonoid glycoside, termisoflavone D (**1**), and six known isoflavonoid glycosides (**2**-**7**) were isolated from the culture extract of *Streptomyces* sp. RB1. The chemical structure of new compound (**1**) was elucidated by extensive spectroscopic methods including 1D and 2D NMR as well as LC/MS analysis. In addition, antiviral effects of the isolated compounds were studied in cell culture monolayers



1



2



3

O-57

PROCYANIDINS: IDENTIFICATION AND ANALYSIS USING ION MOBILITY MASS SPECTROMETRY

Emily A. Rue,[1] Jan A. Glinski[2] and Richard B. van Breemen[1]

[1] UIC/NIH Center for Botanical Dietary Supplements, University of Illinois College of Pharmacy, Chicago, IL 60612; [2] Planta Analytica LLC, New Milford, CT 06776

An ion mobility electrospray mass spectrometry method was developed for the rapid separation of mixtures of oligomeric procyanidins. First, this separation was demonstrated for a mixture consisting of catechin (monomer), procyanidin A1 (dimer) and cinnamtannin-D (trimer). As more complicated mixtures were evaluated, not only were oligomers separated according to their chain length, but A-type and B-type procyanidins of the same chain length could be resolved using ion mobility. For example, this was demonstrated for a mixture of procyanidins B1 and A1 (dimer), C1 and peanut trimer B (trimer), as well as peanut tetramer E and cocoa tetramer D (tetramer). Coupled with ion mobility separations that required only milliseconds, accurate mass measurements and product ion tandem mass spectra were obtained for additional structural characterization. Compared with conventional HPLC or NMR, IMS MS/MS was many orders of magnitude faster for procyanidin separation and characterization.

O-58

ANTI-STRESS ACTION OF AN ORALLY-GIVEN COMBINATION OF RESVERATROL, B-GLUCAN, AND VITAMIN C

Vaclav Vetvicka, and Jana Vetvickova

Department of Pathology, University of Louisville, Louisville, Kentucky, 40292, USA

Natural plant-derived products, useful in preventing and/or treating various diseases, have been sought after throughout the history of mankind. β-Glucans are structurally complex homopolymers of glucose, isolated from various sources including yeast, fungi, and wheat. Their role as biologically active immunomodulators has been well documented for more than 50 years. Stress has repeatedly been found to reduce the abilities of the immune system to fight against individual attacks. The current dissatisfaction with classical medications has led to more attention being focused on natural molecules. As recent studies have suggested that some bioactive molecules can have synergistic effects in stimulation of immune system and reduction of stress, we have evaluated the stress-reducing effects of the resveratrol-β-glucan-vitamin C combination. We found that compared to its individual components, this combination was the strongest reducer of stress-related symptoms, including corticosterone levels and IL-6, IL-12 and IFN-γ production. Conclusion: combination of these three compounds showed a strong anti-stress properties most probably manifested via stimulation of immune reactions. A study attempting to reveal the exact mechanisms of these effects is currently under progress.

O-59

COMPUTER-AIDED DRUG DISCOVERY OF SELECTIVE GSK3B INHIBITORS INSPIRED BY NATURAL PRODUCTS FOR ALZHEIMER'S DISEASE

Zhibin Liang and Qing X. Li

Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu, HI 96822, United States

Alzheimer's disease (AD) is the most common form of dementia globally that cannot be prevented, cured or even slowed. Hyperphosphorylation of tau proteins resulting in neurofibrillary tangles plays a pivotal role in AD pathology. Glycogen synthase kinase-3β (GSK3β) is a key enzyme responsible for hyperphosphorylation of tau proteins and is a promising thera-

peutic target of AD. *C*-Glycosylflavones omnipresent in plants have shown diverse therapeutic potential to treat human diseases including AD. Our previous studies demonstrated that the *C*-glycosylflavone isoorientin, from corn silk (*Zea mays*), is a selective, non-ATP competitive, substrate-competitive, and reversible GSK3β inhibitor [1]. In the present study, we applied computer-aided drug design and a SAR-based lead optimization approach. Semi-synthesis was used to produce analogs of isoorientin targeting the substrate-binding site on GSK3β. We have demonstrated that the newly developed analogs not only show increased potency against GSK3β but also improved drug-like physicochemical properties in comparison with the natural product counterpart. Additionally, these new analogs have distinct kinase selectivity to GSK3β and effectively block GSK3β-mediated tau hyperphosphorylation and amyloid neurotoxicity in molecular and cellular AD models. This investigation elaborates a delicate case study of computer-aided drug discovery inspired by natural products for AD chemotherapy.

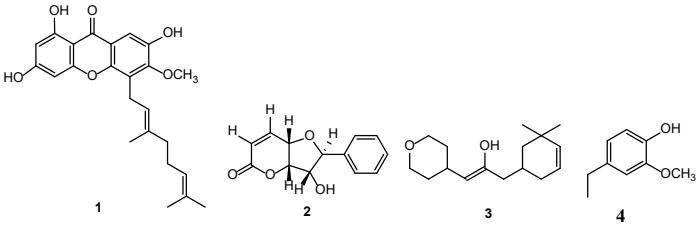
[1] Liang, Z.; Zhang, B.; Su, W. W.; Williams, P. G.; Li, Q. X. *ACS Chem. Neurosci.* **2016**, *7*, 912-923.

O-60

ANTIBACTERIALS FROM THE TROPICAL RAIN FORESTS OF BORNEO

Stephen Teo[1], Sanjib Bhakta[2] and Simon Gibbons[1]
[1]Research Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK, [2]Department of Biological Sciences, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK

The screening of plant extracts from Borneo (Sarawak) led to the isolation of compounds with activity against Gram-positive and Gram-negative bacteria besides the acid-fast group of human and animal pathogens such as the mycobacteria. Among these were a xanthone (**1**), and a pyranone from *Garcinia celebica*, styryl lactones (e.g. **2**) from *Goniothalamus longistipetes,* pyranones (e.g. **3**) from *Horsfieldia grandis* and phenolic compounds (e.g. **4**) from *Gymnacranthera ocellata* (pictured below). The biological activities of the bioactive compounds will be presented.



Gymnacranthera ocellata

O-61

BOTH PHENOLIC AND NON-PHENOLIC GREEN TEA FRACTIONS INHIBIT MIGRATION OF CANCER CELLS

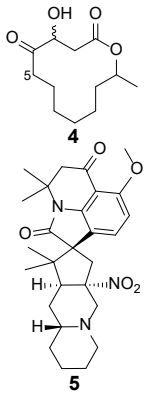
*Ean-Jeong Seo[1], Ching-Fen Wu[1], Zulfiqar Ali[2], Yan-Hong Wang[2], Shabana I. Khan[2,3], Larry A. Walker[2,3], Ikhlas A. Khan[2,3], Thomas Efferth[1]**
[1]Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Staudinger Weg 5, 55128 Mainz, Germany, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA, [3]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Green tea consumption is associated with chemoprevention of many cancer types. While the polyphenols of green tea have been well investigated, it is still largely unknown, whether or not non-phenolic constituents also reveal chemopreventive and anti-metastatic effects. We investigated the effects of a fraction of green tea rich in phenolic compounds (PF), a non-phenolic fraction (NPF), which contains glyceroglycolipids (GGL), and a pure glyceroglycolipid compound isolated from the non-phenolic fraction in human cancer. All three green tea samples did not show significant cytotoxic activity in both HepG2 and AML12 cells. We identified three sets of genes differentially expressed upon treatment with the green tea samples. The genes were associated with cytoskeleton formation, cellular movement and morphology. HepG2 and U2OS cells treated with green tea extracts showed the delayed closures. Besides, the number of distinct tubulin filaments decreased upon treatment with green tea samples. We identified not only PF, but also glyceroglycolipids in NPF as contributing factors to the chemopreventive effects of green tea. Both PF and NPF of green tea inhibited cancer cell migration by the disassembly of microtubules, even though they were not cytotoxic.

O-62

IDENTIFICATION OF NEW AND BIOACTIVE COMPOUNDS FROM FERMENTATION OF THE ENDOPHYTIC FUNGUS *PENICILLIUM CONCENTRICUM*

Tehane Ali[1], Choon Yong Tan[1], Gerardo D. Anaya-Eugenio[1], Chad Rappleye[2], Thomas Wieboldt[3], Esperanza J. Carcache de Blanco[1], Harinantenaina L. Rakotondraibe[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, [2]Department of Microbiology, Center for Microbial Interface Biology, The Ohio State University, Columbus, OH 43210, USA, [3]Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA



In our continuing systematic investigation to find cytotoxic compounds from endophytes, we isolated and evaluated seventeen endophytic fungi of the liverwort *Trichocolea tomentella*, for cytotoxicity against hormone-dependent breast (MCF-7) and colorectal (HT-29) cancer cell lines. *P. concentricum* was selected due to the potent activities of its ethyl acetate extracts

with ED_{50} values ranging from 2.2-7.0 and 2.5-12.0 ***μg/mL against*** MCF-7 and HT-29, respectively. From these extracts, new compounds including: 6-chloro-3,8-dihydroxy-1-methylxanthone (**1**), 2-bromo-gentisyl alcohol (**2**), a mixture of 6-dehydroxy-6***β***- and 6-dehydroxy-6***α***-bromogabosine C (**3a**, **3b**), 5-dehydroxy pandangolide 1 (**4**) and a prenylated clopiamine-type alkaloid with a rare alicyclic nitro group (**5**) together with twenty four previously reported compounds have been isolated. The structure elucidation and antiproliferative evaluation of the isolated compounds will be presented.

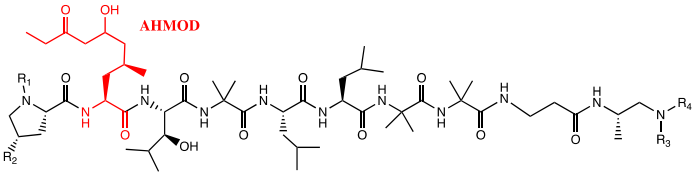
O-63

EVOLUTIONARY METABOLOMICS IN *TOLYPOCLADIUM* FUNGI TO GUIDE NATURAL PRODUCTS DISCOVERY

Richard Tehan[1], Rheannon Arvidson[2], Ryan Goold[2], Joseph W. Spatafora[2], Kerry L. McPhail[1]

[1]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331. [2]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331

Fungi in genus *Tolypocladium* produce biologically active secondary metabolites (SMs), including the clinically important immunosuppressant, cyclosporin A. Genome sequencing and genome mining enable a census of secondary metabolic potential, which may be induced by varying culture conditions. To this end, thirteen *Tolypocladium* spp. grown in twelve different media conditions were surveyed by parallel metabolomics (LC-MS/MS) and transcriptomics (RNASeq) analyses, to guide the discovery of new SMs, and to trace the evolution of secondary metabolism in hypocrealean fungi. These analyses revealed new peptaibiotics in several *Tolypocladium* spp. which were subsequently paired with their BGCs. An expansive series of linear decapeptides containing the unique amino acid, 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD), is produced by a cohort of wood endophytes. The scope, bioactivity, and biosynthetic context of AHMOD-containing peptides produced by *Tolypocladium* spp. is being systematically investigated to reveal the overarching patterns and processes of production.



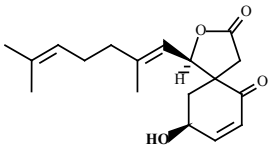
O-64

ANTICANCER EFFECTS OF MILIUSANES ON COLORECTAL CANCER CELLS

Yi-Xuan Xia[1], Wen-Hui Pan[1], Siu Wai Tsang[1], and Hong-Jie Zhang[1,]*

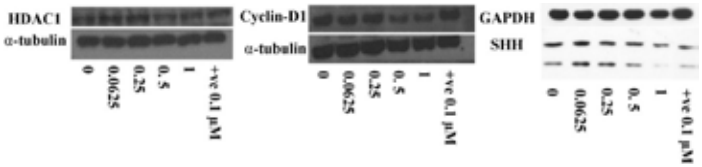
[1]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, P.R. China.

In our previous study, we isolated a series of miliusanes from *Miliusa sinensis* Finet and Gagnep, a medicinal plant found in southern Asia. Several of the miliusane compounds including miliusol showed cytotoxic activity against various types of cancer cells (Zhang, et al., 2006, Miliusanes, a class of cytotoxic agents from *Miliusa sinensis*. *J. Med. Chem.*, **2006**, *49*, 693-708.). Miliusol was investigated for its antitumor potency in a xenograft mouse model. Further study showed that miliusol effectively inhibited the expression levels of the sonic hedgehog signaling pathway components and some phosphoinositide 3-kinase (PI3K)/serine-threonine kinase (AKT) signaling pathway effectors, which suggested that the anti-cancer mechanism of miliusol may be associated with attenuation of PI3K/ AKT signaling pathway. *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).*



Miliusol

A)



B)

Figure 1. A) Chemical structure of miliusol; **B)** Miliusol attenuated apoptosis mediators *in vitro* [HCT116 cells were treated with miliusol in various of concentrations (μg/mL) for 48 h prior to Western blotting analysis, +ve indicated the positive control drug]: α-tubulin and GAPDH were served as a loading reference, the protein levels of apoptosis mediator HDAC1 and cyclin D1, and the hedgehog signaling pathway mediator SHH were down-regulated by treatment of miliusol.

Poster Presentations

P-001

A UNIVERSAL PLATFORM FOR CONNECTING BIOSYNTHETIC GENE CLUSTERS AND THEIR COGNATE PRODUCTS IN ACTINOBACTERIA

Catherine McCaughey[1] and Roger Linington[1]
[1]Department of Chemistry, Simon Fraser University, 8888 University Dr. Burnaby, BC.

Recent advances in genomics have revealed that, despite the trend of re-discovering known chemistry in natural products, microorganisms have great potential to generate novel chemistry hidden within their genomes. Approaches for connecting biosynthetic gene clusters (BGCs) with the products they produce are limited by the complex deconvolution of mass spectra (MS) and inefficiencies in structure prediction algorithms. We have developed a method for connecting natural products to BGCs by utilizing semi-targeted stable isotope labeling (SIL) experiments to identify MS features belonging to specific biosynthetic classes. Using a set of sequenced model organisms we demonstrate that carefully controlled SIL incorporation into polyketide products can be interpreted to decipher compound size, biosynthetic class, and precursor pathways. This analysis effectively deconvolutes polyketide MS features from complex extracts while providing structural information which can be interpreted in the context of the organism's BGCs. We demonstrate that this method may be expanded to identify compounds from other biosynthetic origins, with the goal of connecting all observable natural products with their corresponding gene cluster.

P-002

NMR QUANTITATION AND CACO-2 MODEL PERMEABILITY SCREENING OF A BOTANICAL MARKER IN SURVEY OF *GLYCYRRHIZA* EXTRACTS

Laura Tyler, Alyssa Tonsing-Carter, Richard B. van Breemen, Shao-nong Chen, Guido Pauli
Department of Medicinal Chemistry and Pharmacognosy UIC/NIH Center for Botanical Dietary Supplements Research, College of Pharmacy, University of Illinois at Chicago, IL.

The therapeutic effects of botanical dietary supplements are often attributed the administration of whole plant material. However, quality measures stipulate the standardization of botanical dietary supplements to the presence or concentration of single marker compounds. The central aim of this study is to track the bioavailability modulation of a marker compound co-administered with extracts of diverse polarity and chemical composition. Licorice (*Glycyrrhiza inflata x G. glabra*) extracts were prepared by water infusion, simulated digestion, methanolic percolation, and super-critical fluid as well as serial extraction protocols. The quantitation of licochalcone A was determined for each extract by peak-fitting Global Spectral Deconvolution and Quantum Mechanical QM qHNMR. These quantitative measurements were enabled through the enrichment of licochalcone A by loss free High Speed Counter Current Separation (HEMWat 0, KHSCCC=0.89) to optimize dispersion in the NMR spectra. Extracts were applied to the Caco-2 bioassay to measure the rate of permeability of licochalcone A across a membrane in variable chemical matrices. The results obtained call for the standardization of botanical dietary supplements according to the chemical matrix that promotes the bioavailability of the active constituent.

Research was supported by the National Center for Complementary & Integrative Health of the National Institutes of Health under Award Number F31AT008535

P-003

NMR-BASED STANDARDIZATION OF AN ISOFLAVONE AGLYCONE-ENRICHED SOYBEAN BOTANICAL

Isoo Youn, Gonzalo R. Malca-Garcia, Rasika Phansalkar, Charlotte Simmler, Caitlin E. Howell, Birgit M. Dietz, Judy L. Bolton, James B. McAlpine, Shao-Nong Chen, and Guido F. Pauli.
UIC/NIH Center for Botanical Dietary Supplements Research, Dept. of Med. Chem. and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois, USA.

Soybean (*Glycine max* Merr., Fabaceae) contains the isoflavones daidzein (DA), genistein (GE), and glycitein, which are known for their *in vitro* and *in vivo* estrogenic effects. Especially DA and GE have shown beneficial effects through activation of chemopreventive properties. The objective of this study is to optimize soybean extraction to yield an extract that is enriched in isoflavone aglycones. First, the beans were extracted by maceration at room temperature (5 g/ 150 mL) with EtOH, MeOH, and hydro-alcoholic mixtures. Secondly, an auto-hydrolysis method was developed that utilizes the enzyme naturally present in the beans to increase the concentration of isoflavone aglycones. The auto-hydrolysis was performed by mixing bean powder (10 g) with water (150 mL) under slow rotation at 25 °C during 2, 4, and 6 days. After hydrolysis, the samples were defatted with n-hexane and neat EtOH, successively. The ^{1}H iterative full spin analysis (HiFSA) method was utilized for qHNMR analysis to identify and quantify the target compounds, along with HPLC-UV analysis. Although maceration with MeOH and EtOH yielded extracts with higher concentrations of total isoflavones, aglycones were not detectable (LOD 0.13%). Only the auto-hydrolyzed soy extracts contained isoflavone aglycones at levels around 10%, as determined by a 100% qHNMR method. As DA and GE have estrogenic and chemopreventive properties, this enriched-isoflavone soy extract will optimize these beneficial soy bioactivities.

P-004

INVESTIGATION OF ABSOLUTE PURITY DETERMINATION OF STANDARD HERBAL MEDICINES USED IN JAPANESE PHARMACOPOEIA BY QNMR

Toru Miura
Laboratory and Specialty Chemicals Research Laboratories, Wako Pure Chemical Industries, Ltd., 1633, Matoba, Kawagoe, Saitama, Japan

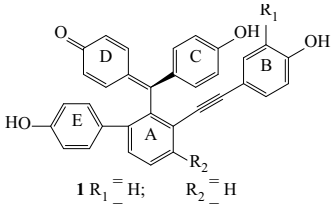
To date, the Area Normalization Method of HPLC has been broadly used to determine the purity of standard crude drug active ingredients listed in the Japanese Pharmacopoeia. However, this method is not absolute purity determination method, including low reliability due to the presence of undetectable impurities and the difference in sensitivity between the main component and the impurities. To solve this problem, our group has developed an alternative method that can efficiently and accurately determine the absolute purity of standard crude drug active ingredients. We have used quantitative Nuclear Magnetic Resonance (qNMR) as a method to obtain absolute purity of crude drug active ingredients, and examined its application to Japanese Pharmacopoeia. As a result, from 2014 to 2017, we have developed qNMR analytical methods for eight crude drug active ingredients and included them in the Japanese Pharmacopoeia. In addition, we were able to develop products that conform to these eight chromatographic standards of crude drug active ingredients and supplied them to the market. The application of qNMR to the Japanese Pharmacopoeia will continue to be examined in the future.

P-005

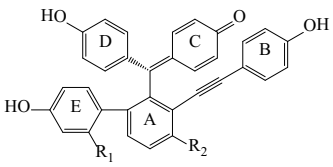
PTP1B INHIBITORY COMPOUNDS FROM THE AERIAL PARTS OF *SELAGINELLA TAMARISCINA* (BEAUV.) SPRING

Ducdat Le[1], Bingtian Zhao[1], Duchung Nguyen[1], Byungsun Min[1], Jaesue Choi[2], and Mihee Woo[1].
[1]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu,Gyeongsan 38430, Republic of Korea, [2]Department of Food Science & Nutrition, Pukyong National University, Busan 48513, Republic of Korea

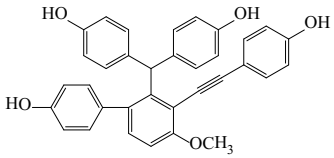
We investigated the protein tyrosine phosphatase 1B (PTP1B) inhibitors isolated from the aerial parts of *Selaginella tamariscina* (Beauv.) Spring. This study described the isolation and structure elucidation of four new (**1**–**4**, selaginellins T–W) and three known (**5, 6,** and **7** as selaginellin, selariscinin D, and selariscinin A, respectively) unsaturated alkylyl phenols from the methanolic extract of *S. tamariscina*. Then, the protein tyrosine phosphatase 1B (PTP1B) inhibitory effect of these isolates was evaluated. The results indicated that compounds **2**–**7** displayed the strong inhibitory effects (IC_{50} values ranging from 4.8 to 15.9 μM). Whereas, compound **1** showed the moderate inhibitory effect with an IC_{50} of 57.9 μM. This study may be beneficial for the treatment of diabetic disease.



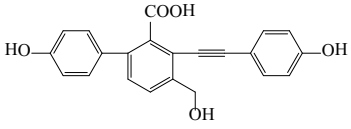
1 R_1 = H; R_2 = H
2 R_1 = H; R_2 = CH_3
3 R_1 = OCH_3; R_2 = CH_2OH



4 R_1 = OH; R_2 = H
5 R_1 = OH; R_2 = CH_2OH



7



6

P-006

VALIDATION OF AN LC-MS/MS METHOD FOR ANALYSIS OF SYNTHETIC DRUGS IN BOTANICAL DIETARY SUPPLEMENTS

Jun Ma, Rahul S. Pawar, and Erich Grundel
Office of Regulatory Science, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740, USA

Many botanical dietary supplements which carry label statements related to blood sugar management are available over the internet. Potential adulteration of such dietary supplements with synthetic drugs is of concern. In this study, we developed and validated an LC-MS/MS method to detect and quantitate 19 synthetic drugs in botanical dietary supplements sold for blood sugar management. Dietary supplements were extracted with methanol. No significant matrix effects were observed over a wide range of analyte concentrations. Mean recoveries of all 19 analytes from a single-product homogenate ranged from 88 to 113% at spike concentrations from 0.5 to 2000 μg/g. Mean recoveries of three analytes (metformin, phenformin, and sibutramine) from composites prepared from mixtures of four or five products ranged from 93 to 115% at a spike concentration of 100 μg/g. The relative standard deviations (RSDs, %) of intra-day analyses ranged from 0.2 to 13 for all recovery studies. Eighty botanical dietary supplements obtained in the U.S. and carrying label statements related to blood sugar management were analyzed using this method and none were found to be adulterated with the above 19 compounds. Two products obtained outside of the U.S. and known to be adulterated were also analyzed by this method and found to contain the target analytes (phenformin, glibenclamide, and sibutramine). This method can be used to analyze dietary supplements for possible adulteration with these compounds.

P-007

IDENTIFICATION OF NEW QUORUM SENSING PEPTIDE FROM *STAPHYLOCOCCUS CAPRAE*

Nadjali A. Chung[1], Dr. Daniel A. Todd[1], Dr. Alexander R. Horswill[2], and Dr. Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA

Many Gram-positive bacteria contain an accessory gene regulator (agr) system, which is a quorum sensing system regulated by a small cyclic peptide called the auto-inducing peptide, or AIP. Although the structure of each AIP is similar across species, each has a different sequence, which can allow AIPs to act as quorum sensing inhibitors when introduced to another species. This has been shown in previous studies. To continue exploring such cross communications of quorum sensing systems—and the agr system in general—the sequence and structure of each AIP must be known. It was the goal of this study to use high-resolution mass spectrometry to identify the AIP produced by *Staphylococcus caprae* and explore its potential to inhibit the production of AIP by *Staphylococcus aureus*. A synthetic peptide of the same structure predicted for the *S. caprae* AIP caused potent inhibition of the *S. aureus* quorum sensing system.

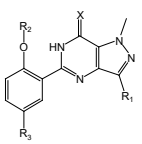
P-008

ILLEGAL PHOSPHODIESTERASE TYPE 5 INHIBITORS (PDE-5I) FOUND AS ADULTERANTS IN SEXUAL ENHANCEMENT DIETARY SUPPLEMENTS

Chee Leong Kee, Min Yong Low, Yun Zeng, Xiaowei Ge
Pharmaceutical Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078 Singapore.

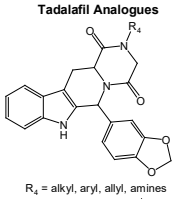
Adulteration of dietary supplements with unapproved PDE-5i is a serious issue in product safety nowadays. The high adulteration rate is an alarming risk to consumers as the toxicity and potency of these derivatives are often not studied, even though they may retain the same pharmacological effects like the approved PDE-5i. Up-to-date, there are already 80 synthetic analogues found as adulterants in sexual enhancement dietary supplements. Sildenafil, tadalafil and vardenafil analogues are the most commonly detected adulterants. The trend of adulteration and challenges in the analysis will be presented in this study.

Sildenafil Analogues

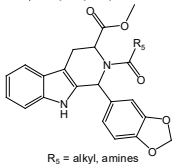


R_1 = $CH_2CH_2CH_3$, $CH_2CH(CH_3)_2$
R_2 = CH_2CH_3, $CH_2CH_2CH_3$
R_3 = sulphonylated, acetylated, and carbonylated groups
X = O, S

Tadalafil Analogues

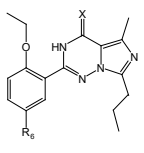


R_4 = alkyl, aryl, allyl, amines



R_5 = alkyl, amines

Vardenafil Analogues



R_6 = sulphonylated and acetylated groups
X = O, S

P-009

TOWARDS ENHANCED DYNAMIC RANGE IN NATURAL PRODUCT MIXTURE ANALYSIS: A TEST CASE USING GOLDENSEAL (*HYDRASTIS CANADENSIS*)

Bety Rostandy[1], Emily R. Britton[1], Cassandra N. Naphen[1], Scott J. Richter[2], and Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, The University of North Carolina at Greensboro, North Carolina 27402, United States. [2]Department of Mathematics and Statistics, The University of North Carolina at Greensboro, North Carolina 27402, United States. E-mail: nbcech@uncg.edu.

In the identification of metabolites in complex mixtures via electrospray-ionization mass spectrometry, studies have shown that the response of one analyte can be influenced by the presence of others in the sample matrix. The analyst often encounters linear dynamic range limitations, in which metabolites present at low levels are masked by other more highly abundant metabolites. We predict that dynamic range limitations can be overcome by simplifying a complex mixture, thus enabling more ions to be detected. Our model of complex mixture is the botanical extract of the medicinal plant goldenseal (*Hydrastis canadensis*). The crude extract and subsequent fractions are analyzed using Acquity Ultra Performance Liquid Chromatograph® (Waters Corporation, USA) coupled to a Q-Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (ThermoFisher Scientifics, USA). Multivariate statistical approaches to determine the number of ions present in complex versus simplified mixtures are being developed. Ultimately, this project will be beneficial for compound identification in complex botanical extracts, as compound identification relies on the ability to detect the ions of interest.

P-010

MINING OF STRONGLY DESHIELDED HYDROXYLS ENABLES METABOLOMIC STANDARDIZATION OF BOTANICALS

Yang Yu,[‡] Lingyi Huang,[*] Li-She Gan,[†] David C. Lankin,[*] James B. McAlpine,[*] Richard B. van Breemen,[*] Dian-Peng Li,[‡] Guido F. Pauli,[*] and Shao-Nong Chen[*]*
[*]UIC Botanical Center, MCP, Coll. of Pharmacy, Univ. of IL at Chicago, Chicago, USA; [†]Coll. of Pharm. Sciences, Zhejiang Univ. Hangzhou, China; [‡]Guangxi Institute of Botany, CAS, Guilin, China

As mass spectrometric techniques have advanced metabolomic analysis using MS, and despite the steady enhancement of MS databases, it remains a challenge to determine the identity of, or even quantify, 2° metabolites that are not present in the databases, directly from complex natural product mixtures. This study developed a new metabolomic methodology based on a combination of 1D and 2D NMR techniques. The method can qualify and (semi-)quantify the flavonoid and chalcone metabolome of a botanical based diagnosis partial structures that have a 5/2' hydroxyl motif in common. The method works in complex mixtures, fractions, and extracts. The chemical shift of the low field OH signals at 11.8–14.5 ppm reflects the strong H-bond effects of these protons. Using 2D HMBC experiments, these ^{1}H resonances exhibit unique patterns that correlate with their nearby carbons. The patterns are affected by the C-ring structure and the substitution of the A-ring, which was used to develop classification of these 2° metabolites into sub-classes and determine their abundance in the complex mixtures using a qHNMR approach. This new 2° metabolite classification also enables the distinction of the botanical source. *Glycyrrhiza uralensis* was selected to establish the proof of concept for this methodology. A qHNMR based quantitative radar graph of eight sub-classes of 2° metabolites can distinguish the three pharmacopoeial *Glycyrrhiza* species.

P-011

IN VIVO FATE OF EPIMEDIN C IN RAT

Liu Yang[1] and Shunjun Xu[2]
[1]Guangdong Provincial Hospital of Traditional Chinese Medicine, Provincial Academy of Chinese Medical Sciences, Guangzhou 510120, P. R. China, [2]Guangzhou ImVin Pharmaceutical Co., Ltd., Guangzhou 510663, P. R. China

A rapid LC-MS/MS method for the determination of epimedin C in rat plasma, various tissues and urine was developed and validated for pharmacokinetics, tissue distribution and excretion studies of this compound, respectively. The analyte was separated on an Agilent Eclipse XDB-C18 column (2.1×150 mm, 5μm and detected with a triple quadrupole mass spectrometer using negative ion ESI in the multiple reaction monitoring (MRM) mode. Calibration curve ($1/x^2$ weighted) offered a satisfactory linearity (r>0.99) over the concentration range 1-500 ng/mL. The accuracy and precision, extraction recovery, matrix effect and stability were satisfactory in all the biological matrices examined. The assay was further successfully applied to the pharmacokinetic, distribution and excretion studies of epimedin C in rat. After a single oral administration epimedium extraction, epimedin C was rapidly absorbed with T_{max} of approximately 0.5 h. Besides, epimedin C rapidly distributed with high concentrations found in liver without significant accumulation or persistence. The cumulative excretion of epimedin C in urine within 36 h of the dose was less than 0.297‰. And the results indicated that the epimedin C is hardly eliminated through urine. Moreover, metabolic pathway after oral and intramuscular administration of epimedin C were significant different.

P-012

PURIFICATION OF BIOACTIVE NATURAL PRODUCTS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY (CPC)

Tsvetelina Mandova[1,2], Grégoire Audo[2], Sylvie Michel[1], Raphaël Grougnet[1]
[1]Laboratoire de Pharmacognosie, Université de Paris Descartes, Sorbonne Paris Cité, Faculté de Pharmacie de Paris, UMR-CNRS 8638 COMETE, 4 avenue de l'Observatoire, 75006 Paris, France [2]Gilson Purification SAS, Application laboratory, 22 rue Bourseul, F-56890 Saint-Avé France,

The unique mechanism makes Countercurrent chromatography (CCC) a magic and powerful separation technique in which two-phase solvent system plays a crucial role. Basically, the Centrifugal Partition Chromatography (CPC), a hydrostatic CCC column, is a continued liquid-to-liquid solvent partition where the target compounds are competitively distributed between the two-phase solvents due to their different partition coefficients (K values).Using a centrifugal force, one phase is kept stationary (stationary phase) in chambers while the other phase (mobile phase) is pumped through the stationary one.

Few examples of applications on the purification of natural products will emphasize the great potential of the CPC technique. Methanolic leaf extract of Argentinean spiny, deciduous tree from *Prosopis* spp. (Mimosaceae) where cyclitols and indolizidine alkaloids were purified by CPC. By the same technique compounds like kaurane diterpene and his glycoside were purified from the above ground parts of *Achillea clypeolata* Sibth. et Sim (Asteraceae) a Balkan endemic species commonly known as "yellow yarrow" and also seco-irioids from the worldwide distributed traditionally used in the folk medicine *Centaurium erythraea* Rafn. (Gentianaceae) by the new generation QuantumCPC and CPC column coupled to a mass spectrometry (MS) detector.

P-013

RAPID PURIFICATION OF HOP ACIDS USING ION EXCHANGE CHROMATOGRAPHY

Jack E. Silver, Inga Henderson
Teledyne Isco, 4700 Superior Street, Lincoln NE 68512 USA

Hop acids have bacteriostatic effects and may inhibit COX-2 expression. The purification of these compounds is presented as a model for related compounds that may exhibit similar biological activities. The use of an ion exchange column allows capture of the compounds from the crude extract; the purified acids are then ready for preparative liquid chromatography to resolve the individual compounds. Techniques for preparing the ion exchange column and loading the samples is described.

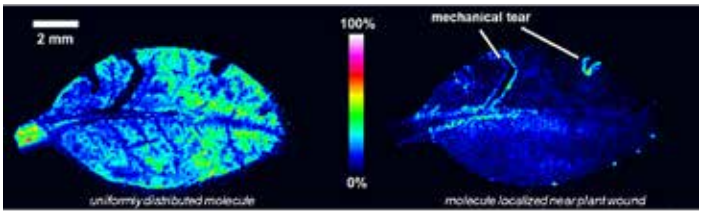
P-014

MOLECULAR IMAGING OF PLANT TISSUE BY MASS SPECTROMETRY

Bindesh Shrestha, Giorgis Isaac, Jimmy Yuk
Waters Corporation, 34 Maple Street, Milford, MA 01757

Distribution of molecules in plant tissue can provide an understanding of spatial metabolomics, plant-environment interactions, and aid compound screening. For example, location information of medically important compounds within a plant will strengthen fundamental understanding of their metabolic origins, as well as, improve their extraction. Mass spectrometry (MS) imaging provides a spatial distribution of a large variety of endogenous and exogenous analytes from plant surfaces without sample extraction or addition of any external label. Molecular images provided by MS are complementary to the structural information provided by optical microscopy.

Here, we share workflow for imaging of plant tissue using matrix-assisted laser desorption ionization (MALDI) or desorption electrospray ionization (DESI). As an example of a workflow, MS imaging was utilized to find molecular markers after mechanical stress in a plant, which is a typical problem throughout their life cycle. MS imaging of a leaf with mechanical damage showed localized molecules near the site of the tears that can shed insight into the protective wound-healing mechanism.



P-015

A TALE OF TWO CULTURES: CO-CULTURING A FUNGUS WITH MRSA

Diana Kao[1], Huzefa A. Raja[1], Daniel A. Todd[1], Nadja B. Cech[1], Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA.

Penicillium restrictum has been reported to produce polyhydroxyanthraquinones, which have been shown to inhibit the quorum sensing mechanism of methicillin-resistant *Staphylococcus aureus* (MRSA). Of these polyhydroxyanthraquinones, ω-hydroxyemodin was not only produced in the highest quantity but was also shown to have the most potent antivirulent properties. Further studies were carried out with ω-hydroxyemodin in a mouse model of MRSA demonstrating and asserting potential *in vivo* activity. Utilizing droplet-liquid microjunction-surface sampling probe (d-LMJ-SSP), we can investigate the interactions between these two microorganisms *in situ* to observe how the secondary metabolites of *P. restrictum* affect the growth and development of MRSA. A technique utilizing d-LMJ-SSP coupled to UPLC-MS provides a new manner by which we can explore and evaluate the chemistry of two entwined microorganisms *in situ*.

P-016

INTEREFERENCE WITH IRON ACQUISITION AS EXPOSED THROUGH IMAGING MASS SPECTROMETRY OF MICROBIAL INTERACTIONS

Ying-Ning Ho, Chi-Ting Hsieh, Yu-Liang Yang
Agricultural Biotechnology Research Center, Academia Sinica, 11529 Taipei, Taiwan

Microbes employ a diverse array of bioactive natural products to mediate interactions with their neighbors, competitors, and predators. However, the natural roles of natural products in the ecosystem are not entirely understood. Understanding the complex biochemical processes that occur in microbial interactions requires the elucidation of structures and spatial distribution of the natural products involved in these processes. Here I will introduce how we employed imaging mass spectrometry to explore the microbial interactions between plant fungal pathogen *Phellinus noxius* and antagonistic microbes. The results demonstrated *P. noxius* is capable of interfering with iron acquisition of *Pseudomonas* and *Burkholderia* by modifying their siderophore structures. What we learned from nature may lead to a better understanding of the ecological niche occupied by those microbes, and improve production and formulation of microbes and their metabolites to enhance the efficacy in therapies and agriculture.

P-017

A MALDI-TOF MS PLATAFORM TO DISCOVER UNDERSTUDIED ACTINOMYCETES FROM ICELANDIC WATERS

Maria Sofia Costa[1], Chase Clark[2], Sesselja Omarsdottir[1], Laura M. Sanchez[2], Brian T. Murphy[2, 3]
[1]University of Iceland, Reykjavik, Iceland, [2]Department of Medicinal Chemistry and Pharmacognosy, [3]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago

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 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 August 2015 we embarked on a collection trip to Iceland, which has a unique geology and geographical position in the North Atlantic Ocean. Using SCUBA and sampling off of vessels, we collected greater than one hundred samples of sediment from forty sites. After purifying 400 strains from the samples that we collected, we acquired protein and specialized metabolite data 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 bioinformatics pipeline. From this collection of strains, we selected eight minor outlying groups, which we postulated to represent understudied actinomycete genera and generated specialized metabolite networks to visualize which strains contain unique chemical profiles. Analysis of 16S ribosomal RNA gene sequencing data of these strains confirmed that our method rapidly highlighted understudied strains directly from colonies on

a plate, and we are currently investigating whether this unique taxonomy will afford new chemistry.

P-018

ABSOLUTE CONFIGURATION AND BIOLOGICAL ACTIVITIY OF PHANTASMIDINE

Richard W. Fitch[1], Anshul A. Pandya[2], Jerrel L. Yakel[2], Thao T. Olson[3], Nour Al-Muhtasib[3], Yingxian Xiao[3], Kevin D. Welch[4], Kip E. Panter[4], Quan Zhou[5] and Barry B. Snider[5]
[1]Department of Chemistry and Physics, Indiana State University, Terre Haute, IN 47809. [2]Neurobiology Laboratory National Institute of Environmental Health Sciences, NIH/DHHS, Research Triangle Park, NC 27709. [3]Department of Pharmacology and Physiology, Georgetown University, Washington, DC 20057. [4]Poisonous Plant Research Laboratory, United States Department of Agriculture, Agricultural Research Service, Logan, UT 84341. [5]Department of Chemistry, Brandeis University, Waltham, MA 02453.

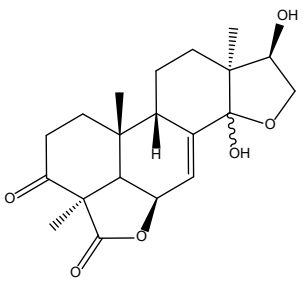
Phantasmidine is a tetracyclic analog of the better known epibatidine, a highly potent nicotinic receptor agonist from the poison frog *Epipedobates anthonyi* (formerly *E. tricolor*). Phantasmidine was isolated from the same frog in vanishingly small quantities (20 ug) and structurally characterized some time ago. Unfortunately, the absolute configuration could not be established at the time, but the initial biological activity seemed promising. Following the successful total synthesis of the racemate and resolution thereof, absolute configurations of the enantiomers were established. In this study, we have followed up on the biological activity and identified the natural product as the (2a*R*,4a*S*,9a*S*) enantiomer (shown). This enantiomer is more active than its antipode by a factor of 30-90. The absolute configuration was established by HPLC-HRMS, which was the only method that was able to detect and establish unequivocally the vanishingly small amounts of phantsmidine present in the remaining natural extract which also contained the nominally isobaric N-methylepibatidine, differing by only 0.0364 mass units.

P-019

ICACINA TRICHANTHA REVISITED FOR NMR ANALYSIS OF 9BH-PIMARANES

Brian Guo,[1] Ming Zhao,[1] Michael M. Onakpa,[1,2] and Chun-Tao Che[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612, USA, [2]Department of Veterinary Pharmacology and Toxicology, University of Abuja, Abuja 920001, Nigeria.

Icacina trichantha Oliv. (Icacinaceae) is a plant native in Nigeria and other regions of western Africa with a history of traditional medicinal uses. We previously reported a number of novel (9βH)-pimarane, 17-*nor*-(9βH)-pimarane and 17-*nor*-pimarane derivatives from the tuber. Further investigation has allowed comparative NMR analysis of the diterpenoid structures and the identification of a new (9βH)-pimarane, 14-hydroxyicacinlactone I. Presence of 9βH corresponds with stronger cytotoxic activity compared to the related pimaranes lacking the 9H.



14-OH-Icacinlactone I

P-020

HIFSA SEQUENCING BIOACTIVE PEPTIDES BY ^{1}H-NMR FOR QUALITY ASSURANCE

Mary P. Choules[1,2], Jonathan Bisson[2], Wei Gao[1,2], David C. Lankin[2], James B. McAlpine[1,2], Matthias Niemitz[3], Birgit U. Jaki[1,2], Scott G. Franzblau[1], Guido F. Pauli[1,2]
[1]Institute for Tuberculosis Research, [2]Dept. of Med. Chem. & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612, USA; [3]NMR Solutions, 70110 Kuopio, Finland

The assurance of identity, purity, and reproducibility is equally essential during the drug discovery process as for the final pharmaceutical product. Many API peptides are larger molecules (≥800 amu) that require great efforts to fully characterize and standardize. However, many compendial methods (HPLC, MS, chemical analysis) have limited ability to identify small structural changes that could plausibly affect biological or safety outcomes. ^{1}H iterative Full Spin Analysis (HiFSA) concurrently yields definitive identity and purity information allowing for QA of the API. HiFSA sequencing lends itself to research and commercial applications as ^{1}H 1D NMR is the most sensitive and basic NMR experiment permitting automation and microgram scale analysis. HiFSA profiles need only to be generated once per API and can be adopted for external calibration. Coupled with QM-qHNMR, it is possible to quantify mixtures and/or determine the ratio of peptide conformers. As HiFSA profiles achieve exhaustive structural characterization, even small changes within larger molecules can be identified. The methodology behind HiFSA Sequencing, its use in enhancing reproducibility of biological studies and clinical usage, will be discussed, as will specific applications of increasing complexity, including standard amino acids (75-204 amu), aspartame (294 amu), oxytocin (1006 amu), and the anti-*M.tb* antibiotic, rufomycin (1041 amu).

Acknowledgments. T32AT007533 (ODS, NCCIH); U41AT008706 (ODS, NCCIH)

P-021

NMR BASED STUDY OF THE METABOLITE FLUX THROUGH THE FAST LOOP OF THE SURFACE OCEAN CARBON CYCLE.

Charalampos G. Panagos[1], Khan Hekmatyar[1], Christa B. Smith[2], Frank Ferrer-Gonzalez[2], Zhang, Sicong[1], Mary Ann Moran[2], Arthur S. Edison[1]
[1] Complex Carbohydrate Research Center, The University of Georgia, 315 Riverbend Road, Athens, GA 30602,USA, [2] Department of Marine Sciences, Marine Sciences Building, University of Georgia, Athens, GA 30602-3636

The study of the diatom-bacteria model system is of paramount importance in order to understand the cycle of the CO_2 absorbed by the oceans. Almost a quarter of Earth's primary production is cycled within days to weeks of fixation by the billion marine bacteria living in each litre of surface seawater. However, surprisingly little is known about the metabolites that sustain their growth. In this study, we present the real-time flux measurements of the transport and process of important carbon currencies (pyruvate, acetic acid and DHPS) by the bacteria of the diatom-bacteria model. Dynamic Nuclear Polarisation (DNP) is employed to follow the metabolic fate of these molecules, when injected to bacteria. Bacteria grown in variable conditions and the effect to their metabolism, were also investigated.

In addition to the study of the metabolic fate of known carbon currencies, we present here the initial steps towards the untargeted metabolomic analysis of the diatom-bacteria system. The main goal of this analysis is to identify novel carbon cycle metabolites, as well as highlight differences between the endometabolomic and exometabolomic footprint of diatoms grown with and without their synergistic bacteria. Challenges common with this kind of analysis include the great number of metabolites present in the system, as well as the high salinity of the samples, which impedes further spectroscopic analysis (NMR, MS). Chromatographic, chemical and spectroscopic

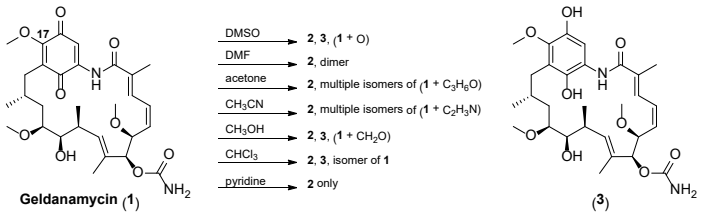
solutions for desalting, fractionating and analysing the metabolites of the system are described.

P-022

PHOTODEGRADATION PRODUCTS OF GELDANAMYCIN

Sloan Ayers, Jonathan Marshall, and Yande Huang
Chemical and Synthetic Development, Bristol-Myers Squibb Co., New Brunswick, NJ 08903, USA.

Geldanamycin (**1**) is a naturally occurring macrocyclic quinone with potent cytotoxic activity (average GI_{50} = 0.18 μM in the NCI human tumor cell line panel). Geldanamycin has previously been shown to be unstable in solution when exposed to light, but to our knowledge, no in-depth studies on the actual structures of the degradants have been reported. In a study covering a variety of solvents, the 17-*O*-desmethyl derivative (**2**) was present in varying levels in all solvents, and **1**+2H (possibly the hydroquinone **3**), was present in three of the seven solvents tested. However, virtually every solvent tested (except pyridine) generated unique degradants as well (according to LC-HRMS), most interestingly acetone and acetonitrile in which the solvents formed multiple products with **1**. Preliminary results from an investigation of the photodegradation of **1** in seven solvents will be presented.

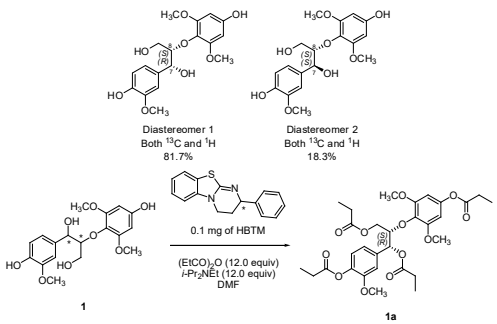


P-023

HIGHLY SENSITIVE, SIMPLE, AND COST/TIME EFFECTIVE METHOD TO DETERMINE ABSOLUTE CONFIUGURATION OF A SECONDARY ALCOHOL

Seoung Rak Lee[1], Jae Sik Yu[1], Seulah Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Hae Min So[1], Sil Kim[1], Dahae Lee[1], Won Se Suh[1], Kyoung Jin Park[1] and Ki Hyun Kim[1]
[1]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

We report a simply optimized new chemical derivative method which utilizes the competing kinetic resolution (CKC) coupled with LC/MS analysis and a successful application to determine the absolute configuration of a secondary alcohol in natural products with multiple reactive functional groups. Compound **1** was isolated from the barks of *Acer tegmentosum* and it was speculated as a new phenylpropanoid derivative to be stereochemically unreported. The absolute configuration of **1** was determined by newly developed method and verified by analysis of circular dichroism (CD) data as well as employing the gauge-including atomic orbital (GIAO) NMR chemical shifts calculation.



P-024

BIOSYNTHESIS OF YM-254890, A SELECTIVE G PROTEIN INHIBITOR FROM *CHROMOBACTERIUM* SP. QS3666

Masatoshi Taniguchi[1], Keita Amagai[4], Ikuko Kozone[3], Junko Hashimoto[3], Takuya Hashimoto[2], Hikaru Suenaga[2], Satoshi Sasamura[1], Shunji Takahashi[4], Haruo Ikeda[5], Kazuo Shin-ya[2], Koji Nagai[1]
[1]Taiho Pharmaceutical Co., Ltd., Ibaraki 300-2611, Japan, [2]National Institute of Advanced Industrial Science and Technology, Tokyo 135-0064, Japan, [3]Japan Biological Informatics Consortium, Tokyo 135-0064, Japan, [4]RIKEN Center for Sustainable Research Science, Saitama 351-0198, Japan, [5]Kitasato Institute for Life Sciences, Kitasato University, Kanagawa 252-0373, Japan

Heterotrimeric G proteins, G_s, G_i and $G_{q/11}$, transduce signals from various receptors to intracellular effectors. We previously reported the discovery of a specific $G_{q/11}$ inhibitor YM-254890, which has been a valuable tool in understanding the role of $G_{q/11}$. YM-254890 is a cyclic depsipeptide containing uncommon amino acids and there are two similar compounds, FR900359 and sameuramide, which differ from YM-254890 in an amino acid constituent and acyl groups. Interestingly both of them were not isolated from bacteria but from the plant *Ardisia crenata* and the marine organism *Didemnidae*, respectively. Recently, Carlier *et al.* reported *Candidatus* B. crenata which is the simbiont of *A. crenata* is responsible for the biosynthesis of FR900359. In order to reveal the biosynthetic pathway of YM-254890, the genome of *Chromobacterium* sp. QS3666 was sequenced. Bioinformatic analysis indicated that a NRPS gene cluster which possesses five NRPS genes encoding a total of eight modules is responsible for the biosynthesis of YM-254890. The comparison between biosynthetic gene clusters of YM-254890 and FR900359 showed they share similar gene clusters.

P-025

BIOSYNTHETIC INVESTIGATION OF A UNIQUE NITROGENATED POLYPHENOL

Calista Horta, Nina Shah, Andrew Whiteley and Katharine Watts.
Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, CA 93407, USA

With antibiotic resistance on the rise, the discovery and production of novel antibiotics is crucial. The antibiotic of interest, TLN-05220, displays bioactivity against methicillin-resistant Staphylococcus aureus (MRSA) in the same therapeutic range as vancomycin, a last resort antibiotic. TLN-05220 is a pentangular aromatic polyketide that contains a unique nitrogenated (piperazinone) ring, for which there have been no prior biosynthetic studies. TLN-05220 was originally isolated from Micromonospora echinospora ssp. challisensis NRRL 12255, and the original publication hypothesized that the molecule was biosynthesized by a hybrid type II polyketide/non-ribosomal peptide (T2PK-NRP) synthase. The long-term goal of our project is to map the biosynthetic pathway of TLN-05220 and identify the genes and enzymes that construct its unique structural features.

Our group has identified an alternate strain of Micromonospora echinospora that synthesizes TLN-05220. Using the publically available genome sequence, literature searches and comparative bioinformatic analysis, a clustered set of genes within the genome have been identified that are likely responsible for production of TLN-05220. Alternative to the original hypothesis, there are no known NRPS genes in the proposed gene cluster that encode for the production of the nitrogenated ring. We hypothesize that the polyketide core is assembled by a type II polyketide synthase, and the extension of the polyketide core into the piperazinone ring is carried out by two amide synthetase enzymes that install the amino acids glycine and alanine. We are currently performing stable isotope feeding studies using glycine and alanine and working towards cloning and expression of the entire proposed gene cluster in a heterologous host, using transformation-assisted recombination.

P-026

BIOSYNTHESIS OF FLUORINATED PEPTAIBOLS USING A SITE DIRECTED BUILDING BLOCK INCORPORATION APPROACH

José Rivera-Chávez[1], Huzefa A. Raja[1], Tyler N. Graf[1], Joanna E. Burdette[2], Cedric J. Pearce[3], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina 27412, [2]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Illinois 60612, [3]Mycosynthetix, Inc., 505 Meadowlands Drive, Suite 103, Hillsborough, North Carolina 27278

Synthetic biological approaches, such as site directed biosynthesis, have contributed to the expansion of the chemical space of natural products, making possible the biosynthesis of unnatural metabolites that otherwise would be difficult to access. Such methods may allow the incorporation of fluorine, an atom rarely found in nature, into complex secondary metabolites. Organofluorine compounds and secondary metabolites have both played pivotal roles in the development of drugs; however, their discovery and development are often via non-intersecting tracks. In this context, we used the biosynthetic machinery of *Trichoderma arundinaceum* (strain MSX70741) to incorporate a fluorine atom into peptaibol-type molecules in a site selective manner. Thus, fermentation of strain MSX70741 in media containing *ortho*- and *meta*-F-phenylalanine resulted in the biosynthesis of two new fluorine containing alamethicin F50 derivatives. The fluorinated products were characterized using 1D and 2D NMR (including ^{19}F), and HRESIMS/MS^n, and their absolute configurations were established by Marfey's analysis. Fluorine containing alamethicin F50 derivatives exhibited potency analogous to the non-fluorinated parent when evaluated against a panel of human cancer cell lines. Importantly, the biosynthesis of fluorinated derivatives was monitored *in situ* using a droplet–liquid microjunction–surface sampling probe.

P-027

BIOCATALYTIC SYNTHESIS OF NOVEL OXIDIZED AROMATIC COMPOUNDS AS POTENTIAL ANTI-BACTERIAL AND ANTI-CANCER AGENTS.

Qwebani-Ogunleye, T.; Kolesnikove, N. I.; Steenkamp, P.; de Koning, C.; Brady, D.; Wellington.

An overwhelming evidence has shown association between certain bacteria and cancer.[1] The number of drugs available for treating malignant tumours and bacterial infections has been reduced by the development of chemo-resistant and antibiotic resistance. Polyhydroxylated compounds have attracted much attention due to their broad spectrum of pharmacological activities such as anti-cancer and anti-bacterial activities and one classical example is usnic acid as previously mentioned. The structural motif of a great number of these compounds contain fused aromatic rings, quinones and hydroxyl moieties. These moieties have the ability to undergo redox cycling, this process is when compounds catalytically cycle and generate ROS such as hydrogen peroxide and superoxide which damages the cell. ROS also inhibit active efflux which is responsible for moving antibiotics out of the cell. The scope of this overview is broad therefore a wide range of reports is presented. Synthesis and mode of action of selected antibiotics, search for new drug candidates, cell circle in cancer cells, cancer in Africa and cancer treatment will be discussed.

REFERENCES

1. Mager, D. L. Journal of Translational Medicine. 2006, 4, 14.

P-028

EVALUATION OF CYP716A12 PROMISCUITY IN THE TRITERPENE FRIEDELIN

Keylla Utherdyany Bicalho[1], Tatiana Maria de Souza-Moreira[1], Sandro Roberto Valentini[2], Cleslei Fernando Zanelli[2], Maysa Furlan[1]
[1]Institute of Chemistry, São Paulo State University, Araraquara, Brazil, [2]School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil.

Friedelin, a pentacyclic triterpene, is andis converted by cytochrome P450 enzymes into the potent antitumor quinonemethides triterpenes maytenin and pristimerin. P450 enzymes are known to show a high degree of functional promiscuity and CYP716A family is frequently reported in literature by its ability to oxidize different terpenoid substrates.*i* In that view, we evaluated the promiscuity of CYP716A12 by coexpression with friedelin synthase in *Saccharomyces cerevisiae.* The coding sequence of friedelin synthase was previously cloned into the pSP-GM1 *URA3*-based yeast expression plasmid [P_{TEF1}-MiFRS, P_{PGK1}-t*HMG1*] and the oxidoreductase sequences were cloned into the pRS423 *HIS3*-based plasmid [P_{TIP1}-CYP716A12, P_{ADH1}-*CPR1*]. Both plasmids were transformed into a *S. cerevisiae* modified strain (*ERG7*-DAmP, VZL 1303). generated by backcrossing CEN.PK2 strain and the *ERG7* Decreased Abundance by mRNA Perturbation (DAmP) strain(VZL1303). Single clones were grown in synthetic complete medium without uracil and histidine for 24 h at 30 °C 200 rpm, when it was replaced by a solution of KH_2PO_4 (0.1 M, pH 7,.0, glucose 3%) for another 24 h of incubation. Cells were collected, dried and extracted with $CHCl_3$:MeOH (2:1) solution by sonication for 10 min. After addition of NaCl (0.73%) solution, the organic phase was collected, dried, ressuspended in ACN and silylated for GC-MS analysis. Mass spectra for peaks in 21.2 and 22.1 min confirmed, respectively, the production of friedelin and a derivative with a carboxyl group at C-28, which confirms the CYP716A12 promiscuity to friedelin substrate and the potential in using P450 enzymes from literature to oxidize friedelin in a combinatorial biosynthesis approach.

P-029

TRANSCRIPTIONAL REGULATION OF PACTAMYCIN BIOSYNTHESIS IN *STREPTOMYCES PACTUM*

*Abdullah R. Alanzi, Wanli Lu, Mostafa E. Abugrain, Andrew R. Osborn and Taifo Mahmud**
Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331-3507, U.S.A

Pactamycin is an aminocyclitol natural product that has potent antibacterial, anti-tumor and anti-protozoal activities. This highly unique natural product is produced by the soil bacterium *Streptomyces pactum*. Despite its high biological potential, the production yield of pactamycin and its genetically engineered congeners under normal laboratory conditions is low. In this study, we investigated factors that regulate pactamycin biosynthesis by interrogating putative regulatory genes within the *S. pactum* genome and altering the culture conditions. Bioinformatics analysis of the pactamycin biosynthetic gene cluster revealed two putative pathway specific regulatory genes, *ptmE* and *ptmF*. Interestingly, inorganic phosphate concentration in the cultures plays a role in regulating pactamycin biosynthesis, possibly through the pleiotropic two-component *PhoR-PhoP* regulatory system. Global regulatory genes *afsA* and *arpA* which are involved in A-factor cascade may also play a role in the regulation of pactamycin biosynthesis. Since pathway specific and global regulators are widely distributed in other microorganisms, better understanding of these regulatory systems in *S. pactum* may not only lead to yield enhancement of pactamycin production but may also be useful for increasing the production of other secondary metabolite biosynthesis in different microorganisms.

P-030

NCI PROGRAM FOR NATURAL PRODUCT DISCOVERY: BIOINFORMATICS-GUIDED APPROACHES TO NATURAL PRODUCT-BASED DRUG DISCOVERY

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

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

P-031

NEW UNTARGETED METHODOLOGIES FOR IDENTIFYING SYNERGISTS IN COMPLEX BOTANICAL MATRICES

Emily R. Britton[1], Joshua J. Kellogg[1], Olav M. Kvalheim[2], Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412, USA, [2]Department of Chemistry, University of Bergen, Bergen 5020, Norway

Botanical medicines are highly complex, and their biological activity is often due to multiple constituents that work together additively or synergistically. Thus, when preparing and standardizing botanical dietary supplements, it is not always desirable to focus on single "bioactive" compounds. One strategy for identifying synergists and overcoming the bias inherent to bioassay-guided fractionation is to utilize an untargeted biochemometrics-guided fractionation approach. This methodology serves as an improvement upon existing techniques in that the entire mass spectral profile is statistically correlated with bioactivity across a set of fractions. The result is selectivity ratio (SR) plots that represent retention time-mass pairs with varying degrees of correlation to the biological activity being evaluated. As a case study, we sought to employ this approach to identify synergists in the medicinal plant *Hydrastis canadensis*. This botanical is known to contain multiple flavonoids that potentiate the activity of the antimicrobial alkaloid berberine against *Staphylococcus aureus*. After three stages of fractionation, LC-MS analysis and antimicrobial screening, mass spectral ions representing known synergists were identified as correlating with bioactivity. Additionally, a new tentatively active flavonoid, 3,3′-dihydroxy-5,7,4′-trimethoxy-6,8-C-dimethyl-flavone, was isolated, and synergistic activity confirmation in combination with berberine is currently underway. The workflow demonstrated here can be applied to any complex mixture to aid in the identification of putative bioactive compounds.

P-032

NATURAL PRODUCT TARGETOME IN CANCER: DEFINITION AND APPLICATION

Steve Chamberlin, ND[1], Aurora Blucher, BS[1], Gabrielle Choonoo, BS[1,2], Molly Kulesz-Martin, PhD[2], Shannon McWeeney, PhD[1,2]
[1]Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University, Portland, OR, [2]Knight Cancer Institute, Oregon Health and Science University, Portland OR

Targeted therapies for cancer act on molecular targets considered to be specific drivers for the disease, theoretically causing little damage to healthy cells and promising fewer adverse effects than cytotoxic approaches. However, limitations of these therapies include acquired drug resistance, limited treatment options for some cancers and for children, and the expense and difficulty with synthesis of effective molecules for some targets. Natural products may address some of these challenges and have also been shown to have synergistic effects with some cancer drugs. Network pharmacology offers a framework to explain and guide targeted therapies that include combination therapies of natural products and FDA approved drugs by using knowledge of critical cancer pathways.

The objective of this study is to comprehensively define the natural product targets for pan-cancer critical disease pathways by leveraging earlier work assessing the coverage of the "cancer targetome" based on FDA approved drugs. **It is our hypothesis that natural products, defined as compounds from living sources (plant, animal, microbial), can substantially increase the coverage of critical aberrational cancer pathways and assist in identifying novel therapeutic strategies**. This work lays a critical foundation needed to predict synergistic combination therapies using natural products.

P-033

INVOLVEMENT OF FILAMENTOUS FUNGI IN PLANT STOMATAL OPENING

Silas A. Rasmussen[1], Peter K. Bjørk[2], Anja T. Fuglesang[2], Thomas O. Larsen[1]
[1]Department of Bioengineering and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark, [2] Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark, E-mail: silan@dtu.dk

Stomata are important for the ability of plants to regulate gas transport and osmotic pressure. In contrast, the stomata pore is considered an important entry site for plant pathogens, why plants with open stomata are more susceptible to a pathogen infection.[1]

Fusicoccin, which is a fungal metabolite, is an example of a compound that irreversibly can cause stomatal opening by modulating the plama membrane (PM) H^+-ATPase.[1] In an ongoing project we have tested several extracts of plant associated filamentous fungi for their ability to modulate plant H^+-ATPase activity. Preliminary results have indicated that among others *Trichoderma harzianum* exhibited activation of ATPase hydrolysis. Dereplication using UHPLC-HRMS/MS tentatively identified this extract to be rich in peptaibols, why ongoing bio-guided fractionation aims at identifying if these peptides are indeed the compounds involved in the modulation of PM H^+-ATPase.

[1]Falhof J., Pedersen J.T., Fuglsang A.T., and Palmgren M. (2016). Plasma Membrane H^+ -ATPase Regulation in the Center of Plant Physiology. Mol. Plant. 9, 323–337.

P-034

UNDERSTANDING THE CHEMICAL ECOLOGY OF ANTARCTIC *PLOCAMIUM CARTILAGINEUM* THROUGH METABOLOMIC ANALYSIS OF HALOGENATED MONOTERPENES

Andrew J. Shilling[1,2], Ryan M. Young[1], Jacqueline L. von Salm[1], Sabrina Heiser[3], Margaret O. Amsler[3], Charles D. Amsler[3], James B. McClintock[3], Bill J. Baker[1,2]
[1]Department of Chemistry; [2]Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL 33620, USA; [3]Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Plocamium cartilagineum is a red macroalgal species found in the shallow waters of Antarctica known to produce many cytotoxic polyhalogenated monoterpenes thought to serve as feeding deterrents to sympatric algal consumers. Individuals around Palmer Station on Anvers Island can be classified into two distinct genotypes, each able to produce varying combinations of these chemical defenses linked with site specificity suggesting each alga is able to tailor its defenses to better suit its unique set of environmental conditions [1]. In an attempt to assess if depth is also a factor in determining the chemotype of an individual at a given site, a larger scale field collection was undertaken in the 2016 Antarctic summer season with both shallow and deep collections at most sites. Metabolomic data linked with genomic analysis from this collection show that both genotype and depth seem to play a role determining the constituency of the chemical feeding deterrents produced by *P. cartilagineum*.

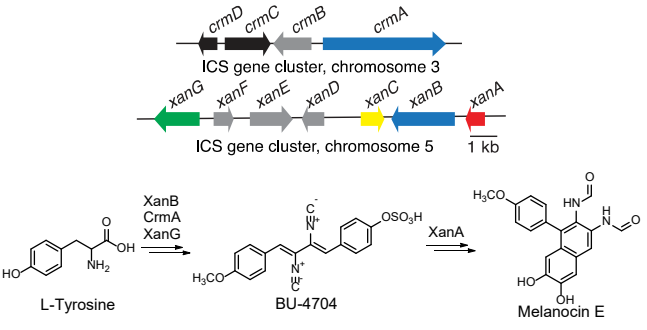
[1] Young RM, von Salm JL, Amsler MO, Lopez-Bautista J, Amsler CD, McClintock JB, Baker BJ. Site-specific variability in the chemical diversity of the Antarctic red alga *Plocamium cartilagineum*. Mar. Drugs 2013, 11: 2126-2139.

P-035

FUNGAL ISOCYANIDE SYNTHASES: AN UNEXPLORED NICHE OF EUKARYOTIC SECONDARY METABOLISM

Tae Hyung Won[1], Fang Yun Lim[2], Joshua A. Baccile[1], Jen Wisecaver[3], Antonis Rokas[3], Nancy P. Keller[2,4], Frank C. Schroeder[1]
[1]Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA, [2]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI, [3]Department of Biological Sciences, Vanderbilt University, Nashville, TN, [4]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI

Although the first naturally-occurring isocyanide (xanthocillin) from the fungus, *Penicillium notatum*, was isolated over half a century ago, nothing is known about isocyanide biosynthesis in eukaryotes. We present the discovery of four fungal isocyanide synthases (ICS) from the opportunistic human pathogen, *Aspergillus fumigatus*. Comparative metabolomics of ICS mutants identified two distinct ICS biosynthetic gene clusters (*xan* and *crm*) contributing to xanthocillin biosynthesis. Phylogenetic examination of ICSs spanning the tree of life reveals wide distribution of ICS homologs in fungi including genera not previously known to harbor secondary metabolite-producing capabilities.



P-036

IN SILICO SCREENING OF TARGET GENES OF PHAMANUTRIONAL PURPOSES IN HORTICULTURAL CROPS

Geung-Joo Lee, Saminathan Subburaja
Dept of Horticulture, Chungnam National University, Daejeon 34134, Korea

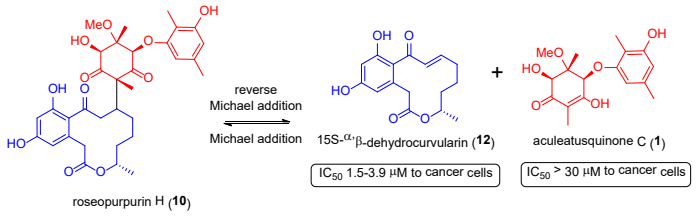
Carotenoids including lycopene, carotenes, lycopene, lutein and xanthins have been recognized to be abundant in various vegetable, fruit and flowering crops. Starting from the genranylgeranyl pyrophosphate (GGPP) ending up to final carotenoids, many structural genes and regulatory factors are involved. One of the strategies to increase the carotenoid yield is to explore the target genes in the pathway and to find elicitors or factors (cultural or genetic) to maximize the expression of the genes. In horticultural crops, various colored cultivars accumulated different major or minor carotenoids. Though the in silico screening approach, we were able to isolate diverse alleles of the LCY (lycopene cyclase) gene, encoding enzymes with altered activities, which allows finding out the mechanisms involved in the accumulation of the final carotenoid (i.e. lycopene). Also determination of allele specific variations in 5' UTR regions in the carotenoid biosynthesis rendered us to develop reliable DNA marker to discriminating LCYB alleles for red and yellow flesh fruits. Large number of transcriptional factors including RIN, SISGR1, PIF-1, AP2a, ERF6, etc was found to be positively or negatively interacting with some structural genes in the carotenoid pathway. Target genome editing of the regulatory genes will be further discussed as one of the way to modify carotenoid content in the pigmentation process.

P-037

ROSEOPURPURINS: CHEMICAL DIVERSITY ENHANCED BY CONVERGENT BIOSYNTHESIS AND FORWARD AND REVERSE MICHAEL ADDITIONS

Zhuo Shang[1#], Zeinab Khalil[1], Li Li[2], Angela A. Salim[1], Michelle Quezada[1], Pabasara Kalansuriya[1], and Robert J. Capon[1]
[1]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD 4072, Australia, [2]Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China.
[#]Current affiliation: Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92037, USA

Culture of an estuarine fungus *Penicillium roseopurpureum* (CMB-MF038) yielded two new Michael adducts (**10** and **11**) and a diverse array of polyketides (**1**–**9**, **12**–**22**), many of which were related via a highly convergent biosynthetic pathway. The structures inclusive of absolute configurations of these molecules were elucidated on the basis of detailed spectroscopic analysis, chemical derivatization, calculated ECD, and biosynthetic considerations. The Michael adducts **10** and **11** are considered as *pro*-drugs, which undergo *in situ* reverse Michael addition to generate the cytotoxic Michael acceptor 15*S*-α,β-dehydrocurvularin (**12**) in cancer cells. Our results provide an inspiration to enhance the therapeutic potential of bioactive Michael acceptors, by masking them as enzyme-activated, reversible Michael adducts with suitably substituted cyclohexanediones.



P-038

SCREENING THE NPDI LIBRARIES FOR J&J: A RICH SOURCE OF "ACTIVE NATURALS"

Matthew Todd[1, 2], Teena Varghese[1], Kahlid Mahmood[1], Bill Kinney[2], Michael Goetz[2], Jason Clement[2], Janet Sigmund[2]
[1]Janssen Pharmaceutical Companies of Johnson & Johnson, Discovery Sciences - Lead Discovery, PO Box 776, Spring House, PA 19477-0776.
[2]Natural Products Discovery Institute of the Baruch Blumberg Institute for Hepatitis B Research, 3805 Old Easton Rd., Doylestown, PA 18905.

A collaboration between Janssen Pharmaceuticals-Lead Discovery, Johnson and Johnson Consumer, and the Natural Products Discovery Institute (NPDI) was begun in early 2013, to probe Natural Product Extract (NPE) libraries for novel molecules affecting molecular targets. The NPDI was gifted the Merck/Schering NPE library in 2010. A collection of targets of potential interest for consumer products was chosen, and progressed through a pharma discovery pipeline. Target selection criteria included:

- Targets of low abundance using whole cell assays, and receptors
- Emphasis on label-free technologies
    - As secondary assays, if not primary
- Broad selectivity profiling prior to deconvolution
    - Cytotox, isozymes, etc.
    - Reversibility (removing alkylating agents)
    - Identify Fluorescence follow-on

A summary of results is consistent with novel chemical matter arising for many targets.

P-039

PROGRESS IN METABOLIC ENGINEERING PRODUCTION OF VALERENADIENE

S. Eric Nybo[1], Johnson Tran[2], Jennifer T. Lamberts[1], and Sean P. McCormick[2]
[1]Department of Pharmaceutical Sciences, Ferris State University, Big Rapids, MI 49307, [2]Department of Chemistry, Ferris State University, Big Rapids, MI 49307

Valeriana officinalis synthesizes a suite of terpenoid and alkaloid compounds in the roots with important anti-anxiety and anti-insomnia properties. The sesquiterpene valerenic acid, and to a lesser extent its intermediate valerena-1,10-diene, exhibit much of the pharmacologically significant GABA-A activity. However, the roots of V. officinalis produce scant quantities of valerenic acid and valerena-1,10-diene, which inhibits further drug development efforts. These molecules are biosynthesized from the substrate farnesyl pyrophosphate (FPP) by valerenadiene synthase (VDS). Recently, an Escherichia coli production platform was developed to synthesize elevated titers of FPP and valerena-1,10-diene. Design of a codon-optimized terpene synthase and heterologous expression of the yeast mevalonic acid (MVA) pathway have enhanced terpene titers to 61 mg/L in shake flask fermentations. Recent progress on combinatorial biosynthesis of the MVA pathway has been used to optimize metabolic flux to FPP and valerena-1,10-diene.

