

American Chemical Society
Division of Medicinal Chemistry
ABSTRACTS

227th ACS National Meeting

Anaheim, CA
March 28-April 01, 2004

D. L. Flynn, Program Chair

SUNDAY MORNING

• **Challenges for the Chemical Sciences in the 21st Century: Health and Medicine**

P. Gund, Organizer

Papers 1-5

• **Potassium Channels, Sponsored by Procter & Gamble**

J. M. Janusz, Organizer

Papers 6-10

SUNDAY AFTERNOON

• **First Time Disclosures**

B. Balasubramanian, Organizer

Papers 11-14

SUNDAY EVENING

• **Poster Session and Social Hour**

D. L. Flynn, Presiding

Papers 15-164

MONDAY MORNING

• **Neuroprotective Agents**

S. Ananthan, Organizer

Papers 165-168

MONDAY AFTERNOON

• **NMDA Receptors**

D. G. Brown, Organizer, Presiding

Papers 169-172

TUESDAY MORNING

• **General Oral Session**

D. L. Flynn, Presiding

Papers 173-182

• **General Oral Session**

D. L. Flynn, Organizer

Papers 183-193

TUESDAY AFTERNOON

• **Reactive Metabolites in Drug Design- Enhancing Drug Safety**

B. Balasubramanian, Organizer

Papers 194-198

WEDNESDAY MORNING

• **Diabetes**

B. Wang, Organizer

Papers 199-204

• **Engineered G-Protein Coupled Receptors**

K. A. Jacobson, Organizer; J. Wess, Presiding

Papers 205-209

WEDNESDAY AFTERNOON

• **Engineered Enzymes**

D. L. Flynn, Organizer

Papers 210-214

• **SERMs**

R. Weatherman, Organizer; R. A. Gibbs, Presiding

Papers 215-219

WEDNESDAY EVENING

• **Poster Session**

D. L. Flynn, Presiding

Papers 220-341

THURSDAY MORNING

• **General Oral Session**

D. L. Flynn, Presiding

Papers 342-352

DIVISION OF MEDICINAL CHEMISTRY

1.

PURPOSES OF THE NRC REPORT “CHALLENGES FOR THE CHEMICAL SCIENCES IN THE 21ST CENTURY”. *Douglas J. Raber*, Self, 4838 Butterworth Pl. NW, Washington, DC 20016, dlraber@verizon.net

The Workshop on Health and Medicine is one of six workshops held as part of the National Research Council's study "Challenges for the Chemical Sciences in the 21st Century." The workshop topics reflect areas of societal need—materials, energy and transportation, national security and homeland defense, health and medicine, information and communications, and environment. The charge for each workshop was to address the four themes of discovery, interfaces, challenges, and infrastructure as they relate to the workshop topic. These themes were the subject of breakout sessions at the workshop: * Discovery: What major discoveries or advances related to health & medicine have been made in the chemical sciences during the last several decades? * Interfaces: What are the major biomedical discoveries and challenges at the interfaces between chemistry/chemical engineering and other disciplines, including biology, information science, materials science, and physics? * Challenges: What are the biomedically-related grand challenges in the chemical sciences and engineering? * Infrastructure: What are the issues at the intersection of health & medicine and the chemical sciences for which there are structural challenges and opportunities—in teaching, research, equipment and instrumentation, facilities, and personnel? Published reports for the overall study and for each of the workshops are available from National Academies Press (www.nap.edu).

2.

STRUCTURAL PROTEOMICS AND DRUG DISCOVERY. *Stephen W. Kaldor*, Syrrx, Inc, 10410 Science Center Drive, San Diego, CA 92121, steve.kaldor@syrrx.com

Considerable advances have been made over the last fifty years in determining the structures of proteins and other naturally occurring macromolecules, providing key insights into structure-function relationships. This has been complemented with a major thrust toward target and mechanism-based approaches to disease modulation. Despite these accomplishments, it is sobering to note that the structures of less than five percent of the known protein families in the human genome have been solved, and that very few drugs have been brought to the marketplace that are true products of structure-based drug discovery. It is interesting to examine our current ability to access and use macromolecular structure and to speculate on what the future will hold. The field of structure-based drug discovery will be used to illustrate opportunities for twenty-first century chemists to advance the basic science in the area of macromolecular structure and simultaneously have a pronounced positive impact on human health.

