Screening of plant extracts for anti-inflammatory activity

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American Society of Pharmacognosy 2015 Annual Meeting

Natural Products Rising to the Top

July 25 - 29, 2015 • Copper Mountain, CO

ASP 2015 Annual Meeting
Copper Mountain, CO

Dear Fellow Natural Product Enthusiasts,

"*Natural Products Rising to the Top*," was selected as the theme for the 2015 American Society of Pharmacognosy (ASP) Meeting. This topic symbolizes both the fact that this year's meeting will take place at the highest altitude of any ASP meeting held to date, as well as the profound rise in interests in natural products across many disciplines. The last half decade has witnessed a revolution in natural product research throughout the world and particularly in the United States. What makes these developments so exciting is the divergent evolution of how natural products are currently studied and used in research. This year's meeting reflects these broadened and escalating interests in natural products by highlighting a rich variety of topics that will be presented by our symposium speakers, in parallel sessions, by award winners, throughout poster presentations, and within workshops. Scanning the enclosed abstracts reveals a breadth of topics including natural product drug discovery and development, medicinal and synthetic chemistry, genomics, ecology, botanicals, health and beauty, law, neuroscience, instrumentation, microbiome studies, and much more. These topics are sure to stimulate the hearts and minds of young and mature researchers alike as we join together to witness the wonderful science of our colleagues and friends.

A few additional special features are notable about this year's meeting of the ASP. First, special efforts have been made to highlight the wide range of perspectives on natural products offered by colleagues from academic, government, and industrial backgrounds. Second, this year's lineup of parallel session speakers was selected solely by each session leader to best reflect the broadest possible spectrum of scientific interests and viewpoints. Notably, many of the parallel session leaders have been assembled from a growing number of 'junior' faculty (recently appointed assistant and research professors) whose voices are important to the growth of the ASP. And third, we are introducing a new session this year, *ASP Younger Members Research Spotlight* led by James Fuchs, which is devoted solely to the contributions of some of our most outstanding graduate and postdoc researchers.

Such a wonderful meeting would not be possible without the help of many outstanding individuals and organizations. Special thanks are given first and foremost to our terrific sponsors and vendors who have helped us achieve an all-time record for meeting support. Appreciation is also given to our presenters for sharing their original work and ideas about natural products. The great assistance of our scientific organizing committee is also acknowledged, especially my co-chair, Susan Mooberry who has helped recruit speakers and provide advice about session topics. It is important to note the enduring support and organizational acumen of Ms. Candace Coker who is the project manager for the Institute for Natural Products Applications & Research Technologies at the University of Oklahoma, which is the host of this year's meeting. She has been at the heart of all matters related to the development, planning, and organization of the meeting and her dedication is greatly appreciated. Finally, it is vital that thanks and recognition be given to all of you who will be attending this year's meeting. Without your comradery and willingness to share your time and thoughts concerning natural products, this meeting would not be possible. It is because of you that natural products are truly rising to the top and the view towards the future of the field looks spectacular.

Sincerely,

Robert H. Cichewicz, Ph.D.
Chair of ASP 2015
Regents' Professor and Director
Institute for Natural Products Applications &
Research Technologies at the University of Oklahoma

ASP 2015 Annual Meeting
Copper Mountain, CO

July 25 – 29, 2015

General Information

Oral Presentations

All oral presentations will be held in the Big Horn Room(s) and/or the Ptarmigan Room in the Copper Mountain Conference Center. Please make sure to check in with your session leader in the room of your talk 15 minutes prior to the session. Presentations will be loaded onto the PC in your session room provided by the ASP meeting. Please have your presentation ready on a USB flash drive. If you would prefer to use your own MAC or your own PC, you may do so, but please notify your session leader in advance. Please note: all data will be removed from the session computers at the conclusion of the meeting.

Poster Presentations

All posters sessions will take place in the Copper Pavilion. Poster Session 1: Posters should be set up for this session on Saturday, July 25 between the hours of 3:00 - 6:00 PM only. Posters should be removed on the same day between the hours of 10:00 - 10:30 PM. Poster Sessions 2 & 3: Posters should be set up on the day of your session between the hours of 7:00 - 8:00 AM only. Posters should be removed on the same day between the hours of 5:30 - 6:00 PM. Posters not removed by the assigned time will be discarded.

Exhibitors

An exhibition of instrumentation, publications, and services will be located in the Copper Pavilion. We encourage everyone to visit these exhibits which will be open during every breakfast, coffee break, and poster session, Saturday, July 25 – Tuesday, July 28.

Registration

The 2015 ASP registration and information desk will be located in Kokopelli Trail, just outside of the Big Horn Room in the Copper Mountain Conference Center.

Name Badges and Attire

Please wear your name badge for admission to the scientific program sessions and all social events. Casual attire is perfectly acceptable for all scientific program sessions and all social events. Average daytime temperature for Colorado in July is a mild 88 degrees Fahrenheit with mornings and evenings being cooler. Rooms in the conference center may be cool as well, so we recommend that you bring a sweater or dress in layers. Copper Mountain is a very walkable community with many outdoor options to enjoy. You may consider wearing comfortable shoes. The Monday evening event and Tuesday afternoon Younger Member's Event will both take place outside.

Extra Banquet Tickets

Extra banquet and event tickets must be purchased prior to 5:00 PM on Saturday, July 25. If you do not plan to attend the banquet, we would appreciate if you would return the ticket to the registration desk so that we may maintain an accurate count for the banquet.

Photography

No photos of presentations or posters will be permitted unless permission has been directly obtained from the presenter. Anyone in violation of this may be asked to leave.

Thank you to our 2015 Committee Members

Local Organizing Committee

Robert Cichewicz, University of Oklahoma (Meeting Chair)
Anthony Burgett, University of Oklahoma
Candace Coker, University of Oklahoma
Adam Duerfeldt, University of Oklahoma
Phil Proteau, Oregon State University (Ad Hoc)
Indrajeet Sharma, University of Oklahoma
Brad Stevenson, University of Oklahoma

Scientific Organizing Committee

Robert Cichewicz, University of Oklahoma (Chair)
Susan Mooberry, UT Health Science Center, San Antonio (Co-Chair)
Cindy Angerhofer, Aveda
Emily Balskus, Harvard University
Lou Barrows, University of Utah
Sean Brady, Rockefeller University
Anthony Burgett, University of Oklahoma
John Cardellina, National Institutes of Health
Jim Fuchs, Ohio State University
Stefan Gafner, American Botanical Council
Mark Hamann, University of Mississippi
Liva Harinantenaina, Ohio State University
Scott Lokey, University of California, Santa Cruz
Nicholas Oberlies, University of North Carolina at Greensboro
Melany Puglisi-Weening, Chicago State University
Tom Prisinzano, University of Kansas
April Risinger, UT Health Science Center, San Antonio
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2015 Award Recipients

Norman R. Farnsworth Research Achievement Award
Raymond Andersen, University of British Columbia

Varro Tyler Prize
Cindy Angerhofer, Aveda

Matt Suffness Young Investigator Award
John MacMillan, UT Southwestern Medical Center

2015 Waters Award for Excellence in Natural Products
Paul Jensen, Scripps Institution of Oceanography

Research Starter Grant

Jason Kwan, University of Wisconsin-Madison

Student Research Award

Mayuramas Sang-Ngern, University of Hawaii

Undergraduate Research Awards

Nicole Nightingale, University of Pittsburgh

Andrea Romanowski, Palm Beach Atlantic University

Paul Scesa, Florida Atlantic University

ASP Student Travel Awards

Maryam Elfeki, University of Illinois at Chicago

Krista Gill, University of Prince Edward Island, Canada Brittany Graf, Rutgers University

Kyuho Moon, Seoul National University, Republic of Korea

Jessica Ochoa, University of California, Santa Cruz

Andrew Osborn, Oregon State University

Chris Thomas, University of Wisconsin-Madison

Active Member Travel Grant

Emily Mevers, Harvard University

Waquar H. Bhatti Student Travel Award

Ashley West, University of Connecticut

Lynn Brady Student Travel Award

Mary Choules, University of Illinois at Chicago

Ben Naman, Ohio State University

Yun Seo-Kil, Ewha Womans University, Seoul, Korea

David Carew Student Travel Award

Bailey Miller, University of California, San Diego

D. John Faulkner Travel Award

Sandra Loesgen, Oregon State University

Jerry McLaughlin Travel Award

Ian Miller, University of Wisconsin-Madison

ASP 2015 Annual Meeting
Copper Mountain, CO

Program Schedule

Saturday, July 25, 2015

8:00 AM – 7:00 PM Registration Open *(Kokopelli Trail)*

Pre-Meeting Workshops | Registration required. Onsite registration available.

9:00 AM – 12:00 PM Bioassays and Pharmacology of Natural Products *(Bighorn C2-3)*
9:00 AM – 12:00 PM LC/MS: The Practical Aspects for Natural Product Analysis *(Ptarmigan C)*
9:00 AM – 12:00 PM Peer Review at NIH: Grantsmanship and the Peer Review Process at NIH; Strategic Funding Priorities for Basic and Mechanistic Research *(Ptarmigan B)*
9:00 AM – 4:00 PM Advanced Analytical Technologies as Applied to Natural Products for Drug Discovery *(Ptarmigan A)*
1:00 PM – 4:00 PM Entrepreneurship and Natural Products; Early Stage Opportunities for Your Discoveries *(Ptarmigan C)*
1:00 PM – 4:00 PM The Nuts and Bolts of Contemporary NMR Spectroscopy *(Ptarmigan B)*

7:00 PM – 10:00 PM **Opening Reception**, **Poster Session 1**, Exhibits Open ***(Copper Pavilion)***
Hors d'oeuvres and cash bar. The Opening Reception is a ticketed event. One ticket is provided to all registrants, except One-Day registrants. Extra tickets can be purchased at the registration desk.

Sunday, July 26, 2015

7:15 AM – 8:00 AM Continental Breakfast, Exhibits Open *(Copper Pavilion)*
7:15 AM – 5:00 PM Registration Open *(Kokopelli Trail)*

General Session *(Bighorn)*

Conference Welcome by Meeting Chair, Robert Cichewicz (University of Oklahoma)

8:00 AM – 8:50 AM **Jonathan Baell** (Monash University): *Your Natural Product Contains a Promiscuous PAINS Motif: Is it Useful as a Biochemical Probe or in Drug Discovery?*
8:50 AM – 9:40 AM **Ben Shen** (Scripps Florida): *Microbial Genomics: New Opportunities for Natural Product Biosynthesis, Engineering, and Drug Discovery*

9:40 AM – 10:15 AM Coffee Break, Exhibits Open *(Copper Pavilion)*

Sunday Morning Parallel Sessions

Session 1 - The Brave New World of Natural Product Total Synthesis *(Bighorn B)*
Chair: Anthony Burgett (University of Oklahoma)

10:18 AM **Jason Chen** (Iowa State University): *Oxylipin Total Synthesis Uncovers Stereochemistry-dependent Conformations of Masked Diols*
10:35 AM **William Maio** (New Mexico State University): *The Taumycin a Macrocycle: Asymmetric Total Synthesis and Revision of Relative Stereochemistry*
10:52 AM **Jeremy May** (University of Houston): *Synthetic Discoveries from Polycyclic Natural Products*
11:09 AM **Joshua Pierce** (North Carolina State University): *Marine Natural Products Synthesis as a Driving Force for Chemical and Biological Discovery*

11:26 AM **Thomas Poulsen** (Aarhus University): *Total Syntheses of Rakicidin A and BE-43547A1*
11:43 AM **Jennifer Stockdill** (Wayne State University): *Enabling Reactivity of Neutral Aminyl Radicals in Polycyclic Heterocycle Synthesis*

Session 2 - Chemical Transformations in the Biosynthesis of Natural Products *(Bighorn C)*
Chair: Katherine Ryan (University of British Columbia)

10:15 AM **Wilfred van der Donk** (University of Illinois – Urbana/Champaign): *Biosynthesis of Cyclic Peptide Antibiotics*
10:45 AM **Alessandra Eustaquio** (University of Bergen): Spliceostatin Biosynthesis in Burkholderia spp.
11:10 AM **David Zechel** (Queen's University): *A Cure for Baldness and Cryptic Biosynthesis in Streptomyces Calvus*
11:35 AM **William Gerwick** (Scripps Institute of Oceanography, University of California, San Diego): *Discovery of Novel Cholorinated Acyl Amides from a Marine Cyanobacterium Using Inegrated Technologies*

Session 3 - Natural Products and HIV, Progress and Directions *(Ptarmigan)*
Chair: Lou Barrows (University of Utah)

10:15 AM **Kirk Gustafson** (Center for Cancer Research, National Cancer Institute): *Mining the Extensive Chemical Diversity of the NCI Natural Products Repository for New Agents that can Target HIV*
10:40 AM **Vicente Planelles** (University of Utah School of Medicine): *Ingenol 3,20 Dibenzoate Efficiently Reactivates Latent HIV*
11:05 AM **Prem Rai** (University of Papua New Guinea School of Medicine and Health Sciences): *Medicinal Plants Used by Traditional Medicine Practitioners for the Treatment of HIV/AIDS and Related conditions in Papua New Guinea*
11:30 AM **Sandra Loesgen** (Oregon State University): *News from Fungi – Targeting Viral Sweetspots*
11:45 AM **Mary Choules** (University of Illinois at Chicago): *A Rufomycin Analogue is an Anti-tuberculosis Drug Lead Targeting CLPC1 with No Cross Resistance to Eumicin*

12:00 PM – 1:45 PM Boxed Lunches *(Kokopelli Trail)*

Sunday Afternoon Parallel Sessions

Session 4 - ASP Younger Members Research Spotlight *(Bighorn B)*
Chair: Jim Fuchs (Ohio State University)

1:50 PM **Jessie Ochoa** (University of California, Santa Cruz): *Phylogenetic and Metabolomic Analysis of Marine Mammal Microbes Contributes to Emerging Spirotetronate Polyketides*
2:00 PM **Cong-Mei Cao** (University of Kansas): *Unusual Withanolides from Physalis Hispida (Waterf.) Cronquist*
2:10 PM **Jie Li** (Scripps Institution of Oceanography, University of California, San Diego): *Discovery of Fatty Acid Synthase Inhibitors and their Biosynthetic Pathways by a Novel Target-directed Genome Mining Strategy*
2:20 PM **Weijing Cai** (University of Florida): *Discovery, Synthesis, and Biological Evaluation of Apratyramide, a Marine-derived Transcriptional Stimulator of Vegf-A*
2:30 PM **Joshua Kellogg** (University of North Carolina at Greensboro): *Chemometric-directed Bioexploration of Natural Products*
2:40 PM **Alyssa Sprouse** (University of Illinois at Chicago): *Pharmacokinetic Interactions Between Drugs and Botanical Dietary Supplements*
2:50 PM **Tyler Olsen** (University of Oklahoma): *Selectivity and Mechanistic Inquiries into the Reactivity of Pericosine A, an Electrophilic Chlorinated Shikimate Analogue*
3:00 PM **Susanna Chan** (Molecular Targets Laboratory, Centre for Cancer Research, National Cancer Institute): *Isolation and identification of Novel Natural Products that Inhibit P300/HIF-1α Interaction*
3:10 PM **Jaqueline von Salm** (University of South Florida): *Targeting Bioactive Chemical Space with a Small Natural Products Library: Expanding Diversity and Predicitability*
3:20 PM **Antonius Ola** (University of Texas Health Science Center at San Antonio): *Design, Synthesis and Biological Evaluation of New Bulky Acyloxy Taccalonolides as Potent Microtubule Stabilizers*

Session 5 - The Evolving Role of Natural Products in Neuroscience *(Bighorn C)*
Chair: Tom Prisinzano (University of Kansas)

1:50 PM **Brian Blagg** (University of Kansas): *Natural Product Inspired Hsp90 Inhibitors*
2:10 PM **David Horgen** (Hawaii Pacific University): *Advances in the Validation of TRPM7 as a Drug Target Using Natural Products – Update on Waixenicin A*
2:30 PM **Scott Runyon** (Research Triangle Institute): *Development of Novel Neuroactive Steroids for the Potential Treatment of Neurological Disorders*
2:50 PM **Kevin Tidgewell** (Duquense University): *Panamanian Marine Cyanobacterial Extracts with In Vivo Activity in Models of Anxiety and Depression*
3:10 PM **Lyndon West** (Florida Atlantic University): *Discovery of Neuroprotective Marine Natural Products Using a Bioimaging/Optogenetics Approach and Drosophila Melanogaster*

Session 6 - Recent Advances in Marine Chemical Ecology *(Ptarmigan)*
Chair: Melany Puglisi-Weening (Chicago State University)

1:50 PM **Marcy Balunas** (University of Connecticut): *Functional and Biosynthetic Analyses of Secondary Metabolites in Host-Microbe Symbioses*
2:10 PM **Jason Kwan** (University of Wisconsin at Madison): *Integrated De Novo Metagenomics and Metatranscriptomics to Study Natural Products and Microbial Ecology In Situ*
2:30 PM **Amy Lane** (University of North Florida): *Biosynthesis and Ecological Functions of Diketopiperazine Natural Products from Marine Actinomycetes*
2:50 PM **David Rowley** (University of Rhode Island): *Mechanisms of Microbe-Microbe-Host Interactions in a Probiont-Pathogen-Bivalve Model*
3:10 PM **Jennifer Sneed** (Smithsonian Marine Station at Fort Pierce): *Macroalgae May Interrupt Important Cues for Coral Larval Settlement*

3:30 PM – 5:30 PM **Poster Session 2**, Coffee Break, Exhibits Open ***(Copper Pavilion)***

General Session *(Bighorn)*

7:00 PM – 7:50 PM **Amy Wright** (Harbor Branch Oceanographic Institute of Florida Atlantic University): *Discovery and Investigation of Therapeutically Important Marine Natural Products*
7:50 PM – 8:40 PM **Leslie Fischer** (Novartis Pharmaceuticals): *The Patent Eligibility of Natural Products in the United States: Understanding the Issues and Navigating the New Waters*

MONDAY, JULY 27, 2015

7:15 AM – 8:00 AM Continental Breakfast, Exhibits Open *(Copper Pavilion)*
7:15 AM – 5:00 PM Registration Open *(Kokopelli Trail)*

General Session *(Bighorn)*

8:00 AM – 8:50 AM **Paul Jensen** (Center for Marine Biotechnology & Biomedicine, Scripps Institution of Oceanography): *New Approaches to Microbial Natural Product Discovery*. 2015 Waters Award for Excellence in Natural Products Innovation, **Sponsored by Waters Corporation**
8:50 AM – 9:40 AM **Carole Bewley** (NIDDK, NIH Intramural Research): *Beyond Structure – Diverse Mechanisms of Anti-infective Natural Products*. **Sponsored by Sequoia Sciences**

9:40 AM – 10:15 AM Coffee Break, Exhibits Open *(Copper Pavilion)*

Monday Morning Parallel Sessions

Session 7 - Natural Products and the Human Microbiota *(Bighorn B)*
Chair: Emily Balskus (Harvard University)
Sponsored by Procter & Gamble & Takeda Pharmaceuticals International, Inc.

10:30 AM **Emily Balskus** (Harvard University): *Gut Reactions: Natural Products and the Human Microbiota*
11:00 AM **Peter Turnbaugh** (University of California, San Francisco): *Food, Drugs, and Bugs: A Metagenomic View of Pharamacology*
11:30 AM **Gerry Wright** (McMaster University): *Biotransformation of Anticancer Drugs by Human and Environmental Microbiomes*

Session 8 - Natural Products Anticancer Drug Lead Discovery in Honor of Richard G. Powell *(Bighorn C)*
Chair: Susan Horwitz (Yeshiva University). Organizer: Douglas Kinghorn (Ohio State University)
Sponsored by the Journal of Natural Products

10:15 AM **Richard Powell** (National Center for Agricultural Utilization Research, USDA, Peoria, IL; Journal of Natural Products): *Historical Aspects of Antitumor Compounds from Plants, including Homoharringtonine (Omacetaxine Mepesuccinate, Synribo TM)*
10:30 AM **David Kingston** (Virginia Tech Center for Drug Discovery): *Development of Taxol as an Anticancer Drug*
11:00 AM **Susan Moobery** (University of Texas Health Science Center at San Antonio): *North American Plants Remain an Excellent Source for Compounds with Anticancer Potential*
11:30 AM **David Newman** (National Cancer Institute): *Are Natural Products Isolated from Plants, Products of EPI- and/or Endo-phytic Microbial Interactions with/within the Host?*

Session 9 - Bumpy Road to Beauty: Natural Products in the Cosmetic Industry *(Ptarmigan)*
Chair: Cindy Angerhofer (Aveda)

10:20 AM **Jon Anderson** (Actives International LLC): *Paving the Way for Skincare*
10:45 AM **Chia Wen Chen** (Estee Lauder): *Ergothioneine, the Amazing Amino Acid*
11:10 AM **Vince Gruber** (Sensient Color Technologies): *From the Berry to the Barrier: Development of Natural Products for Skin and Hair Care*
11:40 AM **Artyom Duev** (AkzoNobel Surface Chemistry): *Selection of Prospective Ficus Species and Development of Novel Multifunctional Zeta Fractions for Personal Care*

12:00 PM – 1:45 PM Lunch on your own

Monday Afternoon Parallel Sessions

Session 10 - Microbial Interactions in Humans and Other Animals *(Bighorn B)*
Chair: Eric Schmidt (University of Utah)

1:45 PM **Jason Crawford** (Yale University): *Discovering and Deciphering the Pathogenic and Probiotic Activities form the Bacterial Colibactin Pathway*
2:10 PM **Nicole Lopanik** (Georgia State University): *Interactions Between the Marine Bryozoan Bugula Neritina, its Endosymbiont, and Symbiont-produced Bryostatins*
2:35 PM **Mohamed Donia** (Princeton University): *Small-molecule-mediated Interactions in Complex Microbial Communities*
3:00 PM **Margo Haygood** (University of Utah): *Versatile Bacterial Symbionts of Shipworms Contribute to Wood Digestion, Fix Nitrogen and Produce Secondary Metabolites*

Session 11 - Novel Screening Strategies for the Identification of Therapeutic Lead Compounds *(Bighorn C)*
Chair: April Risinger (UT Health Science Center at San Antonio)

1:45 PM **Daniel Hoeppner** (Lieber Institute for Brain Development): *Pluripotent Stem Cell Colonies Provide a Developmental Landscape for Pharmacogenomic Drug Development*

2:20 PM **Babu Tekwani** (University of Mississippi): *Neurotrophic and Neuritogenic Drug Leads from Natural Products: A Combines In Vitro Assay for Evaluation of Cell Viability and NGF-Stimulated Neuritic outgrowth in Neuroscreen 1 Cells*
2:37 PM **April Risinger** (University of Texas Health Science Center, San Antonio): *Utilizing Differential Cytotoxicity Screening to Identify Lead Compounds for Rare Adult and Pediatric tumors*
2:54 PM **Danielle Demers** (University of South Florida): *New Antimicrobials from an Epigenetics Based Fungal Metabolite Screening Program*
3:11 PM **Daniel Todd** (University of North Carolina at Greensboro): *A Novel bioassay to Idenify Anti-Virulence Leads Against Gram-Positive Bacterial Pathogens*

Session 12 - New Avenues in Botanical Research *(Ptarmigan)*
Chair: Stefan Gafner (American Botanical Council)

1:45 PM **Muriel Cuendet** (University of Geneva, Switzerland): *HDAC Inhibitors for Cancer Chemoprevention*
2:10 PM **Vladimir Shulaev** (University of North Texas): *Global Lipidomics Profiling of Cotton Seed Oil Genotypes Using CO2-based Chromatography Coupled to Mass Spectrometry*
2:35 PM **Nanjoo Suh** (Rutgers University): *Natural Tocopherol Mixtures As Promising Cancer Preventive Agents*
3:00 PM **Birgit Dietz** (University of Illinois at Chicago): *Can Women's Health Botanicals prevent Estrogen Carcinogenesis?*
3:15 PM **Clinton Dahlberg** (Nature's Sunshine): *Clinical Evaluation of Formula F105, Designed and Developed to Address Elevated Oxidized LDL Cholesterol (oxLDL) Levels*

3:30 PM – 5:30 PM **Poster Session 3**, Coffee Break, Exhibits Open ***(Copper Pavilion)***

Off-Site Group Event

5:45 PM – 10:00 PM **An Evening at Keystone Stables**
Join your colleagues for an evening of western-filled-fun at beautiful Soda Ridge Stables in Keystone, Colorado. Specially chartered buses will depart from Copper Circle at 6:00 PM. This is a ticketed event. One ticket is provided to all registrants, except One-Day registrants.

TUESDAY, JULY 28, 2015

7:15 AM – 8:00 AM Continental Breakfast, Exhibits Open *(Copper Pavilion)*
7:15 AM – 1:00 PM Registration Open *(Kokopelli Trail)*

General Session *(Bighorn)*

8:00 AM – 8:50 AM **Derek Tan** (Memorial Sloan-Kettering Cancer Center): *Natural Product-based Strategies in Diversity-Oriented Synthesis*
8:50 AM – 9:40 AM **John Tallarico** (Novartis Institute for BioMedical Research): *Discoveries at the Interface of Pathways, Phenotypic Screening, and Chemical Biology*

9:40 AM – 10:15 AM Coffee Break, Exhibits Open *(Copper Pavilion)*

Tuesday Morning Parallel Sessions

Session 13 - Applications of Mass Spectrometry to Natural Products Drug Studies *(Bighorn B)*
Chairs: Zhibo Yang (University of Oklahoma) and Si Wu (University of Oklahoma)

10:15 AM **Neil Kelleher** (Northwestern University): *Metabologenomics: Discovery of New Natural Products and their Biosynthetic Gene Clusters by Genome-Informed Metabolomics*
10:55 AM **Markus Schirle** (Novartis Institutes for BioMedical Research, Inc., Cambridge, MA): *Mass Spectrometry-based Chemoproteomics for Target Deconvolution of Bioactive Natural Products*
11:15 AM **Giorgis Isaac** (Waters): *State of Art Analytical Technologies to Solve Natural Products Challenges*

11:30 AM **Zhibo Yang** (University of Oklahoma): *The Single-probe Mass Spectrometry for Single Cell Analysis and Biological Tissue Imaging*
11:45 AM **Laura Sanchez** (University of California, San Diego): *Visualizing Diverse Chemical Families with Molecular Networking*

Session 14 - Enhancing Natural Product Leads via Synthetic Manipulation: In Celebration of Dr. Mansukh Wani's 90th Birthday *(Bighorn C)*
Chairs: Nicholas Oberlies (University of North Carolina at Greensboro) and John Cardellina (National Institutes of Health)

10:15 AM **James Fuchs** (Ohio State University): *Development and Optimization of the Phyllanthusmin*
10:45 AM **Thomas Prisinzano** (University of Kansas): *Synthesis of both Enantiomers of the Meta, Meta-bridged Diarylheptanoid Myricanol*
11:15 AM **Dale L. Boger** (Scripps Research Institute): *Vinblastine: Synthetic and Mechanistic Studies*

Session 15 - Translational Studies of Natural Products *(Ptarmigan)*
Chair: Mark Hamann (University of Mississippi)

10:15 AM **William Fenical** (Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography): *Challenges and Successes in the Development of Natural Product Drugs*
10:45 AM **Guy Carter** (Biosortia): *Measures of Success in Translational Research*
11:05 AM **Trevor Castor** (Aphios): *Development of Zindol®* Through Multi-center Clinical Trial for Chemotherapy Induced Nausea and Vomiting
11:25 AM **Bruce Littlefield** (Eisai): *Discovery and Development of Eribulin, a Macrocyclic Ketone Analog of Halichondrin B, for Treatment of Advanced Breast Cancer*
11:45 AM **Mark Butler** (Institute of Molecular Bioscience, University of Queensland): *Natural Products in Clinical Trials: Current State of Play and Future Directions*
12:05 PM **John Beutler** (National Cancer Institute): *Development of Englerins as Cancer Therapeutics*

Younger Member's Event

12:30 PM **Younger Member's Event**
This is a ticketed event. One ticket is included with each students and postdoc registration. Junior faculty may attend by purchasing a ticket. Attendees will take the American Eagle Lift up the mountain to Solitude Station where they will enjoy lunch surrounded by the scenic views of Copper Mountain. Following lunch, attendees may choose to participate in a short guided hike on the Hallelujah Loop. Participants may then enjoy the American Eagle chair lift ride back down into Copper Village to enjoy discussion sessions tailored specifically to younger members.

4:00 PM **Younger Member's Session *(Ptarmigan)***

WEDNESDAY, JULY 29, 2015

7:15 AM – 8:00 AM Continental Breakfast *(Jack's)*
7:15 AM – 5:00 PM Registration Open *(Kokopelli Trail)*

General Session *(Bighorn)*

8:00 AM – 8:50 AM **Daniel Romo** (Texas A&M University): *Bioactivity-Guided Retrosynthesis: 'Upping the Ante' for Natural Product Total Synthesis*
8:50 AM – 9:40 AM **Peter Senter** (Seattle Genetics): *Potent Antibody-Based Conjugates for Cancer Therapy: From Early Stage Research to a Clinically Approved Drug*

9:40 AM – 10:15 AM Coffee Break *(Jack's)*

Wednesday Morning Parallel Sessions

Session 16 - Beyond The Rule of 5 in the Design of Next-Generation Therapeutics: What Can We Learn From Macrocyclic Natural Products? *(Bighorn B)*
Chair: Scott Lokey (University of California, Santa Cruz)

10:15 AM **Roger Linington** (Scripps Institution of Oceanography, University of California San Diego): *Natural Products: Are We Close to the End?*
10:40 AM **Scott Lokey** (University of California, Santa Cruz): *Interrogating Cyclic Peptide Natural Products from an ADME Perspective: Beyond Cyclosporine A*
11:05 AM **David Craik** (University of Queensland): *Pharmaceutical Applications of Cyclotides*
11:30 AM **Andrei Yudin** (University of Toronto): *Peptide Macrocycles: From Structural Studies to Oral Bioavailability*

Session 17 - Microbial Metabolites and Bioactive Compounds From Plant Microbial Associates (Epiphytes and Endophytes) *(Bighorn C)*
Chair: Liva Harinantenaina (Ohio State University)

10:15 AM **Nicholas Oberlies** (University of North Carolina at Greensboro): *Endophytic Fungi of Medicinal Herbs: Basic Science and Source of New Drug Leads*
10:45 AM **Leslie Gunatilaka** (University of Arizona): *Exploring Plant and Lichen-Associated Microbial Diversity for Discovery of Small-Molecule Bioactive Agents*
11:15 AM **Marc Stadler** (Helmholtz Centre for Infection Research): *Biologically Active Secondary Metabolites from Epiphytic and Endophytic Fungi*
11:45 AM **Althar Ata** (University of Winnipeg, Canada): *Novel Bioactive Compounds from Endophytic Fungi and Aboriginal Medicinal Plants*

Session 18 - Diverse Approaches for Finding and Making Natural Products *(Ptarmigan)*
Chair: Sean Brady (Rockefeller University)

10:15 AM **Frank Schroeder** (Cornell University): *Comparative Metabolomics Reveals a Modular Library of Signaling Molecules in Nematodes*
10:40 AM **Jeremy Owens** (Rockefeller University): *Contemporary Strategies for Targeted Discovery and Transcriptional Activation of Natural Product Biosynthetic Gene Clusters*
11:05 AM **Gavin Williams** (North Carolina State University): *Harnessing the Promiscuity of Natural Product Biosynthesis: A Platform for Engineering Pathways with New Specificities*
11:30 AM **Greg Challis** (University of Warwick): *Manipulation of Actinobacterial Transcriptional Regulation to Discover New Specialized Metabolites*

12:00 PM – 1:00 PM Lunch on your own

Award Symposium *(Bighorn)*

1:30 PM – 2:10: PM **Norman R. Farnsworth Research Achievement Award: Raymond Andersen** (University of British Columbia): *Sponging Off Nature for New Drug Leads*
2:10 PM – 2:50 PM **Varro Tyler Prize: Cindy Angerhofer** (Aveda): *The Ins and Outs of Pharmacognosy: Plants for Health and Beauty*
2:50 PM – 3:30 PM **Matt Suffness Young Investigators Award: John MacMillian** (UT Southwestern Medical Center): *Use of High Content Screening for the Discovery and Biological Characterization of Natural Products*

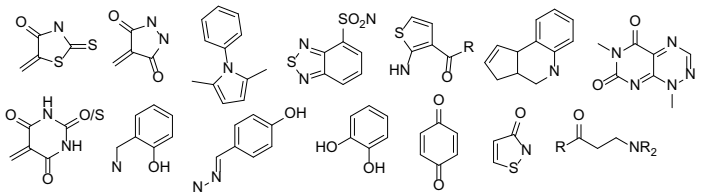
3:30 PM – 5:30 PM ASP Business Meeting *(Ptarmigan)*
6:00 PM – 7:00 PM Reception *(Kokopelli Trail)*
7:00 PM – 10:00 PM **Annual Banquet (Copper Pavilion)**
Ticketed Event.

General Sessions

YOUR NATURAL PRODUCT CONTAINS A PROMISCUOUS PAINS MOTIF: IS IT USEFUL AS A BIOCHEMICAL PROBE OR IN DRUG DISCOVERY?

Jonathan Baell
Professor of Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences (MIPS), Monash University

With increasing access to high throughput screening, academic drug discovery is being accompanied by a plethora of publications that report screening hits as good starting points for drug discovery or as useful tool compounds, whereas in many cases this is not so. These compounds may be protein-reactive but can also interfere in bioassays via a number of other means, many of which may remain unknown, and it can be very hard to prove early on that they represent false starts.[1-5] We have termed such compounds **P**an-**A**ssay **In**terference Compound**s**, or PAINS. Examples of such compound cores are shown below. Some of these cores are prevalent in natural products. PAINS were defined from HTS libraries devoid of natural products. So how should one view PAINS-containing natural products in terms of useful biochemical probes or potential therapeutics? This presentation will delve into such issues.



1. Baell JB & Holloway GA. New substructure filters for removal of pan assay interference compounds [PAINS] from screening libraries and for their exclusion in bioassays. *J. Med. Chem*. **53**, 2719-2740 (2010).
2. Baell JB. Observations on Screening-Based Research and Some Concerning Trends in the Literature. *Future Med. Chem.* **2**, 1529–1546 (2010).
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4.Baell JB, Ferrins L, Falk H, Nikolakopoulos G. PAINS: Relevance to Tool Compound Discovery and Fragment-Based Screening. *Aust. J. Chem*. **66** (2013) 1483-1494.
5. Baell J & Walters MA. Chemical con artists foil drug discovery. *Nature* **513** (2014) 481-483.

BEYOND STRUCTURE - DIVERSE MECHANISMS OF ANTI-INFECTIVE NATURAL PRODUCTS

SPONSORED BY SEQUOIA SCIENCES

Carole Bewley
Senior Investigator and Chief of the Natural Products Chemistry section in NIDDK, NIH.

During the past two decades Drs. Newman and Cragg have eloquently described the indispensable role that natural products and their derivatives have played as sources of new drugs (*J. Nat. Prod.* 2007, ~3000 citations). This is especially true in the area of anti-infectives where synthetic libraries have been less effective than natural products libraries in yielding new lead compounds and drugs. In addition to the discovery of new antibiotics and antivirals, we have been especially interested in identifying the targets of promising inhibitors and defining the chemical and structural basis for their activity. By taking a multidisciplinary approach that may involve microscopy, high-resolution structural and biophysical techniques, or comparative genomics we continue to be surprised at the diverse mechanisms that natural products use to exert their antimicrobial and antiviral effects. Recent progress in these areas including proteins and small molecules from Nature will be presented.

THE PATENT ELIGIBILITY OF NATURAL PRODUCTS IN THE UNITED STATES: UNDERSTANDING THE ISSUES AND NAVIGATING THE NEW WATERS

Leslie Fischer
Senior Patent Attorney, Specialty Care Patent Group, Novartis Pharmaceuticals Corporation

United States (U.S.) patent law has historically held that purified and/or isolated natural products are eligible for patenting. Subject matter as diverse as vitamins, proteins, DNA, yeast, bacteria, and antibiotics have all been the focus of valid U.S. patents. But, in June 2013, the U.S. Supreme Court introduced uncertainty into the natural products' patent arena, by holding that genomic DNA was not eligible for patenting "merely because" it is isolated. The U.S. Patent and Trademark Office and various U.S. federal courts have exacerbated this uncertainty with a series of official patent examination guidelines and decisions extending the Supreme Court's limited genomic DNA holding to other types of natural products. In this lecture, we will explore the concept of what it means to be "patent eligible", the historical analysis of patent eligibility in the natural products field, the new legal doctrines and how they are negatively impacting the patent eligibility of natural products, and how to best navigate this transitional legal period.

NEW APPROACHES TO MICROBIAL NATURAL PRODUCT DISCOVERY

2015 WATERS AWARD FOR EXCELLENCE IN NATURAL PRODUCTS INNOVATION

Paul R. Jensen
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego

Microbial natural products have provided some of today's most important medicines. Despite an industrial downturn in natural product research, recent advances in the field have provided a new impetus to re-visit this resource for drug discovery. These advances have been driven in large part by ready access to genome sequence data, which has revealed that most bacteria maintain considerably more genetic potential than the compounds discovered to date would suggest. Coupled with advanced bioinformatic tools and analytical approaches, it has become possible to mine genome sequences and link pathways to products in unprecedented ways. The results have provided unprecedented insight into the evolutionary processes that generate structural diversity and rapid methods to detect known compounds and target new molecules for characterization. Working with a model group of marine bacteria, it has been possible to apply these approaches to natural product discovery in ways that have provided insight into the relationships between secondary metabolism, biogeographic origin, and taxonomic diversity. The results are providing new opportunities to explore microbial diversity and capitalize on the wealth of biosynthetic potential that has yet to be exploited for natural product discovery.

BIOACTIVITY-GUIDED RETROSYNTHESIS: "UPPING THE ANTE" FOR NATURAL PRODUCT TOTAL SYNTHESIS

Daniel Romo
Texas A&M University

In recent years, synthetic chemists have made fantastic strides toward improving the synthetic efficiency of complex bioactive natural product synthesis through atom and step economy, avoidance of protecting groups, and the development of complexity-generating cascade processes that minimize step count and purifications. Our continued interest in under-

standing the mechanism of action of bioactive natural products, including identification of their putative cellular receptors, has led us to consider adding another requirement to our retrosynthetic strategies. Namely, we have sought to introduce the hypothesized pharmacophore of a natural product early in the synthetic sequence to enable SAR studies at an early stage of a total synthesis effort and continue these studies with increasingly complex intermediates as the natural product itself is approached. This strategy is most readily applicable to natural products that are readily hypothesized to covalently modify their putative cellular receptors (*e.g.* epoxide, β-lactone, exo-methylene carbonyl-containing natural products). Several cases studies implementing this retrosynthetic strategy will be presented along with biological studies that may substantiate this approach.

POTENT ANTIBODY-BASED CONJUGATES FOR CANCER THERAPY: FROM EARLY STAGE RESEARCH TO A CLINICALLY APPROVED DRUG

Peter Senter
Seattle Genetics, 21823 30th Dr. SE, Bothell WA

Monoclonal antibodies (mAbs) have played a major role in cancer medicine, with active drugs such as trastuzumab (Herceptin), cetuximab (Erbitux), bevacizumab (Avastin) and rituximab (Rituxan) in a wide range of therapeutic applications. The mechanism of activity of these agents once they bind to tumor associated antigens may involve direct signaling, interactions with Fcγ receptor positive cells on effector cells, and complement fixation. Several approaches have been explored to improve antibody-based therapies for cancer treatment by optimizing these activities and by using antibodies as delivery agents for highly potent cytotoxic drugs. These areas have advanced significantly in the past few years, leading to the approval of two antibody drug conjugates (ADCs) and a glyco-engineered antibody with enhanced binding to endogenous natural killer cells. New insights into how ADCs can be effectively developed have been gained through studies on cancer antigen targets and their expression on normal tissues, drug potency and mechanism, and linker stability and conditional drug release. Adcetris (brentuximab vedotin, SGN-35) is an example an ADC that has been designed with these parameters in mind. In August 2011, this drug was approved by the FDA for use in relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma, two diseases with significant unmet medical needs. An overview of how Adcetris was developed and how the technology is being extended to include new antigen targets, new drugs, and new linker technologies will be provided.

MICROBIAL GENOMICS: NEW OPPORTUNITIES FOR NATURAL PRODUCT BIOSYNTHESIS, ENGINEERING, AND DRUG DISCOVERY

Ben Shen
Professor at the Departments of Chemistry and Molecular Therapeutics, Vice Chairman of the Department of Chemistry, and Director of Natural Products Library Initiative at The Scripps Research Institute (TSRI), TSRI, Jupiter, Florida.

Natural products are among the best sources of drugs and drug leads and serve as outstanding small molecule probes for dissecting fundamental biological processes. Natural product biosynthesis continues to push the frontier of modern chemistry, biochemistry, and molecular biology by revealing novel chemical reactions, complex enzyme systems, and intricate regulatory mechanisms. The progress made in the last two decades in connecting natural products to the genes that encode their biosynthesis has fundamentally changed the landscape of natural products research and sparked the emergence of a suite of contemporary approaches to natural products discovery. Genetic manipulation of natural product biosynthetic machineries offers a promising alternative to generate natural product structural diversity. Selected examples from our current research will be presented to highlight the opportunities for natural product biosynthesis, engineering, and drug discovery

DISCOVERIES AT THE INTERFACE OF PATHWAYS, PHENOTYPIC SCREENING, AND CHEMICAL BIOLOGY

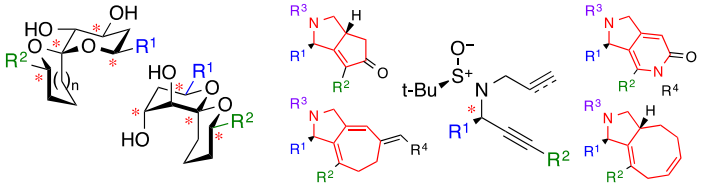
John Tallarico
Executive Director, Novartis Institutes for Biomedical Research

In situations where the genetic bases of diseases are established and the biology is amenable to intervention, we can often develop a therapy. More frequently, understanding the genetics of disease does not yield a specific target and instead points us at specific signaling pathways and processes that are misregulated. For these situations, we have brought the expertise and technologies of several scientific disciplines to bear on the challenge of identifying new targets and mechanisms as potential therapies. A particular area of attention is small molecule phenotypic screening as an approach towards discovering new targets, new mechanisms, and new therapies. We will describe several of our technologies and how we applied them to particular aspects of disease biology. We will also share several examples where our research has uncovered new targets and new mechanisms that may be amenable for therapies.

NATURAL PRODUCT-BASED STRATEGIES IN DIVERSITY-ORIENTED SYNTHESIS

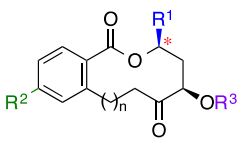
Derek S. Tan, Ph.D
Member and Tri-Institutional Professor, Molecular Pharmacology & Chemistry Program, Memorial Sloan Kettering Cancer Center, 1275 York Ave., Box 422, New York, NY 10065

Genome sequencing and an increasingly molecular understanding of biology have revealed myriad new biological targets of both fundamental and potential therapeutic interest. However, the identification of highly specific small molecules to address these targets remains a significant challenge in chemical biology and drug discovery. To address this challenge, we are developing discovery libraries based on privileged structural motifs from natural products. Such structures have a demonstrated ability to bind multiple classes of biological targets, but have distinct structural and physicochemical properties compared to existing drugs. Thus, these libraries are designed to access complementary regions of chemical structure space and spectra of biological targets. Notably, many of the existing approaches to synthesizing these structures are unsuitable for use in diversity-oriented synthesis, due to its stringent requirements for reaction efficiency and flexibility. Thus, we are presented with numerous opportunities to develop new chemical methodologies with broader applications in organic synthesis. Our current synthetic targets include spiroketals and other oxygen heterocycles, macrocycles and medium rings, and polycyclic alkaloids. Our libraries are being screened against a wide range of targets through multidisciplinary collaborations with biologists, with the long-term goals of elucidating complex biological processes and exploring new therapeutic opportunities in cancer and infectious diseases.

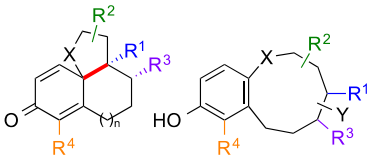


spiroketals
stereochemical diversity (*)

alkaloids/terpenoids
scaffold diversity



macrocycles
conformational restriction



cyclohexadienones and medium rings
scaffold reorganization

DISCOVERY AND INVESTIGATION OF THERAPEUTICALLY IMPORTANT MARINE NATURAL PRODUCTS

Amy E. Wright Ph.D.
Director for the Center of Excellence in Biomedical and Marine Biotechnology, Harbor Branch Oceanographic Institute of Florida Atlantic University

For many years Harbor Branch Oceanographic Institute operated the Johnson-Sea-Link human occupied submersible allowing for the collection of organisms from depths up to 3000 feet of seawater. Use of the submersible coupled to more standard collection methods such as scuba and snorkeling has led to a collection of over 18,000 marine macro organisms and 19,000 microbial isolates that are used in our research. As part of an NIH funded National Cooperative Drug Discovery Group, we began creating an "enriched peak library" to overcome the issues related to screening crude extracts and the need for extensive bioassay-guided fractionation. With funding from NCCAM we greatly expanded our enriched fraction library. The library has been created using a Combiflash™ Companion™ Flash Chromatography System which separates crude extracts into highly enriched fractions, many of which can be up to 90% of a single component. Screening of the library has allowed us to find new modulators of gamma secretase; inhibitors of NFκB; compounds that modulate mast cell function related pancreatic cancer; antiplasmodial compounds and compounds selectively active against the intra-macrophage form of TB, to name a few activities. The presentation will highlight the use of the peak library in discovery of active natural products as well as some of our recent findings related to applications of natural products to cancer drug development.

Award Winners

SPONGING OFF NATURE FOR NEW DRUG LEADS

NORMAN R. FARNSWORTH RESEARCH ACHIEVEMENT AWARD

Raymond Andersen
Department of Chemistry and EOAS, University of British Columbia, Vancouver, B.C., Canada

The secondary metabolites found in marine organisms represent an extremely rich source of novel chemical diversity for academic drug discovery and chemical biology programs. Among the marine invertebrates, marine sponges have historically been one of the most prolific sources of new natural products. Our group at UBC has amassed a sizable library of crude extracts from marine sponges, other marine invertebrates, and cultured marine microorganisms collected in many of the world's oceans. In collaboration with biologists, this crude extract library has been screened for activity in cell-based and pure enzyme assays designed to identify promising marine natural product lead compounds for the development of drugs. Bioassay-guided fractionation of crude extracts and extensive spectroscopic analysis has been used to identify the structures of pure natural products active in the assays. Biology-oriented chemical synthesis has been undertaken to probe the SAR for new natural product pharmacophores that we have discovered and to provide material for *in vivo* testing in animal models. Several new drug candidates for the treatment of cancer, inflammation, cystic fibrosis, and infectious diseases have emerged from this research program. Three of them have progressed to phase II clinical trials in humans and others are in preclinical evaluation/development. The lecture will present highlights from our academic 'Drugs from the Sea' and chemical biology research.

THE INS AND OUTS OF PHARMACOGNOSY: PLANTS FOR HEALTH AND BEAUTY

VARRO TYLER PRIZE

Cindy Angerhofer
Executive Director of Botanical Research, Aveda

Implicit in the word pharmacognosy is the concept that plants provided the first source and recognition of medicinal substances. For thousands of years, sages and healers, shamans, herbalists and clinicians have been honing the use of plants as medicines for internal and external applications. While modern medicine and even many dietary supplements often pursue highly purified single compounds, profound lessons are still to be discovered in crude plant materials. Relatively unrefined plant extracts like buriti oil and turmeric are complex in their chemistry and are used traditionally as foods and as effective topical treatments for the skin. Modern ingredients created from such plants for supplements or cosmetics range from pure compounds to minimally-refined botanicals, and cGMP requirements are vital to help guide production of quality products, but it is imperative to start the process with a sustainable, high quality plant. This presentation will emphasize the roles a pharmacognosist can play in the sourcing supply chain of botanicals as well as their effective application from the perspective of basic academic research as well as the cosmetic industry.

USE OF HIGH CONTENT SCREENING FOR THE DISCOVERY AND BIOLOGICAL CHARACTERIZATION OF NATURAL PRODUCTS

MATT SUFFNESS YOUNG INVESTIGATOR AWARD

John MacMillan
Department of Biochemistry, University of Texas Southwestern Medical Center

Over the past eight years our laboratory has focused on the use of high-content phenotypic screens for the discovery and biological characterization of natural products from a collection of marine-derived bacteria. Profound advances in the physical and functional annotation of the protein-coding elements of the human genome have had a transformative impact on discovery science directed at the mechanistic basis of human disease. Taking advantage of this wealth of information, we have developed a technology platform to investigate the mechanism of action of entire natural product libraries and crude mixtures in the context of cancer cells. This strategy is based on perturbations of cells with miRNA, siRNA and natural products to produce a functional signature of ontology (FUSION) that link bioactive molecules to the proteins and biological processes that they engage in cells. We have used this platform to characterize compounds with functional roles in autophagy, chemotaxis mediated by discoidin domain receptor 2, and activation of the kinase AKT.

In this presentation I will describe the FUSION platform and additional bioinformatics tools we have developed to characterize both known and new naturalproducts . This includes our efforts to elucidate the mechanism of action of didemnin B, the discovery of the discoipyrroles and the identification of selective toxins for non-small cell lung cancer.

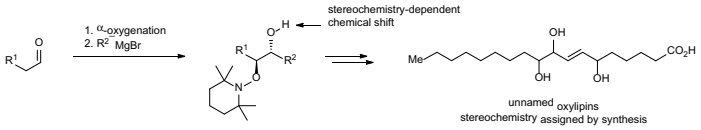
Parallel Sessions

Session 1 - The Brave New World of Natural Product Total Synthesis

OXYLIPIN TOTAL SYNTHESIS UNCOVERS STEREOCHEMISTRY-DEPENDENT CONFORMATIONS OF MASKED DIOLS

Shreyosree Chatterjee, Gayan A. Abeykoon, and Jason S. Chen
Department of Chemistry, Iowa State University, Ames, IA 50011, USA.

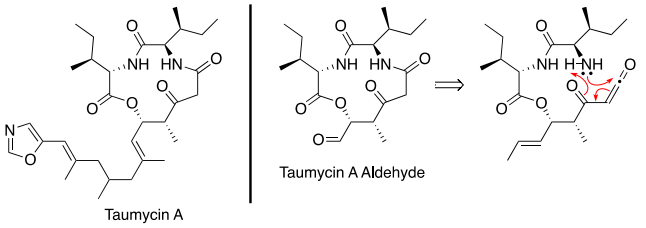
A two-step *anti*-1,2-diol synthesis was developed using aldehyde α-oxygenation followed by Grignard addition. This method was highlighted in the synthesis and stereochemical assignment of two unnamed oxylipins from *Dracontium loretense*. Unexpected NMR chemical shift signatures in the differentially-masked 1,2-diols led to identification of unexpected stereochemistry-dependent conformational biases in 2,2,6,6-tetramethylpiperidinyl-masked 1,2-diols.



THE TAUMYCIN A MACROCYCLE: ASYMMETRIC TOTAL SYNTHESIS AND REVISION OF RELATIVE STEREOCHEMISTRY

William A. Maio[1]
[1]*Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA*

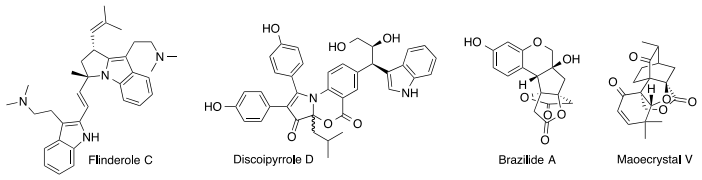
The symbiotic association of marine microorganisms with their host sponges continues to be an extraordinary source of hybrid polyketide / non-ribosomal secondary metabolites that often possess unique structures in conjunction with interesting biological activity. Recently, taumycin A was isolated from a Madagascar sponge of genus Fascaplysinopsis. This work describes the first asymmetric total synthesis and revision of the relative configuration of the 12-membered macrocycle via the synthesis of taumycin A aldehyde. Key to the success of this work is a novel alpha-keto ketene macrocyclization that provided an efficient means by which to access two diastereomers of the desired macrolide without the need to employ additional coupling agents or unnecessary oxidation state adjustments.



SYNTHETIC DISCOVERIES FROM POLYCYCLIC NATURAL PRODUCTS

Jeremy May, Jiun-Le Shih, Thien S. Nguyen, Phong Le, Ravikrishna Vallakati, Chris Huynh, Tho Tran, Santa Jansone-Popova, Brian J. Lundy
Department of Chemistry, University of Houston, 112 Fleming Building, Houston, Texas 77204-5003, United States

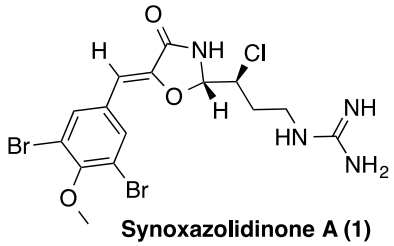
Complex polycyclic natural products present daunting challenges to the synthetic organic chemist and thus drive innovation. We have been inspired by the challenges presented by chiral indole alkaloid and bridged polycyclic natural products. We present new experimental results for enantioselective conjugate additions to generate alpha-chiral heterocycles and carbene cascade reactions to generate functionalized bridged bicycles. We also show the application of these methods to the total synthesis of these natural products.



MARINE NATURAL PRODUCTS SYNTHESIS AS A DRIVING FORCE FOR CHEMICAL AND BIOLOGICAL DISCOVERY

Joshua G. Pierce[1*], *Nataliia V. Shymanska*[1], *and Grant A. Edwards*[1]
[1]*Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA.*

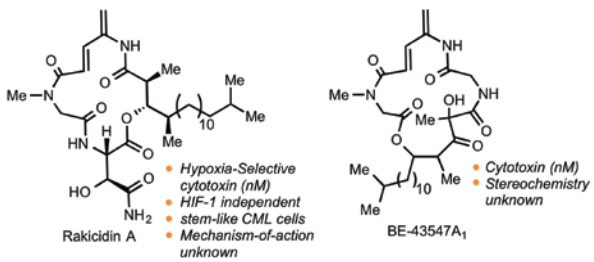
Natural products bearing complex structures and potent biological activities have long been the target of chemical synthesis and have served as successful lead molecules for drug discovery. As part of our program centered on the synthesis and chemical biology of marine natural products we have achieved a rapid synthesis of synoxazolidinone A (**1**) and structural analogs. Through these efforts we have revealed new chemical reactions and potent lead structures for infectious disease research. The synthesis and chemical biology of the synoxazolidinones will be presented.



TOTAL SYNTHESES OF RAKICIDIN A AND BE-43547A$_1$

Thomas B. Poulsen
Department of Chemistry, Aarhus University, Aarhus C, Denmark

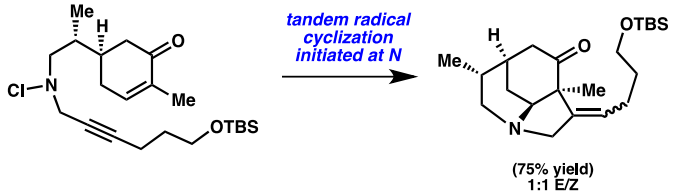
Rakicidin A, a macrocyclic depsipeptide isolated from *micromonospora sp.*, exhibits selective cytotoxicity towards hypoxic cancer cells and induces apoptosis in quiescent stem-like CML cells. Cancer stem cells are suspected to be a major driver of cancer progression and relapse. The hypoxia selectivity of rakicidin A is independent of HIF-1 and the cellular target remains unknown. BE-43547A$_1$ is a related natural product that also displays potent cytotoxicity. The macrocyclic systems of these molecules constitute significant synthetic challenges due to the presence of both congested structural elements and a labile vinylogous dehydroalanine functionality. The latter is unique to this class of natural products. I will present our efforts aimed at the syntheses of both rakicidin A and BE-43547A$_1$ with the further objective to facilitate biological investigations.



ENABLING REACTIVITY OF NEUTRAL AMINYL RADICALS IN POLYCYCLIC HETEROCYCLE SYNTHESIS

Ahmad A. Ibrahim, Alberto M. Lopez, Jennifer L. Stockdill
Wayne State University, 5101 Cass Avenue, Detroit, MI 48072, USA

Tertiary aliphatic amines are a privileged functional group in pharmaceuticals. For example, 51% of FDA approved nervous system drugs possess a tertiary aliphatic amine. However, there is only one neurologic agent with this functionality disposed at a ring junction. This imbalanced representation of structural space likely points to a need for more efficient strategies for the synthesis of these compounds, rather than an inherent problem with these structures as drug agents. We have devised a general strategy to potentially access a variety of such compounds using radical cascade reactions of N-centered radicals. In this seminar, a successful example of our approach that employs neutral dialkyl aminyl radicals will be discussed in the context of our efforts toward the total syntheses of the plant alkaloids daphniyunnine C and daphnicyclidin A.



Session 2 - Chemical Transformations in the Biosynthesis of Natural Products

BIOSYNTHESIS OF CYCLIC PEPTIDE ANTIBIOTICS

Wilfred van der Donk
Department of Chemistry, Howard Hughes Medical Institute and University of Illinois at Urbana-Champaign

Research in the 20th century identified several large families of natural products including terpenoids, alkaloids, polyketides, and non-ribosomal peptides. The genome sequencing efforts of the first decade of the 21st century have revealed that another major class is formed by ribosomally synthesized and post-translationally modified peptides (RiPPs). These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthipeptides are examples of this growing class and many members are highly effective peptide-derived antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) against many pathogenic bacteria. These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity. This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as their use for the generation of non-natural cyclic peptides.

SPLICEOSTATIN BIOSYNTHESIS IN *BURKHOLDERIA* SPP.

Alessandra S. Eustáquio[1,2], Jeffrey E. Janso[1], Anokha S. Ratnayake[1], Li-Ping Chang[1], Christopher J. O'Donnell[1], and Frank E. Koehn[1]
[1]Natural Products Laboratory, Worldwide Medicinal Chemistry, Pfizer Worldwide Research and Development, Groton, CT 06340, USA, [2]Currently at: Department of Biology, University of Bergen, Norway.

Spliceostatins are a suite of bacterial natural products that have been shown to target the spliceosome, an emerging mode of action in cancer therapy[1]. Spliceostatin biosynthesis in *Burkholderia* species is catalyzed by a hybrid nonribosomal peptide synthetase–polyketide synthase system of the trans-acyl transferase type[2, 3]. In this presentation, genetic and biochemical evidence for hemiketal biosynthesis via oxidative decarboxylation – rather than the previously hypothesized Baeyer-Villiger oxidation – will be described[4]. In addition, roles for a cytochrome P450 and a flavin-dependent monooxygenase are proposed based on genetic studies. Understanding late steps in spliceostatin biosynthesis was instrumental to achieve gram-scale production and nearly single-component fermentation of a stable analog (thailanstatin A), which has enabled pre-clinical development of this class of natural products as chemotherapy[5].

References
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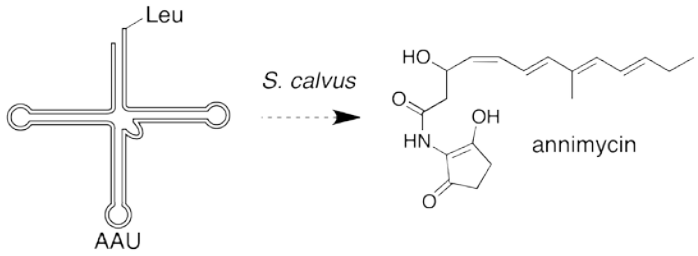
(3) Liu, X. *et al.* (2013) *J Nat Prod 76*, 685-693.
(4) Eustáquio, A. S. *et al.* (2014) *Proc Natl Acad Sci U S A 111*, E3376-E3385.
(5) Dirico, K. J. *et al.* (2014) Patent application WO 2014068443.

A CURE FOR BALDNESS AND CRYPTIC BIOSYNTHESIS IN *STREPTOMYCES CALVUS*

David L. Zechel
Department of Chemistry, Queen's University, Chernoff Hall, 90 Bader Lane, Kingston, Ontario, K7L 3N6, Canada

Streptomyces calvus was first isolated by American Cyanamid in 1956 based on its ability to produce nucleocidin, a mimic of adenosine that contains unusual fluorine and sulfamate substituents. *S. calvus* also differs from most *Streptomyces* in its inability to form spores on solid media, giving colonies a 'bald' appearance. The genome sequence of *S. calvus* revealed the presence of a point mutation in the *bldA* gene, which is predicted to encode a misfolded and nonfunctional Leu-tRNA^{UUA} molecule. The *bldA* gene is well known to play a key role in sporulation and the expression of biosynthetic genes in *Streptomyces*. Upon complementation of *S. calvus* with a functional copy of the *bldA* gene, sporulation was restored and new secondary metabolites were observed in culture extracts. Recent work in identifying these new secondary metabolites and their cryptic biosynthetic genes will be presented.



DISCOVERY OF NOVEL CHLORINATED ACYL AMIDES FROM A MARINE CYANOBACTERIUM USING INTEGRATED TECHNOLOGIES

Karin Kleigrewe[1], Jehad Almaliti[1], Isaac Yuheng Tian[1,2], Robin B. Kinnel[3], Anton Korobeynikov[4,5,6], Emily A. Monroe[1,7], Brendan M. Duggan[10], Vincenzo Di Marzo[8], David H. Sherman[9], Pieter C. Dorrestein[10], Lena Gerwick[1] and William H. Gerwick[1,10]
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An innovative approach was developed for the discovery of new natural products by combining mass spectrometric metabolic profiling with genomic analysis, and resulted in the discovery of the columbamides, a new class of di- and tri-chlorinated acyl amides with cannabinomimetic activity. By genome analysis, a presumed regulatory domain was identified upstream of several previously described biosynthetic gene clusters in two cyanobacteria, and a similar regulatory domain was identified in the *M. bouillonii* PNG genome. A corresponding downstream biosynthetic gene cluster was located and carefully analyzed. Subsequently, MS-based molecular networking identified a series of candidate products, and these were isolated and their structures rigorously established.

Session 3 - Natural Products and HIV, Progress and Directions

MINING THE EXTENSIVE CHEMICAL DIVERSITY OF THE NCI NATURAL PRODUCTS REPOSITORY FOR NEW AGENTS THAT CAN TARGET HIV

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Extracts from a wide variety of terrestrial plants, marine organisms, and microbial isolates have been screened for anti-HIV properties using a cell-based, infectious virus assay format. This type of phenotypic screening can identify inhibitory samples that target virtually any step in the virus infection or replication process. Assay-guided fractionation of active extracts has provided an array of novel, HIV inhibitory compounds that span many different structural classes. These discoveries include new small molecule inhibitors such as alkaloids, terpenoids, quinones, and coumarins, as well as some larger anti-HIV peptides. Biological characterization of lead compounds revealed these metabolites can impact critical processes such as virus entry into host cells, membrane integrity, or reverse transcription of viral RNA. Several of these agents have undergone extensive preclinical evaluations and one has advanced to a Phase II clinical study. The combination of a large, diverse library of natural product samples and a new antiviral screening platform, has provided a valuable resource for discovery of novel HIV inhibitors.

INGENOL 3,20 DIBENZOATE EFFICIENTLY REACTIVATES LATENT HIV

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The HIV-1 latent reservoir represents a major barrier to viral eradication in aviremic HIV-1$^+$ patients taking antiretroviral therapy (ART). Small compounds and drugs capable of latency reversal that are active in vivo are scarce. Here, we describe a careful characterization of the promising reactivation properties of an emerging candidate, ingenol 3,20 dibenzoate (IDB), in cells from aviremic patients, including the effects on cellular activation. Ingenol, is a protein kinase C (PKC) agonist found in *Euphorbiacea*, a family of succulent plants from semi-desertic areas of Brazil.

To be able to test candidate drugs in cells from HIV infected, aviremic patients, we formulated a rapid *ex vivo* assay as follows. After phlebotomy, resting CD4$^+$ T cells are isolated via negative magnetic bead purification and cultured in aliquots of 5x10^6 cells/mL RPMI-based culture medium. Cell aliquots are exposed to medium alone (negative control), candidate drug, or antibodies against CD3/CD28 to induce T cell receptor stimulation (positive control). After 48 hours in culture, quantitative rtPCR is performed using culture supernatant HIV-1 viral RNA.

IDB, demonstrated viral reactivation comparable to CD3/28 antibody stimulation (median reactivation = 49% of positive control). CD69, an early marker of T cell activation known to be up-regulated by ingenol, increased in all cell aliquots exposed to ingenol (median florescence intensity = 81% of positive control).

Ingenol represents an exciting latency-reversing agent because it combines a potent reactivation ability with a very low toxicity profile, which sets it apart from other PKC agonists.

MEDICINAL PLANTS USED BY TRADITIONAL MEDICINE PRACTITIONERS FOR THE TREATMENT OF HIV/AIDS AND RELATED CONDITIONS IN PAPUA NEW GUINEA

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Papua New Guinea (PNG) has the highest burden of HIV/AIDS in the Pacific region. An estimated 33,000 people in PNG are currently living with HIV and Aids. Since the beginning of the AIDS epidemic, patients have consulted both medical doctors and traditional medicine practitioners (TMPs) for all kinds of physical and emotional ills. Access to antiretroviral (ARV) drugs has been irregular, and is virtually non-existent in the rural areas of PNG. Moreover, resistance of opportunistic microbial pathogens to conventional medicines and the side effects associated with antiretroviral drugs are also a major drawback to the management of HIV/AIDS in the country. Due to these factors, many people opt to use of traditional medicines. Traditional medicine practitioners (TMPs) use range of botanicals in the management of different opportunistic infections in people living with HIV/AIDS (PLHIV), namely antithrush, antifever, antidiarrhoea and antidysentery, anticough and anti-infective against various skin pathogens. In a pilot study conducted in the Oro and Milne Bay provinces of PNG it was established that traditional healers were indeed playing an important role in providing medical care and in alleviating suffering of PLHIV. Their main contribution was in improving quality of life by effectively treating many of the associated symptoms and helping patients regain energy and appetite. Conditions such as diarrhoea, skin infections, weight loss, sores and wounds were treated by selective administration of different herbal

preparations. In this study, an ethnobotanical survey was conducted to record the various plant families, species, and plant parts used to manage different HIV/AIDS-related opportunistic infections.This presentation examines and provides an account of most commonly used herbs by TMPs in management of HIV/AIDS in PNG.

NEWS FROM FUNGI - TARGETING VIRAL SWEETSPOTS

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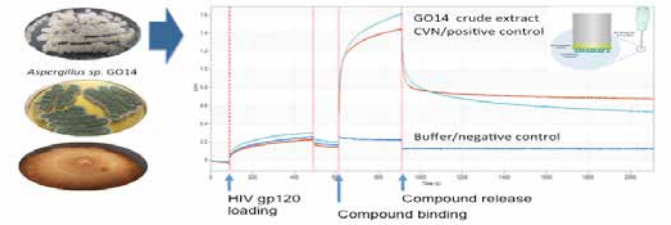
As we enter the post-antibiotic era, there is an increasing demand for new anti-infective compounds. To address this need, we employed a two-pronged drug discovery approach to identify anti-viral compounds from fungal extracts. To increase the probability of isolating unknown compounds, we activated cryptic secondary gene clusters by environmental challenge, epigenetic modifiers, and gene manipulation. To increase throughput, we screened extracts for binding to the HIV viral envelope protein gp120 using a rapid protein-based biosensor assay supported by an *in vitro* cell assay. Here we present the fungal metabolites that were identified using this novel screening approach.



A RUFOMYCIN ANALOGUE IS AN ANTI-TUBERCULOSIS DRUG LEAD TARGETING CLPC1 WITH NO CROSS RESISTANCE TO ECUMICIN

Mary Choules[1,2], Yang Yu[2], Sang-Hyun Cho[1], Jeff Anderson[1], Wei Gao[1,2], Larry Klein[1], David C. Lankin[2], Jin-Yong Kim[4], Jinhua Cheng[3], Seung Hwan Yang[3], Hanki Lee[3], Joo-Won Suh[3,4], Scott G. Franzblau[1], and Guido F. Pauli[1,2]
[1]*Institute for Tuberculosis Research and* [2]*Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL, 60612, USA;* [3]*Center for Nutraceutical and Pharmaceutical Materials;* [4]*Division of Bioscience and Bioinformatics, College of Natural Science, Myongji University, Cheoin-gu, Gyeonggi-Do 449-728, Korea*

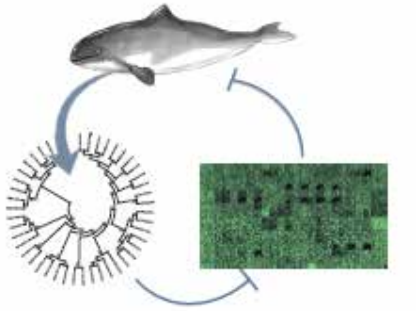
Our recent efforts at finding new anti-tuberculosis drug leads from actinomycetes have led to the discovery of ecumicin (ECU, MW 1599.22, MIC 0.16 uM) and; more recently, a rufomycin analogue (RUF-I, MW 1041.55, MIC 0.019 uM). Although both of these cyclic peptides target the currently unexploited chaperone protein ClpC1 ATPase complex, there is no observed cross resistance. In this study, the structures of RUF-I and other rufomycin analogues, heptapeptides, were elucidated by ^{1}H, ^{13}C, 2D NMR, MS and HiFSA. To understand the lack of cross resistance, *clpC1* from spontaneously generated resistant clones from both leads were sequenced. This revealed several distinct single point mutations, suggesting these compounds bind differently to ClpC1. Checkerboard assay indicated a lack of antagonism between ECU and RUF-I and potential synergistic effects. Preliminary data indicates that ClpC1 could be a multifaceted drug target for multidrug resistant *M. tuberculosis.* Research pertaining to the peptide drug leads, associated analogues, and their mechanism of action against ClpC1 will be presented.

Session 4 - ASP Younger Members Research Spotlight

PHYLOGENETIC AND METABOLOMIC ANALYSIS OF MARINE MAMMAL MICROBES CONTRIBUTES TO EMERGING SPIROTETRONATE POLYKETIDES

Jessica Ochoa, Laura Sanchez, Roger Linington
University of California, Santa Cruz, Department of Chemistry and Biochemistry, 1156 High Street, Santa Cruz, CA, 95060

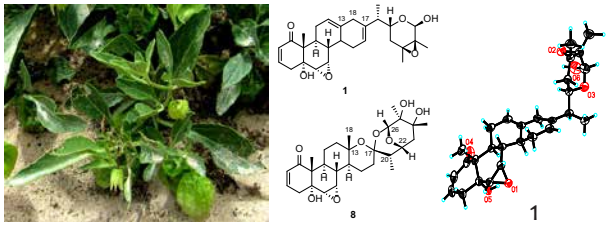
Constant exposure to varying environments along migrations routes suggests that the marine mammal microbiome may be a unique environment for sample collections for natural product discovery. The organisms isolated in this study were prioritized through sequencing and metabolomics profiling to create a highly bioactive screening plate that possesses new molecules produced by marine microbiomes. Molecules elucidated in this study, both known and new provide detailed insight into the chemistry involved in signaling and/or defense within commensal bacteria as well as the capability of these molecules to be used to regulate pathogenesis for one of the worlds best sentinel organisms.



UNUSUAL WITHANOLIDES FROM *PHYSALIS HISPIDA* (WATERF.) CRONQUIST

Cong-Mei Cao[1], Huaping Zhang[1], Robert J. Gallagher[1], and Barbara N. Timmermann*[,1]
[1]*Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045, United States*

Withanolides are a group of modified C_{28} ergostane-type steroids with a C-22, C-26 δ-lactone side chain. As part of our continuing search for unusual withanolides for SAR studies, we isolated and characterized nine new withanolides (**1**–**9**), withahisolides A–I, as well as nine known compounds (**10**–**18**) from the aerial parts of *Physalis hispida*. The structures of **1**–**9** were elucidated through a variety of spectroscopic techniques, while those of **1** and **2** were further confirmed by X-ray crystallographic analysis. Among the eight new withanolides (**1**-**7**, **9**) with an unusual six-membered ring D, **1**–**3** are the first withanolides possessing non-aromatic six-membered ring D moieties. In addition, withanolide **8** represents a novel withanolide skeleton due to the absence of a C-13–C-17 bond within its steroidal nucleus.



DISCOVERY OF FATTY ACID SYNTHASE INHIBITORS AND THEIR BIOSYNTHETIC PATHWAYS BY A NOVEL TARGET-DIRECTED GENOME MINING STRATEGY

Jie Li[1], Xiaoyu Tang[1], Jia Jia Zhang[1], Ellis C. O'Neill[1], Simone M. Mantovani[1] and Bradley S. Moore[1,2]
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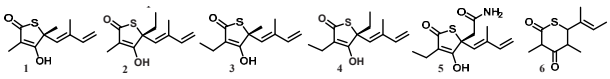
In order to avoid self-toxicity, many antibiotic-producing microbes have developed self-resistance mechanisms, with target modification being one frequent example. We propose that extra copies of an essential housekeeping gene found within or adjacent to a biosynthetic pathway may indicate that the compound(s) produced will target the protein encoded by the corresponding housekeeping gene. Thus, a novel and rational target-directed genome mining strategy was developed, by which potential self-resistance genes were analyzed bioinformatically, followed by a PCR-independent cloning and heterologous expression of the intact gene clusters for rapid production of compounds with desired bioactivities. As a proof-of-principle study, two related orphan gene clusters with potential *fabF* resistance genes were identified and expressed, which led to the isolation of a group of unique thiotetronic acid natural products that inhibit bacterial fatty acid synthase (**1**–**9**). A notable advantage of this genome mining strategy is that specific molecular targets can be hypothesized in the absence of priori knowledge of the structures of the molecules biosynthesized, and may streamline mechanism of action studies for the products obtained.



Compounds **7**–**9** are new with structures not shown here

DISCOVERY, SYNTHESIS, AND BIOLOGICAL EVALUATION OF APRATYRAMIDE, A MARINE-DERIVED TRANSCRIPTIONAL STIMULATOR OF VEGF-A

Weijing Cai[1,2], Lilibeth A. Salvador-Reyes[1], Wei Zhang[1], Susan Matthew[1], Ranjala Ratnayake[1,2], Valerie J. Paul[3], Long H. Dang[2,4], Hendrik Luesch[1,2]
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A collection of the marine cyanobacterium *Moorea bouillonii* from Apra Harbor in Guam afforded apratyramide, a linear depsipeptide consisting of four amino acid residues and one hydroxy acid moiety. The structure was elucidated by a combination 1D/2D NMR spectroscopic and mass spectrometric analysis. The absolute configuration of the stereocenters was determined by LC-MS analysis of the acid hydrolyzate. Apratyramide was then synthesized and tested for bioactivity. Apratyramide induced the transcription and secretion of vascular endothelial growth factor A (VEGF-A) in multiple cell types. This activity is being explored for various applications where VEGF-A upregulation would be beneficial.

CHEMOMETRIC-DIRECTED BIOEXPLORATION OF NATURAL PRODUCTS

Joshua Kellogg[1], Daniel A. Todd[1], Joseph M. Egan[1], Huzefa A. Raja[1], Nicholas H. Oberlies[1], Olav M. Kvalheim[2], and Nadja B. Cech[1]
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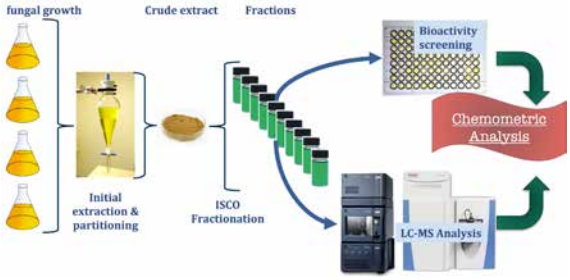
A workflow for the chemometric analysis of a chromatographic fractionation series was developed, integrating partial least-square (PLS) statistical modeling with high-resolution mass spectrometry to yield an associative model that correlated chemical composition and bioactivity. Goldenseal (*Hydrastis canadensis*) fungal endophytes were evaluated to determine their secondary metabolite profile and their influence on growth of *Staphylococcus aureus* strain SA1199. *Pyrenocheata* sp. extract and fractions inhibited the growth of SA1199 as much as 95.8 ± 1.7%, and the resulting PLS model predicted a single active ion (*m/z* 343) as the purported bioactive component. Follow-up analysis tentatively identified the *m/z* 343 ion as the known antimicrobial macrosphelide A.



PHARMACOKINETIC INTERACTIONS BETWEEN DRUGS AND BOTANICAL DIETARY SUPPLEMENTS

Alyssa A. Sprouse[1,2] and Richard B. van Breemen[1,2]
[1] Department of Medicinal Chemistry and Pharmacognosy, [2] UIC/NIH Center for Botanical Dietary Supplements Research University of Illinois at Chicago, Chicago, IL

The use of botanical dietary supplements has grown steadily over the last 20 years despite incomplete information regarding active constituents, mechanisms of action, efficacy, and safety. An important but under-investigated safety concern is the potential for popular botanical dietary supplements to interfere with the absorption, transport and/or metabolism of pharmaceutical agents. Clinical trials of drug-botanical interactions are the gold standard and are usually carried out only when indicated by unexpected consumer side effects or, preferably, by predictive in vitro studies. For example, Phase I clinical trials have confirmed clinical observations and in vitro studies that St. John's wort (*Hypericum perforatum*) induces cytochrome P450 3A4/5, whereas Phase I studies did not substantiate in vitro predictions that milk thistle (*Silybum marianum*) would inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. Here, we highlight discrepancies between in vitro and in vivo data concerning drug-botanical interactions and critically evaluate why some in vitro models overestimate or sometimes underestimate the potential for drug-botanical interactions. Gaps in our knowledge are also highlighted for the potential of some popular botanical dietary supplements to interact with therapeutic agents with respect to absorption, transport and metabolism.

Supported by grant P50 AT000155 from the NIH ODS and NCCIH

SELECTIVITY AND MECHANISTIC INQUIRIES INTO THE REACTIVITY OF PERICOSINE A, AN ELECTROPHILIC CHLORINATED SHIKIMATE ANALOGUE

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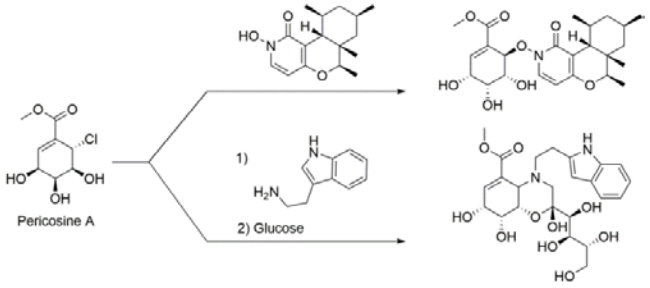
Natural Products Discovery Group, Institute for Natural Products Applications and Research Technologies, University of Oklahoma, Department of Chemistry and Biochemistry, University of Oklahoma, Norman OK 73019

Microorganisms are constantly competing for limited resources in their environment as characterized by secondary metabolite production and enzymatic inactivation of toxins. Pericosine A, a shikimate analogue produced by a *Tolypocladium* sp., has been shown to directly differentiate and inactivate nucleophilic toxins in complex chemical environments. Herein we detail the selectivity of pericosine A towards a diverse set of nucleophiles, as well as the computational and structural studies of the mechanistic

pathways under which it operates. We also show reactivity of pericosine A adducts with monosaccharides to produce novel heterobicyclic scaffolds.



ISOLATION AND IDENTIFICATION OF NOVEL NATURAL PRODUCTS THAT INHIBIT P300/HIF-1α INTERACTION

Susanna T. S. Chan,[1] Paresma R. Patel,[2,3] Gary E. Martin,[4] Robert T. Williamson,[4] Josep Saurí,[4] Alexei V. Buevich,[4] Tanya R. Ransom,[1] Curtis J. Henrich,[1,5] Tawnya C. McKee,[1] William D. Figg,[6] James B. McMahon,[1] Martin J. Schnermann,[2] and Kirk R. Gustafson[1]

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Hypoxia-inducible factor-1 (HIF-1) is an important transcription factor for initiating a response against low oxygen environments in many solid tumors. Under hypoxic conditions, subunit HIF-1α dimerizes with subunit HIF-1β and binds to the multi-domain protein and transcriptional coactivator, p300. Inhibition of the p300/HIF-1α interaction results in the suppression of HIF-1 transcriptional activity during hypoxia. An extract of the marine ascidian *Eudistoma* sp. was identified as active in a high throughput screen for inhibitors of the p300/HIF-1α interaction. Three novel heterocyclic alkaloids were isolated from the extract and their structures elucidated using both spectroscopic analyses and synthesis. The core scaffold of these alkaloids contains an unprecedented fused ring system with embedded guanidine and amidine functionalities. These compounds showed activity inhibiting the binding domains of p300 and HIF-1α.

TARGETING BIOACTIVE CHEMICAL SPACE WITH A SMALL NATURAL PRODUCTS LIBRARY: EXPANDING DIVERSITY AND PREDICTABILITY

Jacqueline L. von Salm[1,2], Daniel Santiago[1], Nerida G. Wilson[3], Laurent Calcul[1,2], Dennis E. Kyle[4], Wayne C. Guida[1,2,5], and Bill J. Baker[1,2]

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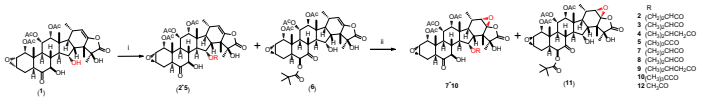
Diverse compound libraries have become a necessity to drug discovery efforts around the world. Target specific computational methods and high-throughput screening programs have aided the search for bioactive compounds, however, inefficiencies remain for neglected and tropical diseases. Detailed knowledge of mechanisms of action and target proteins is limited for parasitic diseases like malaria and leishmaniasis. In order to effectively lead research programs in these areas, our lab has developed a small compound library with diversity that mimics the NCI Diversity Set and the AntiMarin natural products database. Specific activity exhibited by each compound against *Plasmodium falciparum* and *Leishmania donovani* has been overlain to create activity "hotspots", which we hope to improve activity prediction methods for unknown natural products isolated in the future. Limitations in diversity remain in all three libraries, and appear to have properties resembling small (MW < 400) hydrophobic molecules like terpenes. Here we use active antileishmanial terpenoids isolated from Antarctic marine organisms as models to show expansion of overall diversity and future predictability of the library as a bioassay dereplication tool.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW BULKY ACYLOXY TACCALONOLIDES AS POTENT MICROTUBULE STABILIZERS

Antonius R. B. Ola,[1] April L. Risinger,[1,2] Jiangnan Peng,[1] Cynthia L Zamiello,[1] and Susan L. Mooberry[1,2]

[1]Department of Pharmacology, [2]Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, Texas 78229, USA

The taccalonolides are a new class of microtubule stabilizers isolated from plants of the genus *Tacca*. As part of our structure-activity relationship study (SAR) to identify taccalonolides with optimal microtubule stabilizing and antitumor actions, we conducted semi-synthetic reactions to modify C-15 of taccalonolide B (**1**). In this study, five novel bulky acyloxy taccalonolides were synthesized via DMAP catalyzed esterification and DMDO epoxidation. The new bulky acyloxy taccalonolide mono esters were further tested for their antiproliferative potency against HeLa cells. Remarkably, all bulky acyloxy taccalonolide esters demonstrated better potency than taccalonolide AF (**12**), including two with subnanomolar potencies (IC_{50} of 0.6 nM for **11** and 0.8 nM for **9**). *In vivo* antitumor activities were also evaluated in a murine xenograft model.



Session 5 - The Evolving Role of Natural Products in Neuroscience

NATURAL PRODUCT INSPIRED HSP90 INHIBITORS

Blagg, B.S.J.

Department of Medicinal Chemistry, The University of Kansas.

Natural products continue to play a key role in drug discovery, an example of which is highlighted by Hsp90 inhibitors. The 90 kDa heat shock proteins (Hsp90) are responsible for the conformational maturation of nascent polypeptides, many of which are critical to the maintenance of cell signaling networks that contribute to the six hallmarks of cancer. Thus, through Hsp90 inhibition, one can simultaneously derail multiple signaling networks through inhibition of a single biological target. The natural products, geldanamycin and radicicol, have served as lead compounds to develop multiple inhibitors that have entered clinical trials for the treatment of cancer that manifest their activity through inhibition of the Hsp90 N-terminal ATPase domain. In contrast, the natural products, novobiocin, EGCG, and silybin have served as lead compounds that regulate Hsp90 protein folding activity through allosteric modulation of the chaperone via the C-terminal dimerization domain. In addition, the natural products, cruentaren A, celastrol and gedunin, have shown to provide a unique opportunity to modulate Hsp90 activity through disruption of co-chaperone interactions with Hsp90. In this presentation, the utilization of natural products to modulate the Hsp90 protein folding machinery will be discussed along with their potential therapeutic applications.