P-040

INNOVATING MICROBIAL LIBRARIES FOR DRUG DISCOVERY USING MALDI-TOF-MS AND THE CULTIVABLE FRESHWATER SPONGE MICROBIOME.

Chase Clark, Michael Mullowney, Antonio Hernandez, Milan Patel, Laura Sanchez, Brian T. Murphy
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607

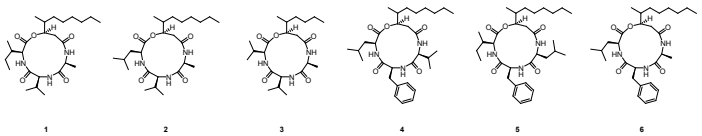
Aquatic sponges (phylum Porifera) are sessile, filter feeding organisms that are among the oldest animals on Earth and harbor diverse microbial communities that can comprise 35% of the sponge biomass. Consequently, they are among the most prolific sources of natural products to date, with nearly 5,000 new small molecules reported in literature. However, most investigations have focused on marine sponges as opposed to their freshwater relatives. Furthermore, creating diverse microbial libraries from environmental sources such as sponges has historically been a blind, cumbersome process that relies on evaluation of colony morphology rather than phylogenetic identity and chemical phenotype. Since 2015 our lab has collaborated with citizen scientists to collect over sixty freshwater sponges from diverse locations across the Great Lakes. We indiscriminately isolated sponge associated bacteria and analyzed the strains using IDBac: an innovative mass spectrometry proteomics and metabolomics platform our lab developed to profile hundreds of cultivable bacteria from agar diversity plates. This rapid, semi-automated method has allowed us to group sponge-associated bacteria by phylogeny, similar to 16S rRNA gene sequencing analysis, while simultaneously providing information about small molecule production *in situ*. This represents a significant advance in creating microbial libraries rich in taxonomic diversity and functional chemistry; and has facilitated detailed studies on the cultivable microbiome of an underexplored natural product source.

P-041

NEW CYCLIC DEPSIPEPTIDES FROM THE ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*

Seoung Rak Lee[1], Jae Sik Yu[1], Seulah Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Hae Min So[1], Sil Kim[1], Won Se Suh[1], Dahae Lee[1], Kyoung Jin Park[1], and Ki Hyun Kim[1]
[1]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

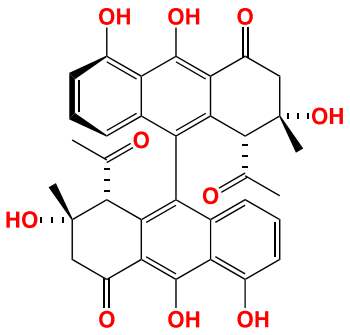
In our endeavor to find structurally novel secondary metabolites from various natural sources, we have focused on the secondary metabolites from the entomopathogenic fungus, *Beauveria bassiana*. Six new cyclic depsipeptides (**1**-**6**) along with four known cyclic depsipeptides (**7**-**10**) were isolated from the MeOH extract of the culture of *B. bassiana*. The chemical structures of new compounds were determined based on comprehensive spectroscopic methods including 1D and 2D NMR as well as HRESIMS data.



P-042

ADVANCING MICROBIAL BIODISCOVERY: MICROBIOREACTOR (MATRIX) PROFILING AS A TOOL TO EXPLORE BIOSYNTHETIC POTENTIAL

Osama G. Mohamed[1], Zeinab Khalil[1,2], Antje Blumenthal[2,3], Robert J Capon[1]
[1]Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, QLD 4072, Australia. [2]The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, 4102, Australia. [3]Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, 4072, Australia



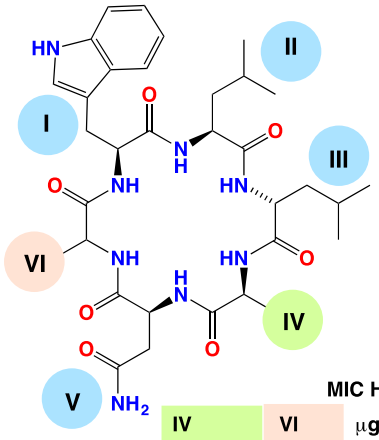
lincolnenin A

Although it is known that bacterial and fungal secondary metabolite production is responsive to culture media and conditions (eg nutrient composition, solid vs liquid phase, oxygen levels etc…), to fully explore these variables using traditional flask cultivation can be prohibitively expensive in consumables, space and time – particularly in an academic laboratory. To address these limitations we implemented a 24 well plate microbioreactor (MATRIX) methodology, optimized for 1.5 mL shaken and static broth, and solid phase cultivations, coupled with *in situ* extraction and UPLC-DAD/UPLC-QTOF profiling. This poster describes the MATRIX as applied to a reference strain of *Streptomyces lincolnensis*, leading to the discovery of new antibacterial atropisomeric bis-anthracence polyketides, evident in only 2 of 34 trialled culture conditions. We provide an account of MATRIX profiling, leading to the prioritisation, production, isolation, spectroscopic characterisation and structure elucidation, and a chemical and biological evaluation of lincolnenins A-D.

P-043

WOLLAMIDES: EXPLORING THE ANTITUBERCULAR POTENTIAL OF CYCLIC HEXAPEPTIDES FROM A REMOTE AUSTRALIAN DESERT STREPTOMYCES

Zeinab Khalil[1,2], Timothy Hall[1], Luis De Leon Rodriguez[3], Rink-Jan Rohman[1], Huy Hoang[1], Norbert J. Reiling[4], David Fairlie[1], Margaret Brimble[3], Antje Blumenthal[2] and Robert Capon[1]
University of Queensland, [1]Institute for Molecular Bioscience and [2]Diamantina Institute, [3]University of Auckland, Maurice Wilkins Centre for Molecular Biodiscovery, [4]Research Center Borstel, Germany.



	IV	VI	MIC H37Rv μg/mL
wollamide B	L-Val	D-Orn	0.81
wollamide B1	L-Val	D-Lys	0.82
wollamide B2	L-Val	D-Arg	0.23
wollamide B3	L-Val	L-Lys	inactive

Tuberculosis is one of the most globally significant infectious diseases, with increasing prevalence of multidrug-resistance demanding an ongoing commitment to discover and develop new antibiotics. This poster presents an account of our investigations into the anti-mycobacterial cyclic hexapeptides, wollamides A and B, first reported in 2014 from a remote Australian desert soil *Streptomyces*. We confirm for the first time that selected wollamides are active against *Mycobacterium tuberculosis* H37Rv, and effective against both drug sensitive and resistant *Mtb*. This study used >50 synthetic wollamides to explore structure activity relationships, to probe and define the wollamide pharmacophore, while simultaneously confirming that wollamides reduce *Mtb* burden in infected macrophages, are not cytotoxic towards mammalian cells, and are a promising lead for the development of next generation *Mtb* antibiotics.

P-044

SCREENING OF MANGROVE-ASSOCIATED FUNGAL EXTRACTS AGAINST PATHOGENIC *CANDIDA ALBICANS.*

Alison H. Hughes,[1] Bill J. Baker [1]
[1]Department of Chemistry and Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL33620.

Candida spp. are the 4th leading cause of nosocomial bloodstream infections in the U.S., with approximately 46,000 cases per year. *C. albicans* is part of our natural microbiome that live symbiotically within us, and thus drug resistance is prevalent when opportunity allows this yeast to manifest into infection. As evolution has shown, microorganisms are far more adroit at competing amongst each other than we have ever been. With that in mind, we aim to screen a library of crude extracts obtained from Floridian mangrove-associated fungi. It is believed that cryptic genes within these environmental fungi are responsible for their production of defensive secondary metabolites in response to stressors. Our lab has developed a method of culturing epigenetically modified fungi to create three distinct

extracts from each fungus, which has led to the compilation of a library of ~9000 extracts. This screening program aims to determine activity of fungal crude extracts against a panel of 14 standardized drug-resistant *C. albicans* strains, obtained from the American Type Culture Collection (ATCC). Results suggest the epigenetic modulated cultures act as independent screening samples, displaying unique activity profiles relative to one another and to non-treated cultures. Progress toward the discovery of compounds active against these *C. albicans* will be presented.

P-045

MICROBIAL TRANSFORMATION OF FLAVONOIDS FROM PROPOLIS

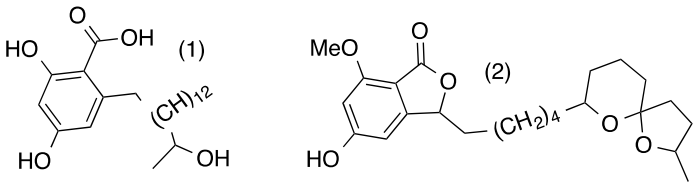
Fubo Han[1], Deborah K.B. Runyoro[2], Olipa D. Ngassapa[2], and Ik-Soo Lee[1]
[1]College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 61186, Republic of Korea, [2]School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65013, Dar-Es-Salaam, Tanzania

Propolis, a sticky resinous substance collected by honeybees from various plant sources, has been widely used as a traditional medicine to prevent and treat colds, wounds, heart disease, diabetes and rheumatism due to its biological and pharmacological properties inclusive of immunomodulatory, antitumor, antimicrobial, anti-inflammatory, and antioxidant effects. Its pharmacological activities are mainly contributed by flavonoids, one of the main groups of phenolic constituents in propolis. However, flavonoid glycosides, the common forms in clinical applications for most flavonoid drugs, are very rare in propolis. And, it is notoriously complicated to accomplish glycosylation of flavonoids at the specific position in a regioselective manner by chemical methods. Hence, bioconversion method using microorganisms as biocatalysts was employed for the production of glycosylated derivatives in order to improve water solubility and potential biological activities of the flavonoids in propolis. Of the twenty-one microorganisms screened, the fungal strain *Mucor hiemalis* was found to perform glycosylation process in a meaningful yield for a series of flavonoids including morin, fisetin, chrysin, and cardamonin. The glycosylated derivatives were isolated and purified following subsequent scale-up fermentation procedures and their structures were identified by spectroscopic methods.

P-046

CHANGES IN VOLATILE AND NON-VOLATILE METABOLITES FROM *PHANEROCHATE CHRYSOSPORIUM* RESULTING FROM VARYING THE GROWTH SUBSTRATE

Kirk P. Manfredi
Department of Chemistry and Biochemistry, University of Northern Iowa, Cedar Falls, IA 50614



Phanerocheate chrysosporium is a wood rot fungus from the phylum basidiomycota. The fungus produces lignin and manganese peroxidases which allows it to degrade lignin to gain access to cellulose and hemicellulose from a mulitude of food sources. We previously showed that growing the organism on wet paper produced the two known metabolites phanerosporic acid (1) and spiroaxine (2).

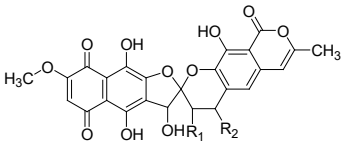
Additionally, we showed that the organism would grow on a number of different substrates. HPLC analysis of the non-volatile metabolites indicated that some substrates would produce(1) and (2) while others would not. Our interest in this organism has been to establish if it could be induced to produce different secondary metabolites by varying its carbon source. Secondly, we are interested in determining if the organism could biotransform the secondary metabolites of its food sources into useful natural products. In this study we chose to monitor volatile compounds using solid phase micro extraction (SPME) with GC/MS. Monitoring volatiles allows for a faster, non-invasive way to view differences in metabolism. Changes in volatile compounds should reflect changes occurring in non-volatile metabolites. In this study *P. chrysosporium* was grown on synthetic media and a number of plant substrates. The volatile metabolites were monitored and identified during fungal growth using GC/MS. The identity and differences of volatiles will be discussed.

P-047

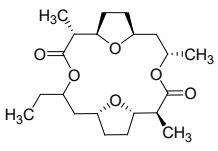
POLYKETIDES PRODUCED BY *STREPTOMYCES PUNICEUS* AB10 ASSOCIATED TO THE FUNGUS-GROWING ANT *ACROMYRMEX RUGOSUS RUGOSUS*

Humberto E. Ortega[1], Timothy Bugni[2], Jon Clardy[3] and Mônica T. Pupo[1]
[1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil, [2]University of Wisconsin, Madison, USA, [3]Harvard Medical School, Boston, USA

This work is part of the ongoing ICBG-Brazil project. This strain was selected for chemical studies for the capability to produce compounds that inhibit the parasite *Leishmania donovani* and the specialized pathogenic fungus *Escovopsis* sp. A few and interesting chemical entities have been reported from this species: for example the antibiotics viomycin, clazamycin B and gougerotin. Griseorhodin A (**1**), C (**2**) and G (**3**) were produced in solid culture using ISP-2 agar. These antibiotics are from the rubromycin family. An interesting group of polyketide pigments published with wide spectrum of biological activities. The antibiotic and antineoplastic compound dimeric dinactin (**4**) and analogues were produced in liquid culture using A-medium. Compound **4** and analogues showed activity against the specialized pathogenic fungus *Escovopsis* sp. The actinobacteria associated to fungus-growing ants can produce an arsenal of compounds to protect these colonies against pathogenic microorganisms, and they could be an interesting source of active natural products.



1: R_1, R_2 = 
2: R_1, R_2 = OH
3: R_1 = OH, R_2 = H



4

P-048

FACILITATING INTERMODULAR INTERACTIONS OF A NON-RIBOSOMAL PEPTIDE SYNTHASE USING COMMUNICATION-MEDIATING DOMAINS

Nick Wauer[1], Josh Keilty[1], Madeline Dennis[1], and Katharine Watts[1]
[1]Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, CA 93407

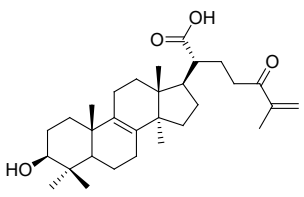
Epoxomicin is a natural product of the bacterial strain *Goodfellowiella coeruleoviolacea* and is biosynthesized by a hybrid Non-Ribosomal Peptide (NRP) Polyketide-Synthase. Epoxomicin is a selective inhibitor of the 20S proteasome and a synthetic derivative, Carfilzomib®, is now marketed as an anti-myeloma drug. The overarching goal of this project is to engineer a bacterial strain for the biosynthesis of the Carfilzomib pharmacophore. The NRPS has 4 modules that each incorporate one amino acid into the peptide core of the molecule. The current goal of the project is to deconstruct the massive NRPS into 4 separate modules, and then re-capitulate the activity of the NRPS *in vitro*. *In vivo*, the modules are covalently linked by 13-15 amino acids, which facilitate interactions and turnover between modules. To enable interactions between deconstructed modules, complementary communication-mediating (COM) domains will be engineered on the termini of each module. The COM domains are expected to create specific inter-modular interactions when expressed in vitro. By PCR amplifying the modules from the wild-type gene cluster and installing overlaps with the vector backbone and the COM domains, Gibson assembly can be performed to create individual modular constructs. We have designed a series of primers that will amplify the desired regions from the epoxomicin gene cluster and subsequently install the overlaps for Gibson assembly. We will present preliminary data on our pilot study with constructs of NRPS modules 1 and 2, including measurement of intermodular interactions using a fluorescence polarization assay.

P-049

CHAGA MUSHROOM (*INONOTUS OBLIQUUS*) INDUCING APOPTOSIS IN HUMAN LUNG CANCERS AND ITS CYTOTOXIC CHEMICAL CONSTITUENTS

Jiwon Baek, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Inonotus obliquus (Hymenochaetaceae), also known as chaga mushroom, has been used in folk medicine for cancer treatment. As part of our ongoing search for new bioactive metabolites from Korean wild mushrooms, we found that the methanolic extract of *I. obliquus* exhibited significant cytotoxicity against human lung adenocarcinoma cells (A549, H1264, H1299, Calu-6 and NIH3T3). Chemical investigation of the methanolic extract was carried out and resulted in the isolation of a new lanostane-type triterpenoid (**1**), and seven known compounds (**2-8**). The structure of the new compound (**1**) was elucidated based on spectroscopic analysis, using 1D and 2D NMR experiments (^{1}H-^{1}H COSY, HMBC, HSQC, and NOESY) and HR-MS data. All the isolates were tested for cytotoxic effects in the human lung cancer cell lines tested.



(**1**)

P-050

CALOTHRIXAMIDES A AND B, TWO ACYL AMIDES FROM THE FRESHWATER CYANOBACTERIUM *CALOTHRIX* SP. UIC 10520

Camila M Crnkovic[1,2], Aleksej Krunic[1], Daniel S May[1], Joanna E Burdette[1], Jimmy Orjala[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA; [2]CAPES Foundation, Ministry of Education of Brazil, Brasília - DF 70040-020, Brazil

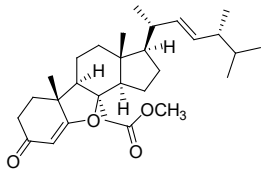
Freshwater cyanobacteria have been known to produce unique metabolites with potential applications to human health. Fractionation of the cyanobacterial extract and subsequent screening in an antiproliferation assay led to the identification of two new compounds named calothrixamides A and B produced by the freshwater strain *Calothrix* sp. UIC 10520. The planar structure of the compounds was elucidated by the use of NMR spectroscopy and mass spectrometry, revealing an alaninol moiety connected to a methylated diene and a long-chain fatty acid modified by a carbamate. Relative configuration was determined by *J*-based configurational analysis along with NOE correlations. Degradation and derivatization methods were utilized to determine the absolute configuration of the stereocenters. Both compounds were tested *in vitro* for growth inhibition of MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines. Calothrixamide B displayed moderate activity in all assays with IC_{50} values in the micromolar range.

P-051

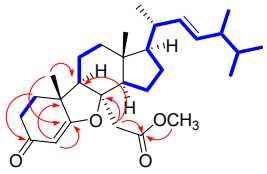
A NOVEL STEROL WITH A CONTRACTED TETRAHYDROFURAN B-RING FROM THE RARE MUSHROOM *CALVATIA NIPPONICA*

Seulah Lee[1], Jae Sik Yu[1], Seoung Rak Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Sil Kim[1], Hae Min So[1], Dahae Lee[1], Won Se Suh[1], Kyoung Jin Park[1], and Ki Hyun Kim[1]
[1]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Calvatia nipponica (Agaricaceae) is one of the most rare species in the *Calvatia* genus. As part of our continuing search for structurally novel and bioactive metabolites from Korean edible mushrooms, chemical investigation of the MeOH extract of the fruiting bodies of *C. nipponica* was carried out and resulted in the isolation of a novel steroid (**1**) which possesses rarely reported [6-5-6-5]-fused rings with a contracted tetrahydrofuran B-ring, along with four known steroids (**2**-**5**). The structure of **1** was determined by spectroscopic analysis, using 1D and 2D NMR spectra and HR-MS. All compounds (**1**–**5**) were tested for the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated RAW264.7 macrophages.



1



1

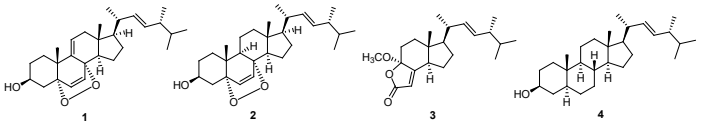
P-052

ANTI-GASTRITIS EFFECTS OF *ARMILLARIELLA TABESCENS* (SCOP.) SING. AND THE IDENTIFICATION OF ITS ANTI-INFLAMMATORY CONSTITUENTS

Seulah Lee[1], Jae Sik Yu[1], Seoung Rak Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Sil Kim[1], Hae Min So[1], Dahae Lee[1], Won Se Suh[1], Kyoung Jin Park[1], and Ki Hyun Kim[1]

[1]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Armillariella tabescens (Tricholomataceae) is a medicinal mushroom traditionally used to treat cholecystitis in China. We found that the MeOH extract of the fruiting bodies of *A. tabescens* showed anti-gastritis activity in ethanol-induced gastric damage in rats, where the extract reduced the gastric damage index in a concentration-dependent manner. Chemical investigation of the MeOH extract led to the isolation of 4 steroids (**1**-**4**), 3 alkaloids (**5**-**7**), 2 nucleic acids (**8**-**9**) and 4 fatty acids (**10**-**13**). All isolated compounds (**1**-**13**) were tested for the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated RAW264.7 macrophages. Compound **10** inhibited NO production with the IC_{50} value of 24.71 **μ**M, and its anti-inflammatory effect was found to be mediated through the inhibition of iNOS and COX-2 expression via downregulation of NF-kappaB.

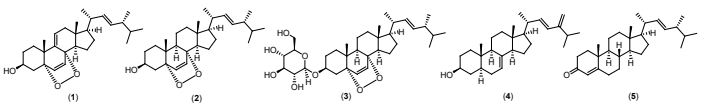


P-053

MORCHELLA ESCULENTA INDUCING APOPTOSIS IN HUMAN LUNG CANCERS AND THE IDENTIFICATION OF CYTOTOXIC CONSTITUENTS

Jiwon Baek, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Morchella esculenta, commonly known as morel and sponge morel, is one of the most highly prized of all the edible mushrooms belonging to the Morchellaceae family. This mushroom has also been used in traditional Chinese medicine to treat indigestion, excessive phlegm, and shortness of breath. As part of our continuing search for structurally novel and bioactive metabolites from Korean wild mushroom, we found that the methanolic extract of the fruiting bodies of *M. esculenta* showed significant cytotoxicity against human lung adenocarcinoma cells. Based on the bioactivity-guided fractionation, chemical investigation of the methanolic extract resulted in the isolation of seven compounds (**1**-**7**), including five steroids (**1**-**5**) from the most active fraction. All the isolates were tested for cytotoxic effects in human lung adenocarcinoma cells, including A549, H1264, H1299 and Calu-6.



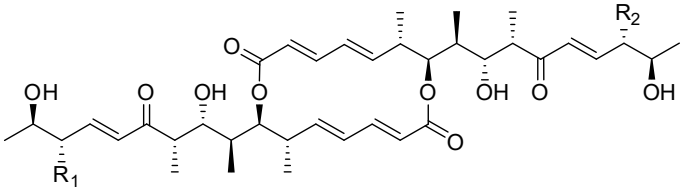
P-054

TERMITELAIOPHYLINS A AND B, ELAIOPHYLIN DERIVATIVES FROM TERMITE-ASSOCIATED *STREPTOMYCES* SP.M56

Seulah Lee[1], Jae Sik Yu[1], Seoung Rak Lee[1], Tae Kyoung Lee[1], Jiwon Baek[1], Sil Kim[1], Hae Min So[1], Dahae Lee[1], Won Se Suh[1], Kyoung Jin Park[1], and Ki Hyun Kim[1]

[1]School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Two new elaiophylin derivatives, termitelaiophylins A (**1**) and B (**2**) and six known elaiophylin derivatives (**3**-**8**) were isolated from the termite-associated *Streptomyces* sp. M56 isolated from the fungal comb material collected from a termite (*Macrotermes natalensis*) nest in South Africa. The structures of new compounds were established by spectroscopic methods including 1D and 2D NMR and HR-ESIMS analysis as well as comparison of their CD data with those of related elaiophylin derivatives. The antifungal and antibacterial properties of all compounds were tested against human-pathogenic bacteria.



1 R_1= CH_3 R_2 = CH_2CH_3
2 R_1= CH_3 R_2 = CH_3

P-055

PROBING THE DIVERSITY OF ENVIRONMENTAL CYANOBACTERIA USING A NEW 16S RRNA CYANOBACTERIAL SPECIFIC PRIMER SET

Daniel S. May[1], George E. Chlipala[1,2], Jimmy Orjala[1]

[1]Department of Medicinal Chemistry & Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, USA; [2]Center for Research Informatics, Research Resource Center, University of Illinois at Chicago, Chicago, Illinois, USA

Cyanobacteria have proven to be a reliable source of biologically active and structurally diverse secondary metabolites. Novel 16S rRNA primers specific for cyanobacteria and amenable to next generation sequencing were developed to investigate the diversity of cyanobacteria in the environment. Amplicon sequencing analyses from samples with macroscopic cyanobacterial growth revealed that we are able to isolate and culture the most prominent cyanobacteria in each collection. Amplicon sequencing analyses of soil samples with no macroscopic cyanobacterial growth indicated that the developed primers were capable of enriching low-abundance cyanobacterial 16S rRNA. The soil amplicon sequencing analyses revealed a greater diversity of cyanobacterial taxa than we currently find in our strain library. Some of these taxa have been reported to produce bioactive secondary metabolites. To enrich for these less frequently isolated cyanobacteria, soil samples were placed into three liquid media with cycloheximide and placed under artificial light. The resulting "artificial blooms" were sequenced to determine if certain media enrich for specific cyanobacteria. Studies using these new primers will allow us to identify and culture less frequently isolated cyanobacteria, which could lead to the discovery of new bioactive compounds.

P-056

A GENERALIZABLE GENOME-GUIDED DESIGN OF SELECTION MEDIA FOR THE GENUS *BURKHOLDERIA*

F.P. Jake Haeckl[1], João L. Baldim[2], Kenji L. Kurita[1], Marisi G. Soares[2], Roger G. Linington[1]
[1]Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 3Z1, [2]Chemistry Insitute of Federal University of Alfenas, Alfenas, Minas Gerais, Brazil.

Bacterial natural products have been an invaluable source for drug discovery efforts since the 1940s. Numerous campaigns in both the pharmaceutical industry and academia have focused on isolating bioactive chemistry from these sources, but increasing rediscovery of known compounds has decreased interest in this area. A recent study revealed that biosynthetic potential is unevenly distributed in bacterial taxonomic space, concentrated primarily in only a few phyla. Based on these results, we hypothesize that investigation of environmental Proteobacteria, particularly from the genus *Burkholderia*, will yield new opportunities in natural products discovery. This is supported by targeted investigation of biosynthetic gene clusters from a selection of *Burkholderia* type strains which has yielded a wealth of novel natural products. Alternatively, investigating many target organisms from the environment yields a biologically diverse library to sample, but requires the challenging step of isolation from complex microbial assemblages. We employed a computational approach using genomic information on antimicrobial resistance, metal tolerance, and carbon and nitrogen source metabolism of 45 strains of *Burkholderia* and 55 strains of untargeted organisms to develop selective media for the rapid, one-pass isolation of this specific genus. We experimentally validated these three key factors that impact bacterial growth as well as pH tolerance and the necessity of other complex nutrients, to systematically develop five new media combinations selective for *Burkholderia.*

P-057

METABOLISM OF XANTHOHUMOL AND 8-PRENYLNARINGENIN BY INTESTINAL MICROBIOTA

Ines L. Paraiso[a,b], Ryszard Zielke[a], Aleksandra E. Sikora[a], Jan F. Stevens[a,b]
[a]College of Pharmacy, [b]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331

Xanthohumol (XN), a flavonoid found in hops (*Humulus lupulus*), exerts mitigating effects on the metabolic syndrome. XN has several pharmacological targets *in vitro* but appears to be active at comparatively low concentrations *in vivo*, suggesting the involvement of bioactive metabolites. To fully understand XN's mechanism of action *in vivo*, it is necessary to gain more information on its fate in the body following ingestion, as a certain proportion of ingested secondary plant constituents might escape absorption in the small intestine and undergo colonic microbial transformation. To study the metabolism of XN and related prenylated flavonoids, XN and 8-prenylnaringenin (8PN) were incubated with *E. ramulus*, a strictly anaerobic bacterium detectable in the gastrointestinal tract of most individuals. Evidence from our study shows that both XN and 8PN are extensively transformed by this gut microbe, producing metabolites with similar pharmacological properties. Moreover, significant changes in the gut microbiota composition occur after treatment with XN, supporting the potential of the flavonoid as a prebiotic. Degradation pathways of XN are proposed based on the intermediates detected by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS).

P-058

DISCOVERY OF ANTIMICROBIAL AGENTS FROM DUAL-CULTURE CONDITIONS USING MULTI-OMICS APPROACH

Pi-Yu Chen, Ying-Ning Ho, Han-Jung Lee, Yu-Liang Yang
Agricultural Biotechnology Research Center, Academia Sinica, 11529 Taipei, Taiwan

A microbe produces various natural products in response to different environmental stimulation. The biosynthesis of such natural products is strictly controlled and depends on various internal and external cues, ensuring a competitive advantage when environmental conditions change. Accordingly, we can manipulate the production of natural products by varying culturing conditions. Dual-culture by simply growing a microbe together with one another is one of straight-forward and effective approaches to monitor natural products generation. Here we employed a multi-omics approach, including RNA-seq transcriptomics, LCMS-based metabolomics, together with imaging mass spectrometry to discover new antimicrobial agents produced by a Gram negative bacterium YMA4 in dual-cultures with *Candida albicans* and *Staphylococcus aureus*, respectively. The bacterium YMA4 is capable of generating different antimicrobial agents including polyynes and cyclic peptides against pathogenic fungi and bacteria in different culture conditions.

P-059

TARGETING QUORUM SENSING SIGNAL BIOSYNTHESIS IN MRSA WITH THE FUNGAL METABOLITE AMBUIC ACID

Cassandra N. Naphen, J. Stempin, Nadjali A Chung, Lindsay K. Caesar, Daniel A. Todd, Huzefa A. Raja, Nicholas H. Oberlies and Nadja B. Cech
Department of Chemistry and Biochemistry, The University of North Carolina, Greensboro, North Carolina 27402, United States E-mail: nbcech@uncg.edu.

Natural products and their derivatives have provided antimicrobial medicines for decades and currently account for up to two-thirds of antibiotic agents on the market. The kingdom of Fungi is a highly diverse group of organisms with an estimate of 5.1 million fungal species to exist, with approximately 135,000 species being described in literature. As such, this represents a chemically diverse and untapped resource for natural product discovery. Methicillin-resistant *Staphylococcus aureus* (MRSA), is responsible for over 80,461 severe infections and 11,285 deaths in the United States in 2015. MRSA possesses an arsenal of virulence factors that are controlled by a density-dependent regulatory system known as 'quorum sensing.' Targeting the pathways that produce these virulence factors is a promising new strategy for anti-infective therapy. This approach could facilitate infection clearance without the need for direct antimicrobial compounds that can lead to the development of resistance. Utilizing a novel mass spectrometry based bioassay, our research has focused on the identification and evaluation of quorum quenchers. Our assay is designed to specifically identify compounds that inhibit the quorum sensing signal biosynthesis mechanism, a process highly conserved in the *Staphylococci* and other Gram-positive pathogens. The only known inhibitor of quorum sensing signal biosynthesis in Gram-positive bacteria is ambuic acid, which inhibits the AgrB segment of the quorum sensing system. This compound is produced by the *Ascomycete* fungus, *Pestalotiopsis microspora*. Using a combination of technologies, including chromatographic separation, molecular networking, mass defect filtering, and synthetic derivatization, we have isolated ambuic acid from *P. microspora* and are in the process of identifying analogs of this compound. Ultimately, our goal is to gain insight into the structural characteristics that govern ambuic acid's ability to inhibit quorum sensing signal biosynthesis, and to improve efficacy.

P-060

CHARACTERIZING ANTIMICROBIAL CONSTITUENTS IN *SALVIA MILTIORRHIZA*

Sabina Nogo[1], Lindsay Caesar[1], Nadja Cech[1], Richo Cech[2]
[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27410, [2]Strictly Medicinal Seeds PO Box 299 Williams, OR 97544

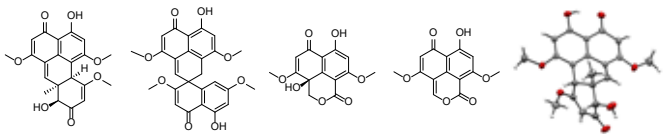
The World Health Organization, the CDC and the NIH all recognize the importance of novel approaches to antimicrobial drug discovery, with a great interest in the principle of anti-virulence, guiding the research of plant secondary metabolites as lead compounds for drug development. *Salvia miltiorrhiza,* also known as red sage, or danshen, is a deciduous perennial plant native to Japan and China, recognized for its numerous medicinal properties in Traditional Chinese Medicine. The red roots of danshen are sought for their antimicrobial activity. The roots were extracted with ethyl acetate and showed 100% growth inhibition of both wild type and methicillin-resistant *Staphylococcus aureus* (MRSA) at 100 µg/mL. When this extract was screened against multi-drug resistant (MDR) *Acinetobacter baumannii* in combination with subinhibitory concentrations of levofloxacin, it inhibited bacterial growth by 100% at 100 µg/mL. The crude extract was fractionated using normal phase flash chromatography, following a bioassay-guided fractionation protocol. Six out of eight first- stage fractions inhibited growth of MRSA at 100 µg/mL. Cryptotanshinone is one known anti-microbial constituent in the botanical, and each fraction from the crude sample was analyzed for its percent composition, using a mass spectrometry generated calibration curve of a cryptotanshinone standard for comparison. High performance liquid chromatography will be utilized to isolate and purify active compounds from the botanical, with the goal of discovering novel antimicrobial compounds or attributing new anti-microbial activity, or potentiation of activity, to known compounds against MDR bacteria.

P-061

A-GLUCOSIDASE INHIBITORS FROM *PREUSSIA MINIMOIDES*

Manuel E. Rangel-Grimaldo, Isabel Rivero-Cruz, Abraham Madariaga-Mazón, Mario Figueroa and Rachel Mata*
Universidad Nacional Autónoma de México, Ciudad de México, 04510, México.

Extensive fractionation of an extract from the grain-based culture of the endophytic fungus *Preussia minimoides* led to the isolation of two new polyketides with novel skeletons, minimoidiones A (**1**) and B (**2**), along with the known compounds, preussochromone C (**3**), corymbiferone (**4**), and 5-hydroxy-2,7-dimethoxy-8-methylnaphthoquinone (**5**). The structures of **1** and **2** were elucidated using 1D and 2D NMR data analysis, along with DFT calculations of ^{1}H NMR chemical shifts. The absolute configuration of **1** was established by a single-crystal X-ray diffraction analysis and TDDFT-ECD calculations. Compounds **1**–**4** significantly inhibited yeast α-glucosidase with IC_{50}'s ranging from 2.9 to 155 µM.



1 2 3 4 1

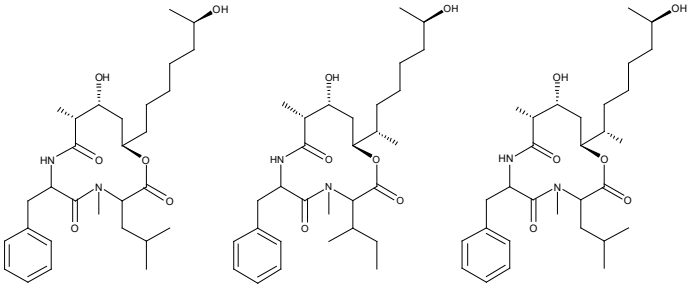
This work was supported by a grant from CONACyT CB-219765.

P-062

NOVEL CYCLIC LIPOPEPTIDES PRODUCED BY AN ENDOPHYTE *COLLETOTRICHUM GLOEOSPORIOIDES* FROM A HALPOPHYTE *SUAEDA JAPONICA*

Changyeol Lee[1], Soonok Kim[2], and Sang Hee Shim[1,*]
[1]College of Pharmacy and Duksung IDC, Duksung Women's University, Seoul 01369, Republic of Korea, [2]National Institutes of Biological Resources, Incheon , Republic of Korea.

Recently endophytes have been recognized as a source of structurally novel and biologically active secondary metabolites. Among the host plants for endophytes, halophytes which live in high saline condition have been reported to carry a variety of endophytes which could produce bioactive secondary metabolites. In this study, a halophyte *Suaeda japonica* was selected as a potential source for endophytes. An endophytic microorganism *Colletotrichum gloeosporioides* JS0417 harbored in *Suaeda japonica* was cultured on a large scale and the extracts of the cultures were subjected to extensive chemical investigation, which resulted in isolation of three new cyclilipopeptides (**1**-**3**) as shown in the following figure. Their chemical structures were elucidated by chemical methods as well as spectral analysis. Stereochemistries of the isolated compounds (**1**-**3**) were established using modified Mosher's method together with advanced Marfey's method.



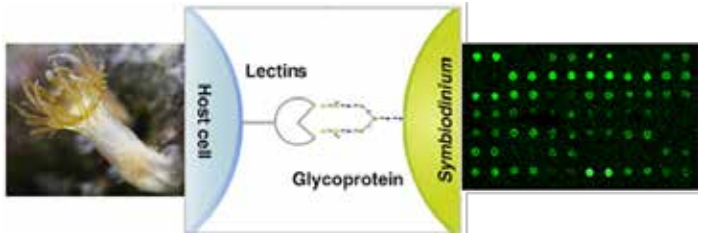
1 **2** **3**

P-063

EXPLORATION OF GLYCAN DEPENDENT RECOGNITION IN CNIDARIANS AND ALGAE

Paige E. Mandelare[1], Donovon A. Adpressa[1], Trevor Tivey[2], John Parkinson[2], Virginia Weis[2], Sandra Loesgen[1]
[1]Department of Chemistry, [2] Department of Integrative Biology, Oregon State University, Corvallis, OR 97331

The sea anemone *Aiptasia* and its *Symbiodinium* symbionts are being examined to understand inter-partner signaling and host cell immune response during onset and breakdown of cnidarian-dinoflagellate symbioses. Our work has shown that treatment of the native *Symbiodinium* symbiont with endoglycosidases reduces the amount of high-mannoside surface glycans resulting in a decrease in the rate of infection of *Aiptasia*. Here we present a comparative study of the algal surface glycans using lectin array technology and *in vivo* infectivity assays to determine what glycan structures make a good or poor algal infector and how this affects onset of symbiosis.



P-064

MARINE SPONGE-ASSOCIATED BACTERIA PRODUCE BIOFILM INHIBITORS IN RESPONSE TO MICROBIAL COMMUNITY PERTURBATIONS

Skylar Carlson[1], Angela Xu[1], Jennifer Ross[1], Tessa Burghardt[1], Samuel French[1], Ian Miller,[1] Melany Puglisi-Weening,[2] Warren Rose,[3] and Jason C. Kwan[1]

[1]Pharmaceutical Sciences Division and [3]Pharmacy Practice Division, School of Pharmacy, University of Wisconsin, Madison, WI [2]Department of Pharmaceutical Science, College of Pharmacy, Chicago State University, Chicago, IL

Marine sponges are filter-feeding invertebrates that harbor complex symbiotic bacterial communities. The water column, where bacteria are present at upwards of 10^6 cells/mL, is actively pumped through marine sponge tissue. The stable microbial communities of marine sponges vary drastically from the bacterial community of the water column, suggesting there is a mechanism to inhibit infection by rival species in host tissues. We hypothesize that small molecules are produced by biosynthetic gene clusters within the genomes of symbiotic bacteria in order to maintain the stable microbial community. To assess our sponge tissue collection, we have identified individuals within morphological groupings (1) capable of inhibiting biofilm formation in *Staphylococcus aureus* and (2) have perturbations in their microbial communities. We have identified 14 sponges from various groups in our sponge tissue collection (N=100) capable of inhibiting biofilm formation. The microbial communities of individual sponges that differed drastically from other members of their morphological grouping also displayed biofilm inhibition activity. Molecules identified from two sponges, FL2015-30 and FL2015-36, are presented. These identified small molecules are used to trace the occurrence of the responsible biosynthetic machinery in other sponges in the same grouping via shotgun sequencing. Through the results of these experiments we can interrogate the impact of infection on microbial community structure in marine sponges. Additionally, the identification of biofilm inhibitors may serve to combat resistance to existing antimicrobial therapies.

P-065

SEARCHING THE IRISH DEEP FOR THE BIOACTIVE MARINE METABOLITES

Ryan Young[1,2] Bill Baker[2,3], Mark Johnson[1] and A. Louise Allcock[1]

[1]Martin Ryan Institute, NUI Galway, University Road, Galway, Ireland, [2]School of Chemistry, NUI Galway, University Road, Galway, Ireland, [3] Department of Chemistry and Center for Drug Discovery and Innovation, University of South Florida, Tampa FL, USA

Ireland has some of the largest territorial waters in mainland Europe (*c.a.* 880 000km^2), about ten times that of its land mass. The Irish continental margin has a high prevalence of submarine canyons and highly diverse corals mounds, dominated by the phyla Porifera and Cnidaria, which account for 80% of novel marine natural products. We are undertaking a 5-year project, funded by Science Foundation Ireland, which includes a targeted deep-sea collection strategy and a program of screening, fractionation and purification of novel marine derived chemistry. We have collected deep-sea fauna including demosponges, hexactinellid sponges, bamboo corals, gorgonians, soft corals, sea pens, and black corals, with two future collecting expeditions funded and scheduled.

Screening assays to identify bioactive deep-sea marine invertebrates are targeted at range of human afflictions such as drug-resistant colorectal cancer, MRSA and malaria. Extracts identified as biologically active will be fractionated and rescreened to identify the active fraction. Bio-guided fractionation will be used to obtain spectroscopically pure bioactive secondary metabolites.

Initial chemical evaluations have begun to yield a series of bioactive alkaloids. Natural product rich fractions have been further purified via HPLC and contemporary spectroscopic methods used to elucidate chemical structures.

P-066

ISOLATION AND CHARACTERIZATION OF THE FISH-KILLING TOXIN FROM THE MARINE DINOFLAGELLATE KARLODINIUM ARMIGER

Silas A. Rasmussen[1], Sofie B. Binzer[2], Per J. Hansen[2], Thomas O. Larsen[1]

[1]Department of Bioengineering and Biomedicine, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark, [2]Marine Biological Section, University of Copenhagen, Helsingør, Denmark

Marine fish farming has the potential to become an economically important industry worldwide. However, blooms of fish-killing harmful microalgae are a recurring phenomenon in coastal waters. The genus *Karlodinium* is known to produce a suite of fish-killing and hemolytic compounds, named karlotoxins.[1] Recently, a new fish-killing *Karlodinium* species was discovered in the Mediterranean Sea, named *K. armiger*.[2] We have isolated and structurally elucidated a new karlotoxin congener that we have named karmitoxin.[3] Using ^{13}C enrichment and high-field 2D NMR spectroscopy the structure of karmitoxin was elucidated. This compound interestingly differs from all other isolated karlotoxins and the related amphidinols from *Amphidinium* sp. by containing a primary amine group. The isolated compound was tested towards the copepod, Acartia tonsa and using a rainbow trout gill cell (RTgill-W1) assay as a proxy for fish toxicity. This showed that karmitoxin was able to immobilize and kill A. tonsa (LC_{50}: 400±100 nM) and lyse RTgill-W1 (LC_{50}: 125±1 nM) cells in the mid nanomolar range, in agreement with the observed concentration in culture. [1]Mooney et al. (2009) J. Phycol. 45:164–175. [2]Bergholtz et al. (2006) J. Phycol. 42:170–193. 3Rasmussen et al. (2017). J. Nat. Prod. (DOI: 10.1021/acs.jnatprod.6b00860)

P-067

ZAMAMIDINE D, A NEW MANZAMINE ALKALOID FROM AN OKINAWAN MARINE SPONGE *AMPHIMEDON* SP.

Takaaki Kubota[1], Shin-ichiro Kurimoto[1], Kenta Nakamura[2], Kanae Sakai[3], Rei Hokari[4], Aki Ishiyama[4], Masato Iwatsuki[4], Kazuhiko Otoguro[4], Tohru Gonoi[3], Satoshi **Ō**mura[4], and Jun'ichi Kobayashi[2]

[1]Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan. [2]Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan. [3]Medical Mycology Research Center, Chiba University, Chiba 260-0856, Japan. [4]Kitasato Institute for Life Sciences, Kitasato University, Minato-ku, Tokyo 108-8641, Japan

The manzamine alkaloids have been reported from several marine sponge genera and are attractive as structurally unique and biosynthetically interesting bioactive natural products. In our continuing search for bioactive natural products from marine sponges, we have previously investigated the extract of an Okinawan marine sponge *Amphimedon* sp. (SS-1231) and isolated a manzamine-related alkaloid, zamamiphidin A, from its EtOAc-soluble materials.[1] Recently, we investigated BuOH-soluble materials of the extract and found a new manzamine alkaloid, zamamidine D, which was the first manzamine alkaloid possessing a 2,2'-methylenebistryptamine unit as the aromatic moiety instead of a β-carboline unit.[2] Zamamidine D exhibited antimalarial and antimicrobial activities.

1. Kubota, T.; Kobayashi, J. *et al. Org. Lett.* **2013**, *15*, 610-612.
2. Kubota, T.; Kobayashi, J. *et al. J. Nat. Prod.* **2017**, doi: 10.1021/acs.jnatprod.6b01110.

P-068

UPREGULATION AND IDENTIFICATION OF ANTIBIOTIC ACTIVITY OF A MARINE *STREPTOMYCES* SP. VIA CO-CULTURES WITH HUMAN PATHOGENS

Anne A. Sung[1], Samantha M. Gromek[1], and Marcy J. Balunas[1,]*
[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, 69 North Eagleville Road, Storrs, CT 06269, USA

Numerous natural products have been discovered from members of the order Actinomycetales, particularly in the genus *Streptomyces*, due to their metabolic diversity in the production of biologically active secondary metabolites. However, many secondary metabolites cannot be produced under laboratory conditions because growth conditions in flask culture differ from conditions in the natural environment. Various experimental conditions (e.g., mixed fermentation) have been performed to increase yields of previously described metabolites, cause production of previously undetected metabolites, and increase antibiotic activity. A marine-derived *Streptomyces* sp. strain PTY087I2 was isolated from a Panamanian tunicate and subsequently co-cultured with human pathogens *Bacillus subtilis*, methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. Minimum inhibitory concentrations (MICs) of these extracts and their spectrometric profiles show upregulation of antibacterial compounds in the challenged extracts, particularly the *Streptomyces* sp. co-cultured with MRSA. Co-culture of *Streptomyces* sp. PTY087I2 with these human pathogens resulted in increased production of two known antibiotics, granaticin and granatomycin D, as well as several derivatives seen via molecular networking. In addition, co-cultures resulted in strongly enhanced biological activity against the Gram positive human pathogens used in these experiments. Expanded utilization of experiments to allow for competitive interactions of co-cultures may enhance metabolite production and further our understanding of microbial interactions.

P-069

KEIKIPUKALIDES: NEW FURANOCEMBRANOIDS FROM THE DEEP-SEA ANTARCTIC SOFT CORAL, *PLUMARELLA DELICTISSIMA*

Santana A.L. Thomas,† Jacqueline von Salm,† Shane Clark,† Steve Ferlita,† Prasanth Nemani†, Ala Alzarian,± Nerida Wilson§, Dennis Kyle± and Bill J. Baker†
†Departments of Chemistry and ±Global Health and Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL 33620, United States and §Western Australia Museum, Perth, Western Australia, Australia

Furanocembranoids are diterpenes produced by plants and soft corals. Neurotoxicity in some members of the family appears to provide protection, especially for soft corals during spawning season to protect their eggs. *Plumarella delicatissima*, a deep-sea feather-like octocoral collected from the Scotia Arc, Antarctica, was found to elaborate five new furanocembranoid diterpenes, the keikipukalides, and two known compounds, pukalide aldehyde and ineleganolide. Keikipukalides A-E was screened for neurotoxic and antiinfective bioactivity. They were found to be active against *Leishmaniasis donovani* and 3 strains of *Candida albicans*.

P-070

EFFECT OF CHEMICAL CROSS-TALK IN BACTERIAL CO-CULTURES ON DIFFERENTIAL GENE EXPRESSION AND ANTIBIOTIC PRODUCTION

Deepa Acharya[1], Navid Adnani[1], Doug Braun[1], Ian Miller[1], Qing Yu[1], Mark Berres[2], Lingjun Li[1], Jason Kwan[1], Tim Bugni[1]
[1]School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, [2]Bioinformatics Resource Center, University of Wisconsin-Madison, 425 Henry Mall, Madison, WI 53706

The phylum Actinobacteria has been found to house numerous cryptic biosynthetic gene clusters (BGCs), encoding possible new therapeutic molecules. Our lab has successfully adopted the approach of interspecies co-culture as a means to tap into this potential. We identified two species of marine sponge associated Actinobacteria, a *Micromonospora* sp. (strain WMMB235) and a *Rhodococcus* sp. (WMMA185) that produce a novel antibiotic, Keyicin, only when co-cultured together. A comprehensive 'omics' based approach has helped us not only identify the gene cluster responsible for the production of the antibiotic in the genome of the *Micromonospora* sp., but also given us promising results on differential gene and protein expression in co-culture versus mono-culture. Moreover, cell-free lysates of *Rhodococcus* sp. were able to activate the production of Keyicin, suggesting the use of small molecule chemical signaling as a means of communication between these species. Taken together, these studies will provide us a roadmap to potentially unlock other cryptic BGC's in Actinobacteria.

P-071

ISOLATION, CHARACTERIZATION AND CYTOTOXICITY OF SECONDARY METABOLITES FROM *LAURENCIA ALFREDENSIS*

Godwin A. Dziwornu[1], Mino R. Caira[1], Jo-Anne de la Mare[2], Adrienne L. Edkins[2], John J. Bolton[3], Denzil R. Beukes[4], and Suthananda N. Sunassee[1,5]
[1]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa, [2]Biomedical Biotechnology Research Unit, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa, [3]Department of Biological Sciences, University of Cape Town, Rondebosch, 7701, South Africa, [4]School of Pharmacy Department of Pharmaceutical Chemistry, University of the Western Cape, Bellville, 7535, South Africa, [5]SAMRC Drug Discovery and Development Research Unit, University of Cape Town, Rondebosch 7701, South Africa.

The marine red algae of genus *Laurencia* are well-known for their structurally diverse and biologically active halogenated and non-halogenated secondary metabolites.[1] *Laurencia alfredensis* is a newly identified species collected outside Port Alfred on the east coast of South Africa.[2] A sequence of silica gel column chromatography, preparative TLC and normal phase HPLC resulted in the isolation of three labdane diterpenes, four triterpenes and three steroidal compounds. The structures of these compounds were elucidated using 1D and 2D NMR, HR-ESIMS, IR and UV spectroscopic methods. All compounds exhibited moderate to weak cytotoxicity when evaluated against the MDA-MB-231 breast and HeLa cervical cancer cell lines.

References

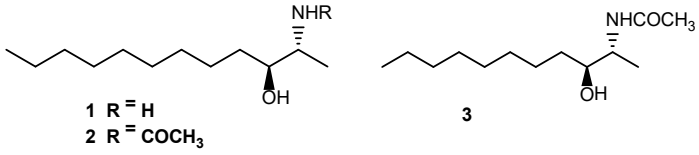
1. Blunt, J. W., Copp, B. R., Keyzers, R. A., Munro, M. H. G., Prinsep, M. R. *Nat. Prod. Rep*., **2012**, *29*, 144-222 and earlier reviews in this series.
2. Francis, C.; Bolton J.J.; Mattio, L.; Mandiwana-Neudani, T.G.; Anderson, R.J. *J. Phycol.* **2017**, *In press*.

P-072

CYTOTOXIC CLAVAMINOLS FROM THE ASCIDIAN *CLAVELINA OBLONGA*

Jessica P. Batista[1], Rhenner N. A. Assis[1], Luis C. Kellner Filho[1], Gustavo M. Dias[2], Iara S. Squarisi[1], Denise C. Tavares[1], Márcio L. A. Silva[1], Wilson R. Cunha[1], Patrícia M. Pauletti[1], Ana H. Januario[1].
[1]Natural Products Research Group, University of Franca, Franca, SP, 14404-600, Brazil
[2]Center for Natural and Human Sciences, Federal University of ABC-São Bernardo do Campo, SP, 09606-070, Brazil

The ascidian *Clavelina oblonga* (CO) is widely distributed in the São Sebastião Channel, São Paulo, Brazil. Reversed-phase column chromatography of the CO extract afforded a mixture of three amino alcohols. This fraction enriched with the 2-amino-3- alkanols known as clavaminols A (**1**), C(**2**) and D (**3**) showed cytotoxicity to normal human fibroblast cells GM07492A with IC_{50} of 121.2 ± 16.6. The structural elucidation of **1**-**3** was established by NMR spectroscopic and mass spectrometric analysis HRESI-MS (+): *m/z* 202.2119 $[M+H]^+$ (**1**, $C_{12}H_{28}NO$); *m/z* 252.1868 $[M+Na]^+$ (**3**, $C_{13}H_{27}NO_2Na$) and HRESI-MS (-): *m/z* 242.2126 $[M-H]^-$ (**2**, $C_{14}H_{28}NO_2$). These compounds were previously isolated from the Mediterranean ascidian ascidian *C. phlegraea* and affected the cell viability of the A549, lung carcinoma; T47D, breast carcinoma, and AGS, gastric carcinoma, being clavaminol A the most active compound in the series.



P-073

ISOLATION OF NEW ALCYOPTEROSIN COMPOUNDS FROM AN ANTARCTIC CORAL

Anne-Claire Limon[1] and Bill Baker[1,2]
[1]Department of Chemistry, University of South Florida, Tampa, FL 33620, [2]Center for Drug Discovery and Innovation, 3720 Spectrum Blvd, Tampa, FL 33612

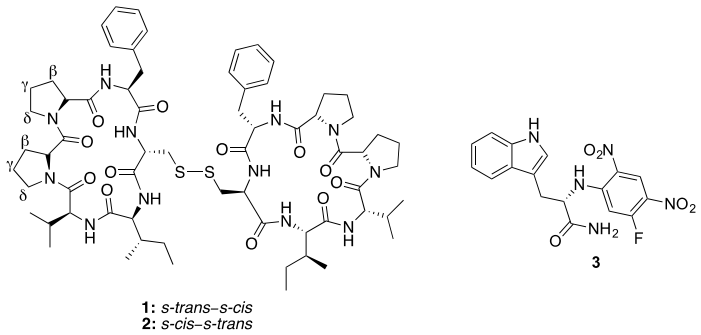
Drug resistance causing the spread of infectious agents and diseases is a global issue, in need of innovative solutions and novel drugs. Marine organisms from Antarctica have been investigated for their potential natural product chemistry. Antarctic corals produce unique secondary metabolites as a defense mechanism to compensate for their immobility. These adaptations persist throughout evolution, accumulating novel secondary metabolites in subsequent generations. The compounds behind these chemical defense systems could be a significant source of novel chemistry to be further developed into new drugs. Alcyopterosins are aromatic sesquiterpenoids of the illudalane class, with modest distribution in nature first isolated from ferns and fungi. In 1999, the subAntarctic soft coral *Alcyonium paessleri* brought forward the first alcyopterosins of marine origin, displaying a nitrate ester moiety never seen before in marine natural products chemistry. The extraction and purification of extracts from an unidentified Antarctic coral, has led to the isolation of several known alcyopterosins alongside new ones. Coral samples collected from Antarctica were freeze-dried, treated to Soxhlet extraction and liquid-liquid partition; then Medium Pressure Liquid Chromatography (MPLC) and High Pressure Liquid Chromatography (HPLC) were performed to purify terpenoids. The purification process was guided by Nuclear Magnetic Resonance spectroscopy (NMR). Once characterized, isolates were subjected to assays against various infectious agents to assess bioactivity and drug potential.

P-074

CYCLIC HEXAPEPTIDE DIMERS, ANTATOLLAMIDES A AND B, FROM *DIDEMNUM MOLLE*

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

Two dimerized cyclic hexapeptides, antatollamides A (**1**) and B (**2**) were isolated from the colonial ascidian *Didemnum molle* collected in Pohnpei. The amino acid compositions and sequences were solved by interpretation of MS, 1D and 2D ^{1}H NMR data. Raney Ni reduction of antatollamide A gave the corresponding monomeric cyclic hexapeptide with replacement of Cys by Ala. The amino acid configurations were established after total hydrolysis and conversion with a new chiral derivatizing reagent, *N*α-(5-fluoro-2,4-dinitrophenyl)-L-tryptophanamide (FDLT, **3**), derived from tryptophanamide, followed by LCMS analysis; all were L- except for D-Ala. FDLT is particularly superior over the traditional Marfey's reagent at resolving polar amino acids and certain *N*-Me-amino acids (e.g. *N*-Me-Ala) as well as derivatives of L- Ile from L- *allo*-Ile, a perennial problem.



P-075

NEW TRICYCLIC DITERPENES FROM A DEEP-SEA ANTARCTIC SEA PEN *ANTHOPTILUM*

Santana A.L. Thomas,[†] Anthony Sanchez±, Nerida Wilson[§], Younghoon Kee± and Bill J. Baker[†]
[†]Department of Chemistry and Center for Drug Discovery and Innovation, ±Department of Cell, Molecular and Microbiology, University of South Florida, Tampa, FL 33620, United States [§]Western Australia Museum, Perth, Western Australia, Australia

Pennatulacea, an order of the subclass Alcyonaria (= Octocorallia), are dominated by terpenes. These sea pen secondary metabolites have proven to exhibit biological properties ranging from cytotoxicity and anti-inflammatory activity to immunomodulation. Pennatulacea commonly produce compounds from the skeletal class of briaranes, a tricyclic diterpene consisting of a γ-lactone fused to a bicyclo[8.4.0] system. This study describes the isolation and structure elucidation of bathyptilolides A and B, briarane diterpenes from the deep-sea Antarctic sea pen *Anthoptilum* sp. In addition to bathyptilolides, this sea pen also produces a dolastane diterpene, bathyptilone A, the first report of this diterpene skeleton from Alcyonaria. Bathyptilone A is a trinor dolastane and represents a new carbon skeleton. Bathyptilolide A shows selective activity in the Ntera2 assay with an MIC of 10ng/mL whereas bathyptilolide B and bathyptilone A are inactive.

P-076

CHEMICAL INVESTIGATION OF SECONDARY METBOLITES FROM ANTARCTIC SPONGES

Matthew A. Knestrick[1,2], Nerida G. Wilson[3], and Bill J. Baker[1,2]
Departments of [1]Chemistry, [2]Center for Drug Discovery & Innovation, University of South Florida, Tampa, FL 33620, [3] Western Australian Museum, Perth, Western Australia, Australia

The waters surrounding Antarctica are cold, geographically isolated, and nutrient rich. This ecosystem contains vast biodiversity, and promotes harsh competition and defensive secondary metabolite biosynthesis, particularly in invertebrate species. Sponges, particularly those native to Antarctica, are well known as a source of new, exciting chemistry. In order to explore this chemodiversity source, a collection of sponges with unidentified taxonomy were collected in the Scotia Arc of the Southern Ocean. A metabolomics approach was utilized to determine chemical similarities between individual sponge extracts, and similar extracts were combined. Iterative rounds of compound isolation were guided by 1D NMR and LC/MS analysis. Here we present the chemical investigation of these sponges extracts, including isolation of several known and potentially new peptides and other secondary metabolites. Structure elucidation and bioactivity data will be presented.

P-077

CHEMISTRY OF MARINE TUNICATES COLLECTED FROM ANTARTICA

Sofia Kokkaliari,[1] Nerida Wilson[2] and Bill J. Baker[1]
[1] Department of Chemistry, University of South Florida and Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL 33620
[2]Marine Biodiversity and Evolution, Western Australian Museum, Welshpool, WA 6106

Marine organisms have attracted the interest of the scientific community in the past few decades. An area of great interest has been organisms native to Antarctica, due to the Antarctic Polar Font, which has isolated the ecosystem leading to high diversity of Antarctic organisms. Many Antarctic invertebrates have been studied, some of which have shown an interesting chemical and/or biological profile. Antarctic invertebrates are of specific interest and especially tunicates have been the source of multiple new and active compounds.[1-4] In this project, we attempted to isolate new compounds from Antarctic tunicates. The tunicates were collected near Nathaniel B. Palmer station in Antarctica and at different depths. After extraction, a sequence of MPLC (medium pressure liquid chromatography) and HPLC (high pressure liquid chromatography) purification procedures were performed. The entire process was guided using proton NMR.

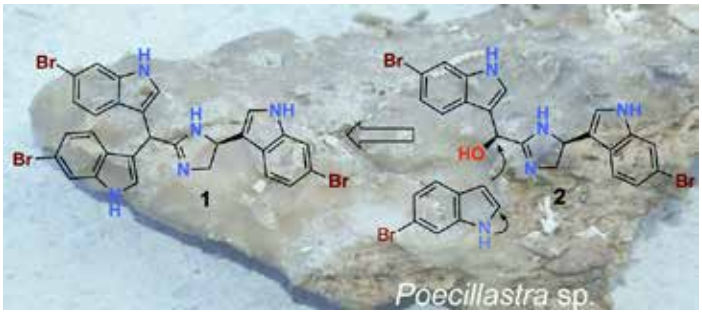
1. Diyabalanage, T.; Amsler, C. D.; McClintock J. B.; Baker, B. J. *J. Am. Chem. Soc.* **128**, 5630–5631 (2006).
2. Noguez, J. H. *et al. Bioorg. Med. Chem.* **19**, 6608–6614 (2011).
3. Lebar and Baker *Aust. J. Chem.* (2010)
4. Lebar et al. *Bioorg Med Chem* **19** (2011) 5756–5762

P-078

POECILLASIRIN A, A NEW TRI-INDOLE ALKALOID FROM A DEEP WATER *POECILLASTRA* SP.

Hong-Bing Liu[1], *Gianluigi Lauro[2], Robert D. O'Connor[1], Katheryn Lohith[1],* Giuseppe Bifulco[2],
and Carole A. Bewley[1]
[1]Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0820, USA, [2]Dipartimento di Farmacia, Università di Salerno, via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy

Antibacterial-guided fractionation of the deep-water marine sponge *Poecillastra* sp. led to the isolation of two new indole alkaloids poecillasirin A (**1**) and poecillasirinol (**2**), along with two known analogues spongotine C and dibromodeoxytopsentin. Their planar structures were determined by 1D and 2D NMR spectroscopy. Their absolute configurations were determined through a combination of experimental and computational analyses. Poecillasirin A (**1**) represents the first example of a di(6-Br-1H-indol-3-yl) methyl group linked to an imidazole core. The presence of alcohol **2** suggested a biosynthetic route to tri-indole **1**. All of the compounds showed strong antimicrobial activity against Staphylococcus aureus, but no inhibitory effect on Escherichia coli. Their anti-HIV activity and cytotoxity were also evaluated.



P-079

NMR CHARACTERIZATION OF COMPLEX HETEROCYCLIC NATURAL PRODUCTS: OPPORTUNITIES AND CHALLENGES IN STRUCTURE ELUCIDATION

Kirk R. Gustafson,[1]*Dennis J. Milanowski,[1] Naoya Oku,[1] Laura K. Cartner,[1,2] Heidi R. Bokesch,[1,2] R. Thomas Williamson,[3] Josep Saurí,[3] Yizhou Liu,[3] Yuanqing Ding,[4] Xing-Cong Li,[4] Daneel Ferreira,[4] Larry A. Walker,[4] Gary E. Martin.[3]*
[1]Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702-1201, USA, [2]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702-1201, USA, [3]NMR Structure Elucidation, Process Research and Development, Merck & Co. Inc., Rahway, New Jersey 07065, USA, [4]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, Oxford, Mississippi 38655, USA

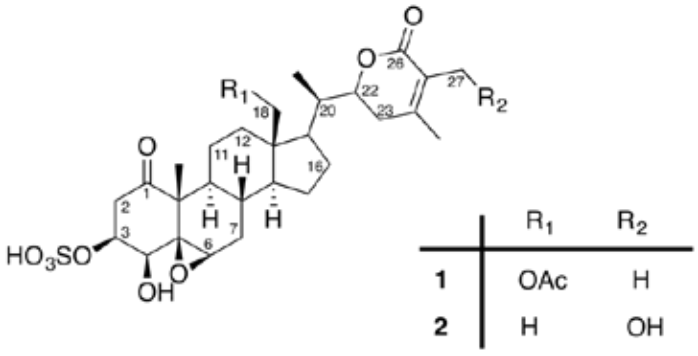
Two novel heterocyclic metabolites, named caulamidines A (**1**) and B (**2**), were isolated from the marine bryozoan *Caulibugula intermis*. These halogenated alkaloids have an intriguing new heterocyclic scaffold and we employed a variety of contemporary NMR techniques to secure their structures. In addition to an array of standard 1D and 2D experiments, new NMR pulse sequences such as LR-HSQMBC, 1,1-HD-ADEQUATE, and HSQMBC-TOCSY provided additional heteronuclear correlation data to define the carbon and nitrogen framework. New band-selective HSQC and HSQMBC experiments, which provided visualization of the $^{35/37}$Cl isotopic effect on Cl-substituted carbons, facilitated halogen assignments. The relative and absolute configuration of these metabolites was established by NOE and ECD analysis. 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. We utilized RDC and RCSA measurements to confirm the structures of the new alkaloids. The application of these new NMR techniques in the structural elucidation of compounds with a novel molecular framework and the potential utility of these experiments for future studies will be presented.

P-080

BIOASSAY-GUIDED ISOLATION OF POTENT ANTI-INFLAMATORY O-SULFATED WITHANOLIDES FROM ACNISTUS ARBORESCENS SCHLTDL.

Juan J. Araya[1], Alfonso García-Piñeres[1], Randall Loaiza[2,3], Clara Schmoelder[4]
[1]Department of Chemistry, University of Costa Rica, San Pedro, Costa Rica 11501-2060. [2]Department of Pharmacy, University of Costa Rica, San Pedro, Costa Rica 11507-2060. [3]Centro de Innovaciones Biotecnológicas, San José, Costa Rica 1174-1200. [4]Department of Pharmacy, Freiburg University, Germany, 79085

Acnistus arborescens Schtdl. (Solanaceae), a common plant species in South and Central America known as "güitite", has been used in popular medicine for a range of ailments. Previous phytochemical investigations of this species have led to the isolation and description of 27 withanolide-type compounds including withaferin A, withacnistin and withaphysalins. Withanolides, oxygenated 28-carbon ergostane-type steroidal lactones, have been actively investigated because their wide range of biological properties including cytotoxic, anti-proliferative, immunosuppressive and anti-inflammatory activities. The main purpose of this work was to isolate anti-inflammatory metabolites with reduced cytotoxicity in the murine macrophages cell line RAW 264.7. In vitro anti-inflammatory potential was evaluated using inhibition of NO production in LPS-stimulated cell assay. Bioassay-guided fractionation of the organic extract of this species afforded two potent anti-inflammatory sulfated withanolides 1 and 2 showing IC_{50} values of 1.3 ± 0.5 and 1.0 ± 0.2 µM respectively. Their mechanism of action and medicinal potential remain to be investigated.



	R_1	R_2
1	OAc	H
2	H	OH

P-081

ISOLATION AND STRUCTURE ELUCIDATION OF A NEW BIFLAVONOID FROM THE LEAVES OF *OCHNA MAURITIANA*

Godwin A. Dziwornu[1], Arvin Moser[2], Dimitris Argyropoulos[3], Ronnett Seldon[4], Audrey Jordaan[4], Digby F. Warner[5], Minu G. Bhowon[6], Sabina Jhaumeer-Laulloo[6] and Suthananda N. Sunassee[1,7].
[1]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa; [2]Advanced Chemistry Development, Inc., Toronto, ON, Canada; [3]Advanced Chemistry Development UK Ltd., Bracknell, UK; [4]H3D Drug Discovery and Development Centre, Department of Chemistry, University of Cape Town, Rondebosch, 7701, South Africa; [5]MRC/NHLS Molecular Mycobacteriology Research Unit, University of Cape Town, Rondebosch 7701, South Africa; [6]Department of Chemistry, University of Mauritius, Reduit 80837, Mauritius; [7]South African Medical Research Council Drug Discovery and Development Research Unit, University of Cape Town, Rondebosch 7701, South Africa

The plant genus *Ochna* is known to produce a plethora of secondary metabolites, of which the most abundant are the structurally diverse ether-linked biflavonoids.[1] *Ochna mauritiana* is a small tree endemic to the island of Mauritius (Indian Ocean).[2] As part of a phytochemical investigation, we have isolated a new biflavonoid molecule, along with three known compounds, from an ethyl acetate extract prepared from the leaves of *O. mauritiana*. The structure of the new biflavonoid compound was unequivocally assigned using a combination of computer assisted structure elucidation (CASE) and band-selective 2D NMR experiments. All four compounds showed moderate to weak anti-tubercular activity against the H37Rv strain of *Mycobacterium tuberculosis*.

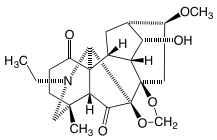
References
1. Bandi, A. K. R.; Lee, D.-U.; Tih, R. G.; Gunasekar, D.; Bodo, B. Chem. Biodivers. 2012, 9, 251-271.
2. Neergheen, V. S.; Bahorun, T.; Jen, L.-S.; Aruoma, O. I. Pharm. Biol. 2007, 45, 9-17.

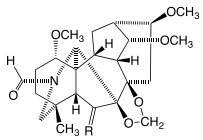
P-082

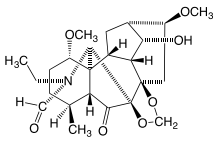
FOUR NEW C_{19}-DITERPENOID ALKALOIDS FROM *DELPHINIUM ELATUM*

Megumi Katoh, Akane Kokubun, Ayano Uchimura, Sakina Mikami, Ayana Takeuchi, Hiroshi Yamashita, Koji Wada.
Medicinal Chemistry, School of Pharmacy, Hokkaido Pharmaceutical University, 7-15-4-1, Maeda, Teine, Sapporo 006-8590, Japan

Four new C_{19}-diterpenoid alkaloids, elapacigine (**1**), *N*-deethyl-*N*-formylpaciline (**2**), *N*-deethyl-*N*-formylpacinine (**3**), and *N*-formyl-4,19-secoyunnadelphinine (**4**), have been isolated from *Delphinium elatum* cv. Pacific Giant together with eleven known C_{19}-diterpenoid alkaloids, delpheline, isodelpheline, 19-oxoisodelpheline, melpheline, pacidine, pacinine, yunnadelphinine, eladine, 6-dehydroeladine, laxicyminine, and delcorine. The structures of these alkaloids were determined by their ms, 1D and 2D-nmr data.


1


2: R = α-OCH3
3: R = O


4

P-083

VOLATILE ALKALOIDS FROM THE LEAVES OF *CARYOTA MITIS* L. BY LC-MS/MS ANALYSIS

Daoud W. Bishay[1], Afaf M. Abdel Baky[1], Ahmed M. Zaher[1,2] and Islam A. Abdelhakim[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt
[2]Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, USA

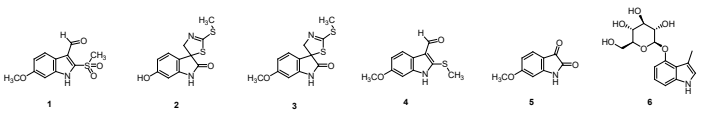
The pure alkaloidal fraction of the leaves of the plant was subjected to LC separation. Extractive ion chromatogram showed 8 different peaks that were identified. Identification and structure elucidation was achieved on basis of accurate mass measurement, molecular formulae, double bond equivalent (DBE), MS/MS fragmentation and comparison with previously available data. These alkaloids are nicotine (**1**), methyl-N-methyl piperidine-3-carboxylate (**2**), propyl-N-methyl piperidine-3-carboxylate (**3**), ethyl-N-methyl piperidine-3-carboxylate (**4**), guvacoline (**5**), ethyl-N-metyl-1,2,5,6-tetrahydropyridine-3-carboxylate (**6**), arecoline (**7**) and ethyl nicotinate (**8**). These 8 alkaloids were reported in *Caryoto mitis* for the first time. The alkaloid fraction showed antimicrobial activity against gram +ve bacteria.