3.

CHALLENGES IN CELL AND TISSUE ENGINEERING. *Linda G. Griffith*, Department of Chemical and Biological Engineering, Center for Biomedical Engineering, and Biotechnology Process Engineering Cent, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Room 66-466, Cambridge, MA 02139, griff@mit.edu

Cell and tissue engineering have captured the imagination of the press and the public. From artificial skin and bone for burn and accident victims, to growing "organs in a box" for organ transplants, the potential medical advances are revolutionary. However, there are many materials science, biochemical, bioengineering, and other challenges standing in the way of turning these opportunities into clinical reality. This talk will review the considerable progress being made on several fronts.

4.

CHALLENGES IN DELIVERY OF DRUGS. *W. Mark Saltzman*, Biomedical Engineering, Yale University, 15 Prospect St., New Haven, CT 06520, Fax: 203-432-0030, mark.saltzman@yale.edu

An impressive number of new medicines have been discovered during the twentieth century, so that some fatal diseases are now in control. Still, premature death and disability from cancer and infectious disease remain world-wide health problems. In some situations, these diseases progress in the presence of effective medication or preventative vaccines, simply because active agents are not administered in an effective manner. Modern engineering presents new opportunities in therapeutics: engineering analysis is essential in understanding the mechanisms of drug transport in normal and diseased tissue. In addition, new materials, such as biocompatible polymers, can be used to produce drug delivery systems that control the rate of delivery, local transport, and metabolism of drugs at tissue sites.

5.

CHALLENGES FOR CHEMISTRY IN HEALTH AND MEDICINE. *Ronald Breslow*, Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027, Fax: 212-9542755

Medicinal chemistry is responsible for most of the incredible 30 year increase in human life expectancy in the U.S. over this past century. Even so, there are many challenges remaining for the current and future generations of chemists to address and solve. This lecture will point out the need for progress in the remaining disease areas, to be achieved by medicinal chemists working Beyond the Molecular Frontier. An example will be described from the authoR's own work.

6.

INTRODUCTION TO POTASSIUM CHANNEL BIOLOGY AND MEDICINAL CHEMISTRY. *Michael J. Coghlan*, Mail Drop Code 0528, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-433-3666, coglan_michael@lilly.com

Potassium channel biology offers the medicinal chemist a variety of attractions and challenges for pharmaceutical intervention. Selectivity and efficacy are priorities for small molecule potassium channel modulators, and many of the tools commonly used by medicinal chemists to evaluate such compounds are specific to the ion channel field. This presentation will provide a perspective of potassium channel modulation, emphasizing the medicinal chemistry associated with several targets. The breadth of potential therapeutic indications enabled by potassium channels for therapy and risk assessment in cardiovascular disease, urology, endocrinology, and immunology will be discussed as a sampling of the progress of this growing research area.

7.

TARGETING THE INHIBITION OF SPONTANEOUS MYOGENIC CONTRACTIONS AS A BASIS FOR KATP OPENERS WITH SELECTIVITY FOR BLADDER. *William A. Carroll¹, Michael E. Brune¹, Steve A. Buckner¹, Murali Gopalakrishnan¹, Michael J. Coghlan², Victoria E. S. Scott¹, Char-Chang Shieh¹, and James P. Sullivan¹.* (1) Neuroscience Research, GPRD, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064-6101, Fax: 947-935-5466, william.a.carroll@abbott.com, (2) Eli Lilly and Company

K_{ATP} channels are expressed in a variety of tissue types (pancreas, bladder, heart, vasculature) and have been explored as drug discovery targets for conditions such as diabetes, airway hyperreactivity, overactive bladder (OAB), hypertension and myocardial ischemia. Molecular targets for the K_{ATP} channel complexes present in each of the aforementioned organs have been identified, with those present in bladder and vasculature being highly homologous.

The discovery of K_{ATP} channel modulators with target organ specificity is contingent upon the utilization of *in vitro* and *in vivo* assays that reflect the heterogeneity of structure and function of the K_{ATP} channels present in different tissue types. The relative susceptibility of spontaneous myogenic bladder

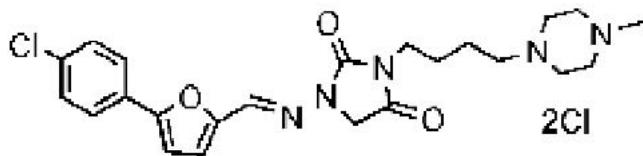
contractions towards relaxation by K_{ATP} openers presents an opportunity to identify bladder selective agents based upon tissue specific regulation by the K_{ATP} channel complex. In vitro and in vivo assays utilized to characterize bladder selective ligands will be described.