ADVANCES IN THE VALIDATION OF TRPM7 AS A DRUG TARGET USING NATURAL PRODUCTS – UPDATE ON WAIXENICIN A

F. David Horgen
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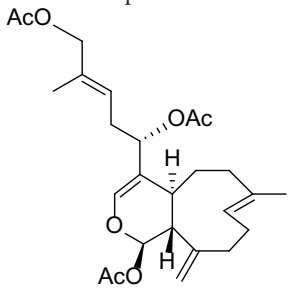
TRPM7 is a ubiquitously expressed divalent cation channel that plays an important role in cell adhesion and migration. TRPM7-dependence on the growth and metastasis of some cancers points to TRPM7 as a potential anti-cancer target. Conversely, in brain ischemia models, dysfunctional activity and up regulation of TRPM7 sustains Ca^{2+} overload in neurons leading to cell death, which implicates TRPM7 inhibition as a potential target for stroke therapy. Motivated by the lack of selective inhibitors, we developed and optimization of a high throughput bioassay (*J. Biomol. Screen.* **2010**, *15*, 498-507) that led to the isolation of waixenicin A, a diterpene from *Sarcothelia edmondsoni*, as a selective and potent inhibitor of TRPM7. In patch clamp experiments, waixenicin A demonstrated Mg^{2+}-dependent inhibition of TRPM7 currents in native cells (*J. Biol. Chem.* **2011**, *286*, 39328), while further chemical investigation of the soft coral provided active and inactive analogues that revealed a preliminary SAR.



Since this recent work, waixenicin A has been employed in numerous on-going *in vitro* and *in vivo* studies aimed at better understanding the functions of TRPM7, particularly in the context of cancer. Most notably, collaborators at the Netherland Cancer Institute (*Cell Calcium* **2013**, *54*, 404) demonstrated that inhibition of TRPM7 by waixenicin A disrupts invadosomes in neuroblastoma cells, a mechanism that appears consistent with the prognosis of TRPM7 expression for breast cancer metastasis and recurrence. Future investigations will seek to assess TRPM7 inhibition in animal tumor and ischemia models. The development of waixenicin A as a successful probe for studying TRPM7's biological functions further demonstrates the value of natural product pharmacophores in advancing new targets for the treatment of disease.

DEVELOPMENT OF NOVEL NEUROACTIVE STEROIDS FOR THE POTENTIAL TREATMENT OF NEUROLOGICAL DISORDERS

Scott Runyon[1], Michael Rogawski[2], Kelvin Gee[3], Nicholas Goeders[4], and Christopher Schmoutz[4]
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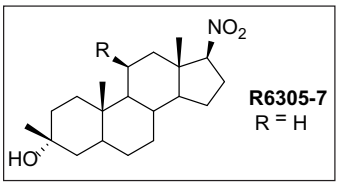

Neuroactive steroids (NS) are defined as steroids active in the central nervous system (CNS). NS were initially identified as metabolites of the hormone progesterone (P) and have been subsequently shown to be potent positive allosteric modulators (PAMS) of extrasynaptic $GABA_A$ receptors. The most potent endogenous NS's at $GABA_A$R's are allopregnanolone (AP) and 5α-THDOC; the principal metabolites of the hormones progesterone and deoxycorticosterone respectively. Considering the physiological importance of AP and THDOC, our laboratory has begun to develop subtype selective and more drug-like analogs of endogenous steroids that have improved potency and reduced hormonal metabolism. Our studies have led to the development of a series of androstane analogs that possess a nitro in the 17-position combined with a 3β-methyl group and a alternate substitutions at the 11β-position. The synthesis of these analogs and potency at select $GABA_A$ subtypes along with the resulting pharmacokinetic properties will be discussed in addition to the in vivo data from anticonvulsant and substance abuse studies.

PANAMANIAN MARINE CYANOBACTERIAL EXTRACTS WITH IN VIVO ACTIVITY IN MODELS OF ANXIETY AND DEPRESSION

Kevin Tidgewell[1,3], Kh. Tanvir Ahmed[1,3], Neil Lax[2,3], and Benedict Kolber[2,3]
[1]Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Mylan School of Pharmacy, [2]Department of Biological Sciences, Bayer School of Natural and Environmental Sciences, [3]Chronic Pain Research Consortium, Duquesne University, Pittsburgh, PA 15282

In the search for compounds with central nervous system (CNS) effects, the oceans are emerging as a largely unexplored source. A number of interesting neurotoxins and ion channel modulators have been discovered from a variety of marine sources. The focus of this research was to screen and identify compounds which could modulate CNS diseases through activity at G-protein coupled receptors (GPCRs). As part of the Panama ICBG and in collaboration with the NIMH psychoactive drug screening program (PDSP) we have screened cyanobacterial fractions against a panel of ~48 GPCRs and transporters involved in CNS disorders. From these initial screening efforts, a number of selective and potent hits are currently being explored to isolate and determine the active components. 2 fractions in particular have been explored because of their ability to bind to the $5\text{-}HT_{2c}$ and $5\text{-}HT_7$ receptors respectively. These fractions and some purified materials have been screened *in vivo* using behavioral models of depression and anxiety in C57Bl/6J mice via intra-cerebroventricular (ICV) injection. Both fractions have shown statistically significant alteration in behavior from single ICV injections. Results and progress to date will be presented.

DISCOVERY OF NEUROPROTECTIVE MARINE NATURAL PRODUCTS USING A BIOIMAGING/ OPTOGENETICS APPROACH AND *DROSOPHILA MELANOGASTER*

Stacee Lee Caplan[1], Ken Dawson-Scully[1], and Lyndon M. West[2]
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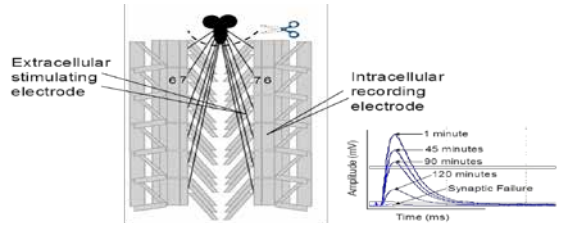
Oxidative stress and cellular excitotoxicity is inherent in the pathophysiology of an array of devastating human ailments. Natural products have played an important role in the treatment of a variety of human diseases and continue to drive the modern drug discovery and development process. The overall goal of this project is to design a novel neuroactivity assay to identify natural products capable of inducing neuroprotection against oxidative stress. This project will focus on a group of natural products, pseudopterosins, known for their potent biological activities with a novel mechanism of action. We hypothesize that these compounds may be of potential therapeutic benefit in protecting neurons and/or neural function both during and after episodes of excitotoxicity in disease states.



Session 6 - Recent Advances in Chemical Ecology

FUNCTIONAL AND BIOSYNTHETIC ANALYSES OF SECONDARY METABOLITES IN HOST-MICROBE SYMBIOSES

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Most if not all animals form benign and/or beneficial relationships with microorganisms. The importance of studying co-evolved beneficial relationships in nature is now being realized (e.g., the recent large-scale effort to sequence the human microbiome). Our lab has been utilizing several well-studied host-microbe systems as unique sources of new biologically active drug leads. We have employed various analytical techniques to interrogate these relationships including molecular networking, screening for biologically relevant activity (e.g., using antimicrobial, anti-cancer, and immunomodulatory assays), as well as isolating and identifying bioactive metabolites. In addition, we have begun characterization of the genomes, metagenomes, and transcriptomes from these host-microbe associations to better understand the biosynthetic capacity of these systems. Recent developments from our chemical ecology investigations will be presented.

INTEGRATED *DE NOVO* METAGENOMICS AND METATRANSCRIPTOMICS TO STUDY NATURAL PRODUCTS AND MICROBIAL ECOLOGY *IN SITU*

Ian J. Miller[1]*, Theodore Weyna[1]*, Christine Mlot[1], Stephen S. Fong[2], Kerry McPhail[3], Grace Lim-Fong[4], Jason C. Kwan[1]
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Biosynthetic pathways that make secondary metabolites are widespread amongst bacteria, and the resulting small molecules presumably confer a selective advantage to producers. Because bacteria live in complex mixed communities in the environment and in association with higher host organisms, a variety of functions have been suggested for secondary metabolites. These include the suppression of competitors, modulation of behavior or communication, as well as functions that align with the interests of a eukaryotic host. Discerning the roles of small molecules in the environment may uncover therapeutically relevant bioactivities, but natural bacterial communities are hard to study as most environmental bacteria are recalcitrant to culture. These uncultured communities can be accessed directly through sequencing of environmental DNA (metagenomics) or RNA (metatranscriptomics). Methods to deconvolute and assemble the genomes of multiple bacteria from metagenomes will be presented, with results from two model systems, the tunicate *Lissoclinum* sp. and the bryozoan *Bugula neritina.* In the latter system we have also integrated metatranscriptomics to give a snapshot of the global gene expression in a complex microbial assemblage.

BIOSYNTHESIS AND ECOLOGICAL FUNCTIONS OF DIKETOPIPERAZINE NATURAL PRODUCTS FROM MARINE ACTINOMYCETES

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Molecules featuring 2,5-diketopiperazine (DKP) scaffolds are common in Nature and have been isolated from a variety of fungi and bacteria. These cyclodipeptides offer a range of medicinal activities, including antimicrobial, anticancer, and immunosuppressant effects. Cyclodipeptide synthases (CDPSs) were recently revealed as a novel family of small (~30 kDa) enzymes that assemble DKPs from aminoacyl-tRNA substrates diverted from primary metabolic pathways. Although bioinformatics analyses of microbial genomes suggest that CDPSs are widespread amongst terrestrial and marine bacteria, fewer than a dozen CDPSs have been biochemically characterized. Here, we present the biochemical characterization of two CDPSs from a marine-derived *Nocardiopsis* sp. actinomycete. Our data reveal that both of these phylogenetically distinct CDPSs are highly selective for tryptophan-charged tRNA substrates to yield exclusively *cyclo*(L-Trp-L-Trp). We also present results from field and laboratory experiments probing *cyclo*(L-Trp-L-Trp) and other microbial DKPs as mediators of interactions between marine microorganisms. Together, our data provide insights into the prevalent, yet understudied CDPS enzyme family and suggest important adaptive functions of corresponding DKP metabolites.

MECHANISMS OF MICROBE-MICROBE-HOST INTERACTIONS IN A PROBIONT-PATHOGEN-BIVALVE MODEL

David Rowley[1], Wenjing Zhao[2], Tao Yuan[1], Christine Dao[1], Saebom Sohn[3], Marta Gomez Chiarri[3], Navindra Seeram[1], and David R. Nelson[2]
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Marine mollusks are filter feeders and thus are continuously exposed to a myriad of microorganisms. While the majority of bacteria likely have no effect on the overall health of the animal, some microbes, including members of the bacteria genus *Vibrio*, can cause infections that lead to rapid mortality, especially in larval and juvenile bivalves. For example, the oyster pathogen *Vibrio tubiashii* causes sporadic crashes of larval oyster production in commercial hatcheries. Alternatively, it is also clear that certain beneficial microorganisms can colonize mollusks and provide disease resistance. Such microbes can potentially be developed as probiotic agents to combat disease outbreaks in bivalve hatcheries. To enable the rational development of such microbes as probiotics, a more comprehensive knowledge of the mechanisms involved in microbe-microbe-host interactions is required. In this study, we investigated how the bacterium *Phaeobacter gallaeciensis* S4Sm reduces mortality of larval oysters when challenged with *V. tubiashii.* A combination of analytical chemistry, genetic mutation, and both *in vitro* and *in vivo* challenge assays were used to interrogate the roles of specific bacterial metabolites and also bacterial behaviors such as biofilm formation. Our results show that the probiotic activity of S4Sm is multifactorial, involving its ability to form extensive biofilms, production of the potent antibiotic tropodithietic acid, and quorum quenching of pathogen virulence genes.

MACROALGAE MAY INTERRUPT IMPORTANT CUES FOR CORAL LARVAL SETTLEMENT

Jennifer M. Sneed, Sarah J. Harrison, Lawrence J. Houk, Valerie J. Paul
Smithsonian Marine Station at Fort Pierce, Florida, USA

Coral reefs are becoming increasingly dominated by fleshy macroalgae. Recovery of reefs is dependent on the recruitment of new corals and the presence of certain macroalgae impedes the settlement of coral larvae di-

rectly through physical and chemical competition. However, there may be a third impediment to the successful recruitment of coral larvae on algal-dominated reefs, namely the interruption of bacterially produced settlement cues. For some corals, the settlement process appears to be dependent on the presence of chemical cues produced by biofilm bacteria. To determine if macroalgae impact bacterial communities on settlement substrata, we examined the effects of two species of macroalgae commonly found on Caribbean reefs (*Halimeda opuntia and Dictyota* sp.) on the bacterial communities associated with the crustose coralline alga (CCA) *Hydrolithon boergesenii*. We attached either a live clump of algae or a fake aquarium plant to CCA pieces and placed them in individual flow-through chambers (n = 5) on the reef. After 48 hours, we sampled the biofilm communities on the CCA and the algae and analyzed them using next-generation sequencing. Both algae caused a shift in the bacterial community found on the surface of the CCA; however *H. opuntia* had the greatest impact. Organic extracts of *H. opuntia* affected growth of bacterial strains isolated from the surface of *H. boergesenii* in laboratory assays and the compound halimedatetraacetate demonstrated antibiotic activity against several strains. This study demonstrates that macroalgae can alter biofilm bacterial communities, some of which may provide chemical cues necessary for coral larval settlement.

Session 7 - Natural Products and the Human Microbiota

Sponsored by Procter & Gamble, Co. **&** Takeda Pharmaceuticals International, Inc.

GUT REACTIONS: NATURAL PRODUCTS AND THE HUMAN MICROBIOTA

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Humans are colonized by vast numbers of microorganisms whose metabolisms are inextricably intertwined with our own. Advances in DNA sequencing over the past twenty years have sparked a revolution in our understanding of the human microbiota and its functional capabilities, causing us to rethink our very notion of our chemical selves. The chemical reactions performed by our microbial companions can be distinct from those of the human host, and there is growing evidence that the products of microbial metabolism may influence both human health and disease. My research seeks to discover and understand enzymes, metabolic pathways, and metabolites from the human gut microbiota. We study reactions involved in transforming natural product-based drugs and dietary components, as well as the chemistry of secondary metabolite biosynthesis. This talk will highlight work from my lab and others that demonstrates how a molecular understanding of gut microbial metabolism can help to reveal the abundance of important functions in these communities and guide strategies for manipulating these activities.

FOOD, DRUGS, AND BUGS: A METAGENOMIC VIEW OF PHARMACOLOGY

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Our gastrointestinal tracts harbor complex microbial communities (the gut microbiome) that are capable of a vast array of enzymatic activities, contributing to the metabolism of our diet and the drugs we take. Yet the molecular mechanisms responsible often remain unknown, making it challenging to translate these findings to new therapies and diagnostics, or to appreciate their broader biological, ecological, and evolutionary implications. We are studying the interactions between gut microbes and xenobiotics, including host-targeted drugs (*e.g.*, the cardiac drug digoxin) and diet-derived bioactive compounds (*e.g.*, polyphenols). I will discuss two ongoing projects in the lab. *First*, we have identified a cytochrome-encoding operon responsible for the bacterial inactivation of digoxin, inhibited by arginine *in vitro* and *in vivo*, absent in nonmetabolizing *E. lenta* strains, and predictive of digoxin inactivation by the human gut microbiome. *Second*, we have shown that food-derived compounds reshape the gut microbiota in a manner that may protect against metabolic syndrome. Ultimately, we aim to obtain a more comprehensive view of human metabolism, yielding fundamental insights into host-microbial interactions, and supporting translational efforts to predict and manipulate the metabolic activities of our resident gut microbes.

BIOTRANSFORMATION OF ANTICANCER DRUGS BY HUMAN AND ENVIRONMENTAL MICROBIOMES

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Many anticancer drugs have toxic effects on microbes in addition to human cells. It is therefore not surprising that many microbes have intrinsic resistance mechanisms that can be deployed to counteract anticancer drug toxicity. This is especially apparent for anticancer agents that are synthesized by soil microbes themselves. We have embarked on an effort to explore the resistomes of anticancer agents in the microbiomes of the human gut and in the environment. We have identified many microbes that can detoxify these anticancer drugs via degradation mechanisms. Understanding the molecular basis of these resistance mechanisms is of value in managing drug concentrations during therapy and possibly in avoiding toxicity and in bioremediation.

Session 8 - Natural Product Anticancer Drug Lead Discovery in Honor of Richard G. Powell

Sponsored by the Journal of Natural Products

HISTORICAL ASPECTS OF ANTITUMOR COMPOUNDS FROM PLANTS, INCLUDING HOMOHARRINGTONINE (OMACETAXINE MEPESUCCINATE, SYNRIBO™)

Richard Powell
National Center for Agricultural Utilization Research (retired), Peoria, Illinois. Current address: 4160 Caddie Dr. E., Bradenton, Florida 34203

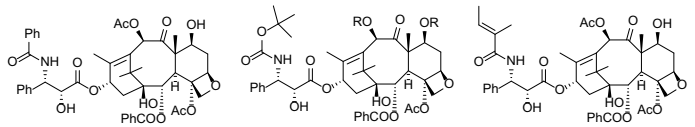
Various preparations derived from vascular plants have been used utilized to treat tumors or cancerous conditions for centuries. The available literature was summarized by Jonathan Hartwell in a remarkable series of papers "Plants Used Against Cancer" published in *Lloydia* from 1967 to 1971. The U.S. National Cancer Institute (NCI) established the Cancer Chemotherapy National Service Center (CCNSC) in 1955. Hartwell transferred to CCNSC in 1957, and under his guidance a major program to discover plant anticancer constituents was developed. Arrangements were made between the NCI and the U.S. Department of Agriculture (USDA) for extensive plant collections in 1960. Many of the collections were from countries that were involved in the Food for Peace Program, Public Law (PL) 480. The Wisconsin Alumni Research Foundation (WARF) was awarded an extraction and screening contract, and research contracts were awarded to Drs. Monroe Wall, Morris Kupchan, Norman Farnsworth, John Cassady and others. When plant seeds were obtained, they were sent to the Northern Regional Research Laboratory (NRRL) in Peoria, Illinois, where they were examined in a program to identify potential new crops. Extracts of many of the seeds

were prepared and screened for antitumor activity and the more significant results obtained from the seed extracts will be summarized.

DEVELOPMENT OF TAXOL® AS AN ANTICANCER DRUG

David G. I. Kingston
Department of Chemistry and Virginia Tech Center for Drug Discovery, Virginia Tech, Blacksburg, VA 24061 USA

The anticancer drug Taxol® (paclitaxel, **1**) and its semisynthetic analogs docetaxel (**2**) and cabazitaxel (**3**) are some of the most effective weapons in the arsenal of drugs for the treatment of breast cancer, ovarian cancer, and various other cancers. The story of how the chemical compound taxol, discovered by Monroe Wall and Mansukh Wani as the major active component of the bark of the Western Yew, *Taxus brevifolia,* became the major drug Taxol® is a fascinating one. It includes the discovery by Richard Powell and colleagues of the closely related compound cephalomannine (**4**), the discovery by Susan Horwitz of its unique mechanism of action, and a summary of the long and tortuous route to clinical approval. Some chemical and biological studies of paclitaxel from the author's laboratory will also be included.



1 Paclitaxel

2 Docetaxel R = H
3 Cabazitaxel R = Me

4 Cephalomannine

NORTH AMERICAN PLANTS REMAIN AN EXCELLENT SOURCE FOR COMPOUNDS WITH ANTICANCER POTENTIAL

Susan L. Mooberry
Department of Pharmacology and Cancer Therapy & Research Center, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229-3900.

One of the most important chemotherapy drugs used clinically today, Taxol®, was discovered from the bark of a North American tree, *Taxus brevifolia*. Our efforts with plants of North America continue to yield new compounds with significant biological activities against cancer cell lines. The use of bioassays as the sole criteria to guide fractionation, combined with modern chemical techniques, allows the rapid identification of these promising molecules. Approaches for discovery, isolation, synthesis as well as biological assay results of 8 new compounds from diverse North American plants including *Amyris texana*, *Verbesina virginica*, *Dalea frutescens* and *Gochnatia hypoleuca* will be discussed. New significant biological activities of known compounds can also be uncovered by these methodologies leading to the potential for discovery of targeted therapies from plants for the treatment of cancer.

ARE NATURAL PRODUCTS ISOLATED FROM PLANTS, PRODUCTS OF EPI- AND/OR ENDO-PHYTIC MICROBIAL INTERACTIONS WITH / WITHIN THE HOST?

David J. Newman[1] and Gordon M. Cragg[2]
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Over the last 15 or so years, there have been multiple reports that "plant-derived" molecules such as paclitaxel, camptothecin, vinca alkaloids, hypericin etc., have been purified from fermented microbes (usually fungal isolates) that were endophytes of the "producing plant". We will discuss these and other reports of the production of molecules as disparate as maytansine and kampferol by microbes isolated from the "producing-plant species". It is tempting to suggest that at this time, we might be at a period in the study of plant metabolites comparable to the one that occurred in the late 1980s with marine invertebrates, when compounds isolated from marine sponges were shown to have structures very similar to those isolated from terrestrial microbes. This work led to the current recognition that a majority of marine invertebrate metabolites are in fact produced by associated microbes, even though in a number of cases, these microbes have not yet been cultivated. Is this now the case with "some" plant-derived compounds?

Session 9 - Bumpy Road to Beauty: Natural Products in the Cosmetic Industry

PAVING THE WAY WITH ACTIVES FOR SKINCARE

Jon Anderson, Young Anderson, and Thushara Diyabalanage
Actives International LLC, Allendale, NJ 07401.

Skin is our body's largest organ and is responsible for many functions including protection from environmental, mechanical, and pathogenic insult, regulation of moisture, sensation, and immune surveillance. The cosmetics and skincare industries focus on the care of healthy or healthy but damaged skin rather than solving disease states. However, contrary to popular opinion, the industry is highly regulated and skincare companies often resort to soft claims to describe the benefits of their products. Nonetheless, extensive research goes into the development of functional ingredients that can impart an observable benefit for the customer. An extensive knowledge of skin biology and a strong background in natural products chemistry are required when developing ingredients for modern skin care products.

During this presentation we will touch on various promising natural products showing unique activities on the skin that will serve by way of example where the skincare industry is trending. Some of the areas of interest range from prevention and repair of photo and chronic aging, reduction of irritation and hyperpigmentation, and skin barrier maintenance.

ERGOTHIONEINE, THE AMAZING AMINO ACID

Chia Wen Chen[1]
[1]Estee Lauder Companies, 125 Pinelawn Road, Melville, NY 11747

Ergothioneine is a naturally occurring amino acid synthesized by microorganism and fungi. Although Ergothioneine cannot be made in human cells, it is present in skin and some tissues at high level as it is absorbed from the diet. The amino acid has good antioxidant properties due to the presence of the thione (C=S) group which is different than other sulfur containing antioxidants. Ergothioneine is called the new vitamin and ergothioneine rich food is often taken for liver and eye health with references for skin health for the reduction of sun damage.

In this presentation, I will share the journey of developing this novel ingredient from concept to cosmetic friendly actives: from sourcing, through in-vitro substantiation, finding the relevance in skin with urban life style and global compliances.

FROM THE BERRY TO THE BARRIER: DEVELOPMENT OF NATURAL PRODUCTS FOR SKIN AND HAIR CARE

James V. Gruber[1]
[1]Sensient Cosmetic Technologies, 107 Wade Avenue, South Plainfield, NJ 07080

The movement of the cosmetic industry away for harsh petroleum-based chemicals and more towards ingredients that originate from natural sources has been accelerating. While many consumers feel that "natural" ingredients are safer and better for the environment, this is not always a truism. Reaching into plants or microorganisms to try and draw out unique ingredients that might have benefits for skin care is not always easy. Often times,

extractions are done using solvents that might themselves not necessarily be considered all that friendly. This talk will examine Sensient Cosmetic Technologies' capabilities in natural extraction technologies including supercritical, subcritical and standard solvent extractions that are employed to develop novel natural ingredients for food, pharmaceutical and cosmetic applications. From these technologies, various types of natural extracts including essential oils, natural colors and antioxidants isolated from proanthrocyanin-rich fruits, and protein hydrolysates from various crop sources are available. The company has begun exploring how these unique ingredients might be better employed in skin and hair care applications. The talk will address some of the pitfalls and difficulties that arise to bring a new ingredient into global compliance and looking at testing techniques that can pinpoint what the active might do when it is topically applied to the skin or scalp.

SELECTION OF PROSPECTIVE *FICUS* SPECIES AND DEVELOPMENT OF NOVEL MULTIFUNCTIONAL ZETA FRACTIONS FOR PERSONAL CARE

Michael Koganov, Artyom Duev, Olga Dueva-Koganov, Li Zhang, Paul Recht, Xiaowen Hou, Steven Micceri, and Alfred Wong
BioMaterials, AkzoNobel Surface Chemistry LLC, 23 Snowden Avenue, Ossining, NY 10562

Underexplored potential of diverse *Moraceae* plant family was investigated by combination of phylogenetic relatedness analysis and novel Zeta Fraction technology. *F. benghalensis, F. elastica, F. microcarpa, F. carica,* and *F. trigonata* were selected amongst over 800 species. Zeta Fraction technology was utilized to obtain multifunctional bioactive ingredients (Zeta Fractions) from living leaves of selected *Ficus* spp. as described in PCT WO2012033989, US 20140199419, US20130243710, and US20130323339. These fractions were analyzed by LC-UV, LC-MS, ToF/MS, and tested for *in vitro* activities related to skin aging, irritation, inflammation, and hyperpigmentation. Compared to respective conventional extracts, these fractions contained a greater variety of compounds and demonstrated superior activities, safety, aesthetics and excellent sustainability. Zeta Fraction from *F. benghalensis* was free of proteins and pheophorbides, showed outstanding activity in selected assays, and is currently used in numerous multifunctional skin and hair care beauty products worldwide. Zeta Fractions from other *Ficus* spp. demonstrated specific activities particularly suited for mitigation of hyperpigmentation (*F. carica*) and inflammation (*F. microcarpa*). Development of these ingredients demonstrates how solvent-free Zeta Fraction technology, based on Density Functional Theory, isolates plant intracellular components in a reproducible and sustainable process. This technology protects integrity of molecular architecture in living cells, produces fractions capable of targeting multiple biological pathways with single ingredient, improves safety by removing the contaminants of concern, and results in minimal environmental impact and no waste. In addition to providing opportunities for product differentiation and improved aesthetics, Zeta Fraction technology is a powerful tool for exploring and discovering new bioactive compounds from plants.

Session 10 - Microbial Interactions in Humans and Other Animals

DISCOVERING AND DECIPHERING THE PATHOGENIC AND PROBIOTIC ACTIVITIES FROM THE BACTERIAL COLIBACTIN PATHWAY

Maria I. Vizcaino[1,2], Eric Trautman[1,2], Philipp Engel[1,2,3], Emilee Shine[4] and Jason M. Crawford[1,2,4]
[1]Department of Chemistry, Yale University, New Haven, CT 06520, [2]Chemical Biology Institute, Yale University, West Haven, CT 06516, [3]Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland, [4]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06510

Select strains of *E. coli* in our gut encode the "colibactin" pathway, a nonribosomal peptide synthetase-polyketide synthase hybrid pathway phenotypically linked to inflammatory bowel disease and colorectal cancer patients. A variety of cell biology and animal model studies have previously been reported for the pathway, but the responsible small molecules driving the phenotypes have remained enigmatic. Employing molecular networking tools, we developed a "pathway-targeted" molecular networking approach to map the colibactin pathway from both the meningitis pathogen *E. coli* IHE3034 and the probiotic *E. coli* Nissle 1917. A combination of bacterial genetics, metabolomic network analyses, system-wide isotopic labeling studies, 1- and 2D-NMR, bioinformatics, and/or synthesis supported the structures and biosynthesis of at least 32 molecules from the colibactin pathway. *In vitro* activity studies – 5-hydroxytryptamine-7 receptor antagonist, bacterial growth-inhibitory, and DNA interstrand crosslinking assays – for some of these molecules provide functional insights and pave the way for connecting bacterial small molecule chemistry to host physiology in animal model systems.

INTERACTIONS BETWEEN THE MARINE BRYOZOAN *BUGULA NERITINA*, ITS ENDOSYMBIONT, AND SYMBIONT-PRODUCED BRYOSTATINS

Nicole B. Lopanik
Department of Biology, Georgia State University.

The invasive marine bryozoan, *Bugula neritina*, is distributed in temperate habitats worldwide. *B. neritina* possesses an uncultured bacterial symbiont, "*Candidatus* Endobugula sertula" that is vertically transmitted from parent to offspring. It is also the source of the bryostatins, twenty bioactive polyketide metabolites with anticancer, anti-Alzheimer's disease, and anti-HIV activity. Antibiotic curing experiments showed that *E. sertula* is, in fact, the true source of the bryostatins, and in vitro biochemical assays with heterologously expressed portions of *bry*, the putative bryostatin polyketide synthase gene cluster, confirmed its role in bryostatin biosynthesis. Interestingly, the bryostatins are ecologically relevant, as some of the bryostatins are distasteful and defend the host from predators such as fish, indicating that this symbiosis is a tritrophic interaction among the host, symbiont, and predator. Bryostatin levels are higher on larval *B. neritina*, which are exceptionally vulnerable to predators, compared to adult colonies which rely more on physical defense. Previous research showed that the host is a complex of three closely related sibling species that vary both in their symbiont and bryostatins, with one (Type N) lacking the symbiont and bryostatins. Our studies along the western Atlantic showed that, instead of being restricted to higher latitudes where predation pressure is presumed to be lower, Type N *B. neritina* can be found at lower latitudes, and Type S colonies at higher latitudes, indicating a more widespread host distribution than previously thought. Curiously, some of those host colonies varied in their symbiotic status, with Type N colonies at low latitudes possessing the symbiont whereas Type S colonies collected from high latitudes are aposymbiotic. Furthermore, the symbiont in Type N colonies at low latitudes

appears to be the same strain as that found in Type S. Together, these data indicate that the symbiont, but not the host, is restricted by biogeography, and that symbiont transmission is more flexible than previously thought. This system reflects the complexity and breadth of biological interactions resulting in symbiont-produced bioactive compounds.

SMALL-MOLECULE-MEDIATED INTERACTIONS IN COMPLEX MICROBIAL COMMUNITIES

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[1]Department of Molecular Biology, Princeton University, Princeton, NJ, 08540

In complex biological systems, small molecules often mediate microbe-microbe and microbe-host interactions. Identifying these molecules, characterizing their biological activity, and explaining their relevance in a native context are challenging endeavors. Here, I will describe our ongoing efforts to develop computational and experimental tools for discovering small molecules encoded and produced in complex host-associated microbiomes. Examples from several systems will be discussed, including human and marine invertebrate microbiomes.

VERSATILE BACTERIAL SYMBIONTS OF SHIPWORMS CONTRIBUTE TO WOOD DIGESTION, FIX NITROGEN AND PRODUCE SECONDARY METABOLITES

Margo Haygood, Marvin Altamia, Sherif Elshahawi, Andrew Han, Zhenjian Lin, Meghan Betcher, Roberta O'Connor, Moriah Sandy, Amaro Trinidade-Silva, Alison Butler, Eric Schmidt, Gisela Concepcion, Daniel Distel

Shipworms, bivalve mollusks that burrow into and consume wood, host intracellular gamma proteobacterial symbionts in bacteriocytes located in the host gill. The bacteria produce cellulolytic enzymes and fix nitrogen, and were proposed to support the host's ability to survive on a food source that is resistant to digestion and poor in nitrogen. Symbiont genomes reveal a surprising number of putative secondary metabolite biosynthesis pathways, leading us to propose that these symbionts may also contribute chemically to the symbiosis. We observed that the wood digestion organ portion of the gut is nearly devoid of microbes, allowing the host to consume the sugars resulting from wood digestion without competition. We demonstrated that cellulolytic enzymes produced by the symbionts in the gill are transported to the wood digestion organ, and we hypothesize that secondary metabolites also follow this route and suppress competing environmental microbes in the wood digestion organ. The candidate compounds found include a new family of catecholate siderophores, the turnerbactins, and tartrolon E, both of which are not only produced by the symbionts in culture, but can be detected by mass spectrometry in extracts of the intact association, suggesting that they may play a vital role in the symbiosis.

Session 11 - Novel Screening Strategies for the Identification of Therapeutic Lead Compounds

PLURIPOTENT STEM CELL COLONIES PROVIDE A DEVELOPMENTAL LANDSCAPE FOR PHARMACOGENOMIC DRUG DISCOVERY

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Human induced pluripotent stem cells (iPSCs) and their differentiated derivatives are now widely used in disease modeling. However, the cellular heterogeneity and inter-line variability of these cultures has inhibited their wide application in drug discovery. To define the constant and variable features of renewal and early differentiation we developed precise methods for cell culture and spatial analysis of unconstrained iPSC colony formation. Time-lapse recording and live-cell immunocytochemistry revealed that after passage, a reproducible set of developmental domains is spontaneously established in monolayer culture: (1) cellular aggregation establishes stable boundaries (2) an epithelial core then emerges on the internal surface with early neural properties of tight cell packing and expression of pro-neurogenic transcription factors. Using this developmental platform, we demonstrate quantitative differences in the kinetics of domain production and associated signaling in iPS cells from multiple donors. Applying high-content analysis with targeted compound libraries, we can measure domain-specific responsiveness revealing differential signaling between donors. We demonstrate a platform for successful drug discovery using human iPSCs if accounting for the critical domains of space, time, and donor genotype.

NEUROTROPHIC AND NEURITOGENIC DRUG LEADS FROM NATURAL PRODUCTS: A COMBINED *IN VITRO* ASSAY FOR EVALUATION OF CELL VIABILITY AND NGF-STIMULATED NEURITIC OUTGROWTH IN NEUROSCREEN 1 CELLS

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Neuritogenesis or neuritic outgrowth is a fundamental cellular differentiation process for formation of new neurons. Recent reports have indicated importance of this process in neuroregeneration and repair of damaged neurons. Neurotrophic and Neuritogenic substances, which repair damaged neurons through stimulation of neuritic outgrowth, may be important for restoration and readjustment of normal neuronal functions of the damaged neurons. Neurite outgrowth assays are important phenotypic methods to screen chemical effects on the neuronal cells, especially for discovery of low molecular weight neurotrophic agents. Bioactive small molecules with neurotrophic and neuritogenic actions hold great promise as therapeutic agents for treatment of neurodegenerative diseases and neuronal injuries by virtue of their ability to stimulate neuritic outgrowth. A combined *in vitro* method that measures and quantifies neurite outgrowth along with the cell viability in a single assay has been described here. This assay is simple, less expensive and a useful tool for identification of new neurotrophic agents. The stimulation of neurite outgrowth was measured by analysis of digital cell images by the NIS software in terms of multiple parameters namely, the effect of test compounds on mean neurite length, neurite length/cell, nodes/cell and number of neurites/cell. An important advantage of this method, which can be performed in a 96-well plate, is the determination of cytotoxicity and neurotrophic activity of the test compounds in a single assay. The assay has been successfully applied for screening natural as well as synthetic compounds libraries. Focused screening of a library of harmala alkaloids and other natural products has identified new natural products leads, which produced significant activation of NGF-stimulated neuritic outgrowth. The lead natural products have been investigated further for interrogation of neurotrophin signaling pathways. **Acknowledgement**- Supported by NIH - National Institute of General Medical Sciences #P20GM104932 (COBRE-*In vitro* Research Core)

UTILIZING DIFFERENTIAL CYTOTOXICITY SCREENING TO IDENTIFY LEAD COMPOUNDS FOR RARE ADULT AND PEDIATRIC TUMORS

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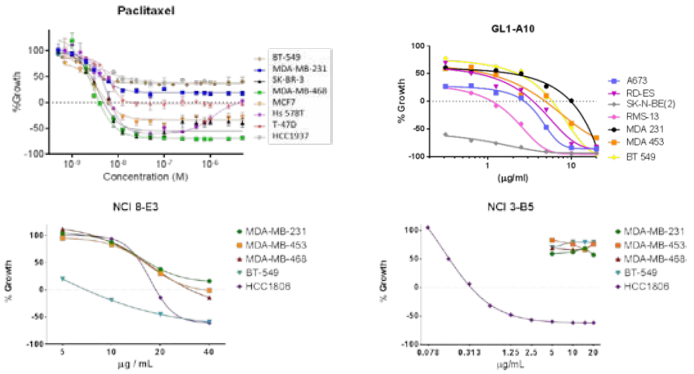
Over the last several decades, natural products have become a mainstay in the treatment of cancers. However, it has become increasingly clear that even agents previously classified as general cytotoxins are only effective in subpopulations of patients, suggesting that these agents may be considered 'targeted therapies'. In addition to understanding the molecular markers that predict response to current therapies, there is a critical need to identify new compounds that specifically target rare, lethal malignancies with limited treatment options, including triple negative breast cancers and some classes of pediatric solid tumors. We have developed an effective method of screening extracts and fractions from fungi and plants that is yielding compounds with selective cytotoxic activities for these cancer subtypes.



NEW ANTIMICROBIALS FROM AN EPIGENETICS BASED FUNGAL METABOLITE SCREENING PROGRAM

Danielle Demers[1], Renee Fleeman[2], Brian Vesely[3], Christopher Rice[3], Beatrice Colon[3], Ala Azhari[3], Ashley Souza[3], Dennis E. Kyle[3], Lindsey N. Shaw[2], Bill J. Baker[1]
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Microbial natural products have historically played an important role in both antibacterial and antiparasitic drug discovery. However, with emerging drug resistance as a global threat to nearly all current antimicrobial treatments on the market, new discovery efforts are needed with increasing urgency. Mangrove endophytic fungi, known as a source of great biological and chemical diversity, were investigated in a large epigenetics based screening program. Cultured under control and epigenetically modified conditions, isolates were extracted and submitted for biological assay against the ESKAPE pathogens, the tropical disease causing parasite *Leishmania donovani*, and the meningoencephalitis causing amoeba *Naeglaria fowlerii*. The chemical investigation of one active organism identified in this way yielded a suite of new antimicrobial compounds. Herein, the structure elucidation and biological profiles of these new compounds will be discussed.

A NOVEL BIOASSAY TO IDENTIFY ANTI-VIRULENCE LEADS AGAINST GRAM-POSITIVE BACTERIAL PATHOGENS

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Infections from drug resistant bacteria result in millions of illnesses and thousands of deaths annually in the US each year, and cost an estimated $55 billion. The anti-virulence approach has emerged as a promising method for combating these infections. In theory, an anti-virulence therapeutic would reduce bacterial pathogenesis, resulting in clearance by the host without promoting resistance. Our lab has developed a novel phenotypical bioassay to identify new broad spectrum anti-virulence therapeutic leads. Utilizing a uniquely engineered strain of *Staphylococcus aureus* coupled to a novel mass spectrometry detection method, we have screened for compounds that interact with a very specific target within the quorum sensing system of Gram-positive bacteria, AgrB. This method has allowed us to efficiently screen over 500 natural product compounds, as well as numerous plant and fungal extracts. We have also employed the new assay to demonstrate that the fungal metabolite ambuic acid targets AgrB, and has broad-spectrum activity against multiple pathogenic bacteria.

Session 12 - New Avenues in Botanical Research

HDAC INHIBITORS FOR CANCER CHEMOPREVENTION

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The treatment of several diseases is highly dependent on natural products and this is especially true in the case of cancer. Most of human cancers seems to be potentially avoidable by controlling exogenous factors (primary prevention), but also by using agents interfering with carcinogenesis. These compounds can be divided into three categories: blocking agents (anti-initiation), agents against the promotion or the progression stages. Epigenetic changes, induced by compounds acting on the methylation or acetylation status of the histones, mostly act in the last stage.

Histone deacetylases (HDACs) are enzymes that deacetylate lysine residues from histones, as well as several other nuclear, cytoplasmic or mitochondrial proteins. In mammals, there are 11 zinc-dependent HDACs classified in three classes: class I (HDACs 1-3 and HDAC8), class II which is subdivided into classes IIa (HDAC4, 5, 7, and 9) and IIb (HDAC6 and 10), and class IV (HDAC11). During the past years, the number of enzyme subtypes has increased and offers the possibility to develop HDAC inhibitors with increased specificity. In the case of cancer, these specific inhibitors should have a better efficacy and decreased side effects. A method using mass spectrometry was developed to measure the inhibitory activity against classes I and II isoforms in a single sample. Various diterpenes and aurones showed promising results and will be discussed. The use of epigenetic modulators could be an optimal intervention to prevent early epigenetic changes and decrease the prevalence of age-related diseases such as cancer.

GLOBAL LIPIDOMICS PROFILING OF COTTON SEED OIL GENOTYPES USING CO_2-BASED CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

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Refined cottonseed oil enjoys widespread applications in the food and chemical industries. Although the major lipids comprising cottonseed oil are well known, there are many diverse lipid species in cotton seeds that occur at much lower levels and have important nutritional or anti-nutritional properties. Lipidomics analysis of complex lipids in seed oil extracts using a single chromatographic technique is challenging due to the diversity of lipid polarity and the large range of concentrations of lipid species in biological samples. Ultra Performance Convergence Chromatography (UPC^2) is a chromatographic system that utilizes liquid CO_2 as primary solvent and co-solvents such as methanol as a mobile phase to leverage the chromatographic principles and selectivity of normal phase chromatography while providing the ease-of-use of reversed-phase LC. We have used UPC^2 coupled to mass spectrometry for the separation of free fatty acids, neutral and polar lipids in a single cotton seed lipid extract. Several seed oil genotypes, including high oleic acid line of *Gossypium hirsutum* cv. Coker 312 with a nonfunctional *Brassica napus* allele of delta-12 fatty acid desaturase (FAD2) and naturally occurring high oleic acid line of *G. barbadense*, were profiled to identify global consequences of modulating seed oil fatty acid composition. Potential markers that discriminate the different genotypes were identified and quantified.

NATURAL TOCOPHEROL MIXTURES AS PROMISING CANCER PREVENTIVE AGENTS

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Naturally occurring vitamin E, a family of fat-soluble antioxidants, exists in eight chemical forms of α-, β-, γ-, or δ-tocopherols and α-, β-, γ-, or δ-tocotrienols. Tocopherols are the major source of vitamin E in the U.S. diet. Tocopherols are widely occurring in corn, soybean, sesame and cottonseed oils and nuts. Vitamin E is also often consumed as a dietary supplement, with α-tocopherol as the most common form available. Numerous epidemiological studies suggest cancer preventive activity of vitamin E. However, recent large-scale randomized human trials with α-tocopherol did not demonstrate a cancer preventive effect. Importantly, each form of tocopherols has varying levels of biological activity. Our extensive studies in animal models of carcinogenesis have shown the cancer preventive activity of γ-tocopherol and δ-tocopherol as well as a naturally occurring mixture of tocopherols, but the lack of cancer preventive activity by α-tocopherol. Here, we present the anti-cancer activity of natural dietary γ-tocopherol rich tocopherol mixtures, γ-TmT, in two different animal models of estrogen-mediated breast cancer. γ-TmT inhibited estrogen-induced mammary tumorigenesis by lowering the levels of circulating estrogen, increasing estrogen metabolism via regulating metabolizing enzyme, CYP1A1, and facilitating the clearance of toxic metabolites and reactive oxygen species by stimulation of Nrf2-mediated antioxidant response. Activation of PPARγ signaling and inhibition of cell proliferation could also contribute to the chemopreventive effects of γ-TmT. Thus, dietary γ-tocopherol rich mixtures could be safe and effective natural agents for the prevention and treatment of estrogen-induced breast cancer.

CAN WOMEN'S HEALTH BOTANICALS PREVENT ESTROGEN CARCINOGENESIS?

Birgit M. Dietz, Tareisha L. Dunlap, Atieh Hajirahimkhan, Shuai Wang, Huali Dong, Charlotte Simmler, Rasika Phansalkar, René F. Ramos Alvarenga, Shao-Nong Chen, Dejan Nikolic, Richard B. van Breemen, Guido F. Pauli, and Judy L. Bolton
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Breast cancer is the second most common malignancy among women becoming even more prevalent with age. Three major pathways are considered to be important in estrogen carcinogenesis: **A**) The hormonal ERα-mediated pathway leading to stimulation of tissue growth; **B**) chemical pathways involving estrogen metabolism to reactive intermediates which damage DNA; and **C**) the inflammatory pathway enhancing the formation of genotoxic estrogen metabolites. The botanicals, *Glycyrrhiza* species, *Humulus lupulus*, and *Angelica sinensis*, are frequently used as alternatives to hormone therapy mainly due to their estrogenic activities. Recent evidence suggests that these botanicals have potential to prevent estrogen carcinogenesis through diverse pathways. *In vitro* and *in vivo* studies have shown that *A. sinensis* constituents can reduce the ERα–mediated effects of estrogens (pathway **A**). All three botanicals induce detoxification enzymes that can facilitate the removal of genotoxic estrogen metabolites (**B**). Interestingly, only one *Glycyrrhiza* species (*G. inflata*) reduces the formation of genotoxic estrogen metabolites (**B**). All three botanicals possess anti-inflammatory activities that might reduce inflammation in estrogen carcinogenesis (**C**). These findings highlight the importance of multi-factorial standardization to all bioactive markers including cytoprotective compounds, in order to achieve prevention of estrogen carcinogenesis.

This research was supported by NIH Grants P50 AT000155 and T32 AT007533.

CLINICAL EVALUATION OF FORMULA F105, DESIGNED AND DEVELOPED TO ADDRESS ELEVATED OXIDIZED LDL CHOLESTEROL (oxLDL) LEVELS

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In 2012, it is estimated that 17.5 million people died from cardiovascular disease (CVD) globally.[1] Meta-analysis reveals that for every 1% reduction in cholesterol an estimated 2.5% reduction in coronary heart disease;[2] in addition emerging science has shown oxLDL to be the most significant predictor of a future cardiac event.[3] In a 12-week open-label clinical study of F105, 8 subjects saw the following changes to major cardiovascular risk factors without dietary intervention or weight loss:

	Median Change	Median % Change	P value*	Hazard Ratio (95% CI)[3]
Total Cholesterol	-23	-10	0.008	1.20 (0.93-1.56)
LDL	-21	-10	0.031	1.90 (1.44-2.51)
Triglycerides	-35	-27	0.039	2.34 (1.79-3.05)
Cholesterol/HDL	-2.5	-45	0.016	6.12 (4.56-8.20)
oxLDL	-14	-19	0.047	8.26 (6.15-11.11)
oxLDL/HDL	-0.3	-25	0.039	13.92 (10.07-19.23)

* P-values were computed using the log-normal distribution of the ratio of change from baseline to 12 weeks using a Wilcoxon Signed Rank test of the median against the null hypothesis.

[1] WHO.int –Cardiovascular Diseases, Fact Sheet No 317.; [2] Holme I. *Circulation* **1990**; *82*:1916-1924.; [3] Johnston, N. et al. *Am. J. Cardiol.* **2006**, *97*: 640–645.

Session 13 - Applications of Mass Spectrometry to Natural Products Drug Studies

METABOLOGENOMICS: DISCOVERY OF NEW NATURAL PRODUCTS AND THEIR BIOSYNTHETIC GENE CLUSTERS BY GENOME-INFORMED METABOLOMICS

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Over the past decade, the genomics revolution has provided a glimpse into the vast, untapped metabolic potential of microbial genomes. Simultaneously, the field of metabolomics, propelled by advances in LC-MS and informatics, now allows for high-throughput, semi-quantitative characterization of metabolites. Focusing on actinomycetes we present a new approach, metabologenomics, marrying metabolomics and genomics to connect exported metabolites to their biosynthetic gene clusters. With the high mass accuracy afforded by FT-Orbitrap instrumentation (<3 ppm), 2,521 metabolite components were identified from the extracts of 178 actinobacterial growths. Of these, 110 were confidently identified as known natural products by searching an aggregated database of 9,817 actinomyctete natural products. Detected metabolites and families of related gene clusters (GCFs) were used for a binary correlation scoring system, to evaluate the likelihood that a cluster could be responsible for the production of a particular secondary metabolite. Output scores ranged from 0 to over 300. Known GCF-metabolite pairings were used to calibrate score interpretation, with 27 known gene cluster families correctly paired with published biosynthetic clusters: these included oxyetracycline (score = 270), actinomycin D (score = 204) and rimocidin (score = 210). From the highest scoring pairs, we characterized a new metabolite and named it rimosamide (score = 264).

MASS SPECTROMETRY-BASED CHEMOPROTEOMICS FOR TARGET DECONVOLUTION OF BIOACTIVE NATURAL PRODUCTS

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Natural products play an important role in phenotypic approaches to drug discovery. Recent years have seen the resurgence of these approaches, in particular due to their pre-selection for cell-active compounds and their ability to identify novel druggable nodes. Therefore, the identification of protein targets of natural products and the elucidation of their mechanism of action remain areas of high interest. Quantitative chemoproteomics has emerged for us as a principal strategy for generation of testable target hypotheses. It typically combines competition-based compound affinity chromatography from cell lysates with mass spectrometry-based protein identification and quantitation using e.g. isobaric labeling tags. These studies enable the identification of the full spectrum of cellular interactors of compounds under conditions approximating the disease-relevant in vivo situation, including the efficacy target(s) and also potential off-targets. However, for unbiased target deconvolution, the immobilization of compound on solid support is required at some stage during the workflow. While the introduction of a functionalized linker at a permissive site, as typically done for synthetic small molecules, is in principle also possible for natural products, it requires extensive knowledge of structure activity relationships which might not always be easily obtainable. Therefore, an extended toolbox of approaches has proven to be valuable, including site-nonselective compound immobilization on photocrosslinker beads. Several variants of chemoproteomics as applied to natural products target deconvolution and validation will be presented.

STATE OF ART ANALYTICAL TECHNOLOGIES TO SOLVE NATURAL PRODUCTS CHALLENGES

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The study of natural products is a daunting task and sometimes intimating for many scientists because of the complex nature of problems at hand. From analytical point of view, there are many analytical challenges to conduct natural product studies. For example: complex samples require comprehensive sample clean-up such as extraction, pre-fractionation, and purification; there is a need to have very high peak capacity and resolving power when it comes to separations; there are wide concentration range of analytes that require detectors with wide dynamic range; the chemical space within NP samples is so vast that an universal detector is strongly desired and yet, unavailable. As a result, having a good set of analytical tools that are fit for purpose and effective is vital for all natural products scientists. As currently, there is not a single "one size fits all" analytical solution available to address the entire spectrum of the natural products challenges. In addition, with the advances in technology, and a variety of analytical solutions available to tackle different analytical problems, another challenge for researchers is to be able to keep up with the pace of the technology advancement, and to understand how to use them effectively to answer the analytical questions at hand quickly and effectively.
The goal of this presentation is to provide updates for the natural products scientists with some example state of art analytical technologies, from sample preparation, to chromatographic separation to mass spectrometry detection to informatics platform through application examples. Examples of technology updates that will be covered by this presentation include mentioned include: supercritical fluid technology (SFX), *nano*-fluidic chromatography, MS detector for routine analysis, Qtof mass spectrometry, as well as ion mobility (IMS) mass spectrometry.

THE SINGLE-PROBE MASS SPECTROMETRY FOR SINGLE CELL ANALYSIS AND BIOLOGICAL TISSUE IMAGING

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Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019

Mass spectrometry (MS) is commonly used to analyze the molecular composition of prepared gas-phase or solution samples. With the recent development, MS has been used to obtain the cellular composition of single cell (i.e. single cell MS) and map the spatial distribution of species on biological tissues (i.e. MS imaging). We have developed a multifunctional device, named as the Single-probe. This device can be coupled with MS for multiple applications, including single cell MS and MS imaging.

To fabricate a Single-probe, a fused silica capillary and a nano-ESI emitter were glued into a dual-bore quartz needle (tip size <10 μm). The Single-probe has been coupled with a X,Y,Z -stage system, digital microscopes, and a LTQ Orbitrap XL mass spectrometer. Single cell MS. We have cultured cancer cells were cultured and treated them with anticancer compounds. Cells were placed on a glass slide that is attached to the stage system. Using microscopes as the visual guide, the tip of the Single-probe was inserted into a cell by precisely lifting z-translation stage. The corresponding anticancer compounds and cellular metabolites were observed. MS imaging. Mouse brain and kidney slices were prepared using a cryomicrotome, and placed on the stage system. We closely approached the Single-probe tip to

the tissue surface, and analytes on the corresponding spot were sampled by the Single-probe followed by MS analysis. We performed point-by-point analysis of the whole tissue slice to reconstruct the spatial distribution of compounds of interest using visualization software. We have obtained high-spatial resolution (8.5 μm) under ambient conditions without sample preparation.

VISUALIZING DIVERSE CHEMICAL FAMILIES WITH MOLECULAR NETWORKING

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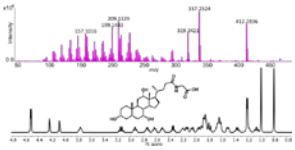

Figure 1. ^{1}H NMR (bottom) and MS/MS spectrum (top) for glycocholic acid, both indicative of a steroid core.

Molecular networking (MN) is an analytical technique that relies on tandem mass spectrometry spectra to serve as a chemical finger print for molecular entities. MN has been shown to improve many areas of natural product workflows including dereplication, discovery, and unveiling environmental niche chemistries. Recently, we have gathered MS/MS data on a thousands of pure compounds received from the NIH, user contributed data to the Global Social Natural Products (gnps.ucsd.edu) library, Massbank, and ReSpect. We have used these library spectra to mine for discernable patterns in the MS/MS data, analogous to structural class identification using ^{1}H NMR. These patterns and other chemical trends have been explored to gather the maximum amount of information from a single MS/MS spectrum while simultaneously using various workflows on GNPS.

Session 14 - Enhancing Natural Product Leads via Synthetic Manipulation: In Celebration of Dr. Mansukh Wani's 90th Birthday

DEVELOPMENT AND OPTIMIZATION OF THE PHYLLANTHUSMIN CLASS OF ARYLNAPHTHALENE LIGNAN NATURAL PRODUCTS

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A series of arylnaphthalene lignans, referred to collectively as the phyllanthusmins, was isolated from *Phyllanthus poilanei* by the Kinghorn lab as a part of a collaborative program project grant directed at the identification of novel anticancer agents (P01 CA125066). These compounds bear a strong structural resemblance to etoposide, a semi-synthetic DNA topoisomerase II (topo II) poison. Although the phyllanthusmins have been shown not to inhibit topo II activity, many of these compounds have demonstrated potent *in vitro* antiproliferative activity against HT-29 and other cancer cells. In an effort to understand the structure-activity requirements and to remedy the limited aqueous solubility of this class of molecules for *in vivo* studies, a series of structural analogues has been synthesized and screened for biological activity, ultimately leading to compounds with improved properties. This iterative process of optimization in collaboration with other members of this program grant will be highlighted.

SYNTHESIS OF BOTH ENANTIOMERS OF THE META,META-BRIDGED DIARYLHEPTANOID MYRICANOL

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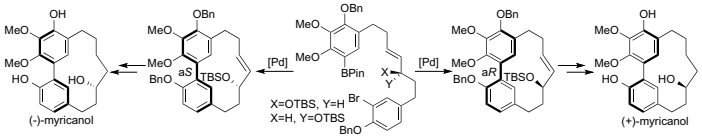
The *meta,meta*-bridged diarylheptanoid myricanol isolated from the stem bark of *Myrica cerifera* is able to lower the levels of tau protein – a protein involved in the progression of Alzheimer's disease and several other neurodegenerative diseases. This biological activity, however, is highly dependent upon the stereochemistry of the sample. Here we report the synthesis of both enantiomers of myricanol. Pivotal to the success of this approach is the use of a remote stereocenter to influence the axial chirality of an aryl-aryl bond formed through an intramolecular Suzuki-Miyaura reaction.



VINBLASTINE: SYNTHETIC AND MECHANISTIC STUDIES

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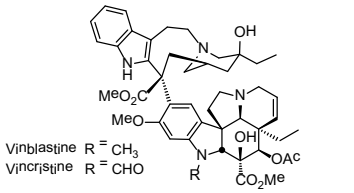
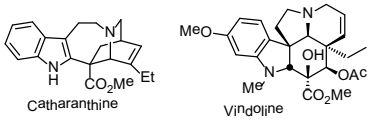
A brief summary of studies providing a first generation and second generation asymmetric total synthesis of vindoline based on the implementation of a powerful [4+2]/[3+2] cycloaddition cascade of 1,3,4-oxadiazoles will be presented along with its extension to the preparation of a series of key analogs. The development of a single-step biomimetic Fe(III)-promoted coupling of vindoline with catharanthine and subsequent in situ oxidation to provide vinblastine, its extension to the total synthesis of related natural products and key analogs, and new mechanistic insights into the reactions will be presented. Use of this work in key studies addressing the structural features of vinblastine contributing to its tubulin binding and antitumor properties, and the synthesis, evaluation, and discovery of potent analogs will be discussed.





Session 15 - Translational Studies of Natural Products

CHALLENGES AND SUCCESSES IN THE DEVELOPMENT OF NATURAL PRODUCT DRUGS

William Fenical
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego.

One of the great challenges in academic drug discovery is finding a pathway for promising discoveries to provide entry into preclinical development. This is particularly challenging, as academics may not be fully aware of the complex characteristics a compound may need to be considered a truly promising lead. Complicating this picture is the perception that academics do not fully understand drug development and thus provide superficial evidence of success at best. But, even with these difficulties, virtually all of the approved marine-derived drugs and most in clinical trials were discovered by academic laboratories. Some examples of success from personal experience, and suggestions on how to overcome these issues will be presented.

MEASURES OF SUCCESS IN TRANSLATIONAL RESEARCH

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The ultimate measure of success in industrial natural products R&D is the launch of a new commercial product. In reality this is a rare event, particularly for those involved in the earliest stages of R&D (Discovery). Regardless of the eventual commercial outcome, which may be governed by business considerations rather than science, there are many achievements in "translational research" that should be recognized as measures of success. Each time an issue that constitutes a roadblock to further product development is overcome, there are valuable lessons learned that may be helpful in subsequent programs. In this presentation I will cover examples of such stepwise problem solving that helped advance natural product R&D projects originating from discoveries made at American Cyanamid Co. (Lederle Laboratories).

- Dioxapyrrolomycin insecticide lead. Issue: preparation of analogs for SAR
- Ganefromycin animal health & growth promotion: stability & supply
- Mannopeptimycin antibiotic lead: IP protection; stability & bioavailability

During this time period the best known examples of translational success were the development of moxidectin (Cydectin) as an antiparasitic agent for animals, tigecycline antibiotic (Tygacil) to overcome resistance to the tetracyclines and the heroic crafting of calicheamicin (Mylotarg) as a payload for antibody-drug conjugate programs.

DEVELOPMENT OF ZINDOL® THROUGH MULTI-CENTER CLINICAL TRIAL FOR CHEMOTHERAPY INDUCED NAUSEA AND VOMITING

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Despite the widespread use of 5-HT_3 receptor antagonist anti-emetics, chemotherapy induced nausea and vomiting (CINV) continues to be reported by up to 70% of adult patients and up to 58% of children receiving highly emetogenic chemotherapy agents. Nausea and vomiting are among the most distressing side effects of chemotherapy. Zindol® is an enhanced ginger product that is standardized by the bioactive constituents, gingerols and shogaols, of ginger (*Zingiber officinale* Roscoe). We utilized polarity-guided supercritical fluid fractionation SFS-CXF technology to establish conditions for the isolation of the active ingredients and then manufactured the API using SFS-CXP following cGMP. The API was then formulated to achieve a specific concentration of gingerols and shogaols with all-natural liquid excipients designed to maximize the stability and bioavailability of the bioactive constituents and encapsulated into gel capsules with a nitrogen head following cGMP. Zindol® was successfully evaluated in a multi-center, 644-patient, dose-finding, placebo-controlled, randomized Phase II/III clinical trial as an adjunctive therapy to conventional 5-HT_3 anti-emetics for nausea in cancer patients undergoing chemotherapy (US Patent). We have since studied the MOA using a newly developed i*n vitro* potency assay (US Patent Pending) and are planning to conduct a pivotal Phase III clinical trial for CINV and file an NDA with the FDA. A sister dietary supplement product, Zindol® DS, has been reported to be safe and effective in motion sickness, and nausea and vomiting related to pregnancy, elective surgery and medications. We plan to conduct rigorous clinical trials for these indications after NDA approval of Zindol® for CINV in adult and pediatric populations.

DISCOVERY AND DEVELOPMENT OF ERIBULIN, A MACROCYCLIC KETONE ANALOG OF HALICHONDRIN B, FOR TREATMENT OF ADVANCED BREAST CANCER

Bruce A. Littlefield
Global Oncology Medical Affairs, Eisai Inc., 4 Corporate Drive, Andover, MA 01810, USA

Marine natural products represent rich sources of novel compounds for drug discovery. Owing to their roles in chemical defense, marine natural products are often remarkably potent in order to overcome highly diluting ocean environments. Halichondrin B (HB), originally isolated from the sponge *Halichondria okadai*, is one such compound. Early reports of HB's remarkable antitumor activity led to significant interest in developing it as a new anticancer drug, but limited natural supplies ultimately foiled such efforts. Fortunately, the total synthesis of HB plus the discovery that its anticancer activity resided in its macrolactone "right half" moiety provided an opportunity to develop structurally simplified, fully synthetic analogs. Eribulin, a synthetic analog of HB's right half, retains HB's high potency with low/sub-nM activity against human cancer cells *in vitro.* Mechanistically, eribulin is a novel microtubule dynamics inhibitor that disrupts mitotic spindle formation, causing apoptosis after 10-12 hours of irreversible mitotic blockade. *In vivo,* eribulin induces tumor regression and long-term survival of nude mice bearing human tumor xenografts. Eribulin (as Halaven®) is now approved for clinical use in the United States, European Union, Japan and many other countries for treatment of certain patients with advanced breast cancer.

NATURAL PRODUCTS IN CLINICAL TRIALS: CURRENT STATE OF PLAY AND FUTURE DIRECTIONS

Mark S. Butler
Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia

The last 10 years have seen massive changes in the pharmaceutical industry with a narrowing of therapeutic focus, continued mergers, a large number of redundancies and a massive increase in outsourcing. During this period, the number of new small molecule drug approvals has been around 15-30 NCE per year despite increased expenditure and more advanced technologies. Companies now predominantly rely upon of high throughput, fragment and virtual screening to identifying new lead compounds with natural product (NP) screening being almost virtually ignored. This is counter initiative as NPs have always played an important role in drug development with a considerable number of marketed drugs being NP-derived. In addition, NPs often also occupy chemical space not usually found in synthetic

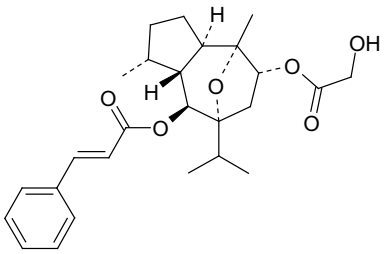
based screening libraries and are often excellent leads for drug development despite their often complex structures.

This talk will summarize the NPs, semi-synthetic (SS) NPs, NP-derived compounds and NP-containing antibody-drug conjugates (ADC) undergoing clinical evaluation or registration, and new NP drug pharmacophores will be analysed. In addition, an insight will be given on how NP-derived compounds move from the bench into clinical trials.

DEVELOPMENT OF ENGLERINS AS CANCER THERAPEUTICS

John A. Beutler
Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702

The sesquiterpene englerin A, obtained from the Tanzanian plant *Phyllanthus engleri* (Euphorbiaceae), is a lead for drug development in kidney cancer and Ewing's sarcoma. Englerin A is available in gm/kg quantities from the bark of the plant, and has been synthesized in excellent yields by several chemistry groups. It is active in xenograft models of kidney and prostate cancer. In kidney cancer cells, we have found englerin A to be selective agonist for protein kinase C isoform θ, an effect which simultaneously induces glucose addiction and down-regulates the glucose transporter. We will report on our recent efforts, which have focused on elucidating structure-activity relationships, investigating pharmaceutical development questions such as formulation, route of administration, and ADME/PK, and conducting further in vivo studies.



Session 16 - Beyond the Rule of 5 in the Design of Next-Generation Therapeutics: What Can We Learn from Macrocyclic Natural Products?

NATURAL PRODUCTS: ARE WE CLOSE TO THE END?

Cameron R. Pye[1], Matthew J. Bertin[2], R. Scott Lokey[1], William H. Gerwick[2], Roger G. Linington[1]
[1]Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064, [2]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92037.

Natural products have historically played a central role in drug discovery and development, but the re-isolation of known scaffolds is reported to increasingly hamper efforts to mine natural products chemical space for novel bioactive molecules. Despite the promise offered by orthogonal discovery strategies such as genome mining and the exploration of new taxonomic space, examination of the literature implies that most natural products being published today bear significant structural similarities to existing scaffolds.

This empirical observation inspired us to systematically explore the state of natural products discovery from the standpoint of both discovery rates and structural novelty. Using a database containing all microbial and marine-derived natural products we determined the Tanimoto structure similarity scores for all database members. We then used these data to examine a number of questions in this area, including: the rates of novel compound discovery over time, the effects of exploring novel source organisms on molecular diversity, and the projected rate of novel discovery for future natural products efforts. Results from these analyses will be presented, along with a perspective on the implications of these findings for future natural products discovery strategies.

INTERROGATING CYCLIC PEPTIDE NATURAL PRODUCTS FROM AN ADME PERSPECTIVE : BEYOND CYCLOSPORINE A.

Cameron Pye and R. Scott Lokey
Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, CA 95064, [2]

In terms of chemical diversity and complexity, the database of known natural products easily trumps the average diversity screening collection. Indeed, most small molecule screening libraries are pre-filtered using classical metrics for drug likeness (e.g., Lipinski's Rule of 5) that necessarily limit the size and complexity of their constituents. These metrics would categorize most natural products as non-druglike, despite numerous clinically prescribed examples to the contrary. Many of these "beyond-Rule of 5" (b-Ro5) success stories include macrocyclic peptides like cyclosporine A (CSA), which has a molecular weight of 1200 and is both cell permeable and orally bioavailable. While CSA has been the poster child representing this new b-Ro5 modality, there are many other natural product cyclic peptides that have structural motifs (e.g., backbone N-methylation, mostly hydrophobic side chains, the presence of D-amino acids) that also suggest the potential for passive permeability. However, the rich chemical landscape of macrocyclic peptides has not been explored from a chemo-informatics perspective, and so we set out to analyze all known cyclic peptide natural products using various quantitative metrics relevant to ADME and pharmacokinetics. To do this we extracted structural information from a comprehensive database of natural products and filtered it for cyclic peptide like species. I will present a survey of the resulting compounds with a focus in particular on structure/property relationships relevant to cell permeability and oral bioavailability.

PHARMACEUTICAL APPLICATIONS OF CYCLOTIDES

David J Craik, Conan Wang, Aaron Poth
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Cyclotides are macrocyclic peptides from plants that are notable for their exceptional stability and broad range of biological applications as well as their uses in indigenous medicines. They comprise a head-to-tail cyclic backbone of around 30 amino acids with a cystine knot arrangement of three disulfide bonds. Together these structural elements make cyclotides impervious to proteolytic breakdown and also exceptionally stable to high temperature and harsh solution conditions. These properties make cyclotides valuable templates in drug design applications. So far, approximately a dozen examples have been reported in which foreign bioactive peptide sequences have been grafted into one of the loops of a cyclotide framework to enhance the stability of the peptide epitope while maintaining the biological activity of that epitope. Applications have included cancer, cardiovascular disease, wound healing, pain, infectious disease, and autoimmune disease, amongst others[1]. This talk will describe the principles of the grafting of cyclotides as well as examining the biophysical[2] and biopharmaceutical properties of cyclotides that underpin their applications as drugs.

[1]Poth A G, Chan L Y, Craik D J: Cyclotides as grafting frameworks for protein engineering and drug design applications. *Biopolymers Peptide Science* (2013) **100**, 480-491.

[2]Wang C K, Northfield S E, Swedberg J E, Harvey P J, Mathiowetz A M, Price D A, Liras S Craik D J: Translational diffusion of cyclic peptides measured using pulsed-field gradient NMR. *J Phys Chem B* (2014) **118**, 11129-11136.

PEPTIDE MACROCYCLES: FROM STRUCTURAL STUDIES TO ORAL BIOAVAILABILITY

Andrei K. Yudin[1]
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Peptides represent a therapeutic modality that has received renewed interest over the past several years. While this surge of activity can be partially explained by the emergence of new technologies for targeted delivery of peptide drugs, there are also reasons to be optimistic about structure-based improvement of key properties of peptides that have traditionally been viewed as liabilities. Large polar surface area is one of these properties. Macrocyclic topology allows one to minimize polar surface area of peptides by increasing the propensity to form intramolecular hydrogen bonds while shielding them from solvation.

We have developed several enabling methods that allow for rapid synthesis of peptide macrocycles and their evaluation as potential therapeutic agents. I will present not only the foundational aspects of this technology, but also the progress we have made in the area of inflammatory bowel disease, wherein our lead molecule recently showed positive efficacy data in a 12-day colitis study. We attribute this and other successes to our emerging understanding of the structure/activity relationship in macrocycles. I will also present our new data in the area of boron-containing peptides. This new class of molecules holds promise for the development of tool compounds to interrogate complex protease targets.

Session 17 - Microbial Metabolites and Bioactive Compounds from Plant Microbial Associates (Epiphytes and Endophytes)

ENDOPHYTIC FUNGI OF MEDICINAL HERBS: BASIC SCIENCE AND SOURCE OF NEW DRUG LEADS

Nicholas H. Oberlies
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Fungi are one of the most diverse organisms on the planet, second only to the insects. Estimates predict a range of 1.5 to 5.5 million fungi in the world, and yet, only about 100 thousand have been ascribed a name, and much fewer have been studied for drug leads. This is somewhat surprising given that the elixir of the 20th century, penicillin, was derived from a fungal Petri dish. Endophytes represent a unique niche of fungi, as they live at least some portion of their life within plants, but do so symbiotically and without imparting disease. Questions abound, such as: Do the endophytes stimulate the biosynthesis of defense compounds in the plant? Does the secondary metabolite profile of the endophytes help the plant? Can the endophyte produce compounds similar to those found from the plant? Can the endophytes serve as a new source for drug leads? Can the endophytes be grown in culture, and if so, will they continue to biosynthesize a similar profile of secondary metabolites? Are the endophytes beneficial to the host, detrimental to the host, or simply saprobes in waiting? While we cannot provide definitive answers to any of these questions, we have amassed data that both probes some of the basic science questions, as well as, demonstrates the generation of new drug leads.

EXPLORING PLANT AND LICHEN-ASSOCIATED MICROBIAL DIVERSITY FOR DISCOVERY OF SMALL-MOLECULE BIOACTIVE AGENTS

A. A. Leslie Gunatilaka[1], *E. M. Kithsiri Wijeratne*[1], *Ya-ming Xu*[1], *Bharat P. Bashyal*[1], *Jianguang Luo*[1], *Jair Mafezoli*[1,2], *Maria C. F. Oliveira*[1,2], *Angela M. Hoffman*[1,3], *Patricia Espinosa-Artiles*[1], *Manping X. Liu*[1], *Scott G. Franzblau*[4], *Luke Whitesell*[5]
[1]*Natural Products Center, School of Natural Resources & the Environment, University of Arizona, Tucson, AZ 85706, USA,* [2]*Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Campus do Pici, Fortaleza, Brazil,* [3]*Department of Chemistry, University of Portland, Portland, OR 97203, USA,* [4]*Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA,* [5]*Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA*

Plant and lichen-associated microorganisms represent a largely untapped resource of small-molecule natural products with chemical structures that have conceivably been optimized by evolution for biological and/or ecological relevance. In our search for natural products with bioactivity and/or novel structures, we have investigated numerous fungal strains living in association with plants and lichens. Extracts and/or pure compounds derived from cultures of these fungal strains have been screened in assays relevant for anticancer, anti-HIV and anti-TB drug discovery, and plant stress tolerance. Organisms producing extracts found to be active in these assays were cultured on large-scale and the derived extracts have been subjected to bioactivity-guided fractionation to obtain a variety of natural products with bioactivity and/or novel structures suggesting the potential of this under-explored niche for small-molecule natural products with novel structural types and/or pharmaceutical and agricultural applications. Isolation, structure elucidation, bioactivity and possible biosynthetic origin of some selected small-molecule metabolites and the significance of their natural occurrence will be presented.

This work was supported by grants from NCI (USA), NIGMS (USA), CNPq (Brazil), and Arizona Biomedical Research Commission.

BIOLOGICALLY ACTIVE SECONDARY METABOLITES FROM EPIPHYTIC AND ENDOPHYTIC FUNGI

Marc Stadler
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Plant-associated fungi, and in particular fungal endophytes, are a prolific source for novel secondary metabolites. However, random isolation/screening approaches of these organisms may easily lead to disappointing results because many species have already been screened or just represent the asexual states in the life cycle of well-known species.

Our studies on the Xylariaceae have shown that there is an alternative: Using chemotaxonomic and ecologial data, as well as molecular phylogeny, as pre-selection criteria has resulted in a rather high discovery rate of novel metabolites. We found various strains that are extremely creative metabolite producers. As exemplified by *Hypoxylon rickii*, extensive optimisation of culture conditions and subsequent scale up of production may even result in the discovery of several dozens of new compounds from a single strain. We are also investigating ecological interactions between fungal endophytes, their host-specific insect vectors and their host plants, where certain secondary metabolites seem to play a pivotal role. Moreover, we are targeting poorly studied plant-associated fungal groups with peculiar ecology, such as the epiphytic capnodialean sooty blotch and flyspeck fungi, and even the Ash dieback pathogen *Hymenoscyphus fraxineus*.

Numerous examples of our recent research will be shown to demonstrate that biodiversity can indeed be translated into chemical diversity if taxono-

mists, ecologists and natural product chemists are working together more intensively than this used to be the case in the past.

NOVEL BIOACTIVE COMPOUNDS FROM ENDOPHYTIC FUNGI OF ABORIGINAL MEDICINAL PLANTS

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Department of Chemistry, College for the Environmental and Science Complex, The University of Winnipeg, 599 Portage Ave. Winnipeg, MB, Canada R3B 2G3

Natural products exhibit interesting anti-microbial, anti-viral, and anti-inflammatory activities. These bioactivities make them an important source for the discovery of new pharmaceuticals. Currently, more than 60% of the drugs available on the market are of natural product origin. One of the aspects of drug discovery process is the identification of small molecules with enzyme-inhibiting activities. Enzymes are essential to human life, mediating biochemical processes including metabolism, cellular signal transduction, cell cycling, and development. Malfunction in these biochemical systems often leads to disease that can be caused either by the dysfunction, overexpression, or hyper-activation of the enzymes involved. An understanding of diseases at the molecular level has provided several enzyme inhibitors in clinics. For instance, galanthamine, a potent acetylcholinesterase (AChE) inhibitor, is used to treat early symptoms of Alzheimer's disease. α-Glucosidase (EC 3.2.1.20) is a membrane bound enzyme and lies at intestinal cells. This enzyme catalyzes the final step of carbohydrates digestion by hydrolyzing the glycosidic bonds in carbohydrates to liberate free glucose. The resulting glucose is a source of an exaggerated rise in blood sugar causing postprandial hyperglycemia. This causes type 2 diabetes mellitus and affects approximately 2.1 billion people worldwide. The potent α-glucosidase inhibitors prevent the breakdown of carbohydrates in small intestine and prolong the absorption of glucose or carbohydrates in blood. These compounds may be used as chemotherapeutic agents in clinics for the treatment of diabetes and obesity. Due to the catalytic role of α-glucosidase in carbohydrate digestion, these inhibitors may also be used as therapeutic target for other carbohydrate mediated diseases including viral infections, cancer, HIV and hepatitis. Our recent chemical investigation of endophytes of Aboriginal medicinal plants of Canada resulted in the identification of natural products exhibiting potent bioactivities including anti-microbial, anti-α-glucosidase and anti-AChE activities. In this presentation, isolation, structure elucidation of new bioactive natural products and their structure-activity relationships will be discussed.

Session 18 - Diverse Approaches for Finding and Making Natural Products

COMPARATIVE METABOLOMICS REVEALS A NATURAL COMBINATORIAL LIBRARY AND A XYLOPYRANOSE-BASED NUCLEOSIDE IN NEMATODES

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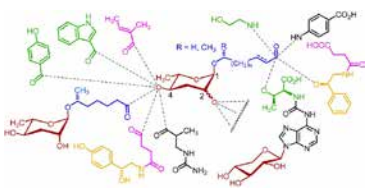
Boyce Thompson Inst./Dept. of Chem. & Chem. Biol., Cornell Univ., Ithaca, NY.

Worms are amazingly skilled chemists: using simple building blocks from conserved primary metabolism and a strategy of modular assembly, nematodes such as the model organism *C. elegans* create complex molecular architectures to regulate almost every aspect of their life history. These compounds are based on the dideoxysugars ascarylose or paratose, which serve as scaffolds for attachment of moieties from amino acid, carbohydrate, neurotransmitter, lipid, and nucleoside metabolism, including an unusual xylopyranose-based adenosine derivative. The resulting signaling molecules can be active at femtomolar concentrations, i.e. encountering just a few molecules is sufficient for worms to respond.



Motivated by this unexpected structural and functional diversity, we are pursuing a systematic characterization of the *C. elegans* metabolome combining mutant screens and 2D NMR/HPLC-MS-based comparative metabolomics. Our screen has produced evidence for several thousand different metabolites of yet undetermined structures, ranging from simple lipids, amino acid derivatives, and nucleosides to complex modular assemblies. Their identification and subsequent quantification in genome-wide mutant screens will, akin to transcriptional profiling, provide a new basis for the study of metabolism and evolutionarily conserved signaling pathways in model organisms.

CONTEMPORARY STRATEGIES FOR TARGETED DISCOVERY AND TRANSCRIPTIONAL ACTIVATION OF NATURAL PRODUCT BIOSYNTHETIC GENE CLUSTERS

Jeremy Owen
Rockefeller University

Soil samples represent some of the most complex microbial consortia known, with upward of 10,000 unique species per gram in some cases. Metagenome libraries constructed from these consortia provide access to an enormous pool of biosynthetic gene clusters that have the potential to yield new, medicinally relevant natural products. This presentation will discuss two approaches aimed at generating an efficient, and broadly applicable metagenome based natural product discovery platform: First, biosynthetic profiling methodologies that use short DNA sequence tags to guide recovery of gene cluster encoding specific compounds of interest will be discussed. Following this, generally applicable synthetic biological strategies for modifying biosynthetic gene clusters to enable the production, isolation and characterization of new chemical entities will be presented.

HARNESSING THE PROMISCUITY OF NATURAL PRODUCT BIOSYNTHESIS: A PLATFORM FOR ENGINEERING PATHWAYS WITH NEW SPECIFICITIES

Gavin Williams
Department of Chemistry, NC State University, Raleigh, NC 27695

Many natural products are biosynthesized in a modular fashion by the selection and condensation of small molecule building blocks. Chimeric biosynthetic apparatus can be constructed in an attempt to produce analogues for drug discovery. Yet, the scope and utility of such approaches is limited by the inherent substrate specificity and poor functional modularity of most biosynthetic components. Here, we show that several types of biosynthetic machinery are more tolerant towards non-natural building blocks than has been previously recognized. Such promiscuity forms a platform for constructing new biosynthetic parts with substrate specificities orthogonal to those found in Nature. Accordingly, we describe a comprehensive program of enzyme engineering, directed evolution, and synthetic biology aimed at constructing artificial bacterial strains capable of producing complex natural products that are regioselectively modified with non-natural chemical functionality. Our synthetic biology approach expands the synthetic capabilities of natural product diversification strategies, and provides an improved understanding of the molecular basis for specificity in complex molecular assemblies.

MANIPULATION OF ACTINOBACTERIAL TRANSCRIPTIONAL REGULATION TO DISCOVER NEW SPECIALIZED METABOLITES

Gregory L. Challis
Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

A key challenge in genomics-driven natural product discovery is the development of rational methods for inducing the expression of gene clusters that are expressed poorly, or not at all, in laboratory cultures. In 2008, we reported that 2-alkyl-4-hydroxymethylfuran-3-carboxylic acids (AHFCAs) induce the production of methylenomycin antibiotics in *Streptomyces coelicolor*. Based on an understanding of the way in which AHFCAs are biosynthesised in *S. coelicolor* and the role they play in regulating methylenomycin biosynthesis, we have identified several putative specialised metabolite biosynthetic gene clusters in other actinobacterial genomes that also appear to be controlled by AHFCA-dependent regulatory networks. Deletion of a repressor gene within such networks upregulates AHFCA production and appears to prolong the expression of biosynthetic genes under their control. These findings have been applied to induction of the expression of putative AHFCA-regulated biosynthetic gene clusters in other actinobacteria that are silent under laboratory growth conditions, resulting in the discovery of novel specialised metabolites.

Posters

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ANTIOXIDANT ACTIVITY AND ISOLATION STUDIES OF EXTRACTS OF SEEDS OF SYZYGIUM CUMINI L.

Khalid Hussain, Muhammad Islam, Naureen Shehzadi, Sohail Amjad, Nadeem I. Bukhari, Hamid Saeed
University College of Pharmacy, University of the Punjab, Allama Iqbal Campus, Lahore-54000, Pakistan

The seeds of *Syzygium cumini* L. (Family: *Myrtaceae*), a well-known medicinal and fruit plant, are used to treat a number of diseases involving free radicals, therefore the present study aims to investigate its extracts for antioxidant activity and isolation of active constituent(s). In 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay, methanol extract and its hexane fraction showed 68.54% and 58.42% free radical scavenging activity, respectively (Fig. 1). The extract and fraction also showed higher antioxidant activity in β-carotene-linoleate assay. From the hexane fraction, caffeic acid (I) was isolated and identified. Methanol extract and hexane fraction were found to contain 74.27 and 40.68 mg/g caffeic acid, respectively. The findings of the present study indicate that the methanol extract of seed of *Syzygium cumini* and its hexane fraction have good antioxidant activity, and contain caffeic acid, a known antioxidant.

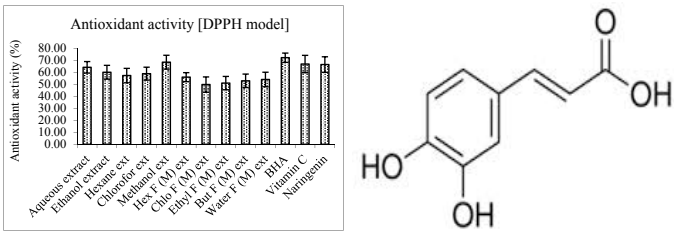

Fig. 1. The antioxidant activity of extracts and fractions (I) of seed of *Syzygium cumini,* and standards using DDPH assay

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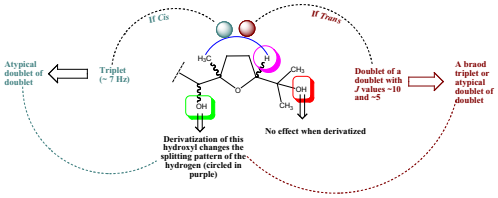
STEREOCHEMISTRY OF 2,2,5 TRISUBSTITUTED TETRAHYDROFURAN RING CONTAINING NATURAL PRODUCTS BASED ON ^{1}H NMR SPECTROSCOPY: SOME OBSERVATIONS

Prabhakar S. Achanta, Raghuram Rao Akkinepally, Ravi Kumar Bobbala, Appa Rao V.N. Achanta
University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009, India.

A hydrogen in 2,2,5 trisubstituted tetrahydrofuran ring of some natural products appears as a triplet when the largest groups were *cis* oriented and as a doublet of doublet when they were *trans* oriented. In compounds containing one THF ring, esterification/etherification of a hydroxyl group in the side chain carbon attached to position 2 of the THF ring was found to convert a triplet (in compounds not derivatized) into an atypical *dd* in the case of *cis* configured compounds and a typical *dd* into an atypical *dd* or a *triplet* in the case of *trans* configured compounds. Esterification/etherification of the hydroxyl elsewhere in the molecules did not affect the splitting pattern.