P-084

INDOLE ALKALOIDS FROM *CAPPARIS HERBACEA* AND THEIR BIOLOGICAL ACTIVITY EVALUATION

Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Jiwon Baek, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Capparis herbacea (Cappariceae) is a perennial shrub distributed around the Mediterranean area and partial region of Central Asia and Middle East. The flower buds and young fruits, also known as capers, are used as seasoning or garnish. It is renowned as a spice, but the chemical investigation is much lacking. Phytochemical investigation of the methanol extract of *C. herbacea* (roots and fruits) was carried out and resulted in the isolation of two new indole alkaloids (**1** and **2**) along with four known compounds (**3**-**6**). Compounds **1**, **3**, and **4** were isolated from the root extract and compounds **2**, **5**, and **6** were isolated from the fruit extract. The chemical structures were elucidated with analyzing the spectroscopic data, as well as extensive 1D and 2D NMR experiments and LC/MS analysis.

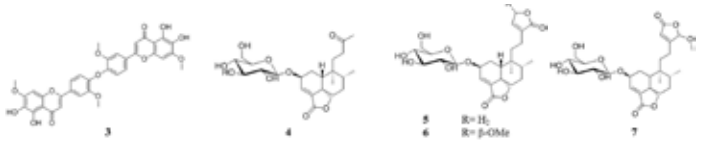


P-085

NEW α-GLUCOSIDASE INHIBITORS FROM *SALVIA CIRCINATA*.

Laura Cecilia Flores-Bocanegra, Martín González-Andrade and Rachel Mata.
Universidad Nacional Autónoma de México, Ciudad de México, 04510, México.

Extensive fractionation of an organic extract of the aerial parts of the anti-hyperglycemic species *Salvia circinata* yielded a new biflavone (**3**) and four new neoclerodane diterpenoids glucosides (**4**–**7**) along with the known compounds amarisolide (**1**), pedalitin (**2**), apigenin-7-*O*-β-D-glucoside (**8**) and flavone 2-(3,4-dimethoxyphenyl)-5,6-dihydroxy-7-methoxy-4*H*-chromen-4-one (**9**). The new compounds were given the trivial names of 6,6",3"'-trihydroxy-7,3',7"-*O*-trimethyl loniflavone (**3**) and amarisolides B–E (**4**–**7**), respectively. The chemical structures of new compounds were elucidated using HRESIMS, 1D and 2D NMR techniques. The absolute configuration at the stereogenic centers of compounds **4**–**7** was established by comparing experimental and theoretical ECD spectra.

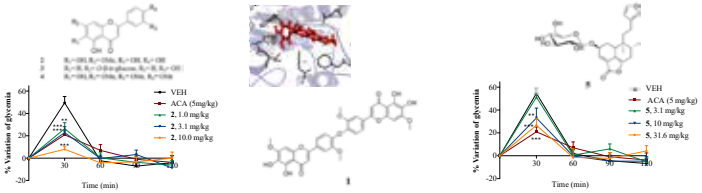


P-086

ANTIHYPERGLYCEMIC POTENTIAL OF *SALVIA CIRCINATA*

Laura Cecilia Flores-Bocanegra, Martín González-Andrade and Rachel Mata.
Universidad Nacional Autónoma de México, Ciudad de México, 04510, México.

An infusion (IS) prepared from the aerial parts of the medicinal plant *Salvia circinata* (Lamiaceae) possessed antihyperglycemic action *in vivo* during an oral sucrose tolerance test (OSTT, 31.6–316 mg/kg), suggesting the presence of α-glucosidase inhibitors in *S. circinata*. Compounds **1**-**5** isolated from the plant were active against mammal α-glucosidase (IC_{50} = 39, 810, 200, 1800 and 500 μM, respectively vs acarbose IC_{50} = 100 μM). Compounds **2** and **5** were also active against a recombinant α-glucosidase from *Ruminococcus obeum* and reduced significantly the postprandial peak during an OSTT in healthy mice. Molecular docking and dynamic studies revealed that compounds **2** and **5** might bind to a-glucosidases at the catalytic center of the enzyme.



P-087

BIOLOGICAL ACTIVITY OF COMPOUNDS FROM *GARCINIA DAEDALANTHERA* PIERRE

Roshamur C. Forestrania[1,2], Ulyana Munoz-Acuña[1,2], Berna Elya[3], and Esperanza J. Carcache de Blanco[1,2]
[1]Division of Pharmacy Practice and Science, [2]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH 43210, and [3]Faculty of Pharmacy, University of Indonesia, Depok, Indonesia 16424.

Garcinia daedalanthera Pierre (Cluseaceae), collected in Indonesia, is an evergreen tree distributed in Asia and Africa [1]. Currently, plants in this genus attract many researchers and pharmaceutical industries due to its hypolipidemic, antioxidant, apoptotic, anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, anti-fungal, anti-ulcer, and anti-protozoal activities [1,2]. Hence, the present study aims to isolate compounds from *G. daedalanthera* Pierre for their cytotoxic potential against MDA-MB-231 triple negative breast and PC-3 prostate cancer cell lines. Briefly, the stem bark of *G. daedalanthera* Pierre was macerated in *n*-hexane and ethyl acetate. The IC_{50} values of hexane and ethyl acetate extracts against MDA-MB-231 were 4.1 and 0.16 μg/mL. The ethyl acetate extract was deemed active at 20 μg/mL on PC-3 cancer cells with 53 % inhibition. Hexane and ethyl acetate extracts of this plant also showed 81% and 92% inhibition in an *in vitro* PARP-1 assay. Active secondary metabolites isolated from this plant species are being identified using spectroscopic techniques such as 1D- and 2D-NMR and absolute configuration will be pursued using MS, Mosher ester reaction, and X-ray crystallography.

References: [1] Hemshekhar M, Sunitha K, *et al*. (2011) Phyto Chem, 10:325-351, [2] Li G, Thomas S, et al. (2013) Ethnopharmacology, 4(80):1-4.

P-088

TOXICOLOGICAL EVALUATION OF *CITRULLUS MUCOSOSPERMUS* (FURSA) FRUIT IN WISTAR RATS.

Ajayi, Temitayo Olayemi[1], Moody Jones Olanrewaju[1], Akintayo, Christopher Oloruntoba[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan. Nigeria [2]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University. Ado-Ekiti, Nigeria.

Citrullus mucosospermus (Fursa.) is popularly known as "Egusi melon" in Southwest Nigeria. It is a pale yellow green and bitter fruit unlike the common red juicy melon *(Citrullus lanatus* subsp. *lanatus)*. Several investigations have been carried out on the whole plant of other species but there are in contrast few references to its toxicity profile. Hence, this study evaluated the toxicity of the fruit pulp of *Citrullus mucosospermus* and the effects on

vital organs of Wistar rats with reference to the biochemical, haematological and histopathological indices.

Daily doses of 100, 200, 400 and 1000 mg/kg body weight methanol extract of *Citrullus mucosospermus* fruit pulp were administered orally to 5 groups of 5 rats per group each for 28 days including the control group. The indices were evaluated by standard methods. Data were analyzed using one way analysis of variance and statistically significant difference was considered at $p<0.05$, $p< 0.01$ and $p<0.001$.

Changes were observed in the heart, liver, lungs, spleen and kidneys of rats treated with the extracts at all doses tested. A significant increase ($p<0.05$) in neutrophil, an insignificant decrease in white blood cell (WBC) and platelet were observed at doses 200, 400 and 1000 mg/kg. A significant decrease in aspartate amino transferase (AST), alanine amino transaminase (ALT) and alkaline phosphatase, (ALP) were observed at all doses tested. These results suggest that the fruit pulp extracts of *C. mucosospermus* are safe at the doses tested. They could also boost immunity and offer hepatoprotective effects.

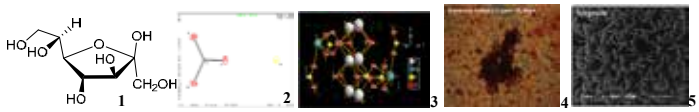
P-089

SEDUM OXYPETALUM. CHEMICAL CHARACTERIZATION AND OSTEOGENIC DIFFERENTIATION BY HUMAN PERIODONTAL LIGAMENT-DERIVED CELLS

Gonzalo R. Lara-Issasi[1], Maria I. Aguilar[1], Higinio Arzate[2], Argelia Almaguer-Flores[2], Nitzine E. Ocampo-Avila[3], Lia Hoz-Rodriguez[2] and Rodrigo Correa-Prado[2]

[1]Pharmacy Department, School of Chemistry, UNAM, Mexico 04510. [2]School of Dentistry, Division of Postgraduate Studies and Research, UNAM, Mexico 04510. [3]Institute of Physics, National Science Laboratory for Research and Preservation of the Cultural Heritage, UNAM, Mexico 04510.

The medicinal plant *Sedum oxypetalum* Kunth (Crassulaceae), is used for the treatment of periodontal disease. The aim of this work was to prove such claim. The aerial parts of the plant were analyzed, their aqueous and organic extracts were chemically characterized as: sedoheptulose (**1**), aragonite (**2**) and syngenite (**3**) in the aqueous extract (AE). The AE was biologically tested and proved to have antimicrobial, ROS-scavenging and anti-inflammatory (anti COX) effects; additionally, the AE was shown to promote the proliferation and the specific differentiation of Human Gingival Fibroblasts (hGHFs) *in vitro* in a specific medium-independent manner (Alizarin red stain **4**, specific alkaline phosphatase and rtPCR detection of markers) and **3** to promote the nucleation of hydroxyapatite crystals *in vitro* **5**.



P-090

A REVIEW OF FOREST MEDICINAL PLANTS PRODUCTION USING TISSUE CULTURE TECHNIQUES AND ITS CURRENT STATUS IN KOREA

Chanhoon An, Jongyun Kim, Hojun Son, Jeong Ho Song, Mahn-Jo Kim. Forest Medicinal Resources Research Center, National Institute of Forest Science, Yeongju 36040

Mass production of forest medicinal plant materials has contributed to commercialize natural products. For plantlet quality in mass production of forest medicinal plants is related to quality control of raw medicinal materials. Plant tissue culture is an important technology to produce high-quality plant materials. Numerous factors are reported to influence the success of *in-vitro* regeneration of medicinal plants. Embryogenesis is known to be the most effective techniques and it has developed in some medicinal plant species. Various *in-vitro* cultural condition for direct and/or indirect somatic embryogenesis systems have developed in *Epimedium koreaum*, *Bupleurum falcatum*, *Paeonia lactiflora*, *Chrysanthemum zawadskii*, *Houttuynia cordata* etc. However, some species including *Paeonia lactiflora* had not been reported to grow in soil. In some species, the acclimation system to soil will have to be improved, and it is able to apply as an efficient system for mass propagation system of medicinal plant raw materials.

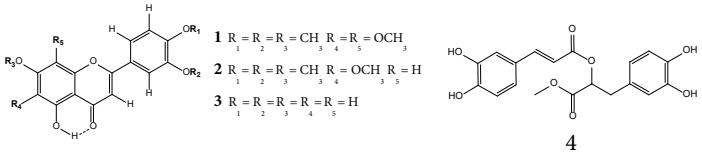
P-091

SECONDARY METABOLITES FROM THE ANTIDIABETIC LEAF INFUSION OF *OCIMUM CAMPECHIANUM*

Javier A. Ruiz-Vargas, Fabiola Escalante-Erosa and Luis M. Peña-Rodríguez Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, México

The leaf infusion of *Ocimum campechianum* Mill. (Lamiaceae) has traditionally been used to control diabetes. Recent evaluations have shown that that this traditional preparation has a strong antidiabetic activity when tested in the inhibition of alpha-glucosidases assay.

In this study, the leaf infusion of *O. campechianum* was partitioned between hexane, dichloromethane and ethyl acetate to produce the corresponding low, medium-low and medium-high polarity fractions. Column chromatographic purifications of these fractions resulted in the isolation of two nobiletin-derived polymethoxyflavones (**1**, **2**), the polyhydroxylated flavone luteolin (**3**) and the rosmarinic acid methyl ester (**4**). The structures of the purified metabolites were established on the basis of their ^{1}H and ^{13}C-NMR spectroscopic data, and the results of their GC-MS analyses.



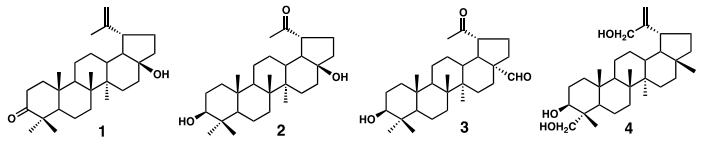
P-092

FOUR NEW TRITERPENOIDS FROM EUONYUMUS 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]

[1]Hokkaido Pharmaceutical University, Sapporo, Hokkaido 006-8590, Japan; [2]Showa College of Pharmaceutical Sciences, Machida Tokyo 194-8543, Japan; [3]Tukuba Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Tukuba, 305-0843, Japan

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. Four new lupane-type triterpenoid, 17β-hydroxy-28-norlup-20(29)-en-3-one (1), 3β,17β–dihydroxy-28,30-bisnorlupan-20-one (2), 3β-hydroxy-20-oxo-30-norlupan-28-al (3) and lup-20(29)-ene-3,23,30-triol (4) were isolated along with twelve known lupane derivatives, lupeol, lupenone, betulin, betulon, 30-oxo-lupeol, (20*S*)-3-hydroxylupan-30-al, (20*R*)-3-hydroxy-lupan-29-al, 30-hydroxylup-20(29)-en-3-one, lup-20(29)-ene-3,30-diol, 3-hydroxy-30-norlupan-20-one, 3,30-dihydroxylup-20(29)-en-28-al and lup-20(29)-en-3,28,30-triol from an ethanol extract of the dried bark of *E. alatus.* These known compounds were found in this plant for the first time.



P-093

IRIDOID GLYCOSIDES FROM THE TWIGS OF *SAMBUCUS WILLIAMSII* AND THEIR BIOLOGICAL ACTIVITIES

Won Se Suh, Chung Sub Kim, Kyoung Jin Park, Joon Min Cha, Tae Hyun Lee, Dong Hyun Kim and Kang Ro Lee
Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

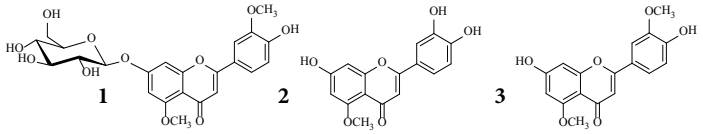
Six new iridoid glycosides, sambucusides A-F (**1**–**6**), and two known derivatives (**7** and **8**) were isolated from the MeOH extract of the twigs of *Sambucus williamsii*. Their chemical structures were elucidated by spectroscopic methods, including NMR (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY) and HRMS. All isolated compounds (**1**–**8**) were evaluated for their antiproliferative activities against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and Bt549), their effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, and their neuroprotective effect through induction of nerve growth factor (NGF) in C6 glioma cells. Among, these compounds **2**, **3**, and **5** showed significant cytotoxicity (IC_{50} 1.27–8.71 μM) and inhibitory effects on NO production (IC_{50} 0.95, 1.25, and 1.22 μM, respectively), and compounds **2**, **4**, and **8** exhibited potent NGF-releasing effects (146.99 ± 5.81, 158.73 ± 5.18, and 152.62 ± 7.29, respectively).

P-094

HPLC QUANTIFICATION OF 5-METHOXYFLAVONES IN *VOCHYSIA DIVERGENS* ASSOCIATED WITH FUNGAL ENDOPHYTES UNDER HYDRIC STRESS

Letícia P. Pimenta[1], Luis C. K. Filho[1], Bruna A. S. Parpinelli[2], Kátia A. Siqueira[2], Marcos A. Soares[2], Márcio L. A. Silva[1], Wilson R. Cunha[1], Patrícia M. Pauletti[1] and Ana H. Januário[1]
[1]Natural Products Research Group, University of Franca, Franca, SP, 14404-600, Brazil, [2]Department of Botany and Ecology, University Federal de Mato Grosso, Cuiabá, MT, 78060-900, Brazil.

Vochysia divergens Pohl is an invasive species that is expanding throughout Pantanal in Brazil. In this work was describes the development and validation of an HPLC-DAD analytical method to quantify 5-methoxyflavones in methanolic extracts of greenhouse-grown *V. divergens* associated with endophytes *Zopfiella tetraspora* and *Melanconiella elegans* and subjected to water stress. The developed method gave good validation parameters and was successfully applied to quantify the flavones 3′,5-dimethoxy luteolin-7-*O*-β-glucopyranoside (**1**), 5-methoxy luteolin (**2**), and 3′,5-dimethoxy luteolin (**3**) in the target extracts. Therefore, the aerial parts of germinated *V. divergens* plants inoculated with fungi responded differently in terms of the production of flavones. The results can cast light on the symbiosis between fungal microorganisms and *V. divergens*, which most likely influences the response of *V. divergens* to changes in the availability of water in Pantanal.

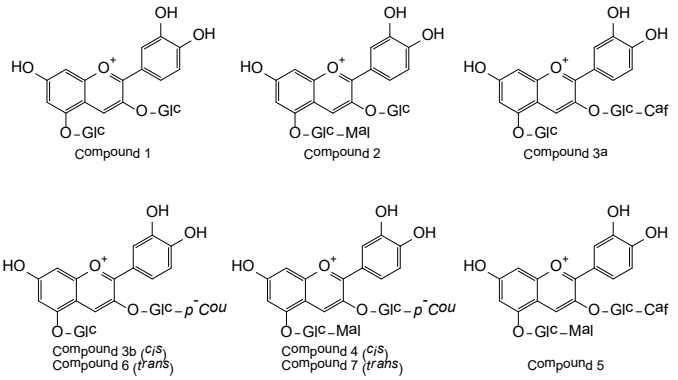


P-095

CHEMICAL SPECIES OF ANTHOCYANINS IN PERILLA PLANTS AND DRIED LEAVES

Yumi Fujiwara[1, 2], Miya Kono[1], Airi Ito[1], Michiho Ito[1]
[1]Department of Pharmacognosy, Graduate School of Pharmaceutical Science, Kyoto University; 46-29 Yoshida-Shimo-Adachi-cho, Sakyo-ku, Kyoto 606-8501, Japan. [2]Department of Pharmacognosy, College of Pharmacy, Kinjo Gakuin University; 2-1723 Ohmori, Moriyama-ku, Nagoya, Aichi 463-8521, Japan.

The reddish color of perilla leaves is due to anthocyanin pigments. Several reports have described the chemical species of anthocyanins in red perilla but a complete analysis of anthocyanins in perilla has not been elucidated. In the present work, the anthocyanins in the leaves of cultivated (green and red perilla) and wild species (*P. citriodora*, *P. setoyensis* and *P. hirtella*) of perilla and those commercially available perilla herbs were studied. Red perilla and most *P. citriodora* strains accumulate compounds 3 to 7. Only one strain of *P. citriodora* comprising compounds 1 to 7 and another strain of *P. citriodora* include compounds 1 and 2. In contrast, most green perilla, *P. setoyensis* and *P. hirtella* samples provided no anthocyanin.

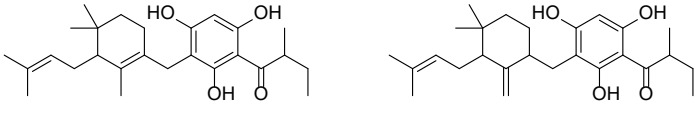


P-096

ANTIPROLIFERATIVE AND ANTIPLASMOIDAL CONSTITUENTS FROM THE ROOTS OF *GARCINIA DAUPHINENSIS*

Rolly G. Fuentes[1,3], Maria B. Cassera[2], Ana Lisa V. Murillo[2], Andriamalala Rakotondrafara[4], Vincent E. Rasamison[5], and David G.I. Kingston[1]
[1]Department of Chemistry and Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061, USA, [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602 USA, [3]Division of Natural Sciences and Mathematics, University of the Philippines Visayas Tacloban College, 6500 Tacloban City, Philippines, [4]Missouri Botanical Garden, BP 3391, Antananarivo 101, Madagascar, [5]Centre National d'Application de Recherches Pharmaceutiques, BP. 702 Antananarivo, Madagascar

The seven new phloroglucinols **1**-**7** together with six known phloroglucinols, δ-tocotrienol, lupeol and a mixture of α- and β-amyrins were isolated from the roots of *Garcinia dauphinensis*, an endemic plant of Madagascar, using various chromatographic techniques. Their structures were elucidated based on their spectroscopic data (NMR, MS, and ECD) and comparison with literature values. Among the compounds tested, **1** and **2** showed the most potent growth inhibitory activity against the A2780 cancer cell line and *Plasmodium falciparum*.

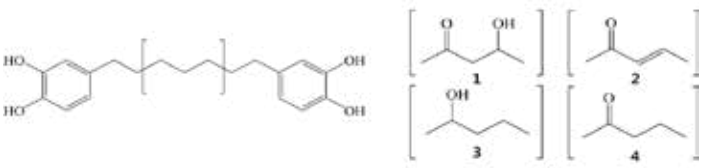


P-097

ANTI-OXIDATIVE AND ANTI-INFLAMMATORY EFFECTS OF PHENOLIC COMPOUNDS FROM BARKS OF *ALNUS SIBIRICA* FITCH. EX TURCZ. PROCESSED ENZYMATIC HYDROLYSIS

Min Won LEE[1], Sung Hye YOON[1], Yoon Jeong HWANG[1], Jun YIN[1], Thi Tam LE[1], Hye Shin AHN[1] and In Hyeok HWANG[1]
[1]Laboratory of Pharmacognosy and Natural Product Derived Medicine, College of Pharmacy, Chung–Ang University, Seoul 156–756, Republic of Korea

The structure elucidation and biological activities of the isolated compounds and extract of *A. sibirica* which were processed enzymatic hydrolysis (EAS) were studied. A chromatographic isolation of EAS yielded hirsutanonol (**1**), hirsutenone (**2**), rubranol (**3**), and muricarpon B (**4**) which are increased aglycone types of diarylheptanoid or newly formed components during the process. EAS showed more potent anti-oxidative and NO production inhibitory activities than AS. In addition, **1**-**4** showed excellent anti-oxidative and NO production inhibitory activities compared with their positive controls. These results suggest that EAS and its isolated compounds might be developed as anti-oxidative and anti-inflammatory agents and enzymatic hydrolysis of natural product is a prominent way to enhance the biological activity of the original plant.

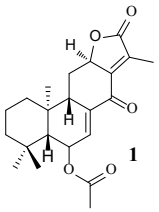


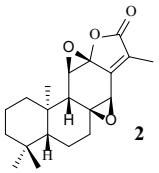
P-098

ISOLATION OF CYTOTOXIC ABIETANE-LACTONE DITERPENOIDS FROM THE *SUREGADA ZANZIBARIENSIS* THROUGH BIOASSAY-GUIDED METHOD

Vuyelwa Jacqueline Tembu[1,2], Mandisa Mangisa[1,2], Gerda Fouche[2], Rudzani Nthambeleni[2] and Moses K Langat[3].
[1]Department of Chemistry, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, RSA, [2]Biosciences, Council for Scientific and Industrial Research, PO Box 395, Pretoria, 0001, RSA, [3]Department of Chemistry, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.

The World Health Organization (WHO) reported that cancer is the second leading cause of death globally, and had claimed over 8.8 million deaths in 2015. Cancer and HIV/AIDS pose a massive threat to the development of South Africa by reducing household incomes and causing many deaths. Most of the cancer drugs and treatments exhibit adverse side effects such as toxicities, therefore the need to develop new alternatives. The rich South African biodiversity could lead to new, affordable and effective anti-cancer leads. Hence, the roots of *Suregada zanzibariensis* were investigated. The dichloromethane extract of the roots of *S. zanzibariensis* was found to be potent. Through bioassay-guided fractionation method, two potent compounds **1** and **2** were isolated. Compounds **1** and **2** were found to exhibit growth inhibition against cancer cell lines, TK10, UACC2 and MCF7. Structures of compounds **1** and **2** (Jolkinolide B) were established on the basis of NMR and other spectroscopic techniques and found to be abietane-lactone diterpenoids.





P-099

BARK ESSENTIAL OILS OF *ZANTHOXYLUM CLAVA-HERCULIS* AND *PTELEA TRIFOLIATA*: ENANTIOMERIC DISTRIBUTION OF MONOTERPENOIDS

Kelly Marie Steinberg, Prabodh Satyal, and William N. Setzer
Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA

Zanthoxylum clava-herculis L., commonly known as toothache tree, Southern prickly-ash, or Hercules's club, is a small deciduous tree, native to southeastern United States. The bark of *Z. clava-herculis* has been used by Native Americans for chronic rheumatism, dyspepsia, dysentery, kidney trouble, heart trouble, colds, coughs, lung ailments, toothache, and nervous debility. *Ptelea trifoliata* L. is commonly known as hoptree or wafer ash. It is a small tree ranging in eastern North America. The wafer-like fruits have been used as a substitute for hops in beer brewing, and the root bark has been used in traditional medicine as an anthelmintic, antibacterial, antiperiodic, stomachic and tonic. The bark essential oils of *Zanthoxylum clava-herculis* and *Ptelea trifoliata* (Rutaceae) were obtained by hydrodistillation and analyzed by both gas chromatography as well as chiral gas chromatography coupled with mass spectrometry. *Z. clava-herculis* bark oil was dominated by sabinene [47.0%, 95% (–)-sabinene], limonene [18.7%, 99% (+)-limonene], and terpinen-4-ol [12.9%, 75% (–)-terpinen-4-ol]. The major components in *P. trifoliata* bark oil were limonene [15.2%, 99% (–)-limonene], sabinene [6.9%, 79% (–)-sabinene], and β-phellandrene [6.2%, 87% (–)-β-phellandrene].

P-100

INHIBITORS OF OAT3 FROM *JUNIPERUS OBLONGA* M. BIEB.

Yilin Qiao[1], Xue Wang[1], Hang Lu[1], Ruicong Ma[1], Youcai Zhang[1], Manana Khutsishvili[2], Vugar N. Kerimov[3], Daniel E. Atha[4] and Robert P. Borris[1]
[1]School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, CHINA. [2]National Herbarium of Georgia, Institute of Botany, Ilia University, Tbilisi, GEORGIA. [3]Institute of Botany, Azerbaijan Academy of Science, Baku, AZERBAIJAN. [4]New York Botanical Garden, Bronx, NY, USA.

Organic anion transporters (OATs) play an important role in the distribution and excretion of numerous endogenous metabolic products and exogenous organic anions, including a host of widely prescribed drugs. The gene for human OAT3 is designated SLC22A8, and the transporter is a member of the solute carrier family of membrane transporters. Studies in mice have found that inhibition of OAT3may also result in lowered blood pressure, raising the possibility that OAT3 inhibitors could be used to treat hypertension [1]. *Juniperus* is a genus composed of evergreen, coniferous shrubs belonging to the Cupressaceae (Cypress family). While *Juniperus oblonga* M. Bieb. does not have an ethnomedical history of use in hypertension, the butanol soluble fraction of a methanol extract of this plant was found to inhibit OAT3 *in vitro*. Bioactivity guided fractionation followed by structure determination based primarily on LC-MS and 1D and 2D-NMR data has led to the identification of two biflavones that are responsible for this

activity. Biological characterization of these compounds *in vitro* and *in vivo* will be presented.

[1] V. Vallon, *et al*., *J. Am. Soc. Nephrol.* (2008) **19**(9) 1732-1740.

P-101

INHIBITORS OF NEUROBLASTOMA FROM *JUNIPERUS OBLONGA* M. BIEB.

Yilin Qiao[1], Micah K. Glasgow[2], Dana-Lynn Koomoa-Lange[2], Ingo Lange[2], Daniel E. Atha[3], Manana Khutsishvili[4], Vugar N. Kerimov[5], and Robert P. Borris[1]

[1]School of Pharmaceutical Science and Technology, Tianjin University, Tianjin CHINA, [2]College of Pharmacy, University of Hawaii at Hilo, Hilo, HI, USA, [3]New York Botanical Garden, Bronx, NY, USA, [4]National Herbarium of Georgia, Institute of Botany, Ilia State University, Tbilisi, GEORGIA, [5]Institute of Botany, Azerbaijan Academy of Science, Baku, AZERBAIJAN.

Neuroblastoma (NB), the most common extracranial solid tumor of childhood, derives from neural crest cells of the peripheral sympathetic nervous system. Despite advances in cancer chemotherapy, as much as 50% of NB patients diagnosed with the high-risk form of the disease will be refractory to treatment or experience relapse after treatment [1]. Preliminary studies in which a library of 500 plant extracts was screened for inhibition of NB cells in culture have shown that extracts of *Juniperus oblonga* M. Bieb. (Cupressaceae) not only inhibited proliferation of these cells, but also induced calcium signaling and apoptosis [2]. In the present study, bioactivity guided fractionation followed by structure determination based largely on LC-MS and 1D and 2D-NMR data have allowed identification of the compounds responsible for the antiproliferative effects of this extract.

[1]. Cole, K.A., and Maris, J.M. (2012) Clinical Cancer Research **18**(9), 2423-2428.
[2]. Lange, I., Moschny, J., Kerimov, V., Kutsishvili, M., Atha, D.E., Borris, R.P., and Koomoa-Lange, D.-L. (2015) J. Pharm Pharm. Scien **1**(1) 1-7.

P-102

INHIBITORS OF OAT1 AND OAT3 FROM *JUNCUS EFFUSUS* L.

Xue Li, Ruicong Ma, Xue Wang, Youcai Zhang, and Robert P. Borris
School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, CHINA.

The organic anion transporters (OATs) play key roles in the distribution and excretion of drugs. Specifically, organic anion transporter 1 (OAT1) and 3 (OAT3) play important parts in the renal elimination of a range of substrate molecules. Additionally, OAT3 has been linked to the development of hypertension and both transporters have been linked to influenza A infection. *Juncus effusus* L. (Juncaceae) is a perennial herb found mainly in the wetlands and coastal marshes in southern China. The stem medullae and whole herb have important applications in Traditional Chinese Medicine (TCM), being used as anxiolytic, sedative, antipyretic, antiphlogistic and detumescence agents. In preliminary studies, the dichloromethane soluble fraction of a methanol extract of *J. effusus* elicited mild inhibition of OAT1 and strong inhibition of OAT3 *in vitro*, and markedly altered the pharmacokinetic parameters of the diuretic, furosemide, a known substrate of both transporters, *in vivo*. As part of our continuing efforts to expand the understanding of the inhibition of OATs by phytochemicals in general, and TCM in particular, a bioactivity guided fractionation was performed on this extract, followed by structure determination based primarily on LC-MS and 1D and 2D NMR data, leading to the elucidation of a series of phenanthrene and dihydrophenanthrene derivatives which are responsible for the observed activity.

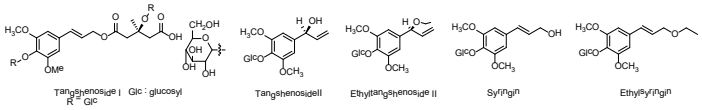
P-103

SECONDARY METABOLITES OF THE ROOTS OF *CODONOPSIS LANCEOLATA*

*Young Eun Du[1], Da Hye Lee[1], Jin Su Lee[1], Jun Lee[2], and Dae Sik Jang[1]**
[1]Department of Life and Nanopharmaceutical Sciences, Graduate School, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Korea [2]Convergence Research Center for Diagnosis, Treatment and Care System of Dementia, Seoul 02792, Korea

Codonopsis lanceolata, one of the *codonopsis* species is a flowering climber which is distributed throughout not only Korea but also Japan and China. Bulked roots of *C. lanceolata* are widely used for medicine or food. *C. lanceolata* has been used for treatment of bronchitis, asthma, cough, tuberculosis, dyspepsia and psychoneurosis.

Repeated chromatography of 50% ethanol extract from the roots of *C. lanceolata* led to the isolation and characterization of one new phenylpropanoid, ethyltangshenoside II. Moreover, four phenylpropanoids, one polyacetylene, three triterpene saponins, four alkaloids and two linear compounds were isolated. The chemical structures of the isolates were identified by using spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR and MS) and by comparison with published data.

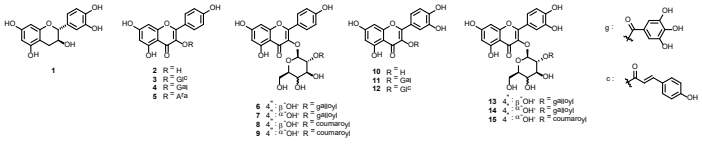


P-104

ISOLATION AND STRUCTURE ELUCIDATION OF A NEW FLAVONOID FROM THE LEAVES OF *DIOSPYROS KAKI*

*Jeong Eun Park[1], Da Hye Lee[1], Jin Su Lee[1], Hak Cheol Kwon[2], Sang Hoon Jung[2], Dae Sik Jang[1]**
[1]Department of Life and Nanopharmaceutical Sciences, Graduate School, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Korea, [2]Natural Product Research Center, Korea Institute of Science and Technology (KIST), Gangneung, 25451, Korea

Persimmon (*Diospyros kaki*) leaves have been traditionally used as a folk medicine for the treatment of angina, hypertension, and some infectious diseases. Phytochemical study on the ethanol extract of the leaves of persimmon resulted in the isolation of a new flavonoid, kaempferol-3-*O*-β-D-2"-coumaroylgalactoside, along with 14 known flavonoids. The structures of compounds were elucidated by physical and spectroscopic data interpretation and by comparison with published data.



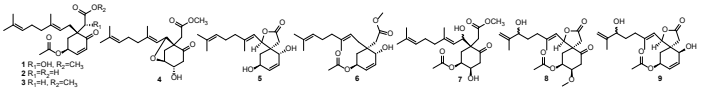
P-105

NEW CYTOTOXIC MILIUSANES FROM *MILIUSA BALANSAE*

Xin-Ya Xu,[1,2] Wen-Hui Pan[1], and Hong-Jie Zhang[1,]*
[1]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, P.R. China, [2]Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, P.R. China

Miliusa balansae (Annonaceae) is an evergreen shrub found in the tropical and sub-tropical regions of Asia. In China, the plant is used as a folk medicine for treatment of gastropathy and glometulonephropathy. Mili-

usanes are a class of characteristic compounds discovered in *Miliusa* sp. They exhibited potent cytotoxicity against various of cancer cells (Zhang, et al. Miliusanes, a class of cytotoxic agents from *Miliusa sinensis*. *J. Med. Chem.*, **2006**, *49*, 693-708.). In our continuing search for anticancer natural compounds, 18 miliusanes including 9 new ones (**1**-**9**) have been identified from the leaves and twigs of *M. balansae*. The compounds demonstrated cytotoxicity against the colorectal cancer cell line HCT116 with IC_{50} values in the range of 4.3-26.9 μM. *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).*

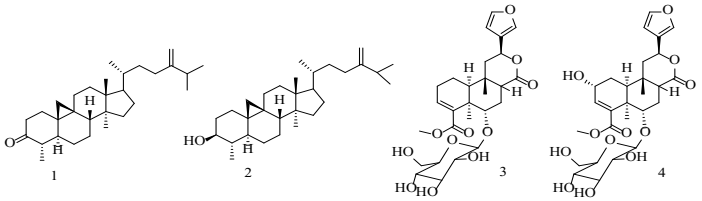


P-106

EXPLORING THE CHEMICAL DIVERSITY OF TWO SPECIES OF *TINOSPORA*

Abidah Parveen[1, 3], Zulfiqar Ali[2], Omer Fantoukh[1], Ikhlas A. Khan[1, 2]
[1]*Department of Biomolecular Sciences, Division of Pharmacognosy,* [2]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS, 38677, USA,* [3]*Abbottabad University of Science & Technology, Havelian, Abbottabad District, Pakistan.*

Tinospora crispa Miers ex Hook.f. & Thomson and *Tinospora sinensis* (Lour.) Merr. (Family Menispermaceae) have similar appearance and the distinction between the two is of critical importance because *T. crispa* has been reported to cause hepatotoxicity. Isolation of secondary metabolites of the two species may aid in exploring the chemical diversity of the two plants and hence help to resolve safety and quality issues. Phytochemical investigation of the stems yielded cycloeucalenone (1), cycloeucalenol (2), borapetoside C (3), and borapetoside B (4) together with several other compounds. The structures of the compounds were elucidated by spectroscopic methods, including 1D and 2D NMR experiments and HRESIMS.

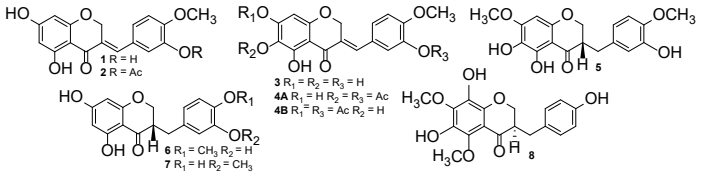


P-107

NOVEL, BIOACTIVE HOMOISOFLAVONOIDS FOR THE TREATMENT OF BLINDNESS CAUSED BY OCULAR NEOVASCULARIZATION

Hannah Whitmore[a], Dulcie A. Mulholland[a], Sianne L. Schwikkard[b], Timothy Corson[c], Walter Knirsch[d], Wolfgang Wetschnig[d]
[a] *Department of Chemistry, University of Surrey, Guildford, GU2 7XH, UK;* [b] *School of Life Sciences, Kingston University, KT1 2EE, UK;* [c] *Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN 46202, U.S.A.;* [d] *Institute of Plant Sciences, Karl-Franzens-University, Graz 8010, Austria.*

Novel homoisoflavonoids **1**, **3**, **5**, **6-8** , were extracted from *Rhodocodon intermedius, R. cryptopodus, R. rotundus* and *Eucomis autumnalis*. The absolute configuration at C-3 of compounds **5-7** was determined by circular dichroism and found to be *S*, unusual for 3-benzyl homoisoflavonoids from the Hyacinthaceae. These compounds, along with acetate derivatives **2**, **4A** and **4B**, have been tested in cell proliferation assays to determine their anti-angiogenic abilities. Compounds which inhibit angiogenesis are of interest in the treatment of eye diseases caused by neovascularization. The structures and biological activity of a selection of novel, known, natural-source and synthetic homoisoflavonoids will be presented.

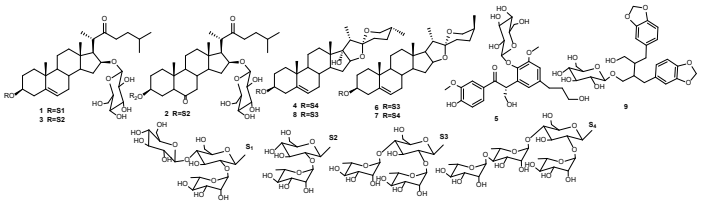


P-108

PHYTOCHEMISTRY OF *PANICUM TURGIDUM* (THE DESERT GRASS IN EGYPT)

Ahmed A. Zaki[1,3], Zulfiqar Ali[3], Yasser A. El-Amier[2], Ikhlas A. Khan[3]
[1]*Pharmacognosy Department, Faculty of Pharmacy,* [2]*Botany Department, Ecology, Faculty of Science, Mansoura University, Mansoura 35516, Egypt.* [3]*National Center for Natural Product Research, University of Mississippi, Oxford, MS 38655, USA*

Panicum turgidum (Poaceae) is a perennial desert grass, occurs in Egypt for many centuries. It is one of the wild plants which used as a supplementary food resource for desert inhabitants in the Middle East. The phytochemical investigation of methanolic extracts of *P. turgidum* resulted in the isolation of phenyl alkanoid glycosides and steroidal saponins (**1**-**9**). The structures of isolated compounds were elucidated on the basis of extensive analyses of spectroscopic data including 1D and 2D NMR and ESI-MS.



P-109

GEOGRAPHICAL AND CLIMATE VARIATIONS OF FLAVONOIDS AND TRITERPENOIDS OF *MICONIA ALBICANS* AND *M. CHAMISSOIS* IN CERRADO.

Valéria M. M. Gimenez[1], Wilson R. Cunha[1], Ana Helena Januário[1], Márcio L. Andrade e Silva[1], Ernane José Xavier da Costa[2], Patrícia M. Pauletti[1]
[1]*University of Franca, São Paulo, Brazil,* [2]*University of São Paulo, São Paulo, Brazil*

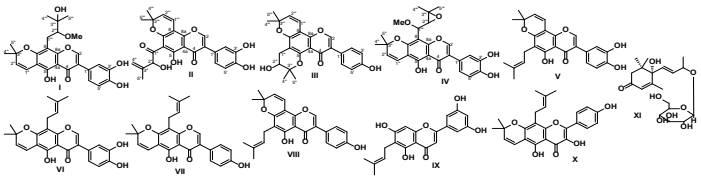
In Brazil, Melastomataceae has a high distribution in the Cerrado, which is considered one of the 34 hotspots worldwide. Biological studies with *Miconia*, its major genus, showed antibiotic, antitumor, analgesic, antimicrobial, and antimalarial activities. The aims of this work were the investigation of the flavonoids and triterpenoids contents between three areas of Cerrado and two climate seasons (dry and wet), using high-performance liquid chromatography coupled to diode array detector (HPLC-DAD) analysis. The leaves of 45 specimens of *Miconia albicans* and *M. chamissois* were collected, extracted with methanol and analyzed by HPLC-DAD in combination with ursolic acid, oleanolic acid, rutin, quercetin, micoside A and micoside B, by two different HPLC conditions. The triterpenoids contents were major in *M. chamissois*, decreasing their concentrations in the wet season of the year. Flavonoids are predominant in *M. albicans* and concentrations increase in the wet period, besides showing the lowest limit of detection (LOD). These data suggested inter and intraspecific variation, also the selected compounds are possible chemical markers of *Miconia*.

P-110

NEW PRENYLATED ISOFLAVONOIDS FROM *MACLURA POMIFERA*

Yerkebulan Orazbekov[1-3], Radhakrishnan Srivedavyasasri[1], Serjan Mombekov[2], Ubaidilla Datkhayev[2], Bauyrzhan Makhatov[3], Samir A.Ross[1,4]*
[1]National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA. [2]Kazakh National Medical University, Almaty, Kazakhstan, [3]South Kazakhstan Pharmaceutical Academy, Kazakhstan. [4]Department of BioMolecular Science, University of Mississippi, University, MS 38677, USA.

Maclura pomifera (Moraceae), is native to Southwestern United States, and known as Osage orange. Various *Maclura* species are worldwide used in folkloric medicine. *M. pomifera* and its components possess several biological activities including cytotoxic, antitumor, antibacterial, estrogenic, antifungal, antiviral and antimalarial activities. Several prenylated flavonoids with potent pharmacological effects were previously reported from *M. pomifera*. Herein, we report the isolation, characterization, and biological evaluation of four new (**I**-**IV**) and nine known compounds (**V**-**XIII**) from the fruits of *M. pomifera* growing in Kazakhstan. The total extract showed significant activity towards cannabinoid receptors (CB1- 103.4, CB2- 68.8 % displacement) and possibly allosteric towards δ and μ opioid receptors (-49.7, and -53.8 % displacement, respectively). Compound **I** exhibited possible allosteric activity in κ and μ opioiod receptors (-88.4, -27.2 % displacement, respectively), and showed moderate activity (60.5% displacement) towards CB1 receptor.

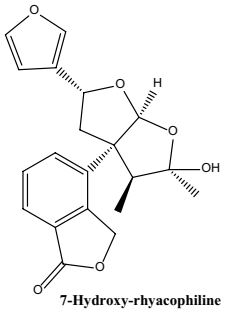


P-111

7-HYDROXYRHYACOPHILINE, A NEW DITERPENOID, AND OTHER DITERPENOIDS FROM *SALVIA REFLEXA* ASSOCIATED WITH HEPATOTOXICITY IN CATTLE.

Dale R. Gardner, Kip E. Panter and Bryan L. Stegelmeier.
USDA-ARS Poisonous Plant Research Laboratory, 1150 E. 1400 N., Logan, Utah, USA

A new diterpenoid, identified as 7-hydroxyrhyacophiline, was isolated along with several other known diterpenoids from contaminated alfalfa hay. Compounds were isolated as part of a bioassay guided fractionation of weedy hay via a mouse hepatotoxic bioassay. The diterpenoids isolated included those compounds identified as salvarin, salvianduline D, and rhyacophine. A new diterpenoid, 7-hydroxyrhyacophiline, was isolated and the structure elucidated by 1D and 2D NMR. The purified diterpenoids were tested in a mouse bioassay and salvarin, salvianduline D and 7-hydroxyrhyacophiline were found to induce sever hepatic nacreous within 48 hours after single oral dosage (~500 mg/kg). At similar dosage levels rhyacophiline was found not to induce the hepatotoxic effects. The identified diterpenoids are known to be found among the different *Salvia* species which led to the identification of dried plant parts (stem and flower pods) of *Salvia reflexa* found among bales of hay. A reexamination of the hay field location found a significant population of *S. reflexa* along the hay field edges and irrigation ditch banks.



7-Hydroxy-rhyacophiline

P-112

TRITERPENES OF THE TWIGS OF *BETULA SCHMIDTII* REGEL AND THEIR BIOLOGICAL ACTIVITIES

Kyoung Jin Park[1], Won Se Suh[1], Joon Min Cha[1], Tae Hyun Lee[1], Dong Hyun Kim[1], Sang Un Choi[2], Kang Ro Lee[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkawn University, Suwon 16419, Republic of Korea, [2]Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea

Betula schmidtii Regel (Betulaceae) is widely distributed in Korea, Japan, and China. This plant source has been used as a Korean traditional medicine for the treatment of stomach disorder. Previous phytochemical research on *B. schmidtii* reported triterpenes, lignans, diarylheptanoids, and flavonoids. Several species in *Betula* genus have been shown to display antioxidant, and cytotoxic activities. Chemical investigation of the MeOH extract of the twigs resulted in the isolation of three new ursane-type triterpenoids (**1**-**3**), along with seven known ones (**4**-**10**). The structures of new compounds (**1**-**3**) were elucidated by spectroscopic methods, including 1D, 2D NMR(^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY) and HR-MS. Cytotoxic study against four cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15) *in vitro* using the SRB bioassay for the isolates (**1**–**10**) are in progress.

P-113

BIOACTIVE LIGNAN DERIVATIVES FROM *ABIES HOLOPHYLLA*

Won Se Suh, Chung Sub Kim, Kyoung Jin Park, and Kang Ro Lee
Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

There are approximately 50 species in the genus *Abies* (Pinaceae) and some have been used in Korean traditional medicine for treating vascular and pulmonary diseases, indigestion, rheumatic diseases, and stomachache. *A. holophylla* Maxim. is a coniferous tree distributed in the mountainous areas of Korea, mainland China, and Russia. Several bioactive monoterpenes, sesquiterpenes, triterpenes, and phenolic compounds have been isolated from this plant. However, few phytochemical and biological investigations on *A. holophylla* have been performed. An extended phytochemical investigation of the trunk of *A. holophylla* afforded four new lignan derivatives (**1**-**4**) together with fifteen known analugues (**5**-**19**). The structures of the new compounds (**1**-**4**) were characterized by extensive NMR methods (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC). The isolates (**1**-**19**) were tested for their cytotoxic activity against A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines in vitro using the SRB bioassay. Also, the effects of the isolated compounds on nitric oxide (NO) levels in lipopolysaccharide (LPS)-stimulated murine microglia BV2 cells were evaluated.

P-114

SECOIRIDOID GLYCOSIDES FROM THE TWIGS OF LIGUSTRUM OBTUSIFOLOUM AND THEIR ANTI-INFLAMMATORY AND NEUROPROTECTIVE ACTIVITIES

Won Se Suh, Oh Kil Kwon, Kyoung Jin Park, Joon Min Cha, Tae Hyun Lee, Dong Hyun Kim and Kang Ro Lee
Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Two new secoiridoid glycosides, obtusifolisides A and B (**1**-**2**), together with 7 known secoiridoid glycosides (**3**-**9**) were isolated from the twigs of *Ligustrum obtusifolium*. The chemical structures of new compounds were determined by a spectroscopic data analysis, including 1D, 2D NMR, HR-MS, and chemical reaction experiments. The isolated secoiridoid glycosides were evaluated for their anti-inflammatory effects in lipopolysaccharide (LPS)-stimulated BV-2 murine microglia cells. Compounds **2**, **5**, **6**, **8**, and **9** significantly reduced the production of NO, with IC_{50} values of 5.45, 11.17, 14.62, 15.45, and 14.96 μM, respectively. None of the compounds were toxic to the cells. Additionally, we evaluated the neuroprotective effects of compounds **1**-**9** on NGF induction in a C6 rat glioma cell line. Compounds **2** and **6** upregulated NGF secretion to 155.56 ± 7.16%, and 139.35 ± 11.65 %, respectively, without significant cell toxicity.

P-115

THREE NEW TRITERPENE GLYCOSIDES FROM THE WHITE FLOWERS OF *IMPATIENS BALSAMINA*

Kyoung Jin Park[1], Chung Sub Kim[1], Won Se Suh[1], Joon Min Cha[1], Tae Hyun Lee[1], Dong Hyun Kim[1], Sang Zin Choi[2] and Kang Ro Lee[1]
[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea, [2]Dong-A ST Research Institute, Kiheung, Yongin 17073, Republic of Korea

Impatiens balsamina L. (Balsaminaceae) is commonly called "Rose Balsam" or "Garden Balsam" and inhabits Korea, China, and India. For centuries, this annual herbaceous plant has been employed as Chinese folk medicine and, in particular, the flowers have been employed for the effective control of dermatitis, lumbago, neuralgia, burns, and scalds. According to published research, the extracts of the *I. balsamina* petals showed antinociceptive, antioxidative, and antitumor activities, and several flavonoids and naphthoquinones were shown to be the predominant constituents exhibiting anti-inflammatory, antipruritic, and anti-anaphylactic activities. As part of the search for bioactive constituents of Korean medicinal plants, we investigated the active constituents of *I. balsamina*. In the present study, we isolated three new triterpene glycosides (**1**-**3**) and six known compounds (**4**-**9**), from the white flower of *I. balsamina*. The structures of the new compounds (**1**-**3**) 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. Isolated compounds (**1**-**9**) were tested for their cytotoxic, anti-inflammatory, and neuroprotective acitivities.

P-116

CHEMICAL CONSTITUENTS FROM *SPIRAEA PRUNIFOLIA* VAR. *SIMPLICIFLORA* AND THEIR BIOLOGICAL ACTIVITIES

Kyoung Jin Park, Chung Sub Kim, Won Se Suh, and Kang Ro Lee
Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

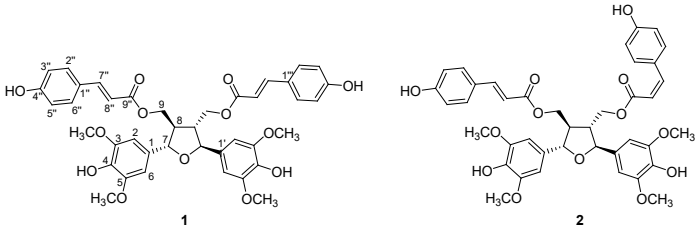
Spiraea prunifolia var. *simpliciflora* Nakai (Rosaceae) is widely distributed in the most parts of Korea. The roots of this plant have been used in Korean traditional medicine to treat malaria, fever, and emetic conditions, whereas its young leaves have been consumed as a salad. As continuing search for bioactive secondary metabolites from Korean medicinal plants, two new phenolic glycosides (**1** and **2**) and 11 known compounds (**3**-**13**) were isolated from the twigs of *S. prunifolia* var. *simpliciflora*. The chemical structures of the new compounds (**1** and **2**) were established using diverse NMR techniques (^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC), HRMS data analysis, and chemical methods. All the purified compounds (**1**-**13**) were evaluated for their cytotoxicity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and BT549), for their anti-inflammatory activity through the measurement of nitric oxide (NO) production levels in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cell line, and for their neuroprotective effects via induction of nerve growth factor (NGF) in C6 glioma cells.

P-117

NOVEL NATURAL PRODUCTS FROM THE ROOTS OF *ORYZA SATIVA* THAT CONTROL ADIPOCYTE AND OSTEOBLAST DIFFERENTIATION

Tae Kyoung Lee, Seoung Rak Lee, Jiwon Baek, Seulah Lee, Jae Sik Yu, Sil Kim, Hae Min So, Dahae Lee, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Oryza sativa L. roots have been used as a traditional medical supplement for protection of the stomach and lungs. We found that the ethanol extract of the roots of *O. sativa* reciprocally regulate adipocyte and osteoblast differentiation. Chemical analysis of the ethanol extract using a bioactivity-guided isolation led to the isolation and identification of two novel lignans responsible for these regulatory activities, oryzativols A and B. Treatment with oryzativol A in the human mesenchymal cell line C3H10T1/2 suppressed gene expression of peroxisome proliferator activated receptor γ (PPARγ), resulting in reduction in adipogenesis. Simultaneously, oryzativol A enhanced the expression of *Runx2* and cellular differentiation into osteoblasts in the same mesenchymal stem cell line.



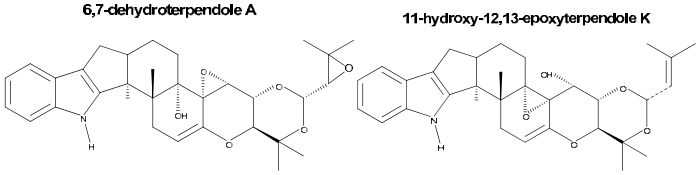
P-118

11-HYDROXY-12,13-EPOXYTERPENDOLE K AND 6,7-DEHYDROTERPENDOLE A: NEW INDOLE DITERPENES FROM *IPOMOEA ASARIFOLIA* AND *IPOMOEA MUELLERI*.

Stephen T. Lee, Dale R. Gardner, and Daniel Cook.
USDA-ARS Poisonous Plant Research Laboratory, 1150 E. 1400 N., Logan, Utah, USA

Ipomoea asarifolia has been associated with a tremorgenic syndrome in livestock in Brazil and was recently reported to contain tremorgenic indole diterpenes. *I. muelleri* has been reported to cause a similar tremorgenic syndrome in livestock in Australia. *I. asarifolia* and *I. muelleri* were investigated by HPLC-HRMS and HPLC-MS/MS for indole diterpene composition. The high resolution mass spectral data in combination with MS/MS fragmentation mass spectral data was used to tentatively identify a number of indole diterpene alkaloids. The previous report of indole diterpenes in *I. asarifolia* was confirmed and the presence of indole diterpenes in *I. muelleri* was found for the first time. The presence of terpendole K and terpendole E in *I. asarifolia* was unequivocally demonstrated by comparison with known standards or by NMR. In addition, two new indole diterpenes were

isolated and their structures determined by 1D and 2D NMR spectroscopy and given the names of 11-hydroxy-12,13-epoxyterpendole K and 6,7-dehydroterpendole A.



P-119

BIOTRANSFORMATION OF *DIOSCOREA NIPPONICA* BY INTESTINAL MICROFLORA AND CARDIOPROTECTIVE EFFECTS OF DIOSGENIN

Tao Yi, and Yina Tang
School of Chinese Medicine, Hong Kong Baptist University, Kowloon, Hong Kong Special Administrative Region, China

Biotransformation of natural products by intestinal microflora is an important approach to identify the active components generated by metabolic activation for drug development. In order to discover the active constituents of the medicinal plant *Dioscorea nipponica* (DN) and explore the therapeutic mechanism of DN in the treatment of myocardial ischemia (MI), biotransformation by intestinal microflora was used to screen for the newly generated metabolites, and the therapeutic effects of the metabolites on myocardial antioxidant levels were adopted to evaluate the cardioprotective efficacy of the metabolites for rat MI in the present study. Our results demonstrated that diosgenin was the main metabolite of DN by rat intestinal microflora, and diosgenin protected the myocardium against ischemic insult through increasing enzymatic and nonenzymatic antioxidant levels *in vivo* (SOD, CAT, GPx and T-AOC), and decreasing the oxidative stress damage caused by MI, thereby explaining the clinical efficacy of DN as anti-MI drugs. This work was supported by the Faculty Research Grant of Hong Kong Baptist University (FRG2/15-16/022).

P-120

AN INDUSTRY APPROACH TO ASSESSING THE SAFETY OF BOTANICALS

Karen M. VanderMolen[1], Jason G. Little[2], Catherine Mahony[3]
[1]The Procter & Gamble Company, 6100 Center Hill Ave, Cincinnati OH 45224, USA. [2]The Procter & Gamble Company, 8700 Mason Montgomery Rd, Mason OH 45040, USA. [3]Procter and Gamble Company Technical Centres Ltd, Egham, Surrey, TW20 9NW, United Kingdom

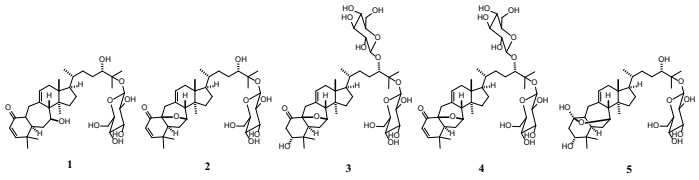
As complex mixtures, botanicals present unique challenges when assessing safe use, particularly when traditional toxicological studies are not available in the literature. Obtaining data for developmental and reproductive toxicity can be particularly difficult. Presented here is an end-to-end, weight of evidence approach to botanical/natural product safety. Assessment begins with an understanding of the history and context of safe use, accurate identity, and constituent-based analysis of the botanical ingredient. The use of *in silico* tools allows us to identify suspected toxicants and assess the potential for deleterious effects using structure activity relationships. Plausible mechanisms can then be tested using *in vitro* models (e.g., receptor binding assays). Using a combination of literature data, *in silico*, and *in vitro* tools can provide assurance of safety while reducing the use of animals.

P-121

NEW TRITERPENE GLYCOSIDES FROM *SUTHERLANDIA FRUTESENCE*

Ahmed A. Zaki[1], Zulfiqar Ali[1], Xing-Cong Li[1], Ikhlas A. Khan[1,2]
[1]National Center for Natural Product Research. [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA.

Sutherlandia frutescens or cancer-bush belongs to family Fabaceae. It is a shrub native to South Africa, Namibia, Botswana and Zimbabwe. In traditional medicine it is known as a medicine for treatment of various diseases. The safety of *Sutherlandia* was confirmed through a long term use as a traditional medicine with no report of toxicity and the study of acute toxicity of *Sutherlandia* aqueous extract and it passed three clinical trials. Furthermore, double-blind study in 2007 revealed that capsules of *Sutherlandia* leaves powder can be tolerated by healthy adults with a daily dose of 800 mg for 3 months. The importance of *Sutherlandia frutescens* in folkloric medicine lead us for further investigation of phytochemistry of the methanolic extract, which resulted in isolation of five new 9,10-seco-9,19-cyclolanostane glycosides (**1**-**5**). The structures of these compounds were confirmed through 1D and 2D NMR and HRESIMS data.

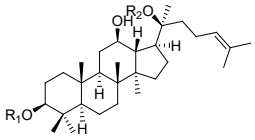


P-122

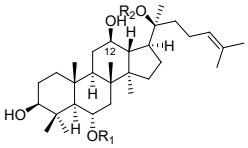
KOREAN RED GINSENG INDUCING APOPTOSIS IN HUMAN LUNG CANCER AND ITS CYTOTOXIC CHEMICAL CONSTITUENTS

Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Jiwon Baek, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Panax ginseng C. A. Meyer, also known as ginseng, was used as a traditional medicine for centuries and among those therapeutic treatments, the steamed and dried root of the ginseng, known as the Korean Red Ginseng (KRG), is known to contribute a variety of pharmacological activities such as anti-inflammatory and antioxidant effects. The refluxed water extract of KRG exhibited significant anti-cancer activity in human lung cancer cell lines. Chemical investigation of the methanol extract of Korean Red Ginseng revealed the isolation of six ginsenosides (**1**-**6**). These isolated constituents were further tested for anticancer activities.



1: R_1 = Glc^2-Glc, R_2 = Glc^6-Glc
2: R_1 = Glc^2-Glc, R_2 = Glc^6-Ara(p)
3: R_1 = Glc^2-Glc, R_2 = Glc^6-Ara(f)
4: R_1 = Glc^2-Glc, R_2 = Glc
5: R_1 = Glc^2-Glc, R_2 = H



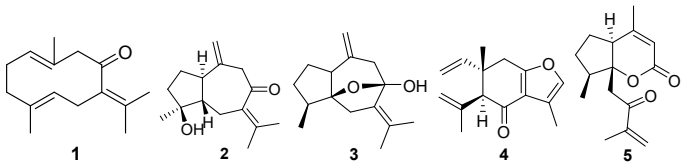
6: R_1 = Glc, R_2 = Glc

P-123

CYTOTOXIC AND ANTI-INFLAMMATORY SESQUITERPENES FROM *CURCUMA ZEDOARIA* ROSC AND THE STUDY OF THEIR MOLECULAR MECHAMISM

Tae Kyoung Lee, Seoung Rak Lee, Jiwon Baek, Seulah Lee, Jae Sik Yu, Sil Kim, Hae Min So, Dahae Lee, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Curcuma zedoaria Rosc (Zingiberaceae) is a kind of turmeric known as zedoary or white tumeric in common. It has been used as ancient spice in India and Indonesia. In East Asia, it is also treated as s traditional medicine for digestive, antidote and circulation. Chemical investigation of the methanol extract of *Curcuma zedoaria* led to the isolation of five sesquiterpenes (**1**-**5**) from the hexane-soluble fraction where cytotoxic effect against AGS human gastric carcinoma cells and anti-inflammatory activity were observed. Among the isolates, curcuzedoalide (**5**) showed the strongest effects of gastric cancer cell death and NO reducing ability. Finally, western blot analysis was performed to identify the mechanism of the active compound on apoptosis and the anti-inflammatory action.



P-124

NYMPHAEA ODORATA EXTRACTS ARE CYTOTOXIC TO THE GASTRIC CANCER CELL LINES AGS AND NCI-N87: CORRELATION TO *HELICOBACTER PYLORI* INFECTION

Nishikant A. Raut[1,2], Temitope O. Lawal[2,3], Shital Patel[2], Gail B. Mahady[2]
[1]Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, India.. [2]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, USA. [3]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

Nymphaea odorata Aiton (Nymphaeaceae; American white water-lily) is an aquatic perennial plant root used in First Nation, Ayurvedic and Chinese traditional medicine for the treatment of various ailments. The dried root and rhizome of the white water lily are used orally for the treatment of gastrointestinal, genital, and bronchial diseases. The leaves and roots have also been used externally, as infusions to treat lesions and inflammation associated with mucous membranes. In this work we have investigated the effects of extracts of *N. odorata* on the growth of *Helicobacter pylori (HP),* the etiologic agent for gastritis, peptic ulcer, gastric MALT lymphoma and gastric cancer, as well as the effects of the active extract on gastric cancer cells *in vitro*. In 17 clinical strains of HP and the ATCC strain 43504, a methanol extract of *N. odorata* inhibited the growth of all HP strains at a concentration of 12.5 µg/ml, with an MIC of 9.25 µg/ml. The second part of the study, the effect of the methanol extract of roots of *N. odorata* was investigated in two gastric cell lines namely, AGS and NCI-N87 at concentrations up to 100 µg/mL. Control cells were treated with vehicle solvent (DMSO 0.02%). Cytotoxicity and cell viability was determined using the CellTiter-Glo® 2.0 assay. The IC_{50} concentration of methanol extract of *N. odorata* root was found to be 26.79 µg/mL in AGS and 35.44 µg/mL in NCI-N87.The results of this study suggest that extracts of *N. odorata* that inhibit the growth of HP also inhibit the growth of gastric cancer cells and may provide a novel mechanism of action for gastric cancer.

P-125

HEPATOPROTECTIVE ACTIVITY OF SEBATIN, A STANDARDIZED BLEND OF MYRISTICA FRAGRANS, ASTRAGALUS MEMBRANACEUS, AND PORIA COCOS

Ping Jiao, Mesfin Yimam, Mei Hong, Qi Jia
Unigen USA, 3005 1st Avenue, Seattle, WA 98121, USA

Traditional herbal medicines have been reported with potent antioxidant properties and could provide significant hepatoprotective activity against liver injuries. An herbal combination Sebatin, comprising three extracts from *Myristica fragrans*, *Astragalus membranaceus*, and *Poria cocos*, was developed for liver protection and evaluated in three different acute liver toxicity models in mice induced by acetaminophen (APAP), carbon tetrachloride (CCl4) and ethanol separately. In APAP and CCl4-induced acute liver injuries model in mice, hepatic functional tests from serum collected at T24, histopathology analysis, and merit of blending three standardized extracts were evaluated in this study with Sebatin administered at doses of 150–400 mg/kg. In alcohol-induced acute hepatotoxicity model, mice received oral doses of Sebatin at 300 mg/kg for four consecutive days. Mice were orally gavaged with 50% ethanol in 12 mL/kg dosing volume following the third dose of Sebatin every 12 h thereafter for a total of three doses. Hepatic functional tests from serum collected at T12, and hepatic glutathione (GSH), superoxide dismutases (SODs), and triglyceride from liver homogenates were evaluated. Histopathology analysis and alcoholic steatohepatitis (ASH) scoring were also determined. Detailed biochemical and histological results and discussions from three animal studies will be presented.

These studies showed that the composition Sebatin could be potentially utilized as an effective hepatic detoxifying agent for the protection of liver damage.

P-126

METABOLOMIC ANALYSIS OF COMMERCIAL CRANBERRY SUPPLEMENTS

Catherine Neto[1], John Turbitt[1], Brian Killday[2] and Kim Colson[2]
[1]Department of Chemistry and Biochemistry, UMass Cranberry Health Research Center, UMass Dartmouth, North Dartmouth, MA 02747 USA, [2]Bruker BioSpin, Billerica, MA 01821 USA

The potential health benefits of cranberries *(Vaccinium macrocarpon)* can be attributed to a variety of secondary metabolites, including proanthocyanidins (PACs), flavonoids, triterpenoids, and organic acids. Commercial cranberry supplements can provide a low-sugar alternative to juices and sweetened fruit products, however the phytochemical content can be expected to vary due to widely differing manufacturing processes. Selected commercial cranberry supplements were analyzed for secondary metabolite profile in comparison to a whole cranberry powder reference standard material, using ^{1}H qNMR with Bruker AssureNMR software. HPLC-DAD and the DMAC assay were employed for total anthocyanin and PAC content respectively. Principal component analysis of ^{1}H NMR spectra showed overlap between several supplements and whole cranberry powder, whereas others varied widely from the standard. Total PAC content varied widely, with four supplements ranging 5 - 10 mg PAC/g dry weight, one at 100 mg PAC/g dry weight, and insignificant PAC content in the rest. Several supplements contained only minimal amounts of organic acids and flavonoids. Cranberry peel constituents ursolic acid (8.0-16.3 mg/g) and oleanolic acid (0.3-5.1 mg/g), were detected in the whole cranberry reference standard but only about half of the supplements. Study results suggest significant variation in phytochemical composition among commercial cranberry supplements, reinforcing the need for reliable industry standards.

P-127

EVALUATING THE EFFICACY OF DIFFERENT SOLVENT SYSTEMS FOR SELECTIVE EXTRACTION OF CAROTENOIDS FROM LYCIUM BARBARUM

Tibebe Z. Woldemariam, Patricia Shakarian, Rita Maria Alajajian, and Katherine Le
California Northstate University College of Pharmacy, 9700 West Taron Drive, Elk Grove, CA 95757

The extraction efficacy of different solvent systems was investigated for extraction of carotenoids from Lycium barbarum, known as Goji berries. Biocompatible extraction solvents were used the extract in the liquid form directly in pharmaceutical formulations that are at least substantially non-toxic and acceptable for human use, topically or orally. Goji berries are full of nutrients including amino acids, carotenoids, and polysaccharides. The optimized solvents selectively extracted the carotenoids from minerals, vitamin C, amino acids, and polysaccharides.

The solvents could offer an environmentally friendly way to isolate the plant compounds without the need for toxic organic solvents. The solvents produced yields similar to those of typical organic solvents including ethanol, ethyl acetate and acetone. The individual carotenoids were further isolated with flash chromatography and identified with nuclear magnetic resonance and mass spectrometry. Chemical and physical data of the extraction and isolation procedure will be presented.

These data further contribute to the identification of optimal and selective extraction and isolation of procedure, which can be used for follow-up in vivo oral and topical administration of carotenoids.

P-128

OSTEOGENIC EFFECTS OF NATURAL PRODUCTS IN TRANSGENIC MEDAKA

Yu Tingsheng[1], Christoph Winkler[1], Nishikant Raut[2], Zhitao Ren[1,3], Simon M. Lee[3], Gail B. Mahady[2]
[1]Department of Biological Sciences, National University of Singapore, Singapore
[2]College of Pharmacy, University of Illinois at Chicago, USA,[3]. University of Macau, Macau, China

Evidence from a combination of observational, experimental, clinical and interventional studies strongly points to a positive link between high consumption of dietary flavonoids and anthocyanins and indices of bone health. However, the mechanism by which these naturally occurring compounds control the activity of osteoclasts and osteoblasts to improve bone health remains unclear. In recent years, medaka (*Oryzias latipes*), have become a popular animal model for skeletal research and to study the molecular basis of human bone disorders. The optical clarity of medaka embryos and larvae allows live imaging of bone cell behavior at high resolution in intact specimen. In this work we are using a previously established set of trangenic medaka lines with fluorescent expression in bone forming osteoblasts and bone resorbing osteoclasts at distinct differentiation stages. Including, a novel triple transgenic line in which expression of the osteoclast-inducing factor Receptor activator of nuclear factor kappa-B ligand (RANKL) is controlled by a heat-shock-inducible promoter. RANKL induction in this model leads to ectopic formation of excess osteoclasts, which results in increased bone resorption and consequently an osteoporosis-like phenotype. In this work, we have tested the effects of dietary anthocyanins and semipurified anthocyanin containing extracts in our double transgenic (dT; osterix:mCherry) and triple transgenic (tT; rankl:HS:CFP/ctsk:mEGFP/osx:mcherry) medaka to assess effects on osteoclastogenesis and osteoblastogenesis. The results demonstrate that incubation of triple transgenic medaka with resveratrol initially increases osteoclast differentiation but reduces the stability of the RANKL-induced osteoclasts and blocks their function, thereby reducing bone resorption, with an optimal dose of 10 μg/ml. The anthocyanins, delphinidin (DEL) and delphinidin-3-glucoside (D3G) reduced the formation of osteoclasts in a dose dependent manner with 5 μg/ml being the optimal dose. Anthocyanin containing extracts and delphinidin and delphinidin-3-glucoside also increased osteoblast formation in hFOB 1.19 osteoblasts and in osterix:mCherry medaka. Consequently, bone integrity was clearly improved in DEL, D3G and resveratrol-treated osteoporotic medaka larvae. Our studies establish medaka as a novel in vivo model for drug screening of naturally occurring bone-modulating compounds.