8.

VOLTAGE-GATED POTASSIUM CHANNEL INHIBITORS AS CLASS III

ANTIARRHYTHMIC AGENTS. *Shengde Wu, and John Janusz, Division of Medicinal Chemistry, Health-Care Center, Proctor & Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45040, Mason, OH 45040, Fax: 513-622-0086, wu.s.3@pg.com*

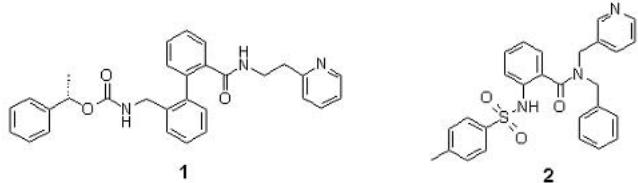
Class III antiarrhythmic agents prolong action potential duration in vitro and effective refractory period in vivo via blockade of repolarizing potassium currents. However, excessive prolongation of cardiac repolarization at slow heart rates has been strongly implicated in the proarrhythmic and potentially life-threatening side effects of existing Class III antiarrhythmic drugs that block IKr. We have taken two approaches towards overcoming these limitations. The first involves compounds with positive rate dependence where the class III effect is present at high heart rates but absent at slow heart rates. The second involves compounds that selectively inhibit Kv1.5, an important repolarizing current that is functional only in human atria. A starting point for these efforts was azimilide, a combined IKr & IKs blocker currently in phase III clinical testing. This talk will describe the evolution of rate dependent class III antiarrhythmic agents and potent, selective Kv1.5 blockers from the azimilide lead.



9.

IDENTIFICATION, SYNTHESIS, AND BIOLOGICAL EVALUATION OF TWO NOVEL CLASSES OF KV1.5 INHIBITORS. *Stefan Peukert¹, Joachim Brendel¹, Bernard Pirard¹, Heinz-Werner Kleemann¹, Thomas Boehme¹, Peter Below¹, Carsten Struebing², Klaus Wirth², and Heinz Goegelein². (1) Medicinal Chemistry, Aventis Deutschland GmbH, Building G 838, D-65926 Frankfurt, Germany, Fax: +49-69-331399, stefan.peukert@aventis.com, (2) DG Cardiovascular, Aventis Deutschland GmbH*

Currently available drug treatment for atrial fibrillation, a common cardiac arrhythmia, is less than satisfactory and fraught with severe difficulties. The voltage-gated potassium channel Kv1.5 is regarded as a promising target for the development of atrial selective drugs with fewer ventricular side effects. In this lecture the identification, synthesis, and biological evaluation of two chemical series of Kv1.5 blockers, namely the bisaryls compounds (e.g. 1) and the anthranilic amides (e.g. 2) will be discussed. The most potent compounds display sub-micromolar inhibition of Kv1.5 and no significant effect on the HERG channel. For selected candidates from both series pharmacological data in pigs and goats are presented which show their usefulness in atrial fibrillation.



10.

STRUCTURE, FUNCTION AND MODULATION OF MAXI-K AND KCNQ

POTASSIUM CHANNELS. *Valentin K. Gribkoff¹, John E. Starrett Jr.², and Steven I. Dworetzky¹. (1) Neuroscience Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, Fax: 203-677-7569, valentin.gribkoff@bms.com, (2) Neuroscience Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute*

Potassium (K^+) channels are a varied group of membrane proteins with a large number of gene families. Most are classified based on structure or some feature

of their regulation. We will examine characteristics of two types of K^+ channel, the large-conductance calcium- (Ca^{2+}) dependent (maxi-K or BK, Slo, or KCa1.1) K^+ channels, and the neuronal voltage-dependent KCNQ channels (KCNQ2-5, or Kv7.2-7.5). Maxi-K channels are both voltage- and Ca^{2+} -regulated, opening as the intracellular $[Ca^{2+}]$ increases or with membrane depolarization. They hyperpolarize the membrane, reducing voltage-dependent Ca^{2+} entry. Maxi-K channels are found in smooth