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PROSPECTING GREAT LAKES BACTERIA FOR DRUG-LEAD DISCOVERY WITH HIGH-THROUGHPUT MICROBIOME SCREENING

*Maryam Elfeki[1,2], Ankur Nakib[4], Stefan J. Green[4], Brian T. Murphy[1-3]**
[1]Department of Medicinal Chemistry and Pharmacognosy, [2]Institute for Tuberculosis Research, [3]Center for Pharmaceutical Biotechnology, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607; [4]DNA Services Facility, Research Resources Center, University of Illinois Chicago, Chicago, IL

Drug discovery ventures have focused on small molecule production from terrestrial actinomycete bacteria for nearly a century, and in the past few decades there has been a similar trend toward studies in the marine environment. Conversely, small molecule production from freshwater-derived actinomycete populations is virtually unexplored. To address this knowledge gap, systematic studies of freshwater actinomycete communities are needed to identify the capacity of these systems to afford both novel taxa and novel small molecules. As part of the current study, we contrasted sediment microbial communities between two of the interconnected Great Lakes (Lakes Huron and Michigan) using high-throughput amplicon sequencing to determine how actinomycete communities were structured with respect to environmental gradients, and if these communities differed significantly as a function of increasing collection depth and geographic location. In both lakes, significant differences in total bacterial and actinobacterial community structure were observed between sediments sampled from shallow and deep locations. Furthermore, by growing bacteria using different media types, we assessed what portion of the *in* situ community was accessible through cultivation, and whether it was possible to retrieve the actinomycete diversity observed from amplicon sequencing. Details of these studies will be presented.

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RESVERATROL/GLUCAN/VITAMIN C COMBINATION OFFERS STRONG STIMULATION OF IMMUNITY

Vaclav Vetvicka, Jana Vetvickova
University of Louisville, Department of Pathology, 511 S. Floyd, Louisville, KY, 40292

Natural products, useful in preventing and/or treating various diseases, have been sought after throughout the history of mankind. The longest history as immunomodulators belongs to beta glucans. Despite the demonstrated activity of various glucans, the search for optimalization of their biological effects still continues. Recently, numerous substances have been shown to have synergistic effects with glucans. We decided to compare the biological activities of insoluble yeast-derived β1,3-D-glucan with a combination of glucan/resveratrol/vitamin C. Our data show that whereas resveratrol or vitamin C alone had only limited (if any) effects on immune reactions, the combination significantly increased the phagocytosis of peripheral blood leukocytes, specific antibody response, nitrite anion production and apoptosis. This combination also showed strong effects on immunosuppression caused by various toxins. In addition, significant inhibition of cancer growth was found using two different experimental models. We can summarize that whereas resveratrol or vitamin C alone had only limited effects on immune reactions, the combination significantly increased all tested immunological reactions. Our data represent further proof that combined preparations of glucan, resveratrol and vitamin C strongly stimulate the immune reactions. We hypothesize that strong anti-cancer properties showed by the combination of these three compounds are manifested via stimulation of immune reactions and apoptosis. A study attempting to reveal the exact mechanisms of these effects is currently under progress.

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SILVESTROL INDUCES AUTOPHAGY AND APOPTOSIS IN HUMAN MELANOMA CELLS

Wei-Lun Chen[1], Joanna E. Burdette[1] and Steven M. Swanson[1,2]
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Silvestrol is a cyclopenta[*b*]benzofuran that was isolated from the fruits and twigs of *Aglaia silvestris,* which is indigenous to the island states of Southeast Asia. Previous testing of silvestrol revealed that it is a potent inhibitor of protein synthesis and has cytotoxic activity similar to or more potent than many FDA approved anticancer agents. Silvestrol is currently under preclinical development at the National Institutes of Health Experimental Therapeutic (NExT) program. The purpose of the current study was to determine if inhibition of protein synthesis caused by silvestrol triggers autophagy and apoptosis in solid tumors. By 24h a clear decrease in cyclin B and cyclin D expression was observed in silvestrol-treated cells relative to control. In addition, silvestrol blocks progression through the cell cycle at the G_2-phase. Silvestrol treatment also induced caspase-3 activation and apoptotic cell death in a time- and dose-dependent manner. Next, DAPI staining of nuclear chromatin showed nucleosomal fragments. Annexin V staining also showed an increase in apoptotic cells after silvestrol treatment. Furthermore, both silvestrol and SAHA enhanced autophagosome formation in MDA-MB-435 cells. Quantitation of the acidic vacuoles measured by flow cytometry further confirmed these results. MDA-MB-435 cells responded to silvestrol treatment with accumulation of LC3-II and dose-dependent p62 degradation. However, bafilomycin A, an autophagy inhibitor, resulted in the accumulation of LC3 in cells treated with silvestrol. Silvestrol represents a natural product scaffold with the potential for the study of autophagy and apoptosis mechanisms in cancer cells. It also highlights the direction of future drug development.

1006

SECONDARY METABOLITES FROM FUNGUS *QUAMBALARIA CYANESCENS*

Bishay DW[1], Abdel-Baki AM[1], Moharram AM[2], Malak LG[1] and Ross SA[3*]
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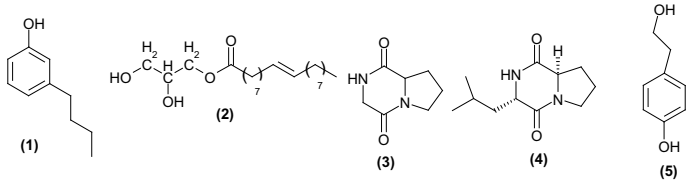
In this study, phytochemical and biological investigations for the fungus *Quambalaria cyanescens* were proceeded. This fungus was provided by Assiut University Mycological Center (Accession No. 4123). It was grown on PDB media and provided the isolation of one new compound; 3-butyl phenol (**1**), along with four known compounds: glycerol-monooleate (**2**), pyrrolopiperazine-2,5-dione (**3**), 3-isobutyl-pyrrolopiperazine-2,5-dione (**4**) and 2-(4'-hydroxyphenyl) ethanol (**5**). The structures of the isolated metabolites were elucidated based on spectroscopic and spectrometric techniques. All the isolated compounds were evaluated for their antimicrobial, antimalarial and antileishmanial activities.



1007

NEW ACIDIC TERPENOIDS FROM PSEUDOPTEROGORGIA ACEROSA

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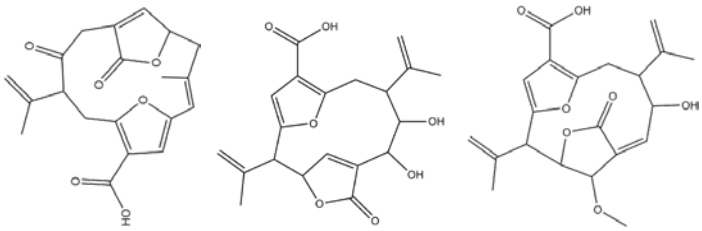
Abstract: One new cembranoid (**1**) and two new pseudopteranoids (**2**-**3**) were found in the Gorgonian coral *Pseudopterogorgia acerosa* collected in the Bahamas. These compounds demonstrate the rare carboxylic acid substitution pattern at C-18. Isolation of these compounds was performed using a combination of reversed phase column chromatography and preparative high pressure liquid chromatography. The structural elucidation was performed using extensive spectroscopic analysis, including mass spectrometry and 1D and 2D NMR spectroscopy.



1008

COMPARATIVE ANALYSIS OF THE ANTIOXIDANT PROPERTIES OF ICELANDIC AND HAWAIIAN LICHENS

Kehau Hagiwara,[1] Patrick R. Wright,[2] Nicole K. Tabandera,[1] Dovi Kelman,[1] Sesselja Ómarsdóttir,[3] and Anthony D. Wright.[1]
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Antioxidant activity of symbiotic organisms known as lichens is an intriguing field of research because of its strong contribution to their ability to withstand extremes of physical and biological stress (e.g., desiccation, temperature, UV radiation, and microbial infection). We present a comparative study on the antioxidant activities of 76 Icelandic and 41 Hawaiian lichen samples assessed employing the DPPH and FRAP based antioxidant assays. Utilizing this unprecedented sample size, we show that while highest individual sample activity is present in the Icelandic dataset, the overall antioxidant activity is higher for lichens found in Hawaii. Furthermore, we report that lichens from the genus *Peltigera* that have been described as strong antioxidant producers in studies on Chinese, Russian and Turkish lichens, also show high antioxidant activities in both Icelandic and Hawaiian lichen samples. Furthermore, we show that opportunistic sampling of lichens in both Iceland and Hawaii will yield high numbers of lichen species that exclusively include green algae as photobiont.

1009

NATURAL PRODUCTS INVESTIGATION OF HAWAIIAN SAMPLES OF *VERBASCUM THAPSUS* – COMMON MULLEIN

Daniela Weingärtener Rosa,[1] Kehau Hagiwara,[2] and Anthony D. Wright.[2]
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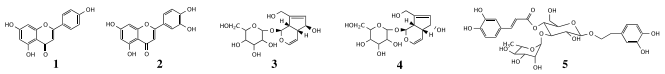
Verbascum thapsus or common mullein has a history of use in Europe that stretches back over 2000 years. It is commonly found in Asia, Africa and Europe and has found its way to Australia, North and South America and Hawaii, where it is classified as a weed. We became interested in this plant since it is only commonly found on the Big Island of Hawaii in areas where the ground has been recently disturbed at higher elevations, 1500 m, and because of its reported antimycobacterial (anti-TB) activity. Although the anti-TB activity of the methanol extract of the leaves proved to be negative we did find it to have some antimicrobial and antioxidant activty. On this basis we undertook fractionation of the extract and isolated and characterized the main components; apigenin (**1**), luteolin (**2**), aucubin (**3**), 6-epi-aucubin (**4**) and verbascocide (**5**). All of these isolates were determined to have antioxidant properties and apigenin (**1**), luteolin (**2**) and verbascoside (**5**) to have antimicrobial activity towards *Staphylococcus aureus*.



1010

MERGING GENOME MINING WITH ANCIENT THREE-WAY SPECIES CO-EVOLUTION MAY OPEN A NOVEL GATE FOR INFORMATIVE NATURAL PRODUCTS DISCOVERY

Walaa Kamel Mousa[1], Charles Shearer[1], Cassandra Ettinger[2], Jonathan Eisen[2] and Manish Raizada[1]
[1]Department of Plant Agriculture, University of Guelph, Guelph, ON Canada N1G 2W1, [2]University of California Davis, Genome Center, Davis, California, USA 95616

Finger millet is an ancient African cereal crop, resistant to many pathogens. It was domesticated 7000 years ago in Ethiopia, reaching India by 3000 BC. Unlike other cereals, finger millet is resistant to the toxigenic fungal pathogen *Fusarium graminearum*. As this fungus is also ancient to Africa, we hypothesized that the crop may host microbes (endophytes) that co-evolved to combat *Fusarium*. Here we describe the first ever discovery of endophytes from finger millet. We report a diverse array of anti-*Fusarium* secondary metabolites isolated from the fungal endophytes. A bacterial endophyte (strain M6) showed potent broad-spectrum antifungal activity including against *F.graminearum*, and was subsequently shown to be a novel *Enterobacter* species. Confocal microscopy showed that GFP-tagged M6 colonized different cereal tissues. Mechanistically, M6 caused cleavage of *F.graminearum* hyphae at septa. At the molecular level, Tn5 mutagenesis aided by whole genome sequencing and gene annotation uncovered 10 operons responsible for the anti-fungal trait, including at least two phenazine biosynthetic ORFs. Real time PCR revealed that most of the candidate genes are inducible by *F.graminearum*. Knockouts of the genes caused loss of cereal resistance to *F.graminearum*. Biochemically, three new phenazine derivatives were discovered for the first time. We conclude, informative search for bioactive compounds opens a new revolutionary era for natural products discovery.

1011

METHOD TO HARNESS BIOACTIVE SECONDARY METABOLITES FROM INTACT QUINOA SEEDS WITH IMPLICATIONS FOR CHRONIC DISEASE PREVENTION

Brittany L. Graf[1,3], Alexander Poulev[1], Peter Kuhn[1], Deborah Esposito[2], Mary Ann Lila[2], and Ilya Raskin[1]
[1]Department of Plant Biology and Pathology, Rutgers University, 59 Dudley Rd, New Brunswick, NJ 08901, USA [2]Plants for Human Health Institute, North Carolina State University, 600 Laureate Way, Kannapolis, NC 28081, USA [3]Centro de Investigación Traslacional, Universidad de Las Américas, Quito, Ecuador

Quinoa (*Chenopodium quinoa* Willd.) is an Andean seed crop rich in bioactive phytochemicals, including phytoecdysteroids (PE) and flavonoid glycosides (FG). Innovations designed to harness the pharmacological value of quinoa through simple, food-grade technologies may facilitate the development of functional foods and nutraceuticals to combat global human health challenges. We optimized a method to leach and concentrate quinoa bioactives from intact (un-macerated) quinoa seeds into aqueous ethanol, yielding a complex phytochemical mixture termed quinoa leachate (QL). QL, comprised of 1.0% PE and 2.6% FG, contained essentially all PE and FG available in the initial seeds compared with traditional extraction of macerated seed powder (567.6 µg PE and 540.9 µg FG/g seed). QL significantly lowered fasting blood glucose in obese, hyperglycemic C57Bl/6J mice and significantly attenuated lipopolysaccharide (LPS)-induced reactive oxygen species (ROS) production in human dermal fibroblasts. Quinoa seed leaching provides an efficient means to produce a food-grade mixture that may be applicable for the treatment and prevention of chronic, complex diseases, while rendering intact post-leached seeds available for additional uses in food.

1012

STRUCTURE ELUCIDATION AND BIOLOGICAL ACTIVITIES OF TWO CLASSES OF PEPTIDES PRODUCED BY THE ACTINOMYCETE, *KITASATOSPORA CYSTARGINEA*

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Department of Chemistry, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada.

Kitasatospora is a rare genus of Actinobacteria that represents an underexplored resource for the discovery of structurally diverse natural products. To highlight the production of unique metabolites produced by twelve members of this genus, the chemical screening method that combines LC-HRMS with Principal Component Analysis (PCA) was used. This approach identified the production of three novel compounds by *K. cystarginea*; which were determined to be a lipopeptide and two β-lactone containing peptide analogs, referred to as cystargamide and cystargolides A and B. Their structures were elucidated using NMR and MS/MS, and absolute configurations using advanced Marfey's Method. The therapeutic potential of cystargamide and cystargolides A and B were evaluated in several bioassays. Most notably, cystargolides A and B displayed inhibition of human 20S proteasome with IC_{50} values of 0.35 and 0.93 µM respectively.

1013

CONFORMATIONAL ANALYSIS OF SOME TYPE XII BISBENZYLTETRAHYDROISOQUINOLINE ALKALOIDS FROM *THALICTRUM ALPINUM* ASSISTED BY QUANTITATIVE NOE, *J* COUPLING CONSTANTS ANALYSIS AND ANISOTROPIC NMR PARAMETERS

C. Benjamin Naman[1], Gaurav Gupta[2], Sanjay Varikuti[2], Heebyung Chai[1], Raymond W. Doskotch[1], Armando Navarro-Vázquez[3], Abhay R. Satoskar[2], A. Douglas Kinghorn[1], and Roberto R. Gil[4]

[1]Division of Medicinal Chemistry and Pharmacognosy, and [2]Department of Pathology, The Ohio State University, Columbus, Ohio 43210, USA, [3]Departamento de Química Fundamental, Universidade Federal de Pernambuco, Cidade Universitária, CEP: 50.740-540 Recife, PE, Brazil, [4]Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213, USA.

Northalrugosidine (**1**), a type XII bisbenzyltetrahydroisoquinoline alkaloid, exhibits *in vivo* efficacy in a murine model of visceral leishmaniasis (*J. Nat. Prod.*, **2015**, *78*, 552-556). The conformational space of the macrocyclic moiety in compounds within the same subgroups of this class should provide more clues on their structure-activity relationships, which will be very beneficial for the potential future medicinal chemistry optimization of **1**. The solution-state conformation of this molecule was investigated by conducting NMR spectroscopic studies on two naturally occurring analogues in the same subclass, thalrugosidine (**2**) and thalidasine (**3**). Residual dipolar couplings (RDCs), collected in compressed PMMA gel, were employed for the determination of relative spatial orientation of ^{1}H-^{13}C bonds, whereas ^{1}H-^{1}H inter-atomic distances were determined by measurement of quantitative nuclear Overhauser effects (qNOEs). The conformation of the studied molecules in solution was determined by simultaneous fitting of these NMR data and $^{3}J_{H,H}$ coupling constants with computed molecular models.

1014

MS, NMR, AND DNA BARCODING, COMPLEMENTARY METHODS FOR IDENTIFICATION AND AUTHENTICATION OF BLACK COHOSH (*Actaea racemosa L.*)

James Harnly, Pei Chen, Kimberly Colson, Joe-Ann McCoy, Danica Harbaugh Reynaud, Peter Harrington

Flow injection mass spectrometry (FIMS), proton nuclear magnetic resonance spectrometry (^{1}H-NMR) and DNA barcoding, two metabolic fingerprinting methods, and DNA barcoding were used to identify and authenticate *Actaea* species. Initially, vouchered *Actaea racemosa* samples from a single sources were distinguished from other *Actaea* species based on principal component analysis (PCA) and soft independent modeling of class analogies (SIMCA) of FIMS and ^{1}H-NMR metabolic fingerprints. The chemometric results for FIMS and ^{1}H-NMR agreed well and showed similar agreements with the identity of some of the non-vouchered samples. DNA barcoding confirmed misidentifications and led to discovery of mislabeling in the laboratory. Differences were observed between vouchered *A. racemosa* samples from four different sources although the within *A. racemosa* species variance was significantly less than the between species variance. A model based on the combined A. racemosa samples still permitted distinction between species. Additionally, the combined *A. racemosa* were distinguishable from commercial root samples and from commercial supplements in tablet, capsule, or liquid form. DNA barcoding verified the lack of authenticity of the commercial roots and was unsuccessful in characterizing many of the supplements due to the lack of DNA.

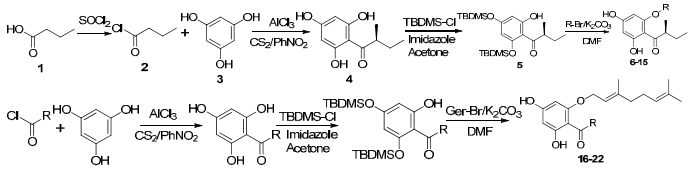
1015

SYNTHESIS OF ACYLPHLOROGLUCINOLS AND THEIR ANTIBACTERIAL ACTIVITIES AGAINST CLINICAL ISOLATES OF MRSA STRAINS

M. Mukhlesur Rahman,[1,2] Winnie Shiu,[1] Simon Gibbons[1] and John P. Malkinson[1]

[1]Department of Pharmaceutical and Biological Chemistry, UCL School of Pharmacy, London WC1N 1AX, UK, [2]School of Health, Sport and Bioscience, University of East London, London E15 4LZ, UK

Bioassay-directed drug discovery efforts on various species of the genus *Hypericum* led to the discovery of a number of acylphloroglucinols including 4,6-dihydroxy-2-*O*-(3",7"-dimethyl-2",6"-octadienyl)-1-(2'-methylbutanoyl)benzene (**6**) from *H. olympicum* with MICs of 0.5-1 mg/L against a series of MRSA strains. Its potential activity prompted us to carry out the total synthesis of **6** and its related analogues in order to assess their structure-activity profile as a new group of antibacterial agents. Total synthesis of **6** and a series of structurally related acylphloroglucinols (**7**-**22**) as well as their antibacterial activities against a panel of MRSA strains will be presented.



1016

PHYTOCHEMICALS AND THEIR ANTI-PROLIFERATIVE ACTIVITY OF *ALOE PERRYI* FLOWERS EXTRACT AGAINST VARIOUS HUMAN CANCER CELL LINES

Ebtesam S. Al-Sheddi[1], Mai M. Al-Oqail[1], Amina S. El-Shaibany[1], Nida N. Farshori[1]

[1]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

In this study, phytochemical screening of petroleum ether, chloroform, ethyl acetate, n-butanol and methanolic extracts of *Aloe perryi* flowers and their antiproliferative activity against seven human cancerous cell lines (namely liver (HepG2), colon (HCT-16), breast (MCF-7), lung adenocarcinoma (A549), prostate cancer (PC-3), human epidermoid cancer cells (HEp-2, and cervical cancer (HeLa)) were studied. The phytochemical screening was performed by Thin Layer chromatography (TLC) and antiproliferative activities by MTT assay. The quantitative tests of phytochemical screening revealed the presence of carbohydrates, glycosides, phytosterols, flavonoids, proteins and amino acids in the different extracts. The results also demonstrated that almost all of the extracts more or less had the capacity to decrease the proliferation of cancer cells. The HCT-16 cells were found more sensitive against all the extracts studied. Among the extracts, the highest activity for petroleum ether extract was found against HCT-16 cell line (IC_{50}=5.61 ug/ml) followed by HeLa (IC_{50}=5.83), A-549 (IC_{50}=7.54), MCF-7 (IC_{50}=7.88), HepG2 (IC_{50}=8.2), PC-3 (IC_{50}=9.51), HEp-2 (IC_{50}=10.1). The results indicated that the extracts used in this study having phytochemicals and anticancer activity against all the cell lines. However, further studies are needed for evaluating their mechanism of action and to isolate the active anticancer compounds responsible for this activity.

1017

A GATEKEEPER ENZYME IN THE BIOSYNTHESIS OF INDOLMYCIN

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[1]Department of Chemistry, University of British Columbia, Vancouver, British Columbia, V6T 1Z1, Canada.

Molecules discovered in the "golden age of antibiotics" are increasingly being revisited in an era where new antibiotics are desparately needed. One of these molecules, the bacterial tryptophanyl-tRNA synthetase inhibitor indolmycin, features a unique oxazolinone heterocycle whose biogenetic origins have remained obscure for over 50 years. We have identified and characterized the indolmycin biosynthetic pathway. Our work revealed the decades-long mystery of how the characteristic core oxazolinone ring is assembled from tryptophan- and arginine-derived metabolites. Our efforts have furthermore shown the key role of a gatekeeper enzyme, which acts to re-route metabolites away from a unique shunt product. Our work establishes the complete pathway for indolmycin formation and sets the stage for using genetic and chemo-enzymatic methods to generate new indolmycin derivatives as potential therapeutic agents.

1018

HONAUCIN A, MECHANISM OF ACTION AND ROLE AS A POTENTIAL CANCER PREVENTION AGENT.

Lena Gerwick[1], Samantha J. Mascuch[1], Gabrial Navarro[1], Paul Boudreau[1], Tristan M. Carland[1], Terry Gaasterland[1], William H. Gerwick[1].
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, California 92093.

Three related natural products, the honaucins A-C, were isolated from a cyanobacterium overgrowing a coral reef in Hawaii. Subsequent biological investigations revealed that these molecules inhibit both prokaryotic quorum sensing and eukaryotic inflammation. The honaucins were originally identified as molecules of interest in an *in vitro* assay that quantified their ability to attenuate nitric oxide production in LPS-stimulated macrophages. Continued experiments using honaucin A displayed a transcriptional down-regulation of IL-6, TNFα, IL-1β, and iNOS in these cells, as well as *in vivo* anti-inflammatory activity in a murine model of ear edema. To uncover the mechanism of action of honaucin A, RNA deep sequencing was performed using total RNA from honaucin A-treated macrophages. Analysis of differentially regulated transcripts strongly suggests that honaucin A is an activator of a pathway of cytoprotective genes. This signaling pathway has recently drawn interest for its potential application in the treatment of neurodegenerative and autoimmune diseases, as well as cancer. Experiments involving reporter assays and protein pull down using a biotinylated probe to validate the proposed target will be discussed.

1019

STAT3 INHIBITORS FROM HAWAIIAN-PLANTS ASSOCIATED FUNGI

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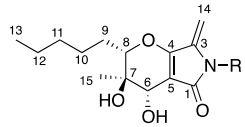
The STAT3 protein is a cytoplasmic transcription factor that is aberrantly activated in many human cancers. Constitutive STAT3 activation is a molecular abnormality that is causally linked to cancer aggressiveness. The STAT3-dependent cell-based luciferase reporter assay captures STAT3 transcriptional activity, based on the binding of active STAT3:STAT3 dimers to the STAT3-specific promoter sequence that drives the luciferase reporter. High Throughput Screening of our fungal natural product library against the STAT3-depedent NIH3T3vSrc cell line showed that some semi-pure fractions were active against STAT3. Assay-guided separation of the active sample FT462 led to the identification of STAT3 inhibitors, which inhibited A2780 and MDA-MB-231. The isolation, structure elucidation, plausible biosynthesis and bioactivity of compounds **1**-**5** will be presented.



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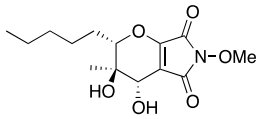

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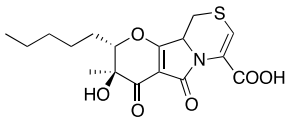

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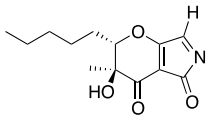

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1020

CYTOTOXIC ACTIVITIES OF THAI LAXATIVE MEDICINAL PLANT RECIPE EXTRACT AGAINST COLON CANCER CELLS

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

Trichintalamaga (TCG) recipe was discovered in Thai Phamacy scripture. This recipe has shown noteworthy lymphatic treat, which is closely related to its anticancer properties. The aim of the present study was to evaluate cytotoxic activities of TCG recipe and its fractions using the SRB assay. The assay was performed on human colon adenocarcinoma cell lines (LS 174T and SW 480). The ethanolic extract of TCG recipe was separated by using vacuum liquid chromatography (VLC). Different fractions were collected and tested for cytotoxic activity. The active fraction was identified for compounds using liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The ethanolic extract of TCG recipe showed the specific cytotoxicity against LS174T and SW 480 cell lines with IC_{50} values of 0.51 ± 0.01 and 0.51 ± 0.02 µg/ml, respectively. After liquid–liquid partitioning sequence, the chloroform fraction showed the highest cytotoxicity against LS174T and SW 480 cells lines with IC_{50} values of 0.45±0.04 and 0.51 ± 0.07 µg/ml, respectively. Fifteen compounds were isolated using LC-ESI-MS/MS guided isolation method. We concluded that major cytotoxic component should be isolated from the chloroform fraction. Therefore, these results support the use of Trichintalamaga recipe as an anticancer drug.

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BIOLOGICAL ACTIVITIES AND TOTAL PHENOLIC CONTENT OF TREGAYSORNMAS FORMULA

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[1]Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

Tregaysornmas formula was discovered in Thai Phamacy scripture including *Jatropha multifida* L., *Nelumbo nucifera* Gaertn. and *Aegle marmelos* L. In Thai traditional medicine, it used to treat fatigue and maintain body balance. The objective of this study was to investigate the anti-allergic, anti-inflammatory and antioxidant activities. The determination of total phenolic content of their extracts were also tested. The results showed that 95%EtOH and water extract of this formula showed the high antioxidant activity (3.861±0.109 µg/ml and 15.997±3.60 µg/ml, respecticely) and total phenolic content (300.236±2.226 mg GAE/g and 184.915±4.328 mg GAE/g, respecticely). Moreover, 95%EtOH extract exhibited a potent allergic activity (93.807±0.831 µg/ml). However, water extract had no anti-allergic activity. Both 95%EtOH and water extract did not show anti-inflammatory activity. We concluded that Tregaysornmas formula showed antioxidant and an-

ti-allergic activities and it was related with the ethnomedical use as maintaining body balance of patients in Thai traditional medicine.

1022

FLAVONOIDS MODULATING c-MET AND ALK ACTIVITY

Sun-Young Han[1], Young Ok Choi[2], Hyuk-Hwan Song[2], Young-Mi Kim[2], and Young-Won Chin[2]
[1]College of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju, Gyeongnam 660-701, Republic of Korea, [2]BK21PLUS R-FIND team and College of Pharmacy, Dongguk University-Seoul, Goyang, Gyeonggi-do 410-820, Republic of Korea,

Bioactivity-guided separation of the roots of *Scutellaria baicalensis*, using c-Met kinase assay, led to the isolation of 19 flavonoids. Of these isolates, norwogonin and baicalin were found to be active in the c-Met assay. Further investigation for c-Met inhibitory compounds in the ALK assay revealed that norwogonin and baicalin were active. Norwogonin and baicalin were found to inhibit both c-Met (IC_{50} 6.24 and 13.4 **μM**, respectively) and ALK kinases (IC_{50} 7.62 and 0.93 **μM**, respectively). Furthermore, the binding mode of norwogonin in the ATP binding site of c-Met was disclosed in docking study.

1023

STUDIES ON THE EXPLOITATION OF *BROUSSONETIA PAPYRIFERA* AND ITS ENDOPHYTIC FUNGI

Baokang Huang, Chunyan Zhang
School of pharmacy, Second Military Medical University, Shanghai, 200433, China

Broussonetia papyrifera (L.) Vent.(Moraceae) is a deciduous tree which is native to Asia. Its fructus is the common TCM "Chushizi" with bioactivities. Its leaves and barks also have medicinal uses. The endophytic fungi from *B. papyrifera* provide new natural products with biological activities. A total of 47 strains were isolated from the different parts of the tree. The leaves have high islation rates (average 0.91) with rich biodiversity. The anti-inflammatory activity of the fermentation products was investigated with mice macrophages RAW 264.7 *in vitro*. The results indicated that 14 endophytes could fully inhibit the growth of inflammatory cell at the concentration of 100 μg/mL. The cytotoxic activity of fermentation products was evaluated by the MTT method, and five endophytic fungi possess significant anti-hepatoma activity in human SMMC-7721 and QJY7023 cell lines. Thirty-six compounds were isolated from the ethyl acetate extract of the two strains *Chaetomium globousm* and *Alternaria* sp. Altertoxin IV is a new compound. Altersolanol A showed significant inhibition to human MG-63 and SMMC-7721 cell lines with IC_{50} values of 0.53 and 2.92 μg/mL. Endophytic fungi of *Broussonetia papyrifera* are promising sources of natural bioactive metabolites.

fungi of *Broussonetia papyrifera* are promising sources of natural bioactive metabolites.

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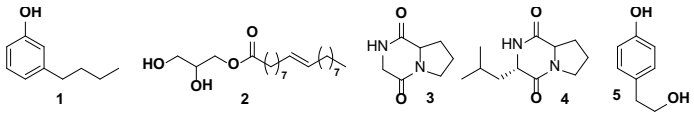
SECONDARY METABOLITES FROM FUNGUS *QUAMBALARIA CYANESCENS*

Dawoud Bishay[1], Afaf Abdel-Baki[1], Ahmed Moharram[2], Lourin Malak [1] and Samir Ross [3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt, [2]Mycological Center, Assiut University, Assiut 71515, Egypt, [3]National Center for Natural Products Research, and Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677

In this study, phytochemical and biological investigations for the fungus *Quambalaria cyanescens* were proceeded. This fungus was provided by Assiut University Mycological Center (Accession No. 4123). It was grown on PDB media and provided the isolation of one new compound; 3-butyl phenol (**1**), along with four known compounds: glycerol-monooleate (**2**), pyrrolopiperazine-2,5-dione (**3**), 3-isobutyl-pyrrolopiperazine-2,5-dione (**4**) and 2-(4'-hydroxyphenyl) ethanol (**5**). The structures of the isolated metabolites were elucidated based on spectroscopic and spectrometric techniques. All the isolated compounds were evaluated for their antimicrobial, antimalarial and antileishmanial activities.



1025

BIPHASIC MODULATION OF HUMAN CATHEPSIN L BY A NOVEL CYANOBACTERIAL DEPSIPEPTIDE

Bailey Miller[1], Matthew Bertin[1], Vivian Hook[2], and William H. Gerwick[1,2]
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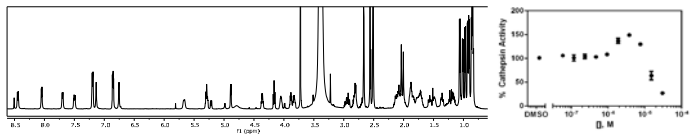
Target-based screening of marine cyanobacterial extracts for small molecule modulators of human cathepsin L, an intriguing target in both cancer and diseases related to the neurosciences, has led to the isolation of a novel depsipeptide natural product. This compound displays an interesting biphasic dose-response against the purified enzyme, characterized by activation at low doses and inhibition at high concentrations. Partial structural determination, based on NMR and MS^2 spectroscopy, has identified multiple modified residues, including the hydroxy acid form of methionine sulfoxide, 3-methyl-2-aminopentanoic acid, and *N-O*-dimethyl tyrosine. A complete structure and kinetic analysis of enzyme modulation will be presented.



1026

ANTI-INFLAMMATORY EFFECTS OF COMPOUNDS DERIVED FROM BOTANICAL MEDICINAL PLANTS IN LPS-STIMULATED THP-1 CELL LINE

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Despite of the importance of inflammation processes in protecting body from the pathogens, chronic inflammation is in the center of multiple important human diseases such as autoimmune and chronic inflammatory

diseases. In this study, we examined the anti-inflammatory effects of compounds derived from botanical medicinal plants on LPS-stimulated macrophages. Cytotoxicity assay, cytokine ELISA, immunofluorescence assay, and western blot analysis were performed to measure the proinflammatory cytokines response, p65 nuclear translocation, and expression of activated NF-κB mediators involved in NF-κB pathway. Out of our plant extract and compound library, we selected 3 best compounds for this presentation and they showed significant inhibition TNF-α, IL-1β, and IL-8 proinflammatory cytokines. Further investigation showed the inhibition of nuclear translocation of NF-κB transcription factor, p65, and significant reduction of inhibitor kappa B kinase, IKK-beta, phosphorylation which is an important regulatory component of NF-κB pathway.

1027

MASS SPECTROMETRY TOOLS FOR SCREENING OF MARINE CYANOBACTERIAL NATURAL PRODUCTS

Tal Luzzatto Knaan[1], Neha Garg[1], Yao Peng[1], Theodore Alexandrov[1], Gabriel Navarro[2], Evgenia Glukhov[2], Lena Gerwick[2], William H. Gerwick [1,2] and Pieter C. Dorrestein[1]
[1]Skaggs School of Pharmacy and Pharmaceutical Sciences, CA, [2]Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093, USA

The marine environment is an extraordinarily rich source of natural products. Here we employed tandem mass spectrometry tools to screen a large-scale dataset from a worldwide collection of marine cyanobacteria, aiming to visualize and map the chemical universe of their metabolites. Molecular networking was utilized to cluster molecules into families based on fragmentation similarities that enabled the dereplication of known compounds, the identification of new derivatives and targeting of novel natural products. Molecular features captured by mass spectrometry were subjected to geographical mapping, revealing the chemical distribution of these natural products and highlighting chemodiversity "hotspots" for potential new chemistries.

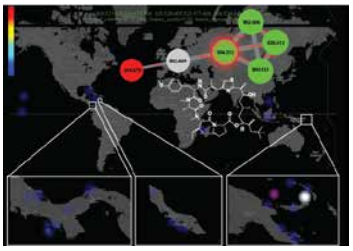
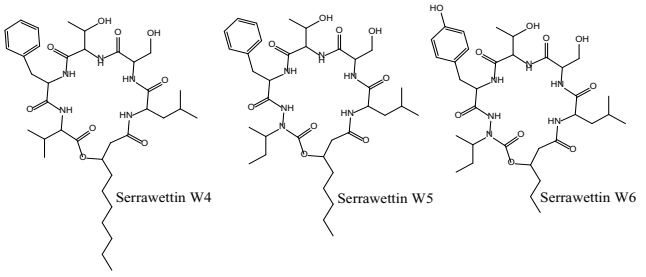


Fig 1: Mapping the distribution of Apratoxins molecular family $[M+Na]^+$ using GNPS molecular networking and "LCMS 2D/3D toolbox".

1028

ACTIVITY COMPARISON OF CYCLIC LIPODEPSIPEPTIDES FROM MAMMALIAN MICROBIOME BACTERIAL SOURCES

Jeremy Lynn Motley, Carter A. Mitchell, Tiffany Culver, Jarrod King, Susan L. Nimmo, Douglas R. Powell, & Robert H. Cichewicz
Department of Chemistry and Biochemistry, University of Oklahoma, 101 Stephenson Pkwy, Norman, OK 73019



Cyclic lipodepsipeptides (CLDP) are produced by bacteria and have been studied extensively for their antimicrobial activities and wetting properties. Bacteria derived from opportunistically sampled mammals, including opossum, skunk, raccoon, boar, and deer, in Oklahoma were cultured and shown to produce CLDPs including viscosin, serratiamycin, and surfactin. Two dimensional NMR studies and MS analysis lead to 3 new serrawettin W2 analogues. Their activities against several biological targets were assessed.

1029

DISCOVERY OF NEUROPROTECTIVE MARINE NATURAL PRODUCTS USING *DROSOPHILA MELANOGASTER*

Stacee Lee Caplan[1], Jennifer Krill[1], Ken Dawson-Scully[1], and Lyndon M. West[2]
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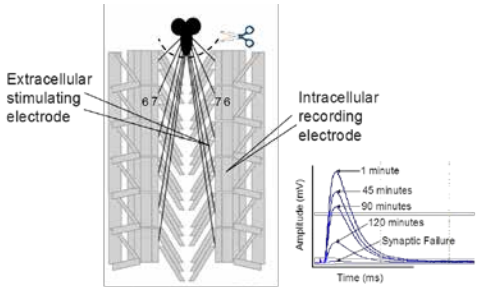
Oxidative stress and cellular excitotoxicity is inherent in the pathophysiology of an array of devastating human ailments. Natural products have played an important role in the treatment of a variety of human diseases and continue to drive the modern drug discovery and development process. The overall goal of this project is to design a novel neuroactivity assay to identify natural products capable of inducing neuroprotection against oxidative stress. This project will focus on a group of natural products, pseudopterosins, known for their potent biological activities with a novel mechanism of action. We hypothesize that these compounds may be of potential therapeutic benefit in protecting neurons and/or neural function both during and after episodes of excitotoxicity in disease states.

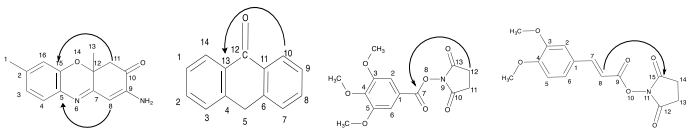


1030

INVESTIGATION OF NEIGHBORING HETEROATOMS AND COUPLING PATHWAY EFFECTS ON LONG-RANGE COUPLING CONSTANTS USING LR-HSQMBC

Ping Guo[1], John D. Anderson[2], Joseph J. Bozell[2], Svetlana Zivanovic[1]
[1]Department of Food Science and Technology, 2510 River Drive, University of Tennessee, Knoxville, TN 37996, United States, [2]Center for Renewable Carbon, 2506 Jacob Drive, University of Tennessee, Knoxville, TN 37996, United States TN 37996

LR-HSQMBC is one of the most recently developed NMR experiments for detection of long-range ($^nJ_{CH}$, n>3) heteronulcear coupling, and for overcoming the challenges of structure elucidation of proton deficient molecules. To investigate the dependencies of long-range coupling constants on the neighboring heteroatoms and coupling pathway between proton and carbon nuclei, LR-HSQMBC was used to visualize $^{4\text{-}6}J_{CH}$ in molecules **1**-**4** below. To aid in the investigation, density functional theory (DFT) was used, and a comparison of the calculated and experimental long-range coupling constant values will be presented.



1. Phenoxazine 2. Anthrone 3. 3,4,5-trimethoxy-acid-NHS ester 4. 3,4-dimethoxy-benzoic cinnamic acid-NHS ester

1031

INVOLVEMENT OF CASPASE-DEPENDENT MITOCHONDRIAL PATYWAY IN CORM-2 INDUCED APOPTOSIS IN NEURONAL CELLS

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Department of Pharmacology, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China

The aim of this study is to investigate the effects of Tricarbonyldichlororuthenium (II) dimer (CORM-2) induced apoptosis and its mechanism in neuronal cells of rats. Optical microscope was applied to observe the morphologic changes of neuronal cells. MTT assay was performed to assess the survival rates of CORM-2 on neuronal cells. Apoptosis was examined by flow cytometric analysis and the expression of relative proteins was measured by Western Blotting analysis. The results suggested that CORM-2 could influence survival rates in a time- and concentration-dependent manner. Survival rates decreased gradually after the cultures subjected to 24 h with 100 μmol·L^{-1}, 200 μmol·L^{-1}, 400 μmol·L^{-1} and 800 μmol·L^{-1} CORM-2. After 24 h treatment, CORM-2 could induce neuronal apoptosis and activate Caspase-3, Caspase-9 and Cytochrome C, the main proteins in Caspase-dependent mitochondrial pathway in a concentration-dependent manner. To sum up, CORM-2 maybe induced neuronal apoptosis through Caspase-dependent mitochondrial pathway.

1032

EFFECTS OF ZXX FROM THE RHIZOME OF *VALERIANA JATAMANSI* ON THE RATS WITH IBS VIA THE REGULATION OF 5-HT PATHWAY

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The effects of 11-ethoxyviburtinal (ZXX) on the rat with irritable bowel syndrome via 7 chronic unpredictable mild stressors in 9 weeks were evaluated. Results show that ZXX treatment for 9 weeks markedly decreased visceral hypersensitivity and prolonged the latency period of defecation in IBS rats. The anxiety- and depression-like behaviors were distinctly ameliorated by ZXX (0.6 and 1.2 mg/kg). Moreover, compared with the vehicle group, ZXX treatment at 0.6 and 1.2 mg/kg increased the contents of 5-HT in the hypothalamus and cortex. ZXX (0.3 and 0.6 mg/kg) elevated the activity of MAO-A in colon, but reduced MAO-A activity in the hippocampus. ZXX (0.3 mg/kg) inhibited the expression level of TPH‖mRNA in the colon, ZXX (0.6 and 1.2 mg/kg) inhibited the expression level of TPH|mRNA in the brain stem. Therefore, ZXX could ameliorate the visceral hypersensitivity, intestinal hyperactivity, and abnormal mental activity in IBS rats. The therapeutic mechanism maybe involved in the synthesis and metabolism of 5-HT pathway in periphery intestinal system and central nervous system.

1033

INVESTIGATION OF *IN VITRO* BIOACTIVITY OF EXTRACTS AND SECONDARY METABOLITES OF CHUMASH NATIVE AMERICAN MEDICINAL PLANTS

Brittany Allison[1], Victoria Hester[1], Matthew Fleming[1], Mark Allenby[1], Shane Bryant[1], and P. Matthew Joyner[1].
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Medicinal plants have historically been a valuable source of new drugs. One reason for this is because many lifetimes of experience have already demonstrated the effectiveness of the chemical constituents of these species in treating human diseases. Unfortunately, there is a severe lack of knowledge regarding the pharmacology and chemistry of many Native American traditional plant-derived medicines. We have investigated the *in vitro* bioactivity of extracts and chemical constituents of medicinal plants of the Chumash Native Americans of Southern California with the goal of establishing essential biological and chemical foundations for future studies of Chumash plant-derived medicines. Extracts of medicinal plants were assayed for anti-inflammatory bioactivity by quantifying cytokine production in murine macrophage cell cultures; extracts were also assayed for antibacterial bioactivity against a panel of representative bacterial species. Bioassay-guided fractionation was used to isolate three flavonoid compounds from *Artemisia californica* with antibacterial activity. Our results indicate that some plants that have traditionally been used by the Chumash people for the treatment of cuts and infections demonstrate *in vitro* anti-inflammatory and antibacterial bioactivity.

1034

VARIATION OF ACTIVE CONSTITUENTS OF AN IMPORTANT TIBETAN MEDICINAL PLANT *SWERTIA MUSSOTII* BETWEEN QINGHAI AND SICHUAN PROVINCE

Yue Liu[1, 2], Li Tang[1], Chunbo Wang[1], Yanxia Huang[1], Tianjiao Wei[1], Linxia Zhang[1], Chuanchuan Chen[1], Fengxian Guo
[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

S*wertia mussotii* Franch is an important Tibetan medicinal plant, which is called "Zangyinchen" in Tibetan region. Concetration of five phytochemical constituents(swertianolin(S_1), 3,7,8- trimethoxy-1-O-β-D- Glucoside (S_2), 1-hydroxy-3,4,7,8-formethoxyxanthone(S_3), 1,7-dihydroxy-3,8-dimethoxy- xanthone (S_4), l,8-dihydroxy-3,7- dimethoxyxanthone(S_5)) were determined and compared in different parts of the title plant, *Swertia mussotii*, collected from Qinghai and Sichuan Province. The concentration of S_1 was the most abundant in the buds of *Swertia mussotii* in Jinchuan county in Sichuan Province. While the concentration of S_2, S_3, S_4 and S_5 was the most abundant in the buds in the plants in Xiaojin county in Sichuan Province, the buds in Jinchuan county in Sichuan Province, the roots in Chengduo county in Qinghai Province, and the buds in Jinchuan county in Sichuan Province, respectively. The concentration of all of the constituents was highest in the buds, and successively reduced in buds, leaves, stems, and roots. Acknowledgment: financial support by project of NSFC-81274158, 81373765, NCET-12-0578, 13-0624, "111"-B08044, URTP2014110018 and YLDX01013.

1035

A POLYPHENOL ENRICHED FRACTION OF ROSE OIL DISTILLATION WATER INHIBITS PROLIFERATION IN HACAT CELLS AND INDUCES APOPTOSIS

Jonas Wedler[1, 2], Eliane Garo[2], Krasimir Rusanov[3], Matthias Hamburger[2], Ivan Atanassov[3], Veronika Butterweck[1]
[1]Institute of Pharma Technology, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Gründenstrasse 40, 4132 Muttenz, Switzerland, [2]Department of Pharmaceutical Sciences, University Basel, Klingelbergstr. 50, 4056 Basel, Switzerland, [3]AgroBioInstitute, 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria

Water steam distillation of rose flowers (*Rosa damascena*) separates the essential oil from the polyphenol containing rose oil distillation waste water (RODW). While the essential oil represents the desired liquid for the cosmetic industry, the polyphenol containing RODW is in the center of our interest. Recently, a strategy was developed to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-

(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects. In the present study, we investigated possible antiproliferative effects of RF20-(SP-207) and fractions thereof F(I)-(IV) in immortalized human keratinocytes (HaCaT). The BrdU cell proliferation assay was used to measure cell proliferation. Cell migration was elucidated by time lapse microscopy. The data demonstrated that from all tested fractions only F(IV) revealed a concentration dependent antiproliferative effect which was comparable to RF20-(SP-207) (IC_{50} of approx. 10 µg/mL). This effect was similar to both positive controls LY294002 (PI3K-inhibitor, 30 % inhibition) and NVP-BEZ235 (dual PI3K/mTOR-inhibitor, 30 % inhibition) and clearly exceeded the anti-proliferative action of quercetin (approx. 20 % inhibition). Time lapse microscopy revealed that cell migration was dramatically decreased under influence of RF20-(SP-207) and F(IV). This effect was comparable to LY294002 and NVP-BEZ235. Fluorescence microscopy images confirmed the qualitative increase of apoptosis under influence of RF20-(SP-207) and (IV).

1036

NEW STRATEGY TO SEARCH LEAD COMPOUNDS WITH UNIQUE STRUCTURES FROM PLANTS

Tom Villani[1,2], Kelsey Gustafson[1,3], Jing Zhen[1,2], James E. Simon[1,2], Qingli Wu[1,2,3]

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For more than a century, natural products have been the most consistent source of lead compounds in drug discovery. Based on biosynthesis pathway and basic structure, natural products found in seed plants (phytochemicals) could be categorized into three major groups including alkaloids, terpenoids and phenolics, and from the viewpoint of previous research and development on natural products chemistry, we now have sufficient data to suggest that most of the therapeutic agents derived from phytochemicals are alkaloids or terpenoids. Whereas the natural phenolic compounds play a vital role in disease prevention. In recent decades, wide application of LC/MS technique enabled us to pre-screen the chemical profile of the matrices and identify the materials containing unique molecules with pharmaceutical interest prior to the time-consuming process of isolation and elucidation. Using the novel strategy upon LC/MS pre-screening, dozens of medicinal plant samples collected from Africa on the basis of bioassay results and traditional application were subjected to in-depth chemical profiling using LC/MS. Under LC/MS guided screening, most species failed to yield unique/new compounds, and only a few plant species were found to contain unique/novel structures. Under this model, we were able to successfully narrow down the plant list to facilitate further natural products investigation, which is presently still very laborious work.

1037

GASTRIC ANTIULCER, ANTISECRETORY AND CYTOPROTECTIVE PROPERTIES OF CELERY (APIUM GRAVEOLENS) IN RATS

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**Departments of Pharmacognosy and Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia, †Department of Pathology, P.O. Box 2925, King Khalid University Hospital, King Saud University, Riyadh 11461, Saudi Arabia, [a]Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia*

Celery seed is generally regarded as safe for human consumption as a spice, natural seasoning and plant extract/essential oil. 500 grams of the shade dried aerial parts of celery was coarse powdered and macerated in 3 liters of 96% ethanol for 72 hours using percolation method**.** Wistar albino rats were used. Celery extract produced a dose-dependent significant protection against the ulcerogenic effect induced by indomethacin. In the ethanol and strong alkali-induced ulcer protocol, it was observed that the treatment with ethanolic extract of celery (250 and 500 mg/kg) significantly reduced the lesion index. A highly significant reduction of ulceration in rats' stomach and intraluminal bleeding was recorded after celery extract pretreatment at the dose of 500 mg/kg orally. In the gastric secretion determination model, using ligated pylorus for 6 h, the treatment with celery extract (250 and 500 mg/kg i.p.), reduced the volume of basal gastric secretion, titratable acidity and ulceration significantly in comparison with control group. The results show that ethanol extract of celery displays gastroprotective activity, as demonstrated by its significant inhibition of the formation of ulcers induced by different experimental models, and its ability to decrease basal gastric acid secretion. This gastric antiulcer capacity of celery extract could be related to its antioxidant properties, resulting in reduction of the lipid peroxidation and elevation of the NP-SH contents, besides, improving mucus coat of the stomach. Therefore, we suggest that due to its antioxidative effects, it may be useful in the prevention of gastric disorders.

1038

ANTI-INFLAMMATORY ACTIVITY OF A NEW CYCLIC PEPTIDE, CITRUSIN XI, ISOLATED FROM THE FRUITS OF *CITRUS UNSHIU*

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In this study, we investigated the active constituents from the fruits of *Citrus unshiu* and evaluated the anti-inflammatory activity in order to support the traditional usage of *C. unshiu*. A new cyclic peptide, citrusinXI (**1**), was isolated and identified from the fruits of *C. unshiu*. The structure of compound **1** was elucidated by spectroscopic analysis, including 1D and 2D NMR, and HR-mass spectrometry, and its absolute configurations were further confirmed by the Marfey's method. Compound **1** decreased NO production in LPS-stimulated RAW264.7cells in a dose-dependent manner with an IC_{50} value of 70 µM. Compound **1** suppressed NO production by decreasing iNOS expression but COX-2 expression was slightly associated with the reduction by compound **1** in LPS-induced RAW264.7 cells. Furthermore, compound **1** inhibited NF-κB activation by blocking IκBα degradation and NF-κB phosphorylation in LPS-stimulated RAW264.7cells. This is the first study to clarify the underlying mechanism of the anti-inflammatory effect exerted by a pure isolated compound from *C. unshiu* in LPS-stimulated RAW264.7 macrophage cells.

1039

YUANHUADINE, A NATURAL DITERPENE, ENHANCES THE DEGRADATION OF AXL TO OVERCOME ACQUIRED GEFITINIB-RESISTANCE IN LUNG CANCER CELLS

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College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib, remains a major problem in non-small cell lung cancer (NSCLC) treatment. Increased activation of AXL has been identified as a novel mechanism for acquired resistance to EGFR-TKIs in NSCLC treatment. However, the cause of uncontrolled AXL expression is not fully understood. Here, we first demonstrate that AXL is overexpressed in an acquired gefitinib-resistant cell line (H292-Gef) as a result of slow turnover and that AXL is degraded by presenilin-dependent regulated intramembrane proteolysis (PS-RIP). Based on the findings, we attempted to enhance AXL degradation to overcome acquired gefitinib-resistance by the treatment of gefitinib-resistant NSCLC cells with yuanhuadine (YD), a potent antitumor agent in NSCLC. Treatment with YD effectively suppressed the cancer cell survival *in vitro* and *in vivo*. Mechanistically, YD accelerated the turnover of AXL by PS-RIP and resulted in the down-regulation of the full-length AXL. Therefore, the modulation of the proteolytic process through degradation of overexpressed AXL may be an attractive therapeutic strategy for the treatment of NSCLC and EGFR-TKI-resistant NSCLC. [Acknowledgements: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (No. 2009-0083533 and No. 2010-0024361)]

1040

EVALUATING HEALTH CLAIMS ASSOCIATED WITH NATURAL PRODUCT CONTENT OF FOODS, SUCH AS EFFECTS OF SOY PROTEIN ON BLOOD CHOLESTEROL

Robin J. Marles[1], Karima Benkhedda[1], Cynthia Boudrault[1], Susan E. Sinclair[2], Chaowu Xiao[3], and Lynne Underhill[1]
[1]Nutrition Premarket Assessment Division, [2]Nutrition Regulations and Standards Division, [3]Nutrition Research Division, Bureau of Nutritional Sciences, Food Directorate, Health Products and Food Branch, Health Canada, 251 Sir Frederick Banting Driveway (Mail Stop 2203E), Ottawa, ON, Canada K1A 0K9

A health claim on the label or advertising of a food states, suggests or implies that a relationship exists between consumption of the food and health. All food health claims are voluntary and must not be false, misleading or deceptive. Claims related to a list, set out in Canadian law, of serious diseases, disorders and abnormal states (e.g., diabetes) are prohibited unless an exemption has been granted based on a pre-market evaluation. While other health claims are subject to post-market federal compliance verification, stakeholders can make a voluntary submission for pre-market evaluation. Proposed health claims are evaluated in a systematic, comprehensive and transparent manner to ensure they are valid. For example, the Food Directorate recently completed evaluation of a voluntary submission for a food health claim regarding consumption of soy protein and cholesterol lowering. Systematic review and meta-analysis showed significant ($P < 0.00001$) reductions in total cholesterol (-0.15 mmol/L, 95% C.I. -0.21 to -0.08) and LDL-cholesterol (-0.15 mmol/L, 95% C.I. -0.19 to -0.11) levels (approximately 2.6% and 4%, respectively) with consumption of soy protein vs. non-soy controls. Epidemiological and intervention data suggest that for every 1% reduction in LDL-C there is a corresponding 1–2% reduction in cardiovascular events, making reduction of elevated LDL-cholesterol an important public health goal.

1041

PLANTAGO ASIATICA EXTRACTS PREVENT SKIN PHOTOAGING IN HAIRLESS MICE

Hee Rae Kang[1], Hee Jeong Eom[1], Seulah Lee[1], Jae Sik Yu[1], and Seoung Rak Lee[1]
[1]Natural Product Research Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

In this study, we examined the anti-wrinkle effects of *Plantago asiatica* seed (PAS) on UV-induced human dermal fibroblasts (HDFs) and hairless mice models to determine if an anti-inflammatory agent could also be developed as an anti-aging treatment. Acute UV irradiation induced matrix metallproteinase (MMP)-1 protein expression levels, but this was suppressed by PAS in HDFs. Next, we investigated the effect of PAS on UV-induced skin changes in hairless mice. Chronic UV exposure of the dorsal skin increased skin thickness and induced wrinkle formation. PAS significantly suppressed UV-induced morphologic skin changes. In addition, MMP-1 expression was dramatically attenuated by treatment with plantainoside D which was purely isolated from PAS, indicating that this is the principle compound inhibiting MMP-1 expression in HDFs. Taken together, our data suggest that PAS can prevent the harmful effects of UV that lead to skin aging.

1042

CREATION OF A HIGH-THROUGHPUT, HIGH-CONTENT, SCREENING ASSAY FOR THE HUMAN PARASITE *TRICHOMONAS VAGINALIS*.

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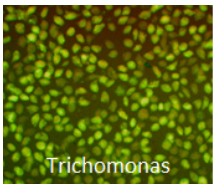
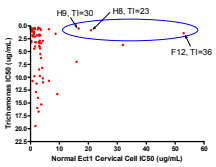
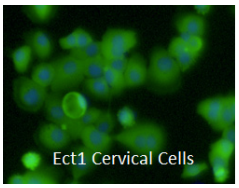
Institute for Natural Products Applications and Research Technologies, University of Oklahoma, Norman, Oklahoma 73019

The human sexually transmitted parasite *Trichomonas vaginalis* infects at least 170 million people worldwide and infections are treated with metronidazole, a suspected human carcinogen. An assay was developed in order to quantify the growth, viability, and morphology of the organism, using the Perkin Elmer Operetta imaging platform. A counter-screen for toxicity was also developed using the normal human cervical cell line Ect1/E6E7 and the Operetta. Initial screening was done on an internal library of pure compounds, followed by screening of our fungal extract library. Pure compounds with up to twenty times the potency of metronidazole and extracts with favorable therapeutic indices up to 36 were found. Active compound isolation for the extracts is in progress.







1043

GENE SCREENING AND METABOLOMICS FOR IDENTIFICATION OF HALOGENATED NATURAL PRODUCTS FROM MARINE SPONGE-ASSOCIATED BACTERIA

S. Goldberg[1], B. Haltli[1,2], F. Berrue[2], R. Kerr[1,2]
[1]Department of Biomedical Sciences, Atlantic Veterinary College, PEI, [2]Department of Chemistry, University of Prince Edward Island, PEI

Marine sponges and their associated microbes are promising sources of halogenated bioactive natural products. In many cases the halogen moiety is required for biological activity. Halogenation reactions are commonly cata-

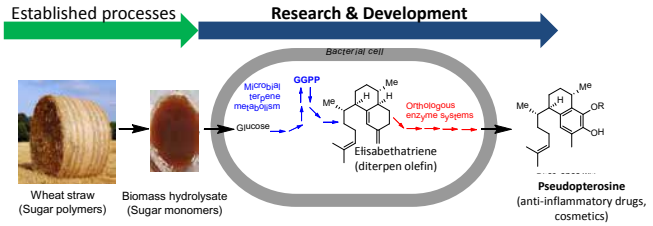
lyzed by flavin-dependent halogenases and vanadium-dependent haloperoxidases. This research addresses how to identify biosynthetically promising bacteria and how to access halogenated metabolites encoded in their genomes. A bacterial library consisting of 980 isolates was established from four Caribbean sponge species. A combinatorial approach utilizing genetic screening and an HRMS-based metabolomics workflow was developed to identify strains capable of producing halogenated metabolites. Small-subunit rDNA sequencing identified 25 OTUs using a similarity cutoff of 99%. Twelve strains represented putatively novel species based on 16S rDNA gene sequences exhibiting show ≤97% identity to known organisms. PCR screening identified 39 strains possessing the potential biosynthetic machinery necessary to produce halogenated metabolites. However, only a few putative halogenated metabolites were detected in a metabolomic analysis of 900 strains cultured under a single fermentation condition. This suggests that gene clusters encoding halogenated metabolites are cryptic in these strains. Investigation of enzyme diversity as well as induction efforts to elicit production of halogenated metabolites for strains positive in the PCR screen will be presented.

1044

ECOEFFICIENT PRODUCTION OF CORAL DERIVED PSEUDOPTEROSIN IN ENGINEERED *E. COLI*

Markus Reinbold[1], *Daniel Garbe*[1], *and Thomas Brueck*[1].
[1]*Division of Industrial Biocatalysis, Department of Chemistry, Technische Universitaet Muenchen, Lichtenbergstr. 4, D-85748 Garching, Germany*

Pseudopterosins are diterpene glycosides, derived from the Caribbean soft coral *Antillogorgia elisabethae*. These anti-inflammatory compounds have a growing market in the cosmetics and pharmaceutical industry. Current production is derived from wild harvested coral, which poses environmental and supply issues for an expanding market. In this project we purify the key biosynthetic enzymes from coral and identify their sequence using proteomic methods. The sequence data will be used to construct a heterologous pseudopterosin production system based on *E. coli*. To regulate metabolic flux, the biosynthetic elements will be integrated into the genome.



1045

IN VITRO ANTITUMOR EFFECTS OF TWO NOVEL COMPOUNDS *Y17* AND *Y18*, ISOLATED FROM A CHINESE MEDICINAL PLANT

Nadin Almosnid[1], *Ying Gao*[1], *Elliott Altman*[1]
[1]*Tennessee Center for Botanical Medicine Research and the Department of Biology, Middle Tennessee State University, Murfreesboro, Tennessee, United States of America.*

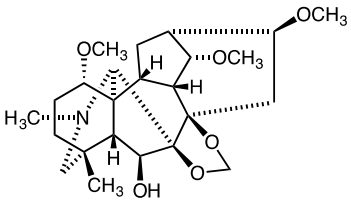
Naturally derived stilbenes have various biological properties, including cytotoxic, anti-ecdysteroidal, anti-mutagenic, anti-oxidative, anti-inflammatory, and anti-tumor activities. Previous phytochemical studies revealed that Chinese medicinal plants are rich in natural stilbenes. The purpose of the present study was to determine the anti-tumor effects and mechanism of action of the novel oligostilbene isomers, *Y17* and *Y18*, isolated from a Chinese medicinal plant. *Y17* and *Y18* exhibited remarkable cytotoxicity against selected human cancer cell lines A549, BT20, MCF7, and U2OS, but showed much less toxicity to two normal human cell lines, HPL1A and HMEC. We also demonstrated that *Y17* and *Y18* exerted their anti-tumor effects by stimulating apoptosis, decreasing membrane mitochondrial potential, inhibiting cell motility, and blocking the nuclear factor-kappa B (NF-κB) pathway in human cancer cells. In addition, we evaluated their respective bioefficacy and found that, interestingly, *Y17* is more potent than *Y18*. Collectively, we showed that both compounds *Y17* and *Y18* could be promising chemotherapeutic agents.

1046

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

Erika Asakawa, Yoshie Tosho, Ayako Nakata, Yuko Hasegawa, 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}-norditerpenoid alkaloids, melpheline (**1**), 19-oxoisodelpheline (**2**), *N*-deethyl-19-oxoisodelpheline (**3**), and *N*-deethyl-19-oxodelpheline (**4**), have been isolated from *Delphinium elatum* cv. Pacific Giant together with twelve known C_{19}-norditerpenoid alkaloids, delpheline, pacinine, pacidine, iminodelpheline, iminoisodelpheline, isodelpheline, yunnadelphinine, *N*-formyl-4,19-secopacinine, delcorine, 6-dehydrodelcorine, laxicyminine, and bonvalotidine C. The structures of these alkaloids were determined by their ms, 1D and 2D-nmr data. 6-Dehydrodelcorine, laxicyminine, and bonvalotidine C were isolated which have not previously been found in this plant.



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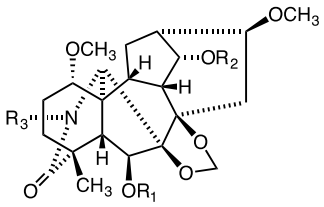


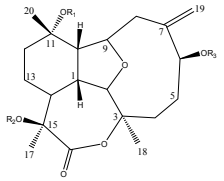
2: R_1=CH_3, R_2=H, R_3=CH_2CH_3
3: R_1=CH_3, R_2=R_3=H
4: R_1=R_3=H, R_2=CH_3

1047

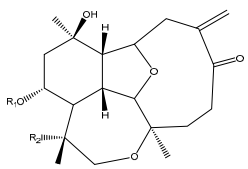
ISOLATION AND STRUCTURAL ELUCIDATION OF SIX TETRACYCLIC DITERPENOIDS FROM MARINE SOFT CORAL *BRIAREUM ASBESTINUM*

Long Zhang[1] *and Lyndon West*[1]
[1]*Department of Chemistry, Florida Atlantic University, Boca Raton, FL 33431, USA*

Six new tetracyclic diterpenoids (**1**-**6**) of the class briarellin were isolated from the methanolic extract of the gorgonian octocoral *Briareum asbestinum* collected off the coast of South Florida. Compounds **1**-**5** are the first series of compounds possessing an unprecedented oxygenated functional group at C-15, and compounds **5** and **6** are briarellins with a longest fatty acid moiety so far, $C_9H_{19}COO$-. The structures of these secondary metabolites were determined by spectral analysis including NMR and MS.



1 R_1=COC_3H_7, R_2=H, R_3=COC_3H_7
2 R_1=$COCH_3$, R_2=$COCH_3$, R_3=$COCH_3$
3 R_1=COC_3H_7, R_2=$COCH_3$, R_3=$COCH_3$



4 R_1=COC_7H_{15}, R_2=$COCH_3$
5 R_1=COC_9H_{19}, R_2=$COCH_3$
6 R_1=COC_9H_{19}, R_2=H

1048

FUNGAL ENDOPHYTES FROM TAXUS FAUNA AS A TREASURE OF BIOACTIVE COMPOUNDS

Hira Mehboob Mirza[1], Ulyana Muñoz Acuña[2], Safia Ahmed[1], Karl A. Werbovetz[2], Esperanza J. Carcache de Blanco[2], Masoom Yasinzai,[1]
[1]Department of Microbiology Quaid-i-Azam International University Islamabad, Pakistan, 45320, [2]Divisions of Pharmacy Practice and Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210

Throughout the ages humans have relied on nature to cater for their basic needs, not the least of which are medicines for the treatment of a wide spectrum of diseases. An increasing number of pathogenic microorganisms and the emergence of new diseases have created great demand for the search for novel chemical entities with improved antimicrobial properties. Among natural sources, recent discovery of microorganisms living asymptomatically in the tissues of higher plants—endophytic microorganisms—are a promising source of pharmaceutically important metabolites. Northern areas of Pakistan have rich biodiversity of *Taxus* species. Crude extracts from endophytic fungi isolated from indigenous species of *Taxus fauna* have been screened in biological assays, as part of a bioassay-guided fractionation scheme. Extracts of *Taxus fauna* have shown promising activities in antibacterial, NF-kB, K-ras and especially in antileishmanial assays. Cytotoxicity of the crude extracts has also been evaluated by the Sulforhodamine B (SRB) assays. Further fractionation of the crude extracts have resulted in partially purified fractions which will be further studied to identify the bioactive metabolites.

1049

MEDICINAL PLANT USE FOR MALARIA: TRENDS AND PROSPECTS

Woon-Chien Teng[1], Ho Han Kiat[1], Rossarin Suwanarusk[2], Cheng-Shoong Chong[1], Hwee-Ling Koh[1]
[1]Department of Pharmacy, National University of Singapore, Singapore 117543. [2]Singapore Immunology Network, Agency for Science Technology and Research, Biopolis, Singapore.

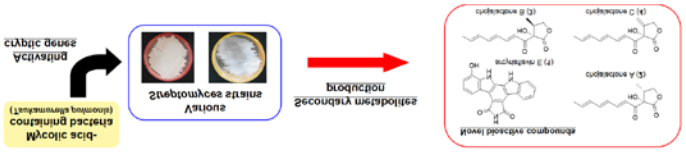
Malaria is a potentially life-threatening disease that affects millions each year, with resistance to most anti-malarial agents documented. Given that two of the widely used agents for malaria today, quinine and artemisinin, are derived from plant sources, medicinal plants are a potentially viable source of novel anti-malarial agents. The objective of this work is to carry out a literature survey on medicinal plants documented for use in malaria. Keywords "ethnobotanical", "survey", "ethnopharmacological", and "malaria" were searched in Scopus, ScienceDirect, and PubMed. Other books and websites were also used. Plants that were reported to be used solely for fever (other than malarial fever), external application, or as insect repellents, were excluded. Data collected were analyzed according to family, genus, location of use, method of preparation, part used, and indication (treatment and/or prevention). A comprehensive database documenting the usage and preparation of these medicinal plants for malaria has been compiled. A total of 1856 plants from 196 families and 1013 genera were reported to be used for malaria worldwide, with 66 plants reportedly used in three or more continents. Based on geographical extent of use, plant family, or genus, the plants for further research can be prioritized. There is evidence of sustained interest in anti-malarial medicinal plant research over the past decade. Detailed results of the survey will be presented, and trends and prospects of medicinal plants for use in malaria will be discussed.

1050

MYCOLIC ACID-CONTAINING BACTERIA INDUCE THE PRODUCTION OF NOVEL SECONDARY METABOLITES IN STREPTOMYCES STRAINS

Shotaro Hoshino[1], Lihan Zhang[1], Takayoshi Awakawa[1], Toshiyuki Wakimoto[2], Hiroyasu Onaka[3] and Ikuro Abe[1]
[1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan, [2]Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo , Japan, [3]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

It was previously reported that several kinds of mycolic acid-containing bacteria interact with various kinds of *Streptomyces* strains and induce the secondary metabolites production (Onaka, H. *et al. Appl. Environ. Microbiol.* 2011, **77**, 400–406). We applied this fermentation method to our collection of *Streptomyces* strains, employing *Tsukamurella pulmonis* as a mycolic acid-containing bacterium. As a result, novel cytotoxic alkaloid arcyriaflavin E (**1**) was isolated from *Streptomyces cinnamoneus* NBRC 13823 and novel cytotoxic butanolides chojalactones A-C (**2**-**4**) were obtained from soil-derived *Streptomyces* CJ-5 respectively. The stereochemistries (including absolute configurations) of **2**-**4** were determined based on careful spectroscopic analysis and the total synthesis of **2** and **3**, together with all of their stereoisomers.



1051

GENOTOXICITY EVALUATION *IN VITRO* BY COMET ASSAY OF COMPOUND AND CRUDE EXTRACT OBTAINED FROM *P.Pubescens* Benth.

Vanessa H.S.Souza[1,3], Ana Paula O.Hohne[2], Rogério Grando[3], Nubia de Cassia de Almeida Queiroz[3], Glaucia M.Pastore[4], Ana L.T.Gois Ruiz[2], João Ernesto de Carvalho, Humbert Moreiro Spindola[1,2], Mary Ann Foglio[1,3]
[1]CPQBA/FOP-Unicamp, Brazil, [2]DFT/CPQBA-Unicamp, Brazil, [3]DQPN/CPQBA-Unicamp, Brazil, [4]FEA-Unicamp, Brazil.

Pterodon pubescens Benth, native of Brazilian Cerrado, is used in folk medicine for pain treatment. Analgesic and anti-inflammatory efficacy was proven with further toxicity studies conducted to ensure safety issues. The genotoxic potential was assessed by Alckaline Comet *in vitro* Assay to detect DNA single-strand damage at level of eukaryotic cell, where CHO-K1 cells were exposed to the crude extract (EBD) and diterpene compound A (Dit-A) in 0.3, 3 e 30µg/ml concentrations for 4 hours. Cell suspensions, in agarose slides, were subjected to electrophoresis and then analyzed with ethidium bromide by fluorescence microscopy. The genotoxic potential was evaluated according to the percentage of DNA fragmentation and Tail Moment. The EBD 30µg/ml (13.11±13.20) and Dit-A 30µg/ml (17.73±9.95), when compared to control MMS 4,7µM (94.17±3.55), did not exhibit genotoxic potential (ANOVA, p<0,001).

1052

A PHENYLPROPANOID AND NEOLIGNANS FROM *MYRISTICA FRAGRANS* HOUTT WITH PARP-1 AND NF-κB INHIBITORY ACTIVITY.

Ulyana Muñoz Acuña[1,2], Peter J. Blanco Carcache[1,2], Susan Matthew[1,2], and Esperanza J. 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, 141N Parks Hall 500 W. 12th Avenue, Columbus, OH 43210.

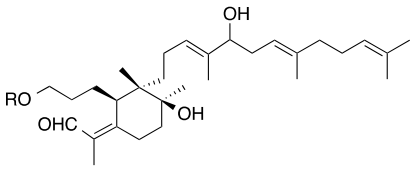
Bioassay guided fractionation of the aril of *M. fragrans* yielded five phenolic compounds, one new acyclic *bis* phenylpropanoid (**1**) and four previously known neolignans. Compound (**1**) (*S*) 1-(3, 4, 5-trimethoxyphenyl)-2-(3-methoxy-5-(prop-1-yl) phenyl)- propan-1-ol, (**2**) benzenemethanol; α-[1-[2,6-dimethoxy-4-(2-propen-1-yl)phenoxy]ethyl]-3,4-dimethoxy-1-acetate, (**3**) odoratisol A, phenol, 4-[(2*S*, 3*S*)-2,3-dihydro-7-methoxy-3-methyl-5-(1*E*)-1-propenyl-2-benzofuranyl]-2,6-dimethoxy, (**4**) 1,3-benzodioxate-5-methanol,α-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-acetate, (**5**) licarin C; benzofuran, 2,3-dihydro-7-methoxy-3-methyl-5-(1*E*)-1-yl-2-(3,4,5-trimethoxyphenyl). A NMR tube Mosher ester reaction was used to determine the absolute configuration of the new isolated chiral alcohol (**1**). PARP-1 inhibitory activity was evaluated for compound (**1**) (IC_{50} = 3.04 μM), compound (**2**) (IC_{50} = 0.001 μM), compound (**4**) (IC_{50} = 22.07 μM) and compound (**5**) (IC_{50} = 3.11 μM). All isolated secondary metabolites were also tested for NF-κB (p65) and K-Ras inhibitory activity. Compounds (**2**) and (**4**) displayed potent NF-κB inhibition, IC_{50} = 1.5 nM and 3.4 nM, respectively.

1053

ANTITRYPANOSOMAL ACTIVITY OF AN IRIDAL FROM *IRIS DOMESTICA* AND INITIAL STRUCTURE-ACTIVITY RELATIONSHIPS

Norma Dunlap[1,3], Anuradha Liyana Pathiranage[1], Jeannie Stubblefield[2], and Anthony Newsome[2,3]
[1]Department of Chemistry, Middle Tennessee State University, Murfreesboro, TN 37132, USA, [2]Department of Biology, Middle Tennessee State University, Murfreesboro, TN 37132, USA, [3]Tennessee Center for Botanical Medicine Research, Middle Tennessee State University, Murfreesboro, TN 37132, USA.

The myristate eseter of iso-iridogermanal was identified as an antitrypanosomal component of *Iris domestica* using bioassay-guided fractionation. This compound has previously been isolated from *I. domestica*, as well as other Iris species, and has been shown to be cytotoxic to tumor cells. Investigation of the antitrypanosomal activity of iso-iridogermanal, as well as semi-synthetic derivatives, will be presented.



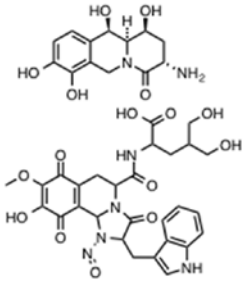
iso-iridogermanal (R = myristoyl)

1054

PLANT-LIKE ALKALOID BIOSYNTHESIS IN FUNGI

Joshua A. Baccile[1], Joseph E. Spraker[2], Eileen Brandenburger[3], Christian Gomez[1], Jin-Woo Bok[2], Dirk Hoffmeister[3], Nancy P. Keller[2], Frank C. Schroeder[1]
[1]Boyce Thompson Institute and Dept. of Chemistry & Chemical Biology, Cornell University, Ithaca, NY. [2]Dept. of Medical Microbiology and Immunology, University of Wisconsin-Madison, WI. [3]Hans-Knöll-Institute, Jena, Germany.

Traditional natural products discovery efforts have focused primarily on biosynthetic genes clusters (BGCs) containing large multi-modular NRPSs; however, sequencing of fungal genomes has revealed a vast reservoir of BGCs containing smaller NRPS-*like* genes whose biosynthetic functions have remained elusive. Using a comparative metabolomics approach, we show that a BGC in the human pathogen *Aspergillus fumigatus* named *fsq*, which contains an NRPS-like gene, produces a family of novel isoquinoline alkaloids, the fumisoquins. Assembly of these compounds is based on an unprecedented carbon-carbon bond formation between L-serine- and L-tyrosine-derived building blocks, followed by formation of the isoquinoline ring system via a sequence that is directly analogous to the biosynthesis of an important family of isoquinoline alkaloids in plants. Fumisoquin biosynthesis requires the *N*-methylase FsqC and the FAD-dependent oxidase FsqB, which represent functional orthologs of coclaurine *N*-methyltransferase and berberine bridge enzyme in plants. Analysis of fungal genomes revealed homologs of *fsq* genes in many species, suggesting that plant-like isoquinoline biosynthesis is widespread in fungi. We corroborated the biosynthetic role of these fungal genes by demonstrating isoquinoline biosynthesis by a different BGC in *A. flavus*. Our results provide a striking example for convergent evolution of a multistep biosynthetic mechanism and show that BGCs containing NRPS-like genes may reveal new biosynthetic paradigms.



1055

AN ETHNOBOTANICAL SURVEY ON THE USAGE OF FRESH HERBS

Yin-Yin Siew[1], Sogand Zareisedehizadeh[1], Wei-Guang Seetoh[1], Soek-Ying Neo[1], Chay-Hoon Tan[2], Hwee-Ling Koh[1]
[1] Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore, [2] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Medicinal plants are important sources of new therapeutics. The objective of this work is to interview users of fresh herbs regarding the usage and their perceptions. Information on demographic data, plant-use methods and perception of usage was collated. Chi-square or Fisher's exact tests were used to study the association between sociodemographic variables and goals/motivations for plant usage. Two hundred users (age 18 to 97) reported the use of 104 species of herbs in the last 5 years. The five most commonly used plants were *Clinacanthus nutans*, *Strobilanthes crispus, Pereskia bleo*, *Aloe vera* and *Zingiber officinale*. The top 3 most commonly cited medical conditions were diseases of respiratory system, neoplasm and diseases of circulatory system. Recommendation by others (150, 75.0%), efficacy (137, 68.5%), and safety (117, 58.5%) were common reasons given for using fresh herbs. 173 users (86.5%) did not consult any healthcare professional for advice about plant usage. Males were more likely to use herbs for treatment while females were more likely to use herbs for both general health and treatment (p = 0.020). Users with lower educational level were more likely than users with higher educational levels to use herbs because they perceived them to be safe and harmless given their natural origin (p = 0.016). Younger users were more likely to use herbs because of their af-

fordability (p = 0.006). Mild, self-resolving symptoms were reported by 28 (14.0%) participants using 16 plant species. Most users (170, 85.0%) were satisfied or highly satisfied with the outcome of plants used. Two hundred users of fresh herbs have been successfully interviewed and the information documented systematically in a database. The results suggest that fresh herbs have a role to play in healthcare in modern society. The information collated will serve as a useful resource for identifying promising plants for future drug discovery efforts.

1056

HERBAL PLANTS FOR ORAL DISORDERS IN CHINA

Chunlin Long[1,2], Yilan Zhou[1], Yuehu Wang[2], Guihua Tang[2]
[1]College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China, [2]Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

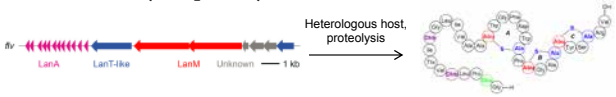
Based on literature studies, ethnobotanical and ethnomedicinal surveys in China, we recorded 1524 taxa of plants used for oral diseases from 36 ethnic groups and Han Chinese. They belong to 164 families and 599 genera. An inventory had been finished with names (local name, Chinese name and scientific name), botanical description, habitat and distribution, taste or smell, functions, parts used, preparing method, other uses and ethnobotanical information. Southwest China is the richest region with most species of medicinal plants, and with plentiful traditional knowledge to use herbal medicine to treat oral disorders. More species will be discovered in southwest China, particularly in Yunnan's ethnic communities. A few species used by ethnic people for treating oral disorders were selected for phytochemical and pharmacological studies. For instance, the stems of *Piper boehmeriaefolium* (Piperaceae) have been used to treat toothache by the De'ang people in southwest Yunnan. We isolated 30 compounds from *Piper boehmeriaefolium*, in which 8 are new ones. Most of theses compounds are alkaloids of amides, which showed good biological activities. This study was supported by National Natural Science Foundation of China (31161140345), the Ministry of Science & Technology of China (2012FY110300), the Ministry of Education of China (B08044) and the Minzu University of China (YLDX01013).

1057

LANTIBIOTICS FROM THE ANAEROBE *RUMINOCOCCUS FLAVEFACIENS* FD-1

Xiling Zhao[1] and Wilfred A. van der Donk[1,2,3]
[1]Department of Chemistry, [2]Biochemistry, University of Illinois at Urbana-Champaign, IL, USA, [3]Howard Hughes Medical Institute, Chevy Chase, MD, USA

Lanthipeptides are a class of ribosomally synthesized and post-translationally modified natural products, a subset of which display antimicrobial activity and are called lantibiotics. Lanthipeptide biosynthetic machinery is conserved, which facilitates discovery efforts. Genome mining revealed a lanthipeptide gene cluster within the anaerobic organism *Ruminococcus flavefaciens* FD-1 that represents an example of lanthipeptide combinatorial biosynthesis in which a pair of enzymes could be responsible for the modification of twelve substrate peptides. In order to systematically assess the structures and bioactivities of the peptides encoded within the cluster, a heterologous host and in vitro production strategy was employed to access the modified peptides. The presence of characteristic post-translational modifications in the processed peptides was confirmed by chiral gas-chromatography mass spectrometry and tandem mass spectrometry. Furthermore, a preliminary assessment revealed the FlvA peptides to be antibacterial and studies are underway to quantify their antimicrobial activity.

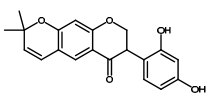


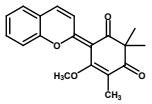
1058

DALEA EMORYI REVISITED: ANTHELMINTIC AND NEW COMPONENTS OF A PREVIOUSLY-STUDIED PLANT

Gil Belofsky[1], Po Keang Kouch[1], Jocelyn McCornack[2], Kaitlin Koppinger[2], and Blaise Dondji[2]
[1]Department of Chemistry, Central Washington University, Ellensburg, WA 98926, USA, [2]Department of Biological Sciences, Central Washington University, Ellensburg, WA 98926, USA.

Detailed chemical studies of *Dalea emoryi* A. Gray (syn: *Psorothamnus emoryi* (A. Gray) Rydb. (Fabaceae) were undertaken using taxonomic rationale. Other *Dalea* spp. have yielded anthelmintic components in our biological testing. *D. emoryi* was the subject of prior studies, and, interestingly, the crude extract was inactive in our bioassay. Nevertheless, new and active compounds **1** and **2**, for example, were revealed upon further purification of the crude *D. emoryi* materials. These findings agree with prior observations regarding the importance of screening partially purified materials in addition to crude extracts. Anthelmintic activities were determined in an *ex vivo* assay with the hookworm *Ancylostoma ceylanicum*. Isolation of compounds was by classical open-column chromatographic methods, with characterization using extensive 1D and 2D NMR spectroscopic methods and by HRESIMS. Details of the bioassay methods, results, and the isolation and characterization of *D. emoryi* compounds will be presented.





1059

BIOTRANSFORMATION OF BIOACTIVE MARINE NATURAL PRODUCTS BY THE MARINE-DERIVED MICROORGANISMS

Keumja Yun and Byeng W. Son
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As part of a program to explore the biological transformation of bioactive marine natural products produced by marine-derived actinomycete bacteria and ascomycete fungi isolated from marine habitats, we have studied biotransformation for some marine-derived fungal and bacterial metabolites. The biotransformation was carried out by some modified Two-Stage Protocol.[1,2] This presentation describes the production, isolation, and identification of new bioactive compounds by biotransformation. We will also show biological activity of the new biotransformed metabolites.

1. Smith, R. V. and Rosazza, J. P. *J. Pharm. Sci.* **1975**, *64*, 1737~1759.
2. Li, X.; Lee, S. M.; Choi, H. D. Kang, J. S.; Son, B. W. *Chem. Pharm. Bull.* **2003**, *51*, 1458-1459.

TOPIC - Microbial Natural Products
KEYWORDS - biotransformation, marine-derived actinomycetes and ascomycetes,

1060

CHEMICAL MYCOLOGY OF FRESHWATER ASCOMYCETES FROM NORTH CAROLINA, USA

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During our ongoing chemical mycological investigations of freshwater fungi in North Carolina, USA, we found two Dothideomycetes taxa from freshwater habitats in the Piedmont region that shared morphological similarities with species of *Minutisphaera.* These new collections prompted a chemical study of the secondary metabolites produced by this genus as well as a molecular phylogenetic study of relationships of taxa within *Minutisphaera*. Based on maximum likelihood and Bayesian analyses of molecular data, as well as examination of morphology, we describe and illustrate two new species of *Minutisphaera.* Chemical analysis of the organic extract *M. aspera* sp. nov. resulted in the isolation and characterization of five known secondary metabolites, of which four were dipeptides (1-4) and one was an aromatic polyketide (5). On the other hand, two aromatic polyketides (5 and 6) were isolated and identified from the organic extract of *M. parafimbriatispora* sp. nov. The isolated compounds were tested for their antimicrobial activity against an array of bacteria and fungi. Compound 6 showed promising activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* with MIC values of 30 and 60 µg/mL, respectively. The *Minutisphaera* clade did not share phylogenetic affinities with any existing taxonomic group within the Dothideomycetes. We therefore establish a new order, Minutisphaerales, and new family, Minutisphaeraceae, for this monophyletic clade of freshwater ascomycetes.