P-129

BLACK COHOSH AND 23-EPI-26-DEOXYACTEIN MODULATE HISTONE DEACETYLASE (HDAC1) AND HAVE BIPHASIC EFFECTS ON GRANULOSA CELLS

Nishikant Raut[1], Shital Patel[1], Temitope O Lawal[1,2], Gail B. Mahady[1]
[1]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago. [2]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

Black cohosh, *Actaea racemosa* L. [syn. *Cimicifuga racemosa* (L.) Nutt., Ranunculaceae] extracts have been used for many years by European and American women for the management of menopausal symptoms. However, one of the primary stumbling blocks to the progression of the clinical science is the lack of a plausible mechanism of action. In this study, a 40% isopropanol extract (BC40) and a 75% ethanol extract of black cohosh (BC75) rhizomes were used, along with the pure compounds actein (AC), 23-epi-26-deoxyactein (DOAc). Granulosa cells (GCs) were cultured in DMEM/F12 media with or without 10% FBS. The results show that BCEs have a biphasic effect on cultured GCs. At very low concentrations (500 ng/ml) the extracts enhance the growth of GCs, but at concentrations >5 μg/ml they inhibit the growth of GCs and induce apoptosis. Interestingly, in colon cancer SW480, CaCo2, and breast cancer MCF-7 cells, BCEs reduced cell proliferation at 0.5-20 μg/ml, with no biphasic effects. BCEs also reversed GC cell cytotoxicity and apoptosis induced by serum starvation, with 0.5 μg/ml restoring cell growth almost as effectively as 10% FBS. Since AC and DOAc are the major triterpenes present in BCEs, we tested the effects of these compounds on the viability cultured GCs. Both AC and DOAc have biphasic effects on cultured GCs with very low concentrations (10.0 ng/ml) enhancing the growth of the cells by 35-80%, but at higher concentrations, both compounds (500 ng/ml) reduced cell viability by 40-55%. In gene expression analysis, BC40 (500 ng/ml) and DOAc (10 ng/ml) significantly reduced the BAX/BCl-2 ratio and increased HDAC1 expression up to 3 fold, while at higher concentrations BC40 and DOAc act as HDAC1 inhibitors and increase apoptosis. Our data suggest both BC40 and DOAc modulate apoptosis of GCs through an epigenetic mechanism involving HDAC1.

P-130

EVALUATION OF THE ANTI-TUMOUR ACTIVITY OF PANAX GINSENG AND TANACETUM PARTHENIUM

Tibebe Z. Woldemariam, Oanh Ma, Christina Stephenson, Artoun Gostantian, and George Talbott
California Northstate University College of Pharmacy, 9700 West Taron Drive, Elk Grove, CA 95757

A library of compounds derived from variety of plants were generated and assessed for possible *in vitro* cytotoxic activity against Human HT-29 colon carcinoma cells. Bioactivity-guided fractionation of the methanol extracts of these plants afforded several fractions and compounds which showed significant reductions in cell viability were observed. Compounds isolated from Panax Ginseng root and Tanacetum Parthenium leaf in particular were found to show significant anti-tumor activities. The most active purified fractions isolated from both Panax Ginseng root and Tanacetum Parthenium leaves contain flavonoids. Efforts are underway to identify and determine the efficacy of individual flavonoids. Observation under a mi-

croscope suggested the cancer cells were dying by an apoptotic mechanism and the exact mechanism of cell death is currently being investigated biochemically. Chemical and physical data for the active compounds, cytotoxic activity and the bioassay-guided fractionation of the methanolic extracts will be presented.

The observed potent anti-tumor activity of flavonoids from both Tanacetum Parthenium and Panax Ginseng make attractive drug candidates for further testing in other tumor cell lines.

P-131

DISCOVERY, STRUCTURE AND FUNCTION OF CYCLOTIDES IN SOME PLANT SPECIES FROM BRAZILIAN TROPICAL FOREST

Meri Emili F. Pinto[1], Antonio Fernandez Bobey[1], Alberto J. Cavalheiro[1], Luma G. Magalhães[2], Adriano D. Andricopulo[2], Norberto P. Lopes[3] and Vanderlan S. Bolzani[1]

[1]Institute of Chemistry, São Paulo State University - UNESP, Araraquara, 14800-060, Brazil, [2]Institute of Physics of São Carlos, The University of São Paulo -USP, São Carlos, 13563-120, Brazil, [3]Faculty of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto-SP, 14040-903 Brazil.

Cyclotides, a novel family of small globular backbone-cyclized micro-proteins (+/-30-residues long) are biosynthesized in some plants species of Angiosperm families as Rubiaceae, Violaceae, Solanaceae, Cucurbitaceae and Fabaceae, which appears to be related to the protection of plants from pests or pathogens[1]. This abstract provides a brief overview of our studies on the isolation, structure elucidation, biological function and distribution of cyclotides in some Brazilian plant species from Atlantic Forest: *Psychotria vellosiana* (Rubiaceae), *Pombalia calceolaria* and *Noisettia orchidflora* (Violaceae). The ethanolic extracts obtained from these species were analyzed by MS and NMR and showed a wide diversity of cyclotides, which after HPLC chromatographic purification and further structure elucidation was possible to identify 8 bracelet cyclotides type. Additionally, **1**-**8** cyclotides isolated were tested to cytotoxic and cell migration activity and only the cyclic peptides **1**-**3** inhibited more than 80% of the cells at 20 μM. The cytotoxic response of **1**-**3** showed some cytotoxicity with IC_{50} values of 1.8, 2.7 and 9.8 μM, respectively. Additionally, the inhibition of cell migration (wound healing assays) showed that only compound **3** showed be able to inhibit cell migration (50%) at a sub toxic concentration (2 μM). Cyclotides are abundant in plants from the families mentioned above, however from Brazilian plants, only few derivatives have been identified, and the discovery of new derivatives from tropical biodiversity may help identifying the mechanism by which cyclotides are processed from linear precursors to mini-cyclic proteins.

Reference

R. Burman, et al. *Journal Natural Products*, **77,** 724–736 (2014).

P-132

LC-TOF-MS ANALYSIS OF CONSTITUENTS FROM DIFFERENT PARTS OF *RHODOMYRTUS TOMENTOSA*

Ronghui Gu[1], Yizhou Wang[1], Shibiao Wu[2], Ping Li[1], Edward J. Kennelly[1,2,], Chunlin Long[1,3,*]*

[1] College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China, [2] Department of Biological Sciences, Lehman College and The Graduate Center, City University of New York, Bronx, NY 10468, USA, [3] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Rhodomyrtus tomentosa is a medicinal and food plant distributed in Southern China. Its fruits have been used as food and medicine, while other vegetative parts, like leaves, branches, and roots, have diverse medicinal values according to local people's knowledge. However, the phytochemical profiles of these different parts have not been well studied for this species. In this research, a high performance liquid chromatography/time-of-flight mass spectrometry (LC-TOF-MS) method was developed for comparative analysis of chemical constituents from different parts of *R. tomentosa*. Principle component analysis (PCA) and partial minimum variance discriminant analysis (OPLS-DA) in Masslynx XS software were used to analyze the obtained data. The PCA results indicated that the constituents present in the leaves were significantly different from those in branches and fruits, while constituents in branches and fruits are similar. Furthermore, based on OPLS-DA, combined with chromatographic retention regulation, accurate molecular mass, isotopic matching and literature searching, four marker compounds from leaves had been found and identified as myricitrin, myricitrin-3-*O*-L-furanoarabinoside, iridin, and 3,3 -didemethyl-9-oxopinoresinol. This research has provided an effective strategy for analyzing chemical difference from different parts of *R. tomentosa*, and may be useful for the study of component difference from different parts of other species.

* Corresponding authors, Email: edward.kennelly@lehman.cuny.edu (EJK), and long.chunlin@muc.edu.cn (CLL)

P-133

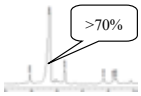
TO DISCOVER ANTICANCER COMPOUNDS IN LINGNAN PLANTS OF HONG KONG

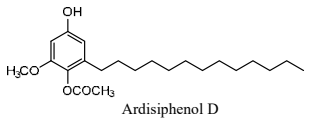
*Chuen-Fai Ku, Xin-Ya Xu, Hong-Jie Zhang**

School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region, PR China

Lingnan is a geographic area referring to the lands in the ancient time of southern China which covers the modern Chinese provinces of Guangdong, Guangxi, Hunan, Jiangxi and Hainan as well as northern Vietnam. Lingnan was once considered as a barbarian land and it had loose contact with the central region of mainland China, which was the cultural cradle of Chinese culture. Because of this, many of the plant resources in Lingnan region remain to be explored. In our drug discovery program to explore the rich diversity of the Lingnan plants, we have collected >100 plant species in Hong Kong and prepared them as extracts using different parts of the plants. These extracts have been tested for their activity against HCT116 (colon) and A375 (melanoma) cancer cells. Among the active plants, the roots of *Ardisia lindleyana* exhibited excellent growth inhibition against HCT116 with an IC_{50} value of 246 ng/mL. Ardisiphenol D is then discovered as the major constituent in the plant, and it displayed potent cytotoxic effect (IC_{50} for HCT116: 590nM) on cancer cells. *Acknowledgements: The work described in this paper was supported the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/15-16/02).*

Ardisia lindleyana





Ardisiphenol D

P-134

POTENTIAL NEW ANTI-INFLAMMATORY COMPOUNDS FROM TIRPITZIA SINENSIS, AN ETHNOMEDICINAL PLANT FROM SOUTHWEST CHINA

Ronghui Gu[1], Yuehu Wang[2], Yeling Wang[1], Ping Li[1], Li Xu[1], Yue Zhou[1], Ze-e Chen[1], Shibiao Wu[1], Edward J. Kennelly[1,3], and Chunlin Long[1,2]

[1]College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, PR China, [2]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, PR China, [3]Lehman College and The Graduate Center, City University of New York, NY 10468, USA

The medicinal plant *Tirpitzia sinensis* is used by the Zhuang ethnic people in Southwest China to treat bleeding, invigorate blood circulation, and treat inflammation and wounds. In order to further explore its traditional medicinal knowledge, we studied this plant phytochemically. Three new

compounds, 1'-trihydroxypropyl-3'-methoxy-8-hydroxymethyl-7-(4-hydroxy-3-methoxyphenyl)-7, 8-dihydro-benzofuran lignin-7'-β-D-apioside (**1**), 3′-*O*-methylluteolin-6-C-[1′′-β-glucoside-(6→1)-α-rhamnoside] (**2**), and (2,6-dimethoxy-4- (methoxymethyl) phenoxy)-tetrahydro-3′,4′-dihydroxy-5- (hydroxymethyl)-pyran-2′-yloxy)-tetrahydrofuran-12,13,14-triol (**3**), respectively, named as tirpitzosides A-C (**1**-**3**), along with five known compounds were isolated from the aerial part of *T. sinensis* for the first time. The structures of tirpitzosides A-C were elucidated by LC-TOF-MS, 1D and 2 D NMR, and other spectrometric methods. A computer-based pharmacophore-based parallel screening approach was employed to predict the potential bioactivities and pharmacological targets of these three new compounds. Screening results suggested tripizoside A may be useful for wound healing, tripitzoside B for anti-inflammation and anti-cancer, and tripitzosides C for anti-inflammation, anti-senescence and wound healing.

*Corresponding authors, Email: edward.kennelly@lehman.cuny.edu (EJK), and long.chunlin@muc.edu.cn (CLL)

P-135

ANTI-*HELICOBACTER PYLORI*, ANTI-INFLAMMATORY AND ANTIOXIDANT EVALUATION OF CRUDE EXTRACTS AND ESSENTIAL OIL FROM *AMOMUM KRERVANH* FRUITS

Bhanuz Dechayont[1], Harit Muangpoolsawad[1], Chantubpapa Liplung[1], Arunporn Itharat[1]
[1]Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Amomum krervanh Pierre ex Gagnep belongs to the family Zingiberaceae. This plant is most commonly used for dyspepsia. The aim of the present study was to determine the anti-*Helicobacter pylori* of crude extracts and essential oil from *A. krervanh* fruits, and evaluate their anti-inflammatory and antioxidant. Ethanolic, aqueous extracts and it essential oil were studied against *H. pylori* standard strain (ATCC 43504) using a disc diffusion test and agar dilution by evaluating the minimum inhibitory concentration (MIC). Anti-inflammatory activity via inhibition of nitric oxide (NO) production was performed in LPS-activated RAW 264.7 macrophages using the Griess assay. For antioxidant activity test, ABTS and DPPH scavenging assays were performed. Then the most effectual extract against *H. pylori* was isolated and elucidated its chemical composition by GC-MS. The results have shown that ethanolic extract produced the widest diameter of inhibition zone (12 mm) and MIC of 250 μg/ml. Interestingly, this extract also displayed moderate nitric oxide scavenging activity (IC_{50} = 49.86 μg/ml), and it also exhibited moderate antioxidant activity using DPPH and ABTS scavenging assays (IC_{50} = 93.78 and 127.63 μg/ml, respectively). GC-MS result the essential oil from ethanolic extract of *A. krervanh* fruits showed euginal strongly inhibited *H. pylori* with inhibition zone = 59.33 mm and MIC values of 2 μg/ml as compared to clarithromycin.

P-136

SCREENING OF THE HERBAL CRUDE DRUGS, FOR INHIBITING ACTIVITY THE DIFFERENTIATION OF REGULATORY T CELLS.

Hitoshi Kotani[1], Tadahiro Yahagi[2], Kazuaki Katakawa[1], Koichi Matsuoka[1], Akimitsu Miyawaki[1], Makoto Inoue[3], Yoshifumi Watanabe[1]
[1]Department of Pharmaceutical Sciences, Musashino University, 1-1-20 Shinmachi Nishitokyo-shi Tokyo, [2]Laboratory of Pharmacognosy, School of Pharmacy, NIHON University, 7-7-1 Narashinodai Funabashi-shi Chiba, [3]Laboratory of Medicinal Resourcesm School of Pharmacy, Aichi Gakuin University,1-100 Kusumoto-cho, Chikusa-ku, Nagoya.

$CD4^+$ T helper(Th) cells play a central role in the immune response. Upon activation by antigens, Th cells follow distinct developmental pathways, through which they develop specialized properties and effector functions. $CD4^+$ T cells were traditionally thought to differentiate into two subsets: Th1 cells and Th2 cells. Recently, an additional subset of Th cells has been identified that is regulatory T cells which is induced by TGF-β and IL-2. Regulatory T cell-mediated suppression serves as a vital mechanism of negative regulation of immune-mediated inflammation and features prominently in autoimmune and inflammatory disorders, allergy, acute and chronic infections, cancer, and metabolic inflammation. In this study, we searched for the herbal crude drugs inhibiting the differentiation of the regulatory T cells which controlled excessive immunity. So, we selected about 150 herbal crude drugs and extracted them with methanol. We screened about 150 methanol extract from herbal crude drugs. Among them, we found that the ingredient included in the crude drug called JINKO methanol extract (*Aquilaria agallocha* extract) inhibited the differentiation of the regulatory T cells from Naïve T cells. Further, JINKO methanol extract promoted IFNγ positive helper Th1 cells from Naïve T cells. These data suggest that JINKO methanol extaract can activate immunity by restraining the differentiation of the regulatory T cells, and anti-tumor activity is expected. We are currently trying to isolate and identify the active compound.

P-137

ANTIOXIDANT ACTIVITIES OF TREGAYSORNMAS FORMULA AND ITS PLANT INGREDIENTS

Pathompong Phuaklee[1], Bhanuz Dechayont[1], Jitpisute Chunthorng-Orn[1], and Arunporn Itharat[1]
[1]Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, 12120, Thailand

Tregaysornmas formula, including *Jatropha multifida* L., *Nelumbo nucifera* Gaertn. and *Aegle marmelos* L., was discovered in Thai Pharmacy scripture. This research aimed to investigate antioxidant activities of the ethanolic and water extracts of Tregaysornmas formula and its plant ingredients using ABTS radical cation decolorization assay, Ferric reducing antioxidant power (FRAP) assay, and Nitroblue tetrazolium (NBT) dye reduction assay, respectively. The results showed that 95%EtOH and water extracts of *Jatropha multifida* and Tregaysornmas formula exhibited strong antioxidant activities by ABTS assay, FRAP assay, and Nitroblue tetrazolium assay. We concluded that Tregaysornmas formula and *Jatropha multifida* showed high antioxidant activities and it was related with the ethnomedical use as maintaining body balance of patients in Thai traditional medicine.

P-138

ALANTOLACTONE IMPROVES PALMITATE-INDUCED INFLAMMATION AND GLUCOSE INTOLERANCE IN BOTH LEAN AND OBESE STATES IN VITRO

Minjee Kim[1], Kwangho Song[1], Yeong Shik Kim[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 08826, South Korea

Obesity is characterized by a massive infiltration of the adipose tissue by macrophages. Adipocytes, together with macrophages create a crosstalk between inflammation and insulin resistance. Excess saturated free fatty acids (FFA), such as palmitate, absorbed via the portal system may cause glucose intolerance and inflammation, which leads to insulin resistance. In this study, we aimed to evaluate the potency of alantolactone (AL), a sesquiterpene lactone isolated from *Inula helenium* in reducing palmitate-induced glucose intolerance, fat accumulation, and inflammation in 3T3-L1 adipocytes (lean state) and adipocyte-macrophage co-culture system (3T3-L1-RAW264.7, obese state). We observed that palmitate reduced glucose uptake and increased fat accumulation, which indicated dysfunctional adipocytes with inadequate lipid storage. However, AL treatment reversed these changes in a dose-dependent manner ($P < 0.05$). Palmitate activated c-Jun N-terminal kinases (JNK) and nuclear factor kappa-B (NF-κB) phosphorylation, and increased the levels of the pro-inflammatory cyto-

kines. AL treatment selectively reduced JNK-associated mitogen-activated protein kinase pathway. In addition, AL decreased the gene expression of JNK upregulating factor, toll-like receptor-4 (TLR4), suggesting inhibition of TLR4-JNK signaling. Moreover, it reduced inflammation-associated cytokines at mRNA levels in both adipocytes and adipocyte-macrophage system. Our study showed that palmitate treatment led to adipocyte dysfunction and macrophage infiltration; however, AL improved palmitate-induced glucose intolerance and inflammation. These findings suggest that AL may inhibit obesity-induced insulin resistance and improve glucose homeostasis and inflammation in insulin target tissues.

P-139

OPTIMIZATION OF EXPANSILE NANOPARTICLES FOR SOLUBILIZING EPIPOLYTHIODIOXOPIPERAZINE ALKALOIDS FOR *IN VITRO* STUDIES.

Chiraz S. Amrine[1], Aaron H. Colby[2], Mark W. Grinstaff[2], Joanna E. Burdette[3], Daniel A. Todd[1], Cedric J. Pearce[4], Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Nanotechnology Innovation Center, Boston University, Boston, MA 02215, USA. [3]Department of Medicinal Chemistry and Pharmacognosy. University of Illinois at Chicago, Chicago, IL 60612, USA. [4]Mycosynthetix, Inc., 505 Meadowlands Drive, Suite 103, Hillsborough, NC 27278, USA.

Verticillins are members of the epipolythiodioxopiperazine (ETP) alkaloid class of fungal metabolites and are known as potent cytotoxic agents. Studies showed that verticillin A has activity as a histone methyl transferases inhibitor with important anticancer properties due to overcoming cancer drug resistance. Verticillin A and Sch52901 were tested against a panel of cancer cell lines and were highly cytotoxic, with IC_{50} values lower than 10 nM. However, like many natural products, these verticillin analogues are poorly soluble, which makes their administration a challenging process. Encapsulating verticillin A and Sch52901 inside expansile nanoparticles (eNPs) enhanced greatly their solubility in physiologic conditions, thereby facilitating *in vivo* studies. eNPs were prepared in two different loading percentages, 1% and 5%, for each eNP (verticillin A and Sch52901) with three replicates for each drug and each percentage. The amount of drug encapsulated inside the eNPs was monitored on a triple quadrupole mass spectrometer. The loaded drugs were within a range of 60-80% of the initial amount encapsulated. From this our team focused on the 5% loaded eNPs for both compounds, which showed promising results *in vitro* against triple negative MDA-MB-231 breast cancer cells.

P-140

MASS SPECTROMETRY AS A TOOL TO QUANTIFY CELLULAR UPTAKE OF ANTIBIOTICS BY MULTI-DRUG RESISTANT *ACINETOBACTER BAUMANNII*

Lindsay K. Caesar[1], Christian Melander[2], Daniel V. Zurawski[3], and Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro NC, 27412, [2]Department of Chemistry, North Carolina State University, Raleigh, NC 27695, [3]Wound Infections Department, Walter Reed Army Institute of Research, Silver Spring, MD 20910

Antimicrobial resistant infections are estimated to cause 700,000 deaths each year, and if the search for new antibiotics does not improve, this number could rise to 10,000,000 by 2050. The paucity of antibiotics is worst for Gram-negative pathogens such as multidrug resistant (MDR) *Acinetobacter baumannii*, which is protected by efflux pumps that extrude toxins. Fluorescence-based methods for monitoring efflux have two critical limitations: (1) the analyte of interest must fluoresce, and (2) fluorescence data can be confounded by quenching. With this project, we aim to produce a *quantitative* tool that can screen complex natural products and identify structural features governing efflux. By measuring extracellular abundance of analytes with mass spectrometry, we can evaluate the extent to which *non-fluorescent* compounds are effluxed. In a preliminary study, incubation with MDR-*A. baumannii* had no effect on extracellular concentration of levofloxacin, suggesting that it was not accumulated by cells. When repeated with heat inactivated cells, however, the extracellular abundance of levofloxacin decreased by 36% (p<0.0001). With living cells, the uptake of levofloxacin is immediately reversed by efflux, but when cells are killed with heat, efflux is disrupted. Thus, the decrease in concentration can be attributed to cellular uptake. Currently, we are optimizing the assay for levofloxacin and modifying it for different analytes of interest. Using quantitative data, we can directly compare cellular uptake of structurally diverse compounds.

P-141

FURANDITERPENES DISCLOSE THEIR POTENTIAL IN CHRONIC PAIN RELIEF

Humberto Spindola[a,b,e],;Rogério Grando[a,b,c] Mariana C Figueiredo[a,b,c] Rosana Basting[a,b,c] Nubia Queiroz[a,b]; João E de Carvalho[a,b]; Angelo de Fátima[d]; Zaijie J Wang[e]; Mary Ann Foglio[a,b,d]
[a]CPQBA, University of Campinas, P.O. Box 6171, 13083-970 [b]Faculty of Pharmaceutical Science University of Campinas P.O Box 6029, 13083-859, [c] Departamento de Ciências Médicas, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, P.O. Box 611, 13083-970 Campinas-SP, Brazil, , [d]GEQOB, ICEx, Federal University of Minas Gerais, 31270-901, Belo Horizonte, MG,Brazil [e]UIC, University of Illinois at Chicago 3320 MBRB, MC 865 Chi, IL, USA

Pterodon genus fruits are used in folk medicine due to their antiinflammatory,analgesic, and anti-rheumatic effects. Previous studies demonstrated that furanditerpenes possessing vouacapan skeleton, possess expressive antinociceptive activities, with promising moiety for the development of new analgesic products. The antinociceptive properties of compounds 6α,7β-6α-hidroxivouacapan-7β-17β-lactone (HVL) and 6α-oxovouacapan-7β-17β-lactone (OVL), semisynthetic analogues of furanditerpenes previously reported as analgesic agents were evaluated on animal experimental models. The main findings were that both compounds were: effective in the writhing test; reduced paw edema in the carrageenan test; effective in the inflammatory phase of the formalin test corroborating their activity against inflammatory pain conditions;effective on reducing pain through the stimulation of vanilloid receptors sensible to capsaicin (an important pathway for chronic pain maintenance); reduced the pain stimulus caused by PGE2 injection (a pathway involved in chronic pain hypersensitivity); effective on decreasing mechanical allodynia in the CFA-model, demonstrating their potential use against chronic pain disorders.

P-142

EFFECT OF *MYRISTICA FRAGRANS* HOUTT SEED (NUTMEG) ON *HELICOBACTER PYLORI*-INDUCED GASTRITIS IN ALBINO RATS: *IN VITRO* AND *IN VIVO* STUDIES

Oyedemi, Temitope O., Lawal, Temitope O., Adeniyi, Bolanle A.
Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

The effect of extracts of *Myristica fragrans* Houtt seed (nutmeg) was studied for anti-*Helicobacter pylori* activity *in vitro* and *Helicobacter pylori* induced gastritis *in vivo*. The choice of the plant was informed by their traditional use in the treatment of gastrointestinal disorders. Anti-*Helicobacter pylori* activities using the agar dilution method with Mueller–Hinton agar, supplemented with 5% defibrinated horse blood was investigated on 38 clinical isolates of *Helicobacter pylori* and *Helicobacter pylori* ATCC 43504. Bactericidal studies were done by the viable counting technique. The effect of 500

mg/kg and 250 mg/kg body weight of the methanol extract of nutmeg on *Helicobacter pylori*-induced gastritis and colonization was investigated in albino rats.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract ranges from 6.25 mg/mL to 25 mg/mL and from 6.25 mg/mL to 100 mg/mL respectively. Bacterial density score of the gastric mucosa reduced from 5.0 ± 7.07 to1.6 ± 1.4 and 3.45 ± 1.4 (mean ± SD, $p < 0.05$) after treatment with concentration equivalent to 500 mg/kg body weight and ofloxacin 400 mg/kg respectively. Analysis of variance (ANOVA) tested the effect of the groups on the treatment days and revealed a significant difference between the treatments at $p< 0.05$.

The results of these studies have proven the anti-*Helicobacter pylori* activities of *Myristica fragrans* seed on *H. pylori* – induce gastritis in albino rats.

P-143

APOPTOSIS INDUCED BY NICLOSAMIDE DERIVATIVES THROUGH HARBORING NF-KB AND CHANGING MITOCHONDRIA MEMBRANE POTENTIAL INTEGRITY

Zonghai Tan[1], Ulyana Munoz Acuna[1], Nelson Freitas Fernandes[1], Tom Li[1], Esperanza Carcache de Blanco[1]
[1]Division of Pharmacy Practice and Science and Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University

As part of our drug discovery program, a series of niclosamide derivatives were synthesized in an attempt to optimize them for the development of new potential anti-leukemic agents. In 2014, leukemia was among the five leading cancer types. This series of compounds were tested in a panel of human cancer cells including: breast cancer cells MDA and MCF7, prostate cancer cells PC3 and DU-145, cervical cancer cells Hela, and acute promyelocytic leukemia cells HL-60. They were also tested in NF-κB, Kras, and mitochondria membrane potential (MTP) assays. Compound 5 was found to exhibit significant cytotoxicity against HL-60 cells. Compound 8 exhibited the most significant activity in the NF-κB assay and compound 17 exhibited the most significant activity in the MTP assay. Analysis of the structural features responsible for the biological activity is being reported for the most promising active derivatives.

P-144

FUNDING OPPORTINITIES IN THE NATIONAL CANCER INSTITUTE'S CANCER DIAGNOSIS PROGRAM

Tawnya C. McKee, Brian Sorg, Miguel Ossandan, and James V. Tricoli
Diagnostic Biomarkers and Technologies Branch, Cancer Diagnostic Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, 9606 Medical Center Dr, 3W512, MSC9728, Bethesda, MD 20850.

The NCI's Cancer Diagnosis Program (CDP) is participating in several funding opportunities related to development of validated biomarker-based diagnostic assays, technology development related to cancer biospecimen, diagnostics and treatment. Information regarding these programs including IMAT, ITCR, Moonshot, Provocative Questions, Analytical and Clinical Validation of Biomarker Assays and a variety of supplements will be provided.

P-145

FUNGAL SECONDARY METABOLITES AS A SOURCE FOR CYTOTOXIC COMPOUNDS

Sonja Knowles[1], Tyler N. Graf[1], Joanna E. Burdette[2], José Rivera-Chavez[1], Huzefa A. Raja[1], Cedric J. Pearce[3], and Nicholas H. Oberlies[1]
[1] Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412, [2] Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, Illinois 60612 and [3]Mycosynthetix, Inc., 505 Meadowlands Drive, Suite 103, Hillsborough, North Carolina 27278. Email: n_oberli@uncg.edu

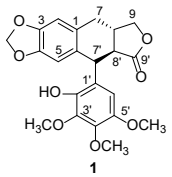
In the United States approximately 40% of individuals will be diagnosed with cancer within their lifetime, and for every 100,000 cases, 171.2 will be fatal, according to the NIH. Fungi are an under investigated source for novel anticancer drug leads. It is estimated that there are between 1.5 and 5 million fungal species that inhabit the earth, and approximately 135,000 have been described, with only a fraction being chemically investigated. In order to study the chemical diversity of fungi, multiple species from around the world were examined, with litter dwelling ascomycetes being the main source. The extraction process was performed using an Accelerated Solvent Extractor; which decreased the overall extraction time, allowing for more samples to be studied. Their bioactivity against human melanoma, human breast cancer, and human ovarian cancer cell lines were assessed. With the aim of decreasing hits from known active compounds, a mass spectrometer based dereplication method was used to correlate with an in house database. This allowed for the isolation and evaluation of new compounds from fungi.

P-146

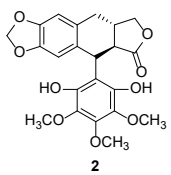
BIOACTIVE DIBENZYLBUTYROLACTONE-TYPE LIGNANS FROM *HERNANDIA VOYRONII*, A MADAGASCAN ENDEMIC AND MEDICINAL PLANT

Dahai He[1,2] and L. Harinantenaina Rakotondraibe.[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA, [2]College of Pharmacy, Southwest University for Nationalities, Chengdu, Sichuan 610041, China

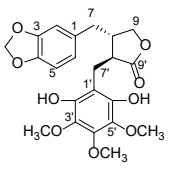
Madagascar possesses one of the most diverse flora in the world and contains about 13,000 plant species, of which 80% are endemic. In the course of our systematic search for antiproliferative (against HT-29 and MCF-7) compounds from endemic plants, we selected a strongly active ethanol extract (IC_{50} <0.16 ug/mL against both cell lines) of the medicinal plant *Hernandia voyronii* (Hernandiaceae), locally called: hazomalany. Bioassay-guided fractionation and isolation work on the extract led to the isolation of three new dibezylbutyrolactone-type lignans named: hernavoyroins A-C (**1**-**3**) together with methylpluviatolide (**4**), deoxypodophyllotoxin (**5**) and atheroline (**6**). The isolation and structure elucidation of **1**-**6** as well as their antiproliferative activities will be presented.



1



2



3

P-147

CYTOTOXIC EFFECTS OF *ANOGEISSUS LEIOCARPUS* EXTRACTS IN COLON CANCER CELL LINES AND INDUCTION OF APOPTOSIS IN SW480 CELLS THROUGH CASPASE 8

TO Lawal[1,2], SR Patel[2], GB Mahady[2]
[1]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

[2]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago.

Anogeissus leiocarpus (DC.) Guill. & Perr. (African birch, Combretaceae) used to treat a wide range of gastrointestinal (GI) disorders including ulcers and cancers were investigated for cytotoxicity activity in colon cancer. The root and stem bark were collected in Ibadan, Nigeria and extracted with methanol by cold maceration. Cell viability and cytotoxicity was determined in a range of colon cancer cell lines including SW480, HCT116, and Caco2 using the CellTiter-Glo® **2.0 assay** at concentrations up to 100 **µg/**mL of the extracts. Control cells treated with vehicle (0.02% DMSO) and drug controls for comparison were incubated simultaneously. Caspase activity for apoptosis was determined with Caspase-Glo® 3/7, Caspase 8, ApoTox-Glo™ Triplex Assay Reagents and flow cytometry. Methanol extracts of *A. leiocarpus* root was active against SW480 and HCT116 with IC_{50} of 15.8 and 20.8 **µg/mL** respectively, while methanol extract of stem bark had IC_{50} of 21.8 and 48.3 **µg/mL** in SW480 and HCT116 respectively. The aqueous partition of the root was found to be the most active with IC_{50} of 15.0 **µg/mL** and explained the activity of the root bark in SW480 and HCT116. Neither extracts nor the partitions were active against Caco2 cells. In SW480 cells *A. leiocarpus* induced apoptosis through the induction of caspase 8. From the results it is concluded that aqueous extracts of *Anogeissus leiocarpus* have activity against colon cancer *in vitro*, thus supporting its traditional medical uses.

Acknowledgements: This research was funded by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

P-148

MECHANISM OF ACTION OF ENGLERIN A IN BT-549 TRIPLE NEGATIVE BREAST CANCER CELLS

Corena V. Shaffer[1], April L. Risinger[1,2], John A. Beutler[3], David J. Beech[4], Susan L. Mooberry[1,2]
[1]Department of Pharmacology and [2]Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, [3]Center for Cancer Research National Cancer Institute, Frederick, MD 21702, [4]Leeds Institute of Cardiovascular and Metabolic Medicine University of Leeds, Leeds LS2 9JT, UK

There remains an unmet medical need to discover new effective therapies for triple negative breast cancers (TNBC). We initiated a screen to discover compounds with selective activity against TNBC cells of molecularly defined subtypes. Englerin A was identified as a selective inhibitor of the mesenchymal BT-549 cell line. Concentration response curves show the growth inhibitory and cytotoxic effects of englerin A are over 200-fold more potent against the BT-549 cell line as compared to other TNBC cells. Englerin A is an agonist of TRPC1/4/5 non-specific cation channels.[1,2] The combination of englerin A with the TRPC1/4/5 inhibitor Pico145[3] cause a rightward shift in the englerin A concentration response curve indicating BT-549 cell sensitivity to englerin A is, in part, due to TRPC1/4/5 activation. Western blot analysis of Na^+/K^+ATPase and treatment of BT-549 cells with ouabain, a Na^+/K^+ATPase inhibitor, suggest BT-549 cells have low levels and/or dysfunctional Na^+/K^+ATPase, which would prevent recovery from englerin A-mediated increases in cellular Na^+. Ongoing studies will measure the time course of englerin A-mediated changes in Na^+ and Ca^{2+} levels in BT-549 cells as compared to TNBC cells that are less sensitive to englerin A. Additional mechanisms, including the activation of PKCθ, are being investigated because Pico145 did not cause a complete rightward shift in the concentration response curve. Identifying the mechanism of englerin A as a selective inhibitor of BT-549 cells is a crucial step in the discovery of new agents and treatment strategies for molecularly defined subtypes of TNBC.

[1] Carson C, et al. 2015. [2] Ludlow, MJ, et al. 2016. [3] Rubaiy, HN, et al. 2017. In Press.

P-149

STRUCTURE AND CYTOTOXICITY STUDY FOR DIGOXIN AND (+)-STREBLOSIDE

Yulin Ren[1], Hennrique Taborda Ribas[1], Qingwei Tan[1], Joshua Henkin[1], Jinhong Ren[2], Wei-Lun Chen[2], Chunhua Yuan[3], Tran Ngoc Ninh[4], Hee-Byung Chai[1], Djaja D. Soejarto[2,5], Joanna E. Burdette[2], Michael E. Johnson[2], and 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]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. [5]Science and Education, Field Museum of Natural History, Chicago, Illinois, USA

Digoxin, a cardiac glycoside obtained from *Digitalis lanata* and used to treat congestive heart failure, was found to show anticancer activity through inhibition of Na^+/K^+-ATPase. In our continuing search for anticancer agents from higher plants, digoxin exhibited potent cytotoxicity toward HT-29 human colon cancer cells, with an IC_{50} value of 380 nM. The present preliminary structure and cytotoxicity study for digoxin showed that the C-12 and C-14 hydroxy group and the C-17 lactone ring are important in the mediation of its cytotoxicity toward HT-29 cells, but the C-3 glycosyl residue is not necessary for such an effect. This has been supported by an analogous study on (+)-strebloside. The saccharide unit, the C-19 aldehyde residue, and the hydroxy groups at C-5 and C-14 together were found to play important roles in the mediation of the cytotoxicity of (+)-strebloside, but introduction of a hydroxy group at C-17 resulted in activity being abolished. Docking files showed that the binding between (+)-strebloside or 17-hydroxystrebloside and Na^+/K^+-ATPase was similar, but the hydroxy group at C-17 of the latter compound was found to be surrounded by several hydrophobic residues. Similar binding poses were observed for the cytotoxic digoxin or the non-cytotoxic 20,22-dihydro-21-hydroxydigoxin and Na^+/K^+-ATPase. The hydrophobic residues around the hydroxy group at C-21 of the latter compound may affect its binding pose and contribute to the lack of activity.

P-150

POTENTLY CYTOTOXIC MAYTANSINOIDS IDENTIFIED FROM THE STEM BARK OF *MALLOTUS BLUMEANUS*

Yulin Ren[1], Hee-Byung Chai[1], Leonardus B. S. Kardono[2,†], Djaja D. Soejarto[3,4], and A. Douglas Kinghorn[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA. [2]Research Center for Chemistry, Indonesian Institute of Science, Serpong, Tangerang, Indonesia. [3]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, USA. [4]Science and Education, Field Museum of Natural History, Chicago, Illinois, USA. †Deceased April 1, 2015.

Maytansine is an *ansa*-macrolide initially discovered as an isolate from a species of the genus *Maytenus* in extremely low abundance. It showed potent antitumor efficacy and has been developed into a new drug, Kadcyla®, approved by the U.S. FDA in 2013 to treat breast cancer, when conjugated

with a monoclonal antibody, trastuzumab. In our continuing search for anticancer agents from higher plants, a chloroform-soluble extract of the dried stem bark of *Mallotus blumeanus* Muell. Arg. (Euphorbiaceae) collected in Indonesia, was found to show potent cytotoxicity against HT-29 human colon cancer cells. Using bioassay-guided column chromatography, two maytansinoids, ansamitocin P-3 and 15-methoxyansamitocin P-3, together with several other types of natural compounds, were characterized from *M. blumeanus*. The structures of these compounds were determined by comparison of their spectroscopic data with literature values, and their cytotoxicity was tested against HT-29 cells. Several of these isolates showed activity, with ansamitocin P-3 found potently active, showing an IC_{50} value of 0.03 nM, much lower than that of the positive control, paclitaxel (IC_{50} 1 nM), and the same as that reported for maytansine (IC_{50} 0.03 nM toward the SK-BR-3 human breast cancer cell line). In a previous *in vivo* study, tumor growth was found to be inhibited significantly, when BD2F1 mice inoculated with murine P388 leukemia cells were treated with ansamitocin P-3, using a dose range of 5–50 μg/kg. Thus, ansamitocin P-3 and its analogues may show promise for development as new antibody drug conjugates, similar to Kadcyla®.

P-151

AN INTRAMOLECULAR C_{AR}–H•••O=C HYDROGEN BOND AND THE CONFIGURATION OF ROTENOIDS AS POTENTIAL ANTICANCER AGENTS

Yulin Ren[1], Judith C. Gallucci[2], and A. Douglas Kinghorn[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, [2]Department of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio, USA.

Over the past 100 years, rotenoids have been used as insecticides or piscicides to contribute to global farming, and many of these natural products have been reported for their promising antitumor efficacy. In our continuing search for anticancer agents from higher plants, (−)-rotenone and several analogous compounds were found to show potent cytotoxicity toward a small panel of human cancer cell lines. The structures and configurations of rotenoids have been established by interpretation of their NMR and electronic circular dichroism spectra and confirmed by analysis of single-crystal X-ray diffraction data, and the H-6' ^{1}H NMR chemical shift was found to be an important indicator of either a *cis* or *trans* C/D ring system. In the present work, structures representing the central rings of a *cis*-, a *trans*-, a dehydro-, and an oxadehydro-rotenoid have been plotted using the *Mercury* program based on the X-ray crystal structures reported previously, with the conformations of the C/D ring system, the local bond lengths or interatomic distances, hydrogen bond angles, and the H-6' chemical shift of these compounds investigated. It has been shown for the first time that a *trans*-fused C/D ring system of rotenoids is preferred for the formation of a potential intramolecular $C_{6'}$–$H_{6'}$•••O=C_4 H-bond, which results in the ^{1}H NMR resonance for H-6' being shifted downfield to indicate the relative configuration of rotenoids for their structure elucidation as bioactive agents.

P-152

HUMAN LEUKEMIA CELL APOPTOSIS INDUCIBLE PARTHENOLIDE ANALOGUES ISOLATED FROM *PIPTOCOMA RUFESCENS*

Yulin Ren[1], Judith C. Gallucci[2], Lichao Chen[3], Hee-Byung Chai[1], Jianhua Yu[3,4], and A. Douglas Kinghorn[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, [2]Department of Chemistry and Biochemistry, [3]Division of Hematology, Department of Internal Medicine, College of Medicine, and [4]Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA.

Previously, four sesquiterpene lactone analogues of parthenolide, namely, goyazensolide (**1**), 15-deoxygoyazensolide (**2**), ereglomerulide (**3**), and rufesolide A (**4**), were isolated from the leaves of *Piptocoma rufescens* collected in the Dominican Republic. The structures of these compounds were determined by interpretation of their spectroscopic data, and their cytotoxicity against HT-29 cells, NF-kB p65 inhibitory activity, and in vivo antitumor potential had also been evaluated. In the present study, the structures of these compounds were confirmed by analysis of their single-crystal X-ray diffraction data, and their apoptosis inducible effects toward the MOLM-13 and EOL-1 human acute leukemia cell lines were investigated. Approximately 50% of late-stage apoptotic cells were induced, when MOLM-13 or EOL-1 cells were treated with **1** or **2** for 24 h at a concentration of 1.1 or 1.7 μM, respectively. However, no apoptotic effects were observed for these cells, when they were treated with **3**, **4**, or the positive control, parthenolide, at a concentration of 5.0 μM for each of these compounds. These results indicate that a 3,10-(1,2-dihydro-4-enone)furan unit is critically important for parthenolide analogues to induce human leukemia cell apoptosis, and both goyazensolide (**1**) and 15-deoxygoyazensolide (**2**) could be promising leads for the development as new antileukemia agents.

P-153

A NEW CARDIAC GLYCOSIDE AND OTHER CYTOTOXIC CONSTITUENTS OF *STREBLUS ASPER*

Yulin Ren[1], Qingwei Tan[1], Jinhong Ren[2], Wei-Lun Chen[2], Tran Ngoc Ninh[3], Hee-Byung Chai[1], Djaja D. Soejarto[2,4], Joanna E. Burdette[2], Michael E. Johnson[2], and 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]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, Illinois, USA.

Previously, (+)-strebloside isolated from the stem bark of *Streblus asper*, was found to exhibit activity in an *in vivo* hollow fiber assay against MDA-MB-231 human breast and OVCAR3 human ovarian cancer cells, and no toxicity was observed in the host mice. Mechanistically, this compound binds to and inhibits Na^+/K^+-ATPase in a similar manner to digitoxin. In the present study, (+)-strebloside and some new and known analogues, along with several other kinds of natural products, were isolated and identified from the flowers, leaves, and twigs of *S. asper* collected in Vietnam. The cytotoxicity of these isolates has been evaluated toward HT-29 human colon cancer cells, and several compounds were found to be potently active. Interestingly, a new cardiac glycoside, (+)-17-hydroxystrebloside, was found to be non-cytotoxic. Comparison of the docking result for this compound and Na^+/K^+-ATPase with that for (+)-strebloside showed that the predicted binding pose for both compounds was similar, with the same hydrogen bonds between their OH-14 and Q111 and between their CHO-10 and T797 observed. However, the hydroxy group at C-17 of (+)-17-hydroxystrebloside was found to be surrounded by several hydrophobic residues, which may affect its binding pose and contribute to its lack of activity.

P-154

CYTOTOXIC CUCURBITACINS AND OTHER COMPONENTS ISOLATED FROM THE ROOTS OF *STEWARTIA CALCICOLA*

Yulin Ren[1], Wei-Lun Chen[2], Tran Ngoc Ninh[3], Djaja D. Soejarto[2,4], Joanna E. Burdette[2], and 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]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, Illinois, USA.

Previously, cucurbitacin D was found to be cytotoxic toward HT-29 human colon cancer cells. It also suppressed proliferation of neurofibromatosis type 2 (NF2)-deficient mouse Sch10545 schwannoma cells and telomerase-immortalized benign Ben-Men-1 human meningioma cells through inhibiting the AKT pathway. In a continuing search for anticancer agents from higher plants, a chloroform-soluble extract of the dried roots of *Stewartia calcicola* T.L. Ming & J. Li (syn. *Hartia yunnanensis* H. H. Hu) (Theaceae), collected in Vietnam, was found to show potent cytotoxicity against HT-29 human colon cancer cells. Using bioassay-guided column chromatography, one new and two known cucurbitacins, together with several other types of natural products, were characterized from *S. calcicola*. The structures of these compounds were determined by comparison of their spectroscopic data with literature values, and their cytotoxicity was tested against the HT-29, MDA-MB-231 human breast, and OVCAR3 human ovarian cancer cell lines. All cucurbitacins isolated showed potent cytotoxicity toward these three cell lines, but none of the other types of isolates were found to be active. To the best of our knowledge, this is the first report for the isolation of cucurbitacins from the family Theaceae.

P-155

PINOCEMBRIN FROM PROPOLIS ATTENUATES METABOLIC DISORDERS IN DIABETIC NEPHROPATHY

Jessica Granados-Pineda[1], Jazmín Pérez-Rojas[2], Blanca E. Rivero-Cruz[1], J. Fausto Rivero-Cruz[1]
[1]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, México, D. F., México.[2]Subdirección de Investigación Básica, Instituto Nacional de Cancerología, México, D. F., México.

Mexican brown propolis has been use in folk medicine for a variety of conditions such as diabetes mellitus. Pinocembrin was isolated from Mexican brown propolis using chromatographic methods. Next, the effect of pinocembrin in a model of diabetic nephropathy (DN) was tested in rats. Two different treatments were assessed, preventive and corrective. In the preventive model a dose of 10 mg/kg was administrated to hyperglycemic rats before the renal damage. It significantly reduced weight loss, LDL (11.5 ± 3.0), cholesterol (67.6 ± 6.1 mg/dL), proteinuria (514.7 ± 208.8 mg protein/24h), urinary volume (76.4 ± 34.68 mL/24h), renal damage markers (KIM 76.02 ± 42.95 pg/mL; N-GAL 11.77 ± 8.37 ng/mL; NAG 14.55 ± 15.07 ng/mL) and apoptosis (5.5 ± 3.4%) in renal tissue compared to hyperglycemic group. Meanwhile, in the corrective model, administrating pinocembrin (10 mg/kg) only leads to the reduction of LDL (8.6 ± 1.6 mg/dL) and apoptosis (0.42 ± 0.40%). Finally, Caco-2 cells were treated with various concentrations of acacetin and pinocembrin. Both flavonoids exhibited high intestinal permeabilities then indicating that the flavonoids tested had reasonable oral absorptions with permeability coefficients of 1.74-1.85 x 10^5 cm/s and 1.80-1.89 x 10^5 cm/s, respectively.

P-156

TOXICOLOGICAL EVALUATION OF HEPACARE®- A NIGERIAN HERBAL FORMULATION

Abiodun H. Adebayo[1], Efejiro E. Ashano[1,2], Olajuwon Okubena[3]*
[1]Medicinal Plant Research Group, Department of Biological Sciences, College of Science and Technology, Covenant University, P.M.B. 1023, Canaanland Ota, Ogun State, Nigeria.
[2]National Biotechnology Development Agency, PMB 5118, Abuja, Nigeria.
[3]Health Forever International, Ikeja, Lagos, Nigeria.

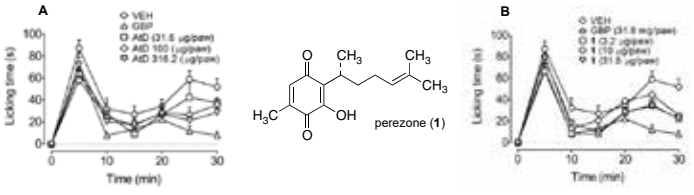
Hepacare® is a widely marketed herbal drug in Nigeria which is used for treating chronic liver ailments and in this study, it is evaluated for its safety as well as pro-inflammatory and genotoxicity effects in mice. The effect of the drug was estimated in a 28-day study in which twenty five mice were randomly divided into five groups and administered orally at 250, 500, 750 and 2500 mg/kg body weight. Biochemical and haematological parameters were determined. The relative organ weights were estimated and histopathology of the liver and kidney tissues were also conducted. Furthermore, mRNA expression of pro-inflammatory cytokines, TNF-α and IL-6 were also estimated by RT-PCR in acute toxicity experiment. The LD_{50} value of the drug was calculated at 3807.89 mg/kg body weight in mice. There was a significant increase ($p < 0.05$) in the activity of ALP in the group treated with 750 mg/kg b.w.; while mice treated with 2500 mg/kg showed significant increase in the levels of AST, ALT, ALP, total bilirubin and total protein as compared to the control group. However, there was a significant dose related increase in monocytes counts in the groups treated with 750 and 2500 mg/kg. There was no significant difference ($p > 0.05$) in mRNA expression of TNF-α, IL-6 and genotoxicity studies in all the treatment groups as compared to the control. However, some hepatic and nephro-pathological derangements were observed in the groups treated with higher doses of the drugs. In conclusion, the study established that the herbal drug may not induce significant pro inflammatory toxic responses and genotoxic effects but prolonged intake may cause severe biochemical and clinical abnormalities especially at higher doses.

P-157

ANTINOCICEPTIVE ACTIVITY OF A DECOCTION AND COMPOUNDS FROM *ACOURTIA THURBERI*

Ana Laura Martínez, Isabel Rivero, and Rachel Mata
Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

Acourtia thurberi (A. Gray) Reveal & R. M. King (Asteraceae) is a medicinal Mexican plant highly valued for the treatment of diabetes mellitus and painful complaints. In this work, the *in vivo* antinociceptive activity of an aqueous extract (AtD) of the plant using a well-known animal model was investigated. AtD (31.6–316.2 mg/paw) reduced the licking time in the formalin test in both neurogenic and inflammatory phases in normal and hyperglycemic-hyperalgesic (**A**) mice. The major component of AtD, namely perezone (**1**) was also evaluated (**B**). All samples were very active in the inflammatory phase; the effect was comparable to that of GBP used as a reference drug. Thus, AtD and its major components possess noted antinociceptive and antihyperalgesic properties valuable for treating painful complaints associated or not with a diabetic condition.



P-158

INFLUENCE OF CO-ADMINISTRATION OF ARTEMISININ AND *RICINODENDRON HEUDELOTII* LEAF EXTRACT ON ANTIOXIDANTS AND LIVER FUNCTION IN RATS

Omolara F. Yakubu[1,2], Abiodun H. Adebayo[1], Emeka S. Okechukwu[1], Oladipupo A. Adeyemi[1], Emeka J. Iweala[1], Ying-Jun Zhang*[2]*
[1]Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria.[2]State Key Laboratory of Phytochemistry and Plant Resources of West China, Kunming Institute of Botany, Kunming China.

Startling rate of malaria parasite resistance to artemisinin and its derivatives has led to possible herb-drug antimalarial combination therapy. This study assessed the effect of co-administration of artemisinin and *Ricinodendron heudelotii* leaf extract on certain antioxidants and liver parameters in rats and also phytochemical analysis of different fractions of the extract. Forty male rats were randomly grouped into four and administered: distilled water (Group A), artemisinin only (Group B), artemisinin with *R. heudelotii* extract (Group C) and *R. heudelotii* extract only (Group D). Selected liver function enzymes were determined using standard methods. Reduced glutathione (GSH), catalase and malondialdehyde (MDA) were determined spectrophotometrically while the qualitative and quantitative phytochemical analysis was also carried out. The results revealed that total protein increased significantly in group C. The activities of ALT and AST were significantly increased ($p<0.05$) in the group administered artemisinin only while their activities were regulated to control level both in group C and D. Similarly, group A exhibited significant increase ($p<0.05$) in MDA. Histological examination showed that few of the hepatocytes were necrotic in the group administered artemisinin only while the group administered artemisinin and extract only showed mild to moderate central venous congestion and periportal cellular infiltration. The study indicates that the bioactive constituents in the *R. heudelotii* extract may either have a regulatory effect on artemisinin toxicity or collaboratively enhance its activity.

P-159

GC-MS ANALYSIS OF *VIBURNUM OPULUS* (L) EXTRACT AND ITS TOXICITY STUDIES IN RATS

Abiodun Humphrey Adebayo, Aristotle B. Alade, Omolara Faith Yakubu Biochemistry and Molecular Biology Unit, Department of Biological Sciences, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria*

This study was aimed at establishing the antimicrobial and phytochemical profiles of *Viburnum opulus* (L) as well as the safety potential of the extract in albino Wistar rats. Ethanol, n-hexane, ethyl acetate, butanol and water fractions were prepared for both phytochemical assessment using gas chromatography-mass spectrum analysis (GC-MS). Five groups of seven rats were used for the study. Group A received distilled water (control), while groups B to E were treated respectively with 250, 500, 1000 and 1500 mg/kg body weight of *V. opulus* extract by abdominal canulisation for 28 days. Blood samples were obtained for biochemical analyses and the liver tissues were further processed for histological studies. The GC-MS spectra revealed the existence of various phytoconstituents such as neophytadiene, germaciene, caryophyllene among others. High density lipoprotein and albumin were significantly ($p<0.05$) elevated in animals administered with 500, 1000 and 1500 mg/kg bw of the leaf extract. Ethanol, butanol and water fractions of the leaf of *V. opulus* showed antimicrobial action against most of the organisms used in this study. The result indicates the *V. opulus* leaf extract contains a wild range of fatty acids and heterocyclic compounds with antimicrobial efficacy and no hepatic damage.

Keywords: *Viburnum opulus*; GC-MS analysis; biochemical; antimicrobial; histology

P-160

USP SAFETY REVIEW OF WILLOW BARK

Hellen A. Oketch-Rabah PhD[1], Tieraona Low Dog MD[2]; Nandakumara D. Sarma, PhD[1] and Gabriel I. Giancaspro, PhD[1]
[1]U.S. Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD, 20852. [2]United States Pharmacopeial Convention's Dietary Supplements Admission Evaluations Joint Standards-Setting Subcommittee, Rockville, Maryland, U.S.A.

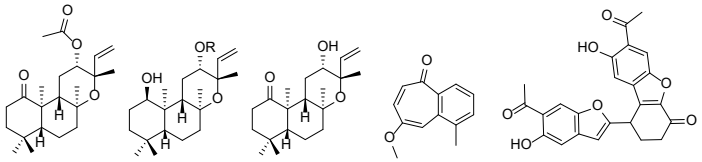
Willow Bark is obtained from several species of *Salix,* and is a dietary ingredient in many dietary supplements (DS) in the USA market. The USP Dietary Supplements Admission Evaluations Joint Standard Setting Subcommittee (USP DSAE JS3) performed an evidence-based review of willow bark to assess any associated health concerns. Clinical trials with extracts delivering 120-240 mg salicin/d for up to 6 weekss, involved adults only; no special populations e.g. pregnant women. Some texts caution: the use of willow bark due to risks of increased bleeding and other effects; concurrent use with aspirin by persons sensitive to aspirin. Common non-serious adverse effects were gastrointestinal. Post-market surveillance data indicated that most adverse events were associated with multi-ingredient products often taken concurrently with other medications; thus causality assignment was uncertain. Importantly, DS products containing willow bark delivering up to 240 mg of salicin (metabolized to ~113 mg salicylic acid) do not required label warning. In contrast the OTC low dose aspirin (80 mg strength) delivering 62 mg salicylic acid is required to include guidelines for use in pregnant women and children, and contraindications pertaining to blood coagulation. USP DSAE JS3 resolved to include in USP quality monographs for willow bark (*Salix* Species Bark, *Salix* Species Bark Powder, and *Salix* Species Bark Dry Extract) a caution labeling statement as follows: "Dosage forms prepared with this article should bear the following statement: Not for use in children, women who are pregnant or nursing, or by persons with known sensitivity to aspirin." DS willow bark products claiming compliance with USP quality standards are required to include this label caution statement on the product label.

P-161

ISOLATION OF NEW CONSTITUENTS FROM *PETRADORIA PUMILA*

Yongle Du[1], Ana Lisa Valenciano Murillo[2], Michael Goetz[3], Maria B. Cassera[2], and David G. I. Kingston[1].
[1]Department of Chemistry and the Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, Virginia 24061, USA. [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602. [3]Natural Products Discovery Institute, 3805 Old Easton Road, Doylestown, Pennsylvania 18902, USA.

An extract of *Petradoria pumila* from the Natural Products Discovery Institute was found to have moderate antiplasmodial activity against the malaria parasite *Plasmodium falciparum*, with an IC_{50} value between 5 and 10 μg/mL. After purification by liquid-liquid partition and chromatography on a silica gel open column and C-18 reverse phase HPLC, four new diterpenoids **1**-**4**, the new benzocyclohepten-5-one **5**, the new lignan **6**, and four known compounds were isolated. The structures of the new and known compounds were determined by interpretation of mass and NMR spectra and ECD spectroscopy. Compounds **1**-**5** showed only weak antiproliferative activities, with IC_{50} values in the A2780 cell line > 20 μg/mL. Diterpenoid **3** displayed the best antiplasmodial activity, with an IC_{50} value of 7.25 μM.



P-162

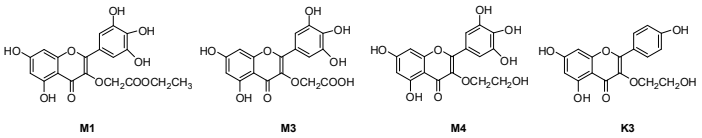
FLAVONOLS DERIVATIVES AS PROTEASOME INHIBITORS: *IN SILICO* DESIGN AND SYNTHESIS

Khaled Orabi[1], Mohamed Abaza[2], Khalid ElSayed[3], Ahmed Elnagar[4], and Samar Faggal[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and [2]Department of Biological Sciences, Faculty of Science, Kuwait University, Kuwait, [3]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, University of Louisiana at Monroe, Monroe, LA, USA, [4]Shire, Los Angeles, CA, USA

A total of forty-four different derivatives of four flavonols; quercetin, myricetin, kaempferol and fisetin, were proposed. Out of those, 18 different ones were selected for chemical synthesis based on their superior docking scores and feasibility of chemical synthesis. Four of them are reported here for the first time; **M1**: 3-carbethoxymethylmyricetin, **M3**: 3-carboxymethylmyricetin, **M4**: 3-hydroxyethoxymyricetin and **K3**: 3-(2-hydroxyethoxy)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one.

Quercetin and myricetin derivatives exhibited differential inhibitory effects on chymotrypsin-, trypsin- and caspase-like activities, while showed very low effect against normal human fibroblasts. On the other hand, quercetin and myricetin showed marked growth inhibitory effects on the normal fibroblasts, while bortezomib exhibited a very potent effect. The efficacy of quercetin and myricetin derivatives to control the growth of the tested male (prostate cancer) and female (breast and ovarian) cancers was tested. Quercetin analogues showed very low anti-proliferative effects on the tested cancers. However, myricetin analogues, in particular **M1** and **M4**, markedly inhibited the growth of the breast and ovarian cancer cell lines.



Acknowledgements: This project was supported by Kuwait University Research Grant SL02/10. Spectral analyses were done at the Faculty of Science, Research Sector Projects Unit (RSPU), Kuwait University; Projects numbers GS01/01 and GS01/03.

P-163

ARRABIDAEA CHICA VERLOT NANOPARTICLE HYDROGEL FOR MUCOSISTIS MANAGEMENT.

Ilza Maria de Oliveira Sousa [1,2] Simone Bussato [3] ; Mariana Cecchetto Figueredo [1,2], Rosanna T. Basting [1,2], Nelson Duran[4], Jose F. Hoffling [3], Mary Ann Foglio[1,2].

[1].Faculty of Medical Science.University of Campinas. São Paulo Brazil.[2].Faculty of Pharmaceuthical Science University of Campinas. São Paulo Brazil.[3]Faculty of Dentistry. University of Campinas. São Paulo Brazil.[4]Chemistry Institute University of Campinas. São Paulo Brazil. Email ilzamo.sousa@gmail.com Postal code 13083-859.

Hydrogels consist of a solid three-dimensional network of polymer chains and can contain over 90% water. They have been used as wound-care coverings, anti-adhesion barriers, drug delivery systems, and tissue engineering scaffolds. In this study, we investigated the effect of a polyvinyl alcohol-hydrogel containing the extracts of *Arrabidaea chica* Verlot (AC) leaves on wound healing. PVA-AC were prepared by method[1].The polymer solution 3% was- mixed with 250 mg of powdered extract of *Arrabidaea chica*. leaves and then homogenized using a sonicators (Qsonica, USA) for 3 cycle of 1 min evaluated zeta potential. After pouring into Petri dishes. After drying, microstructural morphologies were visualized by SEM. Was evaluated antimicrobial activity for different strains candidiasis through the broth microdilution assay[2]. Result show that size at 282,7nm and zeta potential range -11 at 7. Both crude extracts and PVA-AC biofilms exhibited a higher mic of 0.117 mg/mL. Conclusion: the hydrogel containing nanoparticles *A. chica* enhances wound healing and has a potential as a therapeutic cover for mucositis.

References:

1. J. Kim, C.-M. Lee / International Journal of Biological Macromolecules 99 (2017) 586–593.
2. CLSI document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

P-164

EVOLVING STRUCTURAL ANALOGS OF VALERENIC ACID AND IMPROVING ALLOSTERIC MODULATION OF THE GABAA RECEPTOR

Garrett Zinck[1], Scott Kinison[1], and Joe Chappell[1]

[1]Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Lexington, KY 40508

The anxiolytic activity of valerian, *Valeriana officinalis*, has been attributed to the structurally unique 15-carbon sesquiterpenoid that accumulates in the roots. Valerenic acid is reported to allosterically modulate $GABA_A$ receptors – ligand-gated ion channels responsible for fast inhibitory neurotransmission that decrease motor activity and mitigate feelings of anxiety. Through transcriptomic analysis and heterologous gene overexpression, our group previously identified valerenadiene synthase (VDS), the enzyme responsible for catalyzing the conversion of farnesyl diphosphate (FPP) into valerena-1,10-diene (VLD, precursor of valerenic acid). In the current work, our objective is to mutate the VDS enzyme such that it synthesizes more constrained and rigid scaffolds of VLD that possess lower EC_{50} values for the $GABA_A$ receptor, and thus enhanced anxiolytic properties *in vivo*. We will first screen VLD derivatives for their ability to modulate recombinant $GABA_A$ receptors expressed in mammalian cell lines through whole-cell patching techniques, followed by animal behavioral studies with select candidate compounds. To date, we have generated single-point mutants that are either catalytically compromised or produce varying levels of valerena-1,10-diene, indicating it will require additional successive mutations and/or more rationally designed mutations to "unlock" product specificity within the VDS enzyme.

P-165

NCI PROGRAM FOR NATURAL PRODUCT DISCOVERY: PREFRACTIONATED SCREENING LIBRARY METHODS DEVELOPMENT

Christopher C. Thornburg[1], John R. Britt[1], Jason R. Evans[2,3], Rhone K. Akee[1], James Whitt[1], Matthew J. Harris[1], Jerell Thompson,[1] Teresa Ewing[1], Suzanne M. Shipley[1], Paul G. Grothaus[3], David J. Newman,[3] Tanja Grkovic[1] and Barry R. O'Keefe[3]

[1]Natural Products Support Group, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research (FNLCR), [2]Data Management Services, Inc., FNLCR, [3]Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, Maryland 21702.

The Natural Product Repository (NPR) at the US National Cancer Institute 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 active molecules. However, despite the success of natural products in drug discovery, compatibility issues that make natural product extracts challenging for liquid handling systems, coupled with a high rediscovery and false-positive hit rate, have reduced the enthusiasm for high-throughput screening (HTS) of crude natural product extract libraries in targeted as-

say systems. An overview of the prefractionation strategies investigated to address these limitations and make the NPR more amenable to HTS will be presented, along with the development of an automated, high-throughput robotics platform capable of generating a library of 1,000,000 partially purified fractions.

P-166

EXPLOITING CHEMICAL-SYNTHETIC LETHAL INTERACTIONS TO TARGET ANTIMICROBIAL-RESISTANT PATHOGENS

Dennis Yu Liu[1], Bryn Hazlett[2], Alex Wong[2], and Roger G. Linington[1]
[1]Department of Chemistry, Simon Fraser University, 8888 University Dr., Burnaby, BC V5A 1S6 CA. [2]Department of Biology, Carleton University, 1125 Colonel By Dr., Ottawa, ON K1S 5B6 CA.

Antibiotics are a critical component of modern medicine and provide the most effective means of treatment against infectious disease. However, the rise of antibiotic resistance has become a serious and growing challenge in the clinical management of these infections. In order to address these problems, we propose to shift our focus to directly target antibiotic resistance, exploiting a phenomenon known as synthetic lethality. Synthetic lethality is a widespread occurrence whereby a single genetic mutation, such as one conferring antibiotic resistance, reduces the biological fitness of the organism. This reduction in fitness is accompanied by a change in the proteomic and biochemical environment, such that previously non-existing vulnerabilities now arise. We intend to find unique small molecules that selectively target these vulnerabilities as new therapeutics against antibiotic-resistant pathogens. To this end, we plan to screen a large and highly diverse natural product library against a panel of major antibiotic-resistant mutants. Our lab has compiled and refined such a library estimated at approximately 15,000 unique small molecules, derived from marine microbes of the Pacific West Coast. The results of this screen will identify drug-like small molecules that are synthetic-lethal to antibiotic-resistant mutants. Preliminary screens of over 1,300 extracts against 3 drug resistant strains have produced several synthetic-lethal hits, which will be discussed in this work.

P-167

FROM FUNGI TO BENCHSIDE: INVESTIGATING UNTAPPED NATURAL RESOURCES

Mario Augustinovic, Noemi D. Paguigan, José Rivera-Chavez, Tyler N. Graf, Huzefa A. Raja, and Nicholas H. Oberlies
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, 27412. Email: nicholas_oberlies@uncg.edu

In 2016, more than 4,600 individuals in the United States were diagnosed with cancer each day, which culminated in well over a million new cases. At least 35% of these new cases will be fatal, with the highest mortality rates being lung and breast cancers. Natural products have proven to be a continuous and reliable source of cytotoxic drug leads. Fungal natural products are an underrepresented source of potential drug leads, even though there are well over an estimated 1.5 million biologically diverse fungal species, with a fraction of these species being described, and much less explored through their chemical diversity. Natural products from a fungal strain coded G617 have been isolated and characterized, with a number of relevant natural products represented. Vermistatin, dihydrovermistatin, penisimplicissin, and austin are a fraction of compounds found in G617. Of this group, penisimplicissin has shown cytotoxicity against a series of leukemia cancers. Vermistatin also shows moderate inhibition of α-glucosidase, commonly linked to diabetes. Further studies of G617 may prove to be fruitful in the exploration of possible analogues as well as potential drug leads.

P-168

LESSONS LEARNT DURING A SCALED-UP ISOLATION OF Ω-HYDROXYEMODIN FROM *PENICILLIUM RESTRICTUM* (G85) FOR PRECLINICAL EVALUATION

Tyler N. Graf, Huzefa A. Raja, and Nicholas H. Oberlies
Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402

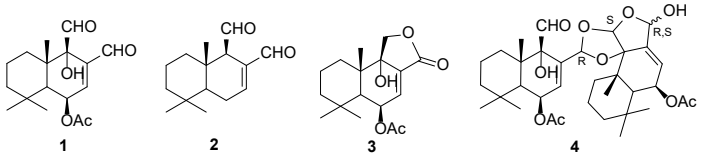
We previously reported the isolation and structural elucidation of a series of polyhydroxyanthraquinones from an organic extract of a solid phase culture of an endophytic fungus, *Penicillium restrictum* (G85). One of these compounds, ω-hydroxyemodin showed promising quorum-sensing inhibition against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in both *in vitro* and *in vivo* models. ω-Hydroxyemodin is currently under preclinical development, and the initial supply was less than 30 mg. Thus we are scaling up isolation of this compound on solid phase cultures for additional *in vivo* studies with the goal of generating 1 gram of material. Valuable insights gained by our research group, both in chemistry and mycology aspects during the scaled-up isolation of ω-hydroxyemodin will be presented.

P-169

BIOACTIVE SECONDARY METABOLITES OF THREE SPECIES OF MADAGASCAN ENDEMIC GENUS *CINNAMOSMA* (CANELLACEAE)

L. Harinantenaina Rakotondraibe,[1,] Edna Alfaro,[2] Peter M. Piermarini,[2] M. Drew,[3] and Nam Lee[4]*
The Ohio State University College of Pharmacy [1]Division of Medicinal Chemistry and Pharmacognosy, [4]Division of Pharmacology, [3]Department of Microbial Infection and Immunity, and Wexner Medical Center, Columbus, OH 43210, [2]Department of Entomology, Ohio Agricultural Research and Development Center, Wooster, OH 44691

Cinnamosma (Canellaceae), a Madagascan endemic and medicinal genus contains three species: *C. fragrans*, *C. macrocarpa*, and *C. madagascariensis* that are characterized by their pungent taste. They are used in traditional medicines to treat many ailments such as malaria, fatigue, and cancer-like symptoms. Our intensive phytochemical and pharmacological investigation of the three plant species led to the isolation of new and known sesquiterpenes, flavonoids, and seco-cycloartane that have cytostatic, angiostatic, antifeedant, antimalarial, and α-glucosidase inhibitory activities. Herein we report the identification of pharmacological properties of four representative sesquiterpenes (**1** - **4**) of the 3 species of *Cinnamosma* and the structure features that contributes to their potent activity.



P-170

A COMMUNITY CALL FOR DATA TRANSPARENCY AND SHARING IN NP RESEARCH

Jonathan Bisson, Charlotte Simmler, James Graham, Guido F. Pauli
Center for Natural Product Technologies (CENAPT), Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, UIC, 833 South Wood Street, Chicago, Illinois, 60612

Natural Product (NP) research is located at the intersection of many scientific disciplines such as biology, biochemistry, botany, ethnobotany, pharmacy, and chemistry. Despite academic publications, most of the in-

formation produced by NP research remains buried in hard drives, printouts, email… Frequently enough, the final results are the only accessible information, presented in the constrained format of publications and often enough behind paywalls. Achievement of greater data transparency requires mechanisms that are formal, accessible, open, and Freely available, allowing the entire NP community to use its own products. At the same time, fundamental limitations and idiosyncrasies limit the mapping of this data to most existing databases available to NP researchers. Thus, it is a timely goal to establish a controlled vocabulary in the form of an ontology that can cross-link existing repositories of bibliographical data, chemical structures, and biological activities, including data outside the NP literature.. The success of the Gene Ontology shows what a formal and logic-based approach to knowledge management can bring to science. Developing a Pharmacognosy/NP Ontology would greatly enhance our capability toexchange information. The presentation is aimed at encouraging the NP research community to collaborate and work (inter-)actively on the development of NP data transparency and sharing mechanisms.

Work funded by NIH-NCCIH grant U41 AT008706

P-171

IDENTIFICATION OF NOVEL MICROTUBULE TARGETING AGENTS FROM DIVERSE NATURAL PRODUCTS

Alison D. Clark[1,2], William Fenical[3], Susan L. Mooberry[1,2]
[1]Department of Pharmacology and [2]Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX. [3]Scripps Institution of Oceanography, University of California, San Diego, San Diego, CA.