1061

REVERSED-PHASE HPLC ANALYSIS STUDIES OF THE SWEET DITERPENE GLYCOSIDES ISOLATED FROM STEVIA REBAUDIANA BERTONI

Venkata Sai Prakash Chaturvedula and Srinivasarao Meneni
Natural Products Research Group, Wisdom Natural Brands, 1203 West San Pedro Street, Gilbert, AZ 85233, USA.

High Performance Liquid Chromatography (HPLC) studies were performed on the nine sweet steviol glycosides reported in Joint Expert Committee on Food Additives (JECFA) namely rebaudioside A, steviolbioside, stevioside, rubusoside, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, and dulcoside A isolated from the leaves of *Stevia rebaudiana*. Using Reversed-Phase (RP) HPLC method, individual retention times and area for nine naturally occurring *ent*-kaurane diterpene glycosides of *S. rebaudiana* have been determined at various temperatures from 20˚C to 79˚C. HPLC results suggested that temperatures between 40˚C and 60˚C would be ideal conditions for better separation of steviol glycosides. Additional HPLC studies at five different temperatures 40˚C, 45˚C, 50˚C, 55˚C, and 60˚C under three different pH 2.4, 2.6, and 2.8 were performed and results suggested that temperatures 50˚C and 55˚C at pH 2.4 would be ideal condition for better separation of steviol glycosides.

1062

POST-TRANSLATIONAL ISOPRENYLATION OF TRYPTOPHAN

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A number of patterns of post-translational modification have been identified at various amino acid residues. One of them is isoprenylation, which generally refers to that of cysteine in eukaryotes. We first identified post-translational isoprenylation in prokaryotes. ComX pheromone from *Bacillus subtilis* had a post-translationally modified tryptophan residue with an isoprenoidal group, and ComX pheromone was biosynthesized by modifying enzyme, ComQ, with either a geranyl pyrophosphate or a farnesyl diphosphate and precursor peptide, ComX.

Here, we report novel tryptophan farnesyltransferase from *desulfitobacteria*, which belongs to the class Clostridia. After homologues of ComQ and ComX were found in *desulfitobacteria*, the modifying enzyme candidate was overexpressed in *Escherichia coli* and the substrate candidate was chemically synthesized by solid-phase peptide synthesis. Using them, *in vitro* farnesylation was carried out in the presence of farnesyl diphosphate and magnesium ion. Consequently, it was confirmed that the tryptophan residue in the peptide was farnesylated by using LC-MS/MS. It was the first report of post-translational isoprenylation of tryptophan in out of bacilli.

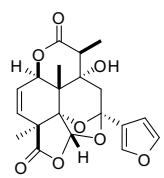
1063

OLD TERPENES AS NEW ANTICANCER LEADS

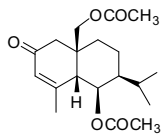
Khaled Orabi[1], Mohamed Abaza[2], and Susan Kurien[2]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and [2]Department of Biological Sciences, Faculty of Science, Kuwait University, Kuwait.

Plants were in the past and still are serving as a good source for providing lots of bioactive compounds including cytotoxic phytochemicals. Doxorubicin, vinca alkaloids and paclitaxel are just few examples.

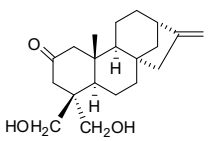
Three terpenes, saudinolide (**1**), plectranthone (**2**) and psiadin (**3**), were isolated from the dried aerial parts of *Cluytia richardiana* L., *Plectranthus cylindraceus* Hochst. ex Benth, and *Psiadia arabica* Jaub. et Spach, respectively. The isolated pure compounds were evaluated for their potential antiproliferative activities. Plectranthone (**2**) and psiadin (**3**) exhibited marked growth inhibition on colorectal and hepatocellular cancer cell lines in time- and dose-dependent manner with minimal cytotoxicity against normal human breast cells. The anticancer effects of psiadin on both colorectal and hepatocellular cancer cells were higher than that produced by saudinolide and plectranthone. Comparison with standard antineoplastic drugs indicated that the effects of **2** and **3** were comparable or even better than the tested cytotoxic drugs including 5FU, doxorubicin, camptothecin and ellipticine.



Saudinolide (**1**)



Plectranthone (**2**)



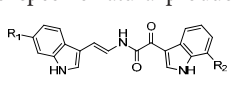
Psiadin (**3**)

1064

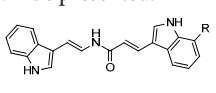
SYNTHESIS OF MARINE NATURAL PRODUCTS THAT CONTAIN A *CIS*-ENAMIDE FRAGMENT.

Hugo K. H. Fong, David Barker and Brent R. Copp
School of Chemical Science, University of Auckland, 23 Symonds St, Auckland, New Zealand

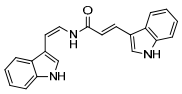
Indolic enamides are a rare class of marine natural products that have demonstrated a broad range of biological activities, including cytotoxicity and antibacterial activities. Examples of this class of compounds include coscinamides A-C, isolated from the marine sponge *Coscinoderma* sp., and chondriamides A-C, isolated from the red alga *Chondria* sp. and *Chondria atropurpurea*. While a number of synthetic routes targeting the *trans*-enamide moiety have been reported, little is known regarding the stereoselective synthesis of the indolic *cis*-enamide fragment. We have made use of a recently reported alkyne hydroamidation methodology to develop a general strategy for the synthesis of *cis*-enamide containing natural products. This strategy, its strengths and weaknesses, as well as approaches to the synthesis of specific natural products will be presented.



Coscinamide A R_1=Br, R_2=H
Coscinamide B R_1=R_2=H
Coscinamide C R_1=Br, R_2=OH



Chondriamide A R=H
Chondriamide B R=OH



Chondriamide C

1065

SYNTHESIS AND CHEMICAL REACTIVITY OF α,β-UNSATURATED CARBONYL FRAGMENTS RELATED TO THE MARINE NATURAL PRODUCT ONCHIDAL.

Melissa M. Cadelis, David Barker and Brent R.
Copp. School of Chemical Sciences, University of Auckland, Auckland, New Zealand.

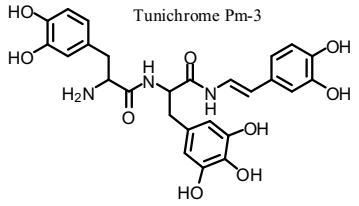
Resurgence of drugs that covalently modify their target has led to an expansion in this therapeutic class with 39 covalent drugs currently approved as treatments for a wide range of clinical applications. Many of these drugs contain electrophilic fragments (Michael acceptors) that are common among several natural products including curcumin, herbimycin A, butein, kaempferol and zampanolide. We have previously examined the electrophilic properties of the dienone-containing alkaloids the discorabdins and the ketofuran containing halenaquinone. As part of our ongoing studies in this area, we recently initiated model studies on the covalent reactivity of the marine natural product onchidal. The reactivity of α,β-unsaturated aldehydes and masked 1,4-dialdehydes were examined utilising model thiol- and amine- based nucleophiles such as 1-pentanethiol and 1-pentylamine. To model potential reactions with cellular protein targets, the reactivity of these compounds towards lysozyme were studied. The synthetic strategy taken towards model compounds and biological studies undertaken on these compounds will be presented.

1066

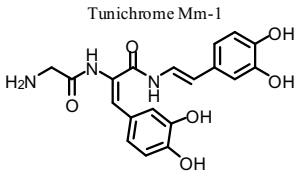
THE SYNTHESIS OF DEHYDRODOPAMINE CONTAINING MARINE NATURAL PRODUCTS

Michael A. Pullar and Brent R.
Copp. School of Chemical Sciences, University of Auckland, NZ

The tunichromes are a family of modified peptide compounds, isolated from the blood cells of various ascidian species, all of which contain a dehydrodopamine moiety within their structure. The biological role of tunichromes is uncertain, with vanadium uptake and protein crosslinking both being proposed. The low natural availability of these compounds, along with their instability, has meant a lack of biological analysis has been performed. By using a copper catalysed coupling of a styryl iodide with an amide, an enamide linkage can be formed to access these dehydrodopamine fragments. The mild reaction conditions have allowed for the synthesis of a library of dehydrodopamine enamides based upon the tunichromes, the synthesis and biological properties of which will be presented.



Tunichrome Pm-3



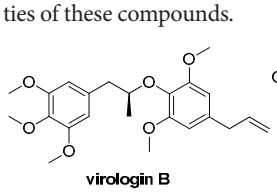
Tunichrome Mm-1

1067

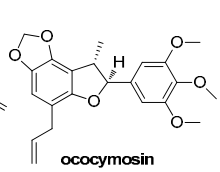
NEOLIGNANS AND OTHER METABOLITES FROM *OCOTEA CYMOSA* FROM THE MAGADASCAR RAIN FOREST AND THEIR BIOLOGICAL ACTIVITIES

Fotso S.,[‡] Xiong Q.,[‡] Graupner P. R.,[‡] Rakotondraibe H. L.,[†], Olson M.,[‡] Wiley J. D.,[§] Krai P.,[§] Brodie P. J.,[†]. Callmander M. W., ⊥Rakotobe E.,[‖] Ratovoson F., ⊥Rasamison V. E,[‖] Cassera M. B.,[§] Hahn D. R.,[‡] and Kingston D. G. I. [†]
[†]Department of Chemistry and Virginia Tech Center for Drug Discovery, [‡]Discovery Research, Dow AgroSciences, Zionsville Road, Indianapolis, [§]Department of Biochemistry and Virginia Tech Center for Drug Discovery, ⊥Missouri Botanical Garden, Madagascar, [‖]Centre National d'Application des Recherches Pharmaceutiques, Madagascar

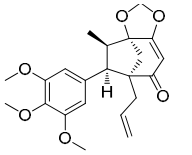
In the course of investigations on plant derived extracts from Madagascar rain forest against agricultural relevant plants pathogens. The bio-guided isolation on *Ocotea cymosa* (Lauraceae) yielded ten new neolignans along with seven known compounds. Three compounds exhibited inhibitory activity against *Aedes aegypti*, while ococymosin, ocobullenone derivative the known ocobullenone and virolongin B showed antiplasmodial activity. This poster highlights the isolation, structure elucidation and biological activities of these compounds.



virologin B



ococymosin



ocobullenone devivative

1068

A NEW ANTI-VIRULENCE STRATEGY AGAINST PATHOGENIC BACTERIA: TARGETING SPREADING FACTORS

Emily R. Britton[1], Carolyn B. Ibberson[2], Martha Leyte-Lugo[1], R. Owen Bussey III[1], Huzefa A. Raja[1], Nicholas H. Oberlies[1], Alexander R. Horswill[2], Nadja B. Cech[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina Greensboro, Greensboro, NC 27412, USA, [2]Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA

The widespread use of antibiotics against bacterial pathogens has caused a surge in the emergence of drug resistant strains. Treating infections caused by drug resistant bacteria costs on average twice as much compared to their drug-susceptible counterparts, and is a burden on the economy and the healthcare system. A promising strategy is the anti-virulence approach, which targets bacterial pathogenicity, thereby facilitating clearance of the infection without pressuring the pathogen to become resistant. Here, we propose to target the enzyme hyaluronidase as an anti-virulence approach against bacterial pathogens such as *Staphylococcus aureus* and *Streptococcus*

agalactiae. Hyaluronidase is an enzyme responsible for degrading hyaluronan in the body and contributes to bacterial growth and penetration. We have designed a mass spectrometry-based assay that can directly assess the anti-hyaluronidase activity of extracts and pure compounds. Additionally, we have identified several endophytic fungal species isolated from the roots of *Anemopsis californica*, a botanical used to treat skin infections, which produce compounds that inhibit hyaluronidase. This presentation will include the results of bioassay-guided fractionation experiments aimed at identifying hyaluronidase inhibitors from *A. californica* endophytes using the new assay.

1069

NOVEL ANTICANCER AGENT, SQAP, BINDS TO FOCAL ADHESION KINASE AND MODULATE ITS ACTIVITY

Jesús Izaguirre-Carbonell, Hirofumi Kawakubo, Hiroshi Murata, Keisuke Ohta, Tomoe Kusayanagi, Senko Tsukuda, Takeshi Hirakawa, Kazuki Iwabata, Yoshihiro Kanai, Sachihiro Matsunaga, Shinji Kamisuki, Kengo Sakaguchi and Fumio Sugawara
Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan.

Sulfoquynovosyl acylpropanediol (SQAP) is a novel and promising anticancer agent that was obtained by structural modifications from a natural compound. SQAP has the ability to trigger antiangiogenesis *in vivo* with very few side effects. In this study, the mechanism by which SQAP modifies the tumor microenvironment was revealed through the application of a T7 phage display screening. We could identify five SQAP-binding proteins including among them the FAT domain of focal adhesion kinase (FAK). Two independent experiments were used to verify the interactions. SQAP decreased FAK phosphorylation and cell migration in human umbilical vein endothelial cells and A549 cancer cells. The obtained data suggest that SQAP antiangiogenic activity may be due the modulation of FAK phosphorylation. Additionally, we hypothesized that SQAP radiosensitizing activity might be at least partly due to ROS generation. Indeed, we observed that SQAP romotes ROS formation on HeLa cancer cells.

1070

BIOCONVERSION OF CURCUMIN AND ITS ANALOGS

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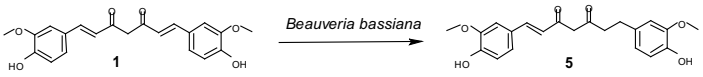
Chemistry, Biochemistry and Physics Department, The University of Tampa, 401 West Kennedy Blvd., Tampa, FL 33606

Reduction of curcumin (**1**), a chemopreventive agent from Turmeric (*Curcuma longa* L.), yielded five products. The three major compounds were identified as 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-dione (**2**), 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptan-3-one (**3**), and 1,7-bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-diol (**4**). Incubation of compound (**2**) with *Beauveria bassiana* ATCC 7159 afforded the compound (**3**) as the sole metabolite. Bioconversion of curcumin (**1**) with *Rhizopus oryzae* ATCC 11145 yielded 1,7-bis(4-hydroxy-3-methoxyphenyl) hept-1-en-3,5-dione (**5**) and metabolites **3** and **4**. Metabolite **2** was not produced. No transformation of curcumin (**1**) was observed with *Aspergillus niger* ATCC 16888. The bioactivities and structural elucidation of these metabolites are reported herein.



1071

PHARMACOPEIAL METHODS FOR POTENTIAL CONFOUNDING HERBAL MEDICINES

Cuiying Ma, Nam-Cheol Kim, Gabriel I. Giancaspro
Department of Foods, Dietary Supplements, and Herbal Medicines, U.S. Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville MD, 20852

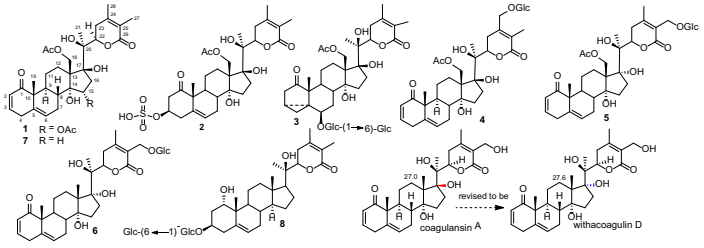
In order to promote the quality of herbal medicines, USP launched Herbal Medicine Compendium (HMC) in May 2013. The monographs in HMC include selective analytical procedures to reduce the possibility of adulteration by substitutes with potential confounders, mainly based on chromatographic techniques, such as, HPLC, UPLC, HPTLC. We present examples in this work based on the monographs developed for *Lonicera japonica* Flower, *Schisandra chinensis* Fruit, *Polygonum multiflorum* Root, *Antrodia camphorata* Fruiting Body and *Terminalia chebula* Fruit. Orthogonality between HPLC and HPTLC procedures provide greater assurances in this matter. The HPLC and HPTLC identification sections of the monograph efficiently distinguish the potential confounding materials for each plant.

1072

WITHANOLIDES FROM *PHYSALIS COZTOMATL*

Huaping Zhang[1], Cong-Mei Cao[1], Robert J. Gallagher[1], Victor W. Day[2], Kelly Kindscher[3], and Barbara N. Timmermann[1,]*
[1]Department of Medicinal Chemistry, School of Pharmacy,[2]The Small-Molecule X-ray Crystallography Laboratory, [3]Kansas Biological Survey, University of Kansas, Lawrence, KS 66047

Six new withanolides (**1**-**6**), as well as the known physachenolide D (**7**) and withanoside VI (**8**), were isolated from the aerial parts of *Physalis coztomatl* (Mociño & Sessé) Ex Dunal (Solanaceae). Structural elucidations of **1**-**6** were achieved through spectroscopic techniques including 2D NMR, while the structure of **1** was confirmed by X-ray crystallography. ^{13}C NMR analysis revealed that coagulansin A in the literature contains a 17α-hydroxy rather than the reported 17β-hydroxy functionality.



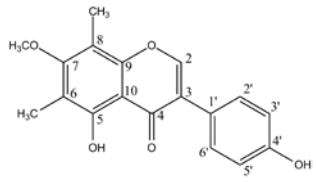
1073

A NOVEL ISOFLAVONE FROM *LEIOPHYLLUM BUXIFOLIUM* (ERICACEAE) AND ITS ANTIPROLIFERATIVE EFFECT

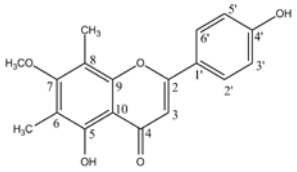
Dan Tian[1], John R. Porter[1]
[1]Program in Pharmacognosy, Department of Chemistry & Biochemistry, University of the Sciences, Philadelphia, PA 19104 USA

A new *C*-methyl-isoflavone, isosideroxylin **1**, and a known *C*-methyl-flavone, sideroxylin **2**, were isolated from the EtOAc extract of the leaves of *Leiophyllum buxifolium*. HR-ASAP-MS and 1D and 2D NMR spectroscopic data were used to determine the structure of **1**. The two compounds were evaluated for their antiproliferative effects against ER$^-$MDA-MB-231 and ER$^+$MCF-7 breast cancer cell lines and the NIH3T3 mouse fibroblast cell line with the sulforhodamine B assay. Compound **2** showed moderate antiproliferative activity on both cancer cell lines, while compound **1** displayed selective antiproliferative effect against MDA-MB-231 cells only, which supports the hypothesis of phytoestrogenic activity of **1**. Neither

compound was inhibitory to the NIH3T3 mouse fibroblasts at the concentrations examined. Since **1** and **2** share the A ring substitution pattern, we concluded the 5-hydroxy-7-methoxy-6,8-dimethyl substitution pattern on the A ring is responsible for the antiproliferative activity. The presence of a *C*-methyl-isoflavone in the Ericaceae supports the presence of a isoflavone synthesis pathway in this family.



1



2

1074

HIT-TO-LEAD OPTIMIZATION OF SIPHOLANES FOR THE CONTROL OF INVASIVE BREAST CANCER THROUGH SUPPRESSION OF BRK AND FAK SIGNALING

Ahmed I. Foudah[1], Mohamed R. Akl[2], Asmaa A. Sallam[2], and Khalid A. El Sayed[2]

[1]Department of Pharmacognosy, College of pharmacy, Sattam bin Abdulaziz University, Al-Kharj, KSA, [2]College of Health and Pharmaceutical Sciences, The University of Louisiana at Monroe, Monroe, LA 71209

Sipholane triterpenes are natural products isolated from the Red Sea sponge *Callyspongia siphonella*. Semisynthetic modifications afforded sipholenol A 4β-*O*-3`,4`-dichlorobenzoate (SPA) as a potent breast cancer migration inhibitor, with an IC_{50} of 1.3 μM in the wound-healing assay, without cytotoxicity to the non-tumorigenic breast cells MCF10A. The effects of SPA on the growth, migration, and invasion of diverse human breast cancer cells were studied. Results showed that SPA inhibited the growth of the human breast cancer cells MDA-MB-231, MCF-7, BT-474, and T-47D in a dose-dependent manner. SPA suppressed breast cancer cell migration, invasion, and decreased Brk and FAK activation in a dose-dependent manner. Molecular docking study suggested a perfect fitting at the FAK's FERM domain, inhibiting the main autophosphorylation site Y397, which was further confirmed by Western blot analysis. In vivo studies showed that SPA treatment suppressed breast tumor growth, CD31, p-Brk and p-FAK expression in orthotopic breast cancer in nude mice. Pharmacophore modeling and 3D-QSAR studies highlighted the important pharmacophoric features responsible for the antimigratory activity and Brk phosphorylation inhibition, including rings A and B (perhydrobenzoxepine) together with substituted aromatic ester moiety, creating a simpler structure and eliminating rings C and D ([5,3,0]bicyclodecane system). This opens new horizons for future design of novel sipholane-inspired active leads with perhydrobenzoxepine-aromatic cores, feasibly and cost-effectively.

1075

NEW TECHNIQUES IN EXTRACT COLUMN SCREENING II

Jack E. Silver

Teledyne ISCO, 4700 Superior Street, Lincoln, NE 68504

Column screening is an integral first step in purifying natural products. Efficient column screening allows facile and rapid purification of active components for further purification. The utilization of automated flash chromatography offers opportunities to streamline the column screenings of extracts. Combining pre-packed columns of a variety of chemistries with wide-polarity range chromatography is the first step in streamlining the process. Useful column chemistries include silica, ion-exchange, alumina, C18, and diol. Coincident to streamlining column screening is the incorporation of simultaneous detection techniques such as UV-Vis, mass spectrometry, and ELSD. Utilizing this enhanced automated procedure not only allows rapid purification and early correlation with previously discovered compounds, but also enables the researcher to focus on potentially novel active targets. The purification of extracts from the leaves of *Camellia sinensis* using ion exchange columns establishes a model protocol for other extracts. The screening of other column types was discussed previously.[1] Using this same example the advantages of simultaneous multiple detection techniques will be illustrated, as well as purification with a combined Flash/Preparative HPLC system.

[1]Silver, J.E.; Bailey, C. New Techniques in Extract Column Screening. Presented at the American Society of Pharmacognosy meeting, August 2014; poster PM3

1076

FURTHER (9βH)-PIMARANES AND DERIVATIVES FROM *ICACINA TRICHANTHA*

Ming Zhao,[1] Michael M. Onakpa,[1,2] Wei-Lun Chen,[1] Bernard D. Santarsiero,[1] Steven M. Swanson,[1,3] Joanna E. Burdette,[1] and Chun-Tao Che.

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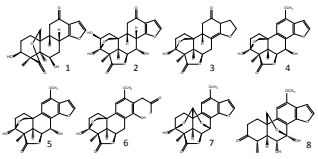

Further to our previous reports on the isolation of (9βH)-pimaranes, (9βH)-17-norpimaranes, and 17-norpimaranes from the tubers of the African medicinal plant *Icacina trichantha*,[1-2] eight additional diterpenoids (**1**-**8**) were identified. Noteworthily, **8** is a 17,19-dinorpimarane. We propose diterpenoids in *Icacina* plants are biosynthesized from (9βH)-pimarane rather than pimarane. All isolates were evaluated for cytotoxic activity against MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines, with humianthenolide C being most active (IC_{50} of 0.7 μM).

[1]Chemistry & Biodiversity (2014), 11(12), 1914-1922.
[2]Journal of Natural Products (2015), DOI: 10.1021/np5010328

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METHOD DEVELOPMENT FOR ACCESSING NOVEL MYXOBACTERIAL CHEMOTYPES: ANALYSIS OF SEPARATE CULTURE CONDITIONS IN THE PURSUIT OF OPTIMIZING SECONDARY METABOLITE PRODUCTION

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Myxobacteria are among the most metabolically talented Gram-negative δ-proteobacteria capable of producing a diverse array of secondary metabolites. Nearly 40% of myxobacteria-derived metabolites possess novel chemical structures. They are among the more intensively studied bacteria because of their unique "wolfpack" predatory lifestyle and cooperative cellular morphogenesis. From a chemical standpoint, the natural products secreted during their micropredatory activities represent an exciting opportunity to explore novel chemical mechanisms used by the myxobacteria to subdue microbial prey. However, pursuing these chemical-ecology-driv-

en studies present one major challenge in that their secondary metabolite production often result in low yields. This project aims to overcome these issues by exploring new culture conditions in hopes to optimize secondary metabolite production.

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METABOLITE AND TRANSCRIPTOME ANALYSIS OF AN AUSTRALIAN EREMOHILA PLANT AND ITS CORRELATION TO ANTIBACTERIAL EFFECTS

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The Australian plant *Eremophila* belongs to the *Myoporaceae* tribe and includes over 200 different species. Some *Eremophila* species have been used in the Aboriginal medicine to alleviate symptoms like skin lesions and sore throat [1]. Various studies showed antibacterial effects of different *Eremophila* species against a wide range of Gram positive bacteria. Interestingly, we also observed an antibacterial effect of semipurified *Eremophila serrulata* fractions against the Gram negative bacterium *Proteus vulgaris*. This study deals with the elucidation of terpene biosynthetic pathways in *Eremophila serrulata*. Different terpene transcripts were identified by applying different bioinformatic approaches in a normalized transcriptome data set sequenced on the Illumina MiSeq system. The poster presents different terpenes identified from this plant and describes the results from sequence analysis of the transcriptome for metabolic reconstruction and of the expression and characterization of biosynthetic enzymes.
[1] Ndi *et al.* (2007). *Phytochemistry*, **68**.

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CHEMICAL INVESTIGATION OF WINTER FIREFLIES (*ELLYCHNIA CORRUSCA*) REVEALS LUCIBUFAGINS

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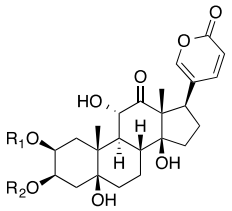
The behavior and coloration of the winter firefly, *Ellychnia corrusca*, are indicative of the presence of chemical defenses. Also, *E. corrusca* is proposed to be phylogenetically related to other species of firefly that are known to exhibit chemical defenses. However, the identity of the defensive chemistry of *E. corrusca* is unknown. Through extraction and partial purification, four compounds were identified by one-dimensional and two-dimensional NMR techniques, including COSY, HMBC and HSQC. The structures of these compounds were determined to be steroidal pyrones in the lucibufagin family. Presence of this class of molecules in *E. corrusca* confirms that it has chemical defenses against predators and supports a recent molecular phylogenetic tree.



1 R_1 = Ac R_2 = Ac
2 R_1 = Ac R_2 = H
3 R_1 = H R_2 = Ac
4 R_1 = H R_2 = H

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ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF ISOLATED COMPOUNDS FROM *GARCINIA BRASILIENSIS*

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Garcinia brasiliensis, a plant native to the Brazilian Amazon Rainforest, is used in traditional medicine to treat inflammation of the urinary tract, peptic ulcers, arthritis and others. The purposes of this study were to analyze the chemical constituents of branches and leaves from *G. brasiliensis* and to evaluate the potential of isolated compounds to act as inhibitors of both the oxidative burst of stimulated neutrophils and oxidative damage in human erythrocyte membranes. The biflavonoids procianidine, fukugetine, amentoflavone and podocarpus flavone isolated from *G. brasiliensis* had potent inhibitory effect on the oxidative burst of neutrophils, reaching 50% inhibition at 1 μmol L^{-1} on total reactive oxygen species (ROS) production and NADPH enzyme. These biflavonoids were also potent inhibitors of hemolysis and lipid peroxidation of human erythrocytes, reaching the malondialdehyde level (a biomarker of oxidative stress) of 8.5 ± 0.3 nmol/mg Hb at 50 μmol L^{-1} for procianidine. These findings indicate that these biflavonoids modulate the oxidative stress, suggesting the use of *G. brasiliensis* extract as antioxidants and anti-inflammatory agents. *Acknowledgements: FAPESP* (grants #2010/52327-5, #2011/03017-6 and #2013/07600-3), *CNPq and CAPES.*

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TRIGGERING EPIPOLYTHIODIOXOPIPERAZINE ALKALOID BIOSYNTHESIS IN FUNGAL ENDOPHYTES OF GOLDENSEAL (HYDRASTIS CANADENSIS)

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Epipolythiodioxopiperazine (ETP) alkaloids are secondary metabolites that are biosynthesized in fungi and have been reported to have notable cytotoxic and antibacterial activities. ETPs are isolated from a wide variety of fungi, and their biosynthesis does not seem to be linked to certain genera. Preliminary LC-HRESIMS dereplication suggested the presence of ETPs in extracts of two fungal endophytes from goldenseal (*Hydrastis canadensis*). However, these secondary metabolites were not encountered when regrown, which is a common challenge with fungal endophytes. Therefore, an evaluation of growth conditions to reactivate and maintain the ETP biosynthetic pathway was conducted by adopting the "One Strain-Many Compounds" (OSMAC) approach to culture-based methods. Both endophytes were plated on solid media that varied in nutritional value and in the presence of autoclaved goldenseal and fungal material to trigger the targeted pathway. When these cultures reached full growth on Petri plates, they were subjected to extraction. Then dereplication and mass defect filtering was carried out to identify the presence of potential verticillin analogues under distinct conditions.

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CONSTITUENTS FROM *LITSEA JAPONICA* LEAVES AND THEIR INHIBITORY EFFECTS ON AGES FORMATION AND ALDOSE REDUCTASE

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In our continuing efforts to identify effective naturally sourced agents for diabetic complications, two new (**1** and **2**) and 15 known compounds (**3**–**17**) were isolated from the 80% EtOH extract of *Litsea japonica* leaves. The structures of the new compounds were established by spectroscopic and chemical studies. These isolates (**1**–**17**) were subjected to an *in vitro* bioassay evaluating their inhibitory activity on advanced glycation end-product (AGE) formation and rat lens aldose reductase (RLAR) activity. Of the tested compounds, flavonoids (**4**, **6**–**8**, **11**, and **12**) markedly inhibited AGE formation with IC_{50} values of 7.42–72.05 μM, compared with that of a positive control, aminoguanidine (IC_{50} = 975.9 μM). In the RLAR assay, consistent with the inhibition of AGE formation, flavonoids (**4**, **6**–**8**, **11**, and **12**) exhibited considerable inhibition of RLAR with IC_{50} values of 1.10–12.5 μM.

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LOCALIZATION OF KABIRAMIDES IN THE SPONGE *PACHASTRISSA NUX* AND SPONGE SURFACE-ATTACHED BACTERIA

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The sponge *Pachastrissa nux* has been reported to be a rich source of cytotoxic macrolides. In our previous study, we found that the *P. nux* sponge has the ability to develop the capitum part, a gorgonian-like structure that sprouts from the substratum-attached base, and accumulate the macrolides predominantly in the protruding capitum. Using kabiramides B, C, and G as markers, here we demonstrate the impact of kabiramides allocation on the sponge surface-attached bacteria. The population density of bacteria that attach to the surface of the capitum part and also on the appendage extending from the core base part was significantly less than that on the surface of the base itself. Selected bacteria were identified, but no unusual bacterial species that may be specific to the sponge were detected. Although too premature to conclude whether the sponge intentionally produces such toxic metabolites as chemical defense against the microbial foulers, or otherwise the settling microbes affect the chemical allocation, the correlation between the macrolide accumulation and the attachment of bacteria on the sponge's surface clearly shows how the sponge has adapted to fit in its specific environment and how it interacts with the microbial cohabitants.

1084

TWO NEW LACTAMS FROM THE HULLED SEEDS OF *COIX LACHRYMA-JOBI* VAR. *MA-YUEN*

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Two new lactams, coixspirolactam F ((2*R*,3*R*)-(+)-2-octanoate-spiro[furan-3(2*H*),3'-[3*H*]indole]-2',5(1'*H*,4*H*)-dione; **1**) and coixspirolactam G ((2*R*, 3*R*)-(+)-6'-hydroxy-2-octanoate-spiro[furan-3(2*H*),3'-[3*H*]indole]-2', 5(1'*H*,4*H*)-dione; **2**) were isolated from the hulled seeds of *Coix lachryma-jobi* L. var. *ma-yuen* (ROM. CAILL.) STAPF (Gramineae). Their structures were elucidated by physical and spectroscopic data analysis, including 1D and 2D NMR, ESI-MS, and CD experiments.

1085

CONSTITUENTS OF *ANGELICA KEISKEI* AND THEIR HEAT SHOCK PROTEIN INDUCING ACTIVITIES

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Seven new compounds, 4,2',4'-trihydroxy-3'-[(2*E*,5*E*)-7-methoxy-3,7-dimethyl-2,5-octadienyl]chalcone (**1**), (±)-4,2',4'-trihydroxy-3'-[(2*E*)-6-hydroxy-7-methoxy-3,7-dimethyl-2-octenyl]chalcone (**2**), 4,2',4'-trihydroxy-3'-[(2*E*)-3-methyl-5-(1,3-dioxolan-2-yl)-2-pentenyl]chalcone (**3**), 2',3'-furano-4-hydroxy-4'-methoxychalcone (**4**), (±)-4-hydroxy-2',3'-(2,3-dihydro-2-methoxyfurano)-4'-methoxychalcone (**5**), (±)-4,2',4'-trihydroxy-3'-{2-hydroxy-2-[tetrahydro-2-methyl-5-(1-methylethenyl)-2-furanyl]ethyl}chalcone (**6**), and (+)-(2'*S*,3'*S*)-2',3'-dihydro-2'-hydroxyisopropyl-3'-methoxyangelicin (**15**), were isolated from the aerial parts of *Angelica keiskei* Koidzumi (Umbelliferae) together with eight known chalcones **7**-**14**, which were identified as 4,2',4'-trihydroxy-3'-[(6*E*)-2-hydroxy-7-methyl-3-methylene-6-octenyl]chalcone, xanthoangelol, xanthoangelol F, xanthoangelol G, 4-hydroxyderricin, xanthoangelol D, xanthoangelol E, and xanthoangelol H, respectively. The isolates, **1**-**15** were evaluated for their promoter activity on heat shock protein 25 (*hsp25*, murine form of human *hsp27*). Among them, compounds **1** and **7** activated the *hsp25* promoter by 21.9- and 29.2-fold of untreated control at 10 μM, respectively. Further protein expression patterns of heat shock factor 1 (HSF1), HSP70, and HSP27 by **1** and **7** were examined. Compound **7** increased the expression of HSF1, HSP70, and HSP27 with 4.3-, 1.5-, and 4.6-fold of untreated control, respectively, without any significant cellular cytotoxicities, whereas compound **1** did not induce any expressions of these proteins. As a result, compound **7** seems to be a prospective HSP inducer.

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PROFILING FUNGAL CULTURES *IN SITU* VIA THE DROPLET-LMJ-SSP COUPLED WITH UPLC-PDA-HRMS-MS/MS

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Ambient ionization mass spectrometry techniques have recently become very prevalent in natural product research due to their ability to examine an organism *in situ*. Profiling a sample directly on an organism without the need for lengthy regrowth and extraction procedures is extremely attractive. However, direct ambient ionization techniques, such as DESI and nanoDESI, lose the valuable capability of chromatographic separation, thus limiting the ability to differentiate isomers and confirm metabolite identities. To overcome this limitation, a droplet-liquid microjunction-surface sampling probe (droplet-LMJ-SSP) can be coupled with UPLC-PDA-HRM-MS/MS, thus gaining the separation, retention times, and UV data. By capturing these mutually supportive data, the identity of secondary metabolites can be assigned confidently and rapidly. Using the droplet-LMJ-SSP, a protocol was constructed to dereplicate fungal cultures directly from the Petri dish.

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THE COMBINATION OF DNA BARCODING AND METABOLOMICS FOR THE MULTI-CONSTITUENTS CHARACTERIZATION OF LICORICE BOTANICALS

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Licorice sold as Botanical Dietary Supplements (BDSs) can be obtained from mainly three *Glycyrrhiza* species: *G. glabra*, *G. uralensis*, and *G. inflata*.[1] Therefore, BDSs are composed of either single *Glycyrrhiza* species, mixtures of *Glycyrrhiza* species, or even hybrids. A DNA barcoding method[2] was optimized and applied for the authentication of 51 licorice acquisitions (capsules, bulk powders, sticks). Complementary chemometric models were developed with samples containing only single authenticated *Glycyrrhiza* species (37 among 51): PCA/SIMCA were performed on ^{1}H NMR spectra, and CDA was carried out using UHPLC-UV data. These models enabled the chemical distinction of source *Glycyrrhiza* species, and the metabolomic characterization of licorice mixtures. Together with DNA barcoding, both models were utilized for the identification of *Glycyrrhiza* hybrids and species outliers. The study demonstrates that the composition in major flavanones and chalcones is specific to each *Glycyrrhiza* species, and can be used for the characterization of licorice sold as BDSs.[3] Additionally, the present work addresses the congruence and complementarity between DNA analysis and metabolomics.

Abbreviations: PCA: Principal Component Analysis, **CDA:** Canonical Discriminant Analysis, **SIMCA**: Soft Independent Modeling of Class Analogy;

Ref: (1) WHO monographs 1999, 1:183–194; (2) Kondo, K. et al. *Biol. Pharm. Bull.* 2007, 30:1497-1502; (3) Simmler, C. et al. *J. Nat. Prod.* 2015 (submitted)

1088

STELLIOSPHAEROLS A AND B, SESQUITERPENE-POLYOL CONJUGATES FROM AN ECUADORIAN FUNGAL ENDOPHYTE

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Endophytic fungi are plant tissue-associated fungi that represent a rich resource of unexplored biological and chemical diversity with potential applications in the fields of medicine, bioremediation, and agriculture. As part of an ongoing effort to explore endophytes from the Amazon forest, numerous fungi were isolated and cultured using plants collected from the rainforest in Ecuador. From these samples, DNA sequencing and scanning electron microscopy (SEM) revealed a previously undescribed fungus in the order Pleosporales that was cultured from the tropical tree *Duroia hirsuta*. Extracts of this fungal isolate displayed modest activity against *Staphylococcus aureus,* and were thus subjected to detailed chemical studies. Two compounds with very modest antibacterial activity were isolated and their structures were elucidated using a combination of NMR spectroscopic analysis, LC-MS studies, and chemical degradation. These efforts led to the identification of stelliosphaerol A (**1**) and B (**2**), new sesquiterpene-polyol conjugates that are responsible, at least in part, for the *S. aureus* inhibitory activity of the fungal extract.

Funded by FNLCR contract HHSN261200800001E

1089

ISOLATION AND STRUCTURE ELUCIDATION OF MEROCYCLOPHANE C FROM THE CULTURED CYANBACTERIUM *NOSTOC* SP. (UIC 10110)

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Cyanobacteria are known to be prolific producers of biologically active natural products. Initial screening of the extract of UIC 10110, a *Nostoc* sp. based on 16S rRNA phylogenetic analysis, found it to be cytotoxic against MDA-MB-435 cells and HT-29 cells in an antiproliferation assay. Dereplication by mass spectrometry and NMR indicated the presence of a potentially novel active constituent which was isolated using semipreparative C18 HPLC. Accurate mass spectrometry allowed for the determination of the molecular formula. This in combination with the proton NMR spectrum revealed the active compound to be a [7,7] paracyclophane. The complete structure of Merocyclophane C was determined by a combination of 1D and 2-D NMR experiments. The absolute stereoconfiguration of Merocyclophane C was determined by CD spectroscopy.

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EXPLORING THE SPECTRUM OF ANTIMICROBIAL ACTIVITY FROM NATIVE NORTH AMERICAN PRAIRIE PLANTS

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For the past three years this lab has been investigating the use of North American prairie plants as sources of natural preservatives and antimicrobial compounds for use in personal care products. The experimental approach was to perform a simple agar-disk assay against a Gram positive bacteria from crude plant extracts. Compounds isolated from active fractions were then quantitatively assayed against Gram positive bacteria to determine their EC_{50} values. Commercial topical antibiotics (trioclosan) and preservatives (parabens) EC_{50} values were determined for the same assay and compared to the isolated plant compounds. To be effective antimicrobials and preservatives compounds must display an array of antimicrobial activity. We have expanded our antimicrobial assay to include organisms associated with non-sterile commercial personal care products (*E.coli, C.albicans, A. niger, P. aeruginosa* and *S. aureus.*). This presentation will discuss the spectrum of efficacy of compounds isolated from *J. virginiana* and *P. argophylla* against the aforementioned microorganisms.

1091

A UPDATE OF BOTANICAL DRUG DEVELOPMENT IN THE UNITED STATES: STATUS OF APPLICATIONS

Kuei-Meng Wu, Charles Wu, Jinhui Dou, Hanan Ghantous, Sau Lee, Lawrence Yu

We conducted a survey of botanical drug applications across the therapeutic divisions within CDER, FDA since 1999. The objective is to evaluate the status and trend of botanical IND submissions under the current U.S. regulatory environment. The overall number of botanical submissions are as follows: 1999: 21(including Pre-IND: 0), 2000: 20(4), 2001: 24(3), 2002: 21(5), 2003: 40(9), 2004: 33(11), 2005: 44(6), 2006: 33(11), 2007: 46(7), 2008: 41(14), 2009: 37(10), 2010: 37(10), 2011: 41(8), 2012: 38(5), 2013: 59(8), 2014: 46(11), 2015: 8 (3). The trend shows a surge in number of submissions after the publication of Agency's Botanical Guidance in 2001, followed by steady submissions, ranging between 40-50 annually (except

in 2013 that peaked at 59). Among the 602 submissions totaled so far, 79% (476) are submitted in original IND and the rest 21% (126) in pre-IND format initially. From the therapeutic perspectives, more than 26% of the INDs have been focused on the unmet cancer-related therapy areas (161), followed by dermatology (71), GI (50), antiviral (43), endocrine/metabolism (37), reproductive & urology (36), neuropharmacology (36), pulmonary/allergy/rheumatology (30), cardiovascular (28), anti-infective (21) and ophthalmology (10), etc. In the last 5 years (2009-2014), 20 out of 217 INDs were placed on clinical holds, indicating ≥90% of the original INDs were evaluated as safe to proceed for the initial human trials. This 10% rate of clinical holds is significantly less than those in the pre-botanical guidance era, during which 34% were placed on clinical hold, suggesting that the regulatory environment for botanical drug development has been becoming more transparent, consistent and harmonious. In summary, based on our analysis of the 602 INDs submitted, the Agency has undertaken a proactive role in this unique drug category in effectively coaching sponsors for botanical drug development, and as a result, two products, namely kunecatechins (Veregen) and crofelemer (Fulyzaq), each achieved and met the human efficacy/safety standards for a new drug, and were approved for market uses in the United States.

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1,2-DEHYDROPYRROLIZIDINE ALKALOIDS IN THE TRADITIONAL ANDEAN HERBAL MEDICINE "ASMACHILCA"

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Asmachilca is a Peruvian herb preparation ostensibly derived from *Aristeguietia gayana* (Wedd.) R.M. King & H. Rob. (Asteraceae). Decoctions are purported to be useful for bronchodilation. Its attractiveness to pyrrolizidine alkaloid-pharmacophagous insects indicated a potential for human toxicity. "Asmachilca" materials were purchased in Lima, Peru and from internet-suppliers. LC-esi(+)MS and MS/MS screening of plant extracts found known and suspected 1,2-dehydropyrrolizidine alkaloids (dehydroPA). Further structure elucidation of isolated alkaloids was based on 1D and 2D NMR spectroscopy. Five asmachilca samples contained rinderine and supinine and their *N*-oxides as the major dehydroPA. A 6th lacked supinine or its *N*-oxide. Small quantities of other dehydroPA monoesters, including echinatine and intermedine, were also detected. In addition, two previously undescribed major metabolites were isolated and identified as dehydroPA monoesters linked to viridifloric and/or trachelanthic acids. Total dehydroPA and *N*-oxide content in the asmachilca ingredients varied from 0.4 – 0.9% (w/dw). The mean dehydroPA content of a hot water infusion of an asmachilca tea bag was 1.7 mg. Morphological and chemical evidence showed that asmachilca is prepared from several plant species. All asmachilca samples and infusions contained 1,2-dehydroPA at levels relevant to human health. Many tasks for future research are obvious, including investigations of incidence of chronic disease associated with asmachilca use and the botany of asmachilca preparations.

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HONEY, BEES, AND A HEPATOTOXIC ALKALOID ECHIMIDINE

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Echimidine is the main pyrrolizidine alkaloid of Echium plantagineum L, (Jane's Salvation, Patersons Curse), endemic to Australia. The plant is a great attractant for bees and as a result echimidine finds its way into honey. Because of its hepatotoxicity, echimidine became a target of new EU regulation requiring testing of imported honey. Under normal RP HPLC conditions echimidine produces always a sharp peak. Only recently, we discovered that this single peak represents actually a mixture of three alkaloids. Using relatively new "core-shell" RP HPLC column (Kinetex EVO C18, Phenomenex) in a buffer system we resolved "echimidine" into two well separated peaks. An NMR analysis proved that later eluting peak belonged indeed to echimidine, while the earlier peak contained two, largely unresolved alkaloids echihumiline (major) and hydroxymyoscorpine (minor). Each of them has been isolated before from other plants. All these alkaloids are isomeric $C_{20}H_{31}NO_7$ and produce in MS a single MH^+, signal at m/e 398, in and their NMR spectra are similar, which explains why they have not been detected before. Examination of several of echimidine samples derived from different plant collections revealed that the contribution of the early peak varied from 13% to 43%. Considering that these alkaloids may contribute unequally to the hepatotoxicity, effects of both, purified echimidine and the mixture of echihumiline and hydroxymyoscorpine were compared in rat hepatocytes.

1094

SCREENING AND IDENTIFICATION OF UNDECLARED SYNTHETIC COMPOUNDS AS ADULTERANTS USING UPLC-QTOF-MS COUPLED TO A NOVEL INFORMATICS PLATFORM

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The wide spread use and increasing demand for herbal products as economical and perceived safe alternatives to prescription drugs has led to the increase of such products being adulterated with undeclared synthetic compounds by unscrupulous manufacturers. LC–MS based methods are widely-used for the characterization of adulterants because of their high sensitivity and selectively for samples from complex matrices. However, from LC-MS sample acquisition to data processing/analysis, critical steps are required in order to effectively determine the answer. In this study, two commercial herbal product samples to relieve pain and inflammation were obtained from India and Taiwan and were analyzed using UPLC and QTof MS coupled to a novel informatics platform. A natural product analytical workflow was used to analyze the mass spectra of the herbal products which utilize tools such as an adulterant database library containing accurate mass fragment ions for confirmation of analytes of interest, structure elucidation module and automatic reports to effectively provide the visualization of the results. For the commercial sample obtained from India, phenylbutazone and oxyphenbutazone were identified as adulterants while, no adulterants were found from the sample purchased from Taiwan. Here, we present a comprehensive streamlined workflow to quickly screen and identify adulterants from a single LC/MS injection using a novel informatics platform.

1095

LAESIMS STUDIES OF ECHINACEA PURPUREA, RUDBECKIA HIRTA, AND HELIANTHEMUM ANNUS

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Laser ablation electrospray ionization mass spectrometry has provided a nearly sample-preparation-free imaging technique that is well suited to biological tissues. While a number of studies have focused on its applications to animal tissues, fewer have used LAESIMS with plants. We present here an investigation into a plant commonly used as an herbal supplement or tea, *Echinacea purpurea*, and two other plants of the Heliantheae tribe: *Rudbeckia hirta* and *Helianthemum annus*. Fresh and dried samples were subjected to LAESIMS and we present the results here. The LC-MS spectra are also presented.

1096

PHYTOTOXIC CONSTITUENTS FROM *CURVULARIA INTERMEDIA* INFECTING *PANDANUS AMARYLLIFOLIUS*

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As part of an on-going effort in search for agrochemicals from natural sources, a fungus from an infeected leaf of *Pandanus amaryllifolius* showing visible necrosis was isolated and identified as *Curvularia intermedia*. The fungus was cultured in potato dextrose agar (PDA) plates and then was grown in potato dextrose broth (PDB) culture medium at 24 ^{0}C for 14 days. The liquid culture broth was extracted with ethyl acetate to afford a brownish solid that showed phytotoxicity on monocots and dicots. Silica gel biotage column chromatography fractionation followed by crystallization afforded two phytotoxic compounds. The structures were determined as curvularin and dehydrocurvularin by spectroscopic techniques. Dehydrocurvularin inhibited seed germination and caused cellular leakage in cucumber cotyledons and the effects were less pronounced by curvularin at the same concentrations. Isolation and biological activity of these constituents will discussed.

1097

HIGH RESOLUTION MASS SPECTROMETRY BASED IDENTIFICATION AND QUANTIFICATION OF CHEMICAL MARKERS IN A TCM FORMULATION

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YANG XIN® is a traditional Chinese medicine (TCM) formulation used for nervous fatigue and consists of a proprietary blend of concentrated extracts from eighteen plants. In this study, liquid chromatography coupled with a quadruple time-of-flight mass spectrometer (LC-QTOF-MS) was used for identification of characteristic chemical markers from the eighteen plants present in the YANG XIN® formulation. LC-MS chromatograms of each plant were compared, the identities of the unique peaks were confirmed, and the structures elucidated by comparing the mass spectra and retention times with those of the reference compounds. The selected chemical markers were ophiopogonanone A, sibiricose A5, poricoic acid A, α-asarone, formononetin, ginsenoside Rf, swertisin, racemic mixture of 3-butylidenephthalide, schizandrol A, α-amyrin, 4,5-dicaffeoylquinic acid, cistanoside D, polygonatine A, forsythoside A, neoxanthin, batatasin III, pinusolide and armepavine. LC-QTOF-MS method was validated to quantify the chemical markers in the three samples of the formulation by constructing a calibration curve for each individual marker. The linear range was investigated up to an analyte concentration of 62.5 µg/ml. The average amount of the eighteen chemical markers was found to be within the range of 0.02-933 ug/g in the TCM formulation samples.

1098

PHENOLIC COMPOUNDS FROM THE LEAVES OF *HOMONOIA RIPARIA* AND THEIR INHIBITORY EFFECTS ON AGES FORMATION AND ALDOSE REDUCTASE

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In searching for novel treatments for diabetic complications from natural resources, we found that the EtOAc-soluble fraction of the 80% EtOH extract of the leaves of *Homonoia riparia* has a considerable inhibitory effects on both AGEs (Advanced glycation end products) formation and rat lens aldose recuctase (RLAR). Further phytochemical study of this fraction resulted in the isolation of 15 phenolic compounds (**1**–**15**). These compounds were evaluated *in vitro* for inhibitory activity against the formation of AGEs and RLAR. In the AGEs assay, compounds **3**, **5**–**7**, **10**, **11**, and **14** significantly inhibit the AGEs formation with IC_{50} values ranging from 2.23 to 15.14 µM, compared to that of positive control, aminoguanidine (IC_{50}, 962 µM). In the RLAR assay, compound **4**, quercitrin gallate, showed the most potent inhibitory activity with an IC_{50} value of 0.064 µM.

1099

AUTHENTICATION IS FUNDAMENTAL FOR THE STANDARDIZATION AND GLOBALIZATION OF HERBAL MEDICINE

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Traditional Chinese medicine (TCM) has earned worldwide recognition for its efficacy. As TCM undergoes rapid globalization, the safety of Chinese medicinals is drawing international concern. Authentication is a key first step for standardizing Chinese medicinals for global markets and research. Authentication methods include origin identification, macroscopic identification, microscopic identification, physical/chemical identification, and molecular biological identification. Many issues related to authentication have remained unresolved since ancient times. Determining the authenticity and quality of Chinese medicinals remains as much a frontier as it is an essential science in guaranteeing the safety and efficacy of Chinese medicinals in clinical use. Authentication is directly connected to the clinical efficacy of TCM. This can be a matter of life and death in clinical practice and will almost certainly influence the fate of Chinese medicinals.

In the past 15 years, the School of Chinese Medicine (SCM) of Hong Kong Baptist University has laid a solid foundation in the field of Chinese medicinal authentication and has made a major impact at home and abroad in this capacity. As a center of international trade, Hong Kong is the source of many Chinese medicinal materials reaching international markets, and the quality of Chinese medicinals in Hong Kong directly reflects the status of herbal markets overseas. SCM will continue to make great efforts to promote Chinese medicinal authentication and the development of medicinal resources, which will in turn further strengthen the competiveness and influence of Chinese medicinals in the international community.

1100

CYTOTOXIC SESQUITERPENOIDS FROM *SIEGESBECKIA GLABRESCENS*

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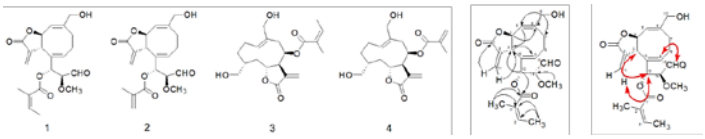
Two new sesquiterpenoids, siegenolides A (1) and B (2), and two known sesquiterpenes 3 and 4 were isolated from *Siegesbeckia glabrescens*. Their structures were elucidated by spectroscopic analyses, and they were further evaluated for their cytotoxic activities against human cancer cells (MCF-7, AsPC-1, SW480, HCT 116, HepG2, HeLa). Compounds 1–4 showed differential cytotoxic effects on the target cancer cells with IC_{50} values in the range of 0.9–33.3 μM.



1101

DEHYDROCOSTUS LACTONE AND COSTUNOLIDE INHIBIT WNT/β-CATENIN PATHWAY IN COLON CANCER CELL

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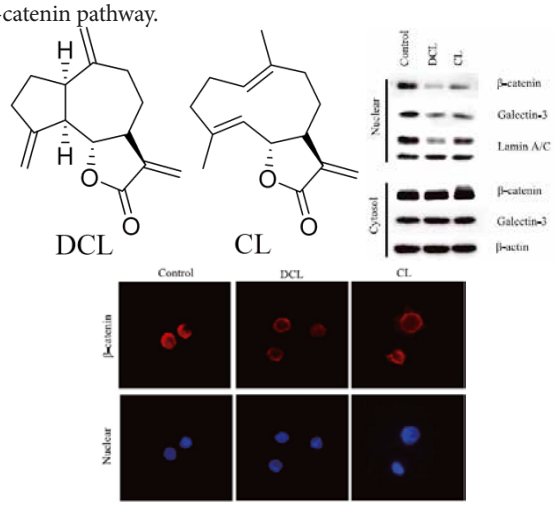
The suppression of abnormally activated β-catenin is one of good strategies for chemoprevention and treatment of colorectal cancer. In this study, we have isolated two main compounds from root of *Saussurea lappa*, dehydrocostus lactone (DCL) and costunolide (CL), and investigated their anti-colorectal cancer activities. DCL and CL suppressed cylcin D1 and survivin through inhibiting nuclear translocation of β-catenin and galectin-3 that is one of coactivators of β-catenin. Furthermore, DCL and CL suppressed proliferation and survival of SW-480 colon cancer cells through inducing cell cycle arrest and cell death. Taken together, DCL and CL from root of *Saussurea lappa* have anti-colorectal cancer activities through inhibiting Wnt/β-catenin pathway.



1102

PHENOLIC COMPOUNDS FROM THE WHITE FLOWERS OF *IMPATIENS BALSAMINA*

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Impatiens balsamina L. is an annual herbaceous plant of Balsamineaceae family and the flowers of this plant have been used to treat lumbago, neuralgia, burns and scalds. Previous phytochemical studies have led to reports of naphthalene derivatives, triterpene glycosides, and flavonoids associated with anti-microbial, antitumor, anti-allergy, antioxidant, antifungal, and antinociceptive properties. In the course of our continuing search for biologically active compounds from Korean medicinal sources, we investigated the white flowers of *I. balsamina*. From their MeOH extract, two new phenolic compounds (**1**-**2**) containing nitrile group and eleven known phenolic compounds (**3**-**13**) were isolated. The chemical structures of new compounds (**1**-**2**) were determined through NMR, HRMS, and CD data. We tested the isolated compounds (**1**-**13**) for their neuroprotective activity by determining their effects on nerve growth factor (NGF) secretion in C6 cells and anti-neuroinflammatory by measuring nitric oxide (NO) production in a lipopolysaccharide (LPS)-stimulated BV-2 cells.

1103

NEW TRITERPENES FROM *ABIES HOLOPHYLLA* AND THEIR BIOLOGICAL ACTIVITES

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Abies holophylla MAXIM. (Pinaceae), also known as Manchurian Fir or Needle Fir, is a evergreen and coniferous tree that is widely distributed in Korea, China, and Russia. Several species in *Abies* genus have been used as a Korean folk medicine for the treatment of colds, stomachache, indigestion, rheumatic diseases, and vascular and pulmonary diseases. Previous phytochemical investigations on *A. holophylla* reported lignans, terpenoids, and phenolic compounds, and their cytotoxic and anti-inflammatory activities. However, few phytochemical and biological investigations on *A. holophylla* have been performed. An extended phytochemical investigation of the trunk of *A. holophylla* afforded five new triterpenes (**1**-**5**) together with eleven known ones (**6**-**16**). The structures of the new compounds (**1**-**16**) were characterized by extensive NMR methods (^{1}H and ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY). The isolated compounds (**1**-**16**) were tested for their anti-inflammatory activity measuring NO production in a LPS-activated murine microglial cells and their effects on NGF secretion from C6 glioma cells. Also, we evaluated the cytotoxicity of isolates (**1**-**16**) against A549, SK-OV-3, SK-MEL-2, and HCT116 cell lines *in vitro* using the SRB bioassay.

1104

CHEMICAL NANO SHIFTS EXPLAIN THE NMR FINGERPRINTS OF DENTIN-ENHANCING OLIGOMERIC PROANTHOCYANIDINS

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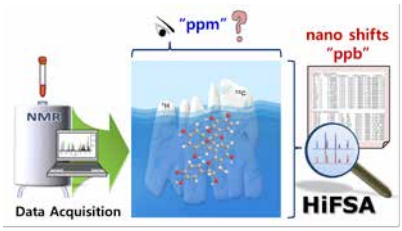
[1]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, and [2]Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, Chicago, IL 60612, USA



1D NMR spectra contain a wealth of vital structural information that can enhance the description of bioactive molecules. The present study demonstrates how quantum-mechanics driven ^{1}H iterative Full Spin Analysis (QM-HiFSA) is capable of distinguishing spectral detail that cannot be interpreted manually or visually, but provides important information of the 3D structure and bonding (re-)activity of the molecules. This approach is established by analyzing 1D NMR spectra of oligomeric proanthocyanidins (OPACs), which exhibit high dentin bioactivity, and were isolated from the inner bark of pine. The higher order coupling and proton-deuterium exchange effects observed in these complex molecules were fully explained and quantified by QM-HiFSA. Dimeric and trimer OPACs provide evidence that high d precision is applicable to ^{13}C, in addition to ^{1}H 1D NMR spectra, requiring reporting to the ppb level and below. Both the nano chemical shifts (ppb) and the associated nano substituent chemical shifts (s.c.s.) are significant properties of the ^{1}H and ^{13}C NMR spectra and enable recognition of structural properties that are relevant to better understanding of the intermolecular interactions between the OPAC pharmacophores and dentin micromolecules triggering enhanced tissue mechanics.

1105

GC/MS METHOD FOR CHARACTERIZATION AND QUANTITATIVE ANALYSIS OF GINKGOLIC ACIDS IN GINKGO BILOBA PLANTS AND DIETARY SUPPLEMENTS

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[1]National Center for Natural Products Research, and [2]Division of Pharmacognosy, Department of BioMolecular Science, School of Pharamacy, University of Mississippi, University, MS 38677, [3]Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808-1610, USA

A high resolution GC/MS with Selected Ion Monitor (SIM) method focusing on the characterization and quantitative analysis of ginkgolic acids (GAs) in *Ginkgo biloba* L. plants, extracts and commercial products was developed. The method involved sample extraction with (1:1) methanol and 10 % formic acid, liquid-liquid extraction with *n*-hexane, and derivatization with trimethylsulfonium hydroxide (THSH). Separation of 2 saturated (C13:0 and C15:0) and 6 unsaturated ginkgolic acid methyl esters with different positional double bonds (C15:1 Δ8 and Δ10, C17:1 Δ8, Δ10 and Δ12, and C17:2) was achieved on a polar (88% cyanopropyl) aryl-polysiloxane HP-88 capillary GC column. The double bond positions in the GAs were determined by ozonolysis. The developed GC/MS method was fully validated according to ICH guidelines, and the quantitation results were verified by comparison with a standard HPLC method. Nineteen *G. biloba* authenticated and commercial plant samples and 21 dietary supplements purported to contain *G. biloba* leaf extracts were analyzed. Finally, the presence of the marker compounds, terpene trilactones and flavonol glycosides, for *Ginkgo biloba* in the dietary supplements was determined by UHPLC/MS and used to confirm the presence of *G. biloba* leaf extracts in all of the botanical dietary supplements.

1106

CYTOTOXIC ROTENOIDS AND ISOFLAVONOIDS FROM THE FRUITS OF *MILLETTIA CAERULEA*

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Three new and seven known rotenoids, along with two new isoflavonoids and a known analogue, were isolated from the *n*-hexane extract of the fruits of *Millettia caerulea* (Graham) Baker (Fabaceae) collected in Vietnam (sample A06946; voucher specimen DDS-14879). The structures of the new compounds were established by analysis of their ECD, IR, UV, NMR, and mass spectra, with those of the known compounds being determined by comparison of their spectroscopic data with literature values. Several of these compounds were found to be cytotoxic toward the HT-29 human colon cancer cells, among which a known compound, 12β-hydroxyrotenone, was the most potently active, showing an IC_{50} value of 0.1 μM. This same compound was active (IC_{50} 3.1 μM), when evaluated in a K-Ras inhibition assay using HT-29 cells.

1107

HEMATOPOIETIC EFFECTS OF PAEONIFLORIN AND ALBIFLORIN ON RADIOTHERAPY AND CHEMOTHERAPY-INDUCED ANEMIA MICE

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Paeoniflorin (PF), a monoterpene glycoside isolated from P. lactiflora, possesses a variety of pharmacological activities. However, albiflorin (AF), another constituent regarded as a characteristic one, has not been well studied. This study aimed to investigate the hematopoietic effects of AF and PF on anemia mice induced by radiotherapy or chemotherapy and to explore the underlying mechanisms. The anemia mice were irradiated at a dose of 2.5 Gy using cobalt-60 gamma resources or intraperitoneally injected with cyclophosphamide (160.0 mg/kg). The numbers of blood cells from peripheral blood were counted. The thymus index and spleen index were also measured. In addition, of the chemotherapy-induced mice, the levels of TNF-α, GM-CSF, IL-3 in serum were measured by RIA. AF and PF significantly increased the numbers of peripheral blood cells and reversed the atrophy of thymus and spleen. Furthermore, AF and PF increased the levels of GM-CSF and IL-3 and reduce the level of TNF-αin serum.. Our results

suggest that AF and PF may promote the recovery of bone marrow hemopoietic function in a myelosuppressed mouse model.

1108

ANTIOXIDANT FLAVONOL GLYCOSIDES FROM HERBA EPIMEDII

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Herba Epimedii (Yinyanghuo in Chinese) is a traditional Chinese herbal medicine, which has been used as tonic, aphrodisiac and anti-rheumatic in China for thousands of years. It was proven to be an effective remedy for cardiovascular diseases, osteoporosis and for improving sexual and neurological functions. And it was verified that the effectiveness of Herba Epimedii is highly related to its antioxidant ingredients. Hence the antioxidant constituents from Herba Epimedii were investigated, and the ethyl acetate fraction of the methanolic extract of Herba Epimedii, which contains a major bioactive component icariin, showed highest DPPH radical scavenging activity. Several known and novel flavonol glycosides were isolated by silica gel open column chromatography and HPLC. Structures of the isolated compounds were identified by analyses of spectroscopic data from 1D- and 2D-NMR as well as UV, IR and MS. Details of isolation and structure elucidation will be discussed.

1109

ANTI-CANCER ACTIVITY OF SOME SYNTHESIZED AROMATIC OXYBUTYNYL AMINE DERIVATIVES

Manas Omyrzakov[1,2,3], Yerkebulan Orazbekov[1,3,6], Guoyi Ma[3], Ubaidilla Datkhayev[1], Madi Omirzak[2], Ahmad E. Mostafa[3,5], Bauyrzhan Makhatov[6], Kuandyk Orazbekuly[7], Shokan Begaliyev[1], Lyazzat Yeraliyeva[1], Zuriyada Sakipova[1], Kulpan Orynbasarova[6], Samir A. Ross[3,4]*
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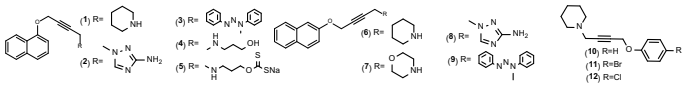
Twelve aromatic oxybutynyl amine derivatives (**1**-**12**) were synthesized via Mannich reaction. All synthesized compounds were tested primarily for their anti-leukemic activity against leukemia K562 cells, in a conc. of 10 μg/ml. Compound **9** showed the highest activity of 100% inhibition. Furthermore, compound **9** showed an IC_{50} value of 0.03 and 0.008 μg/ml against leukemia K562 and HL60 cells respectively, where IC_{50} value of Taxol against both cells was 0.0023 μg/ml.



1110

BIOACTIVE POLYKETIDES FROM A *PENICILLIUM LANOSUM* STRAIN DERIVED FROM A SWAB OF BAT'S WING

Guojian Zhang[1,2], Jarrod King[1,2], Andrew N. Miller[3], and Robert H. Cichewicz[1,2]
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Bacterial infection, particularly from multi-drug resistant strains, remains a serious threat to human lives. Thus, there is a clear and critical medical need for the discovery of novel antibacterial agents. Our recent work within the scope of an anti-multidrug-resistant *Acinetobacter baumannii* screening program led to the isolation of one fungal strain *Penicillium lanosum* 1298-15 from a swab of bat's wing. Bioassay guided fractionation of the secondary metabolites from the strain 1298-15 afforded 4 new polyketide lanoctones A-D (**1**-**4**), along with 3 known compounds: 3-chloro-2,5- dihydroxybenzyl alcohol (**5**), sohirnone A (**6**) and bislongiquinolide (**7**). Compounds **5** and **6** exhibited strong activity with IC_{50} values of 5.7 nM and 45.4 nM. However, compounds **1**-**4** and **7** showed weak or no activity.

1111

METABOLIC GIVE AND TAKE BETWEEN NEIGHBORING FUNGUS AND BACTERIUM

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To understand the soil ecosystem, many studies focus on the secondary metabolites involved in the chemical interaction between microorganisms. However, the interactions initiated by primary metabolites are often overlooked. When we were isolating fungi from soil on Czapek's agar, we found a bacterium, *Pseudomonas alcaligenes*, inhibited the growth of a fungus *Curvularia inaequalis*. This drew our attention, since the bacterium was unculturable when alone on Czapek's agar. Using laser ablation electrospray ionization mass spectrum (LAESIMS), we observed the fungus helped the bacterium to grow on Czapek's agar by digesting sucrose into glucose and fructose with only glucose being used directly by the bacterium as a carbon source. More interestingly, when it started to grow, the bacterium was able to inhibit the growth of the fungus. Bioassay guided purification yielded 2,4-diacetylphloroglucinol as fungal inhibitor. Further determination of sucrose uptake and digestion by LC-MS confirmed our observation on LAESIMS. It is the first report of the interaction of these two soil microbes.

1112

TRANSFERRING FUNGI TO A DEUTERIUM-ENRICHED MEDIUM RESULTS IN ASSORTED, CONDITIONAL CHANGES IN SECONDARY METABOLITE PRODUCTION

Bin Wang[1,2], Elizabeth M. Park[1,2], Jarrod B. King[1,2], Allison O. Mattes[1,2], Susan L. Nimmo[2], Chaevien Clendinen[3], Arthur S. Edison[3], Clemens Anklin[4], Robert H. Cichewicz[1,2,]*

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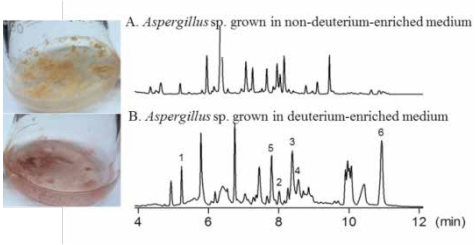

The incorporation of deuterium into organic substrates is known to alter the function of enzymes. Our study tested the extent to which deuterium enrichment would result in the modification of fungal secondary metabolite production. Eight fungal cultures we tested were marked by changes in natural product production. Work-up of one *Aspergillus* sp. grown under deuterium-enrichment conditions resulted in an active fungal extract against MRSA, which was not observed in the crude extract from the fungus grown under non-deuterium enriched conditions. An assortment of NMR and mass spectrometry experiments enable us to identify isotope-labelled brevianamide F (**1**), stephacidin A (**2**), notoamide D (**3**), notoamide L (**4**), notoamide C (**5**), and bacterial inhibitor pigmentosin A (**6**). **1** and **3-6** have not been previously observed from this fungal isolate. Therefore, we propose that deuterium-enrichment might offer an effective method for further expanding a fungus's chemical diversity potential.

1113

ANTIMALARIAL METABOLITES FROM *BUXUS SEMPERVIRENS*

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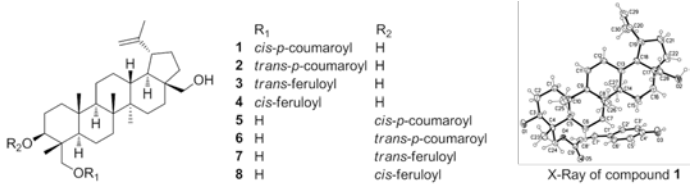

Some malaria parasites (*Plasmodium falciparum*) have developed resistance to all currently available antimalarial drugs including artemisinin derivatives, so there is an urgent need to discover new chemicals with antimalarial activity. Eight new lupinane derivatives (**1**-**8**) were isolated from the extract of the English boxwood (*Buxus sempervirens*) using bioassay guided fractionation and examined for activity against malarial parasites *Plasmodium falciparum*. The lupinane derivatives range in potency from 0.49–3.36 μM against the drug sensitive HB3 parasite and retain efficacy in an artemisinin and multidrug resistant parasite recently isolated in Southeast Asia. Compounds **2** and **3** in particular exhibited greater than 30-fold selectivity for malaria parasites as compared to human cancer cell lines.

1114

QUANTITATIVE DETERMINATION OF FIVE TRITERPENOID GLYCOSIDS AND CHEMICAL PROFILING OF *MOMORDICA CHARANTIA* BY UHPLC-ELSD/MS

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Bitter melon, *Momordica charantia* L. (Cucurbitaceae), is used in traditional medicine of the world and in dietary supplements of the United States. An UHPLC-UV-ELSD/MS method was developed to quantitative determination of five cucurbitane-type triterpenoids from *M. charantia*. The method was validated including extraction solvents, linearity, repeatability, limits of detection (LOD) and limits of quantification (LOQ). Cucurbitane-type triterpenoid saponins in *M. charantia* were classified into three sub-groups according to substitutions and oxidizations at C-3, C-7 and C-19. The key fragments of three sub-groups were characterized and further used for the identification of triterpenoids from *M. charantia*. This method was applied to the analysis and chemical profiling of plant samples of *M. charantia* and related products.

1115

SMALL SCALE PURIFICATION OF CONSTITUENTS FROM COMPLEX NATURAL PRODUCT EXTRACTS USING SUB-2-μM CHROMATOGRAPHY

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Extracts from natural product samples can be complex often containing a large number of diverse compounds. Increased separation performance of sub-2-μm column technology along with low dispersion instrumentation provides a tool that produces sharp, narrow, and more concentrated peaks. When there is a need to collect narrow peaks from these complex mixtures, traditional fraction collection instrumentation designed for preparative HPLC conditions does not provide an adequate solution.

Extracts from several natural product samples were analyzed using sub-2-ìm chromatography. Potential peaks of interest were identified and isolated using a fraction collector designed to overcome the limits of traditional fraction collectors. Collection of narrow peaks generated using modern sub-2 μm chromatography along with closely eluting compounds is demonstrated using a variety of collection modes including time based and peak detection techniques. Analysis of collected fractions to demonstrate purity is shown using UPLC.

1116

DATA MINING OF SIMPLE SEQUENCE REPEATS IN TRANSCRIPTOME SEQUENCES OF MONGOLIA MEDICINAL PLANT *ARTEMISIA FRIGIDA*

Yue Liu[1, 2], Xiaoqing Ma[1], Yi Wang[3], Hongbo Sun[1], Huoli Yang[1], Qin Li[1], Fuxin Chen[1], Linxia Zhang[1], Chuanchuan Chen[1], Hua Li[1], Li Tang[1]
[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] Department of Plant Sciences, University of California Davis, CA 95616, USA

Artemisia frigida Willd. is a Mongolian traditional medicinal plant with pharmacological functions of stanch and detumescence. With the increasing demand for commercial use, selection of germplasm with high pharmaceutical efficacy at molecular level is important and requires the availability of efficient genetic and molecular marker data. In this study, MicroSAtelite software was employed to screen SSRs in 143,700 contigs of *Artemisia frigida* transcriptome sequences. There are 3,747 SSRs distributed in 3,614 contigs which accounted for 2.51% of 143,700 contigs with 123 types of SSR motifs. The most abundant repeat type was tri-nucleotide (56.20%, 2,106), followed by the di-nucleotide (31.60%, 1,184). AAC/TTG and AC/TG were the main types of motif in tri-, di-nucleotide repeats. The most common number of tandem repeats was 5 (38.54%, 1,444). The primer pairs for 3,743 SSRs were designed for further investigation of the potential of these SSRs as genetic markers. These data could be further utilized in functional genomics studies on *Artemisia frigida*. Acknowledgment: financial support by project of NSFC-81274158, 81373765, NCET-12-0578, 13-0624, GCCX2014110021, YLDX01013, and 111-B08044.

1117

A TCM HERBAL FORMULA INCREASES IGF-1 AND IGFBP-1 LEVELS IN THE SERA AND OVARIES IN AN AGED FEMALE RAT MODEL OF MENOPAUSE

Min Wei[1], Sheng Z. Zheng[1], Ye Lu[1], Daniel Liu[2], Hong Ma[3] and Gail B. Mahady[4]
[1] Jiangsu Institute of Botany, Chinese Academy of Science; Nanjing 210014, China; [2] Beijing Clinical Service Center, Beijing, China 100123 [3] College of Basic Medicine, Nanjing University of Traditional Chinese Medicine; Nanjing, 210029, China; [4]Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA.

Menoprogen (MPG), a TCM herbal formula improves menopausal symptoms in clinical trials, however its mechanism has remained elusive. Previous studies show that MPG is not directly estrogenic, thus the goal of this study was to investigate the effects of MPG on IGF-1 and IGFBP-1 levels in an aged female rat model of menopause. In a six-armed study, 14-month-old Sprague-Dawley (SD) female rats (n = 8 per arm) were randomly divided into an untreated aged group, an E2-treated aged group (17β-estradiol), and three arms with increasing doses of MPG groups (162, 324, or 648 mg/kg/day). The sixth arm contained four-month-old SD female rats as a normal comparison group. Four weeks after MPG or E2 administration, the animals were sacrificed after blood draws, and then ovarian tissues were excised. The levels of E2 and progesterone (P4) were determined by radioimmunoassay, and serum and ovarian tissue levels of IGF-1, IGFBP-1, and IGF-1 receptor (IGFR) were determined by ELISA. Compared with the normal group, the aged rats had significantly reduced serum levels of E2, P4, and IGF-1 and increased serum and ovarian tissue levels of IGFBP-1. MPG restored serum IGF-1 and IGFBP-1 levels and down-regulated the ovarian levels of IGFBP-1, which were closely related to increases of E2 and P4 levels in the aged rats. No significant differences were observed between the three doses of MPG for either IGF-1 or IGFBP-1. MPG has a direct in vivo effect in aged female rats by positively regulating serum and ovarian IGF-1 and IGFBP-1 levels.

1118

NOVEL MECHANISM OF ACTION OF A TRADITIONAL CHINESE HERBAL FORMULA IN AN INTACT AGED FEMALE RAT MODEL OF MENOPAUSE

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The effects of Menoprogen (MPG), a traditional Chinese medicine formulation for menopause, were determined on granulosa cell (GC) apoptosis in an aged rat model of menopause. Intragastric administration of MPG or estradiol valerate to 14-month-old female rats for 8 weeks increased plasma estrogen levels and the weight of both ovarian and uterine tissues. Flow cytometry (FCM) analysis of the GCs from treated animals showed reductions in both the G0/G1 ratio and apoptotic peaks. Electron microscopy of the GCs showed an increase in cell size, the numbers of cytoplastic organelles and intracellular gap junctions, reappearance of secretory granules and a lack of apoptotic bodies. Data from the TdT-mediated dUTP nick end-labeling (TUNEL) assay revealed a reduction in GC apoptosis after treatment. Immunohistochemical analysis showed a down-regulation of Bax proteins and an up-regulation of Bcl-2 proteins. In vitro, the addition of MPG medicated serum to the media of isolated GCs with cadmium chloride-induced apoptosis reduced GC apoptosis as measured by FCM analysis in the TUNEL assay. A down-regulation of caspase-3 protein in GCs treated with MPG. This work demonstrates that both MPG and estradiol valerate inhibit GC apoptosis in aged female rats, and represents a novel mechanism of action for this herbal medicine for the treatment of menopause.

1119

ANTIMICROBIAL DEFENSES OF *CAULERPA MEXICANA* AND *UDOTEA LOOENSIS* AGAINST *VIBRIO* SPP. AND *BACILLUS* SP.

Bobby Owens[1], Jordan Ekhoff[1], Elizabeth Philip[1], Jason Kwan[2], Tomasz Jurga[1], and Melany P. Puglisi[1]
[1]Chicago State University, College of Pharmacy, 9501 S. King Dr., Chicago, IL 60628, [2]University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI 53705

In a broad survey of extracts of common marine algae from the Florida Keys against an extensive panel of environmental bacteria, extracts from the green algae *Caulerpa mexicana* and *Udotea looensis* exhibited significant activity against two conspecific strains of *Vibrio* sp. from Florida and one strain of *Bacillus* sp. from the Red Sea. To continue the investigation of antibacterial defenses of marine algae, crude extracts from *C. mexicana* and *U. looensis* were subjected to bioassay-guided fractionation. Extracts were partitioned between ethyl acetate and DI water. The non-polar fractions were separated using Si gel medium pressure liquid chromatography followed by reverse phase high pressure liquid chromatography. Bacterial cultures were maintained on A1 media. Extracts were dissolved in DMSO (600 μg/ml ≤ natural volumetric concentration) and added to each well of a 96-well plate using DMSO (solvent), streptomycin and tetracycline (positive) as controls. Initial and final readings were obtained on a Biotech plate reader at 625nm. *C. mexicana* and *U. looensis* produce metabolites that selectively inhibit the growth of one strain of *Vibrio* sp. or *Bacillus* sp. and several others that show broad spectrum antimicrobial activity against potential pathogens in the marine environment. This study was conducted by PharmD candidates as part of a college wide research training program at Chicago State University.

1120

CHANGE OF CHEMICAL COMPOSITION IN *FLOS LONICERAE*: AN INVESTIGATION OF HERBAL PROCESSING (PAO ZHI) USING NMR SPECTROSCOPY

Jianping Zhao[1], Mei Wang[1], Bharathi Avula[1], Ikhlas A. Khan[1,2,3]
[1]National Center for Natural Products Research, [2]Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA; [3]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Processing of herbal medicine, known as *Pao Zhi* in Traditional Chinese Medicine (TCM), is a unique part of TCM and has been widely used for the preparation of Chinese materia medica. It is believed that processing can alter the properties and functions of remedies, increase medical potency, and reduce toxicity and side effects. Both processed and unprocessed *Flos Lonicerae* (flowers of *Lonicera japonica*) are important drug ingredients in TCM. To gain insights on the effect of processing factors (heating temperature and duration) on the change of chemical composition, NMR combined with chemometric analysis was applied to investigate the processing of *Flos Lonicerae*. The results indicated that the composition changed significantly, particularly when processing at the higher temperature. Five chemical components, *viz.* 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, chlorogenic acid and myo-inositol, whose concentration changed significantly during the processing, were isolated and identified. The changes of their concentrations were analyzed by UHPLC. The study demonstrated that an NMR-based chemometric approach could be a promising tool for investigation of the herbal processing in TCM.

1121

EXTRACTION AND GAS CHROMATOGRAPHY ANALYSIS OF ESSENTIAL OIL FROM GARLIC

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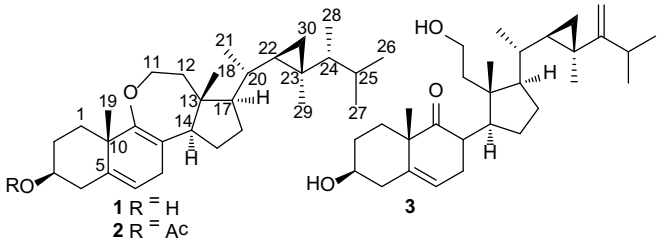
Commercially available garlic (*Allium sativum* L.) has been widely used as spice and condiment for centuries. Extracts of the garlic have been used traditionally in Chinese herbal medicine against various human diseases and disorders. Previous phytochemical investigations have revealed that there are more than 200 compounds identified from garlic. Among them, the essential oil have raised interest because of their wide range of bioactivities such as antioxidant, antifatigue, antiatherosclerotic, antidiabetic and immunomodulation effects. The present study was designed to obtain the optimum extraction condition for essential oil and analysis its major constituents by gas chromatography. An easy and convenient extraction process has been obtained with the 0.419% yield of essential oil by simple distillation, water as the solvent for 60 min at 50 °C. The GC analysis of the essential oil indicated that diallyl trisulfide, dimethyl sulfide, methyl prop-1-enyl disulphide and diallyl thioether as the main constituents.

1122

NEW CYCLIZED 9,11-SECOSTEROLS ENOL-ETHER FROM SOFT CORAL *PSENDOPTEROGORGIA AMERICANA*

Yang-Qing He[1], Stacee Caplan[2], Paul Scesa[2], and Lyndon M. West[2]
[1]Department of Applied Chemistry, Xi'an University of Technology, Xi'an, Shaanxi 710054, P. R. China, [2]Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, Florida 33431, USA

Chemical investigation of the MeOH extract from the gorgonian *Pseudopterogorgia americana* afforded two rare sterols, ameristerenol A (**1**) and B (**2**), both 9,11-secosterols containing a seven-membered cyclic enol–ether in ring C, and ameristerol A (**3**), the first 9,11-secosterol containing a gorgosterol side chain containing an exocyclic methylene group at C-24, along with three related known analogues. Ameristerenol A (**1**) was converted to semi-synthetic sterols **4**–**6**. The structures of compounds **1**–**6** were determined on the basis of extensive spectroscopic analysis and by comparison with literature data.



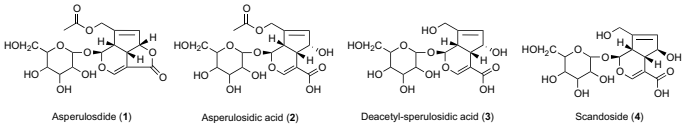
3

2001

THE FIRST NATURAL PRODUCTS INVESTIGATION OF THE HAWAIIAN ENDEMIC PLANT *COPROSMA ERNODEOIDES* OR NENE BUSH ('AIAKANENE)

Myria Lang,[1] Diana Zelta-Pinet,[2] Kehau Hagiwara,[2] and Anthony D. Wright[2]
[1]Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland; [2]DKI-College of Pharmacy, University of Hawaii at Hilo, 34 Rainbow Drive, Hilo, Hawaii 96720

The Nene bush (*Coprosma ernodeoides*) or 'Aiakanene in Hawaiian is a rambling perennial prostrate shrub endemic to Hawaii. Worldwide, mainly the Pacific Islands, there are around 110 species in the genus the majority of which (55) are to be found in New Zealand. The plant has very small leaves (2-3 mm) that grow densely along branches that can be up to three meters long. At various times of the year the bush produces shiny black edible berries (5-8 mm dia) that were and are used by native Hawaiians as laxatives. Our interest was drawn to this plant because of its distinctive berries, the fact that it is endemic to Hawaii and finally because there are no reported natural products investigation of any parts of the plant. The data presented on this poster result from investigations of methanol extracts made from the leaves and from the berries that both demonstrated antioxidant properties in DPPH and FRAP assays. From the berries the three known iridoid glycosides **1** - **3** were isolated and from the leaves the known iridoid glycoside **4**.

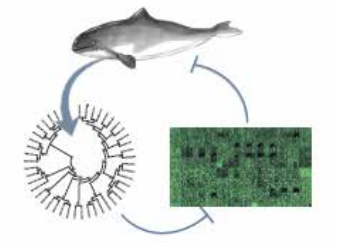


2002

PHYLOGENETIC AND METABOLOMIC ANALYSIS OF MARINE MAMMAL MICROBES CONTRIBUTES TO EMERGING SPIROTETRONATE POLYKETIDES

Jessica Ochoa, Laura Sanchez, Roger Linington
University of California, Santa Cruz, Department of Chemistry and Biochemistry, 1156 High Street, Santa Cruz, CA, 95060

Constant exposure to varying environments along migrations routes suggests that the marine mammal microbiome may be a unique environment for sample collections for natural product discovery. The organisms isolated in this study were prioritized through sequencing and metabolomics profiling to create a highly bioactive screening plate that possesses new molecules produced by marine microbiomes. Molecules elucidated in this study, both known and new provide detailed insight into the chemistry involved in signaling and/or defense within commensal bacteria as well as the capability of these molecules to be used to regulate pathogenesis for one of the worlds best sentinel organisms.

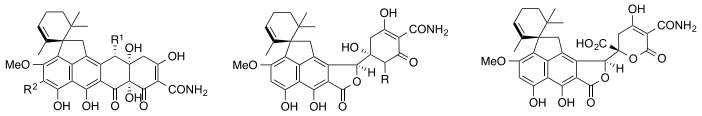


2003

VIRIDICATUMTOXINS: EXPLORING STRUCTURAL DIVERSITY AND ANTIBIOTIC PROPERTIES

Zhuo Shang[1], Angela A. Salim[1], Zeinab G. Khalil[1], Michelle Quezada[1], Paul V. Bernhardt[2] and Robert J. Capon[1]
[1]Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia, [2] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD 4072, Australia

Viridicatumtoxins are a rare class of antibiotics composed of a tetracycline scaffold and a geranyl-derived spirobicyclic ring. Chemical investigation of a fungus *Paecilomyces* sp. obtained from a marine mollusc *Siphonaria* sp. led to the isolation of three novel viridicatumtoxin lactones, named viridicatumtoxins D–F (**4**–**6**), together with two known compounds, viridicatumtoxins A (**1**) and B (**2**). Re-cultivation of this fungus on rice solid media afforded one new compound viridicatumtoxin C (**3**), together with two known metabolites, spirohexaline (**7**) and previridicatumtoxin (**8**). Compounds **4** and **5** were proved to be natural products by the acid stability study of **1**. The antibacterial and cytotoxicity activities of **1**–**8** were also evaluated.

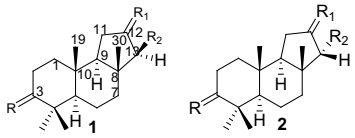


2004

^{13}C NMR SHIFTS IN 13α AND 13β MALABARICANES, THE EFFECT OF CYCLOBUTYL SIDE CHAIN: SOME INTERESTING OBSERVATIONS

Prabhakar S. Achanta, Raghuram Rao Akkinepally, Ravi Kumar Bobbala, and Appa Rao V.N. Achanta
University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506009, India.

13α-malabaricanes are fewer in number when compared with 13β-malabaricanes. In 13α-malabaricanes there is considerable upfield shift of C-30 by about 10 ppm when compared with 13β-malabaricanes. However this observation which is valid in general, does not hold good in all cases. Malabaricanes containing a cyclobutane ring in the side chain caused the same upfield shift despite the fact that H-13 was β oriented. Significant changes observed in ^{13}C NMR shift values at other carbon atoms in epimeric and cyclobutane ring containing malabaricanes are discussed.



R, R_1= H_2/ O/ -OH; R_2= Side chain

2005

ISOLATION AND IDENTIFICATION OF NOVEL NATURAL PRODUCTS THAT INHIBIT P300/HIF-1α INTERACTION

Susanna T. S. Chan,[1] Paresma R. Patel,[2,3] Gary E. Martin,[4] Robert T. Williamson,[4] Josep Saurí,[4] Alexei V. Buevich,[4] Tanya R. Ransom,[1] Curtis J. Henrich,[1,5] Tawnya C. McKee,[1] William D. Figg,[6] James B. McMahon,[1] Martin J. Schnermann,[2] and Kirk R. Gustafson[1]
[1]Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, [2]Chemical Biology Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, [3]National Centre for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20892, [1]NMR Structure Elucidation, Process, and Analytical Chemistry, Merck & Co. Inc., Rahway, NJ, 07065 [5]Basic Science Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, [6]Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892.

Hypoxia-inducible factor-1 (HIF-1) is an important transcription factor for initiating a response against low oxygen environments in many solid tumors. Under hypoxic conditions, subunit HIF-1α dimerizes with subunit HIF-1β and binds to the multi-domain protein and transcriptional coactivator, p300. Inhibition of the p300/HIF-1α interaction results in the suppression of HIF-1 transcriptional activity during hypoxia. An extract of the marine ascidian *Eudistoma* sp. was identified as active in a high throughput screen for inhibitors of the p300/HIF-1α interaction. Three novel heterocyclic alkaloids were isolated from the extract and their structures elucidated using both spectroscopic analyses and synthesis. The core scaffold of these alkaloids contains an unprecedented fused ring system with embedded guanidine and amidine functionalities. These compounds showed activity inhibiting the binding domains of p300 and HIF-1α.

2006

IDENTIFICATION OF CYTOTOXIC AND ANTI-INFLAMMATORY CONSTITUENTS FROM THE BARK OF *TOXICODENDRON VERNICIFLUUM* (STOKES) F.A. BARKLEY

Hee Rae Kang[1], Hee Jeong Eom[1], Seulah Lee[1], Jae Sik Yu[1], and Seoung Rak Lee[1]
[1]Natural Product Research Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

Toxicodendron vernicifluum (Stokes) F.A. Barkley (Anacardiaceae) has traditionally been used as a food supplement and in traditional herbal medicine to treat inflammatory diseases and cancers for centuries in Korea. This study was designed to isolate the bioactive constituents from the ethanol extract of *T. vernicifluum* bark and evaluate their cytotoxic and anti-inflammatory activities. Bioassay-guided fractionation and chemical investigation of the ethanol extract of *T. vernicifluum* bark resulted in the isolation and identification of three new polyphenols (**1–3**) and six flavonoids (**4–9**). The structures of the isolated compounds were elucidated by spectroscopic analysis, including 1D and 2D NMR, and HR-MS, and their absolute configurations were further confirmed by chemical methods and CD data analysis. Compounds **4–9** showed antiproliferative activity against the tested cells, with IC_{50} values of 4.78–28.89 μM. Compounds **5** and **8** significantly inhibited NO production in lipopolysaccharide (LPS)-stimulated BV-2 cells with IC_{50} values of 23.37 and 11.68 μM, respectively.

2007

ALKALOIDS FROM *ACORUS GRAMINEUS RHIZOMES* AND THEIR BIOLOGICAL ACTIVITY

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As part of our ongoing search for bioactive constituents from natural Korean medicinal resources, a bioassay-guided fractionation and chemical investigation of the MeOH extract from the rhizomes of *Acorus gramineus* resulted in the isolation and identification of two alkaloids, including a new aporphine-type alkaloid (**1**), named gramichunosin, and a known pyrrole alkaloid (**2**). Their structures were determined by a combination of 1D and 2D NMR spectroscopic analysis and HRMS. This is the first report of alkaloids from *A. gramineus*. Compounds **1** and **2** showed antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines with IC_{50} values in the range of 7.46-45.23 μM. Moreover, the anti-neuroinflammatory activities of these compounds were determined by measuring the nitric oxide (NO) levels in the medium using murine microglia BV-2 cells. Compound **1** inhibited NO production in lipopolysaccharide-stimulated BV-2 with IC_{50} values of 7.83 μM.

2008

TARGETING BIOACTIVE CHEMICAL SPACE WITH A SMALL NATURAL PRODUCTS LIBRARY: EXPANDING DIVERSITY AND PREDICTABILITY

Jacqueline L. von Salm[1,2], Daniel Santiago[1], Nerida G. Wilson[3], Laurent Calcul[1,2], Dennis E. Kyle[4], Wayne C. Guida[1,2,5], and Bill J. Baker[1,2]
[1]Department of Chemistry and [2]Center for Drug Discovery & Innovation, University of South Florida, Tampa, FL 33620, USA, [3]Western Australia Museum, Perth, Western Australia, Australia, [4]Department of Global Health, University of South Florida, Tampa, FL 33620, USA, [5]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL 33620, USA

Diverse compound libraries have become a necessity to drug discovery efforts around the world. Target specific computational methods and high-throughput screening programs have aided the search for bioactive compounds, however, inefficiencies remain for neglected and tropical diseases. Detailed knowledge of mechanisms of action and target proteins is limited for parasitic diseases like malaria and leishmaniasis. In order to effectively lead research programs in these areas, our lab has developed a small compound library with diversity that mimics the NCI Diversity Set and the AntiMarin natural products database. Specific activity exhibited by each compound against *Plasmodium falciparum* and *Leishmania donovani* has been overlain to create activity "hotspots", which we hope to improve activity prediction methods for unknown natural products isolated in the future. Limitations in diversity remain in all three libraries, and appear to have properties resembling small (MW < 400) hydrophobic molecules like terpenes. Here we use active antileishmanial terpenoids isolated from Antarctic marine organisms as models to show expansion of overall diversity and future predictability of the library as a bioassay dereplication tool.

2009

MICROBIAL MANNOSIDATION OF CHLOROGENTISYL ALCOHOL BY THE MARINE-DERIVED FUNGUS *CHRYSOSPORIUM SYNCHRONUM*

Keumja Yun and Byeng W. Son
Department of Chemistry, Pukyong National University, Busan 608-737, Korea.

The microbial transformation of the biologically active chlorogentisyl alcohol, isolated from the marine-derived fungus *Aspergillus* sp., was studied.

Preparative-scale fermentation of chlorogentisyl alcohol with marine-derived fungus *Chrysosporium synchronum* resulted in the isolation of a new glycosidic metabolite, 1-*O*-(α-*D*-mannopyranosyl)chlorogentisyl alcohol. The stereostructure of the new metabolite obtained was assigned on the basis of detailed spectroscopic data analyses, chemical reaction, and chemical synthesis. Chlorogentisyl alcohol and its mannoside exhibited significant radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 1.0 and 4.7 μM, respectively. Both compounds were more active than the positive control, *L*-ascorbic acid (IC_{50}, 20.0 μM).

TOPIC – Microbial Natural Products
KEYWORDS - microbial mannosidation, 1-*O*-(α-*D*-mannopyranosyl) chlorogentisyl alcohol, *Chrysosporium synchronum*

2010

UNUSUAL WITHANOLIDES FROM *PHYSALIS HISPIDA* (WATERF.) CRONQUIST

Cong-Mei Cao[1], *Huaping Zhang*[1], *Robert J. Gallagher*[1], *and Barbara N. Timmermann*[*,1]
[1]*Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045, United States*

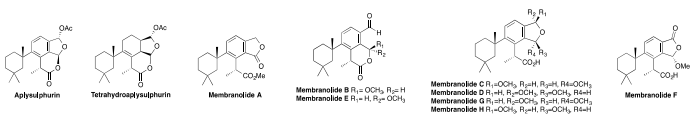
Withanolides are a group of modified C_{28} ergostane-type steroids with a C-22, C-26 δ-lactone side chain. As part of our continuing search for unusual withanolides for SAR studies, we isolated and characterized nine new withanolides (**1**–**9**), withahisolides A–I, as well as nine known compounds (**10**–**18**) from the aerial parts of *Physalis hispida*. The structures of **1**–**9** were elucidated through a variety of spectroscopic techniques, while those of **1** and **2** were further confirmed by X-ray crystallographic analysis. Among the eight new withanolides (**1**-**7**, **9**) with an unusual six-membered ring D, **1**–**3** are the first withanolides possessing non-aromatic six-membered ring D moieties. In addition, withanolide **8** represents a novel withanolide skeleton due to the absence of a C-13–C-17 bond within its steroidal nucleus.

2011

ECOLOGICAL AND ANTILEISHMANIAL ACTIVITY OF DITERPENES DERIVED FROM THE ANTARCTIC SPONGE *DENDRILLA MEMBRANOSA*

Christopher G. Witowski[1,3], *Jacqueline L. von Salm*[1], *J. Alan Maschek*[1], *Margaret O. Amsler*[4], *Brian A. Vesely*[2], *Dennis E. Kyle*[2], *James B. McClintock*[4], *Charles D. Amsler*[4], *and Bill J. Baker*[1,3]
Departments of [1]*Chemistry,* [2]*Global Health, and* [3]*Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL 33612, and* [4]*Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294*

The cold waters of Antarctica harbor bountiful marine life and biodiversity that promotes competition and the biosynthesis of defensive secondary metabolites. One inhabitant, the vibrant yellow sponge *Dendrilla membranosa*, is known to inhibit feeding of Antarctic predators such as sea stars and amphipods. A metabolomics approach was undertaken to identify whether sponges within the amphipod-rich algal canopy adopt different chemical profiles to account for the increased predation pressure. In addition, our group has identified the membranolides from *D. membranosa*. This suite of methoxylated compounds possess potent and selective activity against the leishmaniasis-causing parasite *Leishmania donovani*. The origin of these compounds will be investigated as artifacts from methanolic degradation of aplysulphurin.

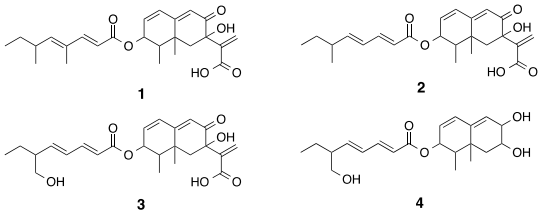


2012

NEW ANTI-PROLIFERATIVE PETASOL ANALOGS FROM FUNGUS FT087

Chunshun Li[1], *Baojun Yang*[1], *James Turkson*[1] *and Shugeng Cao*[1,*]
[1]*Natural Products and Experimental Therapeutics, Cancer Center, University of Hawaii, 701 Ilalo Street, Honolulu, Hawaii 96813, USA*

In our search for anti-proliferative natural products from Hawaiian fungi as part of our Natural Product and Experimental Therapeutics Program, we screen our semi-pure natural product library against a panel of human cancer cell lines. One fraction generated from fungus FT087 was active against both A2780 (cisplatin-sensitive A2780) and A2780cisR (cisplatin-resistant A2780). Bioassay-guided fractionation led to the isolation of four new petasol analogs **1**-**4** together with a few known compounds. The structures of the new compounds were elucidated by spectroscopic methods. Some compounds showed strong antiproliferative activity against A2780S and A2780cisR.



2013

BIOACTIVE LIGNAN CONSTITUENTS FROM THE TWIGS OF *LINDERA GLAUCA*

Hee Rae Kang[1], *Hee Jeong Eom*[1], *Seulah Lee*[1], *Jae Sik Yu*[1], *and Seoung Rak Lee*[1]
[1]*Natural Product Research Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea*

A bioassay-guided fractionation and chemical investigation of the MeOH extract from the twigs of *Lindera glauca* (S_{IEB}. et Z_{UCC}.) B_{LUME} resulted in the isolation and identification of six lignans (**1–6**) including three new lignan derivatives, named linderuca A (**1**), B (**2**), and C (**3**). The structures of the new compounds (**1–3**) were determined on the basis of spectroscopic analyses, including two dimensional NMR and circular dichroism (CD) spectroscopy studies. The cytotoxic activities of the isolates (**1–6**) were evaluated by determining their inhibitory effects on human tumor cell lines. Compounds **1–5** showed antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines with IC_{50} values of 7.79–29.42 ***μ***M. Based on the understanding that inflammation is a crucial cause of tumor progression, we also investigated the anti-inflammatory activities of the isolates (**1–6**) in the lipopolysaccharide-stimulated murine microglia BV-2 cell line by measuring nitric oxide (NO) levels. The new lignans (**1–3**) significantly inhibited NO production with IC_{50} values of 12.10, 9.48, and 9.87 ***μ***M, respectively, without cytotoxicity.

2014

BACTERIAL SYMBIONTS ISOLATED FROM FUNGUS-GROWING ANTS COLLECTED IN SÃO PAULO STATE, BRAZIL, AS SOURCES OF NATURAL PRODUCTS

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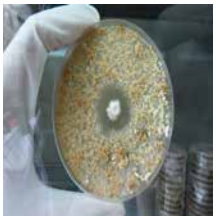
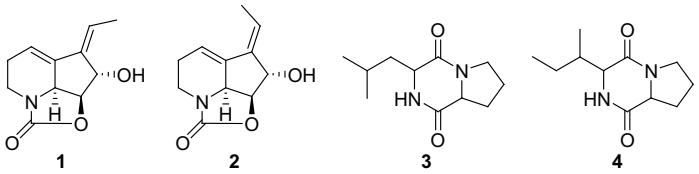
[1]*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP, Brazil,* [2]*University of Wisconsin, Madison, USA,* [3]*Harvard Medical School, Boston, USA*

As part of an ongoing ICBG (International Cooperative Biodiversity Groups) project we have focused collecting efforts on leaf-cutting ants from

different Brazilian biomes aiming the identification of new pharmacologically active natural products from insect bacterial symbionts. The initial collecting efforts have been centered on remaining areas of Atlantic Forest and Cerrado in São Paulo State. Seventy seven bacterial strains were isolated from the bodies and fungal gardens of *Acromyrmex* ants. All bacterial strains were evaluated in antagonism bioassays against the specific pathogenic fungus *Escovopsis* sp. Four of them showed good antagonist activity and presents morphology of actinobacteria. The four bioactive strains were cultured using ISP-2 agar and extracted with EtOAc. One extract has been fractionated by chromatographic methods and the antibiotics streptazolin (**1**) and its E-isomer (**2**) have been identified by NMR and HRESIMS; in addition to the diketopiperazines Cyclo(Leu-Pro) (**3**) and Cyclo(Ile-Pro) (**4**). Compound **1** has also been detected by HPLC-ELSD-PDA in the inhibition zone between the ant symbiont bacteria and *Escovopsis* sp.





2015

STRUCTURE ELUCIDATION OF TWO NOVEL PEPTIDES FROM A MUSHROOM-DERIVED *STREPTOMYCES* SP.

Thomas P. Wyche[1], Antonio C. Ruzzini[1], Laura Schwab[2], Cameron R. Currie[2], and Jon Clardy[1]
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Bacterial-fungal interactions are present in numerous environments, including agriculture, food, and the human body, but in many cases, this relationship is not well understood. To investigate interactions between bacteria and fungi, we examined the metabolites produced by several bacterial strains isolated from a collection of mushrooms. One strain (*Streptomyces* sp. HrubLS-53), isolated from the mushroom *Hymenochaete rubiginosa*, was found to produce enterobactin and paenibactin, as well as two novel peptides. The novel structures were determined by NMR and MS, including a ^{13}C-^{13}C COSY of isotopically enriched compounds.

2016

PHYTOCHEMICAL AND ANTI-BACTERIAL STUDIES OF CISSUS IBUENSIS HOOK (VITACEAE)

Augustine A Ahmadu[1], Adebola Onanuga[2] and A. Agunu[3]
[1]Department of Pharmaceutical and Medicinal Chemistry, Niger Delta University, Wilberforce island,Bayelsa-Nigeria, [2] Department of Pharmaceutical Microbiology and Biotechnology, Niger Delta University,Wilberforce island,Bayelsa-Nigeria, [3] Department of Pharmacognosy and drug development,Ahmadu Bello University, Zaria-Nigeria

As part of our studies on Nigerian ethnomedicinal plants for bioactive plant metabolites,the leaves of Cissus ibuensis was examined. Cissus ibuensis is a climber which belongs to the family Vitaceae and is widely distributed in tropical countries especially Nigeria,Niger,Togo and Ghana (1). Traditionally,the leaves of this plant is used in Northern Nigeria to treat gastrointestinal disturbance,as remedy for bacterial infections and to relieve rheumatism and arthrithis (2). The acetone and ethanolic extracts of the leaves were screened for anti-bacterial activity against clinical isolates of S.aureus,B.subtilis,E.coli and Ps.aeruginosa using the method described by Mendoza (3). The ethanol extract showed significant activity against all the test bacteria isolates. The ethanol extract was then fractionated into ethyl acetate and n-butanol. The n-butanol soluble part of the ethanolic extract showed activity against all the test organisms with zones of inhibition ranging from 16-20mm. The n-butanol extract was then subjected to column chromatography over silica gel and reverse phase HPLC to afford four flavonoids namely: Rutin (15 mg),Kaempferol 3-O-α-rhamnopyranosyl 1-6) β-D-galactopyranoside (II),Kaempferol 3-O-rutinoside (III) and Kaempferol 3-O-dirhamnosyl galactoside (IV). The structures were elucidated using spectroscopic technique and compared with literature (4,5)

Reference
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2.Dalziel,J M(1958). Flora of West Tropical Africa,PP 280-281
3. Mendoza,L;Wilkens M and Urzua A (1997) . Journ of Ethnopharmacol 58(2):246-252
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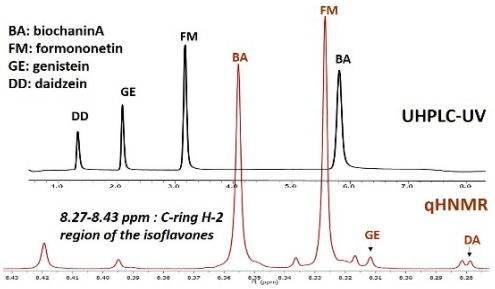
5.Markham KR et al (1978) ^{13}C-NMR spectra of Flavonoids.

2017

ORTHOGONAL LC AND QNMR STANDARDIZATION CONFIRMS STABILITY OF RED CLOVER CLINICAL EXTRACT

Rasika S. Phansalkar, Shao-Nong Chen, Charlotte Simmler, David C. Lankin, James B. McAlpine, Guido F. Pauli
University of Illinois at Chicago, UIC/ NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, 833 S. Wood Street, Chicago, Illinois.



Trifolium pretense L. (red clover) is well known for containing estrogenic isoflavones and is one of the commonly used herbs for the alleviation of post-menopausal symptoms. In this study, we have developed two orthogonal methods, UHPLC-UV and qHNMR, for the quantitation of the two major isoflavones, biochanin A and formononetin, as well as several minor isoflavones including daidzein and genistein, in a 10-year old clinical trial extract. Quantitation by ^{1}H-NMR has been performed using two approaches. The first employs the traditional integral-based quantitation, internal calibration, and linear deconvolution of the signals to resolve the overlap. The second, more advanced approach uses the quantum-mechanical based deconvolution approach of the HiFSA-qHNMR method. Thus, quantitation is performed based on the population ratios of different molecular species in the extract via QM-based spectral simulation. The quantitative results from the LC and the qHNMR methods are congruent, allow conclusions about precision and accuracy in botanical standardization, and demonstrate the chemical stability of the red clover extract over time.

2018

OXYESTEROL-BINDING PROTEIN FAMILY (OSBP/ORP) LIGAND BINDING AND NATURAL PRODUCT DRUG DEVELOPMENT

Juan Nunez, Nicholas Wasinger, Anthony W.G. Burgett
Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Ok 73109, USA

A structurally-diverse class of potent anti-cancer natural product compounds – cephalostatin 1, OSW-1, ritterazine B and schweinfurthin A – exert their anti-proliferative activity through binding to members of the oxysterol-binding protein (OSBP) and OSBP-related protein (ORP) family. Based on their shared OSBP/ORP cellular targets, these natural products are referred to as the ORPphilins. The ORPphilin compounds have been shown to bind with high affinity (K_i values = 20-70 nM) to two members of the OSBP/ORP family; namely OSBP and ORP4. This shared tight binding is remarkable due to the structurally-diversity of the ORPphilin natural product class of molecules, and the fact these compounds were isolated from very different biological sources. The cellular function(s) of the OSBP/ORPs are yet to be fully determined, but some of the family members apparently serve as sterol sensors and/or sterol transporting proteins. There are twelve different OSBP/ORPs in humans that show very different tissue distribution patterns. The expression levels and function of many different OSBP/ORPs have been implicated in in many disease-states, including different cancers, diabetes and cardiovascular disease. Despite their connections to disease, the OSBP/ORPs remain understudied with respect to their ligand binding and resulting biological function. The OSBP/ORP have been shown to experimentally bind oxysterols and phospholipids, but the physiological ligands for the OSBP/ORP members have not been identified. We have launched a systematic study of class-wide OSBP/ORP ligand binding, which includes understanding the binding interactions of the OSBP/ORPs with the ORPphilin natural products. Our 96-well OSBP/ORP ligand binding assay allows screening of many ligands against the complete panel of OSBP/ORP proteins, and the screen will incorporate a diverse set of oxysterols, phospholipids and ORPphilin-derived compounds. Through these comprehensive binding assays and subsequent biochemical studies, we will identify putative physiological ligands for individual OSBP/ORP family members. We will also begin to develop natural product-derived anti-cancer compounds, based on the ORPphilin compound OSW-1, that selectively target the anti-cancer target protein ORP4 over the other members of the OSBP/ORPs family.

2019

BIOACTIVE SALICIN DERIVATIVES FROM *SALIX GLANDULOSA*

Won Se Suh, Chung Sub Kim, Kyoung Jin Park, Oh Kil Kwon, Joon Min Cha, and Kang Ro Lee
Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

The genus *Salix* (willow) includes about 400 species of deciduous trees, and extracts of their bark have been used in folk medicine for the treatment of fever, pain and inflammation. *Salix glandulosa* SEEMEN (= *S. chaenomeloides* KIMURA, Salicaceae), also called "Korean King Willow", is distributed in Korea, Japan and mainland China. In a continuing search for bioactive constituents from Korean medicinal plants, we found that the MeOH extract of *S. glandulosa* twigs showed anti-inflammatory effect. The $CHCl_3$ and EtOAc layers were subjected to repeated column chromatography and semi-preparative HPLC to give two new salicin derivatives (**1**-**2**), along with fourteen known analogues (**3**-**16**). The structures of **1**-**16** were characterized by the use of NMR methods (^1H and ^{13}C NMR, ^1H-^1H COSY, HMQC and HMBC), chemical hydrolysis, and GC/MS. All the isolates (**1**-**16**) were evaluated for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line.

2020

THREE NEW LIGNAN DERIVATIVES FROM THE TWIGS OF *SAMBUCUS WILLIAMSII*

Won Se Suh, Kyoung Jin Park, Joon Min Cha, Oh Kil Kwon, and Kang Ro Lee.
Natural Products Laboratory, School of Pharmacy, SungKyunKwan University, Suwon 440-749, Republic of Korea

As a part of our ongoing search for cytotoxic constituents from natural Korean medicinal sources, we investigated the constituents of the twigs of *Sambucus williamsii* var. *coreana* NAKAI (Caprifoliaceae). This plant is deciduous shrub, which is widely distributed throughout Korea, Japan and China. *S. williamsii* has been used as a Korean traditional medicine for the treatment of bone fracture and osteoporosis. Previous phytochemical investigation on *S. williamsii* reported triterpenes, phenolic compounds, and lignans. Especially, lignans were mainly isolated and some of them showed anti-proliferation activities on osteoblastic-like UMR106 cells. A bioassay-guided fractionation and chemical investigation of the twigs of *S. williamsii* resulted in the isolation and characterization of three new lignan derivatives (**1**-**3**) along with seven known lignans (**4**-**10**). The structures of these new compounds were determined on the basic of spectroscopic analyses including 2D NMR and CD spectroscopic data. The isolated compounds (**1**-**10**) were tested for their cytotoxic activity on the human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using a SRB assay.

2021

BIOACTIVITY-GUIDED ISOLATION AND QUANTIFICATION OF EPISILVESTROL AND SILVESTROL FROM *AGLAIA PERVIRIDIS*

Garima Agarwal,[1] Li Pan,[1] C. Benjamin Naman,[1] Hee-Byung Chai,[1] Tran Ngoc Ninh,[2] Djaja Djendoel Soejarto,[3,4] and A. Douglas Kinghorn.[1]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210; [2]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [3]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612; [4]Science and Technology, Field Museum, 1400 S. Lakeshore Dr., Chicago, IL 60605

Bioactivity-guided isolation, fractionation and quantification from *Aglaia perviridis* Hiern (Meliaceae) (voucher specimen: *Soejarto et al* 14595) demonstrated it to be only the third plant of the genus *Aglaia* found to produce silvestrol and its epimer, 5‴-episilvestrol (episilvestrol). Several fractions from the chloroform extract produced from the whole plant were found to be active against HT-29 human colorectal cancer cells *in vitro*. Further purification and chemical analysis of the active fractions interestingly showed them to contain episilvestrol in a higher amount compared to silvestrol, contrary to the observation of silvestrol being the more abundant compound as in *Aglaia foveolata*.

2022

PHARMACOKINETIC INTERACTIONS BETWEEN DRUGS AND BOTANICAL DIETARY SUPPLEMENTS

Alyssa A. Sprouse[1,2] and Richard B. van Breemen[1,2].
[1] Department of Medicinal Chemistry and Pharmacognosy, [2] UIC/NIH Center for Botanical Dietary Supplements Research University of Illinois at Chicago, Chicago, IL

The use of botanical dietary supplements has grown steadily over the last 20 years despite incomplete information regarding active constituents, mechanisms of action, efficacy, and safety. An important but under-investigated safety concern is the potential for popular botanical dietary supplements to interfere with the absorption, transport and/or metabolism of pharmaceutical agents. Clinical trials of drug-botanical interactions are the gold standard

and are usually carried out only when indicated by unexpected consumer side effects or, preferably, by predictive in vitro studies. For example, Phase I clinical trials have confirmed clinical observations and in vitro studies that St. John's wort (*Hypericum perforatum*) induces cytochrome P450 3A4/5, whereas Phase I studies did not substantiate in vitro predictions that milk thistle (*Silybum marianum*) would inhibit CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. Here, we highlight discrepancies between in vitro and in vivo data concerning drug-botanical interactions and critically evaluate why some in vitro models overestimate or sometimes underestimate the potential for drug-botanical interactions. Gaps in our knowledge are also highlighted for the potential of some popular botanical dietary supplements to interact with therapeutic agents with respect to absorption, transport and metabolism.

Supported by grant P50 AT000155 from the NIH ODS and NCCIH

2023

FUNDING OPPORTUNITIES IN THE NCI'S CANCER DIAGNOSIS PROGRAM

Tawnya C. McKee

Diagnostic Biomarkers and Technology Branch, Cancer Diagnosis Program, DCTD, NCI, Bethesda, MD 20892-9704.

The Cancer Diagnosis Program is part of the NCI's Division of Cancer Treatment and Diagnosis and supports a variety of funding opportunities related to cancer diagnosis and treatment. New funding opportunities include: NCI's IMAT Program, Academic-Industrial Partnerships for Translation of Technologies for Cancer Diagnosis and Treatment, Assay Validation for High Quality Markers for NCI-Supported Clinical Trials, Opportunities in Bio-engineering Grants and Advancing Translational and Clinical Probiotic/Prebiotic and Human Microbiome Research. These and other related funding opportunities will be presented.

2024

BOTANICAL INITIATIVE FOR THE DIETARY SUPPLEMENT INGREDIENT DATABASE (DSID): PRELIMINARY DATA FOR GREEN TEA SUPPLEMENTS

Karen W Andrews[1], Phuong-Tan Dang[1], Sushma Savarala[1], Pavel A Gusev[1] Fei Han[1], Pamela R Pehrsson[1], James M Harnly[2], Pei Chen[2], Johanna T Dwyer[3], Joseph M Betz[3], Leila G Saldanha[3] and Rebecca B Costello[3]

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The Dietary Supplement Ingredient Database (DSID) provides analytically-derived estimates of the ingredient content in dietary supplements (DS). DSID 3.0 now includes 4 categories of DS: adult, children's and non-prescription prenatal multivitamin/mineral products (MVM), and omega-3 fatty acid DS. Green tea was chosen for analysis in the pilot study for the botanical initiative due to high consumption and availability of certified reference materials and validated analytical methods. The goals of this study are to obtain estimates of content for catechins, caffeine and other ingredients in products with and without label information. Green tea DS (n=32) were purchased from multiple sales channels and analyzed by 3 laboratories. A third (33%) of the products had labeled amounts for caffeine (4–195 mg per day) or total catechins (19-700 mg per day). More products (75%) had labeled amounts for epigallocatechin gallate (EGCG) or total polyphenols ranging from 125-1050 mg per day and 28-1200 mg per day respectively. In initial tests, 44% of products failed disintegration testing. Preliminary evaluation of the quality control data showed agreement among the labs for caffeine and EGCG in certified reference materials. The final results will inform the plans for the next botanical study.

2025

THE DISCOIPYRROLES: A MULTIFACETED APPROACH TO UNDERSTAND A NOVEL FAMILY OF MARINE NATURAL PRODUCTS

Colosimo DA[1], Cai F[1], Hu Y[1], Potts MB[2], White MA[2], Ready JM[1], MacMillan JB[1]

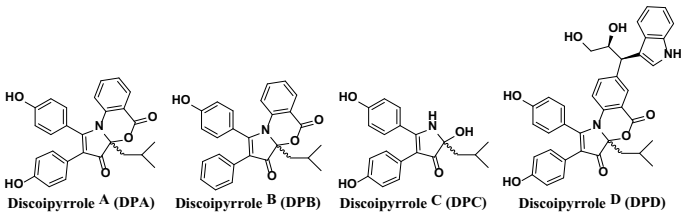
[1]Department of Biochemistry, [2]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75390

Around 4-8% of patients with squamous cell carcinoma, the most prevalent subtype of non-small cell lung cancer (NSCLC), carry genetic mutations in discoidin domain containing receptor 2, or DDR2 (Hammerman, PS, *Cancer Discovery* **2011**). This unique receptor tyrosine kinase binds collagen in the extracellular matrix and initiates signaling for cellular processes such as migration and adhesion. We discovered a novel family of natural products, the discoipyrroles, produced by the marine bacteria *Bacillus hunanensis* that demonstrate potent cytotoxicty towards DDR2 mutated NSCLC cell lines, but not DDR2 wildtype NSCLC cell lines (Hu, Y, *JACS* **2013**). Currently, we are investigating multiple aspects of the discoipyrroles including their optimization as potential therapeutics, the mechanism by which they elicit cell-specific cytotoxicity, and their intriguing biosynthesis which features key non-enzymatic steps.

Cytotoxicity to lung cancer cell lines

	HCC366*	H2286*	A549†	>40 cell lines†
DPA	0.12	0.256	10.2	>24
DPB	0.19	0.276	8.8	-
DPC	0.712	0.796	19.6	-
DPD	0.275	0.412	13.4	-
Dasatanib	0.17	-	9.8	-

*DDR2 mutant cell lines (HCC366: L293R; H2286: I638F).
†DDR2 wildtype cell lines. IC_{50} in µM.



Discoipyrrole A (DPA) **Discoipyrrole B (DPB)** **Discoipyrrole C (DPC)** **Discoipyrrole D (DPD)**

2026

IDENTIFICATION OF NEW THERAPEUTIC LEADS FOR TRIPLE NEGATIVE BREAST CANCER SUBTYPES

Andrew J. Robles[1], Lin Du[3], Shengxin Cai[3], Robert H. Cichewicz[3] and Susan L. Mooberry[1,2].

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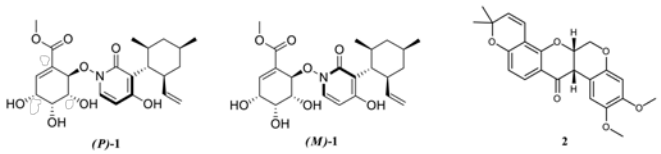

***(P)*-1** ***(M)*-1** **2**

High-content screening of plant and fungal extracts in a panel of cell lines modeling triple negative breast cancer subtypes identified 11 extracts with selective activity against particular cell lines. Maximiscin, ***(P/M)*-1**, was identified as having selective cytotoxic efficacy against the basal-like MDA-MB-468 cell line. Deguelin **(2)** was identified as having selective antiproliferative activity against the MDA-MB-453 cell line, representing the luminal androgen receptor subtype of TNBC. Mechanistic studies showed that ma-

ximiscin caused an accumulation of cells in G_1. Immunoblotting of maximiscin-treated whole cell lysates showed that maximiscin increases levels of P-p53, P-Chk1, P-Chk2, and γ-H2A.X. These results are consistent with the observed G_1 accumulation and collectively suggest that maximiscin induces DNA damage. Preliminary experiments suggest deguelin modulates AR localization and inhibits phosphorylation of Akt, ribosomal protein S6 and 4E-BP1 as soon as 2 h after treatment. These results demonstrate that new compounds with potential therapeutic value for the treatment of TNBC subtypes can be identified from nature.

2027

CHEMICAL EXCHANGE IN ENDOPHYTIC ACTINOBACTERIA COMMUNITIES

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Endophytic actinobacteria from *Lychnophora ericoides* -a Brazilian plant popularly used against wound, inflammation and pain- have not deeply studied yet, resulting in ignorance about the role this microorganisms play in this medicinal plant. Endophytic microorganisms are in a continuous endophyte-endophyte interaction inside plant tissues. For that reason, by simulating actinobacteria communities we were able to correlate phenotypic differences in interacting actinobacteria as a consequence of metabolic exchange detected by recent mass spectrometry techniques. These interesting results, as part of our efforts to understand the biological role of microbial molecules from endophytes and their impact into their hosts, will be presented.

Example of a MALDI-TOF image showing interaction of two endophytic actinobacteria resulting in elicitation of an unknown metabolite detected as the ion of *m/z* 311. Timecourse of coculture: **a.** 24, **b.** 48, **c.** 72 and **d.** 96 hours. White colonies correspond to *Streptomyces albospinus* RLe7 and brown colonies correspond to *Streptomyces mobaraensis* RLe3.

2028

GARCINIA BENZOPHENONES PROMOTE HYPHAL APOPTOSIS AND POTENTIATE ACTIVITY OF FLUCONAZOLE IN *CANDIDA ALBICANS* BIOFILMS

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Xanthochymol and garcinol, isoprenylated benzophenones purified from *Garcinia xanthochymus* fruits, showed multiple activities against *Candida albicans* biofilms. Both compounds effectively prevented emergence of fungal germ tubes, although they did not affect fungal adhesion to substrate. Xanthochymol indefinitely prevented hyphal emergence and thus prevented development of hyphae and subsequent biofilm maturation. Garcinol was a transient inhibitor. Xanthochymol (10^{-5} M) also induced hyphal-specific cell death in forming and mature biofilms. The death had characteristics of apoptosis, including externalization of phosphatydyl serine and DNA fragmentation, as evidenced by TUNEL fluorescence. This activity contributed to inhibition of biofilm maturation, and also led to hyphal death in mature biofilms. Further, xanthochymol was synergistic with fluconazole, reducing the EC_{50} value for the antifungal 80-fold from >1024 μg/ml to 13 μg/ml. Therefore xanthochymol has potential both as a reagent for determining cellular mechanisms of morphogenesis and programmed death, and as an adjuvant for antifungal treatments.

2029

QUALITY EVALUATION OF TERPINEN-4-OL TYPE TEA TREE OILS AND COMMERCIAL PRODUCTS USING GC/MS AND CHEMOMETRICS

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GC/MS, chiral GC/MS and chemometric techniques were used to evaluate a large set (n=104) of tea tree oils (TTO) and commercial products purported to contain TTO. Twenty terpenoids were determined in each sample and compared with the standards specified by ISO-4730-2004. Several of the oil samples that were ISO compliant when distilled did not meet the ISO standards primarily due to the presence of excessive *p*-cymene and/or depletion of terpinenes. Forty-nine percent of the commercial products did not meet the ISO specifications. Four terpenes, *viz*., α-pinene, limonene, terpinen-4-ol and α-terpineol, present in TTOs with the (+) isomer predominant were measured by chiral GC/MS. The results clearly indicated that 28 commercial products contained excessive (+) isomer or contained the (+) isomer in concentrations below the norm. Of the 28 outliers, 7 met the ISO standards. There was a substantial subset of commercial products that met ISO standards but displayed unusual enantiomeric (+)/(-) ratios. A class predictive model based on the oils that met ISO standards was constructed. The outliers identified by the class predictive model coincided with the samples that displayed an abnormal chiral ratio. Thus, chiral and chemometric analyses could be used to confirm the identification of abnormal commercial products including those that met all of the ISO standards.

2030

STRUCTURES OF TWO OLEANANE-TYPE TRITERPENOIDS FROM AN EXTRACT OF *CYRILLA RACEMIFLORA* HOUSED IN A REPOSITORY

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Two new oleanane-type triterpenoids were isolated from a small-scale sample of a dichloromethane partition of an ethanol-soluble extract of *Cyrilla racemiflora* L. (Cyrillaceae) collected in Dominica. This extract repository was donated to BSBI by Merck Research Laboratories, Rahway, NJ, USA. The structures of these compounds were elucidated by interpretation of their spectroscopic data, with an unusual compound containing a di-angelated glucose residue being confirmed by its MS^2 and MS^3 mass spectra. The conformation of the pentacyclic ring system of these triterpenoids was characterized based on the published single-crystal structure of 3-*O*-acetyloleanolic acid, and the absolute configuration of these two compounds was ascertained by analysis of their electronic circular dichroism (ECD) induced with $[Mo_2(OAc)_4]$ and NOESY 2D NMR spectra.

2031

NEW ANTIBACTERIAL COMPOUNDS INHIBITING *STAPHYLOCOCCUS AUREUS* ENOYL-ACP REDUCTASE FROM PENICILLIUM SP.

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Bacterial enoyl-ACP reductase (FabI) is an attractive antibacterial target which catalyzes the final and rate-limiting step in bacterial fatty acid synthesis. Therefore, FabI inhibitors could be interesting lead compounds for treatment of multidrug-resistant bacteria. In the course of our screening for FabI inhibitors from microbial resources, a fungal strain *Penicillium verruculosum* producing FabI-inhibitory and antibacterial metabolites was selected. Five novel metabolites named verrulactones A-E with the known compound, altenuisol, were isolated from the fermentation broth of the fungal strain. Their chemical structures were elucidated by 2D NMR and MS analysis. Verrulactones A and B were dimeric compounds of alternariol. Verrulactones C was a dispiro compound. Verrulactones D and E were highly quaternary and unique compounds. Verrulactones A and B strongly inhibited *Staphylococcus aureus* enoyl-ACP reductase with an IC_{50} of 1 μM. Verrulactones C-E also showed FabI-inhibition even though weaker than Verrulactones A and B. They, however, didn't inhibit *S. aureus* FabG, another reductase of bacterial FAS, even at 300 μM. It indicated selective inhibition for FabI. Consistent with their FabI inhibitory activity, they also showed inhibition on intracellular fatty acid biosynthesis in *S. aureus* as well as antibacterial activity against *S. aureus* and MRSA.

2032

RAPID IDENTIFICATION OF MINOR ACTIVE METABOLITES IN EXTRACTS OF MARINE ORGANISMS: A PLATFORM FOR EFFICIENT DRUG DISCOVERY

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Efficient identification of biologically active metabolites present in complex crude extracts continues to be a significant challenge in natural products discovery. To overcome this obstacle, contemporary drug discovery programs have placed an emphasis on the development of multifaceted approaches to capture comprehensive biological and chemical data early in the isolation workflow. This information can be used to quickly characterize active metabolites and direct research efforts to those with the highest potential for development as drug leads. Here we outline a discovery platform used to rapidly generate and screen a large fraction library from extracts of marine organisms. Fractions are concurrently analyzed by high-resolution LC-MS/MS and assayed against a panel of human cancer cell lines. Analysis of aggregate biological and mass spectrometric data is combined with molecular networking to evaluate the structural diversity of active metabolites and direct future isolation efforts. To highlight the effectiveness of this platform, we describe the identification of dolastatin 10 and symplostatin 1, potent cytotoxic linear peptides, in multiple extracts of phylogenetically diverse organisms collected from a single geographic area. Production of these compounds is attributed to a cyanobacterial strain co-occurring in low abundance with the field collected organisms. Subsequent purification of both compounds, guided by the chemical information collected during library generation, allowed structural verification by NMR.

2033

EFFECT OF PAEONIFLORIN ON NERVOUS SYSTEM: A SYSTEMATIC REVIEW

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Radix Paeoniae Alba, a well-known Chinese herbal, has been widely used in Chinese Medicine for thousands of years. Paeoniflorin (PF) has been extensively studied in the last 5 years and shows multiple pharmacological actions. In this paper, we review the relevant literature to know the effect of PF on nervous system (NS). Data sources of PUBMED from January 2012 to April 2015 were searched. Inclusion criteria clearly described the effect of PF on NS. Among 280 articles identified in the literature search, 22 studies met the review inclusion criteria. According to current findings, PF was found to ameliorate the decline of memory and learning capacities among NS-related pharmacological models. In addition, PF shows protective effects on depression, cerebral ischemia injury as well as Parkinson's disease by reliving the pain and improving neural synapse plasticity. The pharmacological targets involve multiple pathways including adenosine A1 receptor, M cholinergic receptor, opiate receptor calcium ion channel, NF-κB, Akt and NGF.

2034

DISCOVERY OF FATTY ACID SYNTHASE INHIBITORS AND THEIR BIOSYNTHETIC PATHWAYS BY A NOVEL TARGET-DIRECTED GENOME MINING STRATEGY

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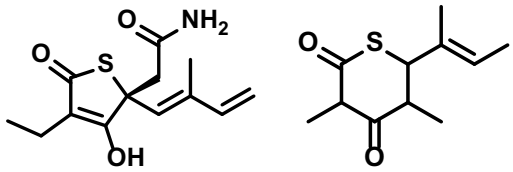

In order to avoid self-toxicity, many antibiotic-producing microbes have developed self-resistance mechanisms, with target modification being one frequent example. We propose that extra copies of an essential housekeeping gene found within or adjacent to a biosynthetic pathway may indicate that the compound(s) produced will target the protein encoded by the corresponding housekeeping gene. Thus, a novel and rational target-directed genome mining strategy was developed, by which potential self-resistance genes were analyzed bioinformatically, followed by a PCR-independent cloning and heterologous expression of the intact gene clusters for rapid production of compounds with desired bioactivities. As a proof-of-principle study, two related orphan gene clusters with potential *fabF* resistance genes were identified and expressed, which led to the isolation of a group of unique thiotetronic acid natural products that inhibit bacterial fatty acid synthase (**1**–**9**). A notable advantage of this genome mining strategy is that specific molecular targets can be hypothesized in the absence of priori knowledge of the structures of the molecules biosynthesized, and may streamline mechanism of action studies for the products obtained.

2035

HEMATOPOIETIC EFFECTS OF PAEONIFLORIN AND ALBIFLORIN ON RADIOTHERAPY AND CHEMOTHERAPY-INDUCED ANEMIA MICE

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Paeoniflorin (PF), a monoterpene glycoside isolated from P. lactiflora, possesses a variety of pharmacological activities. However, albiflorin (AF), another constituent regarded as a characteristic one, has not been well studied. This study aimed to investigate the hematopoietic effects of AF and PF on anemia mice induced by radiotherapy or chemotherapy and to explore the underlying mechanisms. The anemia mice were irradiated at a dose of 2.5 Gy using cobalt-60 gamma resources or intraperitoneally injected with cyclophosphamide (160.0 mg/kg). The numbers of blood cells from peripheral blood were counted. The thymus index and spleen index were also measured. In addition, of the chemotherapy-induced mice, the levels of TNF-α, GM-CSF, IL-3 in serum were measured by RIA. AF and PF significantly increased the numbers of peripheral blood cells and reversed the atrophy of thymus and spleen. Furthermore, AF and PF increased the levels of GM-CSF and IL-3 and reduce the level of TNF-αin serum.. Our results suggest that AF and PF may promote the recovery of bone marrow hemopoietic function in a myelosuppressed mouse model.

2036

IRISH *OSMUNDEA* spp.: FOOD OR SHELTER FOR *APLYSIA* sp.?

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The Irish coastline is approximately 7500 Km long representing one of the most biodiverse and rich-species habitats in Europe. With only few studies conducted in the North East Atlantic region, Irish waters can be a great source of new and unexplored chemical diversity. Four different *Osmundea* sp., commonly found in intertidal zone, have been described from Irish waters. *Aplysia* sp., is a sea hare which has been found to be associated with *Osmundea* algae. This project is focusing on the isolation and characterisation of secondary metabolites from *Osmundea* spp. and *Aplysia* sp. samples collected from the shore of Western Ireland in county Galway. The ultimate aim is to compare the chemistry produced by these two marine organisms and determine whether the sea hares are sequestering the compounds from the algae or they are using the *Osmundea* spp. as a shelter from predators and strong water currents. Thus half the *Aplysia* sp. collected were allowed to fast prior to analysis affording an opportunity to sample the chemistry contained within the sea hares rather than that contained within the digestive tract. The algal and animal samples were extracted separately in organic solvents followed by purification and isolation of secondary metabolites by means of Medium Pressure Liquid Chromatography (MPLC) and High Performance Liquid Chromatography (HPLC). Comparisons between the algal and sea hare extracts were carried out through metabolomics analysis using Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectroscopy (GC-MS). Moreover, the structures of pure metabolites were elucidated by means of 1D and 2D Nuclear Magnetic Resonance (NMR) spectroscopy.

2037

ACAI (EUTERPE OLERACEA MART.) ATTENUATES ALCOHLO-INDUCED LIVER DISEASE IN RATS

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[1]Beijing University of Chinese Medicine, 11 Beisanhuandonglu, Chaoyang Qu, Beijing 100029, China, [2]Chengde Medical University, Hebei province Chengde 067000, China, [3]Department of Psychiatry & Neuroscience, University of Florida, Gainesville, FL 32608, USA.

Alcohol-induced oxidative stress plays a crucial role in the pathological development of alcoholic liver disease. Açaí has been shown with high levels of phytochemicals that exhibit anti-oxidant and anti-inflammatory activities in many studies. However whether these features of açaí make it a good candidate for treating ALD is unknown. The aim of this study is to investigate the effects of açaí on hepatic oxidative stress and consequent liver disfunction in rats. Açaí puree (1mL /100g body weight) was administered to alcohol-induced liver disease rats for 8 weeks. Various biochemical parameters related to oxidative stress and inflammatory factors along with histology of liver were evaluated to assess the effect of açaí against ALD. Our present study has shown that the administration of açaí puree attenuated liver injury caused by alcohol consumption. The production of inflammation mediators, such as TNF-α, TGF-β and IL-8 were elevated in alcohol treated rats but decreased upon the administration of açaí puree. With the administration of açaí, the levels of NF-κB and CD68 were lowered. The results obtained here strongly suggested the protective role of açaí against ALD and these effects are via its effects on oxidant stress and inflammation.

2038

MICROBIAL METABOLISM OF PRENYLATED APIGENIN DERIVATIVES

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Prenylated flavonoids are rich in structural variety and pharmacological activity. It has been reported that the introduction of a prenyl group into the flavonoids could improve their biochemical and pharmacological properties. Many prenylated apigenins have been shown to exhibit significant antitumor, anticonvulsant, anti-inflammatory and vascular protective properties, but their biological activities are often limited by their poor water solubility. Microbial transformation is a useful method to modify chemical structures of natural products to derivatives which are difficult to produce synthetically, as well as to improve the solubility and biological activities. The aim of our study is to identify more polar metabolites of the bioactive prenylated apigenin derivatives through microbial metabolism. The detailed procedure of fermentation, isolation and structure elucidation of these metabolites will be presented.

2039

THE PROCESS OF DESIGNING AND DEVELOPING SPLICEOSTATIN CLASS OF SPLICING INHIBITORS AS PAYLOADS FOR ANTIBODY DRUG CONJUGATES

Anokha S. Ratnayake[1], Edmund Graziani[1], Jesse Teske[1], Kenneth Dirico[1], Haiyin He[1], Alessandra Eustaquio[2], Sujiet Puthenveetil[1], Nathan Tumey[1], Li-Ping Chang[1], Jeffrey E. Janso[1], Christopher J. O'Donnell[1], Chakrapani Subramanyam[1], Frank E. Koehn[1] and Frank Loganzo[3]

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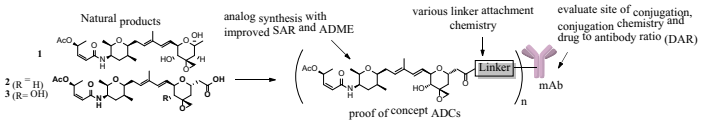
The natural product FR901464 (**1**), spliceostatin C (**2**) and thailanstatin (**3**), are ultrapotent inhibitors of eukaryotic RNA splicing, via binding to the SF3b subunit of the U2 snRNA subcomplex, an essential component of the spliceosome. Manipulation of the biosynthetic genes that control production of these analogs in the bacteria have allowed us to generate sufficient material for a medicinal chemistry approach to evaluating this mechanistic class as potential payloads for antibody drug conjugates (ADCs). This oral presentation will detail some successful ways to leverage spliceostatin analogs to generate potent and efficacious ADCs for the treatment of cancer.



2040

SMALL MOLECULE SCREENING LINKS METABOLIC PLASTICITY WITH THE TARGET ORGAN SELECTIVITY OF TRIPLE-NEGATIVE BREAST TUMOR METASTASES

Yu-Dong Zhou[1], Fakhri Mahdi[1], Kun-Ping Li[1,2], Sandra Milasta[3], Mika B. Jekabsons[4], Yan-Hong Wang[5], Ikhlas A. Khan[1,5], Jieying Gao[1,6], Jing Wu[7], Taosheng Chen[7], Yannan Ouyang[8], Douglas R. Green[3], Kounosuke Watabe[9], Dale G. Nagle[1,6]

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Metastatic disease is responsible for over 90% of cancer mortality. In breast cancer, the major secondary lesion sites are the lungs, bones, brain, and liver. Small molecule screening and comparative flux studies revealed metabolic heterogeneity in human triple-negative breast cancer MDA-MB-231-derived metastatic subclones that were isolated from the lungs (LM), bones (BoM), and brain (BrM). Oxygen consumption was significantly reduced in LM and BoM, relative to MDA-231 and BrM cells. The extracellular acidification/glycolytic rates were comparable among the cell lines. The mitochondria electron transport chain retained functionality in LM cells, but appeared dysfunctional in BoM and BrM cells. Incorporation of oxidative phosphorylation into its cellular metabolic portfolio afforded LM cells an apparent growth advantage and immunity from glucose depletion, but made LM cells more susceptible to mitochondria-targeted metabolic disruptors. The observed metabolic diversity suggests that organ-dependent metabolic plasticity may serve as a new means to selectively target metastases.

2041

GLUCURONIDE METABOLITES OF FLAVONOLIGNANS FROM MILK THISTLE (*SILYBUM MARIANUM*): ENZYMATIC SYNTHESIS

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Milk thistle (*Silybum marianum*), an herbal product used therapeutically for nearly 2000 years, has been studied extensively as treatment for hepatic disorders and as a perpetrator of herb-drug interactions. Relative to drugs, rigorous pharmacokinetic knowledge of herbal product constituents is limited at best and is virtually nonexistent when considering quantification of herbal constituent metabolites. Generation and characterization of authentic metabolite standards are essential to understanding the chemical fate and disposition of natural products both in vitro and in vivo. Regulatory guidelines highlight the importance of identifying metabolites to demonstrate safety and determine if metabolites contribute to the overall pharmacology of the drug.

A preliminary study to address this need for authentic flavonolignan glucuronide standards was conducted to determine the most cost-effective method for generating individual glucuronides of the major flavonolignans (silybin A, silybin B, isosilybin A, and isosilybin B). The following biosynthetic conditions were examined: concentrations of key reagents, enzyme concentration, enzyme source, and time course for each reaction condition. A large scale enzymatic synthesis was carried out under the optimized conditions, obtaining glucuronides in good yield (>52%) and at a substantially reduced cost of synthesis.

2042

ANTIFUNGAL PRENYLATED ISOFLAVONOIDS FROM MACLURA AURANTIACA

Yerkebulan Orazbekov[1,2,3], Ubaidilla Datkhayev[2], Manas Omyrzakov[1,2], Ahmed M. Metwaly[5], Bauyrzhan Makhatov[3], Melissa R. Jacob[1], Bakhyt Ramazanova[2], Zuriyada Sakipova[2], Amir Azembayev[6], Kuandyk Orazbekuly[7], Kulpan Orynbasarova[3], Samir A.Ross [1,4]*

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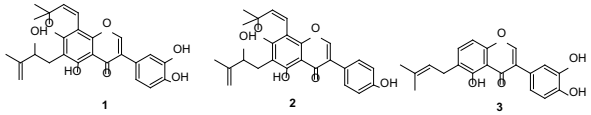
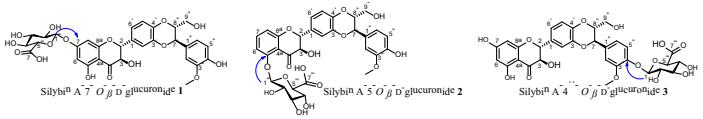
Three prenylated isoflavonoids (**1**-**3**) have been isolated from the ethanolic extract of *Maclura aurantiaca* fruits. The isolated compounds were chemically identified as 3'-hydroxyeuchrenone (**1**), euchrenone (**2**), 5,3',4'-trihydroxy-6-(3-methylbut-2-en-1-yl) isoflavone (**3**). Compounds **1** and **2** showed antifungal activities against *Candida glabrata* with IC_{50} values of 5.7 μg/ml and 1.0 μg/ml, respectively. Compound **3** showed antifungal activities against *Cryptococcus neofomans* and *Candida glabrata* with IC_{50} values of 5.0 μg/ml and 4.9 μg/ml, respectively.



2043

GLUCURONIDE METABOLITES OF FLAVONOLIGNANS FROM MILK THISTLE (*SILYBUM MARIANUM*): STRUCTURAL CHARACTERIZATION

Noemi D. Paguigan[1], Tyler N. Graf[1], Brandon T. Gufford[2], Mary F. Paine[2], and Nicholas H. Oberlies[1]
[1]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro North Carolina, USA; [2]Experimental and Systems Pharmacology, College of Pharmacy, Washington State University, Spokane, Washington, USA



Milk thistle (*Silybum marianum*) is a commonly used herbal product, of which the crude extract, termed silymarin, is a well-known hepatoprotectant. Relative to conventional drugs, rigorous pharmacokinetic knowledge of herbal constituents is limited, largely due to the scarcity of reference standards. Glucuronidation is a conjugation reaction that plays a key detoxifying role in xenobiotic metabolism. Glucuronide metabolites are needed as reference standards to advance the understanding of silymarin metabolism, particularly with respect to situations when silymarin is taken in combination with drugs. The aim of this study was to identify the structures and the glucuronidation sites of chemoenzymatically synthesized glucuronides of one constituent, silybin A, by HRMS and NMR. Glucuronide biosynthesis resulted in three *O*-glucuronide regioisomers (**1**-**3**), with the highest yield from glucuronidation at the 7 position.

2044

NOVEL 22-MER PEPTAIBOLS ISOLATED FROM *TOLYPOCLADIUM* SP. WITH POTENT ANTITUMOR ACTIVITIES

Lin Du[1,2], April L. Risinger[4,5], Carter A. Mitchell[1,2], Blake W. Stamps[3], Ning Pan[2], Jarrod B. King[1,2], Zhibo Yang[2], Bradley S. Stevenson[3], Robert H. Cichewicz[1,2]
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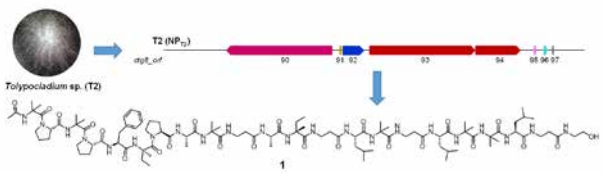

Genome mining of a *Tolypocladium* sp. isolate led to the purification and identification of gichigamin A (**1**), the first 22-mer and longest peptaibol from a natural source. The β-alanine rich sequence of **1** results in the assembly of an unprecedented 3_{10}-like helical structure, which was revealed by single-crystal X-ray diffraction analysis. The unique structural character of **1** was determined to be essential for its unexpected high penetrability through cell membranes. As a result, gichigamin A (**1**) exhibited potent *in vitro* cytotoxicity by direct disruption of mitochondrial function and the capability to inhibit MIA PaCa-2 xenograft tumor growth in nude mice.

2045

QUANTITATION OF MAJOR INDOLE AND OXINDOLE ALKALOIDS FROM DIFFERENT KRATOM (MITRIGYNA SPECIOSA) STRAIN EXTRACTS USING UHPLC-MS/MS

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Kratom (*Mitragyna speciosa*) is a common plant found in Southeast Asia, particularly Indonesia, Malaysia and Thailand. Kratom is high in the alkaloid mitragynine, as well as other minor constituents of the indole and oxindole classes. Mitragynine is an indole alkaloid that acts on opioid receptors and might be a viable alternative to morphine. When taken in conjunction with morphine, mitragynine has been shown to improve analgesia and delay tolerance significantly. This combination would offer analgesic synergism to allow a reduction of the required dosage. When taken alone, mitragynine has similar analgesic activity as codeine. Kratom has a complex extract profile. Published work has analyzed organic extracts but not aqueous extracts, which are the forms of routine human use (such as steeping kratom leaves in water). There are also variations in the chemical profiles between different strains of kratom. However, the only published quantitation of kratom constituents concerned plant material purchased via the internet that was not characterized by strain. In this study, UHPLC-MS/MS was used to quantify and compare 4 major constituents of 5 different strains of kratom.

2046

NEAR REAL TIME *IN SITU* ANALYSIS OF FUNGAL NATURAL PRODUCTS USING LAESIMS

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Laser ablation electron spray ionization mass spectrometry (LAESIMS) can be used to rapidly detect compounds *in situ* with minimal sample preparation at ambient conditions. LAESIMS was explored for use as a dereplication method in a natural products discovery pipeline with a natural product pure compound library. This resource helped test the limits of LAESIMS detection. The resulting data were analyzed for correlations between detection and physical properties (functional groups and ACD calculated variables). Selected fungal compounds were analyzed *in situ* by LAESIMSMS to assess the prospects of using this method for metabolite dereplication from microbial colonies on Petri plates.

2047

COMPARATIVE STUDIES IN THE PHYTOCHEMICAL PROFILE OF *CICHORIUM INTYBUS*

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Bioassay guided fractionation of *Cichorium intybus* collected in East Tennessee yielded two active metabolites in minute quantities. Attempts have been made to optimize active metabolite production from liquid cell culture and hairy root transformation. Quantification of active metabolite profiles from various methods will be presented here.

2048

METABOLOMIC APPROACH TOWARDS STABILITY OF HERBA ANDROGRAPHIDIS

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Herba Andrographidis is the aerial part of *Andrographis paniculata* (Burm. F.) Nees (Acanthaceae). The herb is widely used in traditional medidine throughout several Asian countries. In WHO Monographs on Selected Medicinal Plants (vol. 2), Herba Andrographidis has been listed as a prophylaxis and for the treatment of common cold and upper respiratory infection, as well as for lower urinary tract infection and diarrhea. The herb has a very disadvantageously short shelf life of six months. Here we traced stability of Herba Andrographidis using an NMR-based metabolomics approach. The herb was stored over a period of six months. The ^{1}H NMR spectra of the crude $CHCl_3$ extracts ($CDCl_3$, 500 MHz) showed only two major diterpene lactones, andrographolide and deoxyandrographolide, predominated the NMR spectra. After four months, PCA began to show a cluster due to the decomposition of the chemical constituents in the herb. The NMR spectra agreed with the loading plots and showed that whereas the contents of deoxyandrographolide varied very little, andrographolide, which is also the most active components, have started to disappear. The results were in line with the recommended short shelf life (<6 months); however, we were still unable to trace dideoxy-anhydrodehydroandrographolide, reported previously as the degraded products from the heat-accelerated experiments, nor were other derivatives that might be possible decomposition products of the disappearing andrographolide.

2049

DIGGING DEEP FOR NEW COMPOUNDS FROM *SILPHIUM LACINIATUM*

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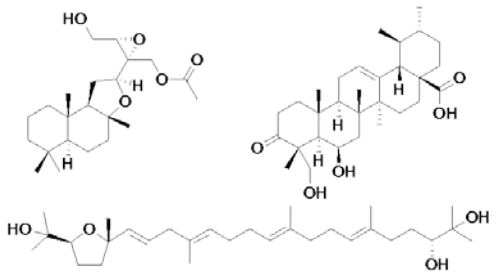

The compass plant, *Silphium laciniatum*, is an iconic perennial plant of the North American tallgrass prairie. The plants of the tallgrass prairie have historically been subjected to a number of biological and environmental stresses. Among the adaptations developed by *S. laciniatum* is a large deep taproot. This study of the chemical constituents of the root of *S. laciniatum* was undertaken in two phases. The first was an examination of the variation in constituents based on depth of the root material. The second was the isolation of compounds with activity against the NCI-H460 human large-cell lung carcinoma cell line. This investigation has led to the identification of fifteen new terpenoids and two new semisynthetic terpenoids.

2050

LICOCHALCONE A AND ISOLIQUIRITIGENIN FROM LICORICE SPECIES DIFFERENTIALLY MODULATE P450 1B1-MEDIATED ESTROGEN METABOLISM

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The risk of breast cancer increases with cumulative exposure to estrogens. Estrogens are metabolized to genotoxic metabolites by P450 1B1, which is up-regulated through inflammation and activation of the aryl hydrocarbon receptor (AhR). Accordingly, cytokine-induced P450 1B1 mRNA levels were inhibited by either a nuclear factor-kappaB (NF-κB) inhibitor or an AhR antagonist in breast cells. Extracts of three pharmacopoeial licorice species, *Glycyrrhiza glabra* (GG), *G. inflata* (GI), and *G. uralensis* (GU), and their constituents were tested to determine their effects on inflammation and P450 1B1-mediated estrogen metabolism. GI, which specifically contains Licochalcone A (LicA) and has the highest levels of isoliquiritigenin (LigC) equivalents, inhibited iNOS activity more strongly compared to the other species. GI and LicA reduced P450 1B1 mRNA levels with and without cytokines, yet GG, GU, and LigC with cytokines additively increased its expression. Similarly, in a sensitive LC-MS/MS assay, GG, GU, and LigC increased genotoxic estrogen metabolites, yet GI and LicA reduced their production. LicA inhibited whereas LigC increased P450 1A/1B activity. LicA also inhibited TCDD-induced XRE-luciferase reporter activity, indicating AhR antagonism. Thus, GI and LicA may protect women against estrogen carcinogenesis by inhibiting inflammation and P450 1B1-mediated estrogen oxidative metabolism. Supported by NIH Grants P50 AT000155 and T32 AT007533

2051

A MODERN APPROACH TO TRADITIONAL MEDICINE: SCREENING, BIOFILMS, AND MECHANISM OF ACTION IN *CANDIDA ALBICANS*

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The Chinese, along with other cultures, have been using plants medicinally to treat infections, inflammation, and cancer for thousands of years. Until recently, modern approaches have not been used to understand the effectiveness of traditional Chinese medicine (TCM). Along with TCM, aurones are naturally occurring heterocyclic compounds in plants such as snapdragons that lend the flowers a yellow pigment. Due to the antifungal activities of a group of related biosynthetic precursor compounds, it is reasonable to believe that aurones may also possess antifungal activity. *Candida albicans* is a polyphenic fungus that is known to cause opportunistic infections of the oral cavity and genitalia in humans in a variety of diseases under the collective term candidiasis, where this particular species is the most prevalent cause. We use standardized screening methods to test extracts and aurones against various infectious organism. The focus of this study is to evaluate the efficacy of these TCM extracts and aurones on *C. albicans*. Of 156 TCM extracts and 26 aurone compounds, 19 exhibited greater than 80% inhibition of *C. albicans* growth. IC50 doses fall below common antifungal treatment concentrations. Preliminary results from treatment with aurones also suggest biofilm degradation and prevention. It is imperative to discover and develop new treatments for fungal infections due to the deleterious effects that current treatments such as amphotericin B have on the body and growing resistance to antifungals. We have already identified several compounds that can be used as alternative treatments and are currently working to understand their mechanisms of action against *C. albicans.*

2052

DEREPLICATION OF NATURAL PRODUCTS BASED ON RATIO ANALYSIS ^{1}H NMR SPECTROSCOPY AND HPLC-DAD-ESI-QToF-MS/MS

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[1]Nucleus of Bioassays, Biosynthesis and Ecophysiology of Natural Products, NuBBE, Department of Organic Chemistry, Institute of Chemistry, Sao Paulo State University, Rua Francisco Degni 55, Araraquara, São Paulo 14800-900, Brazil, [2] Northwest Metabolomics Research Center, Department of Anesthesiology and Pain Medicine, University of Washington, 850 Republican St., Seattle, WA 98109.

A new strategy for dereplication of natural products directly from plant crude extracts was performed by ratio analysis ^{1}H NMR spectroscopy (RANSY) in association with HPLC-DAD-ESI-QToF-MS/MS. ^{1}H RANSY metabolite identification was applied using the ratio between ^{1}H NMR peak heights of multiple spectra and their coefficient of variation values. In this work, fourteen leaves extracts from *Jatropha multifida* (Euphorbiaceae), were analyzed. A selective extraction, using solvents of different polarity, produced a variation of the metabolite concentrations between samples, mandatory for RANSY application. The relationship of spin-correlated peaks, obtained after RANSY, was integrated with the UV and MS data and led to selective dereplication of *C*-glucosylflavonoids, already reported for *Jatropha*. This is the first attempt to integrate NMR, MS and UV data for the *in situ* detection of bioactive natural compounds.

2053

HIGH THROUGHPUT SCREENING OF NATURAL PRODUCTS UTILIZING PULSED ULTRAFILTRATION OR MAGNETIC MICROBEAD AFFINITY SELECTION WITH UHPLC-MS/MS

Michael Rush, Elisabeth Walker, Richard B. van Breemen
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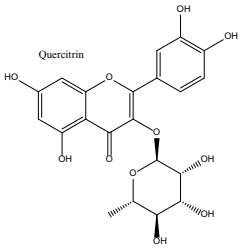

As natural products remain an important resource in the discovery of new potential drugs, it is imperative to develop efficient and sensitive high-throughput screening techniques to find ligands for macromolecular receptors and enzymes. Two techniques developed by our research group, Pulsed Ultra Filtration (PUF) and the higher-throughput Magnetic Microbead Affinity Selection Screening (MMASS), are target-based screening techniques that can be used to identify potential ligands within complex mixtures such as natural product extracts. Based on PUF and MMASS screening of 14 traditional Native American medicinal botanicals for ligands to the anti-inflammation target 15-lipoxygenase (15-LOX), *Proserpinaca palustris* L – Mermaid Weed – showed a hit with an accurate mass of 449.108(+) and 447.095(-). However, this compound could be one of a variety of constitutional isomers of this mass known in this and related species. Given that these constitutional isomers showed similar retention times, MS/MS fragmentation patterns were used to distinguish them. UHPLC was used with a Shimadzu IT-TOF high resolution hybrid mass spectrometer and LCMS-8040 triple quadrupole mass spectrometer to compare the hit with standards. This natural product hit was identified as quercitrin (3-quercetin rhamnose) which is a known inhibitor of 15-LOX.
Supported by grant R01 AT007659 from the NIH NCCIH

2054

FOUR ANTIMALARIAL LIMONOIDS ISOLATED FROM *CARAPA GUIANENSIS* AND TWO ANTIPROLIFERATIVE DITERPENES ISOLATED FROM *HYPOESTES SP.*

Ming Wang[1], Priscilla Krai[2], Seema Dala[2], Maria Cassera[2], Michael Goetz[3], Stéphan Rakotonandrasana[4], Vincent E. Rasamison[4], 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, Virginia Tech, Blacksburg, VA 24061 USA, [3]Natural Products Discovery Institute, 3805 Old Easton Road, Doylestown, PA, 18902, USA, [4]Centre National d'Application de Recherches Pharmaceutiques, Antananarivo, Madagascar.

In a search for antimalarial natural products from plants in collaboration with the Natural Products Discovery Institute we investigated an antiplasmodial extract from *Carapa guianensis* (Meliaceae). This extract gave a DCM fraction with activity against the chloroquine-resistant Dd2 strain of *P. falciparum* with an IC_{50} value about 4 μg/mL. The four active limonoids (**1-4**) were isolated from this fraction and their structures were elucidated by 1D and 2D NMR spectroscopic and MS data. This is the first report of the antimalarial activity of 6α,11β-diacetoxygedunin (**1**). In a separate study as part of our work in the Madagascar International Cooperative Biodiversity Group program, two diterpenes with good antiproliferative activity

against the A2780 human ovarian cancer cell line as well as antimalarial activity were found in the hexanes fraction from the extract of *Hypoestes sp.* (Acanthaceae). This is the first report of the activity of crotonolide G (**5**) towards A2780 cell lines and of its antiplasmodial activity.

2055

ANTIPROLIFERATIVE COMPOUNDS FROM *PENICILLIUM CHRYSOGENUM*, A FUNGAL ASSOCIATE OF THE LIVERWORT *TRICHOCOLEA TOMENTELLA*

L. Harinantenaina Rakotondraibe,[1] Keith Nichols,[1] Hyun-Young Park,[1] Tehane Ali,[1] Hee-Byung Chai,[1] Chad Rappleye[2]
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, [2]Department of Microbiology, Center for Microbial Interface biology, The Ohio State University, Columbus, OH 43210, USA

Our ongoing systematic work on developing new sources of antiproliferative compounds led to the isolation of the bioactive fungus *Penicillium chrysogenum* from the liverwort *Trichoclea tomentella* collected in Newport, Virginia. Bioassay guided fractionation of an Ethyl Acetate extract of a fermentation of this strain using potato dextrose agar led to the isolation of 2,5-dihydroxybenzyl alcohol (**1**) and epiepoxydon (**2**) as the active compounds with IC_{50} values of 6.4 and 24.3 µM, respectively. Fermentation of the same strain in rice medium afforded a bioactive fraction with IC_{50} value of 2.5 mg/mL against the HT-29 colon cancer cell line. New and known methylxanthones together with compounds **1** and **2** and their derivatives have been isolated from this active fraction. The structure determination of the new and bioactive compounds using spectroscopic methods, the bioassay, and the structure-antiproliferative (against HT-29 colon and MCF-7 human breast carcinoma) activity relationship study of the isolated compounds will be presented.

2056

SCREENING BIOACTIVE SECONDARY METABOLITES FROM COSTA RICAN ENVIROMENTAL MICROBIAL COMMUNITIES

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[1] Centro de Investigación en Productos Naturales (CIPRONA) y Escuela de Química, Universidad de Costa Rica, 2060 Costa Rica, [2] Instituto de Investigaciones Farmacéuticas (INIFAR) y Facultad de Farmacia, Universidad de Costa Rica, 2060 Costa Rica, [3] Centro de Investigación en Estructuras Microscópicas (CIEMIC), Universidad de Costa Rica, 2060 Costa Rica, [4] Centro de Investigaciones Agronómicas (CIA), Universidad de Costa Rica, 2060 Costa Rica, [5] Centro de Biología Celular y Molecular (CIBCM), Universidad de Costa Rica, 2060 Costa Rica.

Our group at the University of Costa Rica has started an initiative to explore the microbial diversity of our country making a screening campaign towards biological evaluation of organic extracts from different environmental microbial communities. Our collection of microbial isolates currently has more than 300 microorganisms, including bacteria, actinomycetes and fungi. Those microbes have been isolated from diverse sources like insects, insect's nests, soil, frog skins and extreme environments. We have started the process of small-scale fermentation, extraction and biological evaluation. Preliminary results, including entomopathogenic bacteria *Photorabdus temperata* and *Xenorhabdus sp*, will be presented. Future plans include expansion of our microbial and organic extract collection as well as testing using new biological assays.

2057

ISOLATION OF SESQUITERPENE LACTONES FROM *TRICHOSPIRAS VERTICILLATA*

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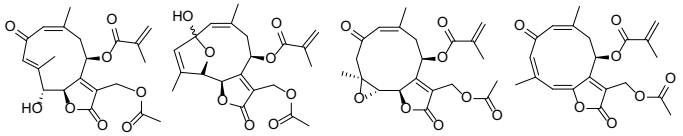
A CH_2Cl_2 extract of *Trichospiras verticillata* from the Natural Products Discovery Institute was found to have good antimalarial activity, with an IC_{50} value of 5 µg/mL. After purification by liquid-liquid partition, chromatography on Sephadex LH-20, and C-18 reverse phase HPLC, the four new sesquiterpenes **1-4** were isolated. The structures of the new compounds were determined by using NMR spectroscopy, MS and CD. Among these four compounds, the conjugated dienone **4** had the most potent antimalarial activity.



1 $10 > IC_{50} > 5$ µg/mL
2 $IC_{50} \sim 10$ µg/mL
3 $IC_{50} \sim 5$ µg/mL
4 $IC_{50} <<< 1.25$ µg/mL

2058

SIMULTANEOUS DETERMINATION OF TEN HIGH-INTENSITY SWEETENERS OF REGULATORY INTEREST USING UHPLC-UV-ELSD/MS

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High-intensity sweeteners have been widely used in food, beverage and drugs. With the purpose of quality assurance and adulterant assessment, an efficient and sensitive method was developed for the quantitative determination of ten high-intensity sweeteners using UHPLC-UV-ELSD/MS. These sweeteners including saccharin, aspartame, acesulfame potassium, sucralose, neotame, advantame, rebaudioside A, stevioside, dulcoside A and neohesperidin dihydrochalcon were baseline separated in a 13 min run. The method was validated according to the validation of analytical procedures of ICH guidelines and applied to quantitation of these analytes and identification of adulterants form various food, beverage and other products.

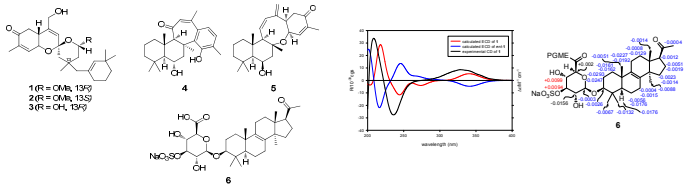
2059

SESTERTERPENES AND A NORTRITERPENE SAPONIN FROM THE SPONGE CLATHRIA GOMBAWUIENSIS

Jung-Kyun Woo[1], Chang-Kwon Kim[1], Seong-Hwan Kim[1], Heegyu Kim[2], Chan-Hong Ahn[2], Dong-Chan Oh[1], Ki-Bong Oh[2], and Jongheon Shin[1]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University, Gwanak, Seoul 151-742, Korea, [2]Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Gwanak, Seoul 151-921, Korea

Six new sesterterpene metabolites (**1-6**) were isolated from the Korean marine sponge *Clathria gombawuiensis*. On the basis of the results of com-

bined spectroscopic analyses, gombaspiroketals A–C (**1**-**3**), tetracyclic sesterterpenes of a novel skeletal class, the structures of phorone B (**4**) and ansellone C (**5**) were determined to be the phorone and ansellone classes, respectively, whereas the saponin gombaside A (**6**) was a nortriterpene sodium *O*-sulfonato-glucuronide of the rare 4,4,14-trimethylpregnane class. Six compounds exhibited moderate cytotoxicity and antibacterial activity.



2060

ISOLATION OF STRONGYLOPHORINES FROM THE MARINE SPONGE *PETROSIA CORTICATA*, AS PROTEASOME INHIBITORS

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Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan

The proteasome is a new oncology target after the approval of Velcade® and we have been searching for new inhibitors from natural sources. Recently, we isolated two new strongylophorines (**1** and **2**) along with six known congeners (**3**-**8**) from the marine sponge *Petrosia corticata* and measured their inhibitory activities against the chymotrypsin-like activity of the proteasome.

2061

MANGROVE ENDOPHYTE METABOLITES AND HERBIVORY- ECOLOGY OF THE BIOACTIVE NICHE

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With such great species diversity, it is unsurprising that the mangrove ecosystem harbors many complex ecological interactions. Even within each mangrove we observe a world teeming with biodiversity- we enter the realm of endophytes. The diverse and innumerable fungal and bacterial species create a unique chemical environment, affecting not only the trees themselves, but also the other residents of the mangrove community. Isolated endophytes produce many bioactive metabolites, with pathogens one would associate with the tropical mangrove environment. We have begun to observe the ecological interactions these endophytes and their secondary metabolites have on the invertebrate herbivores that predate the three species of mangroves found in costal Florida, white (*Laguncularia racemosa*), red (*Rhizophora mangle*), and black (*Avicenna germinans*). Identification and quantification of leaf tissue damage alongside a metabolomic study of the fungal metabolites, allows us to outline the ecological significance for the endophytes.

2062

ADVANCES IN PROFILING OF NATURAL PRODUCTS BY TRIPLE DETECTION TECHNIQUES COMBINED WITH SUPER CRITICAL CO_2 MOBILE PHASES AND SUB-2µM STATIONARY PHASES

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Convergence chromatography (CC) is a separation technique that utilizes both super and sub-critical carbon dioxide (CO2) and sub-2 µm stationary phases to achieve unique selectivity, low solvent usage and high efficiencies. The use of supercritical fluid as a mobile phase provides higher diffusivity and lower viscosity than liquid mobile phases, thereby providing higher throughput and chromatographic efficiencies as compared to liquid chromatography. The analysis of natural products has typically been challenging, not only because of the complex matrices but also the numerous components with varying physical and chemical properties. To address these difficulties, multiple detectors are typically used during a single analysis whereas each detection technique is based on a different physical or chemical property of the molecule. For example, mass detectors and PDA are commonly combined to obtain both mass and UV-spectral information. Evaporative light scattering, a more universal technique, addresses compounds without ultraviolet absorbance (no chromophore) and poor ionization by MS. The combination of these three detection techniques allows for analysis of a wide range of compounds. In this presentation, we will investigate the analysis of a number of natural products using triple detection in combination with CC. Identification and quantitation of compounds will also be illustrated. Guidance combining triple detection with CC will be provided based on the observations attained throughout the analysis. This approach when combined with sub-2 µm column chemistries will allow for the detection of compounds with a wide range of physical and chemical properties.

2063

DISSEMINATION OF ORIGINAL NMR DATA ENHANCES THE REPRODUCIBILITY OF NATURAL PRODUCT RESEARCH

Guido F. Pauli,[1,2] J. Brent Friesen,[1,2] Matthias Niemitz[3], Jonathan Bisson,[1] David C. Lankin,[1] Cristian Soldi,[4,5] Jared T. Shaw,[4] Dean J. Tantillo,[4] Shao-Nong Chen,[1,2] James B. McAlpine[1,2]
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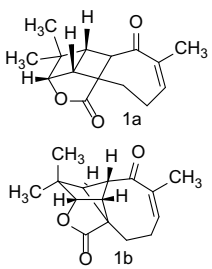

The acquisition of 1D ^{1}H NMR (HNMR) spectra is one of earliest steps in characterizing natural products and other organic molecules. For publication, HNMR information usually is "converted" into a table format, and sometimes spectral plots are provided. However, this transformation is lossy and frequently insufficient for unambiguous dereplication. This ambiguity can even lead to structural revision, such as in the recent case

of aquatolide (**1**), a sesquiterpene lactone from *Asteriscus aquaticus*. Our study demonstrates that public dissemination of original (digital) HNMR data (FIDs) can be a powerful means of enhancing the reproducibility of structural assignments and, thus, any downstream biological studies. Using the archived 800 MHz HNMR spectrum, and employing a semi-automated quantum mechanics-driven spectral analysis (HiFSA), we were able to rule out the initial assignment (**1a**), confirm the revision (**1b**), and achieve the full interpretation of the HNMR fingerprints. Using additional examples of constitutional and diastereomeric isomers which exhibit complex and near-identical HNMR spectra, we show that the public sharing of original HNMR data (FIDs) is not only essential for robust structural assignments, but can enhance the reproducibility of research with bioactive natural products and other organic molecules simply and productively.

Acknowledgement: We appreciate the diligent help of Michael J. Di Maso, UofCA Davis, with NMR data management.

2064

SJN1 A-D, NEW GLYCOLIPIDS FROM THE ANT-ASSOCIATED BACTERIUM, *DEINOCOCCUS GOBIENSIS* SP.

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Bacteria in insect symbiotic systems are considered as an uprising source of novel bioactive small molecules because of the huge biological and chemical diversity of insects and their symbiotic microbes. Our recent studies have focused on the ecosystem of the carpenter ant *Camponotus japonicus*. We isolated bacterial strains from queen specimen of *C. japonicus* and chemically analyzed their secondary metabolites by LC/MS. As a result, four new glycolipids, SJN1 A-D (**1-4**), were discovered by a Gram-negative bacterial strain belonging to *Deinococcus gobiensis*. The structures of SJN1 A-D were determined mainly through NMR and mass spectroscopic data. The relative configuration of amino sugar was elucidated by ^{1}H-^{1}H coupling constants and ROESY NMR spectral analysis. The biological evaluation is still under progress.