Microtubule targeting agents (MTAs) are highly effective drugs used to treat cancer. Diverse MTAs disrupting microtubule dynamics leading to changes in microtubule structures. Microtubule destabilizers inhibit tubulin polymerization resulting in net loss of cellular microtubules. In contrast, microtubule stabilizers promote tubulin polymerization leading to an increase in cellular microtubules. Disruption of microtubule structures inhibits microtubule-dependent events including intercellular trafficking, and signaling. While MTAs have slightly different spectrums of clinical activity, cancers are often resistant to the effects of MTAs because of innate or acquired drug resistance. Clinically relevant mechanisms of drug resistance include expression of the ATP-dependent drug efflux pump P-glycoprotein and the expression of the βIII isotype of tubulin. Identification of novel MTAs that can circumvent clinically relevant forms of drug resistance would be beneficial.

All of the MTAs used to treat cancer are derived from compounds found in nature. To this end, cell-based screening efforts are aimed to identify compounds from diverse natural product extracts and fraction libraries were initiated. A high throughput cell imaging screen with GFP-expressing HeLa cells was used to identify fractions that caused mitotic accumulation and/or aberrant mitotic spindles. Initial screening of 1,600 fractions yielded 11 "hits" in this primary assay. Secondary assays using indirect immunofluorescence assays with HeLa cells confirmed 7 fractions that affected microtubule structure, as indicated by changes in mitotic accumulation and aberrant mitotic spindle formation. Current efforts are underway to isolate the active constituents. This initial study demonstrates the value of the HTS for primary screening and we are optimistic that new MTAs will be discovered. Supported by The Greehey Endowment and K12GM11726.

P-172

ANTIVIRAL SCREENING OF MICROBIAL AND SYNTHETIC LIBRARIES

Ross Overacker, George Neuhaus, Somdev Banerjee, Paul Blakemore, Sandra Loesgen
Department of Chemistry, Oregon State University, 153 Gilbert Hall, Corvallis, OR 97331, USA.

We have developed an HIV-1 antiviral dual-screening method utilizing a protein binding assay in unison with a cell-based single-round infectivity test. Fast and selective binding of small molecules to HIV-1 glycoprotein 120 (gp120), a vital viral surface protein used during the fusion process, were identified using BioLayer Interferometry (BLI) on a biosensor platform. Several extracts derived from a *Streptomyces* sp. bacteria and an *Aspergillus* sp. fungus with potent *in-vitro* HIV-1 inhibitory activity where identified. Bioactivity guided-fractionation of the active microbial extracts resulted in the isolation of terrein (IC_{50} 6.7μM). In addition, three synthetic azaBINOL-type compounds from our synthetic small molecule library were identified and their binding to gp120 characterized.

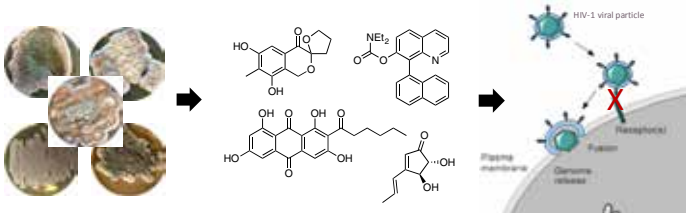


Figure 1: Screening of microbial and synthetic small molecule libraries for entry inhibitors of HIV-1

P-173

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

Chris Thomas[1], Gregory A. Ellis[1], Doug Braun[1], Cole Michel[1], Vlad Vinnik[2], Michael G. Thomas[2], Tim S. Bugni[1]
[1]Pharmaceutical Sciences Division, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI [2]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI

Carbapenem resistant *Enterobacteriaceae* (CRE) infections are an urgent health threat due to its pan-resistance. The New Delhi Metallo β-lactamase 1 (NDM-1) was first identified in 2009 as a member of the metallo β-lactamase (MBL) family of enzymes. MBL enzymes utilize divalent zinc ions to hydrolyze β-lactam antibiotics and are therefore not inhibited by serine β-lactamase inhibitors. Reports from the CDC indicate that NDM-1 is the most prevalent MBL found in bacteria causing CRE infections in the U.S. The CDC also recently confirmed the presence of the NDM-1 gene in a lethal CRE infection resistant to all available antibiotics. Taken together, these reports suggest that inhibition of NDM-1 would be a viable therapeutic strategy to treat CRE. Therefore, our group developed a cell-based screening platform to identify inhibitors of NDM-1. An arabinose inducible expression system was employed in *E. coli* BW27749 cells using a pBAD33 plasmid encoding the NDM-1 gene. In this system, we were able to tightly regulate the expression of NDM-1 for our assay. A fractionated library generated from marine Actinomycete culture extracts was used for screening. An LCMS-based screen was developed as a secondary assay. Preliminary data obtained through bioassay guided fractionation show promise for new inhibitors of NDM-1. Structural characterization and clinical isolate screening is currently underway.

P-174

SIRENAS PLATFORM: A DISRUPTIVE DRUG DISCOVERY ENGINE

Oliver B. Vining, E. Paige Stout, Venkat Macherla, Tamara Schwent, Steven B. Cohen, Rich Schiavi,
Phil S. Baran, Jake Beverage, Eduardo Esquenazi. Sirenas, LLC, 3550 General Atomics Ct, Bldg 02/211, San Diego, CA, 92120

Sirenas, LLC has developed an efficient and proprietary data-driven technology for the discovery and translation of nature inspired small molecules into therapeutics that address unmet need. This approach integrates rare, chemically-rich samples with modern instrumentation and cutting edge metabolomics approaches through our proprietary informatics software, Atlantis™, yielding an optimized, data-centric approach to natural product discovery supported by next generation synthetic chemistry. Thus far, this approach has yielded three promising discoveries including a novel dolastatin (SMD-3117) currently being evaluated in vivo as an antibody-drug conjugate (ADC) payload, an exquisitely potent anti-malarial (SMD-5053) with a novel structure and mechanism of action, and Sirenas' lead molecule, SMD-5033, an anti-cancer agent with a novel mechanism of action currently being evaluated preclinically as an ADC payload. In addition, the company has a pipeline of earlier-stage ADC payloads and has recently expanded into the discovery and translation of first-in-class small molecule therapeutics for immuno-oncology and infectious disease. Here, we describe the discovery of SMD-3117 to highlight the ability of this platform to identify extremely minor but potent metabolites within complex samples, allowing rapid isolation, synthesis, and in vivo characterization of lead molecules.

P-175

SEPARATION OF PYRETHRIN ESTERS BY CENTRIFUGAL PARTITION CHROMATOGRAPHY

Alan Wong, Jan A. Glinski
Planta Analytica, LLC. 461 Danbury Rd, New Milford, CT. 06776

Pyrethrins are potent ion-channel neurotoxins present in the oil extract from *Chrysanthemum cinerariaefolium* flowers. These natural non-derivatized phytochemicals are increasing in popularity as agricultural insecticides because of the environmental safety profile stemming from short half-lives. The natural pyrethrins are six structurally related esters, either of *trans*-chrysanthemic acid (pyrethrins I group) or pyrethric acid (pyrethrins II group). Each group comprise of pyrethrin, cinerin, and jasmolin, which differ in the alcohol moiety. Gram quantities of these phytochemicals and their derivatives are required for studying toxicity and soil degradation; the difficulties stem from lipophilic nature and rapid decomposition. We have developed a two-step purification process to facilitate such studies. The mixture was first separated by normal phase column chromatography by differentiating the two acid moieties. Next, we applied centrifugal partition chromatography to achieve baseline separation of the esters. We explored, evaluated, and optimized solvent systems that were subsequently applied for achieving a multi-gram scale separation of the pyrethrin esters. The pyrethrins I group were separated using a solvent system containing heptane-methanol-acetonitrile (6:1:2, v/v) in ascending mode. The pyrethrins II group were separated using a solvent system containing heptane-methyl tert-butyl ether-acetonitrile-water (8:1:5:1.5, v/v) also in ascending mode. In both cases, gram quantities of individual pyrethrins in purity exceeding 99% were produced by a single run.

P-176

CPC-MS: AN EFFECTIVE VERSATILE TECHNOLOGY APPLIED TO VALORIZATION OF VEGETAL EXTRACTION BY-PRODUCTS

Grégoire AUDO[1], Jérôme MASSON[2], Céline Le QUEMENER[1], Hugues BREVARD[2]
[1]Gilson Purification SAS, Application laboratory, 22 rue Bourseul, F-56890 Saint-Avé France, [2]Robertet SA, Research Division, 37 Avenue Sidi Brahim, F-06130 Grasse, France

Countercurrent chromatography (CCC) is a support-free liquid–liquid chromatography based on the partitioning of solutes between two non-miscible liquid phases. The lack of any solid support provides many advantages over conventional LC such as high selectivity, high loading capacity, total recovery of the loaded sample and easy scale-up.

The Centrifugal Partition Chromatography (CPC), a hydrostatic CCC column, was used to perform a rapid separation, isolation or enrichment of various bioactive compounds from very complex mixtures. Furthermore, the versatility of CPC technology allows to target different objectives in term of applications, whether by compounds differing in polarities and concentrations, by extract matrices, or by process.

In this context, CPC technology presents a great interest for by-product extracts valorization in flavor and fragrances industries. From one example, two CPC methodologies are presented in this poster: The first one is using CPC in batch mode, for an efficient purification of compounds of interests. The second one is performed with CPC in its continuous mode, called True Moving Bed (TMB), for fractionation in two valuable extracts.

In order to optimize the CPC valorization of these kinds of by-products, CPC column is coupled to a mass spectrometry (MS) detector. Thereby fractions analysis and treatments are reduced by driving the collect only according the average mass of the compounds of interest.

P-177

UNBIASED CASES OF COMPUTER ASSISTED STRUCTURE ELUCIDATION (CASE)

David Adams, Dimitris Argyropoulos
Advanced Chemistry Development Inc., 8 King Street East, Suite 107, Toronto, Ontario, Canada, M5C 1B5

Computer Assisted Structure Elucidation (CASE) first appeared almost 50 years ago but it was only during the last 20 years that it gained traction mostly because of the development of computers powerful enough to handle the problem. CASE allows for the very rapid generation of structures that are in agreement with the signals observed in 1D and 2D NMR correlation spectroscopy (HSQC, HMBC, COSY etc.) followed by ranking and selection of the ones which agree more with the observed chemical shifts. Apart from the obvious benefit of reduced time to solve a structure CASE offers also the advantage of being completely unbiased and not falling into mind traps that a human might do. In this presentation we review the variety of almost 60 structures solved as part of an Elucidation of the Month column and show details about the types of structures solved, their sizes (number of heavy atoms), the proton content, the elucidation time and the confidence level on the validity of the proposed structure.

P-178

UNUSUAL BIOACTIVE REARRANGEMENT PRODUCTS FROM AQUEOUS PHOTOLYSIS OF PHARMACEUTICAL STEROIDS

Nicholas C. Pflug[1], Dalma Martinovic-Weigelt[2], David M. Cwiertny[3], and James B. Gloer[1]
[1]Department of Chemistry, University of Iowa, Iowa City, IA 52242; [2]Department of Biology, University of St. Thomas, St. Paul, MN 55105; [3]Department of Civil and Environmental Engineering, University of Iowa, Iowa City, IA 52242

In an ongoing effort to study the environmental fate of potent, endocrine-active steroid hormones, we report the formation of two unusual phenolic rearrangement products with a novel tetracyclic ring system encountered upon aqueous photolysis of the pharmaceutical dienone steroids dienogest and methyldienolone. Reversed phase HPLC was used to isolate the products, while HRMS and 2D NMR techniques were used to elucidate their structures. NOESY data for the products were employed in conjunction with knowledge of the stereochemical features of the parent steroids to assign absolute configuration. The products showed moderate progestin and estrogen receptor activity in *in vitro* nuclear hormone receptor assays.

P-179

DEVELOPMENT OF AN NMR PLATFORM FOR UNIVERSAL CHARACTERIZATION OF NATURAL PRODUCTS MIXTURES

Egan, Joseph M.[1] ; Lewis, Andrew[1] ; Linington, Roger G.[1]
[1] Department of Chemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada, V5A 1S6

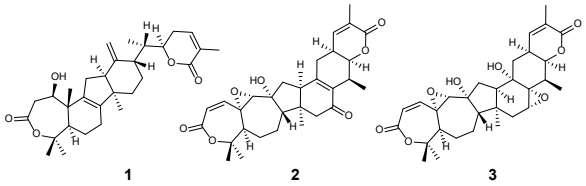
Natural products have historically been a large and important bastion for drug discovery efforts, due in no small part to the chemical diversity and variety of bioactivities that secondary metabolites display. Over the past century, researchers have investigated natural products from a diverse array of sources, including fungi, botanicals, marine invertebrates, and unicellular bacteria. However, due to the complex nature of these natural product extracts, often the most prevalent molecules are isolated and characterized, which leads to problems with large amounts of time and resources ending in rediscovery. Development of methods of analysis that aid in prioritization of new or novel compounds help to circumvent problems of rediscovery. In recent years, there have been approaches to NMR metabolomics that make use of the data that can be acquired through 2D experiments, although most of these approaches use a targeted analysis that require databases. The platform developed and introduced here utilizes features of several orthogonal 2D NMR experiments to derive constituent information across samples in a natural product library, aiding in the prioritization of molecules that have potential to be new or novel, without the need for a database of standards. The goal of the system is to allow for untargeted constituent analysis and feature comparison with additional data sources such as bioactivity assays or mass spectroscopy to aid in efforts of universal characterization.

P-180

NEW TRITERPENOIDS FROM *KADSURA JAPONICA*

Yuan-Bin Cheng[1,2] and Ya-Ching Shen[1]
[1]School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan, [2]Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan,

A phytochemical investigation of *Kadsura japonica* has yielded three new triterpenoids, kadjadilactones A-C (**1**-**3**). Compound **1** has a novel skeleton possessing a 7/6/5/6-fused tetracyclic ring system. Compounds **2** and **3** belong to kadlongilactone-type triterpenoids. The structures of **1**-**3** were elucidated on the basis of spectroscopic methods, especially 2D NMR techniques (COSY, HMQC, HMBC, and NOESY). A plausible biosynthetic pathway for **1** will be discussed.



1 2 3

P-181

SEEING MORE FROM MUCH LESS IN NMR: DETERMINATION OF OPTIMAL FREQUENCIES FOR MULTIPLE SOLVENT SUPPRESSION AND INCORPORATION IN SELECTIVE EXCITATION EXPERIMENTS.

Clemens Anklin
Bruker Biospin Corporation, 15 Fortune Drive, Billerica MA 01821 USA

The determination of optimal frequencies for solvent suppression can in many cases be done by simple peak picking of the solvent signal. Very strong solvent signals in high sensitivity probes are always subject to radiation damping (RD). As shown by Torchia[1], Berger[2] and Lin[3] the position of signals experiencing RD is different from the true resonance frequency. Thus the optimal frequency is different from the observed maximum intensity. The effect is dependent on the concentration of the solvent and the quality factor of the probe. This poster will present methods to correctly determine the frequencies for the suppression of single or multiple resonances, in a simple manual or even automatic fashion. The methods are based on eliminating RD or by using components of the solvent signal that are not affected by it to determine the true position. These optimized solvent suppression schemes can easily be combined with selective excitation experiments to allow the collection of NMR spectra on small quantities of materials in protonated solvents.

REFERENCES:
1. Torchia, D.A. J Biomol NMR (2009) 45: 241.
2. Findeisen M., Brand T., Berger S., Magn Reson Chem. 2007 Feb;45(2):175-8.
3. Susie Y. Huang, Clemens Anklin, Jamie D. Walls, and Yung-Ya Lin, J. Am. Chem. Soc., 126 (49), 15936 -15937, 2004

P-182

FERMENTED NONI JUICE (FNE) AND ITS BIOACTIVE COMPONENTS

Leng Chee Chang,[1] Tamara Kondratyuk,[1] Mengke Zhang,[1] Dejun Zhang,[1] Marisa M. Wall,[2] John M. Pezzuto[1,3]
[1]Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, 96720. [2]United States Department of Agriculture, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, Hilo, HI, 96720, USA. [3]Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY 11201,USA.

Noni (*Morinda citrifolia*) has been extensively used by Polynesians for several thousand years. The ripe fruit and fermented juice are favored as a modern remedy for diabetes, and certain types of cancer. Noni juice is marketed as a novel food ingredient or dietary supplement in the U.S. Consequently, it is important to identify the active components which contribute to its biological activities. We introduced fermentation into the process of making noni juice to produce fermented noni exudates (fNE). Bioassay-guided fractionation of an organic extract of fNE, led to the isolation of

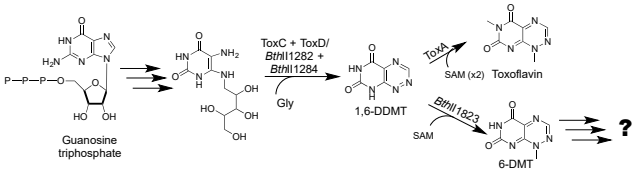
several iridoid glycosides and other components. Their structures were determined by spectroscopic methods, including, spectroscopy and mass spectrometry. Iridoid glycosides exhibited, with some structures activity on quinone reductase-1 (QR1). For example, the iridoids glycosides rhodolatouside, and rhodolatouside B demonstrated considerable QR1 inducing effects. The presence of iridoids in fNE were assessed using liquid chromatography-mass spectrometry (LC-MS) analysis. These results suggest that fNE juices displays both antioxidant activity and induction of phase II enzymes.

P-183

BIOSYNTHETIC AND GENOME MINING APPROACHES TO IDENTIFY TRIAZINE CONTAINING NATURAL PRODUCTS

Khaled H. Almabruk[#], Michael K. Fenwick[§], Steven E. Ealick[§], and Benjamin Philmus[#]
[#]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331, and [§]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853

Triazine containing natural products are of interest because of their attractive biological activities and the unique chemistry involved in their biosynthesis. One of the most studied examples is toxoflavin, which has been isolated from plant pathogenic strains of *Burkholderia*, and plays a key role in virulence. Genome mining has revealed that triazine natural product gene clusters are found in pseudomonads and actinobacteria suggesting that triazine-containing natural products are more prevalent than previously realized. In this study, we present our results from precursor labeling studies in an effort to shed light on the complex biosynthetic steps involved in forming the N-N-bond of the triazine moiety during toxoflavin biosynthesis. We also discuss the crystallographic and the in vitro characterization of two N-methyltransferases that use 1,6-didesmethyltoxoflavin (1,6-DDMT) as a substrate.



P-184

MTMW AND THE CRYPTIC FINAL PRODUCT OF THE MITHRAMYCIN (MTM) BIOSYNTHETIC PATHWAY

Ryan Wheeler, Xia Yu, Jhong-Min Chen, Caixia Hou, Oleg V. Tsodikov, and Jürgen Rohr
Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506, USA

Previous work on the pentyl side chain of mithramycin (MTM) has yielded analogues with improved specificity for Ewing's sarcoma cell lines. MtmW has previously been shown to catalyze the reduction of the 3'-keto group on the pentyl side chain *in vivo*; in order to better characterize the reaction we sought to perform an *in vitro* reaction and obtain the crystal structure of MtmW. The *in vitro* reaction was carried out starting from premithramycin B and required the presence of MtmOIV. This mixture of two enzymes produced an unexpected product, a C2 epimer of MTM. The epimer (iso-MTM) had no detectable bioactivity, but rapidly isomerized to MTM in the presence of divalent metal ions. The crystal structure of MtmW appeared to show that the di-keto precursor of MTM, MTM DK, was used as a substrate, and further interrogations of the crystal structure are ongoing. In short, we have demonstrated that MtmW catalyzes a ketoreduction, but produces iso-MTM, a molecule with no bioactivity that isomerizes in the presence of divalent metal ions. We hypothesize that isomerization of iso-MTM is a regulatory feature indicating the presence of Mg^{2+} in *S. argillaceus*'s environment, particularly its cell membrane, thereby converting an inactive into an active compound while it is channeled out of the organism, which helps to prevent intercellular DNA damage.

P-185

ELUCIDATION OF THE BIOSYNTHESIS OF A NOVEL NUCLEOSIDE ANTIBIOTIC

Jonathan Overbay[1], Zheng Cui[1], Zhaoyong Yang[2], Christian Ducho[3], Steven Van Lanen[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40506, [2]Key Laboratory of Biotechnology of Antibiotics, Institute of Medicinal Biotechnology, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 1000050, China, [3]Department of Pharmacy, Pharmaceutical and Medicinal Chemistry, Saarland University, 66123 Saarbrucken, Germany

Drug-resistant bacterial pathogens are rapidly becoming a widespread problem in the United States and across the globe. Meanwhile, new antibiotics entering the clinic are alarmingly scarce. Natural products have long been a vast source for efficacious drugs. Highly modified nucleoside antibiotics, a class of natural products, target MraY bacterial translocase I, a relatively unexploited enzyme target in peptidoglycan cell wall biosynthesis. This class of secondary metabolites have strong antibacterial activity against Gram-positive pathogens, yet are greatly underexplored. A subset of nucleoside antibiotics contains a characteristic 5'-C-glycyluridine (GlyU) core. The newly discovered nucleoside antibiotic, sphaerimicin, has a novel scaffold that makes it distinct from the highly modified nucleosides known to date. In sphaerimicin, the GlyU core is ultimately appended to a dihydroxylated piperidine that is bridged to the 5" amine of a 5-amino-5-deoxyribosyl moiety. Our study will investigate this modification, which we propose is partially achieved via FMN-dependent deamination of a 3-amino propyl group to create an aldehyde, which can then have a C_2 unit incorporated by a transketolase. Importantly, elucidating how this scaffold is biosynthesized may lead to discovery of new enzymatic chemistries that could power innovative chemoenzymatic synthesis and genome mining to uncover new natural products.

Support for this work was provided by NIH grants AI087849 and UL-1TR000117.

P-186

ELUCIDATION OF THE TIRANDAMYCIN BIOSYNTHETIC PATHWAY AND SUBSTRATE ENGINEERING OF A TIRANDAMYCIN C ANALOGUE

Rosa Vasquez[1,2], Kinshuk Srivastava[1], Jessica L. Stachowski[2], Matthew DeMars[1], Jacob C. Carlson[1], Shengying Li[1], Shamila S. Gunatilleke[1], Yojiro Anzai[1], Douglas A. Burr[1], Larissa M. Podust[3], John Montgomery[2] and David H. Sherman[1].*
[1]Life Sciences Institute and [2]Departments of Chemistry, Medicinal Chemistry, and Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan 48109, USA, [3]Department of Pathology and Sandler Center for Drug Discovery, University of California, San Francisco, California 94158, USA.

Tirandamycin antibiotics are structurally diverse molecules that show a vast array of antibacterial, antiparasitic and antifungal activities. The bicyclic ketal moiety of tirandamycin is key to biological activity. Elucidating the tirandamycin biosynthetic pathway expands our understanding of the formation and modification of potent bioactive compounds, and provides access to unique enzymes that modify complex substrates in a selective manner. Here, we present a review of our previous work[1] on the *in vitro* reconstitution of a multi-step oxidative cascade responsible for the biosyn-

thetic tailoring of the bicyclic ketal moiety. This pathway is mediated by two co-dependent oxidative enzymes, TamI, a multi-functional cytochrome P450 monooxygenase, and TamL, a flavin adenine dinucleotide-dependent oxidase. We also include our current work on substrate engineering of a tirandamycin C analogue to provide insights into the importance of the tirandamycin structure in the enzyme-substrate interaction as well as the substrate tolerance and selectivity of the remarkable P450, TamI.

[1]Jacob C. Carlson et. al., *Nature Chemistry* (2011) 3(8), 628-633.

P-187

TRANSCRIPTOME ANALYSES TO IDENTIFY GENES INVOLVED IN SECOIRIDOID BIOSYNTHESIS IN TIBETAN MEDICINAL PLANT *SWERTIA MUSSOTII*

Yue Liu[1,2§], Yi Wang[3§], Fengxian Guo[1], Lin Zhan[1], Toni Mohr[3], Prisca Cheng[3], Naxin Huo[3,4], Ronghui Gu[1], Danning Pei[1], Jiaqing Sun[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. Secoiridoids are the major bioactive compounds. We generated transcriptome sequences from the root, leaf, stem, and flower tissues, and performed *de novo* sequence assembly. Putative functions could be assigned to 35,029 transcripts (35.52%) based on GO and KEGG analysis. The expression profiles of 39 candidate transcripts encoding the key enzymes for secoiridoid biosynthesis were examined in different *S. mussotii* tissues, validated by qRTPCR, and compared with the homologous genes from *S. japonica*. The examination of the accumulated levels of three bioactive compounds, sweroside, swertiamarin, and gentiopicroside, revealed that there is no significant correlation with the expression profiles of key genes in the pathway. Acknowledgment: financial support by project of NSFC-81274185, 81373765, NCET-13-0624, ydzxxk201618, and 111-B08044.

P-188

THE ANTIBIOTIC RESISTANT TARGET SEEKER (ARTS), AN EXPLORATION ENGINE FOR ANTIBIOTIC CLUSTER PRIORITIZATION AND NOVEL DRUG TARGET DISCOVERY

Mohammad Alanjary[1], Brent Kronmiller[2], Martina Adamek[1],Kai Blin[3], Tilmann Weber[3], Daniel Huson[1], Benjamin Philmus[2], Nadine Ziemert[1]

[1]University of Tübingen, Tübingen, Germany, [2]Oregon State University Corvallis, OR, USA, [3]Technical University of Denmark, Kgs. Lyngby, Denmark

Genome mining has re-invigorated natural product discovery via rapid screening of the wealth of available genomic data. Existing genome mining tools are excellent at identifying a wide range of biosynthetic gene clusters (BGCs) but provide little help in prioritizing the numerous predictions for those with antibiotic potential. One recently developed method, target-directed genome mining, prioritizes clusters with known self-resistance mechanisms as a selector for antibiotic production. However this approach has largely remained a manual process and may result in antibiotic rediscovery. Here we introduce an automated web-server: the "Antibiotic Resistant Target Seeker" (ARTS) available at https://arts.ziemertlab.com. ARTS integrates target-directed methods with "essential gene screening" for rapid prioritization of BGCs encoding for antibiotics with interesting and potentially novel mode of actions. By highlighting known resistance factors and putative novel targets ARTS enriches for promising leads for wet-lab experiments. Additionally, novel target predictions were shown to be a useful orthogonal approach to BGC identification by highlighting regions of the genome with incomplete or absent BGC predictions.

P-189

IN SILICO COMPARISON OF BINDING MODES OF PHENOLIC INHIBITORS OF THE ANTIBACTERIAL DRUG TARGET ENOYL-ACP REDUCTASE

P. Matthew Joyner

Department of Chemistry, Natural Science Division, Pepperdine University, 24255 Pacific Coast Highway, Malibu, California, USA 90263

The enzyme enoyl-ACP reductase (FabI) is a validated target for antibacterial drugs and is inhibited by the antibiotics triclosan and isoniazid. Multiple studies of have identified phenolic plant metabolites that inhibit this enzyme but no experimental structural evidence is currently available to ascertain the binding mode of these inhibitors to enoyl-ACP reductase. The molecular docking software AutoDock was used to predict binding modes of phenolic plant metabolites that have been reported to inhibit this enzyme. Previously reported IC_{50} values and mechanisms of inhibition for these phenolic inhibitors were used in combination with the computational predictions to compare the probable binding interactions between these metabolites and the enzyme's active site and to identify specific amino acid residues that are predicted to be important for binding of these inhibitors to the enzyme.

P-190

CORRELATING PHYLOGENY AND CHEMISTRY TO IMPROVE THE CYANOBACTERIAL NATURAL PRODUCT DRUG DISCOVERY PIPELINE

Peter Sullivan, Daniel May, Jimmy Orjala

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

Cyanobacteria are an emerging source of biomedically relevant natural products. Strains that are closely related have shown to generate the same or similar chemistry. Utilizing the 16S rRNA sequence to establish phylogeny, we carried out an analysis of cyanobacteria across orders from both freshwater and marine environments to identify taxonomic trends in secondary metabolite production. Moving forward, our goal is to sequence and determine how our 800+ strain library clades amongst each other and published strains known to produce natural products. With a better understanding of how chemistry correlates with taxonomic positioning, we can exploit this trend to help expedite our drug discovery pipeline to prioritize which strains to grow. This technique has the potential to categorize cyanobacterial secondary metabolite production in our library based on the 16S rRNA region.

P-191

FUNCTIONAL SIGNATURE ONTOLOGY (FUSION) MAPPING FOR RAPID MECHANISM OF ACTION DISCOVERY

Anam Shaikh[1], Rachel Vaden[2], Elizabeth McMillan[2], Malia B. Potts[2], Michael A. White[2], and John B. MacMillan[1]

Departments of [1]Biochemistry and [2]Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75235, USA.

Natural products play a critical role as tools for advancing the fundamental understanding of human, plant and microbial biology and in the treatment of human disease. Natural product research programs have traditionally placed an emphasis on identifying novel chemical structures without fully exploring their biological activity. Additionally, scientists usually charac-

terize natural products by a small number of bioassays that do not test a diverse set of biological processes. This time-intensive "grind and find" approach to natural product discovery has resulted in the frequent re-identification of known chemical scaffolds as well as an inability to identify molecules with unique bioactivity. As a result, the White and MacMillan labs at UTSW have developed FUnctional SIgnature of ONtology (FUSION), which is a computational "guilt by association" strategy that links bioactive natural products to the molecular entities or biological processes that they engage in cells. FUSION is a broad-scale and disease agnostic approach to define the mechanism(s) of action for natural products against human cancer cells. It employs an information-rich, high-throughput, endogenous reporter gene expression platform that allows quantitative discrimination of cellular responses to genetic and chemical perturbations.

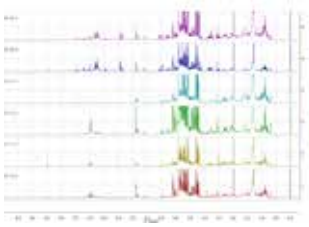
Through iterations of "FUSION-inspired" bioassay-guided fractionation, I have used this platform to characterize secondary metabolites. Currently, I have identified the biological pathways associated with two molecules; one molecule acts as an electron transport chain inhibitor, and the other attenuates NFkB expression levels. Herein, we will describe the chemical and biological characterization of these molecules.

P-192

NMR APPROACH FOR METABOLITE VARIATION OF *SCELETIUM TORTUOSUM* FROM DIFFERENT LOCATIONS

Jianping Zhao[1], Nyasha T. Jambwa[3], Alvaro Viljoen[3], Ikhlas A. Khan[1,2]
[1]National Center for Natural Products Research, [2]Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, 0001, South Africa.

Sceletium tortuosum (L.) N.E. Br. (Mesembryanthemaceae) is indigenous to South Africa and mainly found in the Karroid areas of the Cape region. The plant and its fermented preparation have been used for treatment of CNS-associated conditions. It is also used for quenching thirsty and relief of hunger. *Sceletium* raw materials and various products are currently marketed with claims of psychoactive properties such as mood uplifting and reduction of anxiety. NMR spectroscopy combined with multivariate analysis was applied to explore the extent of chemotypic variation and the characteristic constituents in 145 specimens collected from different locations in South Africa. The geographic location was found to be one of factors that affected the metabolite variations in the samples. The marker compounds were identified for differentiating the specimens from different locations.



P-193

CHEMOTYPIC STUDY OF ESSENTIAL OIL COMPOSITION BY AGGLOMERATIVE HIERARCHICAL CLUSTER ANALYSIS.

Prabodh Satyal[1,2], William N. Setzer[1].
[1]Department of Chemistry, University of Alabama in Huntsville, Huntsville AL-35899, [2]dōTerra International LLC, Pleasant Grove, UT 84062.

The result of our systematic and detailed analyses of essential oil chemical composition has revealed new chemotaxonomic divisions for some of the different species. Plant chemotypes are not only dependent on genetic differences between species but vary among geographical and climatic conditions. Hierarchical cluster analysis is used in some cases through comparison of oil compositions of the same species that are collected from different locations. Similarity or dissimilarity between groups by selected characteristics is observed by using a dendrogram (i.e., a tree diagram). In this study, an agglomerative hierarchical cluster algorithm is used in dendrogram creation of several essential oil chemical compositions such as *Ocimum basilicum*, *Salvia officinalis*, *Cannabis sativa*, *Thymus vulgaris*, *Cryptomeria japonica*, and *Rosemarinus officinalis,* etc. So, this study confirms that the variation of the chemical composition should also be considered in the taxonomy of essential oil bearing plants.

P-194

DO CAULERPIN AND ITS DERIVATIVES PLAY A ROLE IN RECRUTIING *VIBRIO SPP.* TO THE SURFACE OF *CAULERPA SETULARIODES*?

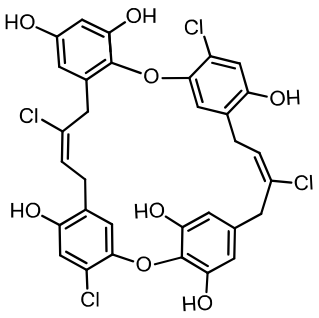
Stanley Budzynski[1], Skylar Carlson[2], Tomasz Jurga[1], Jason Kwan[2], Melany P. Puglisi[1]
[1]Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628. [2]University of Wisconsin, Madison, College of Pharmacy, 777 Highland Avenue, Madison, WI 53705

Thirteen strains of *Vibrio*, including some known pathogens to benthic marine organisms, have been isolated from the surface of *Caulerpa cylindracea* in the Atlantic Mediterranean. High densities of microbial populations on the surface of the algae suggest an algal-bacterial association that may increase the fitness of *C. cylindracea*. In previous study in the Florida Keys, bacteria was isolated from the surface of common algae and used in a panel to screen the algael extracts. Extracts from members of genus *Caulerpa* exhibited broad spectrum growth inhibition and promotion activity in the panel. In this study, bioassay-guided fractionation using *Vibrio* sp., isolated from the surface of *C. mexicana*, was employed to separate the crude extract from *C. sertularioides*. Bacterial cultures were grown in liquid A1 media. Standard bacterial assays were conducted in a 96-well plate format. The crude extract was partitioned between chloroform and 30% methanol/water followed by repeated Si-gel medium pressure liquid chromatography (MPLC) and reverse phase high pressure liquid chromatography (HPLC). Structures were determined by NMR and LC-MS. Bioassay-guided fractionation yielded caulerpin and two derivatives that significantly promote the growth of *Vibrio* sp. (50% above control) below natural concentration (1.8 ug/mL). This is the first report that caulerpin promotes the growth of *Vibrio* sp. Caulerpin has been reported to deter fish feeding and exhibit moderate antimicrobial activity in addition to a number of other biological activities. Further studies to describe *Vibrio* sp. recruitment to the surface of *C. sertularioides* by caulerpin metabolites are warranted.

P-195

ELUCIDATION AND EXPLOITATION OF THE BIOSYNTHESIS OF A NOVEL ANTIBIOTIC IN THE COLONIAL MARINE ALGA *CHRYSOPHAEUM TAYLORI*

Jack R. Davison, Sivakoteswara R. Mandadapu, Carole A. Bewley
Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD, 20892



chrysophaentin A

The chrysophaentins are a series of compounds isolated from the colonial marine alga *Chrysophaeum taylori*, collected in the US Virgin Islands. The compounds show good therapeutic potential because they have potent antibiotic activity against drug-resistant pathogenic bacteria, low eukaryotic cytotoxicity, and a target pathway not shared by any clinical antibiotic class. However, synthetic strategies to obtain a supply of the drugs have so far been unsuccessful. We aim to identify and exploit the biosynthetic pathway controlling chrysophaentin assembly to prepare a reliable supply of the antibiotics or advanced intermediates by synthetic biology. We propose that the chrysophaentins are produced from a phenylalanine-derived starter unit and a type III polyketide synthase, with the linear polyketide product oxidatively cyclized by a cytochrome P450. We will sequence the genome of a laboratory-cultivated strain of *C. taylori*, which we have found to produce low levels of chrysophaentins, and identify potential biosynthetic genes for characterization by heterologous expression.

P-196

EXPLORING GREAT LAKES ACTINOBACTERIAL GENOMES FOR NATURAL PRODUCT DISCOVERY AND DEVELOPMENT

Jana Braesel[1], Brian T. Murphy[1], Alessandra S. Eustáquio[1]
[1] University of Illinois at Chicago, College of Pharmacy, Department of Medicinal Chemistry and Pharmacognosy, Chicago, IL 60612

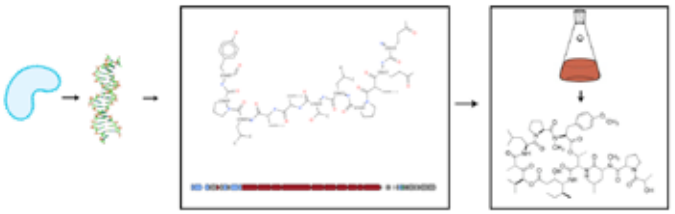
Actinomycetes are known for their ability to produce novel lead compounds of clinical and pharmaceutical importance. In contrast to their terrestrial and marine counterparts, little is known about the capacity of freshwater-derived actinomycete bacteria to produce novel secondary metabolites. The 7 Mb genome of the Great Lakes-derived actinomycete bacterium *Micromonospora* sp. B006 was sequenced using both Illumina technology and Oxford Nanopore. An analysis of the joint assembly with antiSMASH and BlastP revealed 18 secondary metabolite gene clusters, including non-ribosomal peptide synthetases, polyketide synthases, and terpenes. Only four of the predicted gene clusters could be linked to their respective products: the carotenoid sioxanthin, the siderophore desferrioxamine B, the antimicrobial alkaloid diazepinomicin, and the prenylated phenolic lipids alkyl-*O*-dihydrogeranyl-methoxyhydroquinones. The identity of the natural products encoded by the remaining 14 gene clusters remains cryptic. In addition, a putative diazaquinomycin biosynthetic gene cluster was identified using progressive MAUVE alignment and the genome of another diazaquinomycin producer. Diazaquinomycins H and J are the only secondary metabolites that have been isolated from *Micromonospora* sp. B006. We pursue heterologous expression and genetic engineering in the native host to link chosen gene clusters to their natural products.

P-197

ASSESSING THE BIOSYNTHETIC CAPABILITIES OF BACTERIA USING COMPUTATIONAL GENOMICS

Nicholas Lorig-Roach[1], Phillip Crews[1]
[1]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, 95064, USA

There are now over 7,000 bacterial genomes available in NCBI's repository, which represents an invaluable resource for the study of "genes to molecules," translating microbial genetics to new chemical diversity. Many classes of microbial natural products, such as polyketides, non-ribosomal peptides, or hybrid PKS–NRPS molecules, are produced in an assembly line fashion by enzymes with enough homology that their genes can be discovered using hidden-Markov model based search tools (*Nucl. Acids Res.* **2015**, *43*, W237). Here we annotate the biosynthetic gene clusters from a subset of the bacterial genomes available through NCBI (with an emphasis on Gram-negative bacteria available from large culture repositories) in an attempt inform future bacterial isolation efforts and gain insight into the regulation of cryptic gene clusters.



P-198

CYCLIC LIPODEPSIPEPTIDE PRODUCED BY A BACTERIAL STRAIN FROM *CECROPIA-AZTECA* SYMBIOSIS

Taise T. H. Fukuda[1], Cameron R. Currie[2], Jon Clardy[3], Mônica T. Pupo[1]
[1]School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo (FCFRP-USP) Ribeirão Preto, SP, Brazil. [2]Department of Bacteriology – University of Wisconsin, Madison, WI, USA. [3]Department of Biological Chemistry and Molecular Pharmacology – Harvard Medical School (HMS), Boston, MA, USA.

Microorganisms associated with social insects have been extensively studied as sources of new compounds with antimicrobial activity and there are no studies in the literature of the chemical ecology of the microorganisms associated with the myrmecophytic *Cecropia-Azteca* mutualistic interaction. A bacterial strain was isolated from the body of an *Azteca* ant, associated with a *Cecropia* tree, collected at Itatiaia National Park, RJ, Brazil. In pairwise interactions in Petri dishes, this strain showed inhibition properties against *Staphylococcus aureus*, a gram-positive human pathogen, and *Pestalotiopsis clavispora*, a phytopathogenic fungus isolated from *Cecropia* leaves. In order to understand these interactions, the supernatant of the liquid culture of the isolated strain was extracted with ethyl acetate and purified in semi-preparative HPLC furnishing 3.3 mg of a pure compound. 1D and 2D NMR spectra showed signals corresponding to a structure of a cyclic lipodepsipeptide (CLP), such as amide protons (δ 6.61-9.23), α-amino acid protons (δ 3.44-4.57) and an alkyl chain (δ 1.55-0.80). The depsipeptide sequence was determined as Leu-Gln-Thr-Val-Leu-Ser-Leu-Ser-Ile, attached to a β-hydroxy decanoic acid, and this structure was supported by MALDI-MS/MS data. CLPs consist of oligopeptides with cyclization involving a lactone bond between the C-terminus of an amino acid and the alcohol group of a side chain. Several CLPs reported in the literature pres-

ent uncommon or modified amino acids, such as D-amino acids and show a great variety of different biological activities.

P-199

ACTINOBACTERIA ASSOCIATED WITH *ACROMYRMEX* ANTS AS SOURCE OF BIOACTIVE COMPOUNDS

Carla Menegatti[1], Weilan G. da P. Melo[1], Bárbara M. do Prado[1], Cameron R. Currie[2], Adriano D. Andricopulo[3], Jon Clardy[4], and Mônica T. Pupo[1]
[1]School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil, [2]Department of Bacteriology, University of Wisconsin, Madison, WI, USA, [3]Physics Institute of São Carlos, University of São Paulo, São Carlos, SP, Brazil, [4]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Microorganisms and insects are involved in complex relationships and some novel and bioactive natural products have been identified from insect microbial symbionts. One of the best-known symbiotic associations was established over 50 million years between Attini ants and fungi cultivated by them for food. 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 Itatiaia National Park in Brazil. We have screened bacterial strains against the specific fungus *Escovopsis* and the parasites *Trypanosoma cruzi* and *Leishmania donovani*, and selected actinobacteria MP151013-2 for further studies since it showed antifungal activity and its ethyl acetate extract displayed antiprotozoal activity against *T. cruzi* (63% inhibition) and *L. donovani* (83% inhibition). The bioguided fractionation of ethyl acetate extract by SPE and HPLC led to the isolation of two analogous compounds. Analyses of HR-ESI-MS data, 1D and 2D NMR data, and searches on databases, allowed the structure determination of compound **1** $[M-H]^-$ 1181.5 and compound **2** $[M-H]^-$ 1209.6 as the antibiotics chromomycins A_3 and A_2, respectively.

P-200

A *STREPTOMYCES TENDAE* SPECIALIZED METABOLITE INTERFERES WITH QUORUM SENSING IN GROUP A STREPTOCOCCUS

Vanessa M. Nepomuceno[1,2], Tiara Perez-Morales[1], Michael Federle[1], Brian T. Murphy[1,2]
[1]Center for Biomolecular Sciences, [2]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL

Quorum Sensing (QS) is a process where bacteria produce, secrete, and detect chemical signals that trigger specific phenotypic responses including competence, antibiotic production, biofilm formation, and secretion of virulence factors. In group A *Streptococcus* (GAS), this cross-talk between bacteria is believed to play a role in the regulation of virulence. Therefore, finding a natural product regulator of QS in GAS may aid in understanding and manipulating this "switch". A family of transcriptional regulators, the Rgg proteins, have been shown to exhibit regulatory activity on pathogenic behaviors in bacteria, such as lysozyme resistance and biofilm development. Thus, inhibition of Rgg may offer a way to regulate these behaviors. To discover small molecule modulators of QS, a high-throughput luciferase assay was used to screen our actinomycete specialized metabolite fraction library to identify natural product inhibitors of Rgg. A potential QS inhibitor has been identified from a *Streptomyces tendae* strain. The strain, D051, was cultivated in a 28 L fermentation that yielded 1.7 grams of crude extract. Consecutive rounds of chromatographic separation were used to isolate four milligrams of the bioactive molecule from the crude material. High resolution mass spectrometry (HRMS) along with nuclear magnetic resonance spectroscopy (NMR) is currently being employed to elucidate the structure of the compound. Successful structural elucidation allows the use of this molecule as a molecular probe to understand QS mechanisms within GAS.

P-201

SNM55F-I, ANTIANGIOGENIC PEPTIDES FROM AN INTERTIDAL MUDFLAT ACTINOMYCETE

Munhyung Bae[1], Jedo Oh[1], Lee Sang Kook[1], Jongheon Shin[1], Dong-Chan Oh[1,]*
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, 1Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea

A continuous chemical study of the mohangamide producer (*Streptomyces* sp. SNM55) led to the discovery of four new peptides, SNM55F-I, belonging to the WS9326 class. The planar structures of SNM55F-I were established by 1D and 2D NMR, mass, UV, and IR analyses. These compounds bear 6~7 amino acid units with a unique acyl chain. Interestingly, SNM55I incorporates an unprecedented 4-amino-2,4-dihydro-3H-pyarzol-3-one and a sugar-derived moiety. The structure of the pyrazole-derived ring was further confirmed by comprehensive analysis of the ^{15}NHSQC spectrum of SNM55I. In addition, the analysis of COSY and HMBC spectra after selective hydrogenation of the carbon-nitrogen double bond in the ring by using $NaBH_3CN$ clearly supported the structure of 4-amino-2,4-dihydro-3H-pyarzol-3-one. The absolute configurations of amino acids of SNM55F-I were established by advanced Marfey's analysis. The determination of the absolute configuration of SNM55I is in progress. SNM55F-H displayed significant antiangiogenic activity with moderate cytotoxicity against human cancer cells.

P-202

A STRUCTURALLY-INTRIGUING NATURAL PRODUCT PRODUCED BY *METHYLOBACTER TUNDRIPALUDUM*, A METHANE-OXIDIZING BACTERIUM

Emily Mevers[1], Aaron Puri[2], Mary Lidstrom[2], Jon Clardy[1]
[1] Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115; [2] Department of Chemical Engineering, University of Washington, Seattle, WA 98105

Methylobacter tundripaludum 21/22 is an aerobic methane-oxidizing gammaproteobacterium that was originally isolated from the sediment of Lake Washington in Seattle, WA. Upon recent completion of the genome sequencing of this model organism, it revealed a potential to produce several distinct small molecules. One of the biosynthetic gene clusters (BGCs), a putative peptide synthetase cluster, appears to have a role in quorum sensing based on quorum sensing genes, including *mbal* (a *luxl* family member), flanking the putative cluster. This cluster is conserved among all *M. tundripaludum* strains, thus indicating the product of this cluster likely plays an important ecological role. Mass spectrometry based comparative metabolomic analysis between the wild-type and Δ*mbal* mutant strains revealed the product of this BGC has a molecular ion of *m/z* 421.1862, suggesting a molecular formula of $C_{22}H_{28}O_8$. Subsequent large-scale fermentation, targeted isolation, and traditional NMR-based structure determination elucidated the planar structure of this small molecule. Interestingly, it contains a novel carbon skeleton structure that incorporates the fully intact carbon-backbone of chorismic acid but has been uniquely tailored and extended. The function of this molecule is still unknown but it potentially has a role extracellular electron transport as part of a mechanism to reach redox homeostasis.

P-203

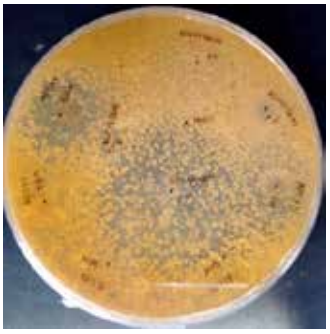
STREPTOMYCES STRAINS ASSOCIATED WITH FUNGUS-GROWING ANTS AS SOURCE OF BIOACTIVE COMPOUNDS

Ana L. Leandrini de Oliveira[1], Weilan G. P. Melo[1], Barbara M. do Prado[1], Adriano D. Andricopulo[2], Cameron R. Currie[3], Jon Clardy[4], Mônica T. Pupo[1]

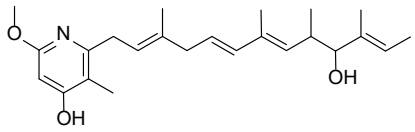
[1]School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil. [2]Institute of Physics of Sao Carlos, University of Sao Paulo, Brazil. [3]University of Wisconsin-Madison, USA. [3]Harvard Medical School, USA.

This work is part of an International Cooperative Biodiversity Group (ICBG) highly focused on the discovery of novel therapeutic agents from bacterial symbionts of Brazilian invertebrates. *Streptomyces* strains ICBG298 and ICBG233, which have been found as active against primary assays using the pathogenic fungus *Escovopsis* sp. and the parasites *Trypanosoma cruzi* and *Leishmania donovani*, were isolated from Attine ants, cultured, organic extracts were prepared and compounds were isolated using chromatographic methods. Besides the compounds **1**, **2** and **3**; several peptide compounds were isolated and are under structure elucidation. All isolated compounds were assayed against *Escovopsis* sp., as shown on Figures **A**: fractions and peptide compounds; and **B**: fractions and compounds **1**, **2** and **3**. In addition, these compounds will be evaluated against *T. cruzi* and *L. donovani*.

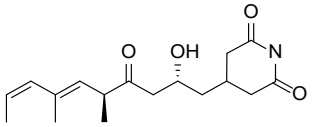
A



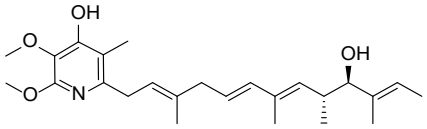
B



1



2



3

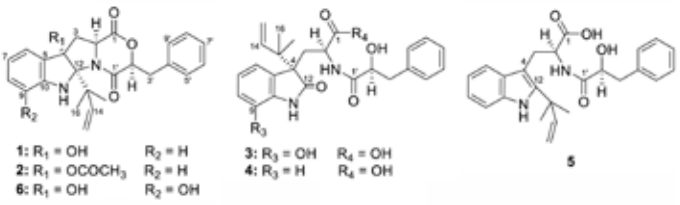
P-204

DIOXOMORPHOLINES AND DERIVARIVES FROM A MARINE-FACULTATIVE *ASPERGILLUS SP.* (MEXU 27854)

Manuel A. Aparicio-Cuevas and Mario Figueroa

Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México 04510, México.

Two new dioxomorpholines **1** and **2**, three new derivatives **3–5**, and the known compound shornephine A (**6**) were isolated from a marine-facultative *Aspergillus* sp. (MEXU 27854). Their structures were established by 1D- and 2D-NMR and HRESIMS data analysis. Absolute configuration of **1** and **2** was elucidated by comparison of experimental and DFT-calculated VCD spectra. Compounds **3**, **5** and **6** were non-cytotoxic on a panel of human cancer cell lines with different functional status for the tumor-suppressor protein p53, but worked as inhibitors of P-glycoprotein by reversing multidrug resistance in a doxorubicin-resistant cell line.



1: R_1 = OH R_2 = H
2: R_1 = $OCOCH_3$ R_2 = H
6: R_1 = OH R_2 = OH

3: R_3 = OH R_4 = OH
4: R_3 = H R_4 = OH

5

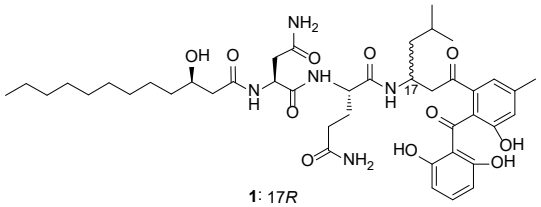
P-205

ASPERPHENINS A AND B, LIPOPEPTIDYL BENZOPHENONES FROM A MARINE-DERIVED *ASPERGILLUS* SP. FUNGUS

Lijuan Liao[1], Song Yi Bae[1], Tae Hyung Won[1], Minjung You[1], Seong-Hwan Kim[1], Dong-Chan Oh[1], Sang Kook Lee[1], Ki-Bong Oh[2], and Jongheon Shin[1]

[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea, [2]Department of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

Asperphenins A (**1**) and B (**2**), novel diastereomeric lipopeptidyl benzophenone metabolites, were isolated from a marine-derived *Aspergillus* sp. fungus. Based on the results of combined spectroscopic analyses, the structures of these compounds were determined to be linear assemblies of three motifs: a hydroxy fatty acid, a tripeptide and a trihydroxybenzophenone. The absolute configurations were assigned using chemical modifications and ECD calculations. The novel compounds exhibited significant cytotoxicity on diverse cancer cells in vitro.



1: 17*R*
2: 17*S*

P-206

INVESTIGATION OF BACTERIA FROM THE *TRACHYMYRMEX SEPTENTIONALIS* FUNGUS GARDEN AS POTENTIAL ANTIBACTERIAL DRUG LEADS

Brendan P. Stewart[1], Rofina Johnkennedy[2], Jonathan L. Klassen[2], and Marcy J. Balunas[1,]*

[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269 USA. [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269 USA

Trachymyrmex septentrionalis is a leaf-cutter ant species in the tribe Attini that grows a cultivar fungus as its primary food source. This cultivar fungus garden is susceptible to microbial pathogens and numerous bacterial strains reside within the cultivar fungus, hypothesized to protect against these pathogens. Our ongoing research has shown that many of the bacterial strains isolated from these fungus gardens possess the biosynthetic capacity to produce secondary metabolites that may protect the cultivar fungus. Following initial screening of fungus garden bacterial extracts, we selected two bioactive strains, *Pseudomonas fluorescens* and *Delftia tsuruhatensis*, for continued investigation. Both strains were isolated from a colony collected from Long Island, NY and both were cultured, extracted, and tested in a suite of antimicrobial assays. Chromatographic and spectroscopic techniques were utilized to isolate and identify the predominant biologically active compounds within each bacterial extract and putative structures of these antimicrobial compounds will be presented. Further studies will be performed to determine general cytotoxicity of these compounds, prioritizing compounds with selective antimicrobial activity and correspondingly low toxicity as potential leads for future novel antibacterial drugs.

P-207

ANTI-*CRYPTOCOCCUS* METABOLITES FROM COPROPHILOUS FUNGI

Nicole M. Krausert[1], Dinith R. Jayanetti[1], Yan Li[2], Qun Yue[2], Gerald F. Bills[2], and James B. Gloer[1]

[1]Department of Chemistry, University of Iowa, Iowa City, IA, 52242, USA, [2]Texas Therapeutic Institute, The Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston, Houston, TX, 77054, USA

Cryptococcus species are among the most common causes of invasive fungal infections worldwide. Current treatments still rely on a limited number of antifungal agents discovered many years ago (e.g., amphotericin B, azoles) which remain unsatisfactory because of their toxicity and the emergence of drug resistance. Natural products from fungi have been important in the development of antifungal agents (e.g., echinocandins), and other promising antifungal leads (e.g., sordarins, enfumafungin) have also originated from fungi. Historically, antifungal screening programs have centered mainly on *Candida* and *Aspergillus* spp., and there is still a need for treatments effective against these pathogens, but *Cryptococcus* spp., have been relatively neglected in such programs. We have initiated a *Cryptococcus*-centric exploration of fungi for antifungal natural products, with an emphasis on fungal sources that remain relatively unexplored to date. Our earlier studies of rarely studied coprophilous (dung-colonizing) fungi afforded a variety of new metabolites with bioactivities that often included antifungal effects against competitor fungi, leading us to specifically target this niche group. Details of this approach will be presented, along with highlights of the isolation and identification of several metabolites with activity against *Cryptococcus neoformans* from coprophilous fungal sources.

P-208

ISOCOUMARINS BASED ON A FUNGAL METABOLITE WITH MOSQUITO LARVICIDAL ACTIVITY

Kumudini M. Meepagala[1], Alden Estep[2,3] and James J. Becnel[2]

[1]USDA-ARS, Natural Products Utilization Research Unit, University, MS 38677, USA.

[2]USDA, ARS, CMAVE, 1600 S. W. 23rd Drive, Gainesville, FL 32608, USA.

[3]Navy Entomology Center of Excellence, NASJAX, Jacksonville, FL 32212, USA.

Aedes mosquitoes are the primary vectors of transmission of Zika virus. As part of continuing joint efforts between USDA and DWFP (Deployed War Fighter Protection) program of Department of Defense, secondary metabolites produced by plant pathogenic fungi and synthetic analogs of natural products were investigated for mosquito control. *Diaporthe eres*, a plant pathogenic fungus, was isolated from infected *Hedera helix* leaves. This fungus was grown in czapek dox broth culture medium for 2 weeks. From the ethyl acetate extract of the culture filtrate, 3,4-dihydro-8-hydroxy-3,5-dimethylisocoumarin was isolated. The natural isocoumarin and its synthetic analogs were evaluated for mosquito larvicide activity against permethrin susceptible strains of mosquito larvae and permethrin sensitive strains of mosquito larvae. The natural isocoumarin was not very active as a larvicide, but the synthesized analogs were significantly active as larvicides. Several of these analogs showed larvicidal activity against a permethrin resistant Puerto Rican strain of *A. aegypti*, as well as the permethrin susceptible Orlando strain *A. aegypti*. These compounds were not active as topical mosquito adulticides. Isolation, synthesis and mosquito larvicidal activities of these isocoumarins will be discussed.

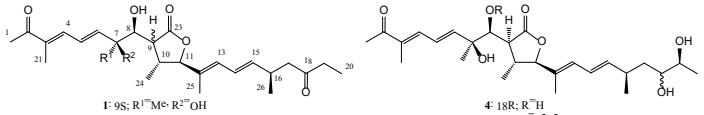
P-209

LINEAR LACTONE DERIVATIVES FROM THE MARINE-DERIVED FUNGUS *FUSARIUM OXYSPORUM*

Jung-Ho Yun[1], Dong-Chan Oh[1], Ki-Bong Oh[2], and Jongheon Shin[1]

[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-742, Korea, [2]Department of Agricultural Biotechnology, College of Agriculture & Life Science, Seoul National University, San 56-1, Sillim, Gwanak, Seoul 151-921, Korea

Six new linear lactones **1**-**6** were isolated from the fermentation broth of the fungus *Fusarium oxysporum* (strain number FF077) obtained from the sponge collected from Geomun-Do, Korea. The structures of these new compounds were determined by combined spectroscopic and chemical analyses. The relative and absolute configurations were also assigned by *J*-based analysis, MTPA method, and CD measurements. The sugar residue was determined to be β-D-mannose by glycolysis and LC analysis.



1: 9S; R1=Me, R2=OH
2: 9S; R1=OH, R2=Me
3: 9R; R1=Me, R2=OH
5: 9S; R1=Me, R2=O-β-D-Man

4: 18R; R=H
6: 18S; R=β-D-Man

P-210

A SYNERGISTIC RELATIONSHIP VITAL TO THE FRAMEWORK REARRANGEMENT IN GILVOCARCIN BIOSYNTHESIS

Redding Gober,[1] Shaimaa M. Salem,[1] Prithiba Mitra,[1] and Jürgen Rohr*[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky, USA

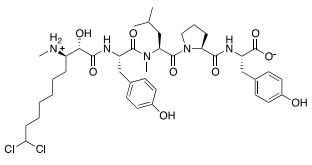
Gilvocarcins are characterized by uncommonly low side effects, which make them interesting potential drug candidates. The gilvocarcin group of anticancer antibiotics is characterized by the unique benzo[*d*]naphtho[1,2-*b*]pyran-6-one chromophore with D-fucofuranose at the 4-position. To achieve this chromophore, polyketide biosynthesis is carried out by a type II polyketide synthase (PKS), and a post-PKS enzyme complex is proposed to convert an angucyclinone intermediate (dehydrorabelomycin) into the defuco-gilvocarcin chromophore. In addition, three enzymes help to decorate the framework: GilOIII (vinyl group generation), GilMT (O-methylation) and GilGT (C-glycosylation). Most interesting are the components of a proposed post-PKS modifying enzyme complex, consisting of GilOII, GilM and GilR. While the basic functions of these enzymes have been determined, a more complete biochemical characterization is still needed to understand their interplay and substrate channeling. These key post-PKS modifications begin with a scaffold rearrangement directed by GilOII, which involves a 5-hydroxylation followed by a Baeyer-Villiger oxidation. GilM, an enzyme with dual functionality, then catalyzes the reduction of a naphthoquinone intermediate to a hydroquinone and subsequently facilitates an O-methylation and hemiacetal formation. GilM has a synergistic relationship with GilR, an oxidoreductase that establishes the lactone core of the gilvocarcins, in which FAD is regenerated. In order to thoroughly understand the interrelationships of these post-PKS modifying enzymes, we attempt to obtain crystal structures and explore substrate specificities.

P-211

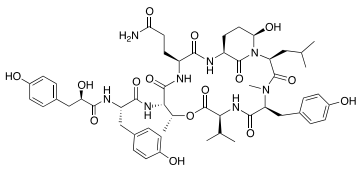
SECONDARY METABOLITES FROM TWO *MICROCYSTIS* BLOOM MASSES COLLECTED IN ISRAEL

Shmuel Carmeli, Anat Lodin-Freedman, and Rawan Hasan
School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

Cyanobacteria, in general, and water bloom-forming genera of cyanobacteria, in particular, are rich source of biologically active natural products. As part of our ongoing research on the chemistry and chemical ecology of cyanobacteria blooms in Israeli water bodies we recently investigated the chemical content of two *Microcystis* spp. bloom biomasses (TAU IL-405 and IL-428) collected in November 2009 and August 2013 from water reservoirs in Israel. The crude extract of the toxic biomass TAU IL-405 afforded 18 new microginins, anabaenopeptins and aeruginosins and 11 known metabolites including microcystin RR, [D-Asp[7]]microcystin RR, microcystins WR, cyanopeptolin S and microppetin KT946. The crude extract of the nontoxic biomass TAU IL-428 afforded two new protease inhibitors, micropeptin TR1058 and aeruginosin TR642. Here we shall present new data on the structure elucidation and inhibition of proteolytic enzymes of the new secondary metabolites.



microginin KR835



micropeptin TR1058

P-212

OPTIMIZATION OF CULTURE CONDITIONS AND BIOREACTOR DESIGN FOR IMPROVED LIPOPEPTIDE PRODUCTION FROM *B. SUBTILIS*

David M. Wright[1], Michael P. Bralkowski[2], Sarah Albertson[2], Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC 27402

[2]Global Bioprotect, High Point, NC 27263

Synthetic surfactants made from a suitable hydrophobe and ethylene oxide have been produced for decades with properties of emulsification and detergency that are unparalleled. However, traditional surfactants are unsuitable for use in medical applications such as pulmonary surfactants to prevent respiratory distress syndrome (RDS). This condition affects approximately 40,000 infants in the US alone each year. RDS is a common diagnosis accounting for 70% of low birth weight infants (< 1500 g). There exists a need to find alternative surfactants to fulfill niche applications. The lipopeptides produced from several strains of Bacillus subtilis have demonstrated the ability to reduce surface tension as well as show efficacy against several phytopathogens. We seek to improve upon the production of surfactin lipopeptides through careful control of culture parameters and an improved bioreactor design using combined static and dynamic oxygenation.

P-213

FUNGAL CO-CULTURES DERIVED FROM THE OCTOCORAL *EUNICEA FUSCA* AS A SOURCE OF NOVEL ANTIMICROBIALS

Logan MacIntyre[1], Bradley Haltli[1], David Overy[2], Douglas Marchbank[3], Hebelin Correa[3] and Russell Kerr[1].
[1]Department of Biomedical Sciences & [2]Department of Pathology and Microbiology, University of Prince Edward Island, Charlottetown, PE, Canada and [3]Nautilus Biosciences Inc., Charlottetown, PE, Canada

Fungal co-cultures have been shown to activate and upregulate silent biosynthetic gene clusters within their constituent organisms, which facilitates detection and isolation of the corresponding cryptic natural products. With the aim of identifying novel cryptic natural products possessing antimicrobial properties, a collection of seven fungal isolates from the Caribbean octocoral *Eunicea fusca* were grown in every possible pairwise co-culture and examined in complementary metabolomic and antimicrobial studies. Extracts from all co-cultures and respective pure cultures were compared by UPLC-HRMS to identify *de novo* induced metabolites (i.e. those present in a given co-culture but absent in respective pure cultures) and then screened for bioactivity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Candida albicans*. The detailed results of this project will be presented in addition to the description of a novel cyclic lipopeptide produced in pure culture that was discovered over its course.

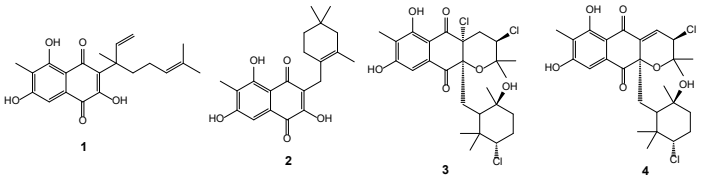
P-214

CHEMICAL INVESTIGATION OF THE DEEP-WATER SEDIMENT-DERIVED *STREPTOMYCES* SP. CP55-76

Scott E Campit,[1] Walter M. Bray,[2] R. Scott Lokey,[2] Frederick A. Valeriote,[3] Taro Amagata[1]
[1]Department of Chemistry & Biochemistry, San Francisco State University, San Francisco, California 94132, [2]Department of Chemistry & Biochemistry, University of California, Santa Cruz, Santa Cruz, California 95064, [3]Division of Hematology & Oncology, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan 48202

As part of our program to identify novel cytotoxic compounds with excellent solid tumor selectivity, we have applied an extract library of acti-

nomycetes separated from marine environments to two kinds of cytotoxic screening systems, HeLa cell-based cytological profiling (CP) and anticancer disk diffusion assay (DDA). The organic extract of the *Stereptomyces* sp. CP55-76 separated from deep-water sediment (-900 m) collected in Santa Monica Basin showed significant cytotoxic effect against HeLa cells and selective cytotoxicity against the human prostate adenocarcinoma cell line (LNCaP), identified by CP and DDA, respectively. Detailed analysis of the secondary metabolites produced by the strain CP55-76 led to the isolation of a new meroterpenoid (**1**) together with three known naphthoquinone meroterpenoids (**2** - **4**). The structure of the new compound was elucidated based on comprehensive 1D and 2D NMR analysis. The cytotoxic effects of the isolated compounds were evaluated.



P-215

ANTIMICROBIAL 1, 4-NAPHTHOQUINONE DERIVATIVES FROM A PALYTHOA-SYMBIOTIC FUNGUS, *FUSARIUM SOLANI*

Chih-Chuang Liaw*[1,2], Po-Yi Cheng[1], Li-Hua Lo[1], and Mo Aqib Raza Khan[1]
[1]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, 70 Lienhai Rd., Kaohsiung 80424, Taiwan,* [2]*Doctoral Program of Marine Biotechnology, National Sun Yat-sen University, 70 Lienhai Rd., Kaohsiung 80424, Taiwan*

The rapid emergence of resistant bacteria becomes a substantial crisis to threaten humans' health benefits. Many research efforts were performed to search novel agents active against resistant bacteria. In addition, marine natural products with structural diversity provide a novel insight into developing potential and useful anti-microbial agents. We tried to screen marine microbes as a source to isolate antibacterial compounds. We isolated a symbiotic fungus from a *Palythoa* sp. and identified as *Fusarium solani* FS-01 by ITS sequencing analysis. Interestingly, the reddish acetyl acetate (EtOAc) extract of FS-01 cultured in the thick medium showed inhibitory effects against *Acinetobacter baumannii* and *Bacillus cereus*, while the yellow EtOAc one in the thin medium only showed the inhibitory effect against *B. cereus*. Based on the molecular networking analysis and the anti-microbial activity-guided fractionation and HPLC isolation, we isolated two new 1,4-naphthoquinone derivatives (**1** and **2**), as well as eight known compounds from the reddish EtOAc extract. The structures of these isolates were elucidated by 1D and 2D NMR and MS data. The anti-microbial activity of these isolates will be presented in the poster.

P-216

COMBINED USE OF DESIGN OF EXPERIMENTS AND METABOLIC ENGINEERING TO ENHANCE PRODUCTION OF NOGALAMYCIN

S. Eric Nybo[1] and Minji Sohn[1]
Department of Pharmaceutical Sciences, Ferris State University, Big Rapids, MI 49307.

Nogalamycin is an anthracycline polyketide with topoisomerase I inhibitory activity. In this work, we describe a combined statistics-driven design-of-experiments (DOE) and metabolic engineering approach for enhancing production of nogalamycin. In the first place, we employed a systematic one-factor-at-a-time (OFAT) experimental design to optimize carbon sources and nitrogen sources for inclusion in a baseline production medium. In the second place, we enhanced nogalamycin production via precursor engineering. To simplify the cloning, we developed BioBricks tools for use in *Streptomyces nogalater.* To enhance carbon flux to malonyl-CoA, we designed constructs overexpressing components of the acetyl-CoA carboxylase. To enhance carbon flux to TDP-deoxysugar donors, we overexpressed NDP-glucose synthase and NDP-4,6-dehydratase enzymes. Lastly, we overexpressed the *snorA* regulatory protein to augment transcription of the nogalamycin biosynthetic pathway. We propose that this study provides a general approach towards systematically enhancing production of important polyketide antibiotics. This combined experimental approach could be beneficial when the underlying philosophy underpinning fermentation medium design or rate-limiting metabolic steps is unknown.

P-217

PROSPECTING FOR BIOACTIVE COMPOUNDS FROM SOIL ACTINOMYCETES OBTAINED FROM AN EARLY ROMAN ARCHEOLOGICAL SITE

Angela Hoffman[1], Edward Valente[1]
[1]*Department of Chemistry, University of Portland, 5000 N Willamette Blvd, Portland OR 97203*

Many useful bioactive natural compounds may be isolated from bacteria and fungi and are proving to be useful antibiotics. A wide variety of bacteria and fungi have been isolated from soils at the early Roman village of Pollentia near Alcudia on the Island of Mallorca (Spain). Bioassay-guided fractionation is being used to isolate and identify potentially useful components with activity against gram negative and gram positive bacteria as well as the oomycete *Pythium ultimum*. Since these soils are as early as 123 BC, some compounds and the organisms that make them may be new to science and may present potentially useful activities.

P-218

DISCOVERY OF MARINE NATURAL PRODUCTS WITH ACTIVITY AGAINST REPLICATING AND DORMANT *MYCOBACTERIUM TUBERCULOSIS*

Carolina Rodrigues Felix[1], Rashmi Gupta[1], Sandra Geden[1], Jill Roberts[2], Priscilla Winder[2], Shirley A. Pomponi[2], Maria Cristina Diaz[2], John, K. Reed[2], Amy E. Wright[2], Kyle H. Rohde[1]
[1]*Division of Immunity and Pathogenesis, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 6850 Lake Nona Blvd. Orlando, FL 32827 USA,* [2]*Harbor Branch Oceanographic Institute, Florida Atlantic University, 5600 US 1 North, Fort Pierce, FL 34946 USA*

Tuberculosis (TB) is one of the leading causes of death by an infectious disease worldwide. The World Health Organization estimates that in 2015, 10.4 million people fell ill from TB while 1.8 million people died of TB. Latent infection with *M. tuberculosis* (*Mtb)* of an estimated 2 billion people coupled to the lack of an effective vaccine hinders attempts to eradicate this disease. The dormant phenotype poses a major challenge in disease treatment since these bacilli are tolerant to front-line drugs. Discovery of agents active against the dormant forms is therefore a high priority. In this project a library of 4,400 enriched marine-derived natural product fractions was assayed against dual-fluorescent *Mtb* under both replicating and non-replicating conditions. Active fractions were further tested for cytotoxicity against the J774 macrophage. This screening program identified 62 non-cytotoxic fractions with activity against replicating *Mtb* and 19 non-cytotoxic fractions with activity against dormant *Mtb.* Hits were further deconvoluted to identify unique pharmacophores active in each screening model. The structures of five pure active compounds were defined by spectroscopic methods. The purification, structure elucidation and biological activity of the active compounds will be presented.