2065

THE SECONDARY METABOLITES FROM HALOPHILIC ACTINOMYCETES FROM A SOLAR SALTERN IN KOREA

Seong-Hwan Kim[1], Yoonho Shin[1], So-Hyoung Lee[2], Won Keun Oh[3], Ki-Bong Oh[2], Sang Kook Lee[1], Jongheon Shin[1], and Dong-Chan Oh[1,]*
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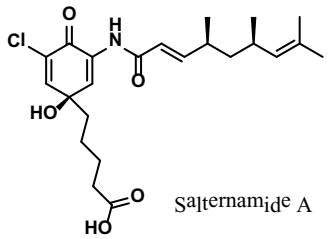
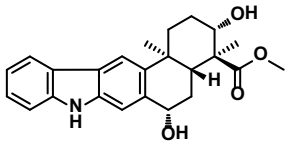


Salternamide A



Xiamycin D

Halophilic actinomycetes from saline environments are distinct from their terrestrial counterparts in terms of their physiology and secondary metabolites. Since the discovery of a novel anticancer drug candidate, salinosporamide A, from an obligate marine actinomycete, studies of halophilic actinomycete-derived secondary metabolites have demonstrated the chemical potential of actinomycetes from saline environments. Salterns may be an extreme representative of saline environments, which possibly harbor halophilic actinomycetes with chemical potential. We selectively isolated actinomycete strains from salterns and investigated their bioactive compounds. During our chemical studies, salternamides A–D (**1**–**4**), the first secondary metabolites from saltern-derived actinomycetes, were discovered from a halophilic *Streptomyces* strain (#HK10) isolated from a saltern on Shinui Island in the Republic of Korea. Salternamide A (**1**), which is the first chlorinated compound in the manumycin family, exhibited potent cytotoxicity against a human colon cancer cell line (HCT116) and a gastric cancer cell line (SNU638) with submicromolar IC50 values. Moreover, xiamycins C-E (**5**-**7**), new indolosequiterpenoids, were identified from another *Streptomyces* sp. strain (#HK18) isolated from a topsoil sample of the saltern. Xiamycins C-E showed potent effects against porcine epidemic diarrhea virus infection via inhibiting viral replication.

2066

DISCOVERY OF NEW BIOACTIVE SECONDARY METABOLITES FROM BACTERIA IN EXTREME HABITATS

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As part of our efforts to explore structurally and biologically novel secondary metabolites from unique environments for drug discovery, we have investigated secondary metabolites of actinomycete strains inhabiting in the Arctic Ocean, tidal mudflats, and Alaskan permafrost. First, arcticoside and C-1027 chromophore-V, were isolated from a culture of an Arctic marine *Streptomyces* strain. These new compounds, bearing a benzoxazine ring, inhibited *Candida albicans* isocitrate lyase. Second, buanmycin and buanquinone, were discovered from the culture of a marine *Streptomyces* strain, from a tidal mudflat. The structure of buanmycin was determined by X-ray crystallographic analysis and NMR experiments including ^{13}C-^{13}C COSY. Lastly, we isolated bacterial strains from Alaskan permafrost samples. Buanmycin strongly inhibited the pathogenic Gram-negative bacterium *Salmonella enterica* (MIC = 0.7 μM). In particular, buanmycin demonstrated inhibition of sortase A, which is a promising target for antibiotic discovery.

2067

A NOVEL TRICYCLIC DILACTONE ISOLATED FROM AN ACTINOMYCETE DERIVED FROM GINESENG-CULTIVATED SOIL

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An actinomycete strain BYK1336, identified as *Streptomyces cinerochromogenes*, was isolated from 4-year-old ginseng farm. While culturing the strain and analyzing its chemical profile by liquid chromatography coupled with electrospray ionization mass spectrometer (LC-ESI-MS), a previously-unreported secondary metabolite BYK1336.360 was detected along with the known compounds belonging to the classes of ansatrienin and naphthomycins. The structure of BYK1336.360 was elucidated by comprehensive analysis of 1D and 2D NMR, mass spectrometric and infrared spectroscopic data. BYK1336.360 is structurally novel by bearing a cyclodecatriene flanked by six-membered and seven-membered lactone ring.

2068

STUDY OF THE CHINESE HERBAL PRESCRIPTION OF TONGFENGKANG IN PROTECTING VASCULAR ENDOTHELIAL FUNCTION IN RATS WITH HYPERURICEMIA

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Tongfengkang (TFK) is the Chinese herbal prescription composed of lysimachia and phellodendron bark, etc, for treatment of hyperuricemia due to heat-dampness and blood stasis. The present study was designed to evaluate the anti-hyperuricemia effects of TFK and the possible active mechanisms. SD male rats were randomly divided into six groups which recorded as normal group, model group, Benzbromarone group, TFK groups (6.94g/kg, 3.47g/kg, 1.47g/kg). The rats in the model group were prepared by feeding yeact and high-purine for three weeks. The results showed that TFK can decrease the level of UA and ET-1 and increase the level of NO in serum. It demonstrated that TFK could protect the vascular endothelial functions. Furthermore, TFK can decrease the level of TXB2, IL-1β, TNF-α and TGF-β1 and increase the level of 6-keto-PGF1a in serum. It suggested that suppressing various inflammatory cytokines so as to protect the vascular endothelial is the important mechanism underlying the anti-hyperuricemia effects of TFK.

2069

SIX SELAGINELLIN DERIVATIVES WITH PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORY ACTIVITY FROM *SELAGINELLA TAMARISCINA*

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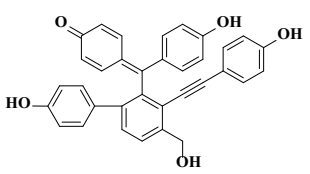
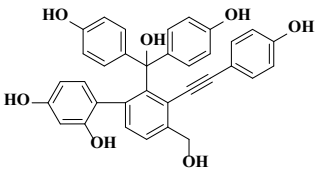
As part of an ongoing search for new PTP1B inhibitors from medicinal plants, the methanol extract of the aerial parts of *Selaginella tamariscina* was found to inhibit 70% PTP1B enzyme activity at a concentration of 30 μg/mL. Thus, bioassay-guided isolation of this active extract yielded six selaginellin derivatives (**1**–**6**), comprising four new compounds (selariscinas A~D; **2**~**6**). These compounds were found to possess inhibitory effect on PTP1B enzyme activity with IC_{50} values ranging from 5.5 ± 0.1 to 21.6 ± 1.5 μM. Furthermore, compound **2** which served as mixed-competitive inhibitor showed the greatest potency, with IC_{50} value of 5.5 ± 0.1 μM, when compared with the positive control (ursolic acid, IC_{50} = 3.4 ± 0.1). This result indicated the potential of these selaginellin derivatives as lead molecules for the development of antidiabetic agents and the beneficial use of *S. tamariscina* against hyperglycemia.



Selariscina A (2)



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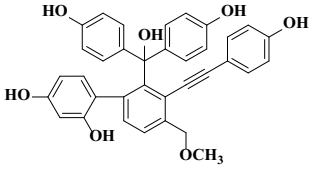

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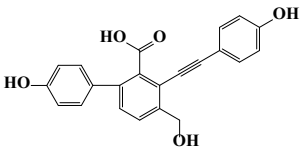

Selariscina D (5)

2070

INVESTIGATION OF ANTIBACTERIAL AND BIOFILM INHIBITION ACTIVITY OF *MICHELIA FIGO* LEAF EXTRACTS AGAINST DENTAL BACTERIUM

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Dental plaque, a complex microbial biofilm, is a pathogenic factor in dental diseases such as dental caries and periodontal disease. The dental diseases usually begin with the formation of dental plaques. *S. mutans*, *S. sobrinus* and *S. sanguinis* are the primary bacterium for the biofilm formation. Some antibiotics and chemical agents could treat oral infections but often accompanied with side effects and drug resistance. To discover agents that can prevent biofilm formation or destroy biofilm is thus considered as a good strategy to avoid drug resistance. Plant natural products are our primary source for discovery of such potential agents. In our study, we have evaluated more than 1,000 plant extracts for their antibacterial and anti-biofilm activity. One plant, *Michelia figo* (Lour.) Spreng., was found to effectively prevent biofilm formation of dental bacteria. We thus re-collected the plant materials (leaves and stems) for further phytochemical investigation. Bioassay-guided separation was carried out to get the active constituents from this plant, which may lead to the potential use of the plant to prevent dental biofilm formation. [The work described in this paper was supported by HKBU Interdisciplinary Research Matching Scheme (RC-IRMS/12-13/03)].

2071

CYTOTOXIC EFFECTS OF ANTHRAQUINONES FROM THE RHIZOME OF *RHEUM TATARICUM* ON HELA AND MDA-MB-435 CELLS

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The effects of anthraquinones, aloe-emodin (TH-4), 1,3,8-trihydroxyanthraquinone (TH-6) and physcion-1-*O*-β-(6'-*O*-acetyl)glucopyranoside (TH-10) isolated from the rhizome of *Rheum tataricum* L., on the growth of HeLa and MDA-MB-435 cells were evaluated. Results show that TH-4, TH-6 and TH-10 inhibited the proliferation of HeLa cells but have no effect on MDA-MB-435 cells up to 100 μM. TH-4 and TH-6 caused HeLa cells to accumulate in G1 with a concurrent depletion of cells in the S and G2/M phases of the cell cycle. After treatment with TH-6, phosphorylation of proteins in the mTORC1 signaling pathway increased. Additionally, phosphorylation of p38 MAPK at T180/Y182 decreased in a time-dependent manner between 2 and 4 hours after treatment with TH-6. What's more, both TH-4 and TH-6 induce cell death at least partially through apoptosis. These findings provide a possible mechanistic explanation for the proliferation inhibitory effect of TH-4 and TH-6 on HeLa cells.

2072

DEVELOPMENT OF A VALIDATED HPTLC METHOD AND QUANTITATIVE ANALYSIS OF SOLANOPUBAMINE IN SIX SPECIES OF *SOLANUM*

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Quantification of biologically active marker compounds in crude drugs facilitates the production of therapeutically effective herbal formulations. The present study is designed to produce maiden work on quantification of a rare alkaloid solanopubamine in leaves and fruits of six different species of genus *Solanum* (*S. schimperianum*, *S. villosum, S. coagulans, S. glabratum, S. incanum and S. nigrum* extracted by two different methods) by a validated high performance thin layer chromatography method. Chromatography was performed on glass-backed silica gel 60 F254 precoated HPTLC plates with solvents $CHCl_3$: CH_3OH: NH_4OH (30:20:1.5 *v/v*) as the mobile phase. After development, the HPTLC plate was derivatized with dragendorff reagent, scanned, and quantified at 500 nm. The system was found to give compact spot for solanopubamine at $R_f = 0.39 \pm 0.01$. The linear regression analyses data for the calibration plot showed good linear relationship with $r^2 = 0.998$ with respect to area in the concentration range of 100- 900 ng. The regression equation for standard solanopubamine was found to be Y= -239.618 + 4.442x. The steroidal alkaloid solanopubamine was found to be present only in leaves extracts of *S. schimperianum* while it was absent in fruit extract of *S. schimperianum* and leaves as well as fruit extracts of *S. villosum, S. coagulans, S. glabratum, S. incanum* and *S. nigrum*. The LOD and LOQ were found to be 35 ng and 110 ng band-1, respectively. The quantity of solanopubamine in leaves extract of *S. schimperianum* extracted by ethanol only and mixture of ethanol and ammonium hydroxide (6:4) was found to be 1.03 % w/w and 2.09 % w/w, respectively. Stress studies of solanopubamine exhibited the maximum (100%) degradation in base and H_2O_2 treated samples and 61.4% in acid treated samples. The UV exposure and photo-oxidation to sample almost caused no damage but 12.91%, 13.83% and 14.08% destruction has been observed while exposed to room temperature, 40 °C and 50 °C, respectively.

2073

DISCOVERY OF NOVEL BIOACTIVE NATURAL PRODUCT-LIKE COMPOUNDS BASED ON DIVERSITY-ENHANCED REACTIONS

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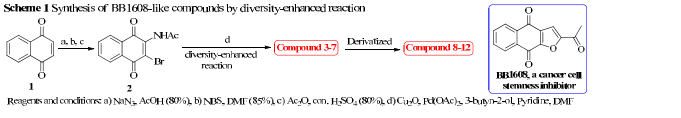
[1]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, P. R. China. [2]HKBU Shenzhen Research Center, Shenzhen, P. R. China.

Diversity-enhanced reaction (DER), a non-selectivity and non-specificity reaction, is proposed to increase the diversified chemical structures of natural products for drug discovery (**Fig. 1**). We have applied this method to quickly synthesize a number of BB1608-like derivatives (**Scheme 1**). **2** underwent DER to afford a mixture. Separation and derivatization provided **3-12**, Compounds **3-12** were evaluated for their anticancer and antiviral activities. The development of more complex DER is in progress. [The work described in this paper was supported by HKBU Interdisciplinary Research Matching Scheme (RC-IRMS/12-13/03) and Natural Science Foundation of China (No. 21402166)].

Figure 1 Diversity-enhanced reaction–a new approach of synthesis of structural diversity of natural product-like

Scheme 1 Synthesis of BB1608-like compounds by diversity-enhanced reaction



Reagents and conditions: a) NaN_3, AcOH (80%), b) NBS, DMF (85%), c) Ac_2O, con. H_2SO_4 (80%), d) $C_{14}O_3$, $Pd(OAc)_2$, 3-butyn-2-ol, Pyridine, DMF

2074

INVESTIGATION OF THE ANTIPROLIFERATIVE ACTIVITY OF *LINOCIERA RAMIFLORA* AND ITS ISOLATES

P. Annécie Benatrehina[1], *Li Pan[1], C. Benjamin Naman[1], Hee-Byung Chai[1], Tran Ngoc Ninh[2], Djaja Djendoel Soejarto[3,4], L. Harinantenaina Rakotondraibe[1], and A. Douglas Kinghorn[1].*
[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA; [2]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam; [3]College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA; [4]Science and Technology, Field Museum, 1400 S. Lakeshore Dr., Chicago, IL 60605, USA.

Following the primary screening of Vietnamese plant extracts against the HT-29 human colon cancer cell line assay, the branch sample of *Linociera* cf. *ramiflora* (Roxb.) Wall. (Oleaceae) (sample A06576; voucher specimen DDS 14374) was selected as a candidate for further study. The methanol extract of this plant sample was subjected to a bioactivity-guided fractionation aiming at the isolation of potential anticancer agents. Liquid-liquid partitions of this extract led to an active chloroform fraction ($IC_{50} = 7.7$ µg/mL). Further separation of this fraction through successive column chromatography afforded numerous active sub-fractions, from which several new and known lignans and arylglycerol-substituted lignan derivatives together with some aromatic compounds were isolated. The structure determination as well as the antiproliferative and quinone reductase inducting activities of these compounds will be described.

2075

PREVALENCE OF ANTIMICROBIAL FUNGAL METABOLITES IN HYDRASTIS CANADENSIS CRUDE EXTRACTS

Joseph M. Egan[1], *Amninder Kaur[1], Huzefa A. Raja[1], Adam R. Brown[1], Nicholas H. Oberlies[1], Nadja B. Cech[1]*
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Goldenseal (*Hydrastis Canadensis* L. Ranunculaceae) is a plant used in Native American traditional medicine to treat infection. This plant has been shown by our laboratory to harbor endophytic fungi that produce antimicrobial secondary metabolites. The aim of this study was to determine whether antimicrobial fungal metabolites could be detected in the crude extracts of *H. canadensis*. A series of extracts were prepared in methanol and subjected to liquid-liquid partitioning to remove fats and sugars. A second extraction technique, using aqueous ethanol, was also employed to improve relevance of this study to the botanical dietary supplements industry. Investigations to identify fungal metabolites from these extracts using ultra-performance liquid chromatography coupled to high resolution mass spectrometry are ongoing.

2076

DIVERSE OLIGOPEPTIDES ISOLATED FROM AQUATIC MICROBIAL CONSORTIA

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[1]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA, [2] Biosortia Pharmaceuticals, 565 Metro Place South, Suite 300 Dublin, Ohio 43017, and National Oceanic and Atmospheric Administration (NOAA), Charleston, South Carolina 29412, USA.

Bioassay-guided purification has conducted on fractions prepared from uniquely harvested biomass of aquatic microalgae using HT-29 human colon cancer cell line, and has led to the isolation of a number of oligo-

peptides, with the most representative sub-types being anabaenopepetins, oscillapeptins and aeruginosins. The structures of the isolated compounds were identified on the basis of spectroscopic data interpretation. A LC-MS dereplication procedure was employed to facilitate the minor cytotoxic agents isolation.

2077

EXTRACTION OF ISOFLAVONOIDS FROM *IRESINE HERBSTII*

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Biliary atresia outbreaks were reported four times in newborn Australian livestock between 1964 and 2013. Epidemiologic data implied that these outbreaks were caused by environmental exposure to an unusual forage plant. We isolated and identified the active toxic compound. Two similar isoflavonoids from *Iresine herbstii* have been reported. We have extracted *I. herbstii*, and are isolating the compounds by column chromatography, flash chromatography and HPLC. We confirmed the presence of the compounds, 2´,2,5-trimethoxy-6,7-methylenedioxyisoflavanone **(1)** and 2´,5-dimethoxy-6,7-methylenedioxyisoflavone **(2)**. Future work will focus on ring opening reactions and testing the toxicities of all compounds in a zebrafish bioassay of biliary function. This is part of our ongoing efforts to determine SAR characteristics of the toxic compound.

2078

TRADITIONAL PREPARATIONS AND METHANOLIC EXTRACTS OF PLANTS FROM PAPUA NEW GUINEA EXHIBIT SIMILAR CYTOCHROME P450 INHIBITION

Erica C. Larson[1], Chris D. Pond[1], Prem P. Rai[2], Teatulohi K. Matainaho[1,2,3], Pius Piskaut[3], Louis R. Barrows[1,2,3], Michael R. Franklin[1]
[1]Department of Pharmacology and Toxicology, University of Utah, 30 S. 2000 E., Salt Lake City, Utah 84112, USA. [2]School of Medicine and Health Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD, Papua New Guinea. [3]School of Natural and Physical Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD, Papua New Guinea.

Human immunodeficiency virus (HIV) and tuberculosis (TB) infections constitute a large portion of the disease burden in Papua New Guinea (PNG). Treatment of HIV and TB require long-term administration of antiretroviral and anti-TB drugs, respectively. In addition to Western medicine, traditional medicine (TM) is widely practiced in PNG. There is increasing concern that traditional medicines may antagonize antiretroviral and anti-tuberculin drug efficacy. Plant-drug, or drug-drug, interactions can occur at the level of metabolism through two major mechanisms: enzyme induction or enzyme inhibition. Our previous study of commonly-used medicinal plants from PNG extracted in methanol found almost one third of TM extracts induced cytochrome P450 (CYP) subtype expression of CYP1A2 or CYP3A4, or both. In addition, almost two thirds inhibited CYP1A2, CYP3A4, or CYP2D6, or combinations thereof. In PNG, the most common route by which traditional medicines are consumed is ingestion of expressed juice, succus. We investigated CYP inhibition of independently recollected medicinal plants (n=17) and compared CYP inhibition by succus to the previous methanolic study through pair-wise comparisons of like genus and species. Results for the recollected succus samples were not significantly different from results obtained with methanolic extracts ($p<0.05$). Therefore, data obtained previously with methanolic extracts likely represents biological activities found in more traditional medicinal preparations. In addition, these results confirm previous *in vitro* data that indicate use of traditional medicines concomitantly with ART or anti-TB drugs could dramatically alter their efficacy.

2079

ANTINOCICEPTIVE ACTIVITY OF THE ESSENTIAL OIL FROM *Artemisia ludoviciana.*

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[1]Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, D.F., 04510, México. [2]Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F., 04510, México.

Artemisia ludoviciana Nutt. is widely used in Mexico for treating gastrointestinal disorders and painful complaints. The antinociceptive action of the essential oil (EO) and its volatile composition were investigated. EO decreased both the first and second phases of formalin-induced nociception. The calculated median-effective dose (ED_{50}) was 269 mg/kg. The effectiveness of EO (ED_{50} = 25.9 mg/kg) for attenuating neurogenic pain was corroborated using the hot plate test. The antinociceptive action of EO was partially blocked by naloxone suggesting that its mode of action involved an opioid mechanism. On the other hand, volatile constituents extracted by hydrodistillation and those obtained by HS-SPME were established by GC-MS; the oil showed high amounts of (±)-camphor, γ-terpineol and borneol, and the major light volatile compounds identified by HS-SPME were 1,8-cineole and (±)-camphor. Altogether, these results support the long-term use of *A. ludoviciana* for treating painful complaints.

2080

HIGH-THROUGHPUT CYTOCHROME P450 INHIBITION COCKTAIL ASSAY FOR EVALUATING POSSIBLE DRUG-BOTANICAL INTERACTIONS: APPLICATION TO LICORICE

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Department of Medicinal Chemistry and Pharmacognosy, University of Illinois College of Pharmacy, UIC/NIH Center for Botanical Dietary Supplements Research, Chicago, IL 60612

Early detection of drug-drug interactions is essential during drug discovery and development, and the investigation of possible drug-botanical interactions is important for the safe use of botanical dietary supplements. Since inhibition of cytochrome P450 (CYP) enzymes is the most common cause underlying drug-drug or drug-botanical interactions, assays that facilitate the in vitro assessment of CYP inhibition are important preliminary tests that need to be carried out as justification for possible clinical trials of safety. Here, an efficient and cost-effective mass spectrometry-based high-throughput CYP cocktail inhibition assay was developed that uses 10 substrates simultaneously against 9 CYP isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4). The method was validated using known inhibitors of each CYP enzyme and then applied to the analysis of potential drug-botanical interactions involving the licorice species *Glycyrrhiza glabra* L., *Glycyrrhiza uralensis* Fisch. Ex D.C., and *Glycyrrhiza inflata* Batalin. Supported by grant P50 AT000155 from the NIH ODS and NCCIH

2081

QUANTIFICATION OF POLYPHENOLS IN FREEZE-DRIED TABLE GRAPE POWDER

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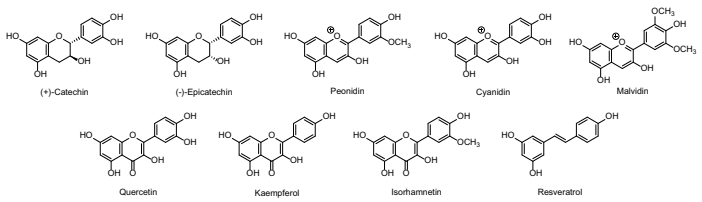
Bioactive polyphenolic compounds such as phenolic acids, cinnamic acids, stilbenes, flavonoids, flavans, flavonols and anthocyanins are associated with the prevention of degenerative diseases such as cardiovascular disea-

se and certain types of cancers. These polyphenols - specifically catechin, epicatechin, peonidin, cyanidin, malvidin, quercetin, kaempferol, isorhamnetin, and resveratrol - are found in various plants such as grapes. In this study, polyphenols in freeze-dried table grape powder prepared from red, green and black grapes (California Table Grape Commission) were extracted using various methods. Polyphenols in the extracts were characterized and quantified using ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The chemically standardized grape powder is suitable for studies of the health benefits of grapes in animal and clinical studies.



2082

RECOVERY OF METABOLITES FROM NATURAL DEEP EUTECTIC SOLVENT MATRICES BY COUNTERCURRENT SEPARATION

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NAtural Deep Eutectic Solvents (NADES) were discovered in 2011 as being mixtures of polar "primary metabolites" that exhibit unexpected solubilizing and stabilizing abilities for hydrophobic and/or bioactive ingredients. However, due to the inherent low vapor pressure of NADES, it is challenging to recover analytes such as "secondary" metabolites from a NADES-analyte(s) matrix by conventional liquid chromatography. The present study shows that countercurrent separation (CCS) can perform this task successfully. The resolution capability of CCS depends on the differential distribution coefficients (*K* values) of the analytes. Compared to most bioactive constituents, the NADES components have extreme *K* values (close to 0 or ∞, in RP or NP, resp.) because of their high polarity. Glucose-choline chloride-water (2:5:5, mole/mole) with rutin, quercetin, kaempferol, or daidzein were chosen as the test matrices. The CCS fraction analysis by UV-UHPLC and qHNMR showed that CCS can recover the target analyte completely from the NADES-analyte matrix, and at the same time yield the NADES quantitatively, allowing further study of "primary" NADES vs. bioactive "secondary" metabolites in botanical extracts.

2083

HIV INHIBITORY ACTIVITY FROM PAPUA NEW GUINEAN MEDICINAL PLANTS

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Papua New Guinea has enormous biological diversity as a result of its topography and tropical latitude. It also has a long and continued use of medicinal plants. In our lab we use bioassay guided discovery and dereplication based on a whole cell HIV assays, p24 flow cytometry, various chromatographic methods, and accurate mass LC/MS/MS. We have explored several collections of medicinal plants gathered by Pharmacy Students under the guidance of UPNG botanists and found several plants with potent anti-HIV activity. Here we report our recent findings on several active plants, including *Macaranga Tanarius, Causarina oligodon* and *Crassocephalum crepidiodes*.

2084

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OILS FROM *PIPER* SPECIES OF PANAMA.

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The chemical composition of the leaf essential oils obtained by hydrodistillation of 6 *Piper* sp.(Piperaceae) from Panama were analyzed by a combination of GC-FID and GC-MS using two capillary columns of different stationary phases (Supelcowax™10 and methylsilicone SE-30). Identification of constituents was achieved by means of their GC retention indices in the two stationary phases and by comparison of their mass spectral fragmentation patterns with those stored in our own library, in the GC-MS database and with literature data. The main components identified in each species were as follows: cembratrienol (25,4%) and derivatives (8,9%), cembrene (15,3%), β-elemene (14,0%) in *P. augustum* Rudge; β-pinene (26,6%), 6-*E*-nerolidol (12,8%), *p*-cymene (8,6%), and limonene (8,2%) in *P. corrugatum* Kuntze; α-pinene (19,4%), β-caryophyllene (13,9%), and limonene (8,1%) in *P. curtispicum* C.DC.; *p*-cymene (43,9%), β-pinene (14,5%) and γ-terpinene (8,0%) in *P. grande* Vahl; linalool (14,5%), α-phellandrene (13,8%), limonene (12,2%), β-pinene (10,1%) and α-pinene (9,6%) in *P. jacquemontianum* Kunth; 6-*E*-nerolidol (8,7%), α-pinene (6,6%), and α-copaene (5,3%) in *P. multiplinervium* C. DC. The ethnobotanical uses and biological activities of the species studies will be presented. Acknowledgement: SENACYT, Panamá.

2085

CHARACTERIZATION OF ENVIRONMENTAL SAMPLES IN THEIR NATURAL STATE USING COMPREHENSIVE MULTIPHASE NMR

Martine Monette[1], Andre Simpson[2], Hussain Masoom[2], Yalda Liaghati Mobarhan[2], Denis Courtier-Murias[2], Blythe Fortier-McGill[2], Ronald Soong[2] and Kimberly L. Colson[1]
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Comprehensive Multi-Phase (CMP) NMR challenges traditional NMR methods by adapting the NMR technology to match natural samples, rather than changing the sample to match a specific NMR technique. Comprehensive Multiphase (CMP) combines all aspects of solution, gel and solid-state NMR into a single approach which allows the study and differentiation of all molecular constituents in-situ and in their natural state. Spectral editing approach permit the differentiation of liquid, gel and solid components in-situ and when combined with molecular interaction studies, binding mechanisms, kinetic transport across phases, bioaccumulation, biotransformation, and binding receptors can be assessed.

This research evaluates the versatility and applicability of CMP-NMR to various disciplines. Both studies of molecular structure and interactions will be presented with respect to soils, algae, contaminant sequestration, and early plant growth. Combined the technique and permits a unique and unprecedented molecular window into intact samples including living organisms and hold great promise to understand biological stress, growth and disease across a wide range of field from environmental chemistry to medicine.

2086

ASTRAGALIN REDUCES OVARIAN FAILURE IN AN AGED RAT MODEL OF MENOPAUSE BY INHIBITING GRANULOSA CELL APOPTOSIS

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A traditional Chinese medical (TCM) formula, containing five medicinal herbs including *Morus alba* L. (mulberry; Moraceae), has been shown to be effective in clinical trials for the management of menopausal symptoms. Mulberry has been used in TCM for thousands of years for the treatment of a wide range of women's reproductive disorders including breast cancer, dysmenorrhea, and menopause. Astragalin (AST), known chemically as kaemperferol-3-O-glucoside is a flavonoid glycoside isolated from mulberry fruits. The in vitro and in vivo activities of AST on ovarian granulosa cell (GC) apoptosis were investigated. The effects of AST on cadmium chloride-induced apoptosis in cultured rat ovary GCs were investigated using the MTT proliferation assay, the proliferation-related index and the Annexin V apoptosis assay. The in vivo effects of AST on 14-month old female rats were examined by measuring serum estrogen and progesterone levels, as well as the apoptosis rate of ovarian GCs by flow cytometry.

The in vitro results showed that AST stimulated GC proliferation, increased 17β-estradiol (E2) and progesterone (P4) secretion, and reduced cadmium chloride-induced apoptosis. In vivo, AST treatment of aged female rats resulted in significant increases of serum E2 and P4 levels, altered levels of serum FSH and LH levels, and reduced apoptosis in ovarian GC cells. Treatment with AST increased anti-apopotic protein Bax and reduced the pro-apoptotic protein Bcl-2 in cultured rat ovarian GCs.

2087

SELECTIVITY AND MECHANISTIC INQUIRIES INTO THE REACTIVITY OF PERICOSINE A, AN ELECTROPHILIC CHLORINATED SHIKIMATE ANALOGUE

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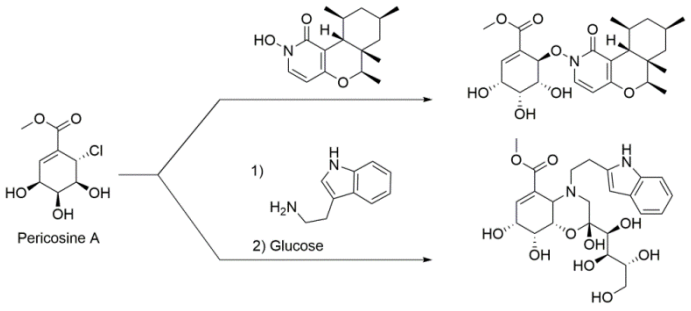
Microorganisms are constantly competing for limited resources in their environment as characterized by secondary metabolite production and enzymatic inactivation of toxins. Pericosine A, a shikimate analogue produced by a *Tolypocladium* sp., has been shown to directly differentiate and inactivate nucleophilic toxins in complex chemical environments. Herein we detail the selectivity of pericosine A towards a diverse set of nucleophiles, as well as the computational and structural studies of the mechanistic pathways under which it operates. We also show reactivity of pericosine A adducts with monosaccharides to produce novel heterobicyclic scaffolds.



2088

RUFOMYCINS - ACTINOMYCETE PEPTIDES WITH POTENT ANTI-TB ACTIVITY

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[1]Department of Medicinal Chemistry and Pharmacognosy, [2]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA, [3]Division of Bioscience and Bioinformatics, College of Natural Science, Myongji University, Cheoin-gu, Yongin, Gyeonggi-Do 449-728, South Korea, [4]Center for Nutraceutical & Pharm. Materials, Myongji University, Cheoin-gu, Yongin, Gyeonggi-Do 449-728, South Korea

The treatment of Tuberculosis (TB), one of most deadly infectious diseases caused by *Mycobacterium tuberculosis*, has become increasingly challenging due to the emerge of multiple and extensively drug-resistant (MDR/XDR) strains. The development of new anti-TB drugs is therefore in urgent demand. In a continuing drug discovery program, based on a high-throughput[1,2] screening of 65,000+ actinomycete extracts, four anti-TB active cyclic heptapeptides, were isolated from a *Streptomyces sp.* MJM3507 extract: three new members of the rufomycin family (**1**-**3**), and one analogue (**4**) with the same constitution and relative stereochemistry of the previously reported rufomycin B_2. The structures of **1**-**4** were determined through extensive analysis of 1D and 2D NMR-as well as mass spectrometric data. Compounds **1** and **2** showed MICs of 0.58 and 1.00 μg/mL, respectively, in the Microplate Alamar Blue Assay (MABA), whereas the mixture of **3** and **4** (ratio *ca* 1:9 based on ^{1}H NMR data) displayed a remarkable low MIC of 0.074 μg/mL (MIC rifampicin = 0.05-0.10 μg/mL) and a selectivity index (IC_{50}/MIC) as high as 675 (IC_{50} rifampicin > 100).
[1] Gao, W., *et al. Org. Lett.* 2014, 16(23), 6044-7. [2] Cai, G. P., *et al. J. Nat. Prod.* 2013, 76(11), 2009-2018.

2089

NEW POLYKETIDES FROM A FUNGICOLOUS ISOLATE OF *PESTALOTIOPSIS DISSEMINATA*

In Hyun Hwang[1], Dale C. Swenson[1], James B. Gloer[1], and Donald T. Wicklow[2]
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In the course of our studies of mycoparasitic and fungicolous fungi, an isolate (MYC-710 = NRRL 62562) was obtained from the surface of a basidioma of *Stereum complicatum* in Georgia. The culture was identified by micromorphology and partial sequence analysis as *Pestalotiopsis disseminata*. *Pestalotiopsis* spp. are known to be prolific sources of structurally diverse secondary metabolites. An ethyl acetate extract of rice fermentation cultures showed antifungal activity and was subjected to chemical investigation. Although the original activity was not recovered, these studies afforded seven new polyketides (disseminins A – E and spiciferones D and E) and a related compound previously known only as a synthetic product. The structures of these compounds were determined mainly by analysis of HRMS and NMR data. Unusual *bis*-tetrahydrofuran and dioxabicyclo[3.2.1]octane ring systems were identified for disseminins A and B, respectively. X-ray crystallographic analysis of the *p*-bromobenzoate derivative of disseminin A confirmed the structure and enabled assignment of its absolute configuration.

2090

THE ABC-AHP-NCNPR BOTANICAL ADULTERANTS PROGRAM'S LABORATORY GUIDANCE ON ANALYTICAL METHODS TO DETECT ADULTERANTS IN BOTANICAL MATERIALS

Stefan Gafner[1], Mark Blumenthal[1], Steven Foster[2], John Cardellina[3], Ikhlas Khan[4], and Roy Upton[5].
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As the market for herbal medicines and dietary supplements increases, so do confirmed reports of undisclosed ingredients being added to botanical raw materials, extracts, essential oils, and finished botanical-based consumer products. These problems not only present a significant challenge to the global botanical medicine and herbal supplement industries but, more importantly, put the health and safety of the consumer at risk. Identifying suitable test methods is crucial for in-house quality control units and third-party analytical testing laboratories. However, the review and assessment of the available analytical methods is an expensive and time-consuming process. The ABC-AHP-NCNPR Botanical Adulterants Program Laboratory Guidance Documents (LGDs) identify the most suitable methods for the authentication of specific botanical materials and the detection of adulterants. The first in the series, a review of methods to authenticate and detect adulteration of skullcap (*Scutellaria lateriflora*), has been published in 2015. Next are the LGDs on bilberry (*Vaccinium myrtillus*) extract, and black cohosh (*Actaea racemosa*). The presentation will give an overview of the Program with a focus on the purpose and content of the LGDs.

2091

PHYTOCHEMICAL AND INSECTICIDAL STUDIES ON *T. ARJUNA*

Alfonso Garcia[1], Ilana Heckler[1], John Hoffmann[1], Renato Lúcio de Carvalho[1], Preeti Dhar[1], Aaron Haselton[2*]*
[1]Department of Chemistry, [2]Department of Biology, 1 Hawk drive, SUNY New Paltz, NY 12561

Plants have co-evolved with insects, developing sophisticated mechanisms to defend themselves. Plants synthesize a wide range of compounds called secondary metabolites that are not directly related to plant metabolism but help the plants defend themselves against pests. *Terminalia arjuna* (TA) is a tree that belongs to the family Combretaceae and is found in abundance throughout India, Burma, Sri Lanka and Mauritius. The bark and fruits from this tree have been used in Ayurveda (the ancient Indian medicine system) for various ailments. Previous studies from our lab have shown the crude ethanolic extract of TA (bark) to show growth inhibition and pupation delay in third instar of *Drosophila melanogaster.* In the current investigation, we have extracted the TA bark sequentially with hexane, ether, ethyl acetate and ethanol and have looked at the sequentially obtained ethanolic extract and its effects on the growth inhibition in larvae of *D. melanogaster*. Phytochemical analysis on the ethanolic extract of TA shows the presence of saponins, flavonoids, and carbohydrates. Results of the bioassays as well as phytochemical analysis will be presented.

2092

ALKALOIDS, FLAVANONES AND OTHER COMPOUNDS NEW TO GOLDENSEAL (*Hydrastis canadensis*)

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Hydrastis canadensis L. (Ranunculaceae), commonly known as goldenseal, is used in traditional and modern herbal medicine to treat a variety of conditions including digestive problems and infections. Previous studies from our laboratory have indicated that leaf extracts from this botanical possess antimicrobial effects against the pathogenic bacterium *Staphylococcus aureus*. The goal of these studies was to identify chemical components of these extracts. Goldenseal leaves were homogenized and percolated in MeOH overnight, and the MeOH extract was concentrated *in vacuo*. The aqueous methanol layer was partitioned with hexane followed by ethyl acetate. Normal-phase flash chromatography yielded eight major primary fractions (FI-FVIII). Fraction II, III and IV was subjected to an additional stage of preparative HPLC, and these fractions yielded several known compounds previously not reported as constituents of goldenseal, two flavanones (**1**-**2**), one isoquinolone alkaloid (**3**), an isoindolobenzazepine alkaloid (**4**), and a spiro compound (**5**). Additionally, fraction FII afforded a new chromenone (**6**) and fraction IV a new methoxycarbonylbenzoic acid (**7**). The structures of the isolates were established by spectrometric and spectroscopic methods. Compound **1** demonstrated efflux pump inhibitory activity against *Staphylococcus aureus*.

2093

TEMPERATURE-DEPENDENT METABOLITE PRODUCTION BY ARCTIC ACTINOMYCETES

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Actinomycetes are a well-established source of natural products with therapeutic potential, particularly for the treatment of infectious diseases. The exploration of microbes from underexplored habitats and the application of novel cultivation conditions are proven strategies for the discovery of novel bioactive metabolites. We set out to explore the metabolic diversity of cold-adapted actinomycetes isolated from marine sediments collected from Canada's Arctic. To screen for metabolites differentially regulated by temperature, 45 strains were fermented at 15 °C and 30 °C and the resulting chemical extracts were tested for antimicrobial activity. Seventy-six percent of the strains exhibited antimicrobial activity against one or more pathogens. Strikingly, extracts prepared from fermentations incubated at 15 °C generated double the number of hits than fermentations conducted at 30 °C. Overall, the majority (44 %) of strains exhibited a greater frequency of antimicrobial activity when fermented at 15°C. Metabolomic analysis (LC-HRMS) was utilized to assess the differential production of metabolites in response to temperature. The results of these experiments will be presented.

2094

DISCOVERY, SYNTHESIS, AND BIOLOGICAL EVALUATION OF APRATYRAMIDE, A MARINE-DERIVED TRANSCRIPTIONAL STIMULATOR OF VEGF-A

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A collection of the marine cyanobacterium *Moorea bouillonii* from Apra Harbor in Guam afforded apratyramide, a linear depsipeptide consisting of four amino acid residues and one hydroxy acid moiety. The structure was elucidated by a combination 1D/2D NMR spectroscopic and mass spectrometric analysis. The absolute configuration of the stereocenters was determined by LC-MS analysis of the acid hydrolyzate. Apratyramide was then synthesized and tested for bioactivity. Apratyramide induced the transcription and secretion of vascular endothelial growth factor A (VEGF-A) in multiple cell types. This activity is being explored for various applications where VEGF-A upregulation would be beneficial.

2095

FINGERPRINT ANALYSIS COMBINED WITH MULTI-RESPONSES EXTRACTION OPTIMIZATION OF *COMASTOMA PEDUNCULATUM*

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A quality evaluating method using high-performance liquid chromatography-diode array detector-electrospray ionization-tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) was proposed for *Comastoma pedunculatum* (Rogle ex D. Dou) Holub, a traditional Tibetan herbal medicine. The optimum extraction conditions for herbal samples were firstly optimized using multi-responses optimization based on response surface methodology (RSM), and achieved by Derringer's desirability function and experimental validation. In the fingerprint analysis, 17 common peaks was developed among 10 batches of *C. pedunculatum* samples. 14 common peaks were identified using HPLC-DAD-ESI- MS/MS method based on their spectral characteristics or comparison with the authentic standards. Chemometrics techniques including similarity analysis, and hierarchical clustering analysis were implemented to discriminate the 10 batches of samples collected from different origins. The results indicated that the 10 batches of samples shared two principle chromatographic patterns as well as differences. These results provided an effective and comprehensive quality analysis method for *C. pedunculatum*. Acknowledgements: Thank the supporting grant for NSFC-81274158, 81373765, NCET-12-0578, 13-0624, SEAC-12ZYZ019, 111 Project B08044 and YLDX01013.

2096

POSSIBLE INVOLVEMENT OF NO/CGMP PATHWAY IN THE ANTIDEPRESSANT ACTIVITY OF PAEONIFLORIN

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Paeoniflorin is a water-soluble monoterpene glycoside isolated from the root of Paeonia lactiflora Pall.. Paeoniflorin (7-14 mg/kg, i.p.) produced a reduction in immobility period in tail-suspension test. The antidepressant-like effect of Paeoniflorin (10 mg/kg, i.p.) in tail-suspension test was prevented by pretreatment with L-arginine (750 mg/kg, i.p.) [substrate for NOS]. Pretreatment of mice with 7-nitroindazole (25 mg/kg, i.p.) [a specific nNOS inhibitor] produced potentiation of the action of subeffective dose of Paeoniflorin (4 mg/kg, i.p.). In addition, treatment of mice with methylene blue (5 mg/kg, i.p.) [direct inhibitor of both NOS and sGC] potentiated the effect of Paeoniflorin (4 mg/kg, i.p.) in the tail-suspension test. Furthermore, the reduction in the immobility period elicited by Paeoniflorin (10 mg/kg, i.p.) was also inhibited by pretreatment with sildenafil (5 mg/kg, i.p.) [phosphodiesterase 5 inhibitor]. Our findings demonstrated that the antidepressant-like effect of Paeoniflorin in the tail-suspension test involved an interaction with the NO-cGMP pathway.

2097

NEW ANTIINSECTAN DIOXOMORPHOLINES FROM *ASPERGILLUS ALABAMENSIS* NRRL 29810

Nicole M. Krausert,[1] Patrick F. Dowd,[2] Stephen W. Peterson,[2] Donald T. Wicklow,[2] and James B. Gloer.[1]
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In the course of our studies of mycoparasitic and fungicolous fungi, an isolate (MYC-1193 = NRRL 29810) was obtained from the surface of a basidiomycete growing on a dead hardwood branch in a sabal palm swamp near the Econfina River in northern Florida. This fungal isolate was identified as *Aspergillus alabamensis,* a relatively new member of *Aspergillus* section *Terrei,* based on morphology and sequence analysis. The crude extract of a rice fermentation showed potent antiinsectan activity and was therefore selected for chemical investigation. Two new dioxomorpholines, along with the known compounds terrein and asteltoxin, were isolated using silica gel column chromatography and reversed phase HPLC. The structures of these metabolites were established by analysis of HRESIMS and 2D NMR data. Relative configurations were assigned using NOESY data, and the structure assignments were supported by NMR comparison with similar compounds. The absolute configuration was determined on the basis of the optical rotation of the hydroxyacid obtained upon acid hydrolysis. The major dioxomorpholine metabolite showed moderate activity in an antiinsectan assay against *Spodoptera frugiperda*.

2098

ISOLATION OF ANNONACOUS ACETOGENINS FROM *ANNONA MURICATA* FRUIT AND THEIR ANTIPROLIFERATIVE ACTIVITY ON HUMAN PROSTATE CANCER CELL PC-3

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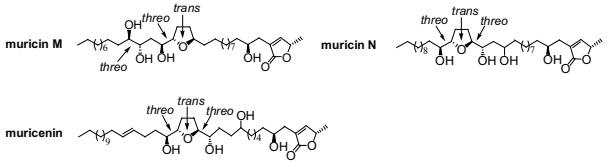
The fruit of *Annona muricata* is an edible and medicinal tropical fruit and commonly available in local markets. The previous work was continued, bioassay-guided fractionation of the fruit powder of *A. muricata* yielded three more novel bioactive compounds: C-35 annonaceous acetogenins, muricins M and N, and C-37 annonaceous acetogenins, muricenin. They all contain a mono-tetrahydrofuran ring and four hydroxyl groups. The structures were elucidated by spectral methods and chemical modification after isolation via open column chromatographic separation and HPLC purification. Especially, murices M and N demonstrated more potent antiproliferative activities against human prostate cancer PC-3 cells.

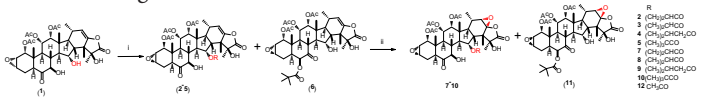


2099

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW BULKY ACYLOXY TACCALONOLIDES AS POTENT MICROTUBULE STABILIZERS

Antonius R. B. Ola,[1] April L. Risinger,[1,2] Jiangnan Peng,[1] Cynthia L Zamiello,[1] and Susan L. Mooberry[1,2]
[1]Department of Pharmacology, [2]Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, Texas 78229, USA

The taccalonolides are a new class of microtubule stabilizers isolated from plants of the genus *Tacca*. As part of our structure-activity relationship study (SAR) to identify taccalonolides with optimal microtubule stabilizing and antitumor actions, we conducted semi-synthetic reactions to modify C-15 of taccalonolide B (**1**). In this study, five novel bulky acyloxy taccalonolides were synthesized via DMAP catalyzed esterification and DMDO epoxidation. The new bulky acyloxy taccalonolide mono esters were further tested for their antiproliferative potency against HeLa cells. Remarkably, all bulky acyloxy taccalonolide esters demonstrated better potency than taccalonolide AF (**12**), including two with subnanomolar potencies (IC_{50} of 0.6 nM for **11** and 0.8 nM for **9**). *In vivo* antitumor activities were also evaluated in a murine xenograft model.



2100

TWO NEW POTENT ANTIMALARIAL ALKALOIDS, CRIPOWELLINS C AND D, FROM THE SWAMP LILY *CRINUM ERUBESCENS*.

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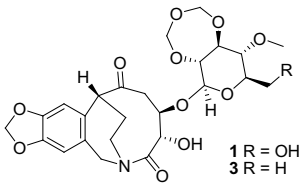
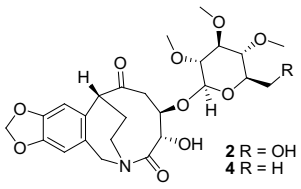
Malaria is a neglected tropical disease that has a disproportionate effect in poor and underdeveloped countries. As part of our ongoing search for new antimalarial compounds, an extract of the Swamp Lily *Crinum erubescens* was selected due to its potent antimalarial activity. Bioassay guided fractionation yielded four potent antimalarial compounds. Compounds **1** and **2** were identified as the known compounds cripowellins A and B, while compounds **3** and **4** were determined to be the new compounds cripowellins C and D. Structures were elucidated using various 1 and 2-dimensional NMR techniques. The isolation and structure elucidation of the cripowellins and their biological data will be presented.





2101

BIOACTIVITY OF POLYPHENOLS FROM JABOTICABA WOOD

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Myrciaria cauliflora (Jaboticaba), native to Brazil, has been traditionally used for the treatment of respiratory problems such as asthma and chronic inflammation of the tonsils. This study was to identify and evaluate major polyphenols from the wood of jaboticaba tree for the first time for their therapeutic relevance to chronic obstructive pulmonary disease (COPD). Antioxidant and anti-inflammatory activity-guided fractionation led to the identification and isolation of 3,3'-dimethyellagic acid-4-*O*-sulphate. The ellagic acid derivative was compared to other potent compounds from the wood and fruits of jaboticaba such as ellagic acid, depside jaboticabin and anthocyanins delphinidin-3-*O*-glucose and cyaniding-3-*O*-glucose. The results indicate that 3,3'-dimethyellagic acid-4-*O*-sulphatate exhibited antiradical activity and significantly inhibited chemokine interleukin-8 production after cigarette smoke treatment of human small airway epithelial cells. Its activity was comparable to that of ellagic acid, but it may be more bioavailable because it is more water-soluble. Bioavailability of jaboticabin, a depside from jaboticaba, was further examined *in vitro* using the human intestinal Caco-2 cell monolayers and *in vivo* with an animal model.

2102

UPLC-qTOF-MS CHEMOMETRIC STRATEGY TO IDENTIFY BIOACTIVE CONSTITUENTS FROM *GARCINIA OBLONGIFOLIA*

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Garcinia oblongifolia is a well-known medicinal plant from southern China, with edible fruits. However, the phytochemistry and bioactivity of the different parts of *G. oblongifolia* are still not well studied. We developed a strategy to identify potential bioactive compounds from the leaves, branches, and fruits of *G. oblongifolia* by combining bioactivity analysis with UPLC-qTOF-MS based chemometrics. Preliminary screening with human breast cancer estrogen receptor positive MCF-7 cells showed that *G. oblongifolia* branch extracts influenced cell growth in a dose-dependent fashion by causing a unique pattern of vacuolization, which was not observed with the leaf or fruit extracts. Cytotoxicity and cell proliferation studies are ongoing in order to further understand the difference in activity of the three extracts. The branch extract also showed strong antioxidant activity in DPPH and ABTS assays. UPLC-qTOF-MS combined with untargeted PCA and OPLS-DA analysis was conducted to analyze and compare the three extracts, and 12 compounds including 2 bioactive xanthones and 4 potential biomarkers were identified as the marker compounds for branches. Imaging and antioxidant results indicate that the branch extract exhibits greater activity than the leaf and fruit extracts. These findings provide a useful strategy to analyze extracts to find potential biomarkers from different parts of medicinal plants.

2103

ACHIEVEMENT OF SUSTAINABLE AGRICULTURAL SOLUTIONS THROUGH MODERN DEREPLICATION TOOLS

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There is an increasing demand to provide safe and sustainably sourced food to the marketplace. With the goal of meeting this need we sought to define a dereplication solution that would allow us to prioritize leads and minimize developing biological crop protection products that did not meet this objective. A dereplication database was constructed composed of natural products from microbial origins, in addition to currently approved human and veterinary antibiotics in both the United States and the European Union. Each entry contains publically available toxicological information, as well as molecular weight, molecular formula, and source organism (if available). In addition, the entries have an assigned score based on over 800 different keywords describing a biological effect. At present our database is composed of over 600 compounds, primarily from the Order Bacillales, that contain compounds with keywords indicating either a potential hazard (score = 2) or need further risk assessment (score = 1). We have also identified additional solutions to aid in the identification of prospective compounds based on predicted logP values and corresponding retention time windows, as well as observed MS/MS fragmentation patterns. Lastly, we have developed an algorithm that provides a probability of identification based on the aforementioned criteria to further aid in the selection process. The dereplication database provides a unique solution for identifying known and potentially hazardous compounds in microbial samples where authentic standards are not readily available.

2104

PHARMACOLOGICALLY ACTIVE EXTRACTS FROM FLORIDA KEYS SPONGES INHIBIT OVARIAN CANCER CELL PROLIFERATION.

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Dept. of Pharmaceutical Sciences, College of Pharmacy; Chicago State University, Chicago, IL

Ovarian cancer is a lethal gynecologic malignancy. Novel chemotherapeutics are urgently needed as the five year survival rate has not dramatically improved in 20 years. Sponges and their associated microorganisms are potential sources of pharmacologically active metabolites.

The purpose of this study is to screen extracts from sponges collected in the near shore habitats in the Florida Keys for growth inhibition of several ovarian cancer cell lines (HeyA8, ES2, and EG, SKPOV3ip1) to identify targets for bioassay-guided fractionation. For our preliminary studies, the common sponges from the seagrass beds in Long Key were extracted in ethyl acetate:methanol. The lipophilic extract was partitioned between ethyl acetate and deionized water, followed by chloroform and 70% methanol. Results from extracts from the fifteen sponges in the first-pass screening demonstrated that the 70% methanol, ethyl acetate, and chloroform partitions reduce cancer cell proliferation via MTT assays at 0.016 - 1 mg/mL in complete media. The collections, extractions and preliminary screening was conducted by PharmD candidates as part of a college wide research training program. Fractions exhibiting excellent IC_{50} values have been identified for the next phase of this study. Active metabolites will be isolated using bio-assay guided fractionation in a high throughput assay system. Reductions in cell proliferation will then be confirmed by examining protein markers specific to the activated apoptotic pathway to elucidate the mechanism of action. Ongoing studies will determine if any of these extracts also inhibit invasion or migration; another cancer cell hallmark.

2105

IDENTIFICATION OF NOVEL INHIBITORS OF BOTULINUM NEUROTOXIN A

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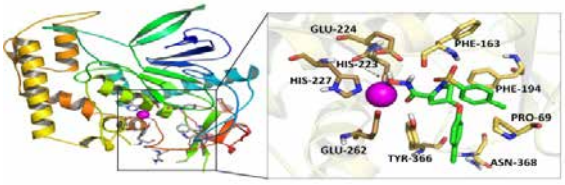
The current post-exposure treatment of botulism using antitoxins is ineffective to treat the already neuron-internalized botulinum neurotoxin. Our aim is to find novel small-molecule 'phytochemical' inhibitors of Botulinum neurotoxin serotype A (BoNT A). Fifteen plants were selected from 'traditional medicine' based on symptoms similar to botulism. Around 850 phytochemicals from these fifteen plants were virtually screened *in silico* in five BoNT A-inhibitor crystal structures. From the *in silico* output, top 50 compounds were selected based on their docking scores, visual inspection and structural diversity. These compounds were screened *in vitro* by HPLC-BoNT A LC protease bioassay. Based on the *in vitro* results, seven compounds were further screened using the mouse phrenic nerve-hemidiaphragm *ex vivo* assay. At 20 µM, NPC-ACA-3 showed marginal protection against BoNT A. The details of the workflow along with the *in vitro* and *ex vivo* data will be presented.



2106

DETECTION OF ADULTERATION IN DIETARY SUPPLEMENTS: MACA (*LEPIDIUM MEYENII*) CASE STUDY BY NMR APPROACH

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Adulteration of dietary supplements has been a potential safety concern for consumers and regulatory agencies. Maca, *Lepidium meyenii* Walpers (Brassicaceae), is a perennial herbaceous plant grown on the high Andean plateaus. Its small turnip-like root has been used as aphrodisiac, energizer, and fertility enhancer, and is so-called a natural Viaga or Peruvian ginseng. In recent years, Maca products have gained increasing popularity throughout the world, and the demand and price have increased dramatically. The global supply shortage of maca plant material has made it prone to be a seriously adulterated dietary supplement. In the present study, a nuclear magnetic resonance (NMR) spectroscopic method was developed to assess the quality of maca products sold on the market. The method can be used as a rapid preliminary tool for detection of maca adulteration.

2107

CHEMICAL INVESTIGATIONS INTO TERMITE-ASSOCIATED ACTINOBACTERIA

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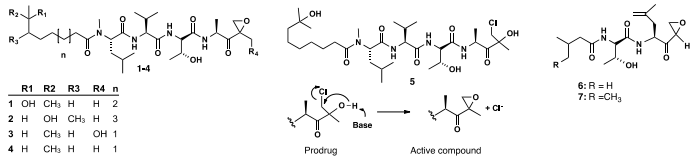
Investigations into numerous strains of termite-associated actinobacteria reveal that they are prolific producers of biologically active small molecules, including over 13 antimicrobial metabolites, such as fungichromin, berninamycin, bafilomycins, and mycotrienin. These metabolites exhibit a range of potencies against gram-positive bacteria, gram-negative bacteria, and/or environmentally relevant fungal pathogens (i.e. *Trichoderma harzianum*). Interestingly, several analogs of the bafilomycin family of metabolites exhibit inhibitory activity against most of the fungal pathogens except *T. harzianum*, where it only slows the growth rate. Furthermore, the bafilomycins visibly induce the production of a bright yellow pigment in the fungal culture. Three analogs of bafilomycin (C1, D, and E) were isolated and evaluated for potency. Bafilomycin C1 shows the greatest ability to elicit the production of this pigment, while bafilomycin E and D exhibit moderate and no activity, respectively. Identification of the major yellow pigments was made possible by growing the *T. harzianum* on solid media that was conditioned with spent liquid media from the bafilomycin-producing *Streptomyces* strain. Three major metabolites were identified, all of which loosely resemble the azaphilone family of metabolites. These metabolites exhibit moderate activity against most of the termite-associated actinobacteria and have no toxicity against the fungal pathogens. This project shows that termite-associated actinobacteria are prolific producers of antimicrobial agents, and that the pathogenic fungus, *T. harzianum*, has the ability to respond to a chemical threat with its own small molecules, inhibiting the growth and sporulation of the actinobacteria.

2108

DISCOVERY OF EPOXYKETONE PROTEASOME INHIBITORS USING METAGENOME MINING

Jeremy G. Owen[1, 2], Zachary Charlop-Powers[1], Alexandra G. Smith[1], Melinda A. Ternei[1] , Paula Y. Calle[1] , Boojala Vijay B. Reddy[1, 2], Daniel Montiel[1] , and Sean F. Brady[1, 2]
[1]Laboratory of Genetically Encoded Small Molecules and [2]Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10065.

This poster describes how short natural product sequence tags derived from conserved biosynthetic motifs can be used profile biosynthetic diversity in the environment and then guide the recovery of gene-clusters from metagenomic libraries. The results of a computational search for epoxyketone proteasome inhibitors within 185 globally distributed soil metagenomes are presented. This screen led to the identification of 99 unique epoxyketone sequence tags that were used to guide the recovery of nine epoxyketone encoding biosynthetic gene-clusters. Seven potent epoxyketone proteasome inhibitors, clarepoxcins A–E (**1**-**5**) and landepoxcins A and B (**6** and **7**), were produced by heterologous expression of two of these pathways.



	R1	R2	R3	R4	n
1	OH	CH_3	H	H	2
2	H	OH	CH_3	H	3
3	H	CH_3	H	OH	1
4	H	CH_3	H	H	1

2109

NEW BENZOPHENONE DERIVATIVES FROM THE COPROPHILOUS FUNGUS *DELITSCHIA CONFERTASPORA*

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During our ongoing studies of coprophilous (dung-colonizing) fungi as sources of new bioactive secondary metabolites, a culture of the coprophilous fungus *Delitschia confertaspora* (ATCC 74209) originally obtained from a sample of rock hyrax dung collected in Namibia was targeted for investigation. Previous studies of this culture by scientists at Merck Research Laboratories yielded the unusual 2,6-diketopiperazine derivative flutimide, which inhibits the cap-dependent endonuclease of influenza virus. This fungus has recently been genome-sequenced as part of the 1000 fungal genome project. The interesting chemistry observed in this earlier work, the limited prior studies of other members of the genus, and the lack of reports about other chemistry produced by this species prompted us to undertake further studies of this organism. Chemical investigation of a *D. confertaspora* fermentation extract resulted in isolation of two new benzophenone derivatives. Both compounds have a somewhat unusual skeleton that incorporates three aromatic rings linked via two ketone carbonyl groups. The structures of these new metabolites were elucidated by analysis of 2D NMR and HRESITOFMS data.

2110

SILYMARIN SUPPRESSES CELLULAR INFLAMMATION BY INDUCING REPARATIVE STRESS SIGNALING

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Departments of Laboratory Medicine[1] and Environmental and Occupational Health Sciences[2], University of Washington, Seattle, WA, 98104, [3]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, 99354, [4]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, NC, 27412.

Silymarin, an extract of the seeds of milk thistle (*Silybum marianum*), suppresses cellular inflammation. To define how this occurs, transcriptional profiling, metabolomics, and signaling studies were performed in human liver and T cell lines. Silymarin altered metabolic pathways; the extract activated adenosine monophosphate protein kinase (AMPK) and inhibited of mammalian target of rapamycin (mTOR) within 4 h of exposure. Metabolomics analyses showed silymarin suppression of glycolytic and amino acid metabolism. Anti-inflammatory effects arose with prolonged (i.e. 24 h) silymarin exposure, with suppression of multiple pro-inflammatory signaling pathways including nuclear factor kappa B (NF-κB). Studies with murine knock out cells revealed that silymarin inhibition of both mTOR and NF-κB was partially AMPK dependent. Other natural products induced similar stress responses, which correlated with their ability to suppress inflammation. Thus, natural products activate stress and repair responses that culminate in an anti-inflammatory cellular phenotype. Natural products like silymarin may be useful as tools to define how metabolic, stress, and repair pathways regulate cellular inflammation.

2111

(HP)TLC-BIOAUTOGRAPHY-MS/NMR – A NEW TOOL FOR THE SEARCH OF ANTI-TUBERCULOSIS LEAD COMPOUNDS

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[1]Inst. for TB Research, [2]Dept. Med. Chem. & Pharmacognosy, COP, UIC, Chicago, IL, 60612, [3]Center for Neutraceutical & Pharmaceutical Materials, [4]Div. of Biosciences & Bioinformatics, Col. Nat. Sci., Myongji University, Gyeonggi-Do 449-728, Republic of Korea

(HP)TLC-Bioautography-MS/NMR is a new method developed to respond to the quest for new anti-tuberculosis lead compounds and to overcome the limitations of the classical bioactivity-guided isolation concept. By combining modern microbiology, chromatographic, and spectrometric methods in a multidimensional setting, it is possible to obtain structural information about the compound of interest in an early purification step, and also overcome the challenges associated with (i) long isolation procedures, (ii) the frequent oversight of minor but highly-active compounds, (iii) the monitoring of anti-TB activity during fractionation, and (iv) the loss of bioactivity due to "synergistic" effects. Exemplary applications involving actinomycete and *Aspergillus* extracts with anti-TB activity demonstrate that the new, integrated methodology enables the identification and/or isolation of new and known anti-TB compounds. Thus, TLC-Bioautography-MS/NMR methodology is fit for the purpose of TB drug discovery from natural sources. [Funding: NIH grant 5 R21 AI093919-02]

2112

MINIMIZING THE PROBLEMS WITH "PIMPS"

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A recent article by Baell[i] on the problems experienced by medicinal chemists with pan-assay interference compounds (PAINS) and Shoichet's work[ii] on the impact of aggregation occurring in high throughput screening libraries, prompts a consideration of how these and other similar problems are experienced by pharmacognosists with promiscuous invalid metabolites as panaceas (PIMPs). Contrary to the classical definition of secondary metabolites as being species specific (or near specific), several natural products, particularly in the more extensively investigated plant kingdom, are common across species, genera, and even families (e.g. β-sitosterol). In the course of bioactivity-guided fractionation, PIMPs have shown up as major components in active fractions of a wide variety of pharmacological assays, i.e., they have been designated as panaceas. As in the case of PAINS, these assay results are almost invariably invalid and lead enthusiastic young scientists down a garden path. Why does this happen and how can it be avoided? Interestingly, the advances in modern methods of structure determination have exacerbated this problem, because it is possible to determine the structure of a compound when it is quite impure, and residual complexity is characteristic of chromatographic fractionation. That these residuals are often the source of the bioactivity is also frequently overlooked. Classic examples where this has occurred and ways to avoid it will be outlined.

[i]Baell, J B, *ACS Med. Chem. Lett.* 2015, 6, 229. [ii]Seidler, J, McGovern, S L, Doman, T N, Shoichet, BK. *J. Med. Chem.* 2003, 46, 4477.

2113

ACCESSING CYANOBACTERIAL NATURAL PRODUCTS: A WORKFLOW FOR SEQUENCING, AND HETEROLOGOUS EXPRESSION.

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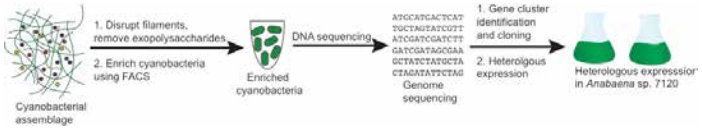
[1]College of Pharmacy, Oregon State University, Corvallis, OR 97331

Cyanobacteria are a great source of structurally diverse bioactive compounds with activities ranging from anti-tumor to anti-malarial to antibacterial. The biggest obstacles to studying cyanobacteria are their low compound yields, slow growth rate, and genetic intractability. We have been working towards a workflow that couples next generation genome sequencing with heterologous expression of cyanobacterial natural products. We have chosen to use the model cyanobacterium *Anabaena* sp. 7120 as a heterologous host for cyanobacterial natural product gene clusters. Using lyngbyatoxin A as an example we show that *Anabaena* sp. 7120 is capable of recognizing marine cyanobacterial promoters and is capable of producing cyanobacterial natural products. We also show that the culture conditions can alter lyngbyatoxin A yield significantly. Using promoter fusions we show that *Anabaena* sp. 7120 has the capacity to recognize a wide variety of promoters from both free living and symbiotic cyanobacteria. Currently we are moving towards identifying and validating the gene clusters for a variety of intriguing cyanobacterial natural products.



2114

ANTIMICROBIAL ACTIVITY OF CONSTITUENTS ISOLATED FROM SEEDS OF *PICRALIMA NITIDA* (STAPF) TH. & H. (APOCYNACEAE)

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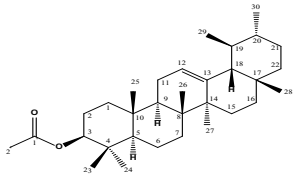
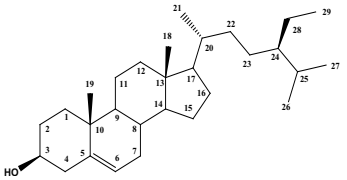
Antimicrobial activity of two compounds isolated from seeds of *Picralima nitida*, namely, Alpha-amyrin acetate and β-sitoseterol were tested against two human Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*) and four Gram-negative ones (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella Pneumoniae*). The compounds isolated, were tested against the fungal strain *Aspergilus niger and Candida albicans.* The structures of the compounds have been confirmed by HR-EIMS, 1H-13C-NMR, COSY, HETCOR spectroscopic methods. The antimicrobial activities were performed by agar well diffusion method. MIC and MBC were carried out by agar dilution method and viable cell count method, respectively. Alpha-amyrin acetate showed maximum antimicrobial activities while β-sitoseterol also showed superior antimicrobial activities when compared to standard antimicrobial drugs.



Alpha-amyrin acetate



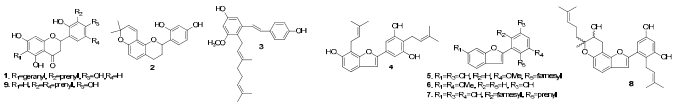
Beta-Sitosterol

2115

POTENTIAL PANCREATIC LIPASE INHIBITORY ACTIVITY OF 2-ARYLBENZOFURAN DERIVATIVES FROM *MORUS ALBA*

Byung Sun Min[1], Ha Manh Tuan[1], Tran Manh Hung[1], and Jeong Ah Kim[2]
[1] College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 712-702, Korea,[2] College of Pharmacy, Kyungpook National University, Daegu 702-701, Korea.

Bioassay-guided isolation of the methanolic extract of *Morus alba* using pancreatic lipase inhibitory activity led to isolation of five new 2-arylbenzofuran derivatives (**4**–**8**), three new flavonoids (**1**, **2**, **9**), one new prenylated stilbene (**3**) and twelve known compounds including moracin B (**10**), moracin E (**11**), isobavachalcone (**12**), morusin (**13**), sanggenol L (**14**), kuwanon T (**15**), kuwanon U (**16**), 5,6-dimethoxy-2-(3-hydroxy-5-methoxyphenyl) benzofuran (**17**), mulberrofuran D (**18**), sanggenofuran A (**19**), mulberrofuran D2 (**20**), and mulberrofuran A (**21**). Their structures were elucidated on the basis of comprehensive analysis of spectroscopic data. Compounds **5**, **7**, **18** and **19** showed potent inhibition against pancreatic lipase with IC_{50} values of 0.564, 0.388, 0.089 and 0.912 μM, respectively.

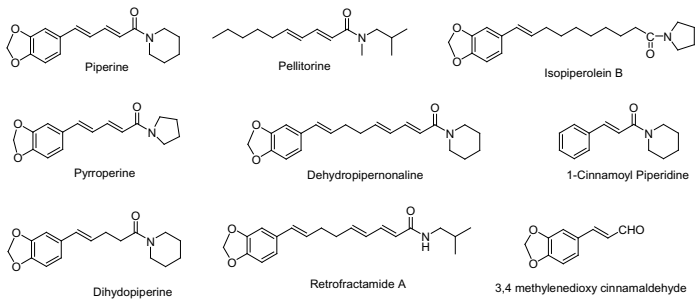


2116

ALKALOIDS CONSTITUENTS FROM THE FRUITS OF *PIPER NIGRUM* L. AND THEIR ANTI-INFLAMMATION ACTIVITY

Bo Mi Lee[1], Quynh Mai N.T[1], Jeong Ah Kim[2] and Byung Sun Min[1]
[1]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 712-702, Korea, [2]College of Pharmacy, Kyungpook National University, Daegu 702-701, Korea.

Eight alkaloids, piperine, pellitorine, isopiperolein B, Pyroperine, dehydropipernonaline, 1-Cinnamoyl piperidine, dihydopiperine, retrofractamide A, along with 3,4 methylenedioxy cinnamaldehyde, were isolated from the fruits of *Piper nigrum* L.. Their structures were elucidated by spectroscopic methods. *In vitro* activity assay showed that pellitorine, isopiperolein B, retrofractamide A and dehydropipernonaline presented remarkable activity on lipopolysaccharide (LPS)-induce NO release.

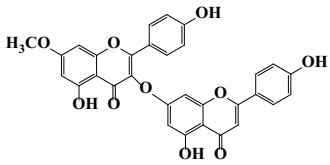


2117

PROTEIN TYROSINE PHOSPHATASE 1B AND ALPHA-GLUCOSIDASE INHIBITORY COMPOUNDS FROM THE LEAVES OF *SMILAX CHINA* L.

Bing Tian Zhao[1], Duc Hung Nguyen[1], Md Yousof Ali[2], Jae-Sue Choi[2], and Mi Hee Woo[1]
[1]College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea, [2]Department of Food Science & Nutrition, Pukyong National University, Busan 608-737, Republic of Korea

Two new flavonoids, bismilachinone (**11**) and smilachinin (**14**), were isolated from the leaves of *Smilax china* L. together with 14 known compounds. The PTP1B and α-glucosidase inhibitory activities for compounds **1**–**16** were evaluated on the molecular level. In the in vitro anti-diabetic assay, compounds **3**, **4**, **6**, **11**, **12**, and **16** showed strong PTP1B inhibitory activity with IC_{50} values of 7.62, 10.80, 0.92, 2.68, 9.77, and 24.17 μM, respectively. Furthermore, compounds **2**–**7**, **11**, **12**, **15**, and **16** showed potent α-glucosidase inhibitory activity with IC_{50} values of 8.70, 81.66, 35.11, 35.92, 7.99, 26.28, 11.28, 62.68, 44.32, and 70.12 μM, respectively. In the kinetic study for the enzyme of PTP1B, compounds **6**, **11**, and **12** revealed competitive inhibition, and compounds **3**, **4**, and **16** showed non-competitive inhibition. These results demonstrate that the leaves of *S. china* are useful as potential functional food ingredients for the prevention and treatment of type 2 diabetes.



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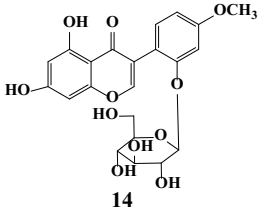

14

2118

EXTRACTION OF LAVENDER ESSENTIAL OIL AND ITS ANTIOXIDANT ACTIIVITY

Qing Zhao[1], Sha-Sha Jin[1]
[1]Department of Applied Chemistry, College of Science, Xi'an University of Technology, Xi'an 710054, China

The flowers and leaves of Lavender (*Lavandula angustifolia* Mill) have been used as a herbal medicine for the treatment of heal burns and insect bites. Previous studies have shown that its exacts exhibited wide range of bioactivities such as analgesic, wound healing, antibacterial, antifungal, sedative and antidepressant effects. Lavender essential oil has attracted a great deal of interest for its potential uses in food, perfume and pharmaceutical industries. It has a soothing and calming effect on the nerves, relieving tension, depression, panic and nervous exhaustion in general and is effective to reduce anxiety in both clinical and preclinical studies. In this study, the details of the optimum extraction condition for lavender essential oil, its chemical constituents and antioxidant properties have been studied. It was found that the most abundant constituents of the lavender essential oil collected in Urumqi (Xiangjiang province, China) are linalool (2.1%) and linalyl acetate (3.0%), both of them have been proposed as topical palliatives of pain in animal models. The antioxidant activity indicated that the lavender essential oil can scavenge hydroxyl radicals.

2119

DIFFERENTIAL INDUCTION OF SECONDARY METABOLITE PROFILES IN ENDOPHYTE FUNGI BY THE ADDITION OF EPIGENETIC MODIFIERS

Victor González-Menéndez, Rachel Serrano, Francisca Muñoz, Fernando Reyes, Olga Genilloud and Jose R. Tormo
Fundación MEDINA, Avda. Conocimiento 34, 18016, PTS, Granada, Spain

Fungal endophytes are known to produce a wide variety of secondary metabolites (SMs) involved in their adaptation and survival within higher plants. The plant-microbe interaction may influence the expression of some biosynthetic pathways, otherwise cryptic in these fungi when grown outside its natural environment. Adding epigenetic small-molecule modifiers of Histone Deacetylase (HDAC) and DNA methyltransferase (DNMT) activities in fungal endophyte fermentations, may induce, among others, the expression of these biosynthetic pathways that occur naturally in plant-microbe interactions.

The addition of these small-molecule epigenetic elicitors since the inocula of several fungi in our study, determined a more consistent activation of fungal endophytes silent biosynthetic pathways, compared to its only addition during their production fermentation. uHPLC analyses showed these differential types of responses, including profiles with few changes in their metabolite production, higher amounts of some or all SMs, or few changes but with new SMs present, proving that the use of these epigenetic modifiers can ensure an extensive generation of new SMs, previously unknown to be produced by this group of endophyte fungi, in laboratory conditions.

2120

DELPHATISINE D AND CHRYSOTRICHUMINE A, TWO RARE NEW DITERPENE ALKALOIDS FROM *DELPHINIUM CHRYSOTRICHUM*

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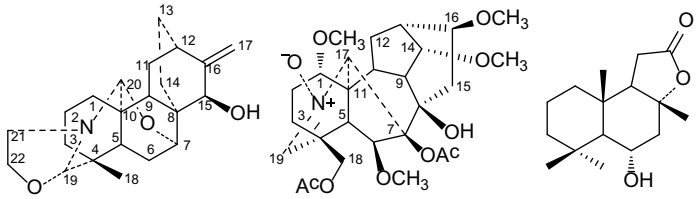
Chemical investigations of the Tibetan folk medicine *Delphinium chrysotrichum* resulted in the isolation of two new diterpenoid alkaloids, delphatisine D (**1**) and chrysotrichumine A (**2**), together with eleven known compounds including one sesquiterpenoid, *3β*,6α-dlphydroxysclareollda (**3**) and ten diterpenoid alkaloids. Compund **1** is a rare isoatisine-type alkaloid from genus *Delphinium* and is the C-15 epimer of spiramine C which bearing an internal carbinolamine ether linkage (N–C–O–C) between C-7 and C-20. Compound **2** is a rare natural C_{19}-diterpenoid alkaloid possessing a nitrone group between C-17 and C-19. Their structures were elucidated on the basis of extensive spectroscopic analysis. Compound **3** is a sesquiterpenoid first isolated from this genus.



1 **2** **3**

2121

INVESTIGATION INTO THE BIOACTIVE METABOLITES OF DEEP SEA FUNGI

Candice L. Bromley[1], Ryan M. Young[1,2], Stephen Jackson[3], Thomas Sutton[3], Alan Dobson[3, 4], Bill J. Baker[1,2]
[1]School of Chemistry, National University of Ireland, Galway, Ireland, [2]Department of Chemistry and Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL 33620, [3]Marine Biotechnology Center, Environmental Research Institute, National University of Ireland, Cork, Ireland,[4] School of Microbiology, University College Cork, National University of Ireland, Cork, Ireland.

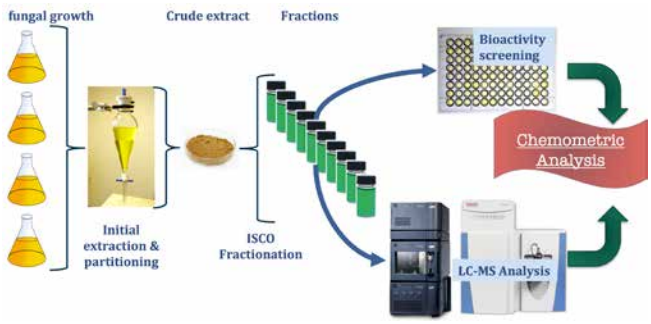
Eleven fungal strains were cultured from deep sea samples collected from Irish waters. The crude organic extracts of the fungi were initially tested for bioactivity against gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, as well as gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. In an attempt to increase the production of bioactive secondary metabolites epigenetic modifiers, capable of activating silenced or attenuated gene clusters in the fungi, were employed. Each of the fungal strains were exposed to two such epigenetic modifiers, namely sodium butyrate and 5-azacytidine, inhibiting histone deacetylase (HDAC) and DNA methyltransferase (DNMT) respectively. The effects of these epigenetic modifications on the bioactivity of the resulting fungal extracts were determined through a range of biological assays. Comparative investigations of the metabolomic profiles produced by fungal strains were conducted using LC-MS as well as NMR spectroscopy to establish any resultant changes in the bioactive secondary metabolites brought about through the epigenetic modification.

2122

CHEMOMETRIC-DIRECTED BIOEXPLORATION OF NATURAL PRODUCTS

Joshua Kellogg[1], Daniel A. Todd[1], Joseph M. Egan[1], Huzefa A. Raja[1], Nicholas H. Oberlies[1], Olav M. Kvalheim[2], and Nadja B. Cech[1]
[1]Department of Chemistry & Biochemistry, The University of North Carolina Greensboro, Greensboro, NC 27402, USA, [2]Department of Chemistry, University of Bergen, Bergen, Norway

A workflow for the chemometric analysis of a chromatographic fractionation series was developed, integrating partial least-square (PLS) statistical modeling with high-resolution mass spectrometry to yield an associative model that correlated chemical composition and bioactivity. Goldenseal (*Hydrastis canadensis*) fungal endophytes were evaluated to determine their secondary metabolite profile and their influence on growth of *Staphylococcus aureus* strain SA1199. *Pyrenocheata* sp. extract and fractions inhibited the growth of SA1199 as much as 95.8 ± 1.7%, and the resulting PLS model predicted a single active ion (*m/z* 343) as the purported bioactive component. Follow-up analysis tentatively identified the *m/z* 343 ion as the known antimicrobial macrosphelide A.



2123

ISOLATION, CHEMICAL PROFILING, AND STANDARDIZATION OF BETAINE, CHOLINE, ACETYLCHOLINE, AND 20-HYDROXYECDYSONE FROM *ATRIPLEX* SPECIES

Mallika Kumarihamy[1], Yan-Hong Wang[1], Francisco Leon[2], Mei Wang[1], Andrew Smesler[1], Stephen J. Cutler[2], Ikhlas A. Khan[1, 2], Ilias Muhammad[1]
[1]National Center for Natural Products Research and [2]Department of BioMolecular Sciences School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA

Atriplex canescens (Pursh) Nutt., is an evergreen, scurfy shrub endemic to the central and western regions of the North America. Native Americans have been used it in folkloric medicine for controlling pain, ant bites and other ailments. From the EtOH extract of *A. canescens* leaves three key dietary ingredients, namely betaine (**1**), choline (**2**), and 20-hydroxyecdysone (**3**) were isolated and identified as major chemical markers, along with oleanolic acid 3-*O*-***β***-D-glucopyranosyl-28-*O*-***β***-D-glucopyranoside (**4**), isovanillic acid (**5**), and **β**-sitosterol-D-glucoside (**6**). Chemical profiling of three major constituents **1**-**3** and acetylcholine (**9**), using RP-HPLC, in different plant parts of *A. canescens*, as well as among other species, namely *A. fruiticulosa, A. fasciculate, A. semibaccata* and *A. lentiformis*, revealed all four compounds are present in the extracts of different parts of *A. canescents*, but 20-hydroxyecdysone is not detected in other species of *Atriplex*. The presence of these dietary and nutritional constituents, particularly in *A. canescens*, explains the implications of this native US plant.

Acknowledgement: Supported by the National Institute of General Medical Sciences (NIGMS), NIH Grant Number P20GM104932, and COBRE, CORE-NPN, Research Core C.

2124

SORDARICINS AND PODOSPORINS FROM *SORDARIA ARANEOSA*

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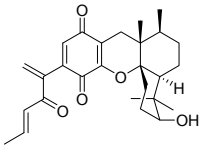
[1]Natural Products Discovery Institute, Baruch S. Blumberg Institute, Doylestown PA, 18902, USA.

During the course of exploring the antifungal constituents produced by the coprophilous fungus *Sordaria araneosa*, two new congeners (**1** and **2**) of podosporin A were isolated, along with podosporin A (**3**), sordarin, and hypoxysordarin. Compounds **1** and **2** showed weak activity against *Staphylococcus aureus*, while **3** was not active. While sordarin and hypoxysordarin were active against *Candida albicans*, **1**-**3** were not active. The compounds were characterized by 1D and 2D NMR analysis and high resolution MS analysis.



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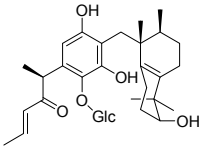

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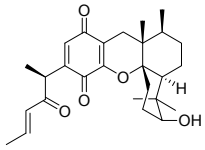


3

2125

SCALE UP ISOLATION OF AAPTAMINE FOR IN VIVO EVALUATION SUGGESTS IN NEUROBIOLOGICAL ACTIVITY IS LINKED TO THE DELTA OPIOID RECEPTOR

Tyler A. Johnson[1,2], Nicole L. McIntosh[1], Eptisam Lambo[1], Nicholas Lorig-Roach[2], Laura Milan-Lobo[3], Li He[3], Philip Crews[2] and Jennifer L. Whistler[3]
[1] Department of Natural Sciences & Mathematics, Dominican University of California, [2] Department of Chemistry & Biochemistry, University of California, Santa Cruz, [3] Department of Neurology, University of California, San Francisco

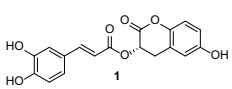
Opioid receptors belong to the large superfamily of seven transmembrane-spanning (7TM) G protein-coupled receptors (GPCRs). As a class, GPCRs are of fundamental physiological importance mediating the actions of the majority of known neurotransmitters and hormones. The Mu, Delta and Kappa (MOP, DOP, KOP) opioid receptors are particularly intriguing members of this receptor family as they are the targets involved in many neurobiological diseases such as addiction, pain, stress, anxiety, and depression. Recently we discovered that the aaptamine class of marine sponge derived natural products exhibit selective agonist activity in vitro for the DOP versus MOP receptor. Our findings may explain reports by others that aaptamine (**1**) demonstrates in vivo anti-depressant effects in mouse models using the Porsolt Forced Swim Test. This project involved the extraction of the sponge *Aaptos* aaptos (a source of **1**), establishing a scale up purification procedure to provide sufficient amounts of **1** (30 mg) for a follow up in vivo evaluation and ultimately confirmation of the structure of **1** using LC-MS and ^{1}H NMR. The results our purification scheme, chemical analysis and in vivo evaluation of **1** using DOP receptor knock out mice in the Forced swim test, Locomotor test and Marble burying test are reported here in and suggest that the in vivo anti-depressant effects of **1** are linked directly to its agonist effects on the DOP receptor.

2126

SECONDARY METABOLITES ISOLATED FROM *SALVIA BOGOTENSIS*

Amer Tarawneh,[1] Francisco Leon,[1] Augusto Rivera,[2] Stephen J. Cutler.[1,*]
[1]Department of BioMolecular Sciences, Division of Medicinal Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677, USA, [2] Departamento de Química, Universidad Nacional de Colombia, Ciudad Universitaria, Carrera 30 # 45-03, Bogotá, Colombia

Salvia is the most diverse genus in the Lamiaceae family, with close to 1000 species distributed around the world. In the Americas the genus is represented by approximately 600 species. Several species of *Salvia* are cultivated for their aromatic characteristics and are used as flavorings, food condiments, cosmetics, perfume additives and as folk medicines. As a part of our continuing search for novel, plant-derived biological agents, *Salvia bogotensis* was studied. Several separations using column chromatography (silica gel, Sephadex LH-20) and preparative TLC were performed to afford two new coumarin derivatives **1**-**2**, a new glucoside monoterpenoid **3** and eleven previously known compounds. The structures of compounds were determined on the basis of HRMS, and 1D and 2D NMR studies, as well as by comparison with data from the literature. The new compounds do not show affinity to opioid and cannabinoid receptors, and showed moderate activity in antibacterial, antifungal, antimalarial and antileishmania assays.



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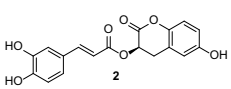

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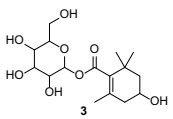


3

3001

DETERMINATION OF ADULTERATION IN *ECHINACEA* SPP. BY PRINCIPAL COMPONENT ANALYSIS OF UV SPECTRA

Mohammed Khan, Frank D'Amelio Sr., Youssef Mirhom, and Rocky Graziose*
Bio-Botanica Inc, 75 Commerce Drive, Hauppauge, NY 11788

Ultraviolet (UV) spectroscopy was used to determine adulteration in *Echinacea* spp. Authenticated samples of *Echinacea angustifolia* and *Echinacea purpurea* roots were used to generate a predictive model using a Principal Component Analysis (PCA) algorithm. Adulterated and unadulterated test samples were used to show the effects of applying the model. Our results show that PCA is effective for distinguishing species of *Echinacea*, as well as determination of adulteration levels in *Echinacea* samples up to at least 25% admixture.

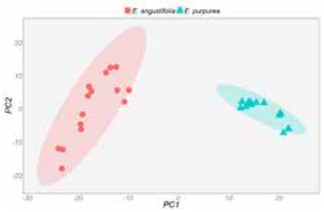


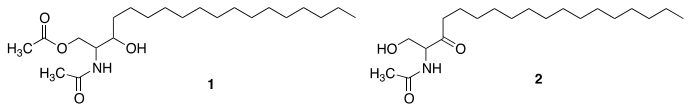
Figure 1: PCA of UV spectra of authenticated samples of *E. angustifolia* and *E. purpurea* showing 95% confidence intervals.

3002

A BRAZILIAN SAMPLE OF THE RED ALGA *LAURENCIA* NSP., YIELDS SPHINGOSINES NEW TO THE MARINE ENVIRONMENT

Erika M. Stein,[1] Pio Colepicolo,[1] Kehau Hagiwara,[2] and Anthony D. Wright[2]
[1]Department of Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, 05508-900 São Paulo, Brazil; [2]DKI-College of Pharmacy, University of Hawaii at Hilo, 34 Rainbow Drive, Hilo, Hawaii 96720

Marine algae belonging to the genus *Laurencia* are the most prolific producers of secondary metabolites compared to any other marine genus. The secondary metabolites produced by these plants are typically halogenated and have a wide variety of biological properties. The most common structural classes encountered being sesquiterpenes, diterpenes, triterpenes, sterols, C_{15} acetogenins and long-chain hydrocarbons. Sphingosines are among the lesser reported compounds isolated from this genus, with only one, (+)-*N*-(2*S*, 3*R*)-2-acetamido-3-acetoxyoctadecan-l-ol, being reported to date. In this poster presentation we report the isolation of additional sphingosines, including **1** and **2**, from the genus *Laurencia*, this time from a new species collected in Brazil.



3003

CYTOTOXIC POTENTIAL OF ESSENTIAL OILS OF *Eucalyptus benthamii* AND ITS RELATED TERPENES ON TUMOR CELL LINES

Paulo V. Farago[1], Patrícia M. Döll-Boscardin[1], Carla C. Kanunfre[2], and Jane M. Budel[1]
[1]Department of Pharmaceutical Sciences, [2]Departament of General Biology, State University of Ponta Grossa, Paraná, Brazil

Eucalyptus L. is traditionally used for many medicinal purposes. In particular, some *Eucalyptus* species have currently shown cytotoxic properties. Local Brazilian communities have used leaves of *E. benthamii* as an herbal remedy for various diseases, including cancer. Considering the lack of available data for supporting this cytotoxic effect, the goal of this paper was to study the *in vitro* cytotoxic potential of the essential oils from young and adult leaves of *E. benthamii* and some related terpenes (**α**-pinene, terpinen-4-ol and **γ**-terpinene) on Jurkat, J774A.1 and HeLa cells lines. Regarding the cytotoxic activity based on MTT assay, the essential oils showed improved results than **α**-pinene and **γ**-terpinene, particularly for Jurkat and HeLa cell lines. Terpinen-4-ol revealed a cytotoxic effect against Jurkat cells similar to that observed for volatile oils. The results of LDH activity indicated that cytotoxic activity of samples against Jurkat cells probably involved cell death by apoptosis. The decrease of cell DNA content was demonstrated due to inhibition of Jurkat cells proliferation by samples as a result of cytotoxicity. In general, the essential oils from young and adult leaves of *E. benthamii* presented cytotoxicity against the investigated tumor cell lines which confirms their antitumor potential. This study was supported by Fundação Araucária (Brazil).

3004

ESSENTIAL OIL FROM LEAVES OF *Eucalyptus benthamii*: SECRETORY STRUCTURES, VOLATILE COMPOSITION AND BIOLOGICAL ACTIVITIES

Jane M. Budel[1], Patrícia M. Döll-Boscardin[1], and Paulo V. Farago[1]
[1]Department of Pharmaceutical Sciences, State University of Ponta Grossa, Paraná, Brazil

Eucalyptus benthamii Maiden et Cambage is a medicinal plant that is also widely used for reforestation in Southern Brazil. However, restricted data about its essential oil are available. The anatomical analysis was performed by the usual light and scanning microtechniques. The identification of volatile constituents was achieved by GC-MS and GC-FID. The antioxidant potential was evaluated by the phosphomolybdenum and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) methods. *In vitro* antimicrobial activity was investigated by the agar disk diffusion technique. Fumigant and repellent activities were carried out against head lice. Secretory ducts containing volatile compounds were observed in mesophyll and petiole. The major volatile components were α-pinene (22.6%), γ-terpinene (16.6%), *p*-cymene (10.6%), aromadendrene (9.9%), and terpinen-4-ol (5.9%). For phosphomolybdenum method, the volatile oil of *E. benthamii* showed a total antioxidant activity (43.15 ± 1.08%) higher than that achieved for rutin (20.70 ± 1.69%). However, a low antioxidant potential was observed by DPPH method (IC_{50} = 3209.66 ± 218.86 μg.mL^{-1}). The essential oil of *E. benthamii* had an antimicrobial effect against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes* strains. In the insecticidal activity against head lice, the investigated volatile oil demonstrated promising fumigant (Knockdown $time_{50}$ = 36.67 ± 2.89 min) and repellent (Repellent index = 35.03 ± 13.13%) effects. This study was supported by Fundação Araucária (Brazil).

3005

ANTITRYPANOSOMAL AND OPIOID RECEPTOR ACTIVITY OF MUSSAENDA LUTEOLA'S SECONDARY METABOLITES

*Shaymaa M. M. Mohamed[1,3], Enaam Y. Backheet[3], Soad A. L. Bayoumi[3], Stephen J.Cutler[1,2] and Samir A. Ross[1,2]**
[1] National Center for Natural Products Research and [2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA, [3] Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Eight new secondary metabolites; five triterpene saponins; heinsiagenin A 3-*O*-*β*-D-glucopyranosyl(1→2)-*O*-*α*-L-rhamnopyranosyl (1→4)-*O*-*β*-D-glucopyranoside (**1**), heinsiagenin A 3-*O*-[α-L-rhamn-

opyranosyl (1→2)-*O*-*β*-D-glucopyranosyl (1→2)]-*O*-*β*-D-glucopyranosyl (1→4)-*O*-*β*-D-glucopyranoside (**2**), 2*α*-hydroxyheinsiagenin A 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*O*-*β*-D-glucopyranosyl (1→4)-*O*-*β*-D-glucopyranoside (**3**), 2α-hydroxyheinsiagenin A 3-*O*-*β*-D-glucopyranosyl (1→2)-*O*-*β*-D-glucopyranosyl (1→4)-*O*-*β*-D-glucopyranoside (**4**) and *N*-(2*S*, 3*R*, 4*R*-3-methyl-4-pentanolid-2-yl)-18-hydroxylanosta-8(9), 22*E*, 24*E*-trien-27-amide-3-*O*-[α-L-rhamnopyranosyl(1→2)-*O*-*β*-D-glucopyranosyl (1→2)]-*O*-*β*-D-glucopyranosyl (1→4)-*O*-*β*-D-glucopyranoside (**5**) , one iridoid glucoside; (5*α*, 9*α*)-8-epiloganin (**7**), and two alkaloids; 10-methoxystrictosidine (**9**) and 10-methoxy pumiloside (**11**) were isolated from the aerial part of ***Mussaenda luteola*** (Rubiaceae) along with four known compounds: (7*α*)-morroniside **(6)**, (7*β*) 7-*O*-methyl morroniside **(8)**, 5*α*-carboxystrictosidine **(10)** and apigenin 7-*O*-neohesperidoside **(12)**. Structural elucidation was based on the analyses of spectroscopic data and HR-ESI-MS. Compounds **1, 2** and **10** showed antitrypanosomal activity while only compounds **1** and **2** showed opioid receptor binding activity.

3006

ANTI-INFLAMMATORY AND ANTITUMOR PHENYLPROPANOID SUCROSIDES FROM THE SEEDS OF *RAPHANUS SATIVUS*

Hee Rae Kang[1], Seulah Lee[1], Hee Jeong Eom[1], Jae Sik Yu[1], and Seoung Rak Lee[1]

[1]Natural Product Research Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea

A bioassay-guided fractionation and chemical investigation of the MeOH extract of *Raphanus sativus* seeds resulted in the isolation and identification of eight phenylpropanoid sucrosides (**1**-**8**) including two new compounds, named raphasativuside A and B (**1**-**2**) from the most active $CHCl_3$-soluble fraction. The structures of these new compounds were elucidated through spectral analysis, including extensive 2D-NMR data, and chemical reaction experiments. We evaluated the anti-inflammatory effects of **1**-**8** in lipopolysaccharide (LPS)-stimulated murine microglia BV2 cells. Compounds **2** and **5** exhibited significant inhibitory effect on nitric oxide production in LPS-activated BV-2 cells with IC_{50} values of 21.63 and 26.96 μM, respectively. All isolates were also evaluated for their antiproliferative activities against four human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15). Compounds **1**–**7** showed consistent cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines with IC_{50} values of 6.71–27.92 μM. Additionally, the free-radical scavenging activity of **1**-**8** was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay where compounds **1**, **3**, and **4** scavenged DPPH radical strongly with IC_{50} values of 23.05, 27.10, and 29.63 μg/mL, respectively.

3007

TILIABISFLAVAN A, A NEW FLAVAN-3-OL DIMER FROM *TILIA AMURENSIS* WITH CYTOTOXIC AND ANTI-INFLAMMATORY EFFECTS

Hee Rae Kang[1], Hee Jeong Eom[1], Seulah Lee[1], Jae Sik Yu[1], and Seoung Rak Lee[1]

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Tilia amurensis Rupr. (Tiliaceae) is commonly known as bee tree and it has been used to treat various diseases as a Korean traditional medicine since ancient times. On the search for biologically active compounds from *T. amurensis*, a bioassay-guided fractionation and chemical investigation of its MeOH extract resulted in the isolation and identification of four flavan-3-ols, including a new flavan-3-ol dimer, tiliabisflavan A (**1**). The isolated compounds were evaluated for their antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines and their inhibitory effects on NO production in a lipopolysaccharide (LPS)-activated BV2 cell line. Compound **1** exhibited the strongest cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} (**1**): 13.37, 14.61, 5.68, and 11.03 ***μM***, respectively). In addition, compound **1** significantly inhibited NO levels with an IC_{50} value of 4.96 ***μ***M, which is more effective than N^G-monomethyl-L-arginine (NMMA), a well-known NO synthase inhibitor.

3008

PRENYLATED ANTHRANOLS FROM THE LEAVES OF *HARUNGANA MADAGASCARIENSIS*

Johnson O. Oluwatosin,[1,2] Ming Zhao,[1] Wei-Lun Chen,[1] Bernard D. Santarsiero,[1] Steven M. Swanson,[1,3] Joanna E. Burdette,[1] Gloria A. Ayoola,[2] H. A. B Coker,[2] and Chun-Tao Che.[1]

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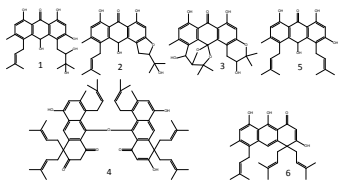

Four new prenylated anthranols (**1**-**4**), along with two known structures kenganthranol (**5**) and harunganin (**6**), were identified from the leaves of the African medicinal plant *Harungana magdagascariensis*. All anthranols were evaluated for cytotoxic activity against MDA-MB-435, MDA-MB-231, and OVCAR3 cell lines. **6** showed activities with IC_{50} values of 6.3-8.7 μM.

3009

METHOD OPTIMIZATION OF AMYLOGLUCOSIDASE ACTIVITY ASSAY AND ITS APPLICATION IN ANALYSIS OF SECRETOME YIELD BY *ASPERGILLUS NIGER*

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Institute for Natural Products Applications and Research Technologies, Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019.

Identifying the active proteoforms of bioconversion enzymes from microbial sources is important for novel compound delivery in pharmaceutical companies. Many of these enzymes are heavily post-translational modified and the modifications are known to regulate the enzymatic activities. Our preliminary glycol-proteomics results suggest that Amylogucosidase in *A. niger* secretome is heavily glycosylated with both *N*-linked and *O*-linked glycans. Here, using Amyloglucisidase as the model system, we develop an integrated proteomics approach to correlate the enzymatic activity with its differently post-translational modified proteoforms. Various LC separation formats (e.g., ion exchange, size exclusive column) are selected to fractionate the total Amyloglucisidase extract. After evaluating the enzyme activities using glucose assay kits, the fractions containing the active proteoforms are further analyzed using the integrated top-down and bottom-up approach employed on an LTQ Orbitrap Velos mass spectrometry. The optimized method will be then applied to analyze the Amyloglucisidase in *A. niger* growing under different conditions (e.g., various pHs and temperatures).

3010

A NEW BIPHENYL DERIVATIVE FROM THE TWIGS OF *CHAENOMELES LAGENARIA*

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Natural Products Laboratory, School of Pharmacy, SungKyunKwan University, Suwon 440-749, Republic of Korea

Chaenomeles lagenaria (LOISEL) KOIDZ. is deciduous shrub with distribution in Korea and China. This plant has been used as Korean traditional medicine to treat diarrhea, edema, and rheumatism. Previous phytochemical investigation on *C. lagenaria* reported triterpenes, phenolic compounds, and flavonoids. In our continuing search for bioactive constituents of Korean medicinal sources, we investigated an 80% MeOH extract of the twigs of *C. lagenaria*. Column chromatographic purification of the $CHCl_3$ fraction resulted in isolation of a new biphenyl derivative (**1**), along with three known biphenyl compounds (**2**-**4**). The chemical structure of the new compound was determined on the basic of spectroscopic analyses including 1D and 2D NMR data. The cytotoxic activities of isolates (**1**-**4**) were evaluated by determining their cytotoxic effects on the human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT-15) using a SRB assay.

3011

ABSOLUTE CONFIGURATION OF THE 1,2-DIOL OF DIGOXIN DETERMINED BY ELECTRONIC CIRCULAR DICHROISM INDUCED WITH $[MO_2(OAC)_4]$

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Digoxin, a cardiac glycoside derived from *Digitalis lanata*, has been long used to treat congestive heart failure. When a number of agents were tested in an early drug repurposing study, digoxin emerged as a potential anticancer agent. The structure of digoxin was determined by analysis of its spectroscopic data and confirmed by the single-crystal X-ray diffraction in previous work. In the present investigation, the conformation of the central ring system of digoxin was characterized based on its published crystal structure, and the absolute configuration was ascertained by analysis of the electronic circular dichroism (ECD) induced with $[Mo_2(OAc)_4]$ and NOESY 2D NMR spectra. When evaluated for its cytotoxicity toward a panel of human cancer cell lines, including HT-29 human colon cancer cells and the human MV4:11, THP-1, and Kasumi-1 myeloid leukemia cell lines, digoxin showed potent activity, exhibiting IC_{50} values of 10 nM at 72 hour incubation, and 100, 59, and 89 nM at 48 hour incubation, respectively.

3012

MICROBIAL TRANSFORMATON OF BAVACHIN BY ABSIDIA COERULEA

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College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Republic of Korea.

Bavachin is one of the major bioactive flavonoid constituents in Fructus Psoraleae (Buguzhi in Chinese), and it displays a broad range of biological activities such as antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor, phytoestrogenic, α-glucosidase inhibitory, and osteoblastic proliferation stimulating activities. In order to improve its bioavailability, we investigated the application of microorganisms as biocatalysts for the transformation of bavachin. Screening studies using twenty two microbes indicated that bavachin could be transformed by several fungal strains including *Absidia coerulea*, *Mucor hiemalis*, and *Cunninghamella elegans*. And our subsequent scale-up fermentation studies of bavachin using *A. coerulea* resulted in the identification of two novel microbial metabolites together with a recently reported compound bavachinone A. Chemical structures of the three compounds were elucidated on the basis of spectroscopic analysis. This is the first report on the biotransformation of bavachin by microbes, which suggested a potential process to prepare novel derivatives from plant secondary metabolites through microbial enzymes.

3013

STRUCTURAL CHARACTERIZATION OF MONACOLIN COMPOUNDS FROM RED YEAST RICE BY LIQUID CHROMATOGRAPHY AND TANDEM MASS

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Monacolin compounds including its acid form such as monacolin K and monacolin K acid are markers of red yeast rice (RYR). With the aim of quality assurance and adulterant assessment, the fragmentation pathways of nine monacolins were investigated using tandem mass spectrometric technique (QToF MS/MS). Nine compounds were classified into three sub-groups according to the substitution at C-8 and whether in acid form at C-1' or forming lactone by cyclization between C-1' and C-5'. The key fragments of three sub-groups were summarized and applied to the identification of monacolin compounds from red yeast rice by UHPLC-QToF MS.

3014

THE GENERALLY USEFUL ESTIMATE OF SOLVENT SYSTEMS METHOD ENABLES THE RAPID SEPARATION OF CURCUMINOIDS BY COUNTERCURRENT SEPARATION

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The Generally Useful Estimate of Solvent Systems (GUESS) method was originally developed for the selection of countercurrent separation (CCS) solvent systems. It was shown if an analyte can be eluted at R*f* value ≈ 0.5 by the organic phase or an equivalent organic-only solvent system on NP-TLC, the analyte will be delivered into the distribution coefficient (*K* value) sweet spot range by the matching solvent system in CCS. However, although >100 research papers have cited the original GUESS publication since 2005, few of them have actually applied the predictive TLC part of the method. The current study demonstrates the rapid prediction power of the GUESS method for CCS solvent systems. It tested three curcuminoids in *n*-hexane-EtOAc-MeOH-Water, $CHCl_3$-MeOH-Water and *n*-hexane-$CHCl_3$-MeOH-Water solvent system families. When developing with the organic phase of *n*-hexane-$CHCl_3$-MeOH-Water (3:7:7:3, v/v), the TLC R*f* values of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were 0.50, 0.46, and 0.38, while their measured *K* values were 0.57, 0.97, and 1.91 in normal phase CCS. The present study indicates that the GUESS method can facilitate natural product separation by CCS.

3015

BOSWELLIA SERRATA AND *OCIMUM SANCTUM* EXTRACTS REDUCE INFLAMMATION IN AN OVA-INDUCED ASTHMA MODEL OF BALB/C MICE

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This study investigated the in vivo anti-inflammatory effects of *Boswellia serrata* and *Ocimum sanctum* extracts in HL-60 cells and in OVA-induced inflammatory lung disease in BALB/C mice. The plant materials were collected in Vidisha (M.P.), India, identified and air-dried. The dried plant materials were pulverized to a powder, extracted in 95% ethanol to exhaustion, dried and fractionated. The extract and fractions were tested for their effects on leukotriene C4 synthase, leukotriene A4 hydrolase and cyclooxygenase-2 in HL-60 cells. The active dried ethanol extracts were then tested in vivo in an OVA-induced asthma mouse model, and inflammation was assessed using 2-D in vivo imaging. Both extracts and some fractions inhibited leukotriene-C4-synthase, leukotriene-A4-hydrolase and/or cyclooxygenase-2 (COX-2) activities in cultured HL-60 cells, IC50 1-10 µg/ml. Intragastric administration of the extracts to OVA-challenged BALB/c mice at a dose of 100 mg/kg body weight led to a significant reduction of OVA-induced lung inflammation as determined by 2-D in vivo imaging using a Xenogen IVIS imaging system. The results showed significant inhibition in vivo and in vitro inflammation suggesting a plausible mechanism of action for the management of asthma.

3016

ADAPTABILITY OF SUBTROPICAL ALGAE TO LOCAL MICROBIAL POPULATIONS

Albert Nelson[1], Thy Nguyen[1], Kaitlin Ferguson[1], Julia Sears[1], Urszula Tanouye[2], Skylar Carlson[2], Theodore Weyna[3], Jason Kwan[3], Brian T. Murphy[2], Melany P. Puglisi[1]

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Marine algae found in the seagrass beds of the Florida Keys appear to be less susceptible to disease and die-offs reported for *Thalassia testudinum*. In the present study, we explore the hypothesis that algal antimicrobial chemical defenses are adapted to the microbial flora in their immediate microhabitat. To explore whether algal chemical defenses have co-evolved within their distinct microenvironments, we evaluated the inhibitory activities of algal extracts from twenty-four common algae in Florida seagrass beds against a panel of diverse marine bacteria. The panel of bacteria included strains isolated from the surface of the algae and from other aquatic and marine locales, including North Atlantic Ocean, Red Sea, and South Pacific Ocean, Iceland and the Great Lakes. Algal samples were placed in sterile seawater and sonicated for 5 minutes. Pieces of algae and seawater were plated separately on nutrient rich A1 or M1 plates. Sediment samples were diluted using sterile DI water, heat shocked at 37° C for 7 minutes, and added directly to nutrient-rich and -limited agar plates and allowed to incubate for 3-5 weeks. Individual colonies were isolated using nutrient rich freshwaterbased-A1 media. We analyzed the pattern of growth inhibition or promotion of algal extracts against co-isolated strains and those from other environments, and found that activity was not patterned on target strain phylogeny, but rather to isolation origin. Non-Floridian strains tended to either strong growth inhibition or promotion in response to many algal extracts, whereas co-isolated strains exhibited more nuanced patterns of activity, suggesting specific bacterial-algal interactions.

3017

TLC-BIOAUTOGRAPHY LINKED WITH GUESS: A TRUELY TARGETED ACTIVE COMPOUND ISOLATION PROCESS

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*[1]Inst. for TB Research, [2]Dept. Med. Chem. & Pharmacognosy, COP, UIC, Chicago, IL, 60612, [3]Center for Neutraceutical & Pharmaceutical Materials, [4]Div. of Biosciences & Bioinformatics, Col. Nat. Sci., Myongji University, Gyeonggi-Do 449-728, Republic of Korea *Equal contributors*

In order to optimize the isolation procedure of anti-TB lead compounds, a truly targeted isolation approach was developed, which aligns (HP)TLC-bioautography with GUESS[1] based countercurrent separation (CCS). (HP) TLC-bioautography was specifically developed for *Mycobacterium tuberculosis*, using the mc^2 7000 lux ABCDE strain, while the GUESS method is used to rapidly determine the optimal CCS solvent system. Optimal CCS conditions target the separation of the inhibition zones within a range of optimal CCS partition coefficients referred to as the "CCS sweet spot", with a corresponding TLC R*f*-value ~0.5. Hereby, the CCS solvent system is chosen on the basis of the R*f*-value of the bioactive component(s) rather than the fraction as a whole. This approach accelerates the isolation process and prevents the isolation of non-active metabolites and byproducts. The new TLC-bioautography-GUESS-CCS workflow is demonstrated with the fractionation of an extract from an anti-TB active actinomycete strain. [Funding: NIH R21 AI093919-02]

[1]Friesen & Pauli, G.U.E.S.S. - A Generally Useful Estimate of Solvent Systems for CCC. *Journal of Liquid Chromatography & Related Technologies*. 2005, Volume 28, Issue17, 2777-2806

3018

LIGNANS FROM SAMBUCUS WILLIASMII HANCE AGAINST OSTEOPOROSIS: A PHARMACODYNAMIC AND PHARMACOKINETIC STUDY

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There are approximately 20 families in the *Sambucus* genus, distributed widely in temperate and subtropical areas in the world. As part of Traditional Chinese Medicine, *Sambucus williamsii* Hance (SWH) has been used for the treatment of bone and joint diseases for thousands of years. Use of the plant was first recorded in *Tang Materia Medica*, completed during the Tang Dynasty (618 -907 A. D.) and also described in the *New Materia Medica*, a book for herbal clinical use, whereby SWH was distributed to joints, and used to reunite fractured tendons and bones. Although SWH has a long history of clinical use, its chemical components were not fully characterized until recently.

We have shown the major phytochemicals in SWH are lignans, including several new compounds. This lignan-rich fraction obtained from SWH,

exerts protective effects against bone loss induced by ovariectomy in aged rats. Eighty five compounds to date have been isolated and several bioactive lignans have been identified exhibiting *in vitro* and exert oestrogen-like effects on osteoblasts. In our continuing study on SWH we undertook an oral PK study of SWH which exhibited a profile with very rapid absorption and fast elimination in blood. These PD and PK studies, the identification of its components and possible mechanism will be presented.

3019

THE DEPIGMENTING EFFECT AND MECHANISM S OF KUWANON O AND SANGGENON T FROM THE ROOTS OF *MORUS AUSTRALIS*

Mingfu Wang, Shuting Hu
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Kuwanon O and sanggenon T, two resorcinol-type polyphenols from the root extract of *Morus australis,* were found to reveal significant depigmenting effects in both murine b16f10 and melan-a cell lines. But their depigmenting mechanisms are slightly different in two cell systems. In b16 cells, kuwanon O and sanggenon T, together with other two phenolics, induced post-transcriptional degradations of MITF without suppressing its mRNA expression, leading to significant decreases of TRP-1 and TRP-2 production. While in melan-a cells, the levels of tyrosinase families were suppressed via MITF downregulation at both transcription and translation level by them, with kuwanon O inducing the greatest suppression. Further evaluations in artificial skin model demonstrated the outstanding depigmenting effects of kuwanon O and sanggenon T. Meanwhile, according to the structure-activity relationship study of theses compounds, to screen or synthesize resorcinol flavonone derivatives with isoprenyl group in the Diels-Alder substituent might be a new direction for the search of potent hypopigmenting agents

3020

ISOLATION OF BIOACTIVE CHEMICAL CONSTITUENTS FROM *JUSTICIA PROCUMBENS*

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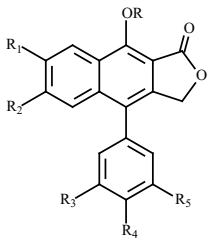
School of Chinese Medicine, Hong Kong Baptist University, Hong Kong SAR, China.

Justicia procumbens L. (Acanthaceae) is a traditional herb used in Chinese medicine (Juechuang) for the treatment of fever, pain, diarrhea and hepatitis. The methanol extract of the plant displayed potent anti-HIV activity in our study. In order to identify the bioactive compounds of the plant, we re-collected the plant materials from Fujian province, China. Phytochemical investigation of the methanol extract led to the isolation of a number of aryl naphthalide lignans containing sugar units. Further LC-MS analysis revealed that abundant number of aryl naphthalide lignans were present in the extract and the sub-fractions of the plant. Aryl naphthalide lignans have been reported to have plenty of biological activities including antiviral and anticancer activities. We thus look forward to isolating many different lignans of this type and conducting their biological evaluation against different disease targets including cancers and other viral diseases. [The study was supported by grants from the RGC of the Hong Kong SAR, China (HKBU 262912), HKBU Interdisciplinary Research Matching Scheme (RC-IRMS/12-13/03), and Mr. Kwok Yat Wai and Madam Kwok Chung Bo Fun Graduate School Development Fund].



Aryl Naphthalide Lignans (R = sugar)

3021

TURNING A NEGATIVE INTO A POSITIVE: USING BACTERIAL SELF-RESISTANCE TO IDENTIFY NEW NATURAL PRODUCTS.

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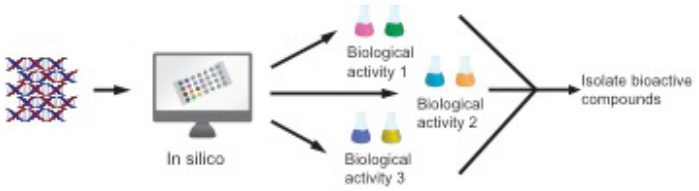
Antibiotic resistance in microbial pathogens is poised to become a problem throughout the world. This necessitates the discovery of new compounds and drug leads. Genome sequencing has revealed that microbial genomes harbor biosynthetic capacity greatly in excess of known compounds for a particular strain. Due to the enormous amount of sequencing data available new ways of identifying gene clusters and compounds of interest are required. Microorganisms that produce biologically active compounds must be resistant to their action or else gain no selective advantage in producing them. These resistance genes co-localize with genes responsible for biosynthesis in biosynthetic gene clusters (BGCs). We have developed a computer program that seeks to identify interesting compounds by their biological mechanism of action called ISMAS (*In silico* Mechanism of Action Screening). ISMAS essentially turns a computer into a high-throughput assay searching for compounds with a particular mode of action and has the advantage of being able to identify natural products from cryptic gene clusters as well as compounds for which an *in vitro* bioassay is not available. Here we present the potential of coupling ISMAS with heterologous expression for the discovery of new natural products.



3022

A DESIGNER LICORICE EXTRACT: SELECTIVE DEPLETION OF GLABRIDIN AND CONGENERIC METABOLITES BY COUNTERCURRENT SEPARATION

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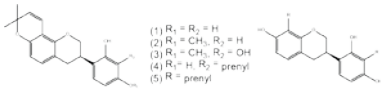
The present study explores **C**ountercurrent **S**eparation (CCS) as a powerful method to **D**eplete and **E**nrich **S**elect **I**ngredients to **G**enerate **N**ormalized **E**xtract **R**esources. A DESIGNER extract of European licorice (*Glycyrrhiza glabra* L.) depleted of glabridin **(1)** and four congeneric metabolites **(2-5)** was prepared for biological testing. **S**olvent **s**ystem (SS) selection was based on partition coefficient (*K* value) determination measured by UV following two-phase distribution. The SS composed of Hexanes/Ethyl Acetate/Methanol/Water 7:5:6:4, *v/v*, was used with the lower phase mobile on a **H**igh **S**peed **C**ounter**c**urrent **C**hromatography (HSCCC) apparatus for the fractionation of *G. glabra* crude extract. An orthogonal CCS step was developed for the continued purification of each compound. The purity and identity of each compound was determined by qHNMR. All other extract resources were recombined to build up the DESIGNER extract. The results obtained highlight that CS enables the si-



multaneous and targeted enrichment and depletion of structurally related metabolites.

This research was supported by NIH Grants P50 AT000155 and T32 5T32AT007533

3023

TWO NEW CYTOTOXIC MEROTERPENOIDS PRODUCED BY THE MARINE SEDIMENT-DERIVED *STREPTOMYCES SP. CP26-58*

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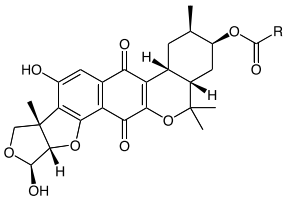
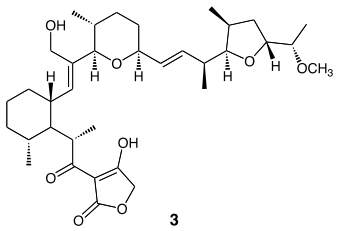
A chemical library containing 474 organic extracts has been applied to an image-based anticancer screening using HeLa cells. This screening identified 41 strains that showed potent cytotoxic effects against HeLa cells. Based on the bioassay-guided fractionation, two new meroterpenoids (**1** and **2**) were isolated from one of the active strains, *Streptomyces* sp. CP26-58, together with the known tetronasin (**3**). The structures of the two new compounds possessing an unprecedented six-fused ring system were firmly assembled by comprehensive 1D and 2D NMR analysis. In addition, their absolute configurations were deduced based on *ab intio* electronic circular dichroism (ECD) spectral calculations.



1: R = -CH(CH$_3$)CH$_2$CH$_3$
2: R = -CH(CH$_3$)$_2$



3

3024

EVALUATION OF THE ANTI-DIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF METHANOL EXTRACT OF *STROPHANTHUS HISPIDUS* ROOTS D.C. (APOCYNACEAE)

Odoh Uchenna E.[1], Ezugwu Christopher O. [1] Ugwuoke, Christopher. E. C., Ezea Sampson C. and Onu Samuel
[1]Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

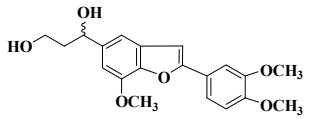
The study is to establish the anti-diabetic effect of the methanol root extract of *Strophanthus hispidus* and to examine the effect of these extracts on the lipid profile of alloxan-induced diabetic rats. The acute toxicity test was carried out using standard methods. Diabetic mellitus was induced in 25 rats by single intraperitoneal injection of alloxan (150 mg/kg body weight). Two days after induction, the hyperglycaemic (>200 mg/dl) rats were treated orally with different doses (200, 400 and 800 mg/kg) of the extract for 14days, glibenclamide (5 mg/kg) was used as the standard drug. The lipid profile was evaluated in diabetic treated groups compared with control. Similar study as above was carried out on normal rats. The extracts were found to be relatively safe. The extract at a dose of 800 mg/kg showed the most significant ($P < 0.05$) reduction (69.43 %) in fasting blood glucose level in the diabetic rats; for normal rats,, it was 41.92 %. The extract at doses of 200 and 400 mg/kg also showed reduction (64.77 and67.75 %) respectively in diabetic rats; (27.5 and 34.2 %) respectively in normal rats. The extract showed a dose- independent decrease in the lipid profile examined at the end of the 14 day period. Phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, reducing sugars, terpenoids, Saponins, proteins, resins, steroids, glycosides and tannins. It can be concluded that the methanol root extract of *S. hispidus* possesses anti-diabetic and hypolipidemic properties on alloxan-induced diabetic rats.

3025

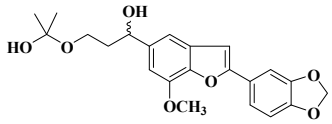
INHIBITORY EFFECT ON NO PRODUCTION OF COMPOUNDS FROM *STYRAX OBASSIA*

Byung Sun Min[1], Thao Quyen Cao[1], Tran Manh Hung[1], and Jeong Ah Kim [2]
[1]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 712-702, Republic of Korea, [2] College of Pharmacy, Kyungpook National University, Daegu 702-701, Korea.

Two new benzofurans, 2-(3,4-dimethoxyphenyl)-5-(1,3-dihydroxypropyl)-7-methoxybenzofuran (**1**), and 2-(3,4-methylenedioxyphenyl)-5-(3-hydroxymethyletoxy-1-hydroxypropyl)-7-methoxybenzofuran (**2**) along with eleven known compounds as syringaresinol (**3**), lariciresinol (**4**), pinoresinol (**5**), *epi*-pinoresinol (**6**), 2-(3,4-methylenedioxyphenyl)-5-(1,3-dihydroxypropyl)-7-methoxybenzofuran (**7**), egonol (**8**), homoegonol (**9**), salicifoliol (**10**), machicendonal (**11**), homoegonol glucoside (**12**), and 3β-acetoxyolean-12-en-28-aldehyde (**13**) were isolated from stem bark of *Styrax obassia*. Their chemical structures have been identified mainly by means of spectroscopic analysis. Their anti-inflammatory activity was evaluated against LPS-induced NO production in macrophage RAW264.7 cells.



(**1**)



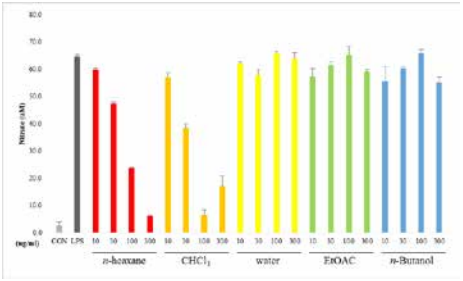
(**2**)

3026

STUDY ON THE ANTI-INFLAMMATORY ACTIVITY FROM *ZIZYPHUS JUJUBA* MILL

Bo Mi Lee[1], Joo-Sang Lee[1], Jeong Ah Kim[2] and Byung Sun Min[1]
[1]College of Pharmacy, Drug Research and Development Center, Catholic University of Daegu, Gyeongbuk 712-702, Republic of Korea, [2]College of Phamacy, Kyungpook National Universiy, Daegu, 702-701, Republic of Korea

In search for anti-inflammatory agents from natural sources, we found that n-hexane and $CHCl_3$ soluble fraction of the methanol extract of the fruits of *Zizyphus jujuba* showed potent inhibitory activity against LPS-induced nitric oxide in RAW264.7 cells. In the experiment, *n*-hexane and $CHCl_3$-soluble fractions were approximately 2.5-fold more potent than EtOAc, BuOH and aqueous soluble fractions at the test concentrations (0~30 μg/mL). Therefore, the two solvent fractions were selected for the further isolation of the active constituents. All the isolated compounds and their inhibitory activities against LPS-induced NO production in the murine macrophage cells will be presented.

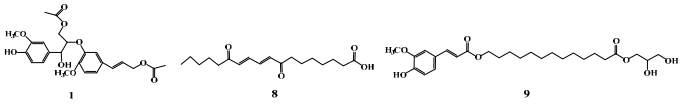


3027

ECHINOCHLORINS A~C FROM THE GRAINS OF *ECHINOCHLOA UTILIS* (BARNYARD MILLET) AND THEIR ANTI-INFLAMMATORY ACTIVITY

Mi Hee Woo[1], Duc Hung Nguyen[1], Bing Tian Zhao[1], U Min Seo[1], Thi Trang Nguyen[1], Do Youn Jun[2], and Young Ho Kim[2]
[1]College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea, [2]Laboratory of Immunobiology, School of Life Science and Biotechnology, College of Natural Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea

Three new compounds (echinochlorins A-C; **1**, **8**, and **9**) together with eight known compounds, isolated form the methylene chloride and ethyl acetate fractions of grains of *Echinochloa utilis*, were examined with regard to their effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells. The results indicated that compounds **3**, **5**, and **9** possessed potent NO inhibitory activity against lipopolysaccharide (LPS)-induced NO release with IC_{50} values of 36.04, 4.80, and 20.78 μM, respectively. In summary, the isolates compounds showed novel biological activity of inhibition in NO production, suggesting that *E. utilis* may be applied as supplemental functional foods having a beneficial effect against inflammation.



3028

EXTRACTION AND ISOLATION OF 6-GINGEROL FROM GINGER

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[1]Department of Applied Chemistry, College of Science, Xi´an University of Technology, Xi´an 710054, China

Ginger (*Zingiber officinale*) is one of the most commonly used as spice food and dietary supplement and has become an important ingredient in Ayurvedic and Chinese herbal medicines for the treatment of various diseases and disorders such as colds, nervous system disorders, asthma, gingival infections, toothache, paralysis, constipation, diabetes and rheumatism. Previous chemical investigations have revealed that the major chemical constituents of this plant are 6-gingerol and its derivatives which are responsible for antioxidant and anti-inflammatory activities. In this paper, the optimum isolation conditions for 6-gingerol were obtained with the 3.61% yields by 80% ethanol for 120 min at 50 °C and the materal-to-liquid ratio of 1:20. The 6-gingerol was analyzed by gas chromatographic, as well as by comparison the retention times of the standard samples.

3029

ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL EVALUATION OF NOVEL CATHEPSIN INHIBITORS FROM MARINE CYANOBACTERIA

Fatma H. Al-Awadhi[†], Valerie Paul[§] and Hendrik Luesch[†,‡]
[†]Department of Medicinal Chemistry, [‡]Center for Natural Products, Drug Discovery and Development (CNPD3), University of Florida, Gainesville. [§]Smithsonian Marine Station, Fort Pierce, Florida, USA.

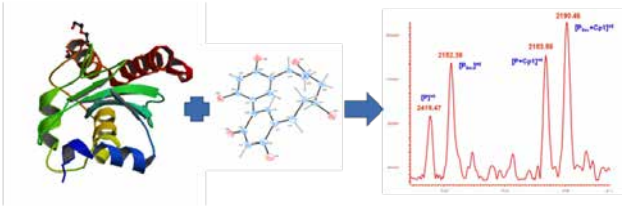
In our continuous search for novel bioactive compounds from marine cyanobacteria, we isolated three novel modified peptides named grassytatins D-F. Their structures were elucidated using a combination of both NMR spectroscopy and mass spectrometry (MS). The hallmark structural feature in those peptides is the presence of statin unit, which led us to suspect that these compounds are aspartic protease inhibitors. The protease inhibitory activity was assessed *in vitro* using cathepsin D and E enzymes. Grassystatins D-F showed a potent cathepsin E inhibitory activity with IC_{50} values of 38, 5, 0.5 nM respectively. Those peptides also inhibited cathepsin D but were less potent compared to their inhibitory activity against cathepsin E. Grassystatins D-F inhibited cathepsin D with IC_{50} values of 1.9, 0.9 μM (Grassystatin D and E), and 50 nM (Grassystatin F).

3030

CYCLIC DIARYLHEPTANOIDS FROM THE AUSTRALIAN ENDEMIC PLANT ALLOCASUARINA LUEHMANNII

Bao N. Nguyen[1], Ngoc B. Pham[1], Peter Healy[2], Hoan T. Vu[1], and Ronald J. Quinn[1]
[1]Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia, [2]School of Natural Sciences, Griffith University, Brisbane, QLD 4111, Australia

We report the isolation of new cylic diarylheptanoids that bind to *Plasmodium falciparum* ubiquitin conjugating enzyme PF10_0330. *Pf* ubiquitin conjugating enzyme PF10_0330 is a potential target for anti-malarial chemotherapy. The X-ray of one of these compounds allowed the absolute configuration to be established. Binding to the *Pf* ubiquitin conjugating enzyme PF10_0330 was demonstrated using bio-affinity mass spectrometry.



3031

DISCOVERY PLATFORM FOR POTENT ADC PAYLOADS FROM MARINE SOURCES

Venkat Macherla, Egle Pociute, Guillermo Cardenas, Paige Stout, Tamara Mayer, Jacob Beverage, Eduardo Esquenazi
Sirenas Marine Discovery, 3550 General Atomics Ct, San Diego, CA 92121

Marine organisms have emerged as an important source of biologically active small molecules, particularly for highly potent cytotoxins. Because of this potent bioactivity, the traditional small molecule, single agent drug discovery approach is severely hampered by a dose-limiting therapeutic window. However, the recent success of Antibody Drug Conjugates (ADC), which offers the targeted delivery of cytotoxins to tumor cells via antibody-binding, provides a renewed path for promising, potent, anti-cancer compounds. ADC's and similar targeting approaches are truly an enabling technology for natural products. In our ongoing drug discovery program, we have developed a unique ADC payload discovery platform which allows a rapid identification of marine-derived cytotoxic payloads. Using this technology, we have discovered potent ADC payloads. The discovery platform and initial results will be discussed.

3032

DESCRIPTION OF BACTERIAL COMMUNITIES IN TWO CARIBBEAN OCTOCORALS AND ASSESSMENT OF THEIR ABILITY TO PRODUCE NATURAL PRODUCTS

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[1]Department of Biomedical Sciences, [2]Department of Chemistry, University of Prince Edward Island, 550 University Ave., Charlottetown, PE C1A 4P3

The octocorals *Antillogorgia elisabethae* and *Erythropodium caribaeorum* are common members of reef communities in the Caribbean and have been shown to contain the bioactive terpenes pseudopterosins and eleutherobin,

respectively. The core microbiome of these octocorals has been identified through the use of culture dependent and culture independent bacterial libraries. The culture independent libraries revel that the genus *Endozoicomonas* is a member of the core microbiome for both octocorals. In addition, their presence in the larvae on *A. elisabethae* suggests that *Endozoicomonas* spp. may be transmitted vertically from parent to offspring. The culture dependent library revealed great taxonomic diversity and led to the identification of a novel bacterium *Pseudobacteriovorax antillogorgiicola,* which was determined to be a member of the novel family *Pseudobacteriovoracaceae*. This bacterium, as well as the others present in culture dependent library were fermented and analyzed using a High Performance Liquid Chromatograph-High Resolution Mass Spectrometer (HPLC-HRMS). The mass and retention time of ions detected by the HPLC-HRMS were coupled with principal component analysis to allow for identification of novel ions in the bacterial fermentations.

3033

ETHNOMEDICINAL PLANTS INVESTIGATION IN HEZHEN NATIONALITY

*Yue Liu[1, 2], Yuanxia Jing[1], Shuixian Zhang[1], Hongbo Sun[1], Li Tang[1], Jinchao Feng[1], Chunlin Long[1], Luqi Huang[2]**

[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

The Hezhen is one of the smallest minorities in China. With a population of 5,354 in 2010, the Hezhen people mainly live in five settlements including Aoqi in Jiamusi, Jiejinkou and Bacha in Tongjiang, Zhuaji in Fuyuan and Sipai in Raohe counties along the rivers of Songhua, Heilongjiang and Wusuli in the northeast of Heilongjiang Province in China. The Hezhen have fished and hunted for generations. Because the Hezhen had little genetic communication with other nationalities in history, Hezhen people have kept their own traditional knowledge for living healthy, especially for the fishing and hunting related illness, such as rheumatism, arthritis, etc. Fifty-four species including medicinal uses, parts and purposes were catalogued based on the ethnobotanical investigation of medicinal plants in five settlements of Hezhen people. These species are belonging to thirty four families, fifty two genera and involved in seventeen different medicinal purposes. The medicinal uses of fourteen species are unique in Hezhen people compared with the Mongolia, Ewenki, and Oroqen nationality. This research explored and recorded the traditional medicinal knowledge of the Hezhen, and the result may be preparing the ground for the development of ethnopharmacology of the Hezhen. Acknowledgment: financial support by project of NSFC-81274158, 81373765, NCET-12-0578, 13-0624, "111"-B08044 and YLDX01013.

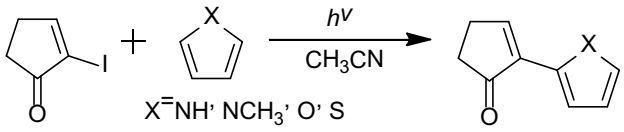
3034

ARYLATION OF α,β-UNSATURATED KETONES *VIA* PHOTOCHEMICAL REACTION

Qian Yang[1], Jie Han,[2] Zun-Ting Zhang[2], Yang-Qing He[1]

[1]Department of Applied Chemistry, Xi'an University of Technology, Xi'an, Shaanxi 710054, China, [2]Key Laboratory of the Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry, and School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, Shaanxi 710062, China

Development of efficient and reliable method to form a C-C bond between heteroaromatics and carbon α to carbonyl group has been a subject of interest in organic chemistry. The use of photochemical reaction to approach the arylation of enones has proven to be a transformation of great unity in pharmaceutical and organic synthesis. In this study, the direct coupling of α-idio substituted unsaturated ketones with heteroaromatics has been achieved *via* photochemical reaction. A variety of α-heteroarylunsaturated ketones were obtained in moderate yields from the corresponding α-idio substituted unsaturated ketones with heteroaromatics such as pyrrole, furan, thiophene. The reaction worked smoothly in acetonitrile under a mercury lampwithout any additives, which provides a catalyst and base-free approach for the arylation of α,β-unsaturated ketones.

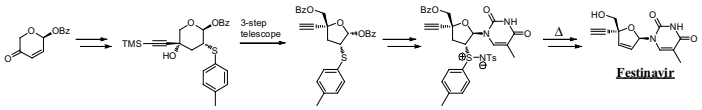


3035

AN IMPURITY INVESTIGATION IN A NEW PROCESS FOR THE SYNTHESIS OF FESTINAVIR

Sloan Ayers[1], Adrian Ortiz[2], Zhongping Shi[2], Tamas Benkovics[2], and Greg Beutner[2]

[1]Analytical and Bioanalytical Development, Bristol-Myers Squibb Co., New Brunswick, NJ 08903, USA, [2]Chemical Development, Bristol-Myers Squibb Co., New Brunswick, NJ 08903, USA.



Festinavir is a synthetic analog of the natural nucleoside thymidine, and is currently under development at Bristol-Myers Squibb (BMS) for the treatment of HIV. A new process was developed at BMS for the synthesis of festinavir, including innovative steps such as a pyranose to furanose ring-chain tautomerization, and an oxidation/thermal elimination step involving an intermediate sulfilimine. Three impurities that formed in the thermal elimination step proved to be troublesome, as they were difficult to purge from the penultimate intermediate, and their resulting daughter impurities were difficult to purge from the final product. These impurities also decreased the yield of the desired products by a significant degree. Elucidating the structures of these impurities was paramount to determining how to adjust the synthetic conditions to minimize their formation. The isolation and structural elucidation of these impurities will be presented, as well as the resulting process changes.

3036

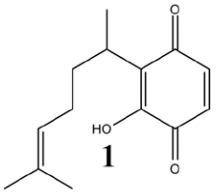
HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITIES OF AN AQUEOUS EXTRACT AND COMPOUNDS FROM *Acourtia thurberi* ROOTS.

Ana Laura Martínez[1], Abraham Madariaga-Mazon[1], Edelmira Linares,[2] Robert Bye[2] and Rachel Mata[1]

[1]Facultad de Química, and [2]Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, 04510, Mexico.

Acourtia thurberi (Asteraceae) is a medicinal plant commonly used for treating diabetes in Mexico. Therefore the present study was undertaken to assess the antidiabetic potential of an infusion (Ati) and compounds from the plant using well known animal and *in vitro* models. In addition, the potential acute toxicity of the infusion and perezone (1), the major component of the plant, was analyzed using the Lorke method. The results indicated that oral administration of Ati (31.6-316.2 mg/kg) or 1 (31.6 mg/kg) decreased postprandial glucose levels in mice during an oral glucose tolerance test. On the other hand, Ati inhibited yeast α-glucosidase in a concentration-dependent manner with an IC_{50} value of 1804.8 ppm. Regarding the toxicity studies, Ati can be considered as non-toxic in the Lorke

model since the calculated LD_{50} was higher than 5000 mg/kg; on the other hand, the LD_{50} for 1 was 1264.9 mg/kg. Altogether, these preliminary results have a tendency to support the alleged antidiabetic properties of *Acourtia thurberi* in Mexican folk medicine.

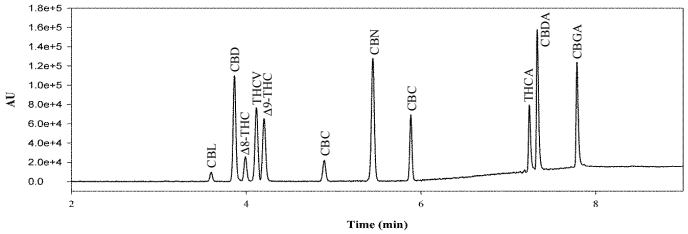


3037

SUPERCRITICAL FLUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF CANNABINOIDS IN EXTRACTS OF CANNABIS SAMPLES

Mei Wang[1], Yan-Hong Wang[1], Bharathi Avula[1], John van Antwerp[3], Jon F. Parcher[1], Mahmoud A. ElSohly[1,2] and Ikhlas A. Khan[1,2]
[1]National Center for Natural Products Research, and [2]Division of Pharmacognosy, Department of BioMolecular Science, School of Pharamacy, University of Mississippi, University, MS 38677, USA, [3]Waters Corporation, Milford, MA 01757, USA

A rapid method is developed for the analysis of cannabinoids in extracts of cannabis samples using supercritical fluid chromatography (SFC) coupled to UV and electrospray ionization-mass spectroscopic (ESI-MS) detection. Eleven cannabinoids, *viz.* CBL, CBD, Δ8-THC, THCV, Δ9-THC, CBC, CBN, CBG, THCA-A, CBDA and CBGA, were well separated in an 8 min run. The developed SFC method was validated according to ICH guidelines and used to quantitate these cannabinoids in 29 different *Cannabis sativa* plant samples. The quantitation results were verified by comparison with a standard UPLC method. The described method offers an alternative means for identification and quantification of cannabinoids in *Cannabis* plants and products. In addition, conditions for decarboxylation of the acid forms of the major cannabinoids were examined at 80 °C, 95 °C, 110 °C, 130 °C and 145 °C with different time intervals in order to select the most appropriate condition for complete decarboxylation.



3038

MODELING CELLULAR RESPONSES IN NON-SMALL CELL LUNG CANCER USING MARINE DERIVED NATURAL PRODUCTS

Oswald NW, McMillan EA, Posner BA, White MA, MacMillan JB
Department of Biochemistry, UT Southwestern Medical Center at Dallas

Using a group of well annotated non-small cell lung cancer (NSCLC) cell lines (mutation status, gene expression profiling, siRNA profiling) selective and non-selective cytotoxic natural products can be identified based upon data of the sensitive cell lines. We have focused our efforts on discovery of natural products from marine-derived bacteria through the screening of a collection of ~6500 natural product fractions. A screen of the 6500 natural product fractions against 27 non-small cell lung cancer cell lines has revealed a number of promising fractions. Target identification predictions can be made based upon cell line data of sensitive and non-sensitive cell lines, thereby allowing for further prioritization of natural product fractions. LCMS and NMR data is combined with the bioinformatics and screening data to allow for better prioritization of natural product fraction leads. The lead fractions are further analyzed to identify known and novel compounds of interest. Biological activities are then followed up with further cytoxicity and mechanistic studies of the natural product against NSCLC cell lines. Representative biomarkers of both resistant and sensitive NSCLC cell lines are also identified. This has led to a number of insights into the sensitivities of cancers based upon their mutation status.

3039

PEROXISOME PROLIFERATOR ACTIVATOR RECEPTOR ALPHA (PPARA) ACTIVATION USING A FREE FATTY ACID LIBRARY; FATTY ACIDS COMPARED.

John F. Rebhun[1], Rodney A. Velliquette[1], Samantha J. Roloff[1], Kelly M. Glynn[1], Jat Rana[1], Kevin W. Gellenbeck[2] and Jeffrey D. Scholten[1]
[1]Analytical Science Department, Amway Corporation, 7575 Fulton St. East, Ada, MI 49355 USA, [2]Nutrilite Division of Amway, Concentrate Development, 5600 Beach Blvd, Buena Park, CA 90622 USA

Peroxisome proliferator activated receptor alpha (PPARA) is a nuclear hormone receptor, of particular interest for its impact on a number of health and wellness issues, including the circulatory system, wound healing, skin inflammatory issues and barrier function. To assess direct and limited indirect PPARA activation, a reporter assay was developed using a Gal4-PPARA Ligand Binding Domain (LBD) fusion construct and the upstream activating sequence (UAS) controlling luciferase production.

Here we describe the evaluation of a compound library containing 68 different free fatty acids, many of them are of current interest in the food and nutrition industry. Their activation of PPARA was evaluated using the Gal4-UAS PPARA reporter assay above. Many of them activated PPARA with some limitations on the size and saturation status of the individual compounds. Oleic acid and linoleic acid were two activating molecules that were selected for further analysis and evaluated side by side. They activated PPARA and this activation was blocked by a known PPARA inhibitor, GW6471 at 10 uM. Activation of specific genes involved with keratinocyte differentiation, wound repair and barrier function were evaluated and discussed.

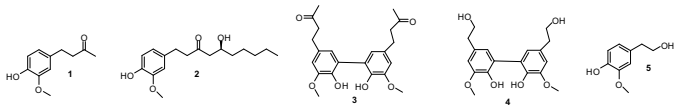
3040

MICROBIAL TRANSFORMATION OF GINGER ISOLATED COMPOUNDS

Roberta Marques Dias de Ávila, João Batista Fernandes, Maria Fátima das Graças Fernandes da Silva, Paulo Cezar Vieira
Departamento of Chemistry, Federal University of São Carlos, São Carlos, SP, CEP 13565-905, Brazil.

Ginger or ginger root, the rhizome of the plant *Zingiber officinale*, has been consumed as a delicacy, medicine, or spice for many years. A number of studies have been conducted on the medicinal properties of ginger against various disorders. Gingerols are among the bioactive compounds isolated from ginger. In order to check for the possibility of obtaining new gingerol derived compounds we have carried out microbial transformation of several gingerols using the fungus *Colletotrichum gloeosporioides*. Cultivation of the fungus in different culture media and having zingerone (**1**) and [6]-gingerol (**2**) as starting material for the biotransformation, dimer derivatives such as **3** and **4** originated from oxidative coupling were isolated. [6]-gingerol was transformed into **5** a product that can be obtained from Baeyer-

Villiger type reaction followed by hydrolysis. Isotope labelling proved that compound **3** is a product from [6]-gingerol biotransformation.



3041

BIOLOGICAL ACTIVITIES OF TRITERPENES ISOLATED FROM THE MEDICINAL MUSHROOM *GANODERMA LUCIDUM*.

Daniel Sliva[1,2,3], Jagadish Loganathan[3], Marta Dudek[4], Michal Glensk[5] and Vitold B. Glinski[5]
[1]DSTest Laboratories, Purdue Research Park, 5225 Exploration Dr., Indianapolis, IN 46241, [2]Indiana University School of Medicine, Indianapolis, IN 46202, USA, [3]Indiana University Health, Indianapolis, IN 46202, [4]Medical University of Warsaw, Dept. of Physical Chemistry, Warsaw, Poland, [5]Planta Analytica, 39 Rose St., Danbury, CT 06810.

Methanol extract of *Ganoderma lucidum* subjected to fractionation on silica gel medium pressure column afforded over 40 fractions. Further fractionation was carried out using Centrifugal Partition Chromatography (FCPC Rousselet Robatel, France), in the hexane-EtOAc-MeOH-Water systems adjusted for the polarity of each fraction. Some isolates were further purified by preparative HPLC. Methyl esters of ganoderic and lucidenic acids were obtained by methylation of the acids. Twelve compounds, including ganoderic acid A (**1**), methyl ganoderate A (**2**), methyl lucidenate A (**3**), methyl lucidenate F (**4**), lucidenic acid D2 (**5**), lucidenic acid A (**6**), lucidenic acid N (**7**), ganoderic acid DM (**8**), ganoderic acid F (**9**), ganoderic acid C (**10**), ganoderiol F (**11**) and ganodermonondiol (**12**) were evaluated for their biological activities. Antibacterial activity was determined against Gram -positive and -negative bacteria, anti-inflammatory effect against endotoxin (lipolysaccharide, LPS) induced inflammatory response in human macrophages, and anti-proliferative effects in human breast and colon cancer cells. We identified ganoderic acid DM as the most potent triterpene with anti-bacterial, anti-inflammatory and anti-proliferative effects when compared to other Ganoderma isolates.

3042

ANTIHYPERALGESIC ACTIVITY OF AN EXTRACT AND COMPOUNDS FROM *ANODA CRISTATA*

Krutzkaya Juárez-Reyes[1], Myrna Déciga-Campos[2] and Rachel Mata[1].
[1]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico, [2]Escuela Superior de Medicina del IPN, Mexico City, Mexico.

Anoda cristata (Malvaceae) is used as food and to treat stomach complaints, fever, cough, wounds and diabetes. In a previous work, the antidiabetic action of a free mucilage aqueous extract (FM-AE) and major compounds [acacetin (**1**) and diosmetin (**2**)] from the plant was reported. This work was undertaken to establish the potential antihyperalgesic action of *A. cristata* using the formalin test in hyperglycemic (NA/STZ: 50/ 130 mg/kg, i.p.) mice. In all cases the treatments were administered subcutaneously in the dorsal surface of the right hind paw. FMAE (10-100 µg/paw), **1** (1-7.5 µg/paw) and **2** (0.1-6 µg/paw), induced significant decrease in licking time. In order to determine the mode of action of **1** and **2,** the animals were pretreated with naloxone (3 µg/paw), flumazenil (6 µg/paw), ketanserin (3 µg/paw), L-NAME (150 µg/paw), ODQ (75 µg/paw) or glibenclamide (3 µg/paw). The effect of **1** was abolished with naloxone and flumazenil, revealing its opioid and GABA mechanisms of action. However, pretreatment with ketanserin was the only one that antagonized the effect of **2**, consistent a serotonergic pathway mediating its pharmacological action. The antihyperalgesic effect of FMAE, **1** and **2** could be of benefit in diabetic patients with neuropathic complications.

3043

TRP CHANNEL ANTAGONISTS FROM A NOVEL TUNICATE-ASSOCIATED FUNGUS

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We have aimed to examine cultivable associates of ascidians as sources of bioactive natural products. A novel filamentous fungus, Eurotiomycetales strain 110162, was identified from the ascidian *Diplosoma* sp. collected in Papua New Guinea (10.0370785 S 145.767741 E). Strain analysis by 18S rRNA, RNA poly-merase II (RPB2) and internal transcribed spacer (ITS) gene sequences indicated that strain 110162 represents a member of the Class Eurotiomycetes, and the strain may represent a novel order within this class. From this novel filamentous fungus, a series of prenylated polyketide metabolites were isolated and showed selective TRPM8 antagonist activity.

3044

ANTIFUNGAL AND ANTIPARASITIC ACTIVITIES OF *LAURUS NOBILIS* (BAY LEAVES)

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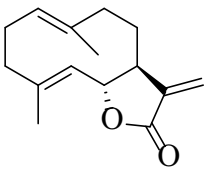
In the course our search for safe antimicrobial agents from natural sources, an extract of leaves and twigs of *Laurus nobilis (LN)*, commonly known as bay leaves, showed activity against *Cryptococcus neoformans* and *Leishmania donovani*. Activity guided fractionation of the extract led to isolation of costunolide as the active compound. Costunolide showed moderate activities against *C. neoformans* (IC_{50} 5.15 µg/mL) and *L. donavani* promastigotes (IC_{50} 4.04 µg/mL) and amastigotes (IC_{50} 1.85 µg/mL). This compound also exhibited good activity against *Trypanosoma brucei* (IC_{50} 1.64 µg/mL). Costunolide has previously been isolated from *L. nobilis* and several other species and a number of other biological activities have been reported for this compound.



Costunolide

3045

SINGLE SHOT DECONVOLUTION OF THE SYMBIOTIC *BUGULA NERITINA* METAGENOME

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With the increasing availability of deeply sequenced data sets, it has become clear that the biosynthetic potential of uncultured microbes, which are believed to account for 99.9% of bacteria, remains largely untapped. Understanding the ecological roles and full biotechnological potential of these uncultivated microbes has historically been limited by our access to complete genomes. Even for systems where biosynthetic pathways have been described, such as with the bryostatin producing endosymbiont of *Bugula neritina, Candidatus* Endobugula sertula, a lack of genomic and ecological context for natural product biosynthesis limits our understanding of secondary metabolite production and its role in the environment. Thus, culture-independent approaches are becoming increasingly important in the study of natural products. Our approach presented here offers a streamlined bioinformatic technique able to unlock the diversity in a single, unenriched environmental sampling and assemble multiple bacterial genomes from the metagenome of the marine invertebrate, *Bugula neritina*. Ultimately, this method represents a powerful tool to explore unculturable bacteria and their biosynthetic potential in complex microbial systems.

3046

EUDISTAMIDES A AND B, NOVEL CYCLIC DEPSIPEPTIDES FROM A MARINE-DERIVED *STREPTOMYCES* SP.

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Addressing antibiotic resistance represents a significant challenge. Methods for rapid structure determination of bacterially produced antibiotics would help streamline discovery pipelines. Therefore, we evaluated application of biomolecular NMR strategies for rapid structure elucidation of peptide-based natural product antibiotics. Two novel peptides eudistamides A and B (**1** and **2**), were isolated from a marine *Streptomyces* sp. Both compounds **1** and **2** contain a unique 3-(2-Methylphenyl)acrylic acid moiety, which was assigned by 1D and 2D NMR experiments. The sequences of the amino residues in **1** and **2** were elucidated on the basis of 3D NMR experiments, and further confirmed by HRESIMS and 1D and 2D NMR data. The absolute configurations were determined by advanced Marfey's method after acid hydrolysis. Compound **2** displays antibacterial activities against strains of Methicillin-resistant *Staphylococcus aureus* (MRSA), *E. coli* and *Bacillus subtilis*.

3047

MARINE NATURAL PRODUCTS DRUG DISCOVERY FROM ACTINOMYCETES AT FUNDACION MEDINA

Jose R. Tormo, Daniel Oves, Rodney Lacret, Catalina Moreno, Mercedes DeLa Cruz, Caridad Díaz, Ignacio González, Jesús Martín, Ignacio Pérez-Victoria, Francisca Vicente, Fernando Reyes and Olga Genilloud

Fundación MEDINA, Avda. Conocimiento 34, 18016, PTS, Granada, Spain

Among microbial environments, marine-derived actinomycetes represent a highly diverse source for finding new NPs that remains still largely underexplored for Human Health Drug Discovery. Fundación MEDINA is devoting strong efforts in evaluating this genuine source for novel leads by collaborating with more than 20 international partners through granted projects, developing a library of NPs extracts and fractions for HTS drug discovery derived from the subset of marine actinomycetes included in our microbial collection of 116.000 strains.

Strategies followed in the generation of this Library of marine NP extracts included: geographical and taxonomic selection of strains, focused fermentation methodologies, and tailored-made semi-automated SPE extraction and fractionation approaches to overcome the special characteristics of marine-like culture media. As a result, MEDINA's Marine Natural Products Program is starting to generate new promising hits with high potential to be developed as Drug candidates. Global screening results, mainly focused on our anti-infective programs will be discussed including preliminary results on the identification of some of the new molecules derived from this Drug Discovery effort.

3048

TO DISCOVER BIOACTIVE COMPOUNDS FROM THE PLANTS IN LINGNAN REGION OF SOUTHERN ASIA

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Lingnan is a geographic region referring to "Five Ranges", which covers five Southern provinces of China and northern part of Vietnam. It is located in a tropical and sub-tropical climate zone with rich rainforests and diversified plant resources. For a decade, our research programs have investigated the chemical and biological properties of several thousands of the terrestrial plant samples in Lingnan region. We have evaluated the plant samples against cancer, parasites, fungi, bacteria and viruses (**Figure 1**). Bioassay-guided fractionation of the selected plant extracts led to the isolation of more than 300 compounds of varying degrees of structural complexity, novelty and/or biological activity (anticancer, antiviral, anti-bacterial, anti-fungal and antimalarial activities). [The work described in this paper was collaborative efforts within multi-disciplinary cooperative programs supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. HKBU 262912 and HKBU12103014), HKBU Interdisciplinary Research Matching Scheme (RC-IRMS/12-13/03), Faculty Research Grants, Hong Kong Baptist University (FRG2/14-15/047 and FRG1/13-14/029), and NIH Grants 3U01TW001015-10S1 and 2U01TW001015-11A1 (administered by the Fogarty International Center as part of an International Cooperative Biodiversity Groups program,

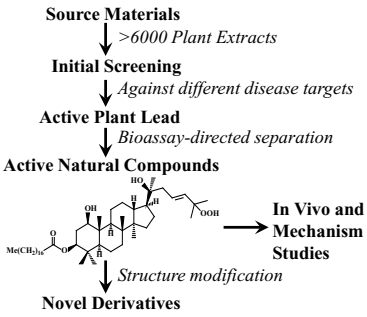

Figure 1. Strategy for discovery of bioactive lead compounds from plant source

through funds from NIH, NSF, and Foreign Agricultural Service of the USDA)].

3049

ALPHA-GLUCOSIDASE INHIBITORS FROM *Sporormiella minimoideS*

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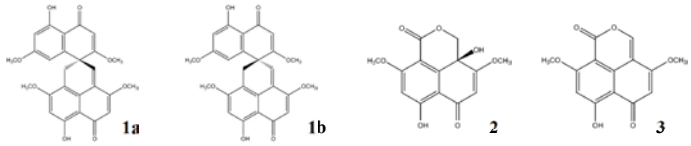
Sporormiella minimoides is an endophytic fungus obtained from *Hintonia latiflora* (Rubiaceae) leaves, a medicinal plant widely used in Mexico to treat diabetes. Bioassay- guided fractionation of the organic extract of the fungus yielded the isolation of a novel metabolite characterized as 5,7'-dihydroxy-2,4',7,9'-tetramethoxy-1'*H*, 4*H*, 6'*H*-spiro[naphthalen-1,2'-phenalen]-4,6'-dione (**1**) obtained as a racemic mixture (**1a** and **1b**). The related known compounds preussochromone C (**2**) and corymbiferone (**3**) were also isolated. Compounds **1**-**3** and acarbose (positive control) inhibited yeast α-glucosidase (IC_{50} values of 2.91, 66.5, 155.5 and 876.9 μM, respectively). Kinetic analysis revealed that **1**-**3** behaved as mixed-type inhibitors (Ki's = 2.7, 52.0 and 163.8 μM, respectively). Finally, docking studies predicted that **1a** could bind to the catalytic site of the enzyme (PDB code 3A4A), whereas **1b**, **2** and **3** to an allosteric site.



3050

DEVELOPMENT OF ANTIOXIDANT PEPTIDES THAT TARGET THE PULMONARY ARTERIES FOR THE TREATMENT OF PULMONARY HYPERTENSION

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Pulmonary hypertension (PH) is a multifaceted disease that affects the lung blood vessels and eventually leads to right heart failure. Therapeutic strategies using antioxidants have shown promising efficacy, but have major limitations, in particular, the lack of site-specific distribution of the antioxidants to the lungs and pulmonary arteries. Targeted drug delivery can overcome this major issue. For this study, we will investigate the efficacy of targeting the delivery of antioxidants to the pulmonary arteries as a treatment for PH.

Botanicals and their extracts are widely used for both traditional medicine and pharmaceutical development. Bioactive peptides isolated from natural sources have been shown to have strong antioxidant activities. For this study, we focused on identifying peptides with superoxide dismutase and catalase activity, two important antioxidants that play a role in PH. Within our library of over 300 botanical species, we have identified several peptide extracts with potent antioxidant activity (EC_{50} = 0.14ug – 2.10ug), which will be further developed as a therapeutic targeting the pulmonary arteries.

3051

TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF LYNGBYASTATIN 7 FOR THE TREATMENT OF PULMONARY DISEASES

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Lyngbyastatin 7 is marine-derived cyanobacterial cyclodepsipeptide that showed better potency towards inhibiting elastase than sivelestat, the only approved drug targeting this enzyme. The discovery of elastase inhibitors is of great importance since this enzyme plays a significant role in several pulmonary diseases, such as chronic obstructive pulmonary disease, the third leading cause of death worldwide. We established the first total synthesis of lyngbyastatin 7 and validated the elastase-inhibitory activity in enzyme assays. Lyngbyastatin 7 also outperformed sivelestat in our cellular assays, where the compound protected human bronchial epithelial cells against elastase-induced antiproliferative effects as well as attenuated the transcription of sICAM-1 and IL1β which are induced by elastase. The synthetic accessibility overcomes the supply issue and paves the way for the synthesis of diverse analogues with the goal of discovering effective therapeutics against elastase-mediated pathologies.

3052

BIOACTIVITY-GUIDED IDENTIFICATION OF BOTANICAL INHIBITORS OF KETOHEXOKINASE

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The excessive consumption of added sugars is epidemiologically associated with rising prevalence of obesity, metabolic syndrome, and cardiovascular diseases in the United States. As a major constituent of added sugars, fructose has been shown to cause a variety of adverse metabolic effects, such as impaired insulin sensitivity, hypertriglyceridemia, and oxidative stress. Recent studies have shown that ketohexokinase isoform C (KHKC) is the key enzyme responsible in fructose metabolism that drive's fructose's adverse effects. The primary objective of this study was to identify botanicals with inhibitory activity against KHKC. Extracts from 406 botanicals were screened initially at 50 μg/mL for their inhibitory activity using a cell free, recombinant human KHKC assay. Dose response evaluations were conducted on botanical extracts that inhibited KHKC by ≥ 30%. *Angelica archangelica*, *Garcinia mangostana, Petroselinum crispum,* and *Scutellaria baicalensis* were the top four botanical candidiates identified with inhibitory activity against KHKC and prevented fructose-induced ATP depletion and elevation in triglyceride and uric acid production.

3053

α-GLUCOSIDASE INHIBITORS FROM *Vauquelinia corymbosa*

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An extract from *Vauquelinia corymbosa* Bonpl. (Rosaceae) showed noted inhibitory effect (IC_{50} = 28.6 μg/mL) against yeast α-glucosidase when

tested with a well-known spectrophotocolorimetric assay. Bioassay-guided fractionation the active extract led to the isolation of prunasin (1), (-)-*epi*-catechin (2), hyperoside (3), isoquercetin (4), quercitrin (5), quercetin-3-*O*-(6′′-benzoyl)-β-galactoside (6), picein (7) and methylarbutin (8). The most active compound was 6 with an IC_{50} of 30 μM; according to a kinetic analysis compound 6 behaved as mixed-type inhibitor (k_i = 50 μM; α= 0.97). The information generated in this study indicates that *V. corymbosa* is a source of new α-glucosidase inhibitors which might delay glucose absorption *in vivo*. The presence of compounds 1-6 in the plant might explain the reputed antidiabetic activity of *V. corymbosa* in Mexican folk medicine. Concerning the occurrence of prunasin (1) in this species, National Health Authorities should provide effective communication to consumers about the risk from its inappropriate usage.

3054

INVESTIGATION OF INTERSPECIES INTERACTIONS BETWEEN MARINE MICROMONOSPORACEAE FOR IDENTIFICATION OF NOVEL ANTIBIOTICS

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Despite the continued rise in antibiotic-resistant infections, discovery of novel antibiotic scaffolds have been on a steady decline. In part, the decline in new antibiotics can be attributed to the repeated use of relatively standardized methods for natural product isolation, leading to the figurative "low-hanging fruit" being picked. Genomic analyses of actinomycetes have highlighted many biosynthetic gene clusters from which natural products have not yet been isolated under standard laboratory growth conditions. Our study investigated whether interspecies interactions, via co-culture, can stimulate production of novel antibiotics. Marine invertebrate-associated Micromonopsoraceae were used based on the high biosynthetic potential, yet relatively low number of reported chemistry. Microscale (500 μL) cultivation followed by bioactivity screening and LCMS-based secondary metabolomics enabled rapid growth and comparative analyses of hundreds of co-culture pairs and monoculture controls simultaneously. Using this approach, we identified a novel antibiotic produced via co-culture of a *Micromonospora* sp. with a *Rhodococcus* sp. In addition to progress towards elucidation of the structure, whole genome sequencing of both bacteria was performed to identify the biosynthetic origin and producing organism of the antibiotic. Furthermore, proteins differentially expressed between co- and monocultures were investigated using comparative proteomic analyses. Overall, our study aims to reinvigorate antibiotic discovery by investigating interspecies interactions.

3055

THE DEFENSIVE CHEMISTRY OF THE IRISH NUDIBRANCH *ARCHIDORIS PSEUDOARGUS* (GASTROPODA OPISTHOBRANCHIA).

Ryan M. Young and Bill J.
Baker. School of Chemistry, National University of Ireland Galway, Galway, Republic of Ireland; Department of Chemistry and Center for Drug Discovery and Innovation, University of South Florida, Tampa, FL, USA.

Historically, marine natural products from the Republic of Ireland have been greatly underrepresented in the literature despite having a coastline of over 4500 miles. *Archidoris pseudoargus* is a soft-bodied, slow moving Dorid nudibranch which inhabits the coastal waters of Ireland and the United Kingdom. Nudibranchs are a good source of new chemical diversity, employing these secondary metabolites to deter predation. In this study we have identified new chemistry as well as used a metabolomics approach to identify the origin of said chemistry.

In early Spring, mature adults come together to reproduce and shortly thereafter to oviposit on the subtidal rocky shoreline. These egg sacs can be brightly colored and exposed to predation, yet none of the many surrounding predators appear to feed on these nutrient rich egg masses. We have investigated whether the defensive chemistry of the parents is responsible for protecting the egg masses.

Left: A photo of a mature *Archidoris psuedoargus* specimen.

Right: The egg mass of *A. psuedoargus* adhered to an algal substrate

3056

CO-CULTURE BETWEEN INSECT-ASSOCIATED MICROORGANISMS STIMULATES POLYKETIDE PRODUCTION

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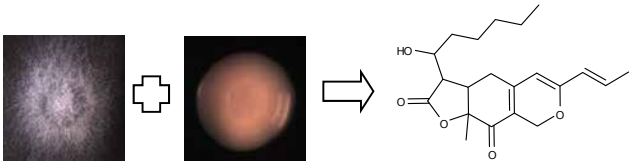
Social insects have been associated with microorganisms that can produce antimicrobial compounds in order to protect their colonies against pathogens. Natural products biosynthesis can be stimulated using co-culture techniques, once in natural conditions microorganisms are competing for nutrients and space. *Monascus ruber* SdFCP01 and *Candida* sp. SdFCP02, both isolated from brood cells of the Brazilian stingless bee *Scaptotrigona depilis*, were co-cultured and the production of the polyketide monascuspiloin by *M. ruber* was elicited. The ecological roles that *M. ruber* and *Candida* sp. play in *S. depilis* colony have been investigated.



M. ruber *Candida* sp. Monascuspiloin

3057

ELUCIDATION OF THE MANGICOL AND NEOMANGICOL BIOSYNTHETIC PATHWAY FROM THE MARINE FUNGUS FUSARIUM EQUISETI CNC-477

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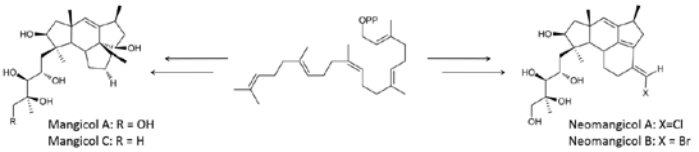
While actinomycetes are often regarded as good resources for the discovery of bioactive metabolites, the recent explosion in whole genome sequencing has brought filamentous fungi back into the spotlight as feasible resources for drug discovery programs. The marine-derived fungal strain *Fusarium*

equiseti CNC-477, characterized by the Fenical laboratory, was 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, respectively, that is crucial for bioactivity. De novo genome sequencing and assembly has revealed candidate biosynthetic clusters responsible for the synthesis of these sesterterpene polyols. These biosynthetic clusters, a proposed biosynthetic route and the development of alternative platforms for the heterologous production of the mangicols and neomangicols will be presented.



3058

THE MANDELALIDES ARE PRODUCED BY A SYMBIONT IN THE PHYLUM VERRUCOMICROBIA IN THE TUNICATE *LISSOCLINUM* SP.

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An increasing number of natural products from higher animals have been found to ultimately derive from a bacterial symbiont. Through connection of pathways with whole symbiont genomes, metagenomics has the potential to yield deeper insights into these symbiotic relationships. In order to investigate the biosynthetic source of mandelalides, a group of macrolides with nanomolar cytotoxicity, we carried out shotgun metagenomic sequencing of DNA extracted from a new *Lissoclinum* species (a tunicate) residing in the waters off the coast of South Africa. As most of the bacterial contigs in the tunicate metagenome were classified as belonging to the phylum Verrucomicrobia, we took all Verrucomicrobia classified contigs and subjected them to an iterative reassembly procedure. The resulting genome was found to contain a complete *trans*-AT PKS pathway. The pathway includes two monooxygenase domains, two *trans*-acting ATs, a glucosyl transferase, an *O*-methyltransferase and a β-methylation cassette. Bioinformatic analysis of this pathway predicted intermediates consistent with the mandelalides final structures. Although we found that one published Verrucomicrobia genome contains secondary metabolite pathways (Opitutus terrae PB90-1), this is the first example of a specific natural product attributed to this phylum.

3059

BIOLOGICALLY ACTIVE NATURAL PRODUCTS FROM *CASSINE VIBURNIFOLIA*

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Plant-sourced natural products have been a generous source of novel anticancer drugs and drug leads. Here, we present the results of a study done on the plant *Cassine viburnifolia* that showed potent NCI-60 cell line growth inhibition as well as activity in the subsequent hollow fiber and xenograft studies. Bioassay-guided isolation led to the identification of three quinone methide-containing natural products. Their structures, activity profiles and relationship to the activity of the crude extract will be presented.

3060

DEVELOPMENT OF A NATURAL PRODUCT-BASED FRACTION LIBRARY FOR IMPROVED PERFORMANCE IN HIGH-THROUGHPUT SCREENING SYSTEMS

Christopher C. Thornburg[1], Tanja Grkovic[1], John R. Britt[1], Rhone K. Akee[1], James A. Whitt[1], Jason R. Evans[2], Paul G. Grothaus[3], Jerry M. Collins[3] and Barry R. O'Keefe[3,4]

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The Natural Products Repository (NPR) within the Natural Products Branch at NCI contains over 230,000 unique extracts derived from more than 75,000 plant, marine and microbial organisms. Importantly, this national resource is available to the research community for screening and isolation of crude extracts. However, compatibility issues that make them challenging for liquid handling systems, coupled with a high rediscovery and false-positive hit rate, reduce the enthusiasm for high-throughput screening of whole extract libraries in targeted assays systems. Furthermore, the chemical complexity inherent to whole extracts may mask active components present as minor metabolites. In order to address these limitations and make the Natural Products Repository more valuable to the extramural research community, we have developed methodology for the pre-fractionation of these whole extracts prior to biological testing. Notably, several marine, microbial and plant extracts examined in the NCI60 cancer cell line screen showed enhanced biological activity after fractionation. Additionally, these methods have recently been adapted for automation and high-throughput processing in order to build a library of pre-fractionated natural products from over 100,000 extracts from the Natural Products Repository.

3061

DETECTION OF HALOGENATED TRYAMINE AND TYROSINE DERIVATIVES IN EXTRACTS OF ALGOA BAY ASCIDIANS

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The crude organic extracts of ascidian species collected from Algoa Bay, South Africa were analysed using liquid chromatography with parallel inductively coupled plasma mass spectrometry/electrospray mass spectrometry (LC-ICP-MS/ESI-MS). The data obtained was used to detect a number of brominated and iodinated tyramine and tyrosine derivatives with in the ascidian extracts establishing this method as a powerful tool for the dereplication of halogenated metabolites in complex mixtures.

3062

A NOVEL NATURAL PRODUCT BLEND EXHIBITS SYNERGESTIC ANTIOXIDANT ACTIVITY

Wei Gao[1], Mohan R. Kaadige[1], Zhe Zhang[1], Clinton J. Dahlberg[1], John G. Babish[1], Xiaolan Kou[1], Matthew L. Tripp[1]
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Oxidative stress plays an important role in the development of many chronic diseases, such as metabolic syndrome and cardiovascular diseases. Antioxidants from foods and dietary supplements help mitigate oxidative stress. The *in vitro* antioxidant activities of apple fruit, bergamot orange, turmeric root and rhizome, green tea leaf, grape seed, grape skin, olive leaf, blueberry, capsicum and mangosteen pericarp extracts were studied individually and in combinations against reactive oxygen and nitrogen species. F105, a unique mixture of these extracts displayed potent antioxidant synergy in these assays (Figure 1).

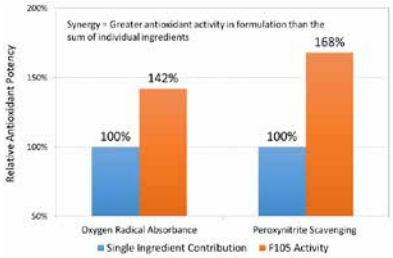

Figure 1. Synergistic antioxidant activity of F105

3063

PROMISING NATURAL PRODUCTS AGAINST GLUCOCORTICOID RESISTANT ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is one of the most common pediatric cancers in developed countries. Although, ALL has a high cure rate (>94%), up to a quarter of patients relapse and at that stage their prognosis is poor. Refractory ALL cases are commonly associated with drug resistance, particularly to glucocorticoid (GC) treatment, an integral component of the therapy. Our main objective is to identify new compound leads from terrestrial natural products to serve as tool compounds and potential therapeutic agents. To evaluate hundreds of natural product fractions, we developed dexamethasone (Dex) pre-B resistant ALL stable cell lines. These Dex resistant cells were characterized via microarray gene expression and protein analysis to interrogate the glucocorticoid pathway. Using CellTiter Glow proliferation assay, we screened our natural product fractions against Dex resistant cell lines and PBMCs. Promising compound leads have been identified and we are current validating them. Our preliminary mechanistic studies on mode of action have focused on the jatrophone sesquiterpenoids and tripterine derivatives. These compound leads will provide insights into GC resistant ALL.