P-219

CHEMOGEOGRAPHICAL ANALYSIS OF A MARINE CYANOBACTERIUM LEADS TO THE DISCOVERY OF A NOVEL SECONDARY METABOLITE

Christopher A. Leber[1], C. Benjamin Naman[1], Lena Keller[1], Eduardo J. E. Caro-Diaz[1], Tiago F. Leão[1], Nathan A. Moss[1], and William H. Gerwick[1]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

The marine benthic filamentous cyanobacterium *Moorea bouillonii* belongs to a genus that is a prolific source of biologically active natural products. This organism, and its associated shrimp symbiont, is widely distributed across the western tropical Pacific and Indian Oceans. However, *M. bouillonii* metabolites have only been described from collections made in a limited array of locations, including Papua New Guinea, Guam, Palau, Palmyra Atoll, the Red Sea, and southern Japan. These collections differed substantially in their metabolite compositions, yielding novel compounds in each case and suggesting that additional metabolites could be accessed through the study of other geographical areas. A novel software tool for the **R**elational **C**omparative **A**nalysis **(o*RCA*)** of LC-MS samples was designed and implemented to determine the chemical similarity of *M. bouillonii* samples from disparate locations. This analysis revealed a strong clustering of samples collected from the same areas, and highlighted a particular MS feature that was present in high abundance in all *M. bouillonii* samples from Saipan, but could be found in little or no abundance in samples from other locales. Targeted isolation and structure elucidation identified this new metabolite, which possesses unusual structural moieties that are suggestive of interesting biosynthetic processes. Structure, absolute configuration, biological properties, and a partial putative biosynthetic gene cluster will be presented.

P-220

TOTAL SYNTHESIS OF NARANJAMIDE AND ANALOGUES FOR EVALUATION AGAINST *P. FALCIPARUM* AND *T. CRUZI*

Kh. Tanvir Ahmed,[1] Laura Pineda,[2] Carmenza Spadafora,[2] and Kevin J. Tidgewell[1]
[1] Division of Pharmaceutical Sciences, School of Pharmacy, Duquesne University, 600 Forbes Ave. Pittsburgh, PA, 15282, USA, [2] Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT-AIP), City of Knowledge , Apartado 0816-02852, Panama City, Panama,

As part of the Panama ICBG efforts to discover novel compounds for the treatment of tropical parasitic diseases, a cyanobacterial extract shown to have activity against the *Trypanosoma cruzi* and *Plasmodium falciparum* parasites was pursued. Naranjamide, an *N*-methylated peptide, was isolated from this orange Panamanian cyanobacterium collected in the Portobelo National Park. The activity of naranjamide was determined to be 79.6% inhibition of growth against *P. falciparum* and 81.51% inhibition against *T. cruzi*. The structure was elucidated using NMR and MS/MS. A total synthesis of naranjamide was undertaken to confirm stereoconfiguration and to provide additional material for mechanistic studies. The synthesis was completed in 7 overall steps with the longest linear path being 6 steps. The overall yield was 4.1% and has been run on milligram scale but is amenable to scale-up. In addition to synthesizing the natural product, a number of analogues with altered methylation, altered peptide sequence, and altered number of amino acids incorporated was also conducted. Based on this preliminary structure activity relationship study, we are planning synthesis of additional analogues and related compounds that might allow us to tune the activity towards either *P. falciparum* or *T. cruzi.*

P-221

A LIPM-LIKE OCTANOYLTRANSFERASE INITIATES MALYNGAMIDE BIOSYNTHESIS IN MARINE CYANOBACTERIUM *OKEANIA HIRSUTA*

Nathan Moss[1], Tiago F. Leão[1], Thomas Bartholow[2], Michael Burkart[2], Lena Gerwick[1], and Bill Gerwick[1,2]
[1]Scripps Institution of Oceanography, University of California, San Diego, CA 92037. [2]Department of Chemistry and Biochemistry, University of California, San Diego, CA 92037. [3]Skaggs School of Pharmacy, University of California, San Diego, CA 92037.

There are 38 characterized malyngamide analogs, distinguished by a 14-C lyngbic acid tail and either a cyclohexyl (type A) or pyrollidinone (type B) head group. Methylations, oxidations, and desaturations of the head group discern analogs, with diverse bioactivity profiles reported. Malyngamides have been isolated pan-globally, primarily from cyanobacterial assemblages, but also sea hares and macroalgae. Genome sequencing paired with stable isotope-labeled feeding studies have revealed the presence of two type A malyngamide PKS/NRPS pathways within cultured cyanobacterium *Okeania hirsuta*. NMR and MS/MS comparison to pure compound standards revealed the pathway products to be malyngamide C acetate and malyngamide I. Interestingly, both clusters feature an unorthodox initiation scheme which utilizes an octanoyltransferase (OT) closely related to bacterial lipoic acid scavenging enzyme lipM to selectively transfer an octanoate moiety onto the first KS module. We will demonstrate this selectivity via two methods: OT-acyl carrier protein (acpP) crosslinking studies, indicating the OT preference for C8-bound acpP, as well as GC/MS and LC/MS analysis of purified OT bound to octanoate, indicating transfer of the C8 moiety to the OT. These findings highlight a unique method by which *Okeania* have evolved to shuttle C8 fatty acid starter units toward secondary metabolism.

P-222

AN UNPRECEDENTED NON-RIBOSOMAL DEPSIPEPTIDE ISOLATED FROM MULTIPLE SOUTH AFRICAN TUNICATES

David Gallegos[1], Xuemei Wan[1], Jeffrey Serrill[1], Jane Ishmael[1], Shirley Parker-Nance[2], Eric Schmidt[3], Kerry McPhail[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA, [2]SAIAB and Nelson Mandela Metropolitan University, Eastern Cape, South Africa, [3]Deparment of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT, 84112, USA

Investigations into new and archived tunicate collections from Algoa Bay, South Africa, have revealed a group of didemnid tunicates with an unusual gelatinous morphology similar to *Lissoclinum mandelai*, producer of the mandelalide class of compounds. Using a bioassay-guided isolation approach, a new "gelatinous didemnid" species, which is in the process of being taxonomically characterized, has yielded the previously discovered natural product vitilevuamide. This compound was never fully characterized presenting the opportunity for a complete determination of its absolute stereochemistry. The pure compound inhibited multiple cancer cell lines with low nanomolar IC50 values and demonstrated differential cancer cell line selectivity towards the SK-N-MC Ewing's sarcoma cell line with an IC50 of 220 picomolar. Vitilevuamide is also known to act as a microtubule polymerization inhibitor and was demonstrated to be 3 fold more potent than paclitaxel against Ewing's sarcoma cells. An NRPS (non-ribosomal peptide synthetase) gene cluster encoding for the production of vitilevuamide has been discovered from the DNA of a bacterial alpha-proteobacterial symbiont isolated from the tissue of the source tunicate. We propose that this compound may be the first example of a lanthionine-containing compound that is biosynthesized non-ribosomally. Additionally, vitilevuamide has been isolated from four different tunicates in multiple geographi-

cal locations, indicating its production by a bacterial symbiont rather than the host animal. The re-discovery of this exciting natural product provides justification for our continued investigation of unique, endemic didemnid tunicates from South Africa as a source of new macrocyclic natural products with cytotoxic, anti-viral or antimicrobial activity.

P-223

ANALYSIS OF SECONDARY METABOLITES FROM CRYPTIC SPECIES OF *SARCOPHYTON GLAUCUM* IN PALAU USING STATISTICAL LEARNING

Elizabeth M. Maloney[1], Jason Chari[1], Ryan T. Botts[1], Taylor S. Davis[2], Oscar A. Alvarado[2], Charlie Brayton[3], Catherine S. McFadden[3], and Katherine N. Maloney[2]

[1]Department of Mathematics, Information, and Computer Sciences, Point Loma Nazarene University, San Diego, CA 92106, [2]Department of Chemistry, Point Loma Nazarene University, San Diego, CA 92106, [3]Department of Biology, Harvey Mudd College, Claremont, CA 91711

Sarcophyton glaucum is an abundant Pacific soft coral that has frustrated natural products chemists due to its idiosyncratic production of several bioactive cembranoid diterpenes. Recently, DNA sequence analysis has revealed that soft coral specimens previously identified as *S. glaucum* are actually members of seven genetically-distinct clades, representing cryptic species. We hypothesized that the observed variation in chemistry isolated from these corals can largely be explained by this distinction between clades. To assess this, we collected dozens of *Sarcophyton* soft corals in Palau, identified them to *S. glaucum* clade D or F using DNA sequence analysis, and performed gas chromatography on organic extracts of each coral specimen to obtain a chemical profile. We used both supervised and unsupervised machine learning algorithms including linear discriminant analysis and principal components analysis to evaluate both the extent to which these genetic differences can explain the observed differences in chemical profiles and how well chemical profiles can be used to distinguish between clades. We present the results of these analyses, which lend support to our hypothesis that the chemical composition of the two species can be explained by distinctions between species.

P-224

THE SEARCH FOR NOVEL BIOACTIVE ACTIOMYCETES FROM FRESH WATER HABITATS

Glenroy Martin, Taylor Harris and Oreoluwa Onabolu.
Department of Life and Physical Sciences, Fisk University, Nashville, TN 37208, USA

Actinomycetes are Gram-positive bacteria that are known to produce bioactive secondary metabolites that are useful in medicine. For over 50 years most of the drugs that were obtained were from terrestrial sources. However, with a decline in the number of new drugs from these sources the ocean was the next source of potentially new pharmaceuticals. The ocean has a rich untapped biodiversity and has been shown to produce new bioactive compounds. In this study, the diversity of cultivable marine sediment-derived microorganisms was examined and their potential as cytotoxic agents against microbial indicators and mammalian tumor cells was investigated. The microorganisms were inoculated on three different isolation media to enhance the diversity of cultivable microbes. Of all the microorganisms isolated, 11 exhibited antimicrobial activities. These 11 microorganisms were grown in large scale fermentations and the identification of the bioactive chemical entities from these extracts are currently under investigation.

P-225

DISCOVERY OF NOVEL ANTICANCER AGENTS FROM MARINE CYANOBACTERIA USING BIOASSAY-GUIDED AND NMR-GUIDED FRACTIONATION

Kara Spencer,[1] Valerie Paul,[2] and Hendrik Luesch[1]
[1]Department of Medicinal Chemistry, University of Florida, Gainesville, FL 32610. [2]Smithsonian Marine Station, Fort Pierce, FL 34949

Marine cyanobacteria are known to produce secondary metabolites that are both chemically diverse and biologically active among various applications. Lyophilized marine cyanobacteria were extracted and fractionated using liquid-liquid partitioning and chromatographic separation techniques. Bioassay-guided and NMR-guided fractionation are used to prioritize and isolate potentially novel anticancer compounds.

P-226

EXPLORING THE CARACOLAMIDES MOLECULAR NETWORK: ION CHANNEL MODULATORS FROM A PANAMANIAN CF. *SYMPLOCA* SP.

C. Benjamin Naman[1], Eduardo J. Caro-Diaz[1], Jehad Almaliti[2], Lorene Armstrong[3], Marsha L. Pierce[4], Evgenia Glukhov[1], Amanda Fenner[5], Carmenza Spadafora[5], Hosana M. Debonsi[3], Pieter C. Dorrestein[6], Thomas F. Murray[4], William H. Gerwick[1,6]
[1]Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093. [2]Department of Pharmaceutical Sciences, The University of Jordan, Amman, 11942, Jordan. [3]Departamento de Física e Química, Universidade de São Paulo, Campus Universitário, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil. [4]Department of Pharmacology, Creighton University School of Medicine, Omaha, NE 68178. [5]Instituto de Investigaciones Científicas y Sevicios de Alta Tecnología, Center of Cellular and Molecular Biology of Diseases, City of Knowledge, Panama 5, Republic of Panama. [6]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093.

Chemical profiling of extracts produced from ten morphologically identified *Symploca* spp. was achieved using LC-MS/MS and Molecular Networking untargeted metabolomics, which revealed several lead candidates for MS-based targeted natural product discovery. Accordingly, the secondary metabolites of the cyanobacterium cf. *Symploca* sp. PAB-6APR13-2, collected near Punta Caracol, Panama, were prioritized for further studies as a result of a unique composition containing chlorine atoms. Subsequent target-directed isolation efforts yielded one new acyl amide metabolite, caracolamide A (**1**), which was shown to have potent in vitro calcium influx and calcium channel oscillation modulatory activity. Compound **1** was synthesized in a three-step process for further pharmacological investigation, and several new analogues that were suggested by the molecular networking have also been generated.

P-227

ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL EVALUATION OF NOVEL CYTOTOXIC MACROCYCLIC DEPSIPEPTIDES FROM A MARINE CYANOBACTERIUM

Danmeng Luo[1,2], Valerie J. Paul[3], and Hendrik Luesch[1,2]
[1]Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610, United States, [2]Center for Natural Products, Drug Discovery and Development, University of Florida, Gainesville, Florida 32610, United States, [3]Smithsonian Marine Station, Fort Pierce, Florida 34949, United States

A novel cytotoxic macrocyclic depsipeptide was discovered from a collection of marine cyanobacterium from Loggerhead Key in Florida. Its struc-

ture was elucidated by detailed analysis of a combination of 1D/2D NMR spectroscopy and mass spectrometry. An intramolecular ester exchange was observed where the 26-membered macrocycle was transferred to a 24-membered compound (occurred at the 1,3-diols unit). This structural transformation was reversible and an equilibrium was considered to exist between these two interconvertible molecules. Both of the molecules displayed low nanomolar antiproliferative activity against HCT116 human colorectal carcinoma cells. The structural transformation from the 26-membered macrocycle to the 24-membered one caused a 7-fold decrease in activity (IC_{50} shifting from 1.79 nM to 12.74 nM). Currently we are investigating multiple aspects of their mechanisms of action and details of these studies will be presented.

P-228

FAGAALUAMIDE A-D, A SERIES OF HYBRID NRPS/PKS METABOLITES ISOLATED FROM CULTURED AND UNCULTURED MARINE CYANOBACTERIA

Yueying Li[1,2], C. Benjamin Naman,[1] Evgenia Glukhov,[1] Huashi Guan[2], William H. Gerwick[1]

[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA, [2]Key Laboratory of Glycoscience & Glycotechnology, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, P.R. China.

A series of hybrid PKS/NRPS metabolites, given the names fagaaluamide A-D, were isolated from two filamentous marine cyanobacteria (an America Samoa field collection and laboratory culture, respectively). The cultured cyanobacterium was grown under a light and temperature controlled environment and with semi-continuous dilution. The samples were harvested after several months of growth. and LC-MS/MS-based molecular networking and bioassay-guided methods, including cytotoxicity assay and calcium influx assay, were used for analyzing the extract and fractions from these two samples. Clusters with molecular ions at *m/z* of 603, 605, 617, and 647 were located in molecular networking diagrams, and three nodes (molecular ions at *m/z* of 603, 617, and 647) were found to share similar UV spectra with identical triple maximum absorptions around 270 nm, indicating the presence of conjugated trienes. Semi-preparative HPLC was used for the final purification of fagaaluamides A-D, and the corresponding structures were determined by detailed analysis of HRESIMS and 1D- and 2D-NMR. The configurations of the amino acids were identified using Marfey's analysis.

P-229

AMELIORATIVE EFFECT OF OSCARELLIN FROM *OSCARELLA STILLANS* ON ALLOXAN-INDUCED PANCREATIC ISLET DAMAGE IN ZEBRAFISH

Hye Been Choi[1], Tong Ho Kang[2], Hyung Sik Kim[1], Jong Hwan Kwak[1], Francis J. Schmitz[3]

[1]School of Pharmacy, Sungkyunkwan University, Suwon 16419, [2]Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University, Gyeonggi-do 17104, Korea, [3]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019, USA

A sponge collected at Honda Bay in Philippines was reported as a new species, *Oscarella stillans*, of the family Plakinidae (phylum Porifera, class Demospongiae, order Homosclerophorida). A new anthranilic acid derivative (**1**) was isolated from *O. stillans*. The structure of compound **1,** named oscarellin, was determined as 2-amino-3-(3'-aminopropoxy) benzoic acid from spectroscopic data and confirmed by synthesis. We examined the anti-diabetic activity of compound **1** in zebrafish model for type 1 diabetes. Type 1 diabetic zebrafish model was induced by alloxan, which cause pancreatic β-cell necrosis. Following alloxan treatment, pancreatic islet size and fluorescence intensity were measured. When compared to the alloxan-induced group, the pancreatic islet size in both the glimepiride- and oscarellin (**1**)-treated groups were significantly increased. Compound **1** treatment led to a greater increase in pancreatic islet size at concentration of 0.1 μM than that of glimepiride treatment as a positive control. Glucose uptake was evaluated in zebrafish treated with compound **1** and glimepiride (positive control) by detecting the uptake of 2-NBDG fluorescence within the pancreatic islets. Oscarellin (**1**)-treated group (at concentration of 0.1 μM) revealed significantly greater glucose uptake than that of the glimepiride-treated group. In conclusion, compound **1** showed potent anti-diabetic activity for type 1.

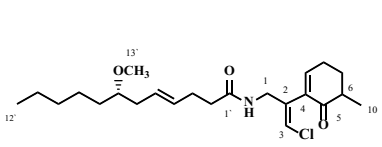
P-230

BIOLOGICALLY ACTIVE NEW METABOLITES FROM *MOOREA PRODUCENS*

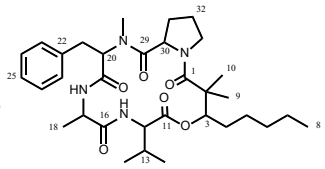
Omar M. Sabry[1,2], Douglas E. Goeger[1] and William H. Gerwick[1,3]

[1]College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, USA, [2]Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt, [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA.

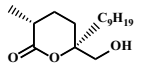
A bioassay guided investigation (cancer cell cytotoxicity) of a *Moorea producens* collection from Key West, Florida, led to the discovery of two novel bioactive natural products [(+)-malyngamide Y and a cyclic depsipeptide, (+)-floridamide]. Their structures were deduced through extensive analysis of 1D and 2D NMR spectroscopic data and supported by HRFAB mass spectrometry. The new cyclic depsipeptide contains four amino acids units, including *N*-methyl phenylalanine (*N*-MePhe), proline (Pro), valine (Val) and alanine (Ala), beside the unique unit, 2,2-dimethyl-3-hydroxy-octanoic acid (Dhoaa). In addition to the discovery of these two new compounds, two previously reported metabolites were also isolated and identified from this cyanobacterial collection; (-)-C-12 lyngbic acid and the antibacterial agent (-)-malyngolide.



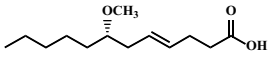
Malyngamide Y



Floridamide



Malyngolide



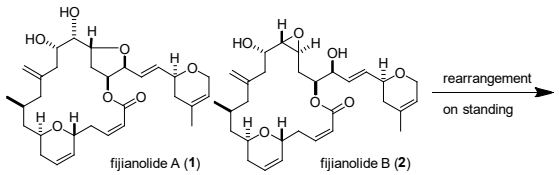
C-12 Lyngbic acid

P-231

PREPARATION AND CYTOTOXICITY EVALUATION OF ACETYLATED FIJIANOLIDES (A.K.A. LAULIMALIDE)

Tyler A. Johnson,[1] David Coppage,[3] Nicole L. McIntosh,[1] Colon V. Cook,[1] Marcos A Ogarrio,[1] Karen Tenney,[3] Frederick A. Valeriote[2] and Phillip Crews.[3]

[1]Department of Natural Sciences and Mathematics, Dominican University of California, San Rafael, CA 94601 USA, [2]Department of Internal Medicine, Division of Hematology and Oncology, Henry Ford Hospital, Detroit, MI 48202, [3]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064



The fijianolides (a.k.a. laulimalides) discovered in the 1980s at UC Santa Cruz and U. Hawaii continue to attract attention as a unique class of sponge-derived polyketides. They've shown low nanomolar cytotoxicity against human tumor cell lines. A mechanism for this activity is their ability to stabilize microtubles at a similar but distinct site to that of the anti-cancer drug Taxol®. Fijianolide B (**2**, aka laulimalide), which is extremely bioactive was the subject of two in vivo studies by different labs and resulted in different outcomes. One study reported significant inhibition of growth of solid tumors over 28 days without toxicity.[1] While another reported minimal inhibition of solid tumor growth accompanied by significant toxicity.[2] We have launched a new campaign to re-examine this dichotomy and create a more stable pre-clinical candidate, because **2** rearranges over time to the much less active **1**. In this poster we describe the preparation of diacetylated analogs of **1** and **2**, and their relative bioactivites against HCT-116 and PANC-1 tumor cell lines.

[1] Johnson et. al., **2007**, *J. Med. Chem. 50*, 3795-3803.
[2] Liu et. al., **2007**, *Anticancer Res. 27,* 1509-1518.

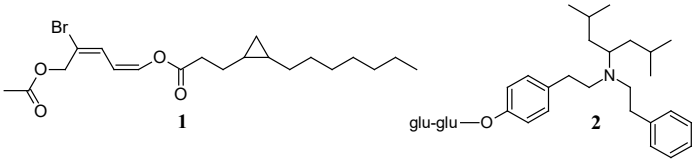
P-232

MARINE CYANOBACTERIA, A SOURCE FOR LEAD COMPOUNDS TO TREAT PAIN AND DEPRESSION

Stacy-Ann J. Parker†, Benedict Kolber‡, Kevin Tidgewell†

†Department of Medicinal Chemistry and ‡Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, United States

In this study we evaluate marine cyanobacterial extracts for lead compounds directed at serotonin (5-HT) G-protein coupled receptors (GPCRs) to treat comorbid pain and depression/anxiety. Approximately 225 fractions were screened, using a radioligand competition-binding assay, for their ability to bind to 5-HT GPCRs and MATs. Eighty-nine of the fractions screened showed greater than 50% inhibition of binding of radioligand to 5-HT GPCRs (50) and MATs (39) – a 40% hit rate. Furthermore, in the high affinity binding assay eight fractions displayed selectivity to the 5-HT_{2c} receptor and three fractions displayed selectivity to the 5-HT_7 receptor, an overall 5% hit rate. Compounds **1** and **2** are representative of the compounds characterized from 5-HT_{2c} and 5-HT_7 active fractions, respectively. The results from this bioassay-guided study will be discussed along with the ongoing research on selective ligands of 5-HT_{2c} and 5-HT_7 GCPRs.



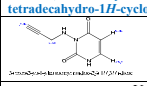
P-233

CHEMICAL STUDIES OF TWO MADAGASCAR MARINE SPONGES (PORIFERA) *HALICLONA SP* AND *CINACHYRELLA VOELTZKOWI* (LENDENFELD, 1897)

Bodosoa H. Rakotonjatovo[1], Isabelle Kerzaon[2], Hanta Andriamanantoanina[1], Marie Geneviève Dijoux-Franca[2] and Marta H. Andriantsiferana[3,*]

[1]Centre National de Recherches sur l'Environnement, BP 1739, Antananarivo 101, Madagascar , [2]UMR 557, Centre d'Étude des Substances Naturelles Faculté de Pharmacie , ISPB CNRS UCBL, Université Lyon1,France, [3]Laboratoire de Chimie des «Produits Naturels» et Biotechnologie (LPNB), Faculté des Sciences Université d'Antananarivo, Madagascar.

With more than 200 new marine natural products, reported each year for the decade between 2001 and 2010, the sponges are recognized as the most prolific marine producers. [1] The selection of the two species was guided by the results issued from preliminary antimicrobial, antifungal and antimalarial activities carried out on methanolic extracts of eighteen sponge species, including *Haliclona sp.* and *Cinachyrella voeltzkowi* (Lendenfeld, 1897). Particularly, *C. voeltzkowi* showed a very interesting antiplasmodial activity against the strain *P. falciparum* with IC50 = 4.62µg/ml. The chemical investigation results are reported below: the occurrence of the compounds discussed.

Sponge	Class of the determined compounds, Number	Note
Haliclona sp	**16** Sterols/Stanols/Oxosterol identified with Δ^5, Δ^7, $\Delta^{5,24(28)}$…	Using GC/MS Techniques
	New: 10,13-dimethyl-17-((*R*)-pentan-2-yl)- 2,3,4,5,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-ol	
Cinachyrella voeltzkowi	 **New compound**	Using UHPLC-UV/DAD-MS QTOF, ^{1}H NMR Techniques
	Uracil, Xanthine, Hypoxanthine	pyrimidine-bases
	Ectoin Natural anti-stress molecule	

Ref.: [1] Mehbub Mohammad Ferdous, Lei Jie, Franco Christopher, and Zhang Wei, Marine Sponge Derived Natural Products between 2001 and 2010: Trends and Opportunities for Discovery of Bioactives *Mar. Drugs* **2014**, *12*, 4539-4577.

P-234

IDENTIFICATION AND CHEMICAL CHARACTERIZATION OF MARINE NATURAL PRODUCTS USING UPLC-QTOF-MS COUPLED TO NOVEL INFORMATICS PLATFORM

Giorgis Isaac[1], Kenji L. Kurita[2], Jimmy Yuk[1], Mark Wrona[1], Rob Plumb[1] and Roger G. Linington[2]

[1]Waters Corporations, 34 Maple Street, Milford, MA 01757; [2]Department of Chemistry, Simon Fraser University, 888 University Drive, Burnaby, BC V5A 1S6, Canada

Despite the advances in hardware and the development of numerous derivatization, labeling, and analytical methods for compound identification, detailed and unequivocal determination of the constitution and configuration of complex natural product is a time-consuming task that requires a significant investment of resources and materials (1). Bioinformatics tools are becoming essential in natural products research, as advances in experimental throughput and the complexity of data obtained from biological profiling make manual interpretation difficult or impossible. In order to address these issues, a natural product analytical workflow was used to analyze the mass spectra of marine extracts which utilize tools such as 3D peak detection, a custom marine natural product database with precursor exact mass, fragment ion information and theoretical isotopic distribution that allows confident identification of compounds from a complex sample. A data-independent acquisition method that provides molecular and structural information from every detectable component in a LC separation was used to ensure maximizing data quality and coverage. This method provides informative mass spectrometry data that includes precursor exact

ion in low energy and corresponding fragment ion spectra in high energy. This semi-quantitative approach requires little prior knowledge of the sample and is unbiased and reproducible. All components not identified from the marine natural product database were then listed as unmatched peaks in the result browser. Further identification was carried on by using the structure elucidation module which utilizes elemental composition determination, online library searches (such as ChemSpider) and fragment ion matching. Structurally related compound was also identified by utilizing tools provided from the informatics platform such as common neutral mass search, common fragment ion search and mass defect filter to connect compounds from a given structural family.

P-235

CONVENTIONAL AND ACCELERATED-SOLVENT EXTRACTIONS OF GREEN TEA FOR METABOLOMICS-BASED CHEMOMETRICS

Joshua J. Kellogg[1], Emily D. Wallace[1], Tyler N. Graf[1], Nicholas H. Oberlies[1] and Nadja B. Cech[1]
[1]Dept. of Chemistry & Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 72412. E-mail: edwallac@uncg.edu

Metabolomics has emerged as an important analytical technique for multiple kinds of applications. However, the value of information obtained from metabolomics analysis highly depends on the degree to which the entire metabolome is present, and the reliability of sample treatment and analysis to ensure reproducibility across the study. Two extraction methodologies, accelerated solvent extraction and conventional solvent maceration extraction, were compared, using commercial green tea (*Camellia sinensis*) products as a test case. The accelerated solvent protocol was first optimized with regards to crucial variables using a D-optimal experimental design study. The accelerated solvent and conventional extraction methods yielded similar metabolite profiles for the green teas studied, however, the accelerated solvent extraction method consumed less solvent and required less active bench time to prepare the samples. This study demonstrated the potential of accelerated solvent as an effective extraction methodology to prepare samples for a metabolomic study.

P-236

IN VIVO PROCESS OF ICARIIN IN RAT

Liu Yang[1] and Shunjun Xu[2]
[1]Guangdong Provincial Hospital of Chinese Medicine, Second Affiliated Hospital, Guangzhou University of Chinese Medicine, Guangzhou 510120, P. R. China, [2]Guangzhou ImVin Pharmaceutical Co., Ltd., Guangzhou 510663, P. R. China.

Icariin is one of the predominant flavonoids contained in Herba Epimedii, a famous Chinese medicine for the treatment of cancers and immune system diseases. In the present study, a liquid chromatographic method combined with electrospray ionization tandem mass spectrometry was developed to quantify the concentration of icariin in rat plasma and various tissues collected at different time points after oral administration of the total flavonoid extract of Herba Epimedii at a dose of 0.69 g/kg (corresponding 42 mg/g icariin). Biological samples were processed by simple protein precipitation. The method was successfully applied to plasma pharmacokinetic and tissue distribution studies of icariin in rat. As a result, it was worth noting that the tissue distribution characteristics of icariin exhibited a significant gender difference. Moreover, a total of 11 potential metabolites were found in rat feces collected in different time periods after oral and intramuscular administration of icariin. *In vivo* metabolic pathways were involved in hydrolysis, demethylation, oxidation and conjugation.

P-237

QUALITY ASSESSMENT OF CHUANKEZHI INJECTION USING HIGH-PREFORMANCE LIQUID CHROMATOGRAPHY

Liu Yang[1], Wesley Chow[1] and Shunjun Xu[2]
[1]Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, P. R. China, [2]Guangzhou ImVin Pharmaceutical Co., Ltd., Guangzhou 510663, P. R. China.

Because traditional Chinese medicine (TCM) is a multi-component system, quality control of TCM always has to confront various difficulties. Although qualifying and quantifying certain compounds contained in TCM has been widely used for quality evaluation, this method does not take the existence of other ingredients into account. Comparatively, the chromatographic fingerprint of TCM is a more suitable approach to holistically assess the quality of herbal drugs. Chuankezhi injection is a well-known Chinese herbal preparation. This study reported a high-performance liquid chromatographic fingerprinting for quality evaluation of Chuankezhi injection. The HPLC profiles of 34 batches of commercial samples were further analyzed using chemometric methods including similarity evaluation and principal component analysis. As a result, the established HPLC fingerprint contained 21 characteristic peaks; therein 11 peaks were unambiguously assigned by comparing their retention time (t_R) and UV spectra with those of reference compounds and 5 peaks were tentatively identified on the basis of their MS/MS fragmentation patterns and UV spectra. Moreover, it could be clearly observed that epimedin C and its analogues predominate in Chuankezhi injection. Except for two samples identified as outliers, other 32 batches of samples displayed similar HPLC profiles, indicating that the producing process and final quality of the injection is stable and consistent and established fingerprinting can be satisfactory for quality control of the injection.

P-238

CHARACTERIZATION OF PHYTOCHEMICALS IN *CENTELLA ASIATICA* EXTRACTS BY LIQUID CHROMATOGRAPHY AND HIGH RESOLUTION MASS SPECTROMETRY

Armando Alcazar Magana[1,2], Maya Caruso[4], Kirsten Wright[4], Amala Soumyanath[4], Jan F. Stevens[2,3], and Claudia S. Maier[1]
[1]Department of Chemistry, [2]Department of Pharmaceutical Sciences, [3]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA, [4]Department of Neurology, Oregon Health & Science University, Portland, OR 97239

Centella asiatica (CA) is an Asian medicinal herb reputed to improve memory. Genetic, geographic and post-harvest processing all influence the secondary metabolite composition of CA products, affecting study reproducibility. Modern mass spectrometry platforms offer accurate mass measurements for structural analysis and quantification of compounds in complex mixtures. We used ultra-performance liquid chromatography (Acquity UPLC) in conjunction with high-resolution tandem mass spectrometry (AB Sciex Triple TOF 5600) in negative ionization mode for the chemical analysis and characterization of dried water extracts from three CA accessions. This method allowed the characterization and quantification, by parent/product ion monitoring, of seven flavonoids, three structural isomers of mono-caffeoylquinic acids (CQAs), five di-CQAs, six caffeic acid derivatives and the two major saponins and related sapogenins. CA extract samples were spiked with 24 available standards at two concentrations (0.25 ng and 5 ng). Recoveries for individual compounds ranged from 91–132 %. For accuracy testing, three standard mixtures of known concentration (low-high) were analyzed in the range of 87-125 %, confirming the procedure's feasibility for quantitative analysis of CA samples. Principal component analysis also revealed differences in components extracted from the three *Centella asiatica* accessions.

P-239

A METHOD FOR SIMULTANEOUS RECOVERY OF *CENTELLA ASIATICA* TRITERPENES AND CAFFEIC ACID DERIVATIVES FROM MOUSE PLASMA

Maya Caruso[1], Kirsten Wright[1], Don Matthews[1], Marguex Hunter[1], Nora Gray[1], Joseph Quinn[1,2] and Amala Soumyanath[1]
[1]Dept. of Neurology, Oregon Health and Science University, Portland, OR 97239, USA, [2]Dept. of Neurology, Portland Veterans Affairs Medical Center, Portland, OR 97239, USA

Centella asiatica is being widely researched for its potential effects as a cognitive enhancer. Triterpene (asiatic acid, madecassic acid and their glycosides) and caffeoylquinic acid (CQA) components have been identified as being neurotropic and neuroprotective. We have developed sensitive liquid chromatography-tandem mass spectrometry methods (LC-MS) to analyze these compounds in order to measure plasma levels in our mouse models of cognitive decline. Due to the small plasma yield obtained from mice, a single work up method to recover these chemically divergent compounds was needed. We have compared several methods utilizing protein precipitation (perchloric acid or organic solvents) or solid phase extraction (Supelco C8, Phenomenex Phree, Agilent Strata X and Strata XA). Mouse plasma (50ul) spiked with the analytes of interest (2.5 to 100 ng/ml), and internal standards, was processed in triplicate by each of the methods. Regression coefficients of calibration curves (ideally >0.95) and relative standard deviations (RSD) of replicates (ideally <15%) were used to evaluate the methods. Phree columns were found to be effective for the CQAs and CQA metabolites, while C8 columns were effective for the triterpenes. Protein precipitation with methanol:acetonitrile 1:3 gave calibration curves ranging from 0.907 to 0.999 regression, and 3% to 16% RSD for triterpenes, CQAs and CQA metabolites, making it the preferred recovery method for these analytes from mouse plasma.

P-240

ACETOGENINS OF GRAVIOLA (*ANNONA MURICATA*) PRODUCTS AVAILABLE COMMERCIALLY IN THE U.S.

Shi Sun, Wenjun Zhu, Fei Yang, Kequan Zhou
Department of Nutrition and Food Science, Wayne State University, 5045 Cass Ave, Detroit, MI, 48202.

Annonaceous acetogenins with strong biological activities were found to specially distribute in plants of family Annonaceae. One tropical fruit, Graviola (*Annona muricata*), also an indigenous medicinal plant, attracts researchers involved in food, agrichemicals, pharmaceuticals, etc. The dietary supplements derived from the fruit and leaf have become popular worldwide. However, the consumptive safety of Graviola and North American Pawpaw (*Asimina triloba)* and their products is unfortunately becoming the customers' concern. There have been reports that acetogenins or alkaloids were possibly responsible for clusters of Parkinsonism/dementia in tropical populations. To know whether there is a possible relationship between acetogenins and the incidence of Parkinson's disease, we firstly investigated acetogenins of Graviola products available commercially in the U.S. In total, 10 samples from different countries showed that there were significant differences in the type and content of acetogenins – 26 acetogenins were detected in one sample, but none of them can be detected in some of the samples by HPLC/UV and UHPLC–MS in SRM mode. Indeed, the anti-proliferation on pancreatic cancer *in vitro* showed significantly positive correlation with acetogenins' abundance in Graviola. As for Parkinso's disease occurring simultaneously with acetogenins' abundance in Graviola, it is necessary to further conduct a full epidemiological survey of the corresponding local Parkinson's incidence.

P-241

APPLICATION OF ^{19}F QNMR TO PHARMACEUTICAL ANALYSIS

Sarah J. Robinson[1], Allison Mattes[1,2], David Russell[1]
[1]Small Molecule Pharmaceutical Sciences, Genentech, Inc. 1 DNA Way South San Francisco, CA 95080 ; [2]Department of Chemistry & Biochemistry, University of Oklahoma 101 Stephenson Parkway Norman, OK 73019.

Quantitative NMR (qNMR) is a well-established technique.[1,2] Historically, most applications of qNMR have focused on the measurement of ^{1}H NMR data due to the relatively high sensitivity and ubiquitous nature of ^{1}H NMR experiments. There are drawbacks to using ^{1}H NMR for this purpose including the relatively narrow band of just 10-12 parts per million (ppm) and overlap of signals when analyzing drug products. In contrast, fluorine is more sensitive than hydrogen to the changes in its local electronic giving this nuclide a useable chemical shift range up to 300 ppm.[3] Additionally, in 2015 28% of the novel approved small molecule drugs contained fluorine.[4] Prestened herein is optimization of several NMR data acquisition parameters to enable accurate and precise ^{19}F qNMR measurements in complex formulation matricies including tip angle, T1, acquisition time, signal to noise, and most importantly for ^{19}F qNMR, excitation bandwidth. Also discussed is application of Mestrenova software for automated data analysis relative to an external standard.

References: [1]Pauli, G. F.; Jaki, B. U.; Lankin, D. C., Quantitative 1H NMR: development and potential of a method for natural products analysis. *J. Nat. Prod.* **2005,** *68* (1), 133-149. [2]Bharti, S. K.; Roy, R., Quantitative 1H NMR spectroscopy. *TrAC, Trends Anal. Chem.* **2012,** *35*, 5-26. [3]Dolbier, W. R., *Guide to Fluorine NMR for Organic Chemists*. John Wiley & Sons, Inc.: Hoboken, NJ, 2009. [4]Novel Drugs 2015 Summary. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugInnovation/.

P-242

OPTIMIZATION OF A SOLID-PHASE EXTRACTION PROTOCOL FOR APPLICATION TO ALKALOID ANALYSIS

Richard W. Fitch[1], Allyson Morris[1], Star Leonard[1], Maria Martinez[2], Rebecca Tarvin,[3] David Cannatella,[3] Santiago Ron[4] and Karina Klonoski.[5]
[1]Department of Chemistry and Physics, Indiana State University, Terre Haute, IN 47809. [2]Terre Haute South Vigo High School, Terre Haute, IN 47802. [3]Department of Integrative Biology, The University of Texas at Austin, Austin, TX 78712. [4]Divison de Amfibios, Museo de Zoologica, Pontificia Universidad Católica del Ecuador, Quito, EC170135, Ecuador. [5]Department of Environmental Science, Policy, & Management, University of California at Berkeley, Berkeley, CA 94720.

In our previous studies of poison frog alkaloids we have relied on aqueous/organic extraction protocols for both small and large scale analyses. In recent years we have relied more on analysis of large numbers of individual animals and analysis of stimulated skin secretions of frogs for ecological studies. Both of these factors have led us to examine higher throughput methods for sample preparation. Solid-phase extraction (SPE), particularly with cation-exchange media is an appealing method. Unfortunately, our first forays into this area were plagued by poor recovery (<5%) based on irreversible adsorption of amines, particularly H-bond donors. We have resolved this largely by pretreatment of media with methanolic ammonia ahead of the usual acid conditioning to preoccupy irreversible binding sites, improving our recoveries tenfold based on GC-MS and LC-MS analysis. In addition, the use of surged splitless injection sharpened peaks substantially for early eluting compounds, improving sensitivity and quantitation. Our application of this method to a stock alkaloid mixture and to alkaloid extracts from South America and Madagascar will be reported.

P-243

MATERIAL VALIDATION BY NMR: SIGNIFICANCE AND IMPLEMENTATION

K. Brian Killday[1], Kaylen Obray[2], Claudia M. Boot[2], Torsten Schoenberger [3], Kristie Adams[4] and Kimberly L. Colson[1]

[1]Bruker BioSpin, Billerica, MA 01821, USA; [2]Colorado State University, Fort Collins, CO 80523; [3]Forensic Science Institute of the Federal Criminal Police Office (BKA), Germany; [4]Dupont, Wilmington, DE, 19803 USA

NMR is an extremely powerful tool that allows for the determination of identity, strength and composition. However, what makes NMR immensely useful is reproducibility in measurements on different instruments at different sites. These characteristics offer great importance for validated NMR methods moving into wide spread use in the near future. Currently, however, it is very common in the industry to associate validation methods with chromatography. Historically, most validated identification and quantification methods have been based on chromatography. For the natural products community, NMR is largely considered a tool for structure elucidation and is underutilized as a quality assessment tool in the GxP environment.

Integral parts of the NMR community are looking to change and expand the current perspective of NMR to include the regulatory environment. To accomplish this goal, a group of concerned NMR citizens initiated a global education campaign and workgroup to establish common NMR material validation guidelines. These guidelines aim to ease the implementation of NMR in a regulatory environment and position the guidelines so that they will be easily recognizable to global regulatory authorities. This poster reports recent progress of this global effort.

P-244

ODS ANALYTICAL METHODS AND REFERENCE MATERIALS (AMRM) PROGRAM: A COLLABORATIVE NIH PROGRAM WHICH CAN ASSIST THE BOTANICAL RESEARCH COMMUNITY

Joseph M. Betz[1], Adam J. Kuszak[1], Catherine Rimmer[2], Laura Wood[2], Barbara C. Sorkin[1], Stephen Wise[1], Paul M. Coates[1]

[1]Office of Dietary Supplements, National Institutes of Health, Bethesda, MD, USA. [2]Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD, USA

The manufacture, scientific investigation, and regulatory oversight of dietary supplements require analytical methods and reference materials that permit the verification of dietary ingredient identity, measurement of key constituents in raw materials and finished products, and the quantification of contaminants. The NIH Office of Dietary Supplements' Analytical Methods and Reference Materials (AMRM) Program was created to support and accelerate the validation of analytical methods and development of reference materials for dietary supplements to meet these needs. The AMRM Program has four main goals: 1) Expand the availability of scientifically valid analytical methods for dietary supplements; 2) Produce and make available certified reference materials appropriate for use in method development, validation, and demonstration of laboratory performance; 3) Support public and private partnerships that emphasize the need for chemical and biological characterization of dietary supplements; and 4) Disseminate information in the peer-reviewed scientific literature. This poster provides an overview of the AMRM Program and how it works to address challenges and gaps in quantitative and qualitative analytical characterization of dietary supplements and their ingredients. The AMRM Program's infrastructure, activities, and products will be outlined, with emphasis on the collaborative efforts of academic institutions, industry, government, and not-for-profit groups, which participate in the Program's priority setting and resource development.

P-245

HIGH THROUGHPUT MASS SPECTROMETRY COMPOUND DEREPLICATION AND DISCOVERY WITH MOLECULAR NETWORKING AND CROWD SOURCED ANNOTATION

Mingxun Wang[1], Pieter C Dorrestein[2], Nuno Bandeira[1]

[1]Computer Science and Engineering, University of California San Diego, La Jolla CA 92093, USA, [2]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego La Jolla CA 92093, USA.

The potential of the diverse chemistries present in natural products (NP) for biotechnology and medicine remains untapped because NP databases are not searchable with raw data and the NP community has no way to share data other than in published papers. Although mass spectrometry (MS) techniques are well-suited to high-throughput characterization of NP, there is a pressing need for an infrastructure to enable sharing and curation of data. We present Global Natural Products Social Molecular Networking (GNPS; http://gnps.ucsd.edu), an open-access knowledge base for community-wide organization and sharing of raw, processed or identified tandem mass (MS/MS) spectrometry data. In GNPS, crowdsourced curation of freely available community-wide reference MS libraries will underpin improved annotations. By leveraging these MS libraries, GNPS enables the community to dereplicate known compounds in a high-throughput fashion. Further, GNPS's molecular networking groups and visualizes molecules with similar MS/MS fragmentation and similar structure together into molecular families even in the absence of known identifications. Thus, dereplication of a single member of a molecular family provides hints at putative structures of the entire molecular family. Applied broadly across over 300 public datasets, molecular networking uncovered nearly 4,000 previously unidentified putative analogs of known molecules that are ripe for further study.

P-246

CHARACTERIZATION OF TRADITIONAL MEDICINE EXTRACTS USING IMPACTOR IONIZATION AND MASS SPECTROMETRIC DETECTION

Kerri M. Smith, Giorgis Isaac, Jimmy Yuk

Waters Corporations, 34 Maple Street, Milford, MA 01757

The analysis of natural products using mass spectrometry has mainly been dominated by the use of electrospray ionization (ESI). Even though ESI offers a wide range of compounds detected, there are developments on other ionization methods that can potentially increase compound coverage. A novel approach called impactor ionization, involves the formation of ions by directing a heated, nebulized spray of liquid onto a surface with an applied voltage. On impact, the ions flow downstream in a path that follows the curvature of the surface (Coand effect). To showcase this new ionization approach, the characterization of a panax ginseng extract by ESI and impactor ionization will be presented.

P-247

HIGH-THROUGHPUT ION MOBILITY MASS SPECTROMETRY SEQUENCING OF CYCLIC PEPTIDES MEDIATED THROUGH OXAZOLIDINONE RING OPENING

Hader Elashal[2], Ryan Cohen[1,2] and Monika Raj[2]
[1]Merck Research Laboratories, Merck & Co., 126 E. Lincoln Ave., Rahway, NJ 07065, USA. [2]Department of Chemistry, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079, USA.

Cyclic peptides are attractive targets for drug discovery due to enhanced proteolytic stability, permeability, and binding affinity versus their linear analogs. However, one hurdle for their drug discovery is sequencing difficulties of both naturally-occurring cyclic peptides and those synthesized via a one-bead one-compound (OBOC) approach. Here we developed a dual ring opening/cleavage methodology for sequencing of cyclic peptides by selective modification of serine, threonine, or cysteine residues to an oxazolidinone moiety. Formation of this moiety increases the susceptibility of the amide bond at the N-terminus towards hydrolysis and leads to the opening of a cyclic peptide to its linear counterpart. The resulting linear peptide can then be sequenced in one minute by a liquid chromatography, ion mobility spectrometry, tandem mass spectrometry (LC-IMS-MS/MS) method. Libraries of cyclic peptides containing different combinations of amino acids were synthesized and sequenced to determine the robustness of this methodology as well as compatibility with free amino acid side chains.

P-248

IDENTIFICATION OF ISOMERIC MARINE NATURAL PRODUCTS BY ELECTROSPRAY ION MOBILITY MASS SPECTROMETRY

Fausto Carnevale Neto[1,2], Norberto Peporine Lopes[1], Leticia V. Costa-Lotufo[3], Roger G. Linington[2]
[1]Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903, Brazil, [2]Department of Chemistry, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada, [3]Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, 05508-900, Brazil.

Metabolomics-based approaches using modern mass spectrometry (MS) techniques have been recognized as one of the most powerful strategies for exploring the chemical diversities in natural products (NP). In the area of marine NP research, issues of access to marine life and elucidation of complex structures at micro-scale demand new tools to improve the identification of bioactive compounds. In this context, dereplication methods using MS have emerged as a rapid way of identifying known compounds in marine extracts. Still, conventional liquid chromatography-mass spectrometry (LC-MS) is often incapable of directly differentiating isomeric and isobaric compounds. This is particularly true in situations where isobaric compounds co-elute, restricting the possibility of prediction and automation in the dereplication process. Here we propose the combination of UHPLC-MS/MS with ion mobility separation (IMS) to separate and identify marine NP isomers. IMS-MS adds an additional dimension of separation based on ion gas-phase conformation. Together with the UPLC separation and high-resolution MS/MS, it is possible to separate isomers and characterize their structures by considering questions related to gas-phase reactivity. This analysis can highlight differential ionization, which includes the identification of possible (de)protonation sites, and fragmentation pathways (e.g. concomitant dissociation processes). We demonstrate the value of IMS-MS as a complement to existing MS techniques through the characterization of different classes of marine and microbially-derived natural products (e.g. erythromycin, staurosporine and phenazine).

P-249

INVESTIGATING INTERSPECIES INTERACTION OF MARINE INVERTEBRATE-ASSOCIATED BACTERIA USING *E.COLI* CHEMICAL GENOMICS

Srikar N. Adibhatla[1], Navid Adnani[1], Jeff Piotrowski[2], Doug R. Braun[1], Deepa Acharya[1], Tim S. Bugni[1]
[1]Pharmaceutical Sciences Division, University of Wisconsin, Madison, Wisconsin, USA. [2] Yumanity Therapeutics, Cambridge, MA, USA

Marine Actinobacteria have been shown to have enormous potential in synthesizing therapeutically relevant secondary metabolites. Our lab has shown that interspecies interactions is an excellent method to access new molecules encoded by cryptic biosynthetic gene clusters that are not active in standard conditions. As part of a high throughput platform we developed, co-cultures and monocultures of marine invertebrate-associated Actinobacteria were grown in microscale cultures, and were analyzed via LC-MS and antibiotic screening methods. Through these methods, we identified combinations that showed numerous metabolites uniquely produced in co-culture, some of which had antibiotic activity. Extracts of fifteen co-culture and their corresponding monocultures were screened against a library of 6000 *E.coli* knockout strains to determine the functions of the potentially novel metabolites unique to co-culture. Based on the mutant strains that are rendered sensitive, this study can help us identify different gene knockout that may be more susceptible to the molecules in the co-culture extracts. Further, based on mutant strains rendered resistant, we can identify molecules helpful in combatting current antibiotic resistant mechanisms. This chemical genomics approach will help characterize potentially novel molecules with unusual activities.

P-250

RECOVERY OF CULTIVATABLE NATURAL PRODUCT BIOSYNTHETIC GENE CLUSTERS FROM SEDIMENT USING NEXT-GENERATION SEQUENCING

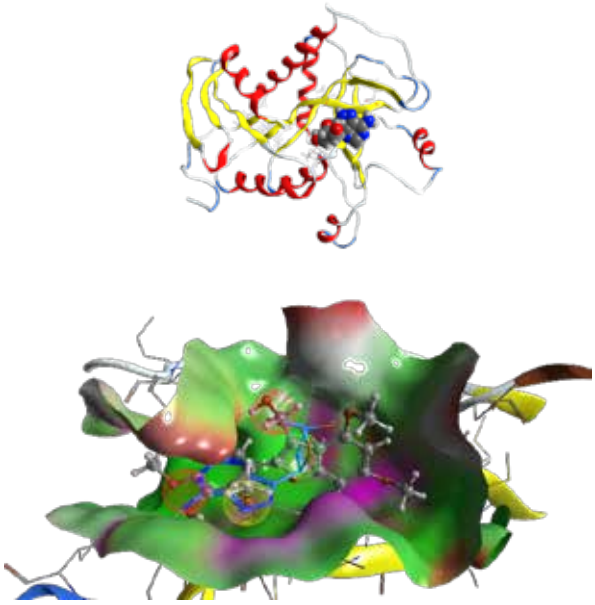
*Maryam Elfeki[1], Mohammad Alanjary[3],Stefan J. Green[1], Nadine Ziemert[3], Brian T. Murphy[1]**
[1]Dept. of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL; [3]Dept. Microbiology and Biotechnology, University of Tübingen, Tübingen, Germany

Despite decades of cultivating microorganisms for drug discovery, and reports of an untapped array of microbial-derived natural product (NP) biosynthetic gene clusters (BGCs) in the environment, few attempts have been made to measure the extent to which common cultivation techniques are accessing this chemical space. Here we employed NGS to assess the 16S rRNA and conserved NP BGCs, directly from DNA isolated from two Lake Huron (LH) sediment samples. We applied the sediment samples to six different nutrient media to select for the growth of spore-forming bacteria. Surprisingly, despite cultivating less than 0.2% of families present in sediment, we calculated the estimated recovery of 28% of polyketide synthase (PKS) ketosynthase operational biosynthetic units (OBUs), 58% of PKS type 2 ketosynthase alpha OBUs, and 10% of nonribosomal peptide synthetase (NRPS) adenylation OBUs from the samples. A large portion of these sequences contained low homology to those in commonly used BGC databases. Taking sequencing limitations into account, our study suggests that a minority of the cultivatable bacteria harbors a disproportionate share of the PKS and NRPS biosynthetic capacity in sediment. Furthermore, this initial insight into the cultivatable chemical space of LH sediment suggests that existing cultivation protocols are yielding several putatively novel NP BGCs, and that it is worthwhile to focus on accessing this chemical space via less serendipitous and biased colony selection methods from environmental diversity plates. These studies are currently underway in our laboratory.

P-251

SCREENING PHYTOCHEMICALS AGAINST TARGETS IN ANTIBIOTIC-RESISTANT PATHOGENS: A COMBINED COMPUTATIONAL APPROACH

Kendall G. Byler[1], Mary Snow Setzer[1], Ifedayo Victor Ogungbe[2], and William N. Setzer[1]
[1]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
[2]Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS 39217, USA



Trichomoniasis, caused by the pathogenic protozoan *Trichomonas vaginalis*, is the most common non-viral sexually-transmitted disease. Metronidazole-resistant strains of *T. vaginalis* have emerged and azole-resistant trichomoniasis infections are on the rise, but there are currently no alternative treatment options. The need to discover and develop new chemotherapeutic alternatives is urgent and plant-derived natural products have long served as sources for new medicinal agents as well as new leads for drug discovery and development. In this work, we use a combined molecular docking, pharmacophore scoring and molecular dynamics annealing to identify strong leads from 952 antiprotozoal phytochemicals with *T. vaginalis* methionine gamma-lyase (TvMGL) and *T. vaginalis* purine nucleoside phosphorylase (TvPNP).

P-252

ALPHAVIRUS NSP3 MACRO DOMAINS AS TARGETS FOR ANTIVIRAL PHYTOCHEMICALS: A COMPUTATIONAL STUDY

B. Danger Davies[1], William N. Setzer[1], Kendall G. Byler[1], and Ifedayo V. Ogungbe[2]
[1]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA; [2]Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS 39217, USA

Alphaviruses are mosquito-borne viruses of the family *Togaviridae*, and include such members as Chikungunya virus (CHIKV), Venezuelan Equine encephalitis virus (VEEV), Sindbis virus (SINV), Barmah Forest virus (BFV), and Aura virus (AURV). Despite their virulence, there are no current anti-viral treatments or vaccinations available, and these are considered neglected diseases. The alphavirus non-structural protein 3 (nsP3) is the macrodomain site. This macrodomain is a known site of viral replication and a binding site for ADP-ribose, therefore, it could be a potential target for antiviral drug discovery. Currently, the crystal structure of the nsP3 macrodomain of the alphavirus has only two available known crystal structures, those of CHIKV and VEEV. In this study, homology models were generated for the nsP3 macrodomains of SINV, BFV, and AURV based on each of the known crystal structures of CHIKV and VEEV and used as targets for a virtual screening method. Of the 2,180 phytochemicals evaluated, a total of 30 exhibited high docking affinity and poses to one or more of the nsP3 homology models.

P-253

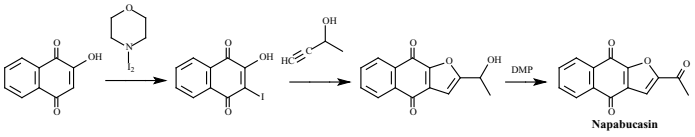
WITHDRAWN

P-254

SYNTHESIS AND BIOACTIVITY EVALUATION OF FURANONAPHTHOQUINONES

Wai I Chik[1], Yi-Fu Guan[1], Yu Zhu[1], Chuen Fai Ku[1], and Hong-Jie Zhang[1,]*
[1]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, P.R. China.

Furanonaphthoquinones (FNQs) are a group of small molecules that can be found in the plants of Bignoniaceae family. They have demonstrated a variety of biological activities including antimicrobial and cytotoxic activities (Wang et al. Bioactive constituents from *Radermachera boniana*. *Chemistry Select* **2016**, *1*, 1575-1579). One of its kind, napabucasin (BBI608), was shown to selectively target cancer stemness and metastasis, and is currently in several active clinical trials according to the National Cancer Institute. The objective of our research is to synthesize analogs of napabucasin and to evaluate their bioactivities against different disease targets in order to analyze the structure activity relationship (SAR) and unravel the mechanism of action. *Acknowledgements: The work described in this paper was supported by the Hong Kong Baptist University (HKBU) Interdisciplinary Research Matching Scheme (RC-IRMS/15-16/02).*



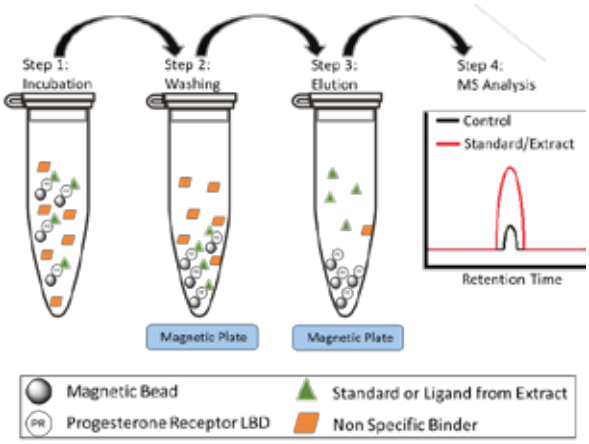
Scheme 1. Optimized synthetic route of napabucasin.

P-255

DEVELOPMENT OF A MAGNETIC MICROBEAD AFFINITY SELECTION SCREENING (MAGMASS) WITH UHPLC-MS ASSAY FOR THE PROGESTERONE RECEPTOR

Daniel G. Nosal, Michael D. Rush, and Richard B. van Breemen
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago College of Pharmacy, 833 S. Wood Street, Chicago, IL 60612, USA.

The progesterone receptor (PR) is a member of the nuclear receptor superfamily which is regulated by progestogens, such as progesterone. If phytoprogestogens exist in products such as botanical dietary supplements and are consumed by women, they could affect endometriosis, fibroids, fertility, and even endometrial cancers. Hypothesizing that some botanical dietary supplements consumed by women might contain phytoprogestogens, we developed a Magnetic Microbead Affinity Selection Screening (MagMASS) UHPLC-MS assay to find PR ligands in complex mixtures such as botanical extracts.



P-256

NUTRITIVE POTENTIALS AND ANTI-SICKLING ACTIVITIES OF TWO NIGERIAN FOOD PLANTS: *PIPER GUINEENSE* AND *GNETUM AFRICANUM*.

Ogunnaike O, [2]Ubani ON, Ajayi TA and Moody JO
Department of Pharmacognosy, Faculty of Pharmacy , University of Ibadan, Nigeria; [2]Nigeria Stored Product Research Institute, Ilorin, Nigeria

Piper guineense Schum et Thon (Piperaceae) and *Gnetum africanum* Welw (Gnetaceae) are widely distributed in high forest zones in Nigeria where they are valued as spices or as ingredients in traditional remedies to treat sickle cell disease. We have examined the nutritive potentials and *in-vitro* anti-sickling activities of the leaf methanol extracts of the two food plants. Determination of physico-chemical parameters were carried out using established AOAC protocols (AOAC, 1999) while *in-vitro* anti-sickling activities were evaluated using the inhibition of sodium metabisulphite - induced sickling of HbSS blood samples with p-hydroxybenzoic acid (5 μg ml^{-1}) as reference standard. *P guineense* exhibited relatively higher crude fiber(8.38 %), crude protein(5.39 %) and zinc (0.98 %) content than *G. africanum* with the corresponding values of 4.13%. 7.56% and 0.83% respectively. While the crude methanol extract and water-soluble fraction of *P. guineense* extract exhibited significant anti-sickling activities for up to 180 minutes incubation, the *Gnetum africanum* leaf extract did not show any anti-sickling activities. The results provide some justification for further investigation of Nigerian food plants for the possible development of dietary supplements and neutraceuticals for sickle cell disease sufferers in the tropics.

P-257

NEURITOGENIC AND NEUROPROTECTIVE ACTIVITIES OF PRENYLATED RESVERATROL DERIVATIVES ON CULTURED P19-DERIVED NEURONS

Thanchanok Puksasook[1], Shinya Kimura[2], Sarin Tadtong[3], Jutamas Jiaranaikulwanitch[4], Jaturong Pratuangdejkul[5], Worawan Kitphati[6], Khanit Suwanborirux[7], Naoki Saito[2], and Veena S. Nukoolkarn[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, 10400, Thailand, [2]Graduate School of Pharmaceutical Sciences, Meiji Pharmaceutical University, Tokyo, 204-8588, Japan, [3]Department of Pharmacognosy, Faculty of Pharmacy, Srinakharinwirot University, Nakhon-nayok, 26120, Thailand, [4]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand, [5]Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok, 10400, Thailand, [6]Department of Physiology, Faculty of Pharmacy, Mahidol University, Bangkok, 10400, Thailand,[7]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

Two series of *O*-alkyl and *C*-alkyl resveratrol derivatives were designed, semisynthesized and evaluated for antioxidant, neuroprotective and neuritogenic activities. The *C*-alkyl showed more potent antioxidant effect than *O*-alkyl resveratrol derivatives due to free OH groups on resveratrol scaffold. This lead to further evaluate their activities on neurotoxic, neuroprotective and neuritogenic using cultured P19-derived neurons. The geranylated resveratrol derivative which is one of *C*-alkyl resveratrol derivatives exhibited neurotoxicity on the P19-derived neurons whereas prenylated resveratrol derivative (**4b**) not only showed no neurotoxicity but also enhanced the survival of the cultured cells. Moreover, this compound showed greater ability to protect neurons from oxidative stress-caused cell death in a serum deprivation model than that of resveratrol and was comparable to quercetin which used as positive control. The phase-contrast micrographs displayed that compound **4b** at 1 nM concentration possessed neuritogenic activity by promoting more branching numbers and longer neurites approximately five- and two-fold than the control, respectively. Collectively, these results suggested that the active compound **4b** might be a potential lead candidate for further development of new drugs for Alzheimer's disease.

P-258

FUNGAL METABOLITES FUEL DRUG DISCOVERY FOR PATHOGENIC FREE-LIVING AMOEBAE.

Cedric J. Pearce[1], Blaise Darveaux[1], Nicholas H. Oberlies[2], Noemi D. Paguigan[2], Bill Baker[3], Danielle Demers[3], Christopher Rice[4], Beatrice Colon[4], Dennis Kyle[4]
[1]Mycosynthetix, Inc. Hillsborough, NC 27278, [2]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27412, [3]Department of Chemistry, University of South Florida, Tampa, FL 33612, [4] Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602

Free-living parasitic amoebae cause life-threatening infections; in the case of *Naegleria fowleri* the chances of survival are very small. A library of 36,119 fungal samples from Mycosynthetix and the University of South Florida's natural products laboratory, including samples from Antarctic microbes and the Everglades in Florida, was assembled and screened for activity against *N.fowleri* and *Acanthamoebae castellanii.* 174 samples showed >67% killing at 5ug/mL against *N.fowlerii* and 63 samples were similarly active against *A.castellanii.* The fungi producing this activity were re-cultured and then evaluated for cytotoxicity against mammalian cells. Forty three had an activity index of >10, *i.e.*, they were greater than 10 times more active against free-living amoebae than mammalian cells. Dereplication of these samples identified known chemistry in eight cases; the remainder are unknown. From a mycological perspective the active fungi represent a broad biodiversity; this will be discussed. We are currently characterizing

the active compounds. The latest developments will be presented together with all relevant background information including the identity of active compounds.

P-259

NEUROTROPHIC ACTION OF BERBERINE IN COMBINATION WITH NERVE GROWTH FACTOR

Narayan D. Chaurasiya[1], Deependra Singh[1,3] and Babu L. Tekwani[1,2]
[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences and [2]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University MS 38677, USA; [3]Institute of Pharmacy, Pandit Ravi Shankar Shukla University, Raipur CG India.

Berberine is a major constituent of many medicinal plants used in Indian and Chinese traditional/native systems of medicine. The plant extracts and products containing berberine are available as over-the-counter dietary supplements and as herbal remedies for different clinical symptoms. Berberine has shown broad range of pharmacological and therapeutic applications. The neuritogenic and neurotropic effect of berberine was tested *in vitro* on Neuroscreen-1 (NS-1) cells, a sub clone of PC-12 cells. NS-1 cells generally stop dividing and differentiate terminally as neuron like-cells, when treated with neurotrophic agents like Nerve Growth Factor (NGF). The neuritogenic effects of berberine on NS-1 cells were evaluated alone and in the presence of suboptimal concentrations of NGF (1.25ng/mL). The neurite outgrowth measurement and quantification were performed by analysis of digital images of treated cells for average numbers of neurites/cell, mean neurite length, nodes/cell and average length of neurites. Berberine alone did not show significant neurotrophic effect. However, berberine significantly potentiated neurotrophic action of NGF on NS-1 cells. The potentiation of neutrophic action of NGF by berberine occurred through activation of relevant signaling pathways. Berberine, with neuritogenic and neurotrophic activity, holds significant potential as therapeutic agent for treatment of neuronal injuries and neurodegenerative diseases due its property to stimulate new neurite outgrowth and regenerate damaged neurons.

Acknowledgements: USDA-ARS Cooperative Scientific Agreement # 58-6408-2-0009: NCRR Grant Number P20GM104932 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH), (CORE C-).

P-260

MARINE MAMMAL MICROBIOTA YIELS NOVEL ANTIBIOTIC WITH POTENT ACTIVITY AGAINST *CLOSTRIDIUM DIFFICILE*

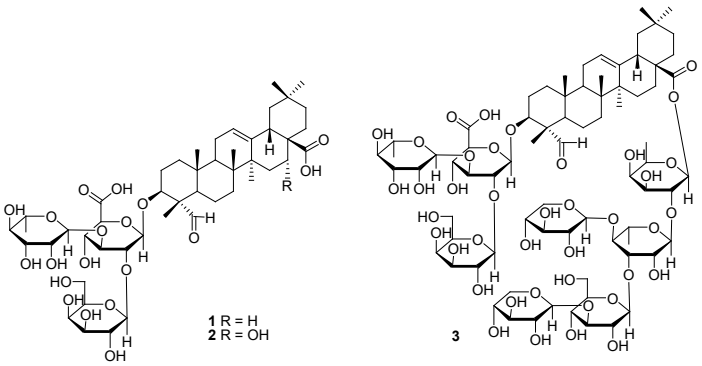
Jessica L. Ochoa[1], Laura M. Sanchez[1,2], Byoung-Mo Koo[3], Jennifer S. Doherty[3], Manohary Rajendram[4], Kerwyn Casey Huang[4], Carol A. Gross[3], Roger G. Linington[1,6]
[1]UCSC, USA, [2]UIC, USA, [3]UCSF, USA, [4]Stanford, USA, [5]SFU, Canada

Research on microbiota has highlighted the important interplay between commensal microorganisms and the health of their cognate hosts. Metabolites isolated from commensal bacteria have been demonstrated to possess a range of antimicrobial activities, and it is widely believed that some of these metabolites modulate host behavior, affecting predisposition to disease and pathogen invasion. Our access to the local marine mammal stranding network (MMSN) and previous successes in mining the fish microbiota poised us to test the hypothesis that the marine mammal microbiota is a novel source of commensal bacteria-produced bioactive metabolites. Examination of intestinal contents from five marine mammals led to the identification of a *Micromonospora* strain with potent and selective activity against a panel of Gram-positive pathogens and no discernable human cytotoxicity. Compound isolation led to the discovery of a new complex glycosylated polyketide, phocoenamicin, with potent activity against the intestinal pathogen *Clostridium difficile*, an organism challenging to treat in hospital settings. Use of our activity-profiling platform, BioMAP, clustered this metabolite with other known ionophore antibiotics. Fluorescence imaging and flow cytometry confirmed that phocoenamicin is capable of shifting membrane potential without damaging membrane integrity. Thus, exploration of gut microbiotas in hosts from diverse environments can serve as a powerful strategy for the discovery of novel antibiotics against human pathogens.

P-261

SRC/SYK-TARGETED ANTI-INFLAMMATORY ACTIONS OF TRITERPENOIDAL SAPONINS FROM GAC (*MOMORDICA COCHINCHINENSIS* SPRENG) SEEDS

Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Jiwon Baek, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, and Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea



Momordica cochinchinensis (Cucurbitaceae), also known as gac, is an indigenous Southeast Asian fruit and the seeds (Momordicae Semen) are known for its use as Traditional Chinese Medicine. Chemical investigation of the ethanol extract resulted in the identification of three triterpenoidal saponins (**1**-**3**), which were investigated for their anti-inflammatory effects. Among the saponins, momordica saponin I (**3**) inhibited NO production and transcriptional activation of inflammatory genes. Furthermore, momordica saponin I suppressed the activation of inflammatory signaling proteins (such as IκBα, Src and Syk) linked to the activation of NF-κB.

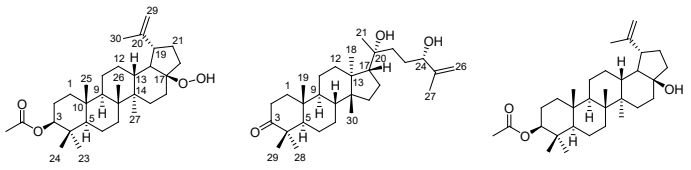
P-262

TWO NEW CYTOTOXIC TRITERPENOIDS FROM THE STEMS OF *CORNUS WALTERI*

Tae Kyoung Lee, Seoung Rak Lee, Jiwon Baek, Seulah Lee, Jae Sik Yu, Dahae Lee, Hae Min So, Sil Kim, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

A new 28-norlupene triterpenoid, 3-acetate-28-norlup-20(29)-en-3β-hydroxy-17β-hydroperoxide (**1**) and a new tirucallane triterpenoid, (20α,24*S*)-dihydroxy-tirucall-25(26)-ene-3-one (**2**), together with one known triterpenoid, 3-acetate-28-norlup-20(29)-en-3β,17β-diol (**3**), were isolated from a methanol extract of the stems of *Cornus walteri*. The chemical structures of the new compounds were elucidated by spectroscopic methods including 1D and 2D NMR and HR-MS techniques. Compound **1** was a relatively rare triterpenoid identified as a 28-norlupane-type triterpene with a 17β-hydroperoxide group and compound **3** was previously reported but only as a synthetic product. Cytotoxicity of the compounds **1**-**3** were mea-

sured by determining their inhibitory effects on human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15).

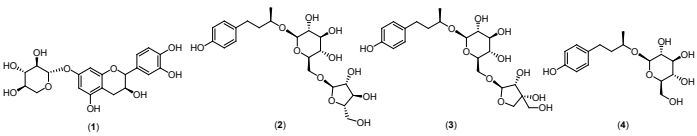


P-263

IDENTIFICATION OF CHEMICAL CONSTITUENTS CONTROLLING ADIPOCYTE DIFFERENTIATION FROM THE BARK OF *BETULA PLATYPHYLLA* VAR. *JAPONICA*

Jiwon Baek, Jae Sik Yu, Seoung Rak Lee, Seulah Lee, Tae Kyoung Lee, Hae Min So, Sil Kim, Dahae Lee, Won Se Suh, Kyoung Jin Park, Ki Hyun Kim
School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea

Betula platyphylla var. *japonica* (Miquel) Hara (Betulaceae), commonly known as Asian white birch, is an endemic species widely distributed in Korea, Japan, China, and eastern Siberia. The bark of *B. platyphylla* var. *japonica* has been used to treat pneumonia, choloplania, nephritis, and chronic bronchitis. As part of ongoing search for new bioactive compounds from Korean traditional medicines, phytochemical investigation of the bark of *B. platyphylla* var. *japonica* resulted in the isolation and identification of four phenolic compounds (**1**-**4**). Treatment of all the isolated compounds (**1**-**4**) in various concentrations in 3T3-L1 preadipocyte resulted in increased cell growth. Moreover, the treatment of the compounds (**1**-**4**) during adipocyte differentiation resulted in inhibition of adipogenesis, with decreased accumulation of lipid droplets.

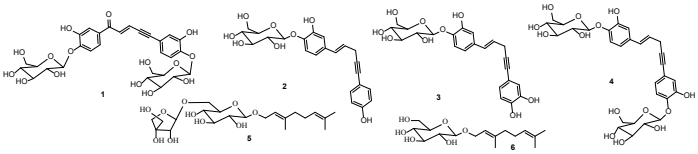


P-264

NORLIGNAN AND MONOTERPENE GLUCOSIDES FROM *HYPOXIS HEMEROCALLIDEA* (AFRICAN POTATO)

Fazila Zulfiqar[1], Samir A. Ross[1, 2], Zulfiqar Ali[1], Ikhlas A. Khan[1, 2]
[1]National Center for Natural Products Research, [2]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA

The corms of *Hypoxis hemeroclladia* have been used as a traditional medicine for centuries in South Africa for the treatment of flu, common cold, diabetes, hypertension, testicular tumors, psoriasis, urinary infections, prostate hypertrophy, cancer, HIV/AIDS, and central nervous system disorders. Two new (**1**, **2**) and two known norlignan glucosides (**3**, **4**) along with two known monoterpene glucosides **(5, 6)** were isolated from the hydroalcoholic extract of the corms of *Hypoxis hemeroclladia*. The isolated norlignan compounds **(1-4)** possess diarylpentanoid carbon skeleton (C6-C5- C6), which were classified as norlignans generated by coupling of C6-C3 and C6-C2 units. Structure elucidation was achieved by means of NMR spectroscopic and mass spectrometric techniques.

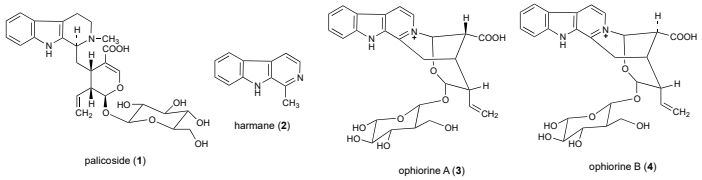


P-265

QUANTITATIVE ANALYSIS OF *SIMIRA MEXICANA* AQUEOUS EXTRACT

Isabel Rivero, Citlaly Valladares, and Rachel Mata
Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

Simira mexicana (Bullock) Steyerm (Rubiaceae) is a medicinal Mexican plant widely commercialized as a substitute of *Cinchona* spp bark. Phytochemical analysis of an aqueous extract from the stem bark of the plant led to the isolation of palicoside (**1**), harmane (**2**), and ophiorines A (**3**) and B (**4**). In order to develop a composition test for quantifying the marker compound **1**, an UPLC–ESI/MS method was developed and validated. The 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 was carried out at 270 nm and with an electrospray source in positive mode. The method was successfully validated in terms of linearity, accuracy and precision. This procedure is appropriated for quality control of the crude drug of *S. mexicana* and preparations.



P-266

CHEMICAL CONSTITUENTS FROM *STACHYS SIEBOLDII* AND THEIR INHIBITORY ACTIVITY ON SOLUBLE EPOXIDE HYDROLASE AND TYROSINASE

Yoo Kyong Han[1], Ji Sun Lee[1], Ya Nan Sun[1], Seo Young Yang[1], and Young Ho Kim[1,]*
[1]College of Pharmacy, Chungnam National University, Daejeon, 34134, Korea.

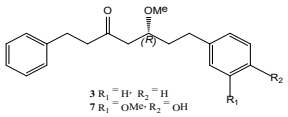
Stachys sieboldii (Labiatae) is used in traditional medicine for the treatment of ischemic stroke and gastrointestinal diseases. Nine compounds were isolated from the MeOH extract of *S. sieboldii* including 8-acetateharpagide (**1**), harpagide (**2**), monomelittoside (**3**), loganic acid (**4**), acteoside (**5**), phlinoside E (**6**), crassifolioside (**7**), *β*-sitosterol glucoside (**8**), and tryptophan (**9**). The structures were eclucidated by ^{1}H-NMR, ^{13}C-NMR spectral data and comparison with reported literatures. All isolated compounds were tested for inhibitory effects on soluble epoxide hydrolase (sEH) and tyrosinase. Compounds **2**-**6** displayed sEH inhibitory activities with IC_{50} values ranging from 26.7 ± 0.2 to 88.0 ± 0.1 μM. Likewise, compounds **1**-**4**, **8** and **9** exhibited inhibitory activity against tyrosinase with IC_{50} values ranging from 1.7 ± 0.3 to 83.0 ± 0.1 μM. The results suggest that these compounds are potential candidates that can be used for further development of preventive and therapeutic modalities for the treatment of cardiovascular risk and pigmentation disorders.

P-267

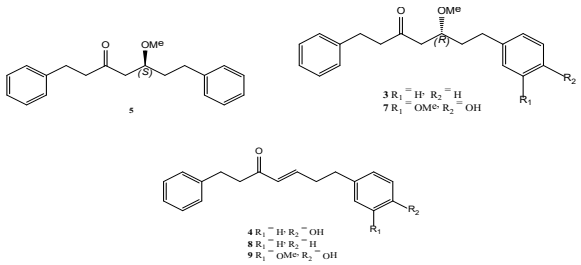
SOLUBLE EPOXIDE HYDROLASE INHIBITORY COMPOUNDS FROM THE RHIZOMES OF ALPINIA OFFICINARUM

Ji Sun Lee[1], Yoo Kyong Han[1], Seo Young Yang[1], Jin Yeul Ma[2] and Young Ho Kim[1]*

[1]College of Pharmacy, Chungnam National University, Daejeon, 305-764, Korea; [2]Center for Herbal Medicine Improvement Research, Korea Institute of Oriental Medicine (KIOM)



Alpinia officinarum (Zingiberaceae) has long been used as an anti-inflammatory and a carminative in traditional medicine. We found that a methanolic extract of the rhizomes of *A. officinarum* significantly inhibited sEH *in vitro*. A phytochemical analysis resulted in the isolation of one new diarylheptanoid compound (**11**), together with ten compounds (**1-10**), three flavonoids (**1**, **2**, **6**), six diarylheptanoids (**3-5**, **7-9**) and isobutyl *β*-D-glucopyranoside (**10**). The structures of compounds were determined based on an extensive analysis using 1D and 2D NMR, and MS spectroscopic methods. All of the isolated compounds inhibited sEH enzymatic activity in a dose-dependent manner, with IC_{50} values ranging from 2.0 ± 0.1 to 21.8 ± 3.0 μM. A kinetic analysis revealed that compound **9** was mixed –type; **7** and **8** were non- competitive-type; **3-6** and **11** were competitive inhibitors. These results proved that herbal drug for cardiovascular disease.



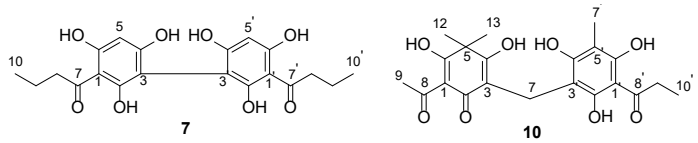
P-268

INHIBITORY EFFECTS OF PHLOROGLUCINOLS FROM THE ROOTS OF DRYOPRERIS CRASSIRHIZOMA ON MELANOGENESIS

Van Cong Pham[1], Okhwa Kim[2], Jeong-Hyung Lee[2], Byung Sun Min[3], and Jeong Ah Kim[1]

[1]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea, [2]College of Natural Sciences, Kangwon National University, Gangwon-do 24341, Korea, [3]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 38430, Republic of Korea

Two new phloroglucinols (**7** and **10**), along with 12 known derivatives (**1-6**, **8-9**, and **11-14**), were isolated from the roots of *Dryopteris crassirhizoma* (Aspiadaceae). Their chemical structures were elucidated by various spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, and HMBC) and high-resolution mass spectrometry. All isolates (**1-14**) were tested for their inhibitory effects on melanin production in B16F10 murine melanoma cells. Norflavaspidic acid PB (**9**) inhibited melanin production with IC_{50} value of 38.9 μM and was also shown to inhibit tyrosinase activity in a dose-dependent manner and melanogenesis with an IC_{50} value of 5.9 μM in B16F10 cells stimulated with 3-isobutyl-1-methylxanthine (IBMX).



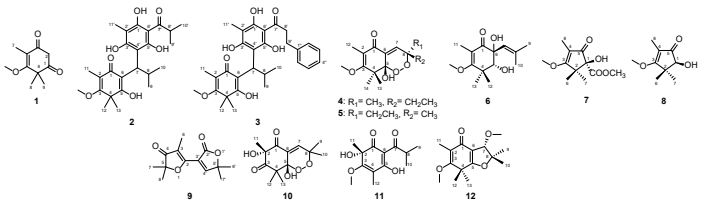
P-269

PHYTOCHEMICAL STUDY ON INDONESIAN BAECKEA FRUTESCENS

Takuya Ito[1], Khoirun Nisa[1,2], Hiroyuki Morita[1]

[1]Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan, [2]Research Unit for Development of Chemical Engineering Processes, Indonesian Institute of Sciences (LIPI), Jl. Jogja-Wonosari Km. 32, Playen, Gunungkidul, Yogyakarta, 55861, Indonesia.

Phytochemical investigation of the $CHCl_3$ extract of the leaves of *B. frutescens* allowed us to isolate twelve new compounds including nine phloroglucinol-type metabolites, baeckenones A–I (**1**–**6**, **10**–**12**), two cyclopentenone derivatives, frutescencenones A and B (**7** and **8**), and a furanone derivative, frutescencenone C (**9**), along with ten known compounds (**13**–**22**). Herein, we report the isolation and structure determination of these compounds, as well as their antibacterial and cytotoxic activities.



P-270

THE LITERATURE REVIEW OF THE EAST ASIAN TRADITIONAL HERBAL MEDICINE ON AUTISM SPECTRUM DISORDER

Sun Haeng Lee[1,2], Sang Min Kim[1,2], Hye Lim Lee[3], Su Hyang Ryu[4], Jin Yong Lee[1,2], Gyu Tae Chang[1,5]

[1]Department of Clinical Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea, [2]Department of Pediatrics of Korean Medicine, Kyung Hee University Korean Medical Hospital, Kyung Hee University Medical Center, Seoul 02447, Republic of Korea, [3]Department of Pediatrics, College of Korean Medicine, Gachon University, Gyeonggi-do 13120, Republic of Korea, [4]Department of Pediatrics, College of Oriental Medicine, Dongshin University, Jeollanam-do 58245, Republic of Korea, [5]Department of Pediatrics of Korean Medicine, Kyung Hee University Hospital at Gang-dong, Seoul 05278, Republic of Korea

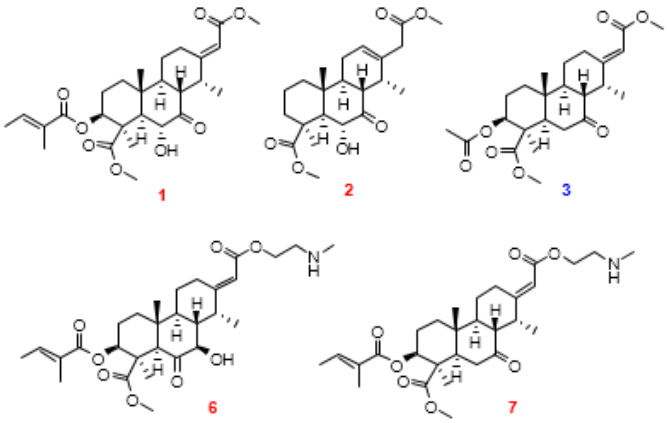
We searched the 13 databases for evaluating use of the East Asian Traditional Herbal Medicine. The 33 clinical studies and 682 cases were reviewed for analysis. The most prescribed herb on autism spectrum disorder was *Poria cocos* (61.73%), and the 10.96 ± 2.24 g was the clinical dose of *P. cocos* for one day in 13 studies reporting whether adverse events mildly occurred or not. *P. cocos* is composed of ß-pachyman, pachymic acid, polyporenic acid, tumulosic acid, eburicoic acid, pinicolic acid, ergosterol, lecithin, histidine, and choline. More than 90% of the constituent in *P. cocos* is ß-pachyman. Pachyman or pachymic acid might have the potential bioactive efficacy on autism spectrum disorder.

P-271

APOPTPSIS-INDUCING ACTIVITY OF CASSAINE DITERPENOIDS FROM *ERYTHROPHLEUM FORDII* AGAINST HUMAN LUNG CANCER CELLS

Manh Tuan Ha[1], Jeong Ah Kim[2], and Byung Sun Min[1]
[1]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 38430, Republic of Korea, [2]College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea

A study of the bark of *Erythrophleum fordii* yielded four new compounds, two new cassaine diterpenoids (erythrofordin T and U, **1** and **2**) and two new cassaine diterpenoid amines (erythroformine A and B, **6** and **7**), together with nine known compounds (**4**-**5**, **8**-**13**). All structures were elucidated using spectroscopic analysis. Cytotoxic activity of the isolated compounds (**1**-**13**) was examined *in vitro* against lung cancer cells. Cassaine diterpene amines (**6**-**10**, **12**, **13**) exhibited potent cytotoxic activity against all three cell lines with IC_{50} values between 0.4 μM and 5.9 μM. Erythroformine B (**7**) significantly induced apoptosis in all three cancer cells in a concentration-dependent manner.



P-272

PHENOLOGICAL CYCLE OF *ELAPHOGLOSSUM PALEACEUM* (HOOK. & GREV.) SLEDGE. AND IN VITRO PRODUCTION OF BIOACTIVE PHLOROGLUCINOLS

María Goretti Arvizu Espinosa[1], María Luisa Villarreal Ortega[1], Amelia Teresinha Henriques[2], Gilsane Lino von Poser[2], Aniceto Mendoza Ruiz[3], Ashutosh Sharma[4] and Alexandre Cardoso Taketa[1]
[1]Centro de Investigación en Biotecnología, UAEM, Cuernavaca, México, C.P. 62209; [2]Universidade Federal do Rio Grande do Sul, Porto Alegre Rio Grande do Sul, Brasil CEP: 90040-060; [3]Universidad Autónoma Metropolitana-Iztapalapa, Ciudad de México, México, C. P. 09340; [4] Instituto Tecnológico y de Estudios Superiores de Monterrey Campus Querétaro, Querétaro, México, C. P. 76130.

Two new dimeric phloroglucinols, Paleacenin A and B with potential antidepressant activity (IC_{50} MAO-A 31.03 ± 0.13µg/mL and 1.26 ± 0.27µg/mL, respectively) were isolated from the rhizomic hexanic extract of the fern Elaphoglossum paleaceum.

Callus cultures of *E. paleaceum* were initiated from the adult stage of the prothallus in order to establish a homogeneous and controlled production of phloroglucinols.

P-273

NATURAL DEEP EUTECTIC SOLVENTS AS POTENTIAL GREEN EXTRATION MEDIA OF CURCUMINOIDS FROM *CURCUMA LONGA* L.

Geonha Park[1,2], Sang cheol Park[1,2], Qianwen Wu[1,2], Young Pyo Jang[1,2]
[1]Department of Life and Nanopharmaceutical Sciences, Graduated School, Kyung Hee University, Seoul 130-701, Republic of Korea; [2]Division of Pharmacognosy, College of Pharmacy, Kyung Hee University, Seoul 130-701, Republic of Korea

Natural deep eutectic solvents (NADES) are standing out as a new green sources to replace risky organic solvents which have been used for lots of chemical reaction, extraction, and synthesis. In this study, an efficient extraction method using ten investigated NADESs was developed and the extraction efficacy on curcuminoids from rhizomes of *Curcuma longa* L. was compared to water and methanol commonly used organic solvent for the extraction of natural plant sources. Results showed that the extraction efficacy of curcuminoids was significantly higher in all of ten NADESs as in water up to a highest of 116.99 mg/g in NADES compare to 3.35 mg/g in water. Two of the investigated solvents showed even higher efficiency than methanol extraction. Based on the results, the investigated NADESs may be used as environmentally friendly solvents improving extraction problem of natural products for various industris.

P-274

EVALUATION OF NATURAL PRODUCT COMPOSITIONS FOR APPETITE SUPPRESSION

Mesfin Yimam,[1] Ping Jiao,[1] Mei Hong,[1] Lidia Brownell,[1] Young-Chul Lee,[2] Eu-Jin Hyun,[2] Hyun-Jin Kim,[2] Tae-Woo Kim,[2] Jeong-Bum Nam,[2] Mi-Ran Kim,[2] and Qi Jia[1]
[1]Unigen Inc., 3005 1st Avenue Seattle,WA 98121, USA; [2]Unigen Inc., No. 450-86, Maebong-Ro, Dongnam-Gu, Cheonan-Si, Chungnam 330-863, S. Korea

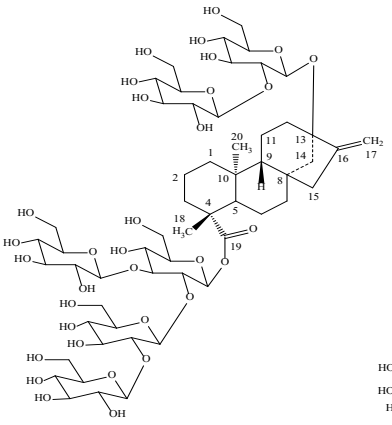
Obesity and its comorbidities continue to challenge the world at an alarming rate. Although the long term solution lies in lifestyle changes, a natural product based intervention could be an inexpensive and relatively safer substitute for prescription drugs to combat obesity and aid in healthy weight management. Many plant based weight management products are available in DSHEA market. However no direct efficacy comparison data are available for the active ingredients to help consumers make an educated choice. In this in vivo acute feed intake study, male Sprague-Dawley rats (Harlan) were used to evaluate the efficacy of well-known natural ingredients in the weight loss market. On the day of testing, 16-hour fasted animals, single housed in cages with grid floors with free access to water and no food, were orally administrated different natural ingredients suspended in 0.5% CMC at designated dosages associated with recommended human consumption. Thirty minutes after oral administration, animals were allowed access to a highly palatable 45% Kcal high-fat diet (TD06415, Harlan). Food consumption was then monitored at 1, 2, 4, 6, 8, 10, and 24 hours after food exposure. We tested pure caffeine, potato skin extract, *Cissus quadrangularis* extract, *Garcinia cambogia* fruit extract, *Crocus sativus* bulb extract, *Morus alba* root bark extract, *Magnolia officinalis* bark extract, *Ilex paraguariensis* leaf extract, Raspberry Ketone from *Rubus idaeus*, one commercial product – Appetrex and one novel composition – UP601 for their impact on acute food intakes of SD rats. The details of the results and discussion will be described.

P-275

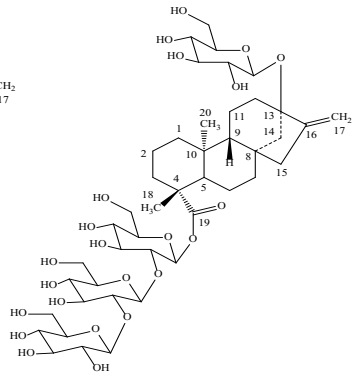
TWO NEW STEVIOL GLYCOSIDES ISOLATED FROM THE *STEVIA REBAUDIANA*

Srinivasarao Meneni and Venkata Sai Prakash Chaturvedula
Natural Products Research Group, Wisdom Natural Brands, 1203 West San Pedro Street, Gilbert, AZ 85233, USA.

Two additional new minor diterpene glycosides have been isolated from the commercial extract of the leaves of *Stevia rebaudiana* Bertoni. Structures of the new compounds have been established as (13-[(2-*O*-**β**-D-glucopyranosyl-**β**-D-glucopyranosyl)oxy] *ent*-kaur-16-en-19-oic acid-[(2-*O*-(2-*O*-**β**-D-glucopyranosyl)-**β**-D-glucopyranosyl-3-*O*-**β**-D-glucopyranosyl-**β**-D-glucopyranosyl) ester] (**1**) and 13-[**β**-D-glucopyranosyloxy] *ent*-kaur-16-en-19-oic acid [2-*O*-(2-*O*-**β**-D-glucopyranosyl-**β**-D-glucopyranosyl) **β**-D-glucopyranosyl ester (**2**) on the basis of extensive NMR spectroscopy (^{1}H &^{13}C, TOCSY, HMQC, and HMBC) and High Resolution (HR) mass spectroscopic data as well as enzymatic and acid hydrolysis studies.



1



2

P-276

PROGESTERONE-ANTAGONISTIC ISOFLAVONES IN RED CLOVER (*T. PRATENSE*)

Jeongho Lee[1], Matthew Dean[1], Joanna E. Burdette[1], Brian T. Murphy[1]
[1]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL

Trifolium pratense (red clover) is taken by millions of women as hormone replacement therapy. Though *T. pratense* contains phytoestrogens, extracts do not increase uterine weight in rats, or the proliferative index in the endometrium of women. This suggests that phytoprogestins may be present in *T. pratense* that are inhibiting the observed estrogenic activity. Few studies have focused on identifying progestogenic components in botanical supplements, particularly in *T. pratense*. Importantly, if specific botanicals contain antagonists of progesterone, this may be a reason for women to avoid their use if they are attempting to get pregnant. Additionally, partial antagonists are being developed for the treatment of fibroids in women, which impact 6.6% of all women and results in infertility, menorrhagia, and pain. Using a progesterone response element (PRE)-luciferase reporter assay in T47D cells, we characterized the agonist and antagonist effects of red clover extracts. Red clover extract had no effect on PRE-luciferase activity in the absence of progesterone, indicating no agonists were present. In contrast, in the presence of 1 μM of progesterone, the red clover extract reduced PRE-luciferase activity by 90%, indicative of an antagonist. Bioassay-guided isolation was performed using HPLC-UV and two fractions showed antagonistic activity (reduced progesterone-stimulated luciferase activity by 94 and 95%). The bioactive compounds were purified and identified as formononetin (**1**) and biochanin A (**2**) through analysis of ^{1}H and ^{13}C NMR, high-resolution MS, and comparison of HPLC retention time with authentic samples. These results indicate that a partial progesterone receptor antagonist has been identified in red clover and may impact hormonal signaling.

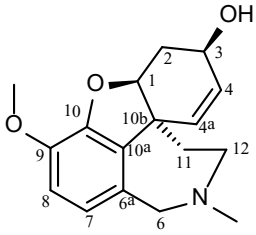
P-277

ACHE VE BCHE INHIBITOR ALKALOIDS FROM *LEUCOJUM AESTIVUM* L.

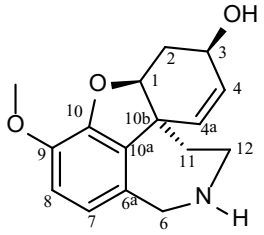
Ozlem Demirkiran[1], Canan Karancula[2]
[1]Trakya University, Faculty of Pharmacy, Department of Pharmacognosy 22030 Edirne, TURKEY, [2]Trakya University, Faculty of Science, Department of Chemistry 22030 Edirne, TURKEY.

A total of four Amaryllidaceae alkaloids were isolated from the dichloromethane extract of the *Leucojum aestivum* L., three of which were galantamine type and one was the lycorin type. One of the galantamine type alkaloids, named dihydroxyhabranthine, was isolated for the first time from plants. The structure elucidation of the compounds achieved by spectral methods such as IR, UV, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, HSQC, COSY, ESI-MS.

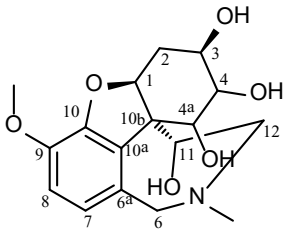
The isolated compounds (**1-4**) were screened to detect AChE and BCHE inhibitory activities. When tested against the AChE, galantamin (**1**), epinorgalanthamine (**2**), dihydroxyhabranthine (**3**), 2-*O*-acetyldemethyllycorenine (**4**) displayed IC_{50} values of 3.5 μM, 7.5 μM, 12.5 μM, 730.0 μM, respectively. The IC_{50} values for BChE inhibition by compounds (**2-4**) were 840.0, 19.3, 197.0 μM, respectively. Standard inhibitor (galanthamine) exhibited AChE and BChE inhibition with IC_{50} value of 3.5 μM and 36.0 μM, respectively.



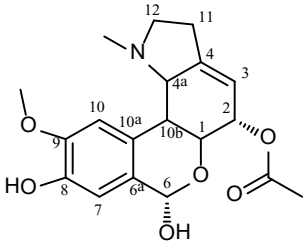
1



2



3



4

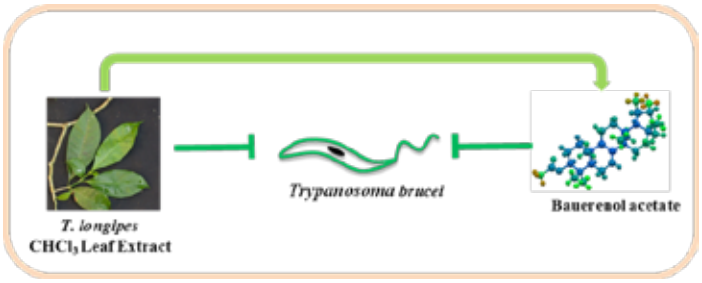
P-278

EXPLORING DIVERSE NATURAL PRODUCTS CHEMICAL SPACE FOR NEW ANTITRYPANOSOMAL AGENTS.

Ifedayo Victor Ogungbe, Simira Carothers, Huaisheng Zhang, Rogers Nyamwihura, Jasmine Collins
Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS, 39217, USA.

Neglected tropical diseases caused by protozoans remain significant burden to public health systems in many developing countries. In this work, the Latin American plant *Tabernaemontana longipes* was studied as a potential source of antiprotozoal agent, and a series of natural products-based compounds and their bioisosteres was designed, synthesized, and evaluated for

antiprotozoal activity. The pentacyclic triterpenoid bauerenol acetate was identified as the antitrypanosomal agent in *T. longipes* while compounds with homomyretenoyl, bornyl, and quinolinyl moiety shows particularly good and selective low-micromolar growth inhibitory activity against *T. brucei* and/or *T. cruzi*. To improve the bioactivity of the compounds, structural analogues and physicochemical congeners are currently being synthesized and evaluated. The isolation, synthesis, and biological assay data will be presented.



P-279

STANDARDIZATION OF *BANISTERIOPSIS CAAPI*: TOWARDS AN FDA-APPROVED PHASE I TRIAL OF *B. CAAPI* IN PARKINSON'S DISEASE

Hayley Prescott, Rebecca Mains, Kaleb Lund, and Leanna Standish

The active constituents of ayahuasca tea are known to be indole alkaloids including *N,N*-dimethyltryptamine (DMT) and the β-carbolines, harmine, harmol, tetrahydroharmine (THH), harmaline and harmalol. These alkaloids have multiple neurotransmitter effects in the human brain that are relevant to the treatment of some neurological and psychiatric diseases including Parkinson's disease, depression, obsessive compulsive disorder and alcoholism. The objective of this project is to make a replicable, stable, standardized, FDA ready hot water extract of *Banisteriopsis caapi* vine (Bc) at a concentration of 2mg harmine/mL.

Plant material was collected from the Big Island of Hawaii and stored at -20°C. Fresh vine was collected by Dr. Leanna Standish in February 2015/2016 and shipped overnight to the lab. Twelve hot water extractions utilizing various methods such as Soxhlet extraction, and 8-12 hour long hot water decoctions were attempted in order to reach the target goal of 2mg/mL of harmine. Triplicate extracts were analyzed by HPLC and the concentrations of β-carbolines were quantitated against known standards. Data are reported as mg/mL alkaloids and will be used to direct future large-scale extractions towards producing the FDA ready extract. Our highest concentration of harmine at 1.5 mg/mL came from a traditional preparation of pounding the vine and extraction via a double aqueous decoction of the same botanical raw material (BRM) that was then concentrated.

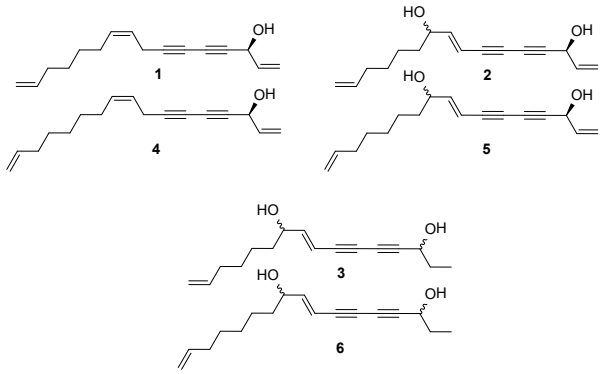
This project was supported by Bastyr Research Seed Grant 2015-16 #5/ Lund-Standish

P-280

THREE NEW C_{16} AND THREE KNOWN C_{17} POLYACETYLENES ISOLATED FROM NCI PLANT SAMPLE 5-C9 (*DESMANTHODIUM GUATEMALENSE*)

Shengxin Cai[1,2], Corena V. Shaffer[3], April L. Risinger[3,4], Barry R. O'Keefe[5], Susan L. Mooberry[3,4], Robert H. Cichewicz[1,2]

[1]Natural Product Discovery Group, Institute for Natural Products Applications and Research Technologies and [2]Department of Chemistry & Biochemistry, Stephenson Life Science Research Center, University of Oklahoma, Norman, OK 73019, [3]Department of Pharmacology and [4]Cancer Therapy & Research Center, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229-3900, [5]Natural Products Branch, National Cancer Institute, Frederick, MD, 21702



Three new C_{16} and three known C_{17} polyacetylenes (**1-3** and **4-6**, respectively) were isolated from NCI plant sample 5-C9 (*Desmanthodium guatemalense*). Polyacetylenes with a C_{17} chain are common products from plant, but analogs with 16 carbons were rarely reported. Mosher's reaction was performed on these compounds, and the absolute configurations of **1** and **4** were established. Compounds **2-3** and **5-6** were confirmed to be mixtures of stereoisomers by Mosher's reaction and chiral HPLC analysis.

P-281

EFFECTS OF ESSENTIAL OIL FROM *LICARIA RIGIDA* ON THE VIABILITY OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS* AND THE HOST CELL

Joyce da Silva[1], Amanda Hage[1], Bruno da Silva[1], Edilene da Silva[1], William Setzer[2]

[1] Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA 66075-110, Brazil [2]Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

Leishmaniasis are neglected emerging diseases in 98 countries caused by several species of protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies. The treatment of leishmaniasis is limited due to the several side effects and the development of protozoan parasite resistance against the drugs used. In recent years, alternative therapies using natural products have emerged, mainly derived from plants species commonly used in herbal medicine. Some plant essential oils (EOs) have immunomodulatory effects applicable to the treatment of infectious diseases, especially those that have no direct adverse effect on host cells. In the present study, we evaluated the cytotoxicity effects *Licaria rigida* essential oil rich in β-caryophyllene (76.1%), α-humulene (6.61%) and viridiflorene (4.65%) on murine macrophage cell line J774G8 and the antileishmanial effects against promastigote form of *Leishmania (Leishmania) amazonensis* (MHOM/BR/26361). The macrophages and parasite were treated for 72 hours and 96 hours, respectively, and the cell viability was performed by MTT assay. Treatment with the sample promoted a reduction in the number of promastigotes of *L. (L.) amazonensis* (IC_{50}= 19.2 µg/mL, SI= 43). No cytotoxic effect was observed when the macrophages were treated with

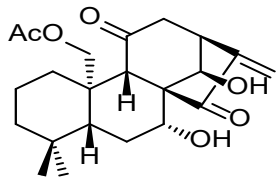
different concentrations of the EO (CC_{50}= 827.6 µg/mL) when compared to the control group. This preliminary results shows that the EO could be useful as alternative source for a new antileishmanial agent.

P-282

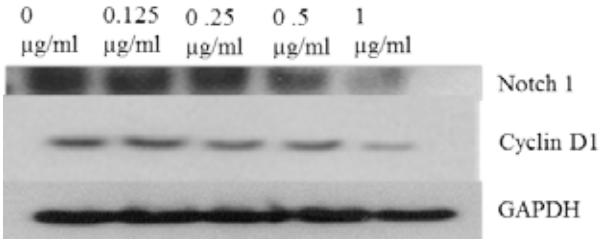
ANTICANCER ACTIVITY AND MECHANISM OF ACTION OF A NOVEL *ENT*-KAURANE DITERPENOID

Md. Shahid Sarwar[1], Siu Wai Tsang[1], Man Shing Wong[2], Hong-Jie Zhang[1*]
[1]*School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, P.R. China.* [2]*Department of Chemistry and Institute of Advanced Materials, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, P.R.China*

Ent-kaurane diterpenoids are considered as a promising source for developing new anticancer agents. The genus *Isodon* is a rich source of *ent*-kaurenes, of which about 1,000 compounds have been reported. By evaluating our diterpene compound library, a novel semi-synthetic *ent*-kaurane diterpenoid (CLS566) was found to exhibit cytotoxic activity with an IC_{50} value of 1.95 µM in prostate cancer cell line PC-3. It induced apoptosis and cell cycle arrest, at least partly by down-regulating the expression of apoptosis and cell cycle markers notch 1 and cyclin D1. *Acknowledgements: This work is part of the cancer projects funded by the Innovation and Technology Fund of Hong Kong Special Administrative Region, China (Project No. ITS/254/16) and the National Natural Science Foundation of China (NSFC 81673476).*



CLS566

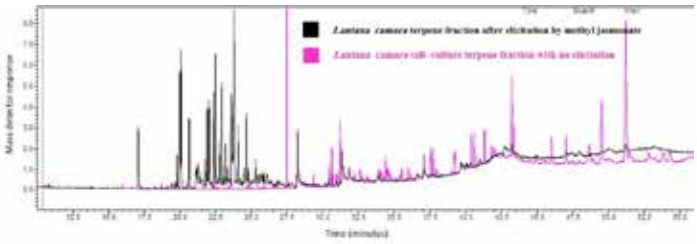


P-283

USING WEEDS AS BIOTRANSFORMATIONAL PLATFORMS FOR SECONDARY METABOLITES PRODUCTION: *LANTANA CAMARA* AS AN EXAMPLE

Maged El-Sayed Mohamed[1,2]
[1]*College of Clinical Pharmacy, King Faisal University, P.O. 55157, Ahsaa 31982, Kingdom of Saudi Arabia.* [2]*Department of Pharmacognosy. Faculty of Pharmacy, University of Zagazig, Zagazig 44519, Egypt*

Lantana camara is a weed, which is common all over the world as an ornamental plant. Weeds can adapt to any type of soil and climate due to their rich cellular machinery for secondary metabolites' production and this does apply to *Lantana camara*. Cell-suspension culture of the plant was produced and their metabolic profile was examined using GC-MS and HPLC-DAD. Application of different external compounds such as caffeine, some essential oils such as wintergreen or elicitation by different hormones such as methyl jasmonate resulted the appearance of s everal new peaks in the GC-MS and HPLC-DAD traces.



P-284

HOPS (*HUMULUS LUPULUS* L.) GROWN IN HOT, ARID ENVIRONMENT HAVE ELEVATED LEVELS OF A GREEN LEAF VOLATILE GLUCOSIDE

Taylan B. Morcol[1,2,3], Paul D. Matthews[4], Adam Negrin[1,2], and Edward J. Kennelly[1]
[1]*Department of Biological Sciences, Lehman College, City University of New York, 250 Bedford Park Blvd. W, Bronx, NY 10468,* [2]*PhD Program in Biology, The Graduate Center, City University of New York, 365 Fifth Ave., New York, NY 10016,* [3]*Institute of Economic Botany, New York Botanical Garden, 2900 Southern Blvd., Bronx, NY 10458,* [4]*S.S. Steiner, Inc., 1 West Washington Ave., Yakima, WA 98903*

1-Hexanol is a green leaf volatile, a type of compound released in response to tissue damage. Plants typically store green leaf volatiles as glucosides and release the aglycones when stressed. We used ultra-high performance liquid chromatography triple quadrupole detector mass spectrometry (UPLC-TQD-MS) to quantify levels of 1-hexanol glucoside—and 28 other compounds—in 23 commercial varieties of hops grown in two different locations in the northwestern United States. Levels of 1-hexanol glucoside were significantly higher in hop varieties grown in the Yakima Valley, Washington than in varieties grown in Bonners Ferry, Idaho. No such trend was observed for the other 28 compounds (phenolics, amino acids, and glycosides of phenolic and volatile compounds). It is suspected that the relatively high temperatures, insect pressure, and drought conditions in Yakima contribute to higher levels of 1-hexanol glucoside in the hops from Yakima. Further experiments are needed to determine if the same trend can be observed across multiple years and for other compounds.

P-285

BENEFICIAL EFFECT OF BIOACTIVE CRANBERRY FLAVONOIDS ON ADIPOGENESIS AND LIPOLYSIS

Yifei Wang[1], Ajay P. Singh[1] and Nicholi Vorsa[1, 2]
[1]*Department of Plant Biology, Rutgers University, New Brunswick, NJ, 08901,* [2]*P.E. Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers University, Chatsworth, NJ,08019*

The effect of individual cranberry flavonoids, including flavonols, proanthocyanidins and anthocyanins, on adipogenesis and lipolysis were investigated using 3T3-L1 preadipocytes/adipocytes model cells, to identify potential health benefits against obesity. A total of 17 flavonoid compounds or fractions were evaluated. Myricetin-3-galactoside and total proanthocyanidins were the most active compounds inhibiting 3T3-L1 pre-confluent preadipocyte proliferation in their respective groups, with IC_{50} values at 99 and 53µg/ml respectively. Anthocyanins showed similar but modest levels of proliferation inhibition (IC_{50} values between 154 and 220µg/ml). Quercetin aglycone and total proanthocyanidins exhibited the strongest inhibition of post-confluent mitotic clonal expansion, with IC_{50} values at 214 and 40µg/ml. In addition they inhibited preadipocyte differentiation (adipogenesis), as 72 h treatment of quercetin algycone (100 µg/ml) or total proanthocyanidins (25 µg/ml) in MDI medium lead to 20% or 39% reduc-

tion on lipid and fatty acid accumulation of differentiated adipocytes, as revealed by Oil Red O staining. Ongoing studies are focusing on these most active flavonoids, e.g., myricetin-3-galactoside, quercetin aglycone and total proanthocyanidins, in determining their effect on lipolysis and the expression of key adipogenesis, lipolysis or triglyceride synthesis regulatory molecules.

P-286

COMPARATIVE STUDY OF TRUE BAY LEAF AND ITS SUBSTITUTES USING GC/Q-TOF COUPLED TO CHEMOMETRIC TOOLS

Mei Wang[1], Vijayasankar Raman[1], Jianping Zhao[1], Bharathi Avula[1], Yan-Hong Wang[1], Philip Wylie[3] and Ikhlas Khan[1,2]
[1]National Center for Natural Products Research, and [2]Division of Pharmacognosy, Department of BioMolecular Science, School of Pharmacy, University of Mississippi, University, MS 38677, [3]Agilent Technologies, Wilmington, DE 19808

Bay leaf is one of the well-recognized culinary leaf spices used in flavoring a wide variety of foods. Botanically, the true 'bay leaf' is the leaf from the tree *Laurus nobilis* distributed mainly in the Mediterranean region. The leaves of several other species, such as *Cinnamomum tamala, Pimenta racemosa, Syzygium polyanthum* and *Umbellularia californica*, are often sold as 'bay leaves'.

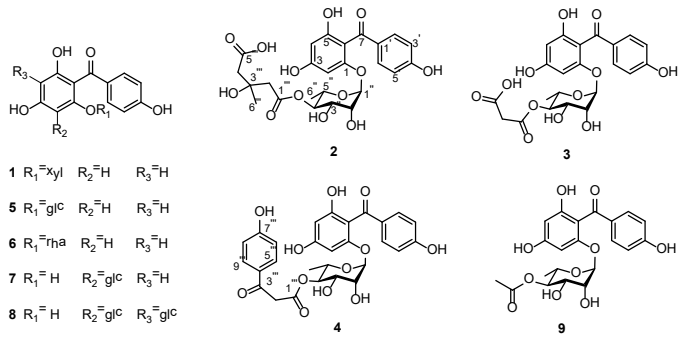
It is clear that efficient analytical methods are essential for quality control of bay leaves. In the present study, 52 authenticated and commercial samples from five species of 'bay leaves' were analyzed by GC/Q-ToF and NMR techniques. The chromatographic and mass spectroscopic data were subjected to chemometric analysis. Different clusters were observed by principal component analysis (PCA). A predictive model was constructed based on partial least square-discriminant analysis (PLS-DA) for classification and differentiation of various bay leaves. The proposed method demonstrated the feasibility of using a predictive model to differentiate the different products marketed as "bay leaf".

P-287

BENZOPHENONE GLYCOSIDES IN FLOWER BUDS OF *AQUILARIA SINENSIS*

Hanwen Yuan,[1,2] Jianping Zhao,[1] Mei Wang,[1] Chunmei Zhai,[1,3] Shabana I. Khan,[1] Qiongming Xu,[4] Wei Wang[2], and Ikhlas A. Khan[1]
[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University of Mississippi, MS 38677, USA. [2]Hunan University of Chinese Medicine, Changsha 410208, P.R. China.[3]Heilongjiang University of Chinese Medicine, Harbin 2193442, P.R. China. [4]College of Pharmaceutical Science, SooChow University, Suzhou 215123, P.R. China

Aquilaria sinensis (Lour.) Gilg (Thymelaeaceae) is an economically important plant and widely planted in southern China. The resinous wood of *A. sinensis,* called 'Cheng-Xiang' in China, plays an important role in TCM for using as digestive, analgesic, sedative and antiemetic agents. The leaves and flowers are consumed as a healthy tea in China due to their health care function. The extract and constituents from the leaves were found to possess anti-inflammatory activity, laxative effect, and inhibitory activity in *vitro* against α-glucosidase. However, no phytochemical and biological studies were conducted on the flower buds of this plant. A phytochemical investigation on the flower buds led to the discovery of four new benzophenone glycosides along with five known ones. The isolates and extracts were evaluated for their anti-inflammatory and cytotoxic activities.



P-288

12A-HYDROXY ROTENOIDS FROM *MIRABILIS MULTIFLORA* (TORR.) GRAY

Vimal K Sharma[1], *SVS Radhakrishnan[2], Zulfiqar Ali[2], Cammi Thornton[1], Kristie L Willett[1], Ikhlas A Khan[2] and Jordan K Zjawiony[1]*
[1]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS, 38677 USA. [2]National Center for Natural Product Research, The University of Mississippi, University, MS, 38677 USA.

The genus *Mirabilis* (four-O'clock) belongs to Nyctaginaceae family and contains about 60 different species. *Mirabilis multiflora* (Torr.) Gray also known as Colorado four o'clock is a perennial herb, found in southwest and western regions of the United States. Traditionally, the Navajos used roots as a poultice to reduce swellings, internally for rheumatism and externally for mouth disorders. The Western Keres used the dried leaves as a tobacco substitute. *M. multiflora* is a well-known Hopi hallucinogen as the Hopi Indians used roots to induce "diagnostic visions". Roots of this plant are also used as an antiseptic, blood strengthener and to induce visions. Twenty eight to fifty seven grams of the root is said to result in "a half hour of gaiety". Till date there are no documented records on the phytochemical and pharmacological studies on this plant. Thus, the present work aims to isolate bioactive moieties from *M. multiflora* roots and to carry out their pharmacological studies. Acetone extract (30 grams) of *M. multiflora* roots was subjected to repeated column chromatography which resulted in the isolation of three new 12a-hydroxy rotenoids and three known rotenoids along with some other known compounds. The structures of all the secondary metabolites isolated from *M. multiflora* were elucidated via 1D and 2D NMR and other spectroscopic techniques. The compounds have been submitted for their different pharmacological potential. The schematics for isolation and bioassay results of these compounds will be presented in the forthcoming ASP annual meeting.

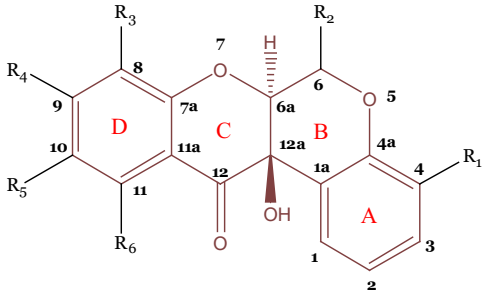


Figure 1: Scaffold of 12a-hydroxy rotenoids isolated from *M. multiflora* roots.

P-289

AN INVESTIGATION OF ANTHELMINTIC SECONDARY METABOLITES OF *DALEA PARRYI* (FABACEAE)

Gil Belofsky[1], Katherine Nash[1], Blaise Dondji[2], Kiah Jones[2], and Victoria McPherson[2]
[1]Department of Chemistry, Central Washington University, Ellensburg, WA 98926, USA, [2]Laboratory of Cellular Immunology and Parasitology, Department of Biological Sciences, Central Washington University, Ellensburg, WA 98926, USA.

Continued investigation of plants of the genus *Dalea* for anthelmintic metabolites was prompted by the discovery of potent inhibitors of hookworm survival from *D. ornata*. *D. parryi* Torr. & A. Gray is an inconspicuous, sprawling plant, native to its collection site in Arizona. Crude methanol extracts of the aerial and root portions of *D. parryi* each exhibited weak activity toward the human pathogenic hookworm *Ancylostoma ceylanicum*, with up to 10% inhibition of worm survival observed after five days at 100 mg/mL. *A. ceylanicum* is a blood feeding intestinal parasite that infects nearly a billion people in tropical and subtropical areas of the world. Hookworm infection often leads to iron-deficiency anemia, weight loss, stunted growth, and malnutrition. Only a few drugs of the benzimidazole class are effective as treatments, and growing resistance to these has been observed. An increase in potency was observed with further purification of the crude extracts, and continued chromatographic work has led to the characterization of four new and ten known phenolic compounds. Structure determinations were accomplished primarily by extensive NMR spectroscopy and by mass spectrometry. Details and results of the *ex vivo* hookworm assay, relative toxicity to healthy cells, and structures of the isolated compounds will be presented.

P-290

ANALYSIS OF PESTICIDE RESIDUES IN CANNABIS USING GC/Q-TOF

Philip L. Wylie[1], Mei Wang[2], Mohamed Radwan[2], Chandrani Gon[2], Mahmoud A. ElSohly[2,3] and Ikhlas A. Khan[2,4]
[1]Agilent Technologies, Wilmington DE 19808; [2]National Center for Natural Products Research, [3]Division of Pharmaceutics, [4]Division of Pharmacognosy, Department of BioMolecular Science, School of Pharmacy, University of Mississippi, University, MS 38677

As of the November 2016 election, 29 states have approved the use of medical cannabis and eight states, representing 65 million people, have approved recreational use by adults. Canada allows medical use and is on a path to full legalization. Because the US government still classifies cannabis as a schedule 1 drug, all legislation controlling the growing, testing and use of cannabis products is done at the state level. There is no uniformity in the regulations and their enforcement. Pesticide use on cannabis plants is very controversial, but is loosely regulated in most states.

It is clear that pesticide residue testing is important for a product that may be eaten or inhaled. This report describes the analysis of cannabis extracts for pesticide residues using an accurate mass high resolution GC/Q-TOF. Chromatograms are analyzed by applying an "all ions" approach using a personal compound database and library (PCDL) containing about 850 pesticide exact mass spectra. The software screens for all compounds in the PCDL in just a couple of minutes. Standards are only needed for those pesticides that are found and need to be quantified. This approach has also been used to identify pesticide residues in food and herbal extracts.

P-291

PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATIONS ON THE SECONDARY METABOLITES FROM PULSATILLA PATENS VAR. PATENS

Vimal K Sharma[1], Grażyna Łaska[2], Aneta Sienkiewicz[2], SVS Radhakrishnan[3], Zulfiqar Ali[3], Cammi Thornton[1], Kristie L Willett[1], Ikhlas A Khan[3] and Jordan K Zjawiony[1]
[1]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677 USA. [2]Department of Environmental Protection and Management, Bialystok University of Technology, 15-351 Bialystok, Poland. [3]National Center for Natural Product Research, The University of Mississippi, University, MS 38677 USA

Pulsatilla patens (L.) Mill. (family: Ranunculaceae) or *Anemone patens* L. is considered as a threatened plant species in many parts of Europe. Subspecies such as *P. patens* var. *multifida*, contains triterpenoid glycosides with hederagenin as an active component. Traditional Chinese herbal extract Fructus Akebiae has been used for depressive disorders and it was found that hederagenin is the active ingredient of the extract. Studies on pure isolated compounds has not been reported yet, hence our current research work aims to perform phytochemical and pharmacological studies on the bioactive secondary metabolites from *Pulsatilla patens* var. *patens*. The phytochemical studies were executed on the ethanolic (EtOH) extract of the roots of *P. patens* var. *patens*. The EtOH extract was first dissolved in dist. water and it was then extracted with chloroform ($CHCl_3$). The Aqueous fraction was subjected to reverse phase column chromatography using sephadex LH 20 and C18-reversed phase silica gel which resulted in the isolation of different triterpenoid glycosides. Phytochemical and pharmacological investigations are still in progress in our lab. The detailed scheme for the isolation and pharmacological studies on the secondary metabolites obtained from *P. patens* var. *patens* EtOH extract will be presented in the forthcoming ASP annual meeting.

P-292

DIFFERENTIATION OF *CUDRANIA TRICUSPIDATA* ACCORDING TO REGIONAL AND SEASONAL VARIANCES USING LC-MS-BASED METABOLOMICS TECHNIQUE

Nahyun Kim, Chanhoon An, Min Sung Lee, Da Som Kim, Su Jin Sim, Jeong Ho Song, Mahn-Jo Kim
Forest Medicinal Resources Research Center, National Institute of Forest Science, Yeongju 36040

Cudrania tricuspidata (Moraceae) is a tree widely distributed in East Asia, mainly in the southern part of Korea. The UPLC analytical method was developed to quantify four major isoflavonoid components including 6,8-diprenylgenistein, 6,8-diprenylorobol, alpinumisoflavone, and 4 -*O*-methylalpinumisoflavone in *C. tricuspidata* fruits. Non-targeted metabolite profiling of immature fruits of *C. tricuspidata* collected from July to October at four different regions in Korea was performed using ultraperformance liquid chromatography–quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS) technique followed by multivariate analyses for the differentiation of regional and seasonal variances of *C. tricuspidata*

fruits. This proposed UPLC-QTOF MS-based analytical method showed the improved efficacy to evaluate *C. tricuspidata* fruits and can be further used for the quality assurance of *C. tricuspidata* to develop as potential dietary supplements.

P-293

ANTICONVULSANT SECONDARY METABOLITES FROM *TAPINANTHUS GLOBIFERUS* (A. RICH.)

Vimal K Sharma[1], Amber Forsman[1], James O Fajemiroye[2], SVS Radhakrishnan[3], Zulfiqar Ali[3], Cammi Thornton[1], Christianah A Elusiyan[4], Kristie L Willett[1], Ikhlas A Khan[3] and Jordan K Zjawiony[1]

[1]*Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS, 38677 USA.* [2]*Center for Studies and Toxicological-Pharmacological Research, Faculty of Pharmacy, Federal University of Goiás, Goiânia, Brazil.* [3]*National Center for Natural Product Research, The University of Mississippi, University, MS, 38677 USA.* [4]*Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-lfe, Nigeria.*

Epilepsy is a neurological condition where the Antiepileptic drugs (AEDs) support people with epilepsy for many years, but they still involve recurrent seizures and undesirable side effects. Nigerian mistletoe, *Tapinanthus globiferus* (Loranthaceae) is a parasitic shrub growing on deciduous trees. Mistletoes find myriad ethnobotanical uses including hypertension, ulcers, **epilepsy**, etc. The present work aims to isolate and carry out bioassays of the compounds obtained from *T. globiferus*. Methanolic extract of the leaves of the shrub was fractionated using hexanes, chloroform and n-butanol. The n-butanol fraction was re-fractionated via column chromatography (CC) using ethyl acetate/chloroform/methanol/water (5:4:1:0.1) as solvent system. Repeated CC of the n-butanol fraction resulted in the isolation of different flavonoid glycosides. Fractions and compounds obtained from *T. globiferus* MeOH extract were bio-assayed for their anti-convulsant potential in the Pentylenetetrazol (PTZ) induced Zebrafish epileptic model. Kaempferol glycoside (AF.1.10.TG.9) at 1 and 5 mg/L concentrations showed a significant decrease in large activity compared to the PTZ alone. Detailed phytochemical and pharmacological investigations on different fractions, sub-fractions and isolated secondary metabolites from *T. globiferus* will be presented.

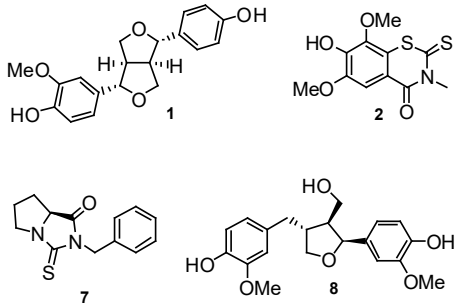
P-294

BIOACTIVE COMPOUNDS FROM THE ROOTS OF *BRETSCHNEIDERA SINENSIS*

Ting-Yuan Tan[1], Yuan-Bin Cheng[2], Tsong-Long Hwang[3] and Ya-Ching Shen[1]

[1]*School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan,* [2]*Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan,*[3]*Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan, Taiwan*

Two new (**1** and **2**) and ten known compounds were isolated from the roots of *Bretschneidera sinensis*. Compound **1** was identified as a new lignan with diarylbutane skeleton, while compound **2** was characterized as a new sulfur-containing alkaloid, which belongs to the skeleton of *N*-benzyl-*S*-methyl-thiocarbamate. Compounds **1**, **2**, **4**–**8** and **10** exhibited significant anti-inflammatory activities. Among them, compound **7** showed the most potent inhibition on superoxide anion release with IC_{50} value of 0.72 ± 0.15 μg/mL.



P-295

STUDIES ON COMPONENTS AND THEIR BIOACTIVITIES FROM *DENDROBIUM TOSAENSE* AND RELATED SPECIES

Li-Jie Zhang[1], Hung-Tse Huang[1], Zhi-Hu Lin[1], Mei-Kung Lu[1] and Yao-Haur Kuo[1,2,3]

[1] *Division of Chinese Materia Medica Development, National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taipei 11221, Taiwan,* [2] *Graduate Institute of Integrated Medicine, China Medical University, Taichung 404, Taiwan,* [3] *Department of Horticluture and Biotechnology, College of Agriculture, Chinese Culture University, Taipei 111, Taiwan*

The physiochemical properties of polysaccharides from *Dendrobium tosaense* at different harvest time (45, 90, 135, 180, 360, and 540 days) have been studied. The influence of ingredients related with harvest time and different parts of *D. tosaense* (stems, leaves, and flower) was investigated. We have also established the HPLC-UV fingerprints and microscopic observation of *D. tosaense* and made the comparisons with other five related species (*D. tosaense*, *D. nobile*, *D. huoshanense*, *Flickingeria fimbriata* and *D. officinale*). The extracts of *D. tosaense, D. nobile,* and *F. fimbriata* and four pure compounds isolated from *D. tosaense* were also evaluated for anti-oxidant and anti-inflammatory effects. These results showed that the extract of *F. fimbriata* possessed the most potent anti-oxidant and anti-inflammatory effects (Anti-NO, IC_{50}=11.40 ± 0.70 μg/mL). Linoleic acid (18:2, *cis*) isolated from *D. tosaense* had anti-NO production with IC_{50} at 8.54 μg/mL, whereas the crude extracts of *D. tosaense* and *D. nobile* were invalid for above bioactivities. The *in vivo* anti-UVB damage assays revealed that the MeOH extract of *D. huoshanense* was good for TV (tear volume) and TBUT (tear film break up time) tests.

P-296

ANTI-DIABETIC ACTIVITY OF METHYL 2-(4'-METHOXY-4'-OXOBUTANAMIDE) BENZOATE ON ALLOXAN-INDUCED DIABETES MODEL IN ZEBRAFISH

Hye Been Choi[1], Youn Hee Nam[2], Tong Ho Kang[2], Hyung Sik Kim[1], Jong Hwan Kwak[1]

[1]*School of Pharmacy, Sungkyunkwan University, Suwon 16419,* [2]*Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University, Gyeonggi-do 17104, Korea*

Methyl 2-(4'-methoxy-4'-oxobutanamide) benzoate (**1**) was isolated from *Helianthus tuberosus* and identified from its spectroscopic data. Compound **1** was previously reported from a dark brown alga, *Jolyna laminarioides*. Compound **1** had inhibitory effect on chymotrypsin, and antibacterial and anti-inflammatory activities in previous biological investigations. Type 1 diabetic zebrafish model was induced by alloxan, which cause pancreatic β-cell necrosis. Following alloxan treatment, pancreatic islet size and flu-

orescence intensity were measured. We evaluated the recovery efficacy of compound 1 on alloxan-induced pancreatic islet damage in zebrafish. Glucose uptake was evaluated in zebrafish treated with compound **1** and glimepiride (positive control) by detecting the uptake of 2-NBDG fluorescence within the pancreatic islets. When compared to the alloxan-induced group, the pancreatic islet size of the compound-treated group was significantly increased at concentration of 5 μM for short treatment (1 h). In the compound **1** long-treated group (12 h), both of pancreatic islet size and glucose uptake were significantly higher compared to the alloxan group (negative control) at concentration of 1 μM. Compound **1** treatment led to a greater increase in pancreatic islet size and glucose uptake, which were similar with the glimepiride-treated group as positive control. In conclusion, compound **1** revealed potent anti-diabetic activity for type 1.

P-297

OPTIMIZED PRODUCTION OF PODOPHYLLOTOXIN BY HAIRY ROOT CULTURES OF *HYPTIS SUAVEOLENS*

Crescencio Bazaldúa[1,2], Alexandre Cardoso Taketa[2], Jesús Arellano[1], Elsa Ventura-Zapata[1], María Luisa Villarreal[2]
[1]Centro de Desarrollo de Productos Bióticos. IPN. Yautepec, Morelos. 62731, México. [2] Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, 62209, México.

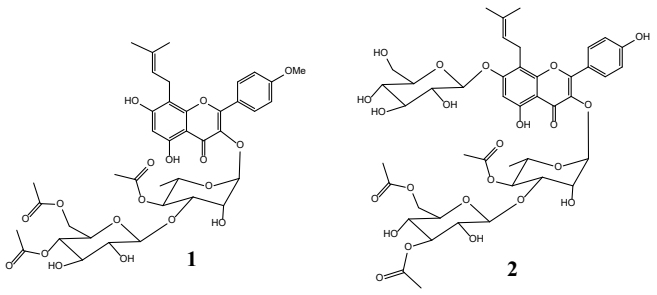
Ten hairy roots lines-cultures of the Mexican species *Hyptis suaveolens* were established by infecting nodal explants with *Agrobacterium rhizogenes* K599+pGus-GFP$^+$ and ATCC15834-pTDT strains. Based on growth rate, cytotoxic activity and production of podophyllotoxin (PTOX), three hairy root lines were selected. Various culture conditions and extraction procedures were explored in the root lines in order to optimize the accumulation of PTOX, which was then verified by HPTLC densitometry, LC-MS and MICRO QTOF II 10392 analyses. The hairy root cell line HsTD-10 reached the maximum accumulation of PTOX (5.39 ± 0.12 mg/ g PS) when cultured in 0.75 MS medium added with 2 mg L^{-1} of thiamine and using a MeOH-CH_2Cl_2 solvent mixture for extraction. This obtained accumulation was 10.6 times higher than that obtained with roots from wild specimens of *H. suaveolens.*

P-298

FLAVONOL GLYCOSIDES FROM *EPIMEDIUM GRANDIFLORUM* AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

Fazila Zulfiqar[1], Shabana I. Khan[1, 2], Zulfiqar Ali[1], Samir A. Ross[1, 2], Ikhlas A. Khan[1, 2]
[1]National Center for Natural Products Research, [2]Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA

Epimedium grandiflorum has been used as a crude drug in China and Japan. Two new flavonol glycosides, epimedigrandiosides A and B (**1**, **2**), and 28 known compounds including flavonoids, lignans, terpenoids, and phenyl alkanes with or without sugar(s), were isolated from the methanol extract of *Epimedium grandiflorum*. Structure elucidation was achieved by means of spectroscopic data analyses including 1D and 2D NMR, and HRESIMS. The methanol extract and the isolated compounds were evaluated for their activity towards several targets related to inflammation and metabolic disorder including NF-kB, iNOS, PPARα and PPARγ. Moreover, their cytotoxic activity against four cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3) and two noncancerous kidney cell lines (LLC-PK1 and Vero) was also evaluated.

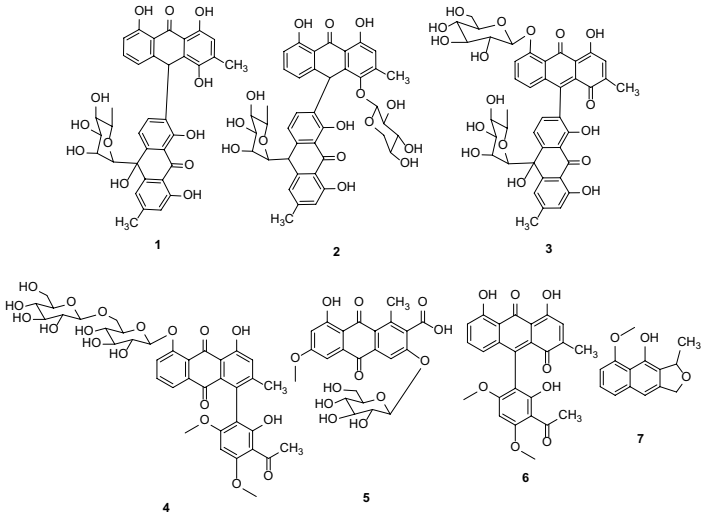


P-299

ANTHRAQUINONE AND NAPHTHALENE DERIVATIVES FROM *BULBINE NATALENSIS*

Ji-Yeong Bae[1], Zulfiqar Ali[1], Ahmed A. Zaki[1], Bharathi Avula[1], Yan-Hong Wang[1], Alvaro M. Viljeon[2], Shabana I. Khan[1,3], Ikhlas A. Khan[1,3]
[1]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Oxford, MS 38655, USA. [2]Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa, [3]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, Oxford, MS 38655, USA.

Bulbine natalensis is traditionally used in the treatment of male sexual dysfunction and immune related ailments. Several *Bulbine natalensis* dietary supplements are in the United States market with the effect of increasing testosterone in men. Six new anthraquinone derivatives, bulbnatalonosides A-E (**1**-**5**) and bulbnatalone (**6**), and one naphthalene derivative, bulbnatalol (**7**) were isolated from the stem of *B. natalensis.* Their structures were determined by 1D- and 2D-NMR spectroscopy. The isolated compounds were tested for the antimicrobial activity and bulbnatalonoside A (**1**) showed moderate inhibition effect (IC_{50} 13 μg/ml) against methicillin-resistant *Staphylococcus aureus* (MRSA).

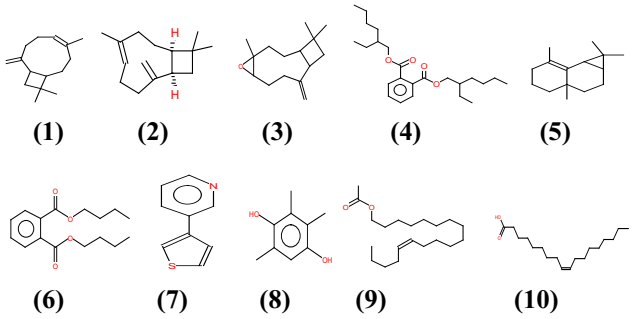


P-300

PHYTOCHEMICAL CONTENTS, TOXICITY AND ANTI-HYPERTENSIVE EVALUATION OF EXTRACTS OF FRUIT RIND OF *PERSIA AMERICANA*

Alaribe Chinwendum, S[1]., Okezua Obinna[1] Iwu Chike[1], Adejare A.A[2]
[1]Dept of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Nigeria; [2]Dept of Physiology, Faculty of Basic Science, College of Medicine, Idi-Araba LUTH

Cardiovascular disease (CVD) is responsible for one third of global deaths and is a leading and increasing contributor to the global disease burden. One of the highly prevalent risk factor for CVD throughout the world is hypertension (Kloet *et al*., 2013). To qualitatively determine some phytochemical contents of the fruit rind (FR) of *Persia Americana,* GC-MS techniques was used. *Invivo* rat model was also used to evaluate the anti-hypertensive activities of both n-hexane and ethanolic extracts of the FR. The chromatographic and spectroscopic analysis results of n-hexane extract of FR showed presence of ten major compounds which include: Caryophyllene **(1)**,11,11-Trimethyl-8-methylenebicycloundec-4-ene **(2)**, Caryophyllene oxide **(3)**, Bis(2-ethylhexyl) phthalate**(4),** 1H-Cyclopropa[a]naphthalene **(5)**, Dibutyl phthalate **(6)**, 3-(3-Thienyl)pyridine **(7),** 1,4-Benzenediol **(8),** 13-Octadecen-1-ylacetate **(9)** , Oleic acid **(10)** among others as shown in figure 1 - 10 below. (Chem Station, NIST, 2015, USA). The ethanolic extract demonstrated promising antihypertensive activity against sodium chloride and cadmium chloride induced hypertensive condition.



(1) **(2)** **(3)** **(4)** **(5)**

(6) **(7)** **(8)** **(9)** **(10)**

Keywords: Cardiovascular diseases**,** GC-MS, *Persia Americana*, anti-hypertension, NaCl, CdCl

References

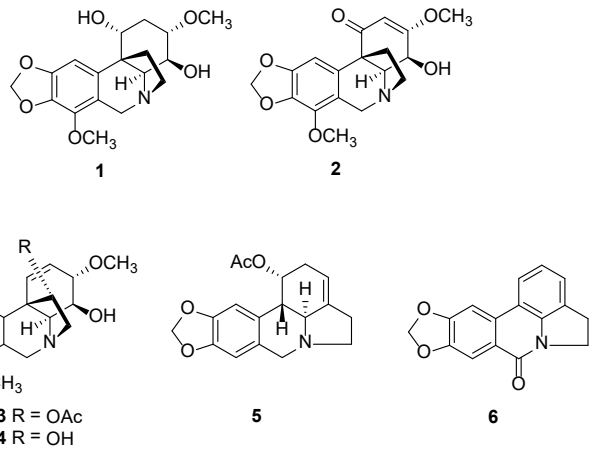
Kolck U, Zaugg C, Erne P (2004). Pharmacological basis of antihypertensive drug therapy. *Praxis*. 93(20):847-56.

P-301

ANTIPLASMODIAL ALKALOIDS FROM BULBS OF *AMARYLLIS BELLADONNA*

Namki Cho[1], Maria B. Cassera[2], Ana Lisa V. Murillo[2], Michael Goetz[3], and David G. I. Kingston[1]
[1]Department of Chemistry and Virginia Tech Center for Drug Discovery, M/C 0212, Virginia Tech, Blacksburg, Virginia 24061, United States, [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602, United States, [3]Natural Products Discovery Institute, 3805 Old Easton Road, Doylestown, Pennsylvania 18902, United States.

A bioassay-guided fractionation of *Amaryllis belladonna* bulbs resulted in the isolation of a new crinane, 1,4-dihydroxy-3-methoxypowellan (**1**), along with the five known amaryllidaceae alkaloids **2**–**6**. The structures were elucidated by their spectroscopic data (HR-ESIMS, ECD, NMR). Among these isolated compounds, the lycorine-type alkaloid acetylcaranine (**5**) exhibited strong antiplasmodial activity, while compounds **3** and **4** were moderately active and compounds **1** and **6** were inactive in this assay.



1 **2**

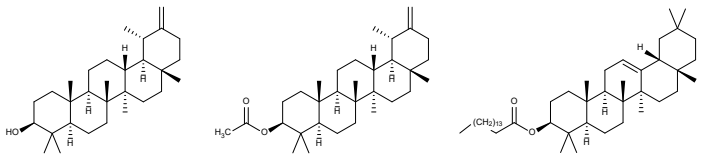
3 R = OAc
4 R = OH **5** **6**

P-302

EFFECTS OF *ANTHEMIS AUSTRIACA* ON POLYCYSTIC OVARY SYNDROME AND COMPOUNDS ISOLATED FROM THE ACTIVE EXTRACT

Mert Ilhan[1,2], Zulfiqar Ali[1], Ikhlas A. Khan[1], Esra Kupeli Akkol[2]
[1]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, 38677, USA.[2]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara, Turkey.

Anthemis austriaca Jacq. is used against abdominal pain, diarrhea, ovarian diseases, hemorrhoids and common colds in folk medicine. Aim of the present study is to evaluate the effects of *A. austriaca* in polycystic ovary syndrome rat model. The flowers of this plant were extracted with hexanes, ethyl acetate and methanol, succesively. Effects of extracts obtained from the plant were evaulated in letrozole-induced polycystic ovary syndrome rat model. The levels of serum gonadotropins, steroids, blood lipid, leptin and glucose and the values of antioxidant parameters were measured. Results of the study demonstrated that hexanes extract showed significantly different activity when compared to the control group and thus proceeded for its chemical constituents investigation. Phytochemical studies of hexanes extract led to the isolation of three triterpene derivatives; namely taraxasterol, taraxasterol acetate and β-amyrin palmitate.



P-303

ESI-MS/MS AND COMPUTATIONAL CHEMISTRY: IMPORTANT TOOLS FOR DEREPLICATION OF BENZOPYRANS FROM PIPERACEAE SPECIES

Amauri A. Souza[1], Ricardo Vessecchi[2], Ian Castro-Gamboa[1] and Maysa Furlan[1]
[1]Instituto de Química, Universidade Estadual Paulista - UNESP, Araraquara, SP 14800-060, Brazil, [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP 14040-901, Brazil

Electrospray ionization tandem mass spectrometry and computational chemistry were used to study the fragmentation mechanisms in gas-phase

dissociation processes of the chromanes and 2*H*-chromenes from Piperaceae species. In this context, we have applied these approaches to assist the analysis of such benzopyrans in high complexity natural matrices. Likewise, computational studies using the B3LYP/6-31++G(d,p) model indicated strong influence of the structural effects in the acid-base equilibrium, such as steric hindrance and hyperconjugative interaction. These results associated to energy-resolved plots have corroborated the major fragmentation mechanisms for chromanes and 2*H*-chromenes, including retro Diels-Alder reaction and intramolecular 1,2-elimination, respectively. Thus, the fragmentation mechanisms for the target compounds were proposed based on the energy-resolved plots and the physical chemistry parameters.