3064

DISCOVERY OF 20-HYDROXYECDYSONE FROM *XEROPHYLLUM ASPHODELOIDES* (TURKEY BEARD)

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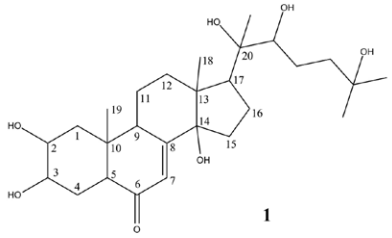
Ecdysterone (20-hydroxyecdysone) is one of the key hormones in insects and crustaceans. A number of higher plants contain ecdysteroids. Phytoecdysteroids play a role in the chemical defenses of plants. We report the discovery of 20-hydroxyecdysone **1** from *Xerophyllum asphodeloides* L. (Nutt.) collected from the NJ pinelands with a brine shrimp assay-guided fractionation approach. The isolated 20-hydroxyecdysone showed modest activity (LD_{50} 12.5 μg/mL) in the brine shrimp assay. The spectroscopic data agree with the reported data. This is the first report of the discovery of 20-hydroxyecdystone in *X. asphodeloides*.



1

3065

AMERICAN INDIAN BOTANICALS: POSSIBLE ALTERNATIVES TO HORMONE THERAPY DURING MENOPAUSE

Tristesse Burton[1], Tareisha Dunlap[1], Huali Dong[1], Guannan Li[1], Judy Bolton[1], Djaja Soejarto[1], and Richard B. van Breemen[1]
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Although pharmaceutical hormone therapy (HT) remains the standard clinical treatment for managing menopausal symptoms, many women seek alternatives such as botanical dietary supplements since HT has been associated with increased risk of breast cancer, coronary heart disease and stroke. The leading botanicals that women take for menopause are black cohosh and red clover, which were also traditionally used by American Indians. Although these two botanicals have been investigated extensively, there are still numerous American Indian plants that lack scientific studies on their safety and efficacy. *Amorpha canescens* Pursh. (Fabaceae) - **leadplant**, *Echinocystis lobata* (Michx.) Torr. & A. Gray (Cucurbitaceae) - **wild cucumber,** and *Silphium perfoliatum* L. (Asteraceae) - **cup plant** inflorescent tissue were examined for estrogenic, chemopreventive, and anti-inflammatory potential in initial screenings based on American Indian ethnobotany and published biological data. **Leadplant** MeOH extract was selected as the best candidate for bioassay-guided fractionation due to high anti-estrogenic activity in the Ishikawa cell-based assay and anti-inflammatory activity in the Griess assay. **Cup plant** and *Lespedeza capitata* Mixch. (Fabaceae) – **roundhead lespedeza** (chosen from the other 12 species) are being pursued as alternatives due to significant anti-estrogenic and estrogenic activity, respectively. This project will identify possible novel and effective drug leads

for menopausal symptoms, cancer, and inflammation and provide support for American Indian traditional knowledge.

3066

ISOLATION AND STRUCTURE ELUCIDATION OF A POSSIBLE ANT TRAIL PHEROMONE, FROM EPOTHILONE B PRODUCING FERMENTATIONS OF *SORANGIUM CELLULOSUM*

Daniel Benigni[1], Douglas Weaver[1], John Baumes[1], Jonathan Karten[2], Mark Kosmoski[1], Linda Phillips[3]
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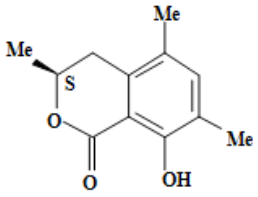
Sorangium cellulosum is a myxobacteria producing epothilone B. During the development of the process to purify epothilone B from fermentation of *Sorangium cellulosum* we identified a possible ant trail pheromone**.** The initial detection, isolation and structure elucidation of S-3,4-dihydro-8-hydroxy-3,5,7-trimethylisocoumarin will be presented as well as literature regarding its biosignificance.



3067

MAGNOLIA BARK EXTRACT IS A POTENT NRF2 ACTIVATOR

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Oxidative and xenobiotic stress have been shown to be causative agents for many diseases including cancer, age-related macular degeneration, cardiovascular diseases, and age-related memory loss. Cells have protection mechanisms against these stressors, in part, through the activation of the Nrf2 pathway. Research suggests that promoting health resilience could be achieved by transiently activating the Nrf2 pathway through an intake of dietary phytochemicals in the absence of oxidative stressors. Magnolia (Magnolia officinalis) bark has been widely used in traditional Chinese medicine they are rich in Magnolol and Honokiol. Here, we demonstrate that magnolia bark extract stimulates the Nrf2 pathway in mammalian liver cells both by reporter and gene expression assay. We also show that Magnolia bark helps protect cells against oxidative stress toxicity. We further carried out bioassay directed fractionation of the extract and show that the NRF2 activation is due to, in part, by the phytochemicals, magnolol and 4 methoxy honokiol, present in the magnolia bark extract.

3068

K-TARGETED ISOLATION OF C-GLYCOSYLFLAVONES FROM VITEX AGNUS-CASTUS BY COUNTERCURRENT METHODOLOGY

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C-glycosylated flavones, including orientin, isoorientin, vitexin, and isovitexin, are minor but biologically significant constituents of fruit extracts of the chaste-tree (*Vitex agnus-castus* L.), a botanical supplement used to treat PMS and postmenopausal symptoms. The partition coefficient, or *K*-value, is the ratio of the concentration of a compound in each phase of a biphasic solvent mixture and is a physicochemical property of a particular compound in a particular solvent system. This value can be used to predict retention volume (V_{ret}) in a countercurrent separation procedure. The *K*-values of C-glycosylflavones present in complex botanical fractions have been determined in a number of solvent system families (HEMWat, EBWat, H*ter*AcWat, *ter*AcWat) using the shake-flask technique combined with relative LC-MS quantification. This *K*-value database has been used to develop targeted centrifugal partition (CPC) and high-speed countercurrent chromatography (HSCCC) methods. In each separation procedure the actual *K* value and V_{ret} was reasonably predicted by the shake-flask partition experiment, confirming the utility of this approach in choosing a solvent system and targeting the fraction that contains the desired compound. This *K*-value database allowed for the efficient isolation of C-glycosylflavones from *V. agnus-castus* using orthogonal CCC and CPC methods.

3069

ANTIOXIDANT PROFILE AND HS-SPME/GC-MS-TOF ANALYSIS OF VOLATILE COMPOUNDS OF *ALNUS ACUMINATA* SSP. *ARGUTA* BARK.

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Alnus acuminata ssp. *arguta* (Schlecht.) Furlow (Betulaceae) (aile) is a widely used wild plant in the Mexican traditional medicine to treat acute inflammation, as astringent, antimicrobial and for treatments of syphilis. *In vivo* studies have demonstrated an anti inflammatory action and diarylheptanoids as components of the methanol extract of the stem bark. This extract exhibited antioxidant activity using four *in vitro* model systems: DPPH$^{\cdot}$, ABTS$^{\cdot+}$, FRAP$^{\cdot}$ radical scavenging activities and inhibition of lipoperoxidation in β-carotene bleaching assay. Total phenol content of the methanol extract was determined using standard spectrophotometric methods (Folin-Ciocalteu, 22.5 ± 1 mg GAE/g of stem bark) and IC_{50} values of 0.04 mg/mL and 0.2 mg/mL in DPPH and ABTS assays were four fold lower and five fold higher than reference compounds respectively. In FRAP test, the extract showed weak antioxidant activity, and good response in the β-carotene bleaching test.

The volatile composition of *Alnus acuminata* stem bark was determined by solid-phase microextraction/mass spectrometry analysis. Forty three compounds, among others, alcohols, aromatic compounds, terpenoids and ketones were identified using this technique. The presence of antioxidant phenolic compounds in this plant may justify some of its medicinal properties.

Objetivos particulares

- Determinación de los compuestos marcadores mayoritarios presentes en la corteza por CCF.

- Determinación de fenoles totales por el método de Folin-Ciocalteu y por fórmula farmacopéica.
- Demostrar la capacidad antioxidante del extracto metanólico mediante los ensayos *in vitro* DPPH, ABTS, FRAP y blanqueamiento de b-caroteno de la materia vegetal para así complementar estudios científicos monográficos tipo OMS.
- Determinación de compuestos volátiles por el método HS-SPME-GC-MS-TOF.

3070

DEVELOPMENT AND VALIDATION OF AN RP-HPLC-PDA METHOD FOR QUANTIFYING JUGLALIN IN *HYDRANGEA SEEMANNII* PHYTOPREPARATIONS

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

Hydrangea seemannii L. Riley (Hydrangeaceae) is a medicinal Mexican plant highly valued for the treatment of type II diabetes mellitus (TII-DM). Recently, we reported the antidiabetic action of an infusion of this plant and isolated the main active components, which included isoquercitrin, hyperoside, trifolin, guaijaverin, astragalin, and juglalin (kaempferol 3-*O*-α-L-arabinopyranoside). Hence, a suitable reversed-phase high-pressure liquid chromatography-photodiode array detection (RP-HPLC-PDA) method was developed for quantifying juglalin, the marker compound. The mobile phase consisted of a linear gradient of water (containing 0.1% formic acid) and acetonitrile (ACN) of 95:5 (0 min) to 100 of ACN (15 min) at a flow rate of 1.2 mL min^{-1} and the detection was carried out at 254 and 327 nm. The method was successfully validated in terms of linearity, accuracy, precision, and detection and quantitation limits. This procedure is appropriated for quality control and elaboration of standardized phytopreparations of *H. seemannii*.

3071

ANTIOXIDANT ACTIVITY OF *MORINGA OLEIFERA* ISOTHIOCYANATES

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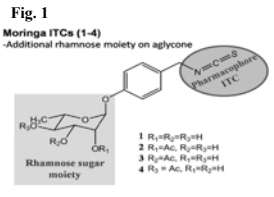
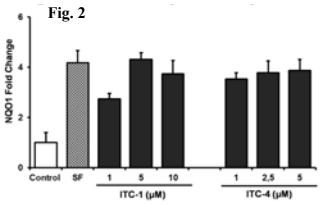
[1]Plant Biology Department, Rutgers, The State University of New Jersey, New Brunswick, NJ-USA. [2]Center for Translational Research, School of Medicine, Universidad De Las Américas UDLA, Quito, Ecuador. [3]Department of Molecular Biology and Genetics, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

Moringa oleifera Lam. (Brassicales) is an edible tropical tree with medicinal properties. Here, we describe a fractionation process of moringa leaves by fast centrifugal partition chromatography (FCPC) to generate polyphenol and isothiocyanate (ITC) rich fractions (Fig.1). The polyphenol-rich fraction showed direct antioxidant activity assayed by oxygen radical absorbance capacity (ORAC), while the ITC-rich fraction demonstrated indirect antioxidant properties assayed by NQO1 activity in Hepa1c1c7 cells. Also, purified 4-[(α-l-rhamnosyloxy) benzyl] isothiocyanate (ITC-1), and 4-[(4'-*O*-acetyl-α-l-rhamnosyloxy)benzyl] isothio-cyanate (ITC-4) were evaluated for their ORAC and NQO1 inducer potency in comparison with sulforaphane (SF) at 5 μM (Fig.2). Both ITCs were as potent as SF in inducing NQO1 activity. These findings suggest that edible *M. oleifera* leaves contain a potent mixture of antioxidants that could explain its health promoting effects.





3072

BRAZILIAN CERRADO BIOME PLANT EXTRACT BANK SCREENING IN INFLAMMATORY DISEASE AND CANCER ENZYMES

Laila S. Espindola[1,2], Brice Wilson[2], John A. Beutler[2], Kirk R. Gustafson[2], Barry R. O'Keefe[2]
[1]Laboratório de Farmacognosia, Universidade de Brasília, Brasília, Brazil
[2]Molecular Targets Laboratory, National Cancer Institute / NIH, Frederick, MD, USA

A total of 702 extracts from the *Brazilian Cerrado Biome Plant Extract Bank* of the University of Brasília, Brazil - produced from 95 species representing 40 families were tested for inhibitory activity against p38 kinase and MALT1 protease. These enzymatic targets are implicated in inflammatory disease and B-cell lymphoma, respectively. Data from both assays were compared directly in order to identify those extracts with specificity for p38. This resulted in the identification of 13 extracts from 6 plant families (Annonaceae, Flacourtiaceae, Hippocrateaceae, Sapindaceae, Sapotaceae and Malpighiaceae) that showed specific p38 inhibition. These extracts were then eluted through polyamide resin to remove polyphenolic compounds. Two extracts (*Pouteria gardneri* and *P. ramiflora*) lost their activity, but an additional extract maintained enzymatic inhibition following polyamide and continues to be investigated. Here we report initial results on the identification of natural product inhibitors of p38.

3073

AZAPHILONE MOLECULES FROM FRESHWATER DERIVED FUNGI

Vanessa M. Nepomuceno, Eoghainin O'hAinmhire, Joanna E. Burdette, Brian T. Murphy**
Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago

Ovarian cancer is the most lethal gynecological malignancy and the fifth leading cause of cancer death among women. This high mortality rate is due to difficult detection, unclear symptoms, and an absence of known precursor lesions. In addition, tumors that are initially responsive to chemotherapeutics often become resistant. Thus, discovery of novel drug leads for ovarian cancer treatment is of paramount importance. To address this need, our lab focuses on secondary metabolites produced by microorganisms in the aquatic environment as a source for new therapeutic leads. In particular, freshwater-derived fungi remain largely uninvestigated for novel chemical scaffolds. Hence, there is untapped potential in this environment for structural uniqueness. In the current study, a fraction library of secondary metabolites produced by freshwater-derived fungi were screened for potential selective toxicity against chemo-resistant ovarian cancer cells. Two new molecules of the azaphilone class have been identified from fungal strain FJ015 in Lake Huron. Herein, we report the structure elucidation of these metabolites by analysis of one- and two-dimensional nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. Details of the biological activity of these molecules will also be discussed.

3074

A NEW BROMINATED INDOLE FROM *HAPLOSCLERIDA* WITH SELECTIVE CYTOTOXICITY AGAINST THE PANC-1 TUMOR CELL LINE

Nicholas Lorig-Roach[1], Tyler A. Johnson[2], Fred A. Valeriote[3], and Phillip Crews[1]
[1]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, 95064, [2]Dominican University of California, San Rafael, CA, 94901 [3]Josephine Ford Cancer Center, Henry Ford Health System, Detroit, Michigan, 48202

An extract of a species of *Haplosclerida* sponge obtained from the National Cancer Institute repository displayed selective cytotoxicity to a pancreatic cancer cell line (PANC-1) relative to the CEM cell line in a disk diffusion assay. Two brominated indoles, the known 6-bromo conicamin (**1**) and the new 2'-oxo derivative (**2**) were identified via bioactivity guided fractionation followed by accurate mass and NMR experiments. Upon purification of these initially co-eluting compounds, the new indole (**2**) was determined to be responsible for the cytotoxic activity of the extract. Its potency and breadth of activity are currently being evaluated.

3075

DRUG INTERACTION POTENTIAL OF *MITRAGYNA SPECIOSA* AND ITS CONSTITUENTS THROUGH PXR MODULATION

Shabana I. Khan[1,2], *Vamshi K. Manda[1], Olivia R. Dale[1], Zulfiqar Ali[1], Larry A. Walker[1,2], and Ikhlas A. Khan.[1,2]*
[1]National Center for Natural Products Research, [2]Department of Biomolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677

The leaves of Mitragyna speciosa (kratum), which are rich in alkaloids, are traditionally consumed for the stimulant and euphoric effects and as a substitute for opium. This study was conducted to predict the drug interaction potential of kratum with concomitantly used conventional drugs by determining the effects of its methanolic extract, an alkaloid enriched fraction and the isolated constituents (indole and oxindole alkaloids) on pregnane X receptor (PXR)-mediated expression of drug metabolizing enzymes (CYPs) and transporters (P-gp). A significant activation of PXR was observed with the extract and the alkaloid fraction with EC_{50} of 18 and 8 µg/mL, respectively. Several of the isolated constituents increased PXR activity with EC_{50} values in the range of 2-6 µM. A significant increase in the expression of mRNA for CYP1A2 and P-gp was observed by the total extract, alkaloid enriched fraction and eight of the isolated constituents while CYP3A4 mRNA was increased only by four constituents. These results indicate that chronic use or high consumption of *Mitragyna speciosa* may affect the pharmacokinetics and pharmacodynamics of co-administered drugs which are substrates of CYPs and P-gp by altering their metabolism and transport. Further in vivo studies are warranted.

3076

PHENOLIC COMPOUNDS FROM *GLYCYRRHIZA URALENSIS* (CHINESE LIQUORICE)

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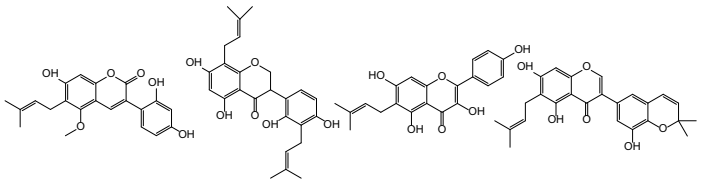
[1]National Center for Natural Products Research, [2]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, MS 38677

Glycyrrhiza uralensis has been used in Chinese traditional system of medicine for many ailments. Over 20 phenolic compounds such as flavonoids, isoflavonoids, flavanoids, isoflavanoids, and coumarins conjugated with prenyl unit(s) were isolated from the methanol extract of *G. uralensis* and characterized by means of NMR and mass spectroscopic techniques. Herein, isolation and structure determination of purified compounds are described.

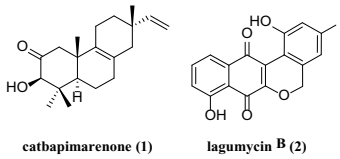


3077

A NOVEL PIMARANE DITERPENE AND CYTOTOXIC ANGUCYCLINES FROM A MARINE-DERIVED ACTINOMYCETE IN VIETNAM.

Michael W. Mullowney,[1] *Eoghainín Ó hAinmhire,[1,2] Urszula Tanouye,[1] Baojie Wan,[3] Sanghyun Cho,[3] Scott G. Franzblau,[1,3] Joanna E. Burdette,[1,2] Pham Van Cuong,[4] Brian T. Murphy[1,2]*
[1]Dept of Med Chem & Pharmacognosy, COP, UIC, Chicago, IL, USA, [2]Center for Pharmaceutical Biotechnology, COP, UIC, Chicago, IL Illinois, USA, [3]Institute for Tuberculosis Research, COP, UIC, Chicago, IL, USA, [4]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

A screening of our actinomycete fraction library against a cisplatin-resistant ovarian cancer cell line (OVCAR5) led to the isolation of catbapimarenone (**1**), lagumycin B (**2**), dehydrorabelomycin (**3**), phananthroviridone (**4**), and WS-5995 A (**5**). These compounds were produced by a *Micromonospora* sp. isolated from sediment in the East Sea of Vietnam. Compounds **2**-**4** were cytotoxic with LC_{50} values ranging from 0.34 µg/mL to 9.12 µg/mL, while **5** exhibited selective cytotoxicity toward the Kuramochi cell line and **1** was inactive. Compound **1** is a novel $\Delta^{8,9}$-pimarane diterpene, representing one of approximately twenty actinomycete-produced diterpenes reported to date while **2** has yet to receive formal characterization in the peer-reviewed literature. The structure elucidation of **1** and **2** was performed by combined NMR, MS, and CD spectroscopic analysis. The isolation and cytotoxicity of **1**-**5**, as well as the structure elucidation of **1** will be presented.



3078

ANTIMICROBIAL PROPERTIES AND CHEMICAL PROFILING OF THE ACESSORY NIDAMENTAL GLAND IN THE SQUID *EUPRYMNA SCOLOPES*

Samantha M. Gromek[1], *Anne A. Sung[1], Allison Kerwin[2], Andrea Suria[2], Spencer V. Nyholm[2], and Marcy J. Balunas[1,*]*
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The Hawaiian bobtail squid, *Euprymna scolopes*, has served as a model for studying host-microbe interactions such as the light organ symbiosis with the bioluminescent bacterium *Vibrio fischeri*. We have previously characterized the composition of the bacterial consortium found in the accessory nidamental gland (ANG), part of the reproductive system of the female host. Our continued research supports the hypothesis that bacteria from the ANG are deposited into the egg jelly coat (JC) where they produce antimicrobial secondary metabolites that protect eggs from harmful microorganisms and degradation throughout their embryonic period, during which time they are physically unprotected. Given that the ANG symbiosis

has naturally evolved to select for bacteria with antimicrobial properties, this provides a source of secondary metabolites that are more likely to have potent antibiotic activity. Bacterial isolates from the ANG and JC have been cultured and extracted, and have shown potent antibacterial activity against a suite of marine and human pathogens. Chemical networking, activity-guided isolation, compound identification, and biological activity of isolates will be discussed. Future studies will involve investigations of the mechanism(s) of antimicrobial activity and medicinal chemistry to probe structure-activity relationships.

3079

FUNGAL METABOLITES AS NOVEL ANTHELMINTICS AGAINST SOIL-TRANSMITTED HELMINTHES.

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It is estimated that over 2 billion people worldwide are suffering from complications due to infections with soil transmitted helminthes (STHs), but very little research is being conducted by human health pharmaceutical companies. In the USA, it is estimated that in distressed areas of poverty, just under 4 million people harbor undiagnosed STH infections.Anthelmintic resistance to broad spectrum compounds has become a worldwide issue. Our initial goal was to screen a unique collection of filamentous fungi and pure isolated fungus metabolites against *Brugia malayi* microfilaria, *Haemonchus contortus* L1 and *Strongyloides stercoralis* L3. We confirmed a number of active fungi against all three targets. Many of these active extracts were produced by cultures not previously shown to produce anthelmintic metabolites. De-replication procedures demonstrated metabolite novelty. Of the 90 pure compounds tested, we identified active metabolites against all three target species. One of the active pure compounds identified was enniatin D which belongs to a structure class known to have anthelmintic activity, thereby validating the approach.

3080

ASSESSMENT OF DRUG INTERACTION POTENTIAL OF *ESCHSCHOLZIA CALIFORNICA* (CALIFORNIA POPPY) THROUGH MODULATION OF CYPs, P-gp AND PXR

Vamshi K. Manda[1], Mohamed A. Ibrahim[1], Olivia R. Dale[1], Mallika Kumarihamy[1], Stephen J. Cutler[2], Ikhlas A. Khan[1, 2], Larry A. Walker[1,2], Ilias Muhammad[1] and Shabana I. Khan.[1, 2]
[1]National Center for Natural Products Research, [2]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.

Eschscholzia californica, a North American plant, is traditionally used for depression, neurasthenia, and various psychiatric conditions. With the rapid rise in the use of herbal supplements in combination with conventional drugs, the risk for potential herb-drug interactions is also increasing. Most of the clinically relevant pharmacokinetic drug interactions occur due to modulation of Cytochrome P450 enzymes (CYPs), P-glycoprotein (P-gp), and Pregnane X receptor (PXR). This study aimed to determine the effects of an EtOH extract, BuOH and $CHCl_3$ fractions and alkaloids of California poppy on the activity of CYPs, P-gp and PXR. The extract and fractions showed strong inhibition of CYP3A4, 2D6, and 2C19 and a weak inhibition of 2C9 and 1A2. Among the alkaloids, escholtzine inhibited 3A4, 2D6 and 2C19 (IC_{50} 3.0, 2.0 and 0.4 μM) and protopine inhibited 2D6 and 2C19 (IC_{50} 0.04 and 1.3 μM). No inhibition of P-gp was observed with the extract, fractions or its constituents. A significant activation (>2 fold) of PXR was observed with the extract and fractions (30 μg/mL) while the alkaloids were not as potent (>1.5 fold at 60 μM). Further studies are in progress to find out if the PXR activation would lead to an increased expression of drug metabolizing enzymes and transporters (CYPs and P-gp). *Acknowledgement:* Supported by the National Institute of General Medical Sciences, NIH Grant Number P20GM104932.

3081

ANTIBIOTIC-PRODUCING BACTERIA ASSOCIATED WITH THE NESTS OF THE LEAF-CUTTING ANT *Atta sexdens rubropilosa*

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The leaf-cutting ants are associated with bacteria that produce antibiotics to protect their colonies against invading microorganisms. Natural products produced by these bacteria were selected by ants during thousands of years of evolution and may be useful for drug discovery. We have been studying the antibiotic-producing bacteria associated with the nests of the leaf-cutting ants *Atta sexdens rubropilosa*. These bacterial strains isolated from the ants and the fungal garden inhibited the growth of the fungal garden parasite *Escovopsis* sp. and the human pathogens *Staphylococcus aureus* ATCC 3538, *Proteus mirabilis* ATCC 29906 and *Candida albicans* ATCC 10231. The antibiotics produced by these bacteria will be presented.

3082

IDENTIFICATION OF CELLULAR PROTEIN TARGETS OF SILYMARIN-DERIVED FLAVONOLIGNANS

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Silymarin, an extract of the seeds of milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] protects liver cells from virus infection, oxidative stress, and inflammation. It was hypothesized that silymarin provides hepatoprotection by binding to cellular proteins. The hypothesis was addressed by establishing an affinity-based system for capturing, identifying, and validating the cellular proteins bound by the flavonolignan mixture silibinin, a major bioactive component of silymarin. Diazirine or diphenylketone photoreactive groups and alkyne or azide moieties were chemically engineered onto eleven derivatives of silybin A or silibinin. Toxicity and anti-hepatitis C virus (HCV) activity testing of probes on human hepatoma cells indicated that all probes had antiviral activity that was separable from compound-induced toxicity. Probes were then used to capture cellular proteins from hepatoma cells. Mass spectrometry and statistical filtering revealed 26 putative cellular protein targets, which specifically bound to a photoaffinity probe. Binding of the probe to protein targets was dose-dependently inhibited by silybin A. Descriptions of the putative targets, along with chemical validation of the interaction of silibinin with the targets, will be presented. The approach should permit further unraveling of the complex biology arising when cells encounter natural products.

3083

A QUARTZ CRYSTAL MICROBALANCE STUDY OF GREEN TEA BINDING TO BOVINE SERUM ALBUMIN

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The development of new label-free assays is important for furthering our understanding of biologically active chemicals in complex mixtures such as botanical extracts. A quartz crystal microbalance (QCM) can be used to measure the change in mass that occurs due to the binding of a ligand to a drug target protein when the protein is immobilized to the surface of a quartz crystal. In this study differences in binding to bovine serum albumin (BSA) between epigallocatechin gallate (EGCG), a natural polyphenol and the most abundant catechin in tea, and a crude green tea extract has been investigated using a QCM. The adsorption of EGCG and green tea onto a surface functionalized with BSA was measured at various concentrations. Langmuir and Freundlich isotherms were used to model the adsorption data. The Langmuir isotherm better described the adsorption behavior with correlations of 0.67 and 0.92 for EGCG and green tea, respectively. The better fit to the Langmuir model indicates that adsorption occurs homogeneously and that aggregation is negligible. Saturation was reached at 0.24 ng/cm^2 for EGCG and at 0.68 ng/cm^2 for green tea. The increased adsorption of the crude extract compared to pure EGCG indicates that EGCG is not the only chemical constituent interacting with BSA and that these constituents likely bind to additional sites since competitive binding is a non-dominant effect. However, the lower rate of adsorption observed for the crude extract indicates that there is some competition for binding to the EGCG binding sites.

3084

IMMUNOMODULATORY DRUG DISCOVERY FROM TUNICATE-ASSOCIATED MARINE BACTERIA

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There is a critical need for new immunomodulatory compounds, including immunosuppressants for use with organ transplantation or to treat autoimmune diseases, as well as immune adjuvants to enhance anticancer or antiviral immunity. We have employed a new phenotypic assay using cytotoxic T cells to identify two kinds of immunologically-relevant small molecules: immunosuppressive compounds that do not work via known cellular signaling pathways, and compounds that enhance the responses of cells stimulated through their T cell receptors, but do not affect cells in the absence of stimulation. Our library of tunicate-associated marine bacterial extracts has been screened using this assay resulting in several extracts prioritized for further investigation. Bioassay-guided fractionation of one of the lead extracts has led to the isolation of a potent immune-enhancing compound (EC_{50} = 0.6 ng/mL). Compound identification and further biological analyses will be discussed. Future studies will include identification of molecular target(s) as well as optimization of compounds for potential preclinical development.

3085

ANTICANCER POTENTIAL OF WITHANOLIDES AND ITS DERIVATIVES FROM PHYSALIS PERUVIANA (POHA)

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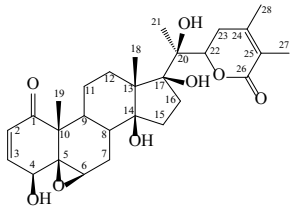
Aberrantly active nuclear factor-κB (NF-κB) and signal transducer and activator of transcription (STAT3) protein are critical factors that regulate tumor processes. Convincing evidence shows the association between NF-κB and STAT3 plays integrated role in promoting cancer development. Consequently, inhibitors of NF-κB and STAT3 isolated from natural sources might be useful as novel anticancer therapeutics. Bioassay-guided fractionation of the organic extracts from both the aerial parts and fruits of *Physalis peruviana* (Pp) led to the isolation of a series of withanolides (WS) including a chlorinated withanolide. Among them, ten compounds inhibited TNF-α-induced NF-κB activity with IC_{50} values in the range of 0.04– 31.2 μM. Seven isolated compounds inhibited nitric oxide (NO) production in lipopolysaccharide-activated murine macrophage RAW 264.7 cells with IC_{50} values in the range of 0.2 – 44.6 μM. The isolate, 4β-Hydroxywithanolide E, differentially inhibited the viability of all three cell lines, with 6-fold preferential effect against human tumor lines (U251MG and MDA-MB-231), with IC_{50} values of 0.3 and 0.1, respectively, compared to effects on normal NIH-3T3, with IC_{50} value of 0.6 μM.



4β-Hydroxywithanolide E
IC_{50} = 0.3 μM (U251MG)
IC_{50} = 0.1 μM (MDA-MB-231)
IC_{50} = 0.6 μM (NIH 3T3)
IC_{50} = 0.04 μM (NF-κB)
IC_{50} = 6.2 μM (NO)

3086

SYNTHESIS OF FIRE ANT VENOM ALKALOIDS, DEHYDROSOLENOPSINS B & C

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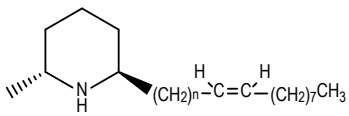
Piperidine alkaloids are the major constituents of the venom of the imported fire ant, *Solenopsis invicta*. Even though the alkaloid fraction is mainly comprised of (2*R*,6*R*)-*trans*-2-methyl-6-alky- and 6– alkenylpiperidines small amounts of (2*R*,6*S*)-cis-analogs are also present. Several procedures have been reported for the stereospecific synthesis of (2*R*,6*S*)-*cis*- and (2*R*,6*R*)-*trans*-2-methyl-6-alkylpiperidine constituents. However, no procedure has so far been reported for the stereospecific synthesis of (2*R*,6*R*)-*trans*-2-methyl-6-alkenylpiperidine constituents. We have developed a procedure for the stereospecific synthesis of the major (2*R*,6*R*)-*trans*-2-methyl-6-alkenylpiperidine fire ant venom alkaloids, (2*R*,6*R*)-dehydrosolenopsin B and C.



(2*R*,6*R*)-Dehydrosolenopsin B n=3
(2*R*,6*R*)-Dehydrosolenopsin C n=5

3087

PHARMACOKINETIC, *IN VITRO* AND *IN SILICO* ASSESSMENT OF ANTI-INFLAMMATORY ALKALOIDS FROM *ISATIS TINCTORIA* L.

Matthias Hamburger[1], Evelyn Jähne[1], Veronika Butterweck[2,3], Mouhssin Oufir[1], Daniela Eigenmann[1], Maxime Culot[4], Roméo Cecchelli[4], Fruzsina Walter[5], Mária Deli[5], and Martin Smiesko[1]
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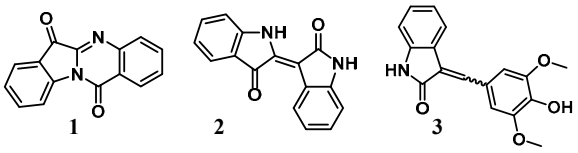
We previously identified the alkaloids tryptanthrin (**1**), indirubin (**2**) and indolinone (**3**) as pharmacologically active compounds in woad (*Isatis tinctoria* L.). They inhibit COX-2, 5-LOX catalyzed leukotriene synthesis, and mast cell degranulation at low μM to nM concentrations, and they possess drug-like physico-chemical properties. A pilot pharmacokinetic study in rats (2 mg/kg i.v. b.w.) showed that **1** and **2** have half-lives of 30-40 min, whereas **3** was rapidly eliminated. *In silico* predictions for **1**-**3** indicated high oral absorption and favourable blood-brain transport. In animal and human *in vitro* blood-brain-barrier (BBB) models **1** and **3** displayed high BBB permeation. In the Caco-2 intestinal absorption model, **1** showed high permeation, while the recovery of **3** was low.



3088

IN VITRO OPIOID RECEPTOR DISPLACEMENT AFFINITY AND *IN VITRO* BEHAVIORAL STUDIES BY TETRAD ASSAY OF *NELUMBO NUCIFERA* FLOWER

Mallika Kumarihamy[1], Francisco Leon[2], Sara Pettaway[2], Janet Lambert[2], Christopher Hill[1], Mei Wang[1], Lisa Wilson[2], Christopher R. McCurdy[2], Mahmoud ElSohly[3], Stephen J. Cutler[1,2], Ilias Muhammad[1]
[1]National Center for Natural Products Research, Departments of [2]BioMolecular Sciences and [3]Pharmaceutics and Drug Delivery, School of Pharmacy, Research Institute of Pharmaceutical Sciences, University of Mississippi, University, MS 38677, USA

Nelumbo nucifera Geartn. (lotus), is a perennial aquatic plant native to South-East Asia. Both white and pink flowers are reported to have a calming effect when smoked or made into a tea. Bioassay-guided isolation and identification of from EtOH extracts revealed eight benzyltetrahydroisoquinoline alkaloids, nuciferine (**1**), *N*-nor-nuciferine (**2**), asimilobine (**3**), armepavine (**4**), *O*-methylcoclaurine (**5**), *N*-methylcoclaurine (**6**), coclaurine (**7**), and neferine (**8**), and as well as linoleic and palmitic acids. Compounds **5**-**8** showed high percent of radioligand displacement in opioid receptors displayed various degrees of displacement activities. Based on the GTPγS functional agonist assay, neferine was determined to be a weak δ-agonist. The acidic and basic partitions, **1**, **7** and mixture of **5**-**7** were subjected to mouse tetrad assay, of which the acidic partition displayed decreased locomotion, and increased catalepsy, hypothermia, and antinociceptive activity at doses of 75-100 mg/kg/ip. This work, along with traditional use and the reported bioactivities of the alkaloids, suggested further studies on *N. nucifera* are needed to understand the roles that the extracts or individual compounds might contribute to the behavioral effects. *Acknowledgement:* Supported by the National Institute of General Medical Sciences (NIGMS), NIH Grant Number P20GM104932, and COBRE, CORE-NPN, Research Core C.

3089

LC-MS METABOLOMICS AND CHEMOTAXONOMY OF *ILEX* SPECIES

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Ilex is a cosmopolitan monotypic genus in the Aquifoliaceae with centers of diversity in Asia and South America. Several species have been used traditionally in the preparation of medicinal teas for treating and preventing various ailments, while others are consumed primarily for their caffeine content. In order to assess the evolution of metabolites in caffeine-containing *Ilex* species, leaf samples of 41 *Ilex* species and hybrids were collected, processed, and extracted for analyses by UPLC-TQD and UPLC-qToF mass spectrometry. Multiple reaction monitoring of compounds and their major mass fragments were developed to detect methylxanthines in leaf extracts at lower limits of detection between 1-10 ng/mL. Three methylxanthines (caffeine, theobromine, and theophylline) and the methyluric acid, theacrine, were quantified in *I. guayusa*, *I. paraguariensis*, and *I. vomitoria*. *Ilex guayusa* showed the highest content of caffeine, followed by *I. paraguariensis* and *I. vomitoria*. Though there have been reports of caffeine content in *I. cassine,* we were not able to detect caffeine nor other methylxanthines using both UPLC-TQD-MS and UPLC-qToF-MS. Chlorogenic acid and three of its isomers were quantified to compare total chlorogenic acid content across all species in this study. The results showed that all caffeine-containing *Ilex* species also had high concentrations of chlorogenic acids. *Ilex guayusa, I. paraguariensis,* and *I. vomitoria* were compared with *Ilex cassine* using UPLC-qToF-MS and the data analyzed using PCA, OPLS-DA, and heatmaps to visualize and identify differences in metabolite profiles among these species. The results revealed triterpenoid saponins as chemotaxonomically informative marker compounds in caffeine-containing *Ilex* species.

3090

A MICROBIAL DRONE SYSTEM FOR TARGETING AND DESTROYING *PSEUDOMONAS* BIOFILMS

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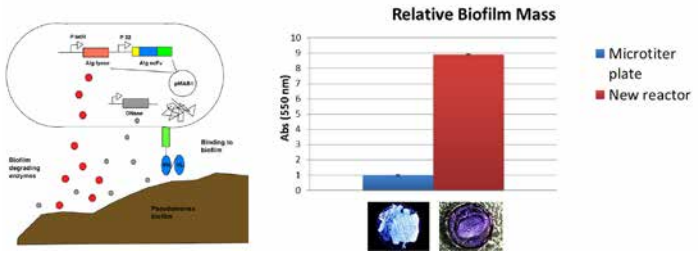
Antibiotics are the primary methods of treatment for biofilm related infections. However, most antibiotics have significantly less potency against pathogens in their biofilm growth state, which can be up to 1000 times more resistant to antimicrobial agents than their planktonic counterparts. There is clearly a need for new, biofilm-specific therapeutic approaches, and here we present the development of an engineered Lactococcus microbial drone that binds to and disrupts Pseudomonas aeruginosa biofilms. This probiotic can be combined with traditional natural products screening approaches to identify synergistic combinations of therapeutics. We will also present an improved flexible bioreactor for growing and testing reproducible, robust biofilms on a variety of clinically and industrially relevant surfaces.



3091

GREAT LAKES-DERIVED FUNGAL EXTRACTS YIELD COMPOUNDS TARGETING PEDIATRIC CANCER.

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There is an unmet medical need to identify more effective and less toxic treatments for rare, but fatal childhood solid tumors including medulloblastoma, rhabdomyosarcoma, Ewing's sarcomas and neuroblastoma. Fundamental to the success of improving therapies for pediatric cancers is the use of screening tools focused on the selectivity/specificity of disease-relevant targets. Tied to this is the need for source material that has chemical diversity and the ability to impact diverse biological pathways. To address this, we have initiated a project aimed at identifying fungal-derived compounds specifically targeting these childhood cancers. We screened fungal extracts acquired from diverse sediment layers of the Great Lakes which have until now, been an untapped resource for new fungal species. A screen, based on the SRB assay, was used to identify extracts with selectivity against a single type of pediatric cancer. Bioassay-guided fractionation was then used to isolate compounds with activity against these cancer types. Mechanistic studies are identifying the modes by which the compounds specifically inhibit childhood cancers.

3092

STRUCTURAL AND EVOLUTIONARY RELATIONSHIPS OF KETOSYNTHASE DOMAINS FROM MODULAR POLYKETIDE SYNTHASES

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To understand how the structures of ketosynthase domains (KS) from hybrid non-ribosomal peptide synthase-polyketide synthase (NRPS-PKS) and acyltransferase-less PKS (AT-less PKS) differ from the paradigm deoxyerythronolide B synthase (DEBS) we used sequence similarity analysis and high-throughput structural biology. DEBS-like PKS KS form a single similarity cluster and hybrid NRPS-PKS and AT-less PKS KS form distinct similarity clusters. Furthermore, AT-less PKS KS display multiple clusters that correlate with incoming substrates. Structures reveal that functional KS domains have identical catalytic residues. Three loops surrounding the active site display unique orientations that correlate with the sequence clustering. Furthermore, residues in the substrate binging pocket correlate with incoming substrate. Together this information reveals how substrate specificity is determined by both large structural changes and subtle sequence variations. This information will be used to reprogram the biosynthetic pathways to enable combinatorial biosynthesis. We also reveal the structural evolution of a NRPS-PKS KS into an AT-less PKS KS.

3093

LCMS-BASED HIERARCHAL CLUSTERING AS A METHOD TO ASSESS BIOSYNTHETIC RELATIONSHIPS AMONG BACTERIA

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With the recent outpouring in whole genome sequencing technologies, natural product chemists now have greater access than ever before to the genomic information surrounding biosynthetic potential. The continued decrease in cost for whole genome sequencing holds great promise as a tool for prioritizing bacterial strains for drug discovery. Nonetheless, rapid methods to prioritize strains for discovery programs are still needed. We sought to investigate hierarchal clustering analysis (HCA) based on LCMS profiles as a method to evaluate metabolomic relationships among strains and examine how these relationships were reflected in genomes using whole genome analysis. To test this method, we analyzed a group of marine *Streptomyces* spp. using LCMS, and developed open-source workflows in R with XCMS to generate hierarchal clusters. We ensured clusters produced using R were comparable to clusters made using proprietary software. Trees from LCMS data were directly compared to 16S rDNA phylogenetic trees, and relationships between these clusters were investigated further using principal component analysis (PCA). A second group of *Streptomyces* spp. with whole genomes assembled were also analyzed by LCMS-HCA. The relationships identified based on metabolites were assessed in tandem with whole genome analysis. Overall, our LCMS-based HCA methods aim to assist the strain selection process for natural product drug discovery platforms, and in addition can offer an alternative "pre-selection" tool to guide future genomic analyses.

3094

BIOCOMPATIBLE POLYMER DELIVERY OF BIOFILM DISRUPTORS OF *VIBRIO CHOLERAE*

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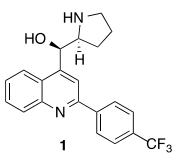
[1]Institute of Textile Chemistry and Macromolecular Chemistry RWTH Aachen University, Aachen, Germany, 52056, [2]Department of Chemistry and Biochemistry, [3]Department of Microbiology and Environmental Toxicology, [4]Chemical Screening Center, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064.

Biofilms are large aggregates of bacterial cells that adhere to surfaces as a survival strategy against antibiotics and harsh environmental conditions. These aggregates contribute to up to 80% of microbial infections, particularly in cases where medical devices are required. In our lab, we have screened a large natural products library for the inhibition of biofilm formation in *Vibrio cholerae*. From lead compounds we synthesized a library of analogs, the most promising of which is compound **1**. Recently, we have developed a system whereby can deliver compound **1** via a polymer coating to delay colonization of *V. cholerae* on non-native substrates in both static and flow conditions. The biological data of this compound and its polymer delivery application will be presented.



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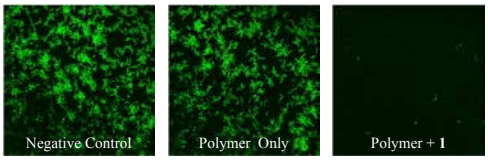

Biofilm images 6 hours post-inoculation

3095

THE ANTIDEPRESSANT-LIKE EFFECTS OF PAEONIFLORIN IN RATS MODEL EXPOSED TO CHRONIC RESTRAINT STRESS

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Beijing University of Chinese Medicine, 11 Beisanhuandonglu, Beijing, 100029, China

The purpose of this sduty was to evaluate the antidepressant-like effects of paeoniflorin (PF) in a rat model of chronic restraint stress (CRS) and the possible active mechanisms. SD male rats were randomly divided into six groups which recorded as normal group, model group, fluoxetine group, paeoniflorin groups (10m/kg, 20mg/kg, 40mg/kg). The rats in the model group were prepared by CRS combined with radiation respectively for 21 days and were fed separately. The results showed that CRS-exposed rats emerged depressive-like behaviour with reduced weight in open field test and step down test, low motor activity as well as reduced consumption of sucrose, with increased concentrations of CORT, ACTH in plasma and CRH in hypothalamus. Furthermore, the contents of NO and cGMP were reduced after PF treated by Griess and RIA methods seperately, and changes with the mRNA expression of sGCα2、sGCβ1、PDE2A、PDE5A、PDE9A. These results suggest that the modulation of the HPA axis and cGMP synthetase and hydrolases are important mechanisms underlying the antidepressant-like effects of paeoniflorin in CRS-treated rats.

3096

FLUORESCENT AZAPHILONOIDAL PIGMENTS FROM THE ENDOPHYTIC FUNGUS *Biscogniauxia* sp.

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

As part of our continuing search for novel cytotoxic fungal compounds, the entophytic fungus *Biscogniauxia* sp. (Xylariaceae) was isolated from a *Bromelia* species collected in the Ecological Reserve on the campus of the National Autonomous University of Mexico. Fungal identification was assessed by nuclear ribosomal internal transcribed spacer (ITS) barcoding. The organic ($CHCl_3$-MeOH) extract from the axenic solid (moisture rice) culture showed promising growth inhibition activity when tested at 20 μg/mL against HCT-15 (colorectal) and HeLa (cervix) cell lines (96.5 and 96.3 % of inhibition, respectively). Dereplication results of the organic extract by UPLC-PDA-HRESI-MS/MS against a database containing more than 200 fungal secondary metabolites revealed the presence of the dimeric tetrahydroxanthone secalonic acid G. From this extract, a series of known and new fluorescent azaphilonoidal pigments were isolated. Their structures were elucidated using a set of spectroscopic and spectrometric techniques. In addition, these compounds were tested for antiproliferative activity against a panel of cancer cell lines.

3097

DEVELOPMENT OF SAFE AND EFFECTIVE BOTANICAL DIETARY SUPPLEMENTS

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Regulated differently than drugs or foods, the market for botanical dietary supplements continues to grow worldwide. The recently implemented US FDA regulation that all botanical dietary supplements must be produced using Good Manufacturing Practice is an important step toward enhancing the safety of these products, but additional safeguards could be implemented, and unlike drugs, there are currently no efficacy requirements. To ensure a safe and effective product, botanical dietary supplements could be developed rigorously in a manner analogous to pharmaceuticals that involves identification of mechanisms of action and active constituents, chemical standardization based on the active compounds, biological standardization based on pharmacological activity, preclinical evaluation of toxicity and potential for drug-botanical interactions, metabolism of active compounds, and finally, clinical studies of safety and efficacy. Although not required, completing as many of these steps as possible would contribute to the translation of botanicals from the field to safe human use as dietary supplements.

3098

SCREENING FOR ANTIDIABETIC COMPOUNDS FROM GOJI BERRIES: IDENTIFICATION OF NOVEL SMALL MOLECULE PPARγ ACTIVATORS

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[1]National Center for Natural Products Research, [2]Divison of Pharmacognosy, Department of BioMolecular Sciences, The University of Mississippi, University, MS 38677, USA, [3]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

The identification of novel antidiabetic PPARγ agents with lesser toxicity profile is of interest since the current PPARγ agonists, glitazones display serious side effects including heart failure and edema. Goji berries are used in the traditional Chinese medicine for the treatment of diabetes mellitus and hypertension and sold in health food products in the western countries. The aim of our study is to identify new PPARγ agonists from Goji berries by *in silico* screening from Goji berries, a followed by *in vitro* testing. Crystal structures of PPARγ with both partial and full agonist were used for *in silico* screening for higher predictivity. The *in silico* screening approach led to the identification of several small molecule amides having the favorable conformation in both partial and full agonist PDB structures. Subsequently, 24 small molecule amides were synthesized and tested using *in vitro* luciferase reporter gene assay. Compounds CA-G-001, CA-G-008 and CA-010 showed selectivity to PPARγ vs. PPARα and good fold activation when compared the positive control, Rosiglitazone. The details of *in silico*, *in vitro* results and other data will be presented.

3099

EPIGENETIC MODIFICATION OF TAMPA BAY FUNGAL STRAIN PRODUCES NEW AND KNOWN COMPOUNDS ACTIVE AGAINST MRSA

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In the face of increasingly resistant bacteria like the ESKAPE pathogens, there is a dire need for new and novel drug candidates. Marine fungal endophytes live in harsh environments, producing secondary metabolites as a survival strategy. In the laboratory, production of secondary metabolites is often down-regulated. Epigenetic modifiers can be used to activate down regulated biosynthetic pathways to access a greater chemical repertoire. Following a high-throughput, epigenetic-based screen of mangrove endophytic fungi, one fungal strain from the Tampa Bay area was identified for its activity against ESKAPE pathogen *Staphylococcus aureus*. It was grown in large scale and in epigenetically modified conditions. Its secondary metabolites were extracted, and the fractionation and purification of extracts was guided by NMR. Epigenetic modification of the fungi caused the production of new and known compounds with one new compound exhibiting activity against *S. aureus*.

3100

A BIOMIMETIC DIVERSITY-ORIENTED APPROACH TO THE PSEUROTINS

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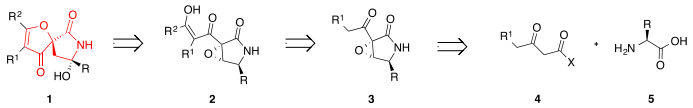
Pseurotins are a small family of secondary microbial metabolites isolated from the fermentation broth of *Pseudeurotium ovalis*. Pseurotin natural products possess a wide range of biological activities including inhibition of chitin synthase, inhibition of monoamine oxidase, antifungal, anti-angiogenic, and dopamine antagonistic activity. Structurally, the pseurotin family contains a highly substituted 1-oxa-7-azaspiro[4,4]non-2-ene-4,6-dione core (highlighted in red) that has been implicated in the biological activities of these natural products. Access to these natural products is limited due to poor fermentation yields or lengthy syntheses. To exploit therapeutic potential of the pseurotins, we are developing a biomimetic approach to the pseurotin core structure starting from readily available amino acids. The biomimetic approach consists of three main features: i) Condensation of a β-ketoester **4** and an amino acid **5** to form an epoxide **3**; ii) α-Acylation to form a diketone **2**; iii) Nucleophilic ring opening of epoxide to form the spiro-core **1**.



3101

DEVELOPMENT OF A SENSITIVE AND RAPID HPLC METHOD FOR QUANTITATIVE MEASUREMENT OF GINKGOLIC ACID IN DIETARY SUPPLEMENTARYS

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Dietary supplements containing extracts of *Ginkgo biloba* are one of the most widely used supplements. These products are used for their purported benefit to improve cognitive functions, blood circulation and in management of neurodegenerative conditions. The beneficial effects of extracts are primarily attributed to flavonoids and triterpene lactones. Ginkgolic acids, a group of structurally-related alkyl phenols, have been shown to have some negative health effects. The European Union currently limits the amount of ginkgolic acid in supplements to a level of 5 parts-per-million (ppm) or less. Recently, the National Toxicology Program reported that *G. biloba* extracts caused cancers of thyroid and liver in rats and mice models. In the light of this report, we intend to measure ginkgolic acids content of Ginkgo dietary supplements in US market.

An HPLC-UV method using a biphenyl column was developed for the separation of three forms of ginkgolic acids (13:0, 15:1, 17:1). The samples were extracted by the QuEChERS method, in which the ginkgolic acids were partitioned in the acetonitrile layer, followed by HPLC-UV analysis. The developed method was validated for linearity, repeatability, accuracy, limits of detection, limits of quantitation and spiked recovery. The method successfully quantified total ginkgolic acid amounts below 5 ppm levels. Quantitative data on the ginkgolic acid content in the dietary supplements will be presented.

3102

CHEMOACTIVATION OF A BACTERIAL PROTEASE: EXPANDING THE MOLECULAR ARSENAL AND EXPLORING THERAPEUTIC POTENTIAL

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Bacterial caseinolytic protease P (ClpP) is a tetradecameric serine protease highly conserved across species. In a healthy bacterial cell, ClpP is responsible for maintaining homeostasis through the highly regulated degradation of misfolded, mistranslated, denatured, and obsolete proteins. In a strain dependent fashion, microbes rely upon properly functioning ClpP for secretion, virulence, reproduction, pathogenicity, and/or survival. Therefore, it is not surprising that aberrant activation of this protease, which results in uncontrolled proteolysis, leads to detrimental bactericidal or bacteriostatic activity. This observation has catapulted ClpP into the spotlight as a promising new antibacterial target.

Although potent ClpP activators have been disclosed, the structural diversity is constrained to one natural product class, the acyldepsipeptides (ADEPs), and a limited collection of small molecules identified in high-throughput screens of commercial libraries. Unfortunately, known ClpP activators suffer from poor bioavailability, limited solubility, effluxation, and/or metabolic weaknesses, which limit applicability and impede clinical utility. To allow for the continued exploration of ClpP as a potential therapeutic target, and provide new chemical probes to investigate the role of ClpP in a variety of bacterial strains, our lab has integrated a multidimensional and collaborative approach to diversify the arsenal of ClpP activators. Progress towards this endeavor will be presented.

3103

A VALIDATED METHOD FOR DETERMINATION OF CANNABINOID CONTENT IN DIETARY SUPPLEMENTS BY UPLC-MS

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Natural products containing cannabinoids derived from *Cannabis sativa* (L.), a medicinal plant with a long history of ethnobotanical uses, are now emerging as popular dietary supplements in the consumer marketplace. In spite of the ambiguous interpretation of the federal legal status, cannabinoid products are now readily available from online outlets and are increasingly being marketed by mainstream retailers. Many states have enacted legislation conferring various levels of legal status to *Cannabis*, and in recent months thirteen states have passed so called "low-THC" or "CBD-only" laws to allow widespread access to certain natural products that are less contentious in terms of the current Schedule I classification by the federal Controlled Substances Act. Although techniques exist for quantification of cannabinoids in crude plant materials as well as in blood and urine, dietary supplements present analytical challenges due to complex matrices and the inherent variability of bioactive forms with oral delivery. Reported here is a validated method for rapid determination of cannabidiol (CBD), cannabidiolic acid (CBD-A), Δ^9-tetrahydrocannabinol (THC), Δ^9-tetrahydrocannabinolic acid (THC-A) and cannabigerol using UPLC with detection by photo-diode array and single quadrupole mass spectrometry.

3104

STUDY OF ANTIOXIDANT ACTIVITY OF AÇAÍ EXTRACTS (EUTERPE OLERACEA)

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Investigation of the potential health benefits of açaí to deliver effective strategies to treat or mitigate a variety of human ailments will require the identification of bioactive chemical constituents, identification of biological targets and associated molecular mechanisms. Reductions in oxidative stress (OS) resulting from increased production of reactive oxygen species (ROS) can be achieved in living cells via two discrete mechanisms. One mechanism, critical to restoration of cellular homeostasis is the induction of cytoprotective anti-oxidant enzymes such as heme oxygenase-01 and NADPH quinine oxidoreductase to eliminate ROS. Induction of such cytoprotective enzymes is dependent on the Nrf2/ARE signaling pathway. Inhibition of toxicologically important cytochrome P450 enzymes such as CYP2A6, CYP2B6, CYP1A1 and CYP2E1 involved in ROS generation presents yet another potential strategy to reduce OS. The current study utilizes a novel bioassay-guided fractionation on açaí berry to study the (a) induction of anti-oxidant enzymes via the Nrf2/ARE signaling pathway in cultured HepG2 cells and (b) inhibition of CYP2A6, CYP2B6, CYP1A1 and CYP2E1 using individually expressed human liver P450s in vitro. Our results indicated the presence of bioactive constituents in açaí with a potential to modulate Nrf2/ARE signaling pathway and subsequent induction of cytoprotective phase II enzymes. Açaí has also been found to have inhibitory constituents towards CYP2B6 and CYP1A1, along with a host of other human P450 isoforms. Interestingly, some of the fractions were found to be active in both the experimental models indicating possible potentiating or synergistic effect. This study promises the identification of cytoprotective constituents of açaí and associated mechanisms of action.

3105

META- APPROACHES TO HARVEST NATURAL PRODUCTS FROM HARMFUL ALGAE BLOOMS.

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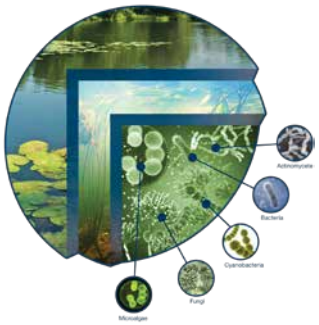

In 2009, Ross Youngs established the company Biosortia Pharmaceuticals based on a new harvesting technique utilizing microalgae natural products chemistry to overcome the lack of efficiency of drug discovery. This approach has focused on the micro-algal consortia using a meta-metabolomics and a mega-scale approach.

Global warming and rising CO_2 concentrations in the atmosphere play a principal role in intensifying phytoplankton blooms in eutrophic and hypertrophic lakes. Harmful algae blooms have pivotal ecological and economical impacts because of the toxic secondary metabolites produced by algae-related microorganisms . However, these metabolites ironically can also serve as a source of new drug leads or in the construction of new drug leads.[a]

Biosortia has developed a unique harvesting technology capable of acquiring a large quantities of biomass containing a wide diversity of microorganisms grown under stress conditions originating from the environmental competition during a bloom where gene mutations and evolution in the related microorganisms are promoted.[b] These approaches may provide access to new complex biology and chemistry. A preliminary screening has already revealed several promising hits for treatment of various diseases including cancer, neurological diseases, metabolic diseases, infectious diseases, and antiparasitic. This uncommon protocol of discovering new natural products may offer new drug-like molecules for many disease targets.

[a]Penn, K. *et al. ISME J* **2014,** *8* (9), 1866-1878. [b]Uchiyama, J. *et al*., *Photosynth Res* **2015**, 1-12.

3106

ANALYSIS OF COUMARIN AND OTHER COMPOUNDS IN COMMERCIALLY AVAILABLE CINNAMON AND CINNAMON SUPPLEMENTS UTILIZING QUANTITATIVE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Cinnamon is an important flavoring agent utilized worldwide. The dried inner bark of *Cinnamomum verum* (Ceylon cinnamon) is referred to as "true" cinnamon whereas the barks of C. *cassia* (Chinese cassia), *C. burmannii* (Indonesian cassia), and *C. loureiroi* (Saigon cassia) (collectively known as cassia cinnamon) are also marketed as cinnamon. Because of its lower cost, Cassia cinnamon and, in particular, Indonesian cassia has replaced Ceylon cinnamon as the species most widely used in the food and beverage industry in the United States, Canada, and Europe.[1] Cassia cinnamon has been shown to contain high levels of the naturally occurring compound coumarin, which can be hepatotoxic to individuals sensitive to it. Because of this, the European Union has set regulatory limits on the amount of coumarin allowed in cinnamon containing foods. A number of clinical trials have shown cassia cinnamon to have modest antidiabetic activity. As a result, there are currently many dietary supplements on the market containing both true and cassia cinnamon. Supplement labels contain suggested usage levels from 1 gram up to 4 grams of cinnamon per day. The amounts of coumarin and other marker compounds in cinnamon bark, powder, foods, and supplements have previously been analyzed with a validated UPLC-UV/MS method.[1] We are in the process of developing an automated high throughput NMR method to quantitate coumarin and other key components in cinnamon containing products. Results with various commercial cinnamon samples and supplements will be presented.

[1]Wang YH, Avula B, Nanayakkara NP, Zhao J, Khan IA. *J. Agric. Food. Chem.* **2013**; 61, 4470-4476.

3107

NOVEL QUASSINOID FROM JAMAICAN *CASTELA MACROPHYLLA* BLOCKS INDUCTION OF CYTOCHROME P450 1 ENZYMES AND LUCIFERASE GENES

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In this study we investigated the potential for a novel quassinoid, glaucarubulone-15-O-β-D-glucopyranoside (Gg) isolated from the endemic Jamaican *Castela macrophylla* plant to inhibit cytochromes P450 (CYPs), in particular CYP1A1 and CYP1B1 enzymes, known to convert polyaromatic hydrocarbons (PAHs) into carcinogenic metabolites. Gg attenuated by nearly 70-fold, the ability of PAH, benzo-a-pyrene(B[a]P) to induce CYP1A gene expression in MCF-7 breast cancer cells as determined by real-time RT-PCR.

Gg was also shown to directly inhibit the enzyme activity of human CYP1A1 and CYP1B1 enzymes (IC_{50}, 6.93 ± 0.31μM and 9.17 ± 0.91μM respectively) according to non-competitive kinetics. Given the ability of Gg to block the induction of phase I gene expression, we investigated its effects on antioxidant response element (ARE)-dependent induction by crude acai berry fractions. Where acai_92H at 50μg/mL caused an approximate five- to seven-fold induction of luciferase gene expression, the inclusion of Gg reduced the induction to below two-fold over the entire range of concentrations, and at 250 nM Gg, complete loss of induction was observed. Taken together, these data suggest that the plant isolate Gg may be an interesting candidate to provide insights into mechanisms of cellular transcription.

3108

PENICILLIUM CHRYSOGENUM, AN ENDOPHYTE FROM PADINA GYMNOSPORA, AS A SOURCE OF DIKETOPIPERAZINES, PEPTIDES AND GRISEOFULVIN

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Endophytic fungi tend to live within the host organism over its entire life. Once associated, the fungi alter nutrient content and enhance or begin production of secondary metabolites which may play important roles in adaptation, defense against predators and represent an important source for bioprospection. Fungi from marine organisms have been shown to produce a variety of bioactive compounds and some are currently under development as antitumoral, anti-inflammatory agents among others. *Padina gymnospora* is a brown alga collected in the Brazilian southeast coast which afforded a fungal strain identified as *Penicillium chrysogenum*. Its crude extract afforded nucleotides, diketopiperazine derivatives in addition to griseofulvin and 7-dechorogriseofulvin. The secondary metabolites identification was carried out mainly by ESIMS, NMR analyses and search in Antimarin2011®, MarinLit® and ChemSpider® databanks. In addition, their antiinflammatory activities were investigated and the results evidenced the dose-dependent inhibition of NO overproduction by hirsutatin A, a cyclohexadepsipeptide obtained from *P. chrysogenum* extract and previously isolated from the insect pathogenic fungus *Hirsutella nivea*.

3109

ABSOLUTE CONFIGURATION ASSIGNMENT OF REMOTELY SEPARATED ALCOHOLS BY THE CSSF-TOCSY-INEPT EXPERIMENT

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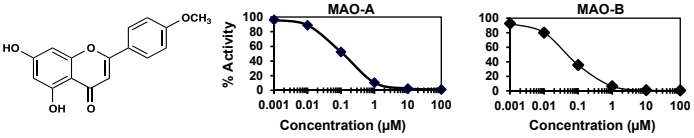
Natural products containing remotely separated secondary alcohols widely exist in marine and terrestrial organisms. Absolute stereochemistry assignment of these alcohols by NMR spectroscopic methods remains challenging. This is mostly because the severely overlapping resonances flanking the stereocenters invalidate the conventional Mosher analysis. Herein, our recently developed 1D selective NMR experiment, CSSF-TOCSY-INEPT, has provided a convenient solution to the stereochemistry of these alcohols. It is achieved via chemical shift selective (CSS) excitation of the oxygenated methine signals, followed by TOCSY and INEPT transfer to generate the ^{13}C NMR subspectra of all carbon segments subtended at each stereogenic center. ^{13}C subspectra consolidation and the extract of anisotropic shift (Δδ) from (R) and (S)-Mosher esters allow the simultaneous configuration assignment of all alcohols. The new approach is unrestricted by the distance between the remote alcohols and also effective for polyhydroxylated compounds, which make it an attractive and unique tool for structure elucidation of macrolide, acyclic lipids, *etc*.

3110

ISOLATION OF ACACETIN FROM *CALEA URTICIFOLIA* AS A POTENT INHIBITOR OF HUMAN MONOAMINE OXIDASE-A AND B

Narayan D Chaurasiya[1], Vedanjali Gogineni[2], Francisco Leon[2], Marvin J Nuñez[3], Stephen J. Cutler[2], Larry A Walker[1,2] and Babu L Tekwani[1,2]
[1]National Center for Natural Products Research and [2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. [3]Laboratorio de Investigacion en productos Naturales, Facultad de Quimica y Farmacia, Av. Heroes y martires del 30 de Julio, San Salvador, El Salvador.

Calea urticifolia (Asteraceae: Asteroideae) has long been used as a traditional medicine in El Salvador to treat arthritis, fever among other illnesses. Phytochemical investigations revealed the presence of sesquiterpene lactones, predominantly germacranolides. The chloroform extract of aerial-parts of *C. urticifolia* showed potent inhibition of recombinant human MAO-A and -B. Further bio-assay guided fractionation led to the isolation of a flavonoid, acacetin, as the most prominent MAO inhbitory constituent. Acacetin is a promising neuromodulator with significant affinity towards serotonin receptors. Follow-up studies showed reversible binding of acacetin with human MAO-A and -B resulting in competitive inhibition. Acacetin was about 4 fold more selective towards MAO-B compared to MAO-A. The results indicate potential for development of acacetin for treatment of psychiatric disorders.



Acknowledgement: This work was supported by NIH-National Institute of General Medical Sciences #P20GM104932 (COBRE-*In vitro* Research Core).

3111

HEAVY METALS IN BACOPA INGREDIENTS COLLECTED FROM THE MARKET IN EUROPE, INDIA AND THE UNITES STATES

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[1]American Herbal Products Association, Silver Spring, MD 20910, [2]Nature's Way Brands, Green Bay, WI 54311, USA

Bacopa monnieri (L.) Pennell (Scrophulariaceae) is a creeping, prostrate, somewhat succulent perennial that grows in moist environments. Bacopa is an important plant in the traditional Indian systems of medicine (Ayurveda, Siddha, and Unani). Traditionally, the entire plant is used as a brain tonic to enhance memory, development, learning ability, and concentration. Bacopa is gaining popularity worldwide and is classified as dietary ingredient under the U.S. Dietary Supplement Health and Education Act of 1994 (DSHEA).

Bacopa has also been recommended as an agent for phytoremediation because of its ability to accumulate heavy metals from contaminated soils. Concerns were raised following a 2013 article published in *Planta Medica* that claimed elevated levels of Cd, Pb, Cu, and Zn in all tested bacopa plant samples collected from peri-urban areas in India.

We analyzed 75 samples of bacopa plant material and extracts acquired from 14 companies in Europe, India, and the United States. This presentation will summarize the results of this market study, which refute the results of the 2013 article.

3112

NOVEL BOTANICAL EXTRACTS AND SMALL MOLECULE INHIBITORS FOR AFRICAN SLEEPING SICKNESS, CHAGAS DISEASE, AND VISCERAL LEISHMANIASIS

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Three of most devastating neglected tropical diseases - African Sleeping Sickness, Chagas Disease and the Leishmaniases - are caused by vector-borne, flagellated protozoa. An estimated 30,000 people are infected and up to 70 million are at risk of developing African Sleeping Sickness. Chagas Disease affects an estimated 7-8 million people primarily in Latin and South America, and is also classified as an emerging infectious disease threat in the United States. The Leishmaniases affect 10-12 million people in almost 100 countries. These diseases can be extremely debilitating and even fatal if untreated. However, current drug therapies have significant issues with toxicity and there are growing concerns about developing resistance. In this study, botanical extracts from plants that are used in traditional Chinese medicine were screened for inhibitory activity against the bloodstream form of *T. brucei*. Selected extracts were fractionated based on bioactivity to identify active pure compounds. In addition, a library of small molecules was synthesized to probe the structure of a botanically-derived compound with known anti-microbial properties. Selected extracts and compounds with low toxicity were further screened against the intracellular forms of *T. cruzi* and *L. donovani*. These screens identified several novel compounds with IC_{50} values in the low micromolar range and low cytotoxicity that have potential to be developed into new drug therapies for these diseases.

3113

STRUCTURE ELUCIDATION OF A PROTON-DEFICIENT NATURAL PRODUCT USING LR-HSQMBC SUPPORTED BY DFT CALCULATIONS

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A severely proton-deficient marine alkaloid ($C_{28}H_{21}N_7O_2$) was intractable to structural characterization using only conventional 1D and 2D NMR techniques including HMBC data, which generally provides primarily two- and three-bond correlations. A sensitive, complementary approach to obtain very long-range ($\geq {}^4J_{CH}$) heteronuclear correlations is afforded by the recently reported LR-HSQMBC experiment. An INEPT pulse sequence element inserted after t_1 evolution, converts anti-phase long-range heteronuclear coupling information back into in-phase magnetization thereby alleviating cancelation of responses emanating from small (<2 Hz) scalar couplings. The role of the LR-HSQMBC experiment in the determination of this natural product structure will be illustrated.

LR-HSQMBC data were employed to gain access to numerous ${}^4J_{CH}$ and several ${}^5J_{CH}$ heteronuclear coupling pathways that were pivotal in linking together segments of the alkaloid structure, which could not be accomplished solely relying on HMBC data. The observed ${}^4J_{CH}$ and ${}^5J_{CH}$ heteronuclear couplings were reasonable and consistent with DFT calculation of the coupling constants and chemical shifts performed on the proposed structure of the alkaloid. The structure was also consistent with the results generated using the ACD Structure Elucidator™ CASE program.

3114

EXTENDING THE STRUCTURAL DIVERSITY OF A MARINE DERIVED FUNGUS SPICARIA ELEGANS KLA03

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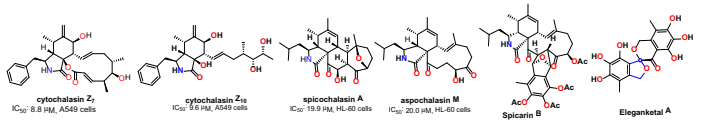
Marine derived fungi are a powerful resource to produce unique bioactive structures. Although thousands of compounds with various chemical structures have been disclosed, most of the gene clusters encoding secondary metabolites are silent under a single culture condition. To maximize the chemical diversity of fungal metabolites, the so called "OSMAC" approach has been used on the marine sediments derived fungus *Spicaria elegans* KLA03. Including the original isolated cytochalasins Z_9-Z_{15} (glucose based medium under static conditions), fourteen new polyketides of this family were further isolated including spicochalasin A and aspochalasins M-Q (starch and soybean based medium upon shaking for 8 days), 7-deoxy-cytochalasins Z_7 and Z_9 (adding a cytochrome P-450 inhibitor to glucose based medium under static conditions), aspochalasins R-T (starch and soybean based medium, shaking for 14 days), and cytochalasins Z_{21}-Z_{23} (adding D- and L-tryptophan to glucose based medium, static conditions), as well as eleganketal A possessing a rare substituted 3H-spiro[isobenzofuran-1,3'-isochroman]-4'(1'H)-one skeleton. In addition, spicarins A-D were also discovered from the acetylated extract of this strain.

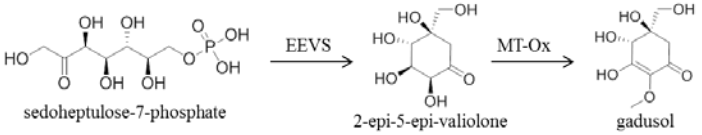


3115

DE NOVO SYNTHESIS OF A VERTEBRATE SUNSCREEN COMPOUND

Andrew R. Osborn,[1] Khaled H. Almabruk,[1] Garrett Holzwarth,[2] Shumpei Asamizu,[1] Jane LaDu,[3] Kelsey M. Kean,[4] P. Andrew Karplus,[4] Robert L. Tanguay,[3] Alan T. Bakalinsky,[2] and Taifo Mahmud[1]
[1]Department of Pharmaceutical Sciences, [2]Department of Food Science and Technology, [3]Department of Environmental and Molecular Toxicology, and [4]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA.

Current paradigms suggest that gadusol, a sunscreen and antioxidant found in fish, is derived from 4-deoxygadusol, a precursor of mycosporine-like amino acids produced by cyanobacteria, fungi, algae, and marine invertebrates. The accumulation of these compounds in marine animals has been proposed to be of dietary or symbiont origin. Here, we report that gadusol is synthesized *de novo* in zebrafish (*Danio rerio*) from a pentose phosphate pathway intermediate, sedoheptulose 7-phosphate, by a novel two-enzyme system, a 2-epi-5-epi-valiolone synthase (EEVS) and a methyltransferase-oxidoreductase (MT-Ox). Heterologous expression of the fish genes in engineered yeast resulted in the production of gadusol in yeast. The product suppresses the UVB-sensitivity of a *rad1Δ* mutant of *Saccharomyces cerevisiae*, confirming the UVB-protective activity of gadusol.



3116

REBIOGRAM ENHANCES THE SEARCH OF BIOACTIVE NATURAL PRODUCTS

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Finding active component(s) from natural sources using traditional chromatographic methods is a time consuming and tedious task. Especially with solid support material, potential loss due to irreversible absorption cannot be avoided during the separation procedure. In contrast, **c**ounter**c**urrent **s**eparation (CCS), a liquid-liquid chromatography, can achieve loss free separation as it does not apply solid support material. This special characteristic deserves more attention when searching for bioactives from complex natural sources via bioassay guided fractionation (BGF) procedures. Considering recent progresses in CCS methodology, a considerable amount of sample can be loaded into a centrifugal partition chromatography (CPC) instrument with a single injection. ReBioGram is a new approach that enhances BGF for the search of bioactive natural products. It takes full advantage of CCS ability to swap the mobile phase in two separated runs to generate a pair of fractions that are exact normal- and reserved-phase mirror images of each other in K-plot. Almost a symmetric ReBioGram could be delineated based on bioactivities of these fractions, which could be used further to validate and prioritize active fraction.

Pueraria montana var. *lobata* (Willd.) (PL) has an isoflavone profile similar to that of *Trifolium pratense* (L.) (TP). However, the content of active components in PL is much lower than in TP. Guided by the alkaline phosphatase in Ishikawa cell assay, and using CPC fractionation and HPLC purification, we demonstrate the efficiency of ReBioGram method in the isolation of active component (*ca* 0.2 mg) from a DCM extract of PL aerial parts in just two separation steps.

3117

NEW AND KNOWN MANDELALIDES FOR BIOLOGICAL MECHANISM AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

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Mandelalides A-D are polyketide macrolides reported from a new *Lissoclinum* species of South African ascidian in 2012. The conformational flexibility of the macrolactone skeleton and the presence of two discrete and independent stereochemical regions confounded the absolute assignments of mandelalides A-D by spectroscopic methods. The absolute structure of mandelalide A was subsequently corrected, through total synthesis, to a configuration where all five stereocenters in the northern hemisphere were inverted. The revision of mandelalide A makes it likely that mandelalides B-D were also misassigned. Further evaluation of the potent biological activity is also needed given that the glycosylated mandelalides A and B showed potent cytotoxicity to human NCI-H460 lung cancer cells (IC_{50}, 12 and 44 nM, respectively) and mouse Neuro-2A neuroblastoma cells (IC_{50}, 29 and 84 nM, respectively), while insufficient amounts of non-glycosylated mandelalides C and D prevented their biological testing as pure compounds. Herein, computational methods have been used to predict ^{13}C and ^{1}H NMR chemical shifts for comparison to the experimental data for mandelalide A and investigate the absolute configurations of mandelalides B-D, which contain an additional furanone compared to mandelalide A. In addition, new members of the mandelalide family have been isolated and characterized from a re-collection of the source tunicate, and provide further insight into the structure-activity relationships of this compound series.

3118

PHENYLPROPANOIDS AND FLAVONES FROM *DESMOS PEDUNCULOSUS* (ANNONACEAE) FROM VIETNAM

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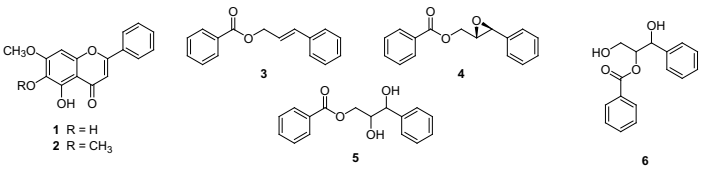
The genus *Desmos* consists of several species of trees and shrubs, mainly found in southeastern Asia. Many of these species have had few or no reports of their chemistry. Phytochemical investigation of the tree *Desmos pedonculosus* (A. DC.) Bân (Annonaceae) from Vietnam led to the isolation and characterization of two flavones and several benzoate esters derived from cinnamyl alcohol. Three of the isolated benzoate esters have not been reported previously from nature. A comparison of the chemistry of different plant parts will be presented, as well as a consideration of the identified components relative to other members of genus *Desmos*. This work illustrates the potential for the discovery of new natural products from the NPDI plant extract collection, in particular the pre-fractionated plant extract set currently being developing.



3119

FIVE NOVEL *O*-METHYLATED BIOPTERIN GLYCOSIDES FROM TWO *MICROCYSTIS* BLOOMS MATERIALS

Marina Lifshits, Dimitry Kovalerchick and Shmuel Carmeli
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Five uniqe biopterins, 2',3',4'-tri-O-methyl-□-mannobiopterin (**1**), 3'-O-methyl-□-glucobiopterin (**2**), 3'-O-methyl-□-rhamnobiopterin (**3**), 3'-O-methyl-□-mannoheptobiopterin (**4**), and 6'-O-methyl-□-glucoobiopterin (**5**), were isolated from the extracts of two bloom materials of Microcystis spp. collected from a fishpond (IL-337) and Lake Kinneret (IL-347), Israel. The structure of the pterins was established interpretation of their UV, CD, 1D and 2D NMR spectra and HR mass measurements. Their antimicrobial and cytotoxic properties were evaluated but all were found not active in both assays. The structure elucidation of the coumpounds will be described.

3120

COMBINATORIAL BIOSYNTHESIS BY THE SPONGE *TEDANIA BRASILIENSIS* OPTIMIZES THE ANTI-PARASITIC ACTIVITY OF BROMOPYRROLE ALKALOIDS

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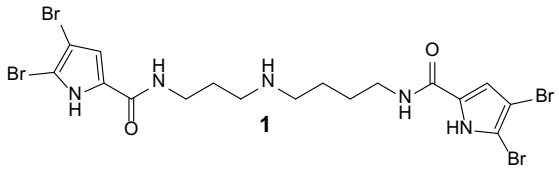
Tedania brasiliensis is a rare species of marine sponge endemic to Brazil, which gave an anti-viral, antileishmanial and antitrypanosomal extract. Bioassay-guided isolation yielded a series of 16 new pseudoceratidine (**1**) derivatives with variable number of bromines and length of the triamine chain, as well as tedamides, unique alkaloids within this series bearing a completely modified pyrrole moiety. The potency against Leishmania infantum and Trypanosoma cruzi parasites of the isolated alkaloids showed influence by the bromination level, results which will be presented and discussed.



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3121

MOLECULAR MECHANISMS THAT UNDERLIE THE SEXUAL STIMULANT ACTIONS OF (*SALVIA LANIGERA*) AND (*OSTEOSPERMUM VAILLANTII*).

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The effects of extracts and subfractions of *Salvia lanigera* rhizomes, *Osteospermum vaillantii* and sildenafil on the sexual behavior of male rats and their effects on the intracavernosal pressure (ICV), intracavernosal cyclic cyclic guanosine monophosphate (GMP) and dihydrotestosterone plasma level were examined. The sexual behavior was followed for four hours using infra-red video cameras to quantify the effects on various male sexual behaviors. The results revealed that the active subfraction in case of *S. lanigera* was the hexane fraction of the chloroformic extracts (C/H) whereas that of *O. vaillantii* was the acetonitrile fraction of the alcoholic extracts (A/Ac). (C/H), (A/Ac) and sildenafil significantly increased the total sexual stimulation index from 53.8 ± 2.7 (control) to 116 ± 3.8, 253 ± 2.7 and 401 ± 30.1, respectively. They significantly increased the index of successful mounting and ejaculation from 2.6 ± 0.5 (control) to 19 ± 0.5, 13 ± 0.6 and 18 ± 1.7, respectively. They significantly increased the cyclic GMP level from 0.94 ± 0.07 (control) to 2.81 ± 0.19, 2.65 ± 0.14 and 3.66 ± 0.19 ng/mg wet weight tissue, respectively. Furthermore, (C/H) increased plasma dihydrotestosterone level. Other treatments did not affect this parameter. (C/H), (A/Ac) and sildenafil increased the (I.CV) pressure. Both extracts and sildenafil acted via an increase in cyclic GMP with an additional increase in dihydrotestosterone release in case of (C/H).

3122

SCREENING OF PLANT EXTRACTS FOR ANTI-INFLAMMATORY ACTIVITY

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In our search for natural products with anti-inflammatory activity, which are comparable to that of the polyphenol resveratrol, we investigated a large number of medicinal plant extracts for their anti-inflammatory activity. Among the tested extracts were the *n*-hexane, dichloromethane, ethyl acetate and methanol extracts produced by sequential extraction of aerial parts and roots of the medicinal plants, *Rhodiola rosea*, *Achillea millefolium*, *Valeriana officinalis*, *Platycodon grandifloras*. Many of these extracts showed strong effect on interleukin-6 (IL-6) production in LPS-stimulated THP-1 macrophages. We found that all four extracts of roots of *V. officinalis* and *P. grandiflorus* as well as the *n*-hexane, ethyl acetate, and methanol extracts of aerial parts of *A. millefolium* were able to inhibit production of IL-6 in LPS-stimulated THP-1 cells comparable to the anti-inflammatory control resveratrol (50 μM). Interestingly some extracts, among them methanol extracts of roots of *R. rosea*, showed an inflammatory effect.

3123

ISOLATION OF CHROMOPHORIC AND NON-CHROMOPHORIC COMPOUNDS IN NATURAL PRODUCTS USING INTEGRATED FLASH AND PREPARATIVE CHROMATOGRAPHY

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Isolation and purification of active components from natural origin is a challenging process, traditionally requiring multiple steps and large amounts of organic solvent. Natural product extracts have been shown to contain both chromophoric and non-chromophoric components. Identifying non-chromophoric compounds in complex mixtures can be difficult because traditional ultraviolet (UV) detection fails to detect targets and impurities that are present at low levels or lack chromophores. Evaporative light scattering detection (ELSD) provides advantages over spectroscopic detectors for detecting compounds that are deficient in a UV-absorbing chromophore. Here we demonstrate the isolation and purification of capsaicins in chili powder to greater than 95% purity using a new "dual mode" flash chromatography and preparative chromatography system capable of detecting both chromophoric and non-chromophoric compounds. Additionally, non-chromophoric Stevioside & Rebaudioside A can be purified to 98.65% & 98.5% purity respectively from other non-chromophoric impurities using the same system. Isolation of these and other active compounds will be reviewed in detail demonstrating simple and effective purification on a single integrated system.

3124

PHYTOCHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITIES OF THE TRADITIONAL HERBAL MEDICINE *MARSDENIA TENACISSIMA*

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Marsdenia tenacissima (Roxb.) Wight et Arn is a traditional herbal medicine, which has been used for centuries in southwest China in the treatment of cancer, neoplasia, and other diseases. The drug made from *M. Tenacissima*, named as antitumor injection, had been proved high clinical effective for hepatocellular carcinoma, lung cancer, and gastric cancer. Previous studies indicated that the main effective components in *M. Tenacissima* were C_{21} steroidals. The aim of the present work is the elaboration of constituents and the cytotoxic activities of C_{21} steroidals in the title herb. Twenty-four compounds were isolated and structurally determined from the ethanol extract under the bioassay-guided procedure, including sixteen C_{21} steroidals, three azulenes, four disaccharide derivatives, and a bisepoxyligan. There are twelve novel compounds among them. The cytotoxic activities of C_{21} steroidals were evaluated using MTT assays on three human non small cell lung cancer cells, NCI-H292, NCI-H460, and NCI-H1975 cell lines. Two C_{21} steroidals, Tenacissoside I and Tenacigenin A, exhibited good potency against the three cell lines with IC_{50} values of 0.32 and 0.97, 0.74 and 2.69, 1.59 and 7.70 μM, respectively.The results support the ethnomedicinal usage of *M. Tenacissima*. Acknowledgements: Thank the supporting grant for NCET-12-0578, 13-0624, 111Project B08044 and YLDX01013.

3125

PHYTOCHEMICAL ANALYSIS OF THE HEPATOPROTECTIVE FRACTION OF *COMASTOMA PEDUNCULATUM* USING HPLC-DAD-ESI-MS/MS

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Comastoma pedunculatum (Rogle ex D. Dou) Holub is a traditional Tibetan herbal medicine used under the name "zangyinchen" for the treatment of liver and gallbladder disease. In the present work, we describe a phytochemical analysis to profile the hepatoprotective fraction (ethyl acetate extract) of the title herb using a high performance liquid chromatography-diode array detector electrospray ionization-tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) analytical method. Seventeen compounds, comprising 6 xanthones, 6 flavonoids, and 5 saponins, were separate and determinate in the hepatoprotective fraction, based on the comparison of their UV and MS data with those of authentic compounds and published data. Carbon tetrachloride-induced acute liver injury model was used for evaluation of antihepatotoxic activity of the ethyl acetate extract of *C. pedunculatum*. The biochemical marker enzymes in serum and liver homogenate of rat were assayed, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin(TB), along with the microscopic pathological examinations for liver tissue.These results may help to gain a better understanding of the therapeutic basis of *C. pedunculatum* and establish a better quality control method for this Tibetan herbal medicine. Acknowledgements: Thank the supporting grant for NSFC-81274158, 81373765, NCET-12-0578, 13-0624, SEAC-12ZYZ019, 111Project B08044 and YLDX01013.

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