Acknowledgments: AAS thanks CAPES and FAPESP (Grant 2014/22239-8) for the Ph.D. scholarships.

P-304

SYNERGISTIC ANTI-TUMOR ACTIVITY OF SESQUITERPENE LACTONES AND FLAVONOIDS: AN IN VITRO STUDY USING HUMAN CANCER CELL LINES

Tibebe Z. Woldemariam, Shawna Evans, Chiderah Ugbaja, DeAndrea Owens, and George Talbott
California Northstate University College of Pharmacy, 9700 West Taron Drive, Elk Grove, CA 95757

In an effort to discover novel bioactive plant natural products with utility in the treatment of cancer, we generated and assessed variety of classes of compounds for possible *in vitro* cytotoxic activity against Human HT-29 colon carcinoma cells. Sesquiterpene lactones isolated from Taraxacum officinale (Dandelion) leaf and flavonoids from Glycyrrhiza uralensis (Licorice) root were found to show significant anti-tumor activities. Chemical and physical data for the active compounds, cytotoxic activity and the bioassay-guided fractionation of the methanolic extracts will be presented.

Bioactivity-guided fractionation of the methanol extracts afforded several fractions and sesquiterpene lactones and flavonoids which showed significant reductions in cell viability were observed. Observation under a microscope suggested the cancer cells were dying by an apoptotic mechanism and the exact mechanism of cell death is currently being investigated biochemically. Combinations of fractions containing the sesquiterpene lactones and flavonoids showed improved IC_{50} values and the results of the synergistic anti-tumor activity of the fractions as well as pure compounds will be presented.

These findings highlight the importance of the use of combining different compounds to augment direct synergistic therapeutic effects when active constituents are combined within and between many medicinal herbs.

P-305

PSORALEA CORYLIFOLIA AND *EPIMEDIUM BREVICORNU* DO NOT ACTIVATE THE ESTROGEN RECEPTOR A OR B IN A MANNER SIMILAR TO 17-B ESTRADIOL

Kelly M. Glynn[1], Molly Hood[1], Jeffrey D. Scholten[1], Feng Tian[2], Min Gui[2], Minjie Li[2], Stephen R. Missler[1] Arun Rajgopal[1] and John F. Rebhun[1]
[1]Amway Corporation, Ada, Michigan, USA; [2]Amway Corporation, Shanghai, China

Phytoestrogens are non-steroidal plant-derived compounds that have been shown to have estrogenic activity. Is the estrogenic activity the result of these compounds binding directly to the estrogen receptor (ER) and directly activating it or is it the result of indirect ER activation through other signaling pathways? This distinction between direct and indirect activation may be important when considering the safety of particular phytoestrogens. Compounds that directly activate the ER, similar to 17-β estradiol, may be less controlled by negative feedback and the lack of negative feedback regulation may be a safety concern. Indirect phytoestrogens may be more negatively regulated and may present less of a concern. To assess direct and indirect ERα or ERβ activation by phytoestrogens, luciferase reporter assays were developed using isolated Ligand Binding Domains (LBD) for ERα and ERβ fused to Gal4. Additionally, full length ERα or ERβ reporter assays were developed. We show that 17-β estradiol activates each of these four assays and we evaluated known phytoestrogens, genistein and diadzein. Next, we selected a number of TCM ingredients that have been described for the treatment of low bone density and assessed their activity. *Psoralea corylifolia* and *Epimedium brevicornu* activated the full length ERα and ERβ. However, only *P. corylifolia* activated the LBD construct. It activated ERβ; it did not activate the LBD of ERα. *E. brevicornu* did not activate either the LBD of ERα or ERβ. Genistein and diadzein were active in each of the 4 assays and ERα or ERβ receptor activation was confirmed using ER inhibitor ICI 182780. Finally, select phytochemicals, kaempferol and luteolin, identified in active fractions of *E. brevicornu* by LC/MS-MS activate full length ERα but not ERα LBD, demonstrating that some phytochemicals activate ERα in a manner different from other phytochemicals. The use of a general term, phytoestrogens, may be misleading.

P-306

THE LICORICE SPECIES, *GLYCYRRHIZA INFLATA*, SHOWS CHEMOPREVENTIVE POTENTIAL BY DOWNREGULATION OF OXIDATIVE ESTROGEN METABOLISM

Birgit M. Dietz, Shuai Wang, Tareisha L. Dunlap, Lingyi Huang, Yang Liu, Charlotte Simmler, Dan Lantvit, Huali Dong, Shao-Nong Chen, Guido F. Pauli, Richard B. van Breemen, Judy L. Bolton
UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, Illinois, USA.

Licorice dietary supplements, such as *Glycyrrhiza inflata* Bat. (GI, Fabaceae), are popular for the relief of menopausal symptoms. Chemopreventive properties have also been demonstrated for GI and its constituent, licochalcone A (LicA), including decrease of oxidative estrogen metabolism *in vitro*. Oxidative estrogen metabolism is one part of estrogen carcinogenesis and mainly involves the hydroxylation of estrogens by P450 1B1 to potentially genotoxic metabolites. This study assessed the influence of GI and LicA on oxidative estrogen metabolism as well as the distribution and metabolism profile of LicA *in vivo*. Vehicle, estradiol benzoate (EB, 1 mg/kg/day, s.c.), GI extract (2 g/kg/day, gavage) plus EB, and LicA (80 mg/kg/day, s.c.) plus EB were administered to August-Copenhagen Irish (ACI) rats for four days. Although LicA was rapidly metabolized through glucuronidation, free LicA was detected in the serum, liver, and mammary glands of the LicA and GI groups. LicA and GI significantly reduced the formation of 2-$MeOE_1$, a marker for overall oxidative estrogen metabolism. Compared to the EB-only group, co-treatment of GI with EB significantly upregulated CYP1A1 and downregulated CYP1B1 expression. GI and LicA also induced the detoxification enzyme, NAD(P)H-quinone oxidoreductase 1 in the liver tissue. These data highlight the chemopreventive potential of GI.

Funded by P50 AT000155

P-307

RED CLOVER ISOFLAVONES HAD NO EFFECT IN MCF-10A CELLS, YET DIFFERENTIALLY MODULATED ESTROGEN GENOTOXIC METABOLISM/ DETOXIFICATION IN MCF-7 CELLS

Tareisha L. Dunlap, Caitlin E. Howell, Shao-Nong Chen, Guido F. Pauli, Birgit M. Dietz, and Judy L. Bolton
Department of Medicinal Chemistry and Pharmacognosy, UIC/NIH Center for Botanical Dietary Supplements Research, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612

Botanical dietary supplement sales are continually increasing; however, the safety and efficacy of many supplements are unknown. This study investigated modulation by red clover/isoflavones [genistein (GN), daidzein (DZ), biochanin A (BA), formononetin (FN)] of estrogen metabolism which involves the P450 1B1 catalyzed genotoxic (4-hydroxylation) and P450 1A1 catalyzed detoxification (2-hydroxylation) pathways. We analyzed gene expression (CYP1A1/1B1) by qPCR and biomarkers of estrogen 2-hydroxylation (2-$MeOE_1$) and 4-hydroxylation (4-$MeOE_1$) by LC-MS/MS in non-tumorigenic (MCF-10A) and tumorigenic (MCF-7) breast cells. Red clover/isoflavones did not modulate estrogen metabolism in MCF-10A cells. In MCF-7 cells, red clover significantly increased both estrogen metabolism and CYP1A1/1B1 expression; however, the 4-hydroxylation pathway was favored. The isoflavones had no effect on 2-hydroxylation, whereas they significantly increased the 4-hydroxylation pathway. For the estrogenic isoflavones (GN, DZ), one mechanism could involve downregulation of CYP1A1 through an ERα epigenetic pathway. In contrast, the AhR agonists (BA, FN) increased CYP1A1/1B1 likely through AhR/XRE signaling. Thus, red clover/isoflavones altered estrogen metabolism to favor the genotoxic 4-hydroxylation pathway in MCF-7 cells and had differential effects on estrogen metabolism according to cell type and nuclear receptor status. These effects should be considered when standardizing botanicals to the bioactive isoflavones to maximize their safety and efficacy, particularly in women with breast cancer. Supported by NIH Grant P50 AT000155

P-308

WITHDRAWN

P-309

SAFETY AND PREVALENCE OF TURMERIC DIETARY SUPPLEMENT USE IN RHEUMATOID ARTHRITIS

Meghan B Skiba[1], Ashley Lukefahr[1], Rachel Groff[1], Chelsea Alfafara[1], Janel C DeSalvo[1], Laura L Hopkins[1], Sean McEvoy[1], Allison L Hopkins[2], and Janet L Funk[1]
[1]Department of Medicine, University of Arizona, Tucson, AZ 85724, [2]Department of Anthropology, Texas A&M University, College Station, TX 77840

A case of turmeric dietary supplement (DS)-induced autoimmune hepatitis (AIH), reported here, leads to a concern about the safe use of turmeric DS, the top selling herbal in the United States, in populations at greater risk for AIH, such as those with autoimmune rheumatoid arthritis (RA). Because the product associated with the index case of AIH could not be identified, a survey of turmeric dietary products sold at local retail outlets (n = 17) was undertaken (Tucson, AZ). A total of 86 unique turmeric products were identified, 44% of which contained additional botanicals, a point of interest as most cases of DS-induced liver toxicity involve products containing multiple botanicals. Labeling indicated enhanced bioavailability on 33% of products, although only 14% of supplements containing piperine were so labeled. As only 2% (n = 2) of products sold had content certified by an independent laboratory, an analysis of selected samples (n = 30) for curcuminoid content and potential contaminants, currently pending, will be presented. To further assess potential population-based risks, individuals self-identified as having RA were surveyed to determine the prevalence of turmeric DS use. Among n = 632 respondents to date, 60% (n = 383) report current use of a natural product DS (NPDS) for RA disease management. Turmeric was the most frequent current NPDS used (55% of NPDS users; n = 210). As consumers are often not aware of possible side effects associated with botanical use, a discussion and consideration of NPDS use by both patients and health care providers is needed, particularly in populations at high risk for drug-botanical interactions, such as those with RA.

P-310

IN VITRO ASSESSMENT OF DRUG INTERACTION POTENTIAL OF LICORICE EXTRACTS

Tonsing-Carter AA[1], Li G[1], Lee A[1], Lopez BR[1], and Richard B. van Breemen[1]
[1]University of Illinois College of Pharmacy, UIC/NIH Center for Botanical Dietary Supplements Research, Chicago Mass Spectrometry Laboratory, Chicago, IL 60612

Although hormone therapy remains the standard of care for women experiencing vasomotor symptoms related to menopause, concerns over increased risk of stroke and breast cancer have women considering botanical dietary supplements such as licorice (*Glycyrrhiza sp*.) for management of these symptoms. To ensure the safe use of licorice dietary supplements, we investigated their potential for drug interactions. Standardized licorice extracts of *Glycyrrhiza glabra*, *G. inflata*, and *G. uralensis,* the three most common species used in dietary supplements, were studied for intestinal permeability, serum protein binding, and possible inhibition or induction of cytochrome P450 enzymes involved in drug metabolism. Licorice compounds showed high permeability in Caco-2 permeability assays and high serum protein binding in rapid equilibrium dialysis assay with human serum. In studies using human liver microsomes, and a cocktail probe substrate assay, all three licorice species showed inhibition of CYP2B6, CYP2C8, CYP2C9 and CYP2C19. *G. inflata* also inhibited CYP1A2, CYP2D6 and CYP3A4. Induction qualified cryopreserved human hepatocytes were used to predict effects of the extracts on cytochrome P450 enzyme induction. CYP3A4 was unaffected by all *Glycyrrhiza sp*, and CYP1A2 and CYP2B6 were induced in hepatocytes by *G. uralensis* and *G. inflata*. Due to these data, dietary supplements containing *G. uralensis* and *G. inflata* are more likely than *G. glabra* to cause clinically relevant drug interactions.

P-311

DRUG INTERACTION POTENTIAL OF BULBINE NATALENSIS AND ITS CONSTITUENTS

Vamshi K. Manda[1], Olivia R. Dale[1], Jiyeong Bae[1], BharathiAvula[1], Zulfiqar Ali [1], Ikhlas A. Khan[1,2] and Shabana I. Khan[1,2]
[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, [2]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.

Bulbine natalensis (Asphodelaceae), an indigenous plant of South Africa, is traditionally used for its wound healing and antidiarrheal properties, and as an aphrodisiac. Due to its androgenic and aphrodisiac properties, the use of this plant in bodybuilding and performance enhancing supplements is on the rise. Chronic use of health supplements along with the conventional drugs always poses a risk for herb drug interactions. The nuclear receptor, PXR (pregnane X receptor) regulates the expression of several drug metabolizing cytochrome P450 enzyme isoforms and the efflux transporters, induction of which is known to be one of the mechanisms responsible for clinically relevant herb-drug interactions. This study was carried out to evaluate the drug interaction potential of *B. natalensis* extract and its constituents (phenylanthraquinones, anthraquinone dimers, and anthraquinone glycosides) by determining their effects on the activity of PXR and its target genes (CYP3A4, CYP1A2, CYP2C9, CYP2B6, and P-gp). A significant activation of PXR (2-8 fold) was seen with the extract and three of its constituents that resulted in an increase in the mRNA expression of CYP3A4 (2-10

fold), CYP1A2 (3-6 fold), CYP2C9 (2-4 fold), and CYP2B6 (2-20 fold). The results suggest that chronic intake of *B. natalensis* may lead to changes in sub-therapeutic plasma concentrations of concomitantly taken drugs that are substrates for CYP3A4, CYP1A2, CYP2C9 or CYP2B6 enzymes.

P-312

ETHNOBOTANY OF MEDICINAL PLANTS IN CLUSIACEAE OF CHINA

*Yuanyuan Ji[1], Edward Kennelly[1, 2, *], Bo Liu[1], Ping Li[1,3], Hang Shu[1], Chunlin Long[1, 4, *]*

[1] College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China; [2] Department of Biological Sciences, Lehman College, and The Graduate Center, The City University of New York, Bronx, NY, 10468, USA; [3] College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China; [4] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

Pharmacological studies revealed the significant biological activities of compounds isolated from Clusiaceae plants, including xanthones, benzophenones, acylphloroglucinols, naphthodianthrone, polyphenols, and flavonoids. In China there are 8 genera and 95 species of Clusiaceae. The ethnic people have rich traditional knowledge about Clusiaceae plants for their values of edible fruits, drinks, herbal medicines, dyes, timber and ornamentals. During our ethnobotanical investigations from 2012 to 2016, we found the local people frequently used Clusiaceae plants as herbal medicines for various purposes. Informants from Han and different ethnic groups including Dai, Miao, Li, Yi, Hani, Bai, Jinuo, Zhuang, Bulang, Yao, Buyi, Dong, and Shui had been interviewed. They collected barks, branches, leaves or fruits of *Garcinia* for treatments of tumors, skin ulcers, or hemorrhoids. The *Hypericum* plants have widely been used to treat hepatitis, dysentery, or jaundice. The decoction of young *Cratoxylum* leaves has been drunk as an herbal tea for treating throat problems, hepatitis, fever, and digestive ailments. Healers are mostly male, and know more about their medicinal values. Almost 50% (48 of 95) of Clusiaceae species are endemic to China, and many of them have not been studied phytochemically or pharmacologically. Further researches to extract chemicals from Chinese Clusiaceae species will be necessary in support of local people's ethnomedicinal uses.

* Corresponding authors, Email: edward.kennelly@lehman.cuny.edu (EJK), and long.chunlin@muc.edu.cn (CLL)

P-313

INVESTIGATION OF AN AMERICAN INDIAN HERB, *AMORPHA CANESCENS* PURSH, FOR IMPROVING WOMEN'S HEALTH

Tristesse Burton[1], Ruth Muchiri[1], Micheal Rush[1], Tareisha Dunlap[1], Huali Dong[1], Judy Bolton[1], Djaja Soejarto[1], and Richard B. van Breemen[1]

[1]UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA.

Some of the leading botanical dietary supplements that women take have traditional uses by American Indians and have been extensively studied. However, there are still numerous American Indian plants that lack scientific investigations on their safety and efficacy for women's health. The purpose of this project was to investigate an American Indian herb, *Amorpha canescens* Pursh (Fabaceae)- leadplant, for its potential estrogenic, chemopreventive and anti-inflammatory activity as it relates to women's health. We used bioassay guided fractionation (BGF) and dereplication techniques to identify the active compounds of leadplant. With BGF, leadplant's methanol extract demonstrated anti-estrogenic, anti-inflammatory, and chemopreventive activity in several cell-based assays. Using dereplication, Pulsed Ultrafiltration (PUF) or Magnetic Microbead Affinity Selection Screening (MagMASS) with the estrogen receptor alpha (ERα: anti/estrogenic), 15-lipoxygenase (15-LOX: anti-inflammation), and retinoid X receptor (RXR: chemopreventive and anti-inflammation) was performed to quickly identify the ligands responsible for these activities. For the RXR and 15-LOX receptors we did not identify any ligands from leadplant's methanol extract. Thus, the anti-inflammatory and chemopreventive activity of leadplant may be contributed by another mechanism of action outside the RXR or 15-LOX pathways. Currently, we are optimizing the MagMASS assay with ERα and completing BGF to determine the active constituents.

P-314

AN ETHNOBOTANICAL STUDY OF *MUCUNA PRURIENS* CULTIVATION IN SOUTHERN INDIA AND ASSESSMENT OF COMMERCIAL MUCUNA PRODUCTS IN THE USA

Tanya Denne[1], Amala Soumyanath[1], Lynn Shinto[1] Amie Hiller[1], and SP Dhanabal[2]

[1]Department of Neurology, Oregon Health and Science University, Portland, OR, USA; [2] JSS College of Pharmacy, Ooty, India.

*Mucuna pruriens (*MP) seeds contain 3-6% levodopa (L-DOPA), and have been used in traditional Ayurvedic medicine to treat diseases resembling Parkinson's disease (PD). Pilot studies in PD show that MP seed powder has similar effects to conventional levodopa/carbidopa medication. A visit to Southern India yielded ethnobotanical data on MP native growing conditions. Three varieties of MP seeds were collected from the 'Silent Valley' and 'Paniya Village' in Kerala and the 'Irula Village' in Tamil Nadu, India. These field sites offered undisturbed native growing conditions and specimens for observation. MP products are frequently used by PD patients in the USA. Samples of six brands of MP products available on the internet in the USA were tested for L-DOPA content. Products were extracted and analyzed using reversed-phase high performance liquid chromatography (HPLC) with fluorescence detection. The claimed L-DOPA content ranged from 25 to 250 mg per dose for the six products. HPLC analysis revealed that only two of the products had L-DOPA values close to the value claimed. Four of six products examined showed a large discrepancy between label claim and L-DOPA content. These studies highlight the need for greater standardization and regulation of MP products. The identification of strains and growing conditions optimizing L-DOPA content is also of interest.

P-315

NEW BIOLOGICAL ACTIVITIES OF THE ISOLATED COMPOUNDS FROM *INONOTUS OBLIQUUS*

Kazumi Sagayama[1], Takatoshi Fukumoto[2], Naonobu Tanaka[3], and Yoshiki Kashiwada[1]

[1]Graduate School of Pharmaceutical Sciences, Tokushima University, 1-78-1, Shoumachi, Tokushima 770-8505, JAPAN, [2]SVENSON Co., Ltd., 1-9-13, Akasaka, Minato-ku, Tokyo 107-0052, [3]Graduate School of Technology, Industrial and Social Sciances, Tokushima University, 2-1, Minamijyousanjima-cho, Tokushima 770-8506

Chaga mushroom (*Inonotus obliquus*), a fungus belongs to the family of Hymenochaetacea, is parasitic on birch tree. Chaga has been used for the treatment of cancer in Russia and Western Siberia for centuries, and therefore, scientific research on Chaga has been focused on biological activities related to its anti-tumor effect so far. In the course of our search of potential natural sources, 50 % EtOH-soluble fraction from the 80 % EtOH extract had the alkaline phosphatase and the antioxidative activities. In contrast, 50 % EtOH-insoluble fraction from the 80 % EtOH extract of Chaga was found to show biological activities related to hair growth, which was more potent than minoxidil used as a positive control, It also showed increasing mRNA expression of the fibroblast growth factor 7 (FGF-7), and the vascular endothelial growth factor (VEGF), related to hair growth cycle. Further separation of this active fraction gave several compounds, these compounds showed biological activities related to hair growth. Since the isolated com-

pounds showed different effect on mRNA expression against FGF-7, VEGF, IGF-1(insulin-like growth factor-l) and HGF (hepatocyte growth factor), they might have different mode of action for hair growth. Identification and biological evaluation of the isolated compounds will be presented.

P-316

INHIBITION OF CYTOCHROME P450 3A4 (CYP3A4) BY A LICORICE EXTRACT

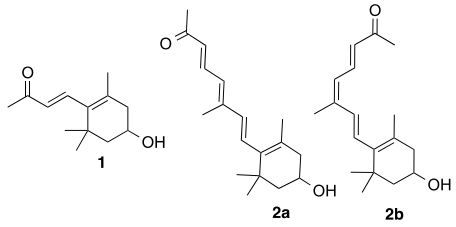
Muchiri R, Huang K, Gauthier L, Lankin DC, Pauli GF, and Richard B. van Breemen
UIC/NIH Center for Botanical Dietary Supplements Research, Dept. Medicinal Chemistry & Pharmacognosy, Univ. Illinois College of Pharmacy, Chicago, IL

Human cytochrome P450 enzymes are essential for the metabolism of many medicines and endogenous compounds. Therefore, the study of drug-botanical interaction is important for safe use of botanical dietary supplements. Most assays for the evaluation of inhibition of cytochrome P450 enzymes by drugs, natural products or botanical dietary supplements address inhibition of one CYP450 isoform at a time. To expedite these assays, a rapid, selective, and sensitive assay using UHPLC-MS/MS was developed and used to monitor simultaneous the metabolism of probe substrates of multiple CYP450 enzymes. Following the FDA drug-drug interaction studies guidance, possible inhibition interactions with nine major cytochrome P450 enzymes were investigated including CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. From the screening assays done with an extract from licorice (*Glycyrrhiza glabra*), the highest inhibitory activity was observed with CYP3A4. The extract was further fractionated and tested with the aim of identifying the compound with the most inhibitory activity. Following isolation and purification, single compounds were tested for time dependent inhibition of CYP3A4. The inhibition of CYP3A4 by a novel compound from *Glycyrrhiza glabra* extract, and its structure elucidation using high resolution mass spectrometry, HPLC-MS/MS, 1D NMR, and 2D NMR data will be presented.

P-317

HYPOCHOLESTEROLEMIC CONSTITUENTS OF THE EDIBLE BLUE-GREEN ALGAE *ARTHROSPIRA PLATENSIS* (SPIRULINA)

Karen C. Tan[1], *Samantha M. Gromek[1], Chai Siah Ku[2], Tho X. Pham[2], Ji-Young Lee[2], Marcy J. Balunas[1,*]*
[1]Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, USA, [2]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA



Arthrospira platensis, more commonly known as Spirulina, is an edible blue-green algae that serves as the active component in various Spirulina supplements available on the market. In our previous research, we established the presence of hypocholesterolemic compounds in *A. platensis*, which down-regulated the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), a key enzyme involved in cholesterol biosynthesis. In the current study, we performed bioassay-guided fractionation of the organic-soluble portion of *A. platensis* to purify and elucidate the structures of compounds in the bioactive fractions. Three compounds have been isolated including compound **1**, identified as 3-hydroxy-β-ionone, and compounds **2a** and **2b**, determined to be all-E and 9-Z isomers of apo-13-zeaxanthinone. All three are apocarotenoids, which are oxidative cleavage products of carotenoids. We have recently completed chemical synthesis of **1** to obtain an adequate amount of pure material for additional biological assays including both in vitro and in vivo testing. Further studies will include evaluation of these compounds for their potential in standardization of Spirulina supplements.

P-318

SPECIFIC INHIBITORS THAT BLOCK THE OREXIGENIC ACTIVITY OF GLUCOCORTICOID AND BSX IN ENERGY BALANCE

Yun Young Lee [1]and Seunghee Lee[1]
[1]College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea

The molecular basis underlying the orexigenic function of glucocorticoid (Gc) is unclear. Brain-specific homeobox factor (Bsx) is a positive regulator of the orexigenic neuropeptide, agouti-related peptide (AgRP), in AgRP-neurons of the hypothalamic arcuate nucleus. Recently, we have shown that in response to fasting-elevated Gc levels, Gc receptor (GR) and Bsx synergize to direct activation of AgRP transcription. Agrp functions as a common orexigenic target gene of GR and Bsx and provide an opportunity to identify specific inhibitors that block the interaction between GR and Bsx and inhibits the transactivation of Agrp gene but not the other GR target genes. We set up a screening system using the Agrp-GRE luciferase reporter and identify small molecules that block the synergistic activation by glucocorticoids and Bsx in HEK293T cells. First, we selected several compounds extracted from Zizyphus jujube and tested them to identify compounds that block the synergistic transactivation by Bsx and Gc but not the activation by Gc alone in Agrp-GRE luciferase reporter. 2 out of 23 compounds resulted in about 70% repression of the AgRP-GRE luciferase activity by Gc and Bsx but they showed marginal repression of the luciferase activity by the treatment of Gc alone. These results define AgRP as a common orexigenic target gene of GR and Bsx and provide an opportunity to identify small molecules that inhibit the orexigenic activity of Gc and Bsx and provide the clinical application for the treatment of the obesity.

P-319

IDENTIFICATION OF ANTI-VIRULENCE COMPOUNDS ACTIVE AGAINST MRSA USING MASS SPECTROMETRY-BASED BIOASSAYS

J. Stempin[1], Noemi D. Paguigan[1], Huzefa A. Raja[1], Nicholas H. Oberlies[1] and Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412. E-mail: nbcech@uncg.edu

Antibiotic resistant organisms are responsible for over 2 million illnesses and 37,000 deaths annually in the United States. Infections from methicillin-resistant *Staphylococcus aureus* (MRSA) alone cause a severe economic burden and have high mortality rates. With a dwindling supply of new antibiotics and a continual increase in the development of antibiotic resistance in bacterial pathogens, there is a high demand for new strategies for fighting bacterial infections. One strategy that has potential is inhibiting the production of virulence factors in the pathogen rather than inhibiting bacterial growth. Targeting virulence factors to fight bacterial infections provides three key advantages: virulence factors are common among many pathogenic strains of bacteria, resistance to virulence factors develops at a much slower rate, and this strategy allows for infections to be cleared naturally by the host immune system. Virulence is regulated in MRSA by the accessor gene regulation (*agr*) system. The inhibition of this system blocks cell-to-cell communication between bacteria, colonization, host cell invasion and apoptosis. With this research, we employed a mass spectrometry based assay to facilitate the identification of compounds that inhibit the *agr* system in MRSA. Using this assay, a newly identified fungal compound and two analogues were determined to act as *agr* inhibitors.

P-320

POTENTIAL ANTI-INFLAMMATORY ACTIVITY FROM *OXYTROPIS FALCATA* BUNGE

Dejun Zhang,[1,2] Mengke Zhang,[2] Tamara Kondratyuk,[2] Leng Chee Chang[2]
[1]College of Ecology and Environment Engineering, Qinghai University, People Republic of China, 810016. [2]Department of Pharmaceutical Sciences, The Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, 96720.

Oxytropis falcata Bunge, locally known as "E Da Xia" was first recorded in the Chinese Pharmacopoeia as an official herbal drug in 1977. As one of the "three anti-inflammatory drugs" and the "king of herbs", it has been widely applied in many Tibetan and Mongolian medicinal prescriptions and with multiple traditional uses for the treatment of conditions or diseases such as anthrax, influenza, haemorrhage, hyperpyrexia, and pain. Recent studies of *O. falcata* have been focused on its hemostasis, antitumor, antioxidant, anti-inflammatory and analgesic activities. To date, compounds including alkaloids, flavonoids, saponins, and polysaccharides were discovered in this plant. Our previous study showed chloroform and ethyl acetate partitions remarkably alleviate myocardial ischemia-reperfusion injuries. These two partitions have protective effects on cardiomyocyte hypoxia/ reoxygenation injuries.

In this study, the chloroform partitions exhibited inhibitory activity *in vitro* against the tumor necrosis factor alpha (TNF-α) activated nuclear factor-kappa B (NF-κB) pathway in transfected human embryonic kidney cell line 293, and potent nitric oxide (NO) inhibitory activity in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells. The result showed that the NF-κB% inhibition ranging from 97.8 to 21.9 without cytotoxic effects. The highest NO % inhibition is 60.9 without cytotoxicity. The isolation, structure identification, and biological data will be presented.

P-321

THE ANTIFUNGAL POTENTIALS OF THE EXTRACT AND CREAM FORMULATIONS OF *COLA MILLENII* K. SCHUM. IN DERMATOMYCOSES-INFECTED ALBINO RATS

Bolanle A. Adeniyi[1], Temitope O. Lawal[1], Taiwo S. Agidigbi[1], Tolulope O. Ajala[2], and Oluwatoyin A. Odeku[2]*
*[1]Departments of Pharmaceutical Microbiology and [2]*Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

The antifungal activities of crude extracts and formulated cream of the bark of *Cola millenii* were tested *in vitro* and *in vivo* against *Trichophyton interdigitalis*, *T. rubrum,* and *Epidermophyton floccosum.* Susceptibility testing and the minimum inhibitory concentration (MIC) was determined by agar diffusion and agar dilution method respectively. The *in vivo* evaluation of anti-fungal activity was made in male Wistar rats with *T. rubrum* to ascertain the efficacy using Empirical Clinical Assessment Score method. The therapeutic efficacy was monitored by histopathology using Periodic Acid Schiff staining technique (PAS) and Haematoxylin and Eosin (H&E) staining techniques. Phytochemical screenings revealed the presence of resins, saponins and alkaloids. The extract demonstrated *in vitro* antifungal activity against the dermatophytes with diameter zone of inhibition of 14±0.2 - 26±0.2 mm and MIC values of 50- 75 mg/mL. Antimicrobial susceptibilities of the extract and cream formulations against dermatophytes species revealed a dose-dependent response. Treatment of *T. rubrum* infected-rat with methanol extract and its cream variables revealed the clearance of fungal hyphae from the skin tissue and wound healing on day 17(8days of treatment). These findings suggest that the extracts of *Cola millenii* contain compounds with anti-fungal activity.

P-322

SAFETY AND FREE TESTOSTERONE BOOSTING EFFICACY OF A NOVEL *CURCULIGO ORCHIOIDES* (FAMILY HYPOXIDACEAE) EXTRACT IN MALE RATS

Kanwaljit Chopra[1], Ravinder Naik Dharavath[1], Anand Swaroop[2], Manashi Bagchi[2], and Debasis Bagchi[2,3]
[1]Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India, [2]Cepham Research Center, Piscataway, NJ, USA, [3]Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX 77002

Curculigo orchioides Gaertn (known as black or kali musli), is an endangered medicinal plant used for diverse medicinal purposes including impotency. 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 either10 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 (*$p < 0.0001$). No effect was observed on total testosterone level. Extensive testicular histopathological analyses were conducted on the seminiferous tubules, spermatogenesis, sperm cell morphology, Leydig cells and Sertoli cells at all doses. Blamus™ demonstrated dose-dependent improvement in structural integrity. Extensive bood chemistry analyses demonstrated the broad spectrum safety. 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-323

RESVERATROL AMELIORATES THE SEVERITY OF FIBROGENESIS IN MICE WITH EXPERIMENTAL CHRONIC PANCREATITIS

Kanglun Liu[1#], Yixuan Xia[1#], Zihao Yin[1], Hong-Jie Zhang[1], Siu Wai Tsang[1,2*]*
[1]School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, China [2]Institute of Research and Continuing Education, Hong Kong Baptist University Shenzhen Research Center, Shenzhen, China
[#]These authors contribute equally to this work.

3,5,4'-Trihydroxystilbene (resveratrol) is a popular medicinal polyphenols since the molecule is scrutinized as an antiaging compound by activating the sirtuin-1 that promotes cellular survival under stress. We aimed to examine whether it ameliorates the severity of fibrogenesis in a model of chronic pancreatitis. The mice were given with repetitive injections of supramaximal cholecystokinin-8 analogue cerulein, and oral administration of resveratrol effectively attenuated PSC activation, reduced ECM deposition and lessened fibrotic parenchyma in the pancreatitic tissues of cerulein-induced mice in addition to a significant reduction of circulating tumor necrosis factor-alpha, a pro-inflammatory cytokine responsible for the maintenance of chronic inflammation. Luciferase assay results revealed the anti-inflammatory effect of resveratrol was associated with a down-regulation of RAR-related orphan receptor gamma-t (RORγt) promoter activity. RORγt indeed plays a crucial role in the differentiation of T helper 17 cells of autoimmune diseases and chronic inflammations. We suggest resveratrol is potentially a remedial agent for the management of pancreatic fibrosis in chronic pancreatitis. This work was supported by the Health and Medical Research Fund (HMRF projects 14150471 and 13144281) of the Food and Health Bureau, Hong Kong SAR, and the Young Scientists Project (81400666) of the National Natural Science Foundation of China.

P-324

ERYTHROFORDINS D AND E, TWO NEW CASSAINE-TYPE DITERPENES FROM ERYTHROPHLEUM SOAVEOLENS

Tanja Grkovic[1], Jason R. Evans,[2,3] Rhone K. Akee[1], Paul G. Grothaus,[3] Liang Guo,[4] Myrtle Davis,[5] David J. Newman,[3] and Barry R. O'Keefe[3]
[1]Natural Products Support Group, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research (FNLCR), Frederick, MD 21702, [2]Data Management Services Inc., FNLCR, Frederick, MD 21702, [3]Natural Products Branch, Developmental Therapeutics Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), Frederick, MD 21702 [4]Laboratory of Investigative Toxicology, Leidos Biomedical Research Inc., FNLCR, Frederick, MD 21702, [5]Toxicology and Pharmacology Branch, DTP, DCTD, NCI, Bethesda, MD 20892.

Two new cassaine-type diterpenoids, namely erythrofordins D and E, sourced from a Cameroon collection of *Erythrophleum suaveolens* were isolated and assessed for anti-cancer activity. In the NCI-60 cancer cell assay, the natural products were found to be cytotoxic in the low micro molar ranges with a mean GI_{50} value of 2.45 and 0.71 μM, respectively. Using the COMPARE algorithm, the compounds were found to have similar NCI-60 response profiles to the known cardiac glycosides hyrcanoside and strophanthin. In an assay examining the viability and contractile function in human cardiomyocytes derived from induced pluripotent stem-cells, erythrofordins showed cardiotoxicity effects similar to cardiac glycosides. The implications and challenges in the development of cardiac glycoside-like compounds for anti-cancer therapeutics are presented and discussed.

P-325

GASTRIC CANCER CELLS ARE SUSCEPTIBLE TO NIGERIAN MEDICINAL PLANTS USED FOR THE TREATMENT OF *HELICOBACTER PYLORI* INFECTION

TO Lawal[1,2], SR Patel[2], GB Mahady[2]
[1]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria; [2]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago.

Nigerian medicinal plants with inhibitory activities against *Helicobacter pylori* - the etiologic agent of gastritis, peptic ulcer disease, gastric MALT lymphoma and gastric cancer were screened for cytotoxicity effects in gastric cancer cell lines- AGS and NCI-N87. The medicinal plants namely *Anogeissus leiocarpus* (DC.) Guill. & Perr. (African birch, Combretaceae), *Terminalia glaucescens* Planch ex Benth. (Combretaceae) and *Dillenia indica* L. (Elephant apple, Dilleniaceae) were identified from an ethnobotanical survey for the treatment of gastrointestinal diseases in Nigeria. The root and stem bark of *A. leiocarpus,* root bark of *T. glaucescens,* as well as the stem bark and leaves of *D. indica* were collected in Ibadan, Nigeria and extracted with methanol by cold maceration. The extracts/partitions were tested at concentrations ranging from 100 to 5 μg/mL to determine cell viability and cytotoxicity using the CellTiter-Glo® **2.0 assay**. Aqueous extracts of *A. leiocarpus* root were active in AGS and NCI-N87 with IC_{50} of 60.0 μg/mL and 76.0 **μ**g/mL respectively, while extracts of the stem bark were not active in concentrations up to 100 **μ**g/mL for both cell lines. Ethyl acetate extract of *T. glaucescens* was active in the AGS cell line with IC_{50} of 57.5 **μ**g/mL. Aqueous extract of the same plant was weakly active in NCI-N87. Aqueous extract of *D. indica* stem bark had IC_{50} of 50 μg/mL in AGS cells, but was not active in NCI-N87 cells. *D. indica* leaf extracts were not active in these cell lines. The results showed that medicinal plants used in Nigeria for gastrointestinal disorders with significant activity against *Helicobacter pylori*, also were cytotoxic to gastric cancer cell lines.

Acknowledgements: This research was supported by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

P-326

INDUCTION OF APOPTOSIS IN MCF-7 CELLS BY EXTRACTS OF *DILLENIA INDICA* L. AND *ANOGEISSUS LEIOCARPUS* (DC.) GUILL. & PERR.

TO Lawal[1,2], SR Patel[2], GB Mahady[2]
[1]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria; [2]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago.

The use of traditional medicinal plants in the treatment of breast cancer is common practice in rural populations in the developing countries including Nigeria. *Dillenia indica* L. (Elephant apple, Dilleniaceae), the mixed juices of bark, leaf and fruits of which is used for the treatment of cancer and *Anogeissus leiocarpus* (DC.) Guill. & Perr. (African birch, Combretaceae) were investigated for anti-cancer effects in MCF-7 breast cancer cells. *D. indica* samples (stem bark, leaves and fresh matured fruits) and *A. leiocarpus* (stem bark and root) were collected in Ibadan, Nigeria. Samples were air-dried and extracted by cold maceration with methanol. Cell viability and cytotoxicity was determined using the CellTiter-Glo® **2.0 assay** in MCF-7 at concentrations up to 100 μg/mL of the extracts. Induction of apoptosis by caspase activation was investigated using Caspase-Glo® 3/7, Caspase-Glo® 8, ApoTox-Glo™ Triplex Assay Reagents and confirmed by flow cytometry in MCF-7 cells treated with aqueous extract. Aqueous and ethyl acetate extracts of *D. indica* stem bark exhibited cytotoxicity activity on MCF-7 with IC_{50} of 65.3 and 60.6 **μg**/mL respectively. Extracts of the leaf and fruits were not active at concentrations up to 100 **μg**/mL. Extracts of *A. leiocarpus* were active with IC_{50} between 12.8 and 64.8 **μg**/mL. The aqueous extracts of root and stem bark were the most active at 15.2 and 20.7 **μg**/mL respectively and explain the activity of the plant in MCF-7 cell. Caspase3/7 was activated in cells treated with aqueous extracts, hence the induction of apoptosis. These findings justify and support the use of these plants for the management of breast cancer in traditional medicine.

Acknowledgements: This research was funded by Schlumberger Foundation Fellowship award to TOL and a First Analysis grant to GBM.

P-327

RADIOSENSITIZING EFFECT OF SG-21, A GALIELLALACTONE ANALOG, IN MDA-MB-468 BREAST CANCER CELLS THROUGH INHIBITION OF STAT3 ACTIVATION

Hyejin Ko[1], Hyun Su Kim[1], Jong Hyun Lee[2], Kwang Seok Ahn[2], Young-Ger Suh[1,3], and Yeong Shik Kim[1]
[1]College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea, [2]College of Korean Medicine, Kyung Hee University, Seoul 02453, [3]College of Pharmacy, CHA University, Gyeonggi-do 13496

Along with standard treatments including surgery and chemotherapy, radiation therapy is an essential modality for treatment of breast cancer, because it significantly decreases breast cancer mortality. Unfortunately, intrinsic and acquired resistance to ionizing radiation limit the utility and efficacy of radiation treatments. In an effort to overcome the radioresistance, we investigated whether SG-21, a novel galiellalactone analog, can enhance the therapeutic effect of radiotherapy on tumor growth in MDA-MB-468 human breast cancer cells. The combination of SG-21 (1.5 μM) treatment with radiotherapy (10 Gy) showed synergistic cytotoxicity effect in MDA-MB-468 cells (CI, 0.71). The combined treatment inhibited constitutive STAT3 phosphorylation at tyrosine 705 and activation of JAK1 and JAK2 kinase. Moreover, the combination treatment also decreased expression of STAT3 downstream target genes including Bcl-2, Cyclin D1, MMP-2, and COX-2. Finally, SG-21 potentiated the therapeutic effect of radiotherapy on the induction of apoptosis via PARP cleavage and caspase-3 activation. This study demonstrated that SG-21 exerts a radiosensitizing effect in MDA-MB-468 human breast cancer cells using an *in vitro* model, and this effect

was mediated by the inhibition of STAT3 activation. These results suggest that SG-21, galiellalactone-based STAT3 inhibitor, may be a promising candidate as a novel radiosensitizer against human breast cancer.

P-328

NEW INSIGHTS INTO THE CYTOSTATIC AND CYTOTOXIC PROPERTIES OF MENSACARCIN

Elizabeth Kaweesa, Birte Plitzko, Sandra Loesgen
Department of Chemistry, Oregon State University, Corvallis, OR 97331, USA.

Mensacarcin is a stereogenic complex polyketide with potent anti-tumor activity produced by a soil-dwelling *Streptomyces bottropensis*. The US National Cancer Institute's 60 human tumor cell line drug screen (NCI60) reveals mensacarcin's cytostatic properties in almost all tested cell lines and distinct cytotoxic properties specifically in eight melanoma cell lines with an average IC50 value of 0.5-1 μM. We show that mensacarcin induces apoptosis in melanoma cells but not in colon cancer carcinoma. Mensacarcin co-localizes in mitochondria, and impairs mitochondrial function in melanoma cells in metabolic flux analysis by either inhibiting mitochondria respiration directly or by causing general mitochondrial dysfunction.

P-329

TARGETING DIENES AND TRIENES IN NATURAL PRODUCTS WITH NITROSO-BASED PROBES

Gabriel Castro-Falcón, Chambers C. Hughes
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA

'Reactivity-guided isolation' has emerged as an approach to access specific classes of natural products from complex mixtures. Taking advantage of the reactivity associated with functional groups found in natural products, the new method utilizes specially designed chemical reagents (or probes) that 1) selectively react with a chosen functional group, 2) facilitate subsequent detection and isolation steps and 3) aid in the process of structural elucidation.

Herein, we report the validation of nitroso-base probes for the discovery of diene- and triene- containing natural products. Using the specially designed nitroso-based probes bromonitrosobenzene and bromonitrosopyridine, both containing conspicuous UV and MS tags, we were first able to show the clean conversion to products under mild conditions using model compounds. The probes adds a degree of crystallinity aided the process of structural elucidation via X-ray crystallography. We then tested the ability of the more reactive bromonitrosopyridine to facilitate the detection and isolation of new natural products with dienes or trienes directly from crude extracts. This led to the discovery of the known compound tylactone in the marine genus *Salinispora*, as well as the new epoxy-quinone containing diene, novodaryamide, two new pyrones, sporapyrones A and B, two new triene-containing natural products, nocarditrienes A and B, from several other marine bacterial extracts.

P-330

GROWTH INHIBITION OF CANCER CELLS INDUCED BY THE EXTRACT AND TRITERPENES OF *SALACIA CRASSIFOLIA*

Laila S. Espindola[1,4], Renata G. Dusi[1,4], Raimundo Braz-Filho[2], Heidi R. Bokesch[3,4], Kirk R. Gustafson[4], John A. Beutler[4]
[1]Laboratório de Farmacognosia, Universidade de Brasília, Brasília, Brazil, [2]FAPERJ/Departamento de Química – ICE – UFRRJ, Seropédica – RJ and Laboratório de Ciências Químicas – CCT - UENF, Campos dos Goytacazes, RJ, Brazil, [3]Molecular Targets Laboratory, Leidos Biomedical Research, Inc., National Cancer Institute, NIH, Frederick, MD, USA [4]Molecular Targets Laboratory, National Cancer Institute, NIH, Frederick, MD, USA

Salacia crassifolia (Mart. ex Schult.) G. Don, Celastraceae is found in the *Brazilian Cerrado Plant Extract Library* of the Universidade de Brasilia, Brazil. Among the extracts screened on tumor cells, the hexane extract of *S. crassifolia* root wood showed GI_{50} values ranging from 1.6 to 5.7 μg/mL in different cells lines. This extract demonstrated an average GI_{50} value of 0.3 μg/mL in the NCI-60 cell screen, with the HCT-15 colon cancer cell line being the most sensitive with a GI_{50} of 0.2 μg/mL. Bioassay-guided fractionation led to the isolation of pristimerin, 6-oxopristimerol and a previously unreported pentacyclic triterpene. In the NCI-60 cell screen, pristimerin exhibited a mean GI_{50} value of 0.2 μM, being UO-31 renal and T-47D breast cancer the most sensitive cell lines. Our research supports conservation of the Cerrado against ever increasing anthropogenic activity by safeguarding biodiversity information for this biome hotspot, with the view to develop molecules capable of inhibiting the growth of cancer cells.

Acknowledgements: The authors wish to thank the Brazilian agency *CNPq* - The National Council for Scientific and Technological Development. Partially funded by FNLCR contract HHSN261200800001E.

P-331

IDENTIFICATION OF ANTICANCER LEADS FROM A FUNGAL PEAK LIBRARY

Jacklyn M. Gallagher[1], José Rivera-Chávez[1], Huzefa A. Raja[1], Gagan Deep[2], Jacques Fournier[3], Tyler N. Graf[1], Daniel A. Todd[1], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC 27402, USA, [2]Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest Baptist Medical Center & Wake Forest School of Medicine, Winston Salem, NC, USA, [3]Las Muros, 09420 Rimont, France

Natural sources have yielded a wide array of compounds leading to advancements in medicine. Specifically, secondary metabolites from fungi often display diverse and dynamic physiological activities to provide therapeutic benefits and it is therefore imperative to continue this research. Hence, we have begun a collaborative project with Wake Forest University to create a natural products peak library for the approximately 950 fresh water fungal isolates, collected from both North America and Europe. The goal is to find new therapeutic leads from identification of either new compounds, or unknown bioactivities of known compounds. This will be done by following a similar protocol previously established in our lab, and proven to be compatible for biological screenings against the African American prostate cancer cell line E006AA-hT. Currently the library is comprised of 96 fungal extracts, 256 fractions constructed of 6 Mother plates, 18 Daughter plates, and 84 Granddaughter plates which yielded hits in 15% of extracts and 8% of fractions when screened against E006AA-hT cell line.

P-332

COMPARATIVE EFFECTS OF TURMERIC (*CURCUMA LONGA* L) SECONDARY METABOLITES ON THE PROGRESSION OF BREAST CANCER BONE METASTASES IN AN ANIMAL MODEL

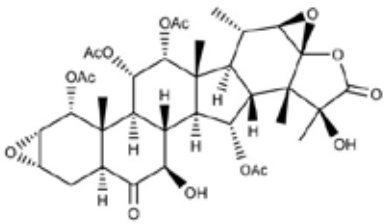
JL Funk[1], LE Wright[2], JB Frye[1], BN Timmermann[3]
[1]Department of Medicine, The University of Arizona, Tucson, AZ,
[2]Department of Medicine, Indiana University, Indianapolis, IN,
[3]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS.

Secretion of TGFβ-induced parathyroid hormone-related protein (PTHrP), a key osteolytic factor in breast cancer bone metastasis (BMET), has previously been demonstrated to be inhibited by purified curcuminoids (CURC), polyphenols isolated from turmeric (*Curcuma longa* L) rhizomes, which also block *in vivo* progression of PTHrP-dependent osteolytic BMET. CURC's previously reported ability to inhibit the formation of bone-resorbing osteoclasts may also contribute to bone protection in this model. In contrast, turmeric essential oils (TEO) actually stimulated tumor cell secretion of PTHrP, suggesting that complex turmeric products containing both secondary metabolites may be less efficacious and/or detrimental in limiting breast cancer BMET progression. To test this hypothesis, the effects of both secondary metabolites on *in vivo* osteolytic BMET formation were determined and relative effects of CURC vs TEO on osteoclastogenesis were compared. In a nude mouse model of PTHrP-dependent human breast cancer BMET (MDA-MB-231 cells), a crude turmeric extract containing both CURC and TEO was more efficacious than purified curcuminoids or essential-oil free extracts in limiting breast cancer BMET progression. *In vitro*, CURC and TEO each directly inhibited osteoclastogenesis and had additive effects when used in combination. These results suggest that both secondary metabolites of turmeric may act in concert to limit the progression of breast cancer bone metastases via effects on tumor cell secretion of osteolytic factors (CURC only), as well as additive effects on bone resorbing osteoclasts (CURC and TEO).

P-333

THE TACCALONOLIDE MICROTUBULE STABILIZERS HAVE POTENT AND PERSISTENT ANTITUMOR ACTIVITY IN DRUG RESISTANT TUMORS

April L. Risinger[1,2], Lin Du[3,4], Robert H. Cichewicz[1,2], Susan L. Mooberry[3,4]
[1]Department of Pharmacology and [2]Cancer Therapy & Research Center, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, [3]Natural Product Discovery Group, Institute for Natural Products Applications and Research Technologies and [4]Department of Chemistry & Biochemistry, Stephenson Life Science Research Center, University of Oklahoma, Norman, OK 73019



1

Microtubule stabilizers are one of the most effective classes of anticancer agents; however drug resistance can limit their clinical efficacy. Each of the clinically approved microtubule stabilizers reversibly binds within the taxane pocket on β-tubulin to elicit similar effects on microtubule stability. The taccalonolides are a novel class of microtubule stabilizers that we have isolated from plants of the genus *Tacca*. The taccalonolides are highly acetylated pentacyclic steroids that bind covalently to β-tubulin, cause profound and distinct microtubule stability, and circumvent the major mechanisms of clinically relevant taxane resistance both in vitro and in vivo. Extensive bioassay guided fractionation combined with semi-synthetic efforts have resulted in the biological characterization of over 100 taccalonolides, some of which are over 5,000-fold more potent than the major plant metabolite, taccalonolide A. Antitumor and pharmacokinetic studies have led to the identification of the lead compound, taccalonolide AF (**1**), which has exquisitely potent and persistent activity against taxane-resistant tumors.

P-334

ANTI-INFLAMMATORY EFFECTS OF MELANDRII HERBA VIA INHIBITION OF NF-KB AND MAPK PATHWAYS AND INDUCTION OF HO-1 IN MURINE MACROPHAGES

Yun Hee Jeong[1], You-Chang Oh[1], Won-Kyung Cho[1], Bohyoung Lee[1] and Jin Yeul Ma[1,]*
[1]Korean Medicine (KM)-Application Center, Korea Institute of Oriental Medicine, 70, Cheomdanro, Dong-gu, Daegu, 41062, Korea

Melandrii Herba (MH) is a traditional herb used to treat breast cancer, anuria, and diseases of lactation in East Asia. The goal of this study was to determine the anti-inflammatory activity and underlying molecular mechanism of MH ethanol extract (MHE) on the LPS-mediated inflammatory response in murine macrophages. The effects of MHE on the production of NO, inflammatory cytokines, related proteins, mRNAs and signaling pathway proteins were investigated using the Griess test, ELISA, Western blotting, and real-time RT-PCR, respectively. Our results showed that MHE treatment significantly inhibited the secretion of NO and inflammatory cytokines, including TNF-α, IL-6, and IL-1β in macrophages without cytotoxicity. Furthermore, MHE treatment suppressed iNOS expression and induced HO-1 expression. Finally, the transcriptional activities of NF-κB and MAPK activation were significantly repressed by MHE treatment in LPS-stimulated macrophages. Therefore, our results suggest the potential value of MHE as candidate to inflammatory therapeutic agent developed from a natural substance based on oriental herbs.

P-335

ANTI-INFLAMMATORY EFFECTS OF SANGUISORBAE RADIX WATER EXTRACT ON THE SUPPRESSION OF STAT-1/JAK-2 ACTIVATION IN HACAT CELLS

Jin Yeul Ma[1], Ju-Hye Yang[1], Jae-Myung Yoo[1], Won-Kyung Cho[1]
[1]Korean Medicine (KM) Application Center, Korea Institute of Oriental Medicine, 70 Cheomdan-ro, Dong-gu, Daegu, 701-300, Republic of Korea

Sanguisorbae Radix (SR) is a well-known herbal medicine used to treat inflammatory disease and skin burns in Asia. In addition, it is used to treat many types of allergic skin diseases, including urticaria, eczema, and allergic dermatitis. SR has been reported to exhibit anti-wrinkle, anti-oxidant, and anti-contact dermatitis bioactivities.

In this study, we investigated the mechanism underlying the anti-inflammatory effects of SR water extract (WSR) using human keratinocyte (HaCaT) cells. Viability assays were used to evaluate non-cytotoxic concentrations of WSR in HaCaT cells. We determined the production of pro-inflammatory chemokines including thymus and activation regulated chemokine (TARC; CCL17), regulated on activation, normal T-cell expressed and secreted (RANTES; CCL5), macrophage-derived chemokine (MDC; CCL22), and interleukin 8 (IL-8; CXCL8) in stimulated human keratinocytes. The ability of WSR to reduce the expression of pro-inflammatory marker proteins was evaluated by Western blotting in HaCaT cells stimulated with tumor necrosis factor (TNF)-α/interferon (IFN)-γ.

WSR suppressed TNF-α/IFN-γ-induced chemokine production and pro-inflammatory molecules *via* a blockade STAT-1, Jak-2, p38, and JNK activation.

P-336

PGB ENHANCES CELL DEATH IN A549 HUMAN LUNG CARCINOMA CELLS VIA MAINLY AMPK/MTOR/AKT SIGNAL-MEDIATED AUTOPHARGY INDUCTION

Nam–Hui Yim[1], Youn–Hwan Hwang[1], Jin Yeul Ma[1]
[1]Korean Medicine (KM) Application Center, Korea Institute of Oriental Medicine (KIOM), Daegu, 701-300, Korea

We investigated the involvement of autophagic cell death and other potential molecular mechanisms induced by the platycoside-containing butanol fraction of PG (PGB) in A549 human lung carcinoma cells. In A549 cells, PGB upregulated LC3-II in a time- and dose-dependent manner, and it redistributed LC3 via autophagosome formation in the cytoplasm. PGB increased the phosphorylation of AMP-activated protein kinase (AMPK) and subsequently suppressed the AKT/mammalian target of the rapamycin (mTOR) pathway. Furthermore, PGB inhibited cell proliferation by regulating the mitogen-activated protein kinase (MAPK) pathways. In conclusion, PGB efficiently induced cancer cell death via autophagy and the modulation of the AMPK/mTOR/AKT and MAPK signaling pathways in A549 cells, therefore, PGB may be an efficacious herbal anti-cancer therapy.

P-337

EVALUATION OF INTERACTION OF HOP BOTANICAL DIETARY SUPPLEMENTS WITH DRUG METABOLISM IN WOMEN

Luying Chen[1], Alyssa A. Tonsing-Carter[1], Richard B. van Breemen[1]
[1]University of Illinois College of Pharmacy, UIC/NIH Center for Botanical Dietary Supplements Research, Chicago Mass Spectrometry Laboratory, Chicago, IL 60612

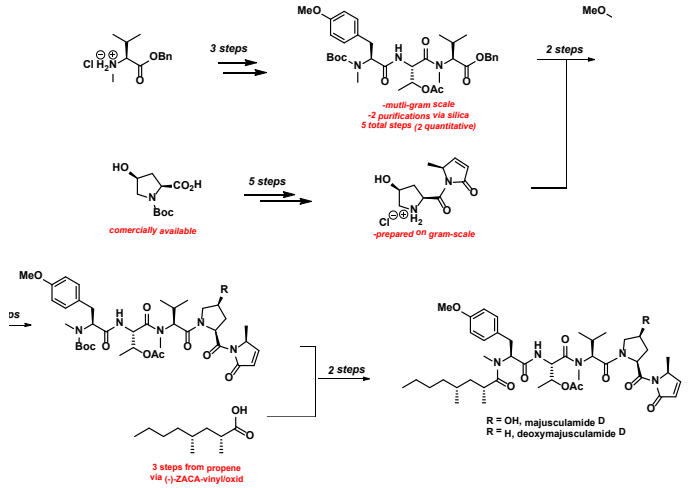
Extracts of hops (*Humulus lupulus* L.) containing prenylated flavanones such as 8-prenylnaringenin (8-PN), a potent phytoestrogen, are being used as botanical dietary supplements consumed by women as alternatives to hormone therapy for the management of menopausal symptoms. In vitro data suggest that hops might induce or inhibit certain cytochrome P450 enzymes involved in drug metabolism. To evaluate this safety issue in the clinic, we tested a botanically authenticated and chemically and biologically standardized hop dietary supplement in peri- and post-menopausal women for possible interactions with the pharmacokinetics of four FDA-approved probe drugs for cytochrome P450 enzymes using UHPLC-MS/MS. Low doses of an oral cocktail of caffeine, tolbutamide, dextromethorphan, and alprazolam (probes for metabolism by CYP1A2, CYP2C9, CYP2D6, and CYP3A4, respectively) were administered to peri- and post-menopausal women at baseline and then again after consuming a hop dietary supplement twice daily for 14 days. Serial blood samples were drawn and analyzed for concentrations of each probe substrate drug over time, and concentration-time curve values were compared. Although in vitro assays predicted in vivo drug-botanical interactions, preliminary clinical data suggest no clinically relevant interactions of hop dietary supplements with drug metabolism highlighting a common problem in botanical supplement research requiring more attention.

P-338

SCALABLE TOTAL SYNTHESIS OF THE ANTICANCER AGENT MAJUSCULAMIDE D

Eduardo J.E. Caro-Diaz[1], Fred Valeriote[2], and William H. Gerwick[1,3]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92037, USA, [2]Division of Hematology and Oncology, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan 48202, United States , [3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, California 92093, USA

We describe our total synthesis of majusculamide D, a lipopentapeptide isolated from *Lyngbya majuscula* that has shown interesting and 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 and the production of large amounts of synthetic intermediates. We also aimed to determine the absolute configuration of the 1,3-dimethyloctanamide motif and achieve a concise synthesis of this buliding block *via* ZACA chemistry.



P-339

TOTAL SYNTHESIS AND ANALOGUE DEVELOPMENT OF THE CYCLIC IMINE NATURAL PRODUCT SCYTONEMIDE A

James R. Fuchs[1], Tyler A. Wilson[1], Robert J. Tokarski II[1], Peter Sullivan[2], L. Harinantenaina Rakotondraibe[1], Jimmy Orjala[2]
[1]College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA, [2]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA

Scytonemide A, a macrocyclic heptapeptide possessing a relatively unique imine linkage was recently isolated by the Orjala lab at UIC from the freshwater cyanobacterium *Scytonema hofmannii* (UTEX 1834) as a part of a collaborative program project grant (P01 CA125066) in an effort to discover novel anticancer agents. This cyclic peptide, which is structurally similar to the nostocyclopeptide natural products, displayed potent inhibitory activity of the 20S proteasome with an IC_{50} value of 96 nM. Unfortunately, a thorough biological evaluation of this compound was made difficult by the low yield of the natural material from culture. From a synthetic perspective, the imine linkage was expected to provide a potential challenge for both the preparation and final purification of this compound. With this in mind, a versatile solid-phase synthesis strategy has been developed that has facilitated the total synthesis of scytonemide A. Utilizing this route, purification of scytonemide A has been accomplished via normal phase column chromatography, enabling the synthesis of several structural analogues for structure-activity relationship studies.

P-340

INHIBITION OF NRF2-MEDIATED DEFENSE MECHANISM TO OVERCOME DRUG RESISTANCE AND INCREASE EFFICACY IN CHEMOTHERAPY

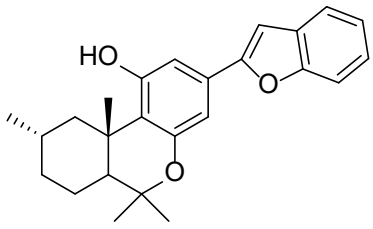
Xiao Liang[1,2], James H. Matthews[1,2], Valerie J. Paul[3] and Hendrik Luesch[1,2]
[1]Department of Medicinal Chemistry, University of Florida, Gainesville, FL 32610, USA, [2]Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, 1345 Center Drive, Gainesville, FL 32610, USA, [3]Smithisonian Marine Station at Ft. Pierce, 701 Seaway Drive, Ft. Pierce, FL 34949, USA

Nrf2 is a transcription factor that regulates the expression of cellular protective genes through antioxidant response elements. Constitutive upregulation of Nrf2 has been found in many types of cancers, including breast cancer, lung cancer and colon cancer. Recently, persistent use of anticancer drugs has been reported to upregulate Nrf2-mediated pathway to contribute to drug resistance in cancer therapy. In our study, three natural product Nrf2 inhibitors have been identified and their role in inhibiting Nrf2-mediated defense mechanism has also been investigated.

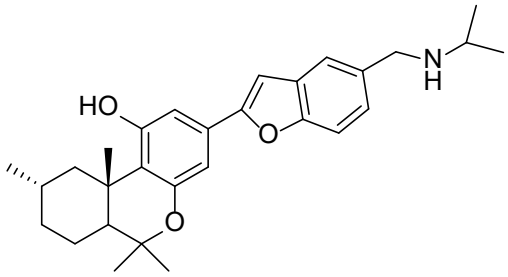
P-341

DESIGN AND SYNTHESIS OF WATER SOLUBLE ANALOGS OF NATURAL ANTIPLASMODIAL COMPOUND, MACHAERIOL B.

H. M T. Bandara Herath, Shabana I. Khan, Babu L. Tekwani, Ilias Muhammad and N. P. Dhammika Nanayakkara
National Center for Natural Product Research, School of Pharmacy, University of Mississippi, University, MS 38677.



Machaeriol B



1

Machaeriol B, (+)-trans-hexahydrodibenzopyran derivative isolated from *Machaerium multiflorum,* has shown strong *in vitro* activity against chloroquine-sensitive (W-2) and –resistant (D-6) *Plasmodium falciparum* clones. Unavailability of sufficient amounts hampered the further study of this compound. In order to overcome the supply problem and to improve its bioavailability, several analogs of machaeriol B were designed and synthesized. Succinic acid salt of compound **1** was found to have comparable antiplasmodial activity and higher water solubility than machaeriol B.

P-342

FUNGAL SHIKIMATE METABOLITE, PERICOSINE A, DEODORIZES SKUNK SPRAY BY MEANS OF A ROBUST NUCLEOPHILIC SUBSTITUTION REACTION PROCESS

Lin Du[1,2], Robert H. Cichewicz[1,2]
[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.

The fungal shikimate metabolite, pericosine A, was reported previously as a chemoreactive natural product that attenuates the antagonistic effects of nucleophilic antifungal agents. In the recent studies, we found that pericosine A reacts robustly with the malodorous thiols and thiol acetates in aqueous environments via nucleophilic substitution mechanisms leading to remarkable deodorizing effects. Optimization of the reaction conditions enabled the discovery of an "all natural" and biocompatible formula which neutralizes (deodorizes) thiols in less than one minute. As thiols and thiol acetates are the major malodorous elements in skunk spray, which are rather difficult to fully remove from skins and hair, our current and future efforts focus on the development of a commercial skunk odour remover with pericosine A as the major active ingredient. This potential product is expected to help pets and their owners effectively deal with the aftermath of unfortunate encounters with skunks.

P-343

CENTELLA ASIATICA AND MITOCHONDRIAL BIOGENESIS IN THE MOUSE BRAIN

Matthews DG[1], Gray NE[1], Meshul C[2,3], Caruso M[1], Moore C[3], Murchison C[1], Harris C[1], Quinn JF[1,4], Soumyanath A[1]
[1]Department of Neurology and [2]Department of Behavioral Neuroscience, Oregon Health & Science University, Portland OR, [3]Research Services and [4]Parkinson's Disease Research Education & Clinical Care Center (PADRECC), VA Portland Health Care System, Portland OR.

Background: Mitochondrial (MT) dysfunction plays a vital role in neuronal loss in Alzheimer's Disease (AD). A water extract from the medicinal herb *Centella asiatica* (CAW) improved cognitive function, and upregulated MT electron transport chain (ETC) genes in hippocampus, cortex, and cerebellum of aged (20mo old) wild type (WT) mice. In neuroblastoma cells *in* vitro, CAW improved MT function and ameliorated the toxic effects of beta amyloid on MT. CAW treatment also elevated ETC genes in rat primary neurons. We therefore hypothesize that CAW may increase MT biogenesis. **Methods:** To examine MT biogenesis *in vivo*, readily available, young (4mo) WT C57BL6 mice were treated for 5wks with CAW (~200mg/kg/d) in their drinking water. Behavioral testing (novel object recognition and Morris water maze) was performed during weeks 2-5 of treatment. At 5 weeks, animals were euthanized and brain tissues fixed for electron microscopy (EM). Sections observed by EM were analyzed via FIJI for MT area and number. **Results:** CAW-treated WT mice showed larger MT area (p=0.04) and larger neural soma cytoplasmic area (p=0.01) than control animals. Behavioral differences were not observed. **Conclusions:** This study supports MT as a target of CAW, in this cognitively intact, young cohort. Future studies will use EM to examine CAW's effects on MT in brains of aged WT and AD model mice, which exhibit greater MT dysfunction and cognitive abnormalities.

P-344

PHARMACOKINETICS OF CAFFEOYLQUINIC ACIDS AND KNOWN METABOLITES FROM CENTELLA ASIATICA WATER EXTRACT IN WILD-TYPE FEMALE MICE

Kirsten Wright[1], Maya Caruso[1], Don Matthews[1], Charles Murchison[1], Joseph Quinn[1,2], and Amala Soumyanath[1]
[1]Department of Neurology, Oregon Health and Science University, Portland, OR 97239, USA, [2]Department of Neurology, Portland Veterans Affairs Medical Center, Portland, OR 97239, USA

Centella asiatica (CA) is a botanical believed, in Eastern medicine, to improve memory. Aqueous extracts (200 - 300 mg/kg/day) improve cognition in several rodent models including aged mice. The extract contains triterpenes (asiaticoside and madecassoside; 0.94-2.41%) and caffeoylquinic acids (CQAs; 0.01-0.46%). CA's CQAs are neuroprotective and neurotropic *in vitro*. Prior pharmacokinetic analyses of CA have focused on triterpene glycosides and aglycones. This study investigated the pharmacokinetics (PK) of three mono- and five di-CQAs and their potential metabolites (caffeic acid, ferulic acid, isoferulic acid and their dihydroderivatives) in mice. A crude aqueous extract (200 mg/kg), as used in previous *in vivo* cognitive experiments, was administered by oral gavage to female C57BL/6J mice. Mice were euthanized at different time intervals over four hours (n = 4 mice per time point). Plasma and brains were collected to determine bioavailability and brain penetration of the analytes, as well as total plasma antioxidant potential. Plasma samples were analyzed using reversed phase liquid chromatography on a C8 column, combined with tandem mass spectrometry. Maximum plasma concentrations (C_{max}) of CQAs and metabolites (10 – 100 ng/ml) occurred within 45 minutes of oral administration. These results demonstrate the oral bioavailability and metabolism of CQAs from a CA water extract. Analysis of brain samples and plasma antioxidant activity is underway.

P-345

CENTELLA ASIATICA ATTENUATES AB-INDUCED BIOENERGETIC DYSFUNCTION AND OXIDATIVE STRESS AND RESTORES SYNAPTIC PLASTICITY IN NEURONS

Gray NE[1], Zweig JA[2], Matthews DG[1], Caruso M[1], Kawamoto C[2], Quinn JF[1,3], Soumyanath A[1]
[1] Department of Neurology, Oregon Health and Science University, Portland OR 97239, [2] Department of Cell, Developmental and Cancer Biology, Oregon Health and Science University, Portland OR 97239, [3] Department of Neurology, Veterans Affairs Portland Health Care System, Portland OR 97239.

Centella asiatica (L.) Urban is used traditionally to enhance cognition. The water extract of the plant (CAW) has been shown to have mitoprotective and antioxidant effects and improve memory in mice. Here we explore the effects of CAW on neuronal morphology, oxidative stress and mitochondrial dysfunction in the context β-amyloid (Aβ) exposure. We also investigated these endpoints *in vivo* and explore the cognitive-enhancing effects of CAW in the context of aged-related cognitive impairment.

Neurons from Aβ expressing mice and their wildtype (WT) littermates were treated with CAW and markers of neuronal health as well as mitochondrial and antioxidant gene expression and mitochondrial bioenergetics were evaluated. Aged WT mice treated orally with CAW were also cognitively evaluated.

CAW attenuated Aβ-induced deficits in dendritic arborization and spine density in isolated hippocampal neurons and improved both endpoints in WT neurons as well. Mitochondrial function and gene expression was also enhanced by CAW in both genotypes. CAW also activated the endogenous antioxidant response pathway and reduced Aβ-induced increases in reactive oxygen species and oxidative damage. CAW similarly increased mitochondrial and antioxidant gene expression and synaptic density in the brains of healthy aged mice. This was accompanied by improved cognitive performance.

CAW improved neuronal health and activated antioxidant and mitochondrial pathways in healthy and those exposed to Aβ. Similar antioxidant and mitochondrial changes were seen *in vivo* in healthy aged animals along with improved cognitive function. Studies are underway to determine how the mitochondrial and antioxidant effects of CAW contribute to its cognitive-enhancing properties.

P-346

CENTELLA ASIATICA TRITERPENES AND CAFFEOYLQUINIC ACIDS SHOW DIFFERENTIAL EFFECTS IN DROSOPHILA MELANOGASTER MODELS OF OXIDATIVE STRESS AND TAU PATHOLOGY

Doris Kretzschmar[1], Maya Caruso[1], Parnian Lak[2], Jan F. Stevens[3,4], Claudia S. Maier[2], Amala Soumyanath[1]
[1]Department of Neurology, Oregon Health & Science University, Portland, OR 97239; [2]Department of Chemistry, [3] Department of Pharmaceutical Sciences, and [4]Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA

Centella asiatica (CA) is a medicinal herb reputed to improve memory. CA water extract (CAW) improves cognition in multiple rodent models. We compared the effects of **CAW**, **A1 (**a subfraction of CAW), **MIX** (purified caffeoylquinic acids in identical concentrations to those in A1), and **CAST**™ (Indena; purified triterpenes in similar concentrations to A1) in *Drosophila melanogaster* flies. Three fly strains were used - Canton S wild type flies (CS), carbonyl reductase deficient flies (Sni) which have high oxidative stress, and flies expressing phosphomimetic human tau (Elav>E14) which leads to neurodegeneration. Test materials were added to food of newly eclosed flies, and performance in fast phototaxis assays (a test for neuronal function) was measured at intervals over their life span. Sni and Elav>E14 flies displayed impaired phototaxis and reduced life span compared to CS flies**. Al**, **MIX** and **CAST** all improved phototaxis in wild type CS flies while **CAW** had a mild effect (though not significant). Only **CAW**, **A1** and **MIX** improved phototaxis in Sni flies, while only **CAW**, **A1** and **CAST** improved phototaxis in Elav>E14 flies. Thus neuroprotective mechanisms may differ for the caffeoylquinic acids (reduced oxidative stress) and triterpene (reduced tauopathy) components of CA.

Author Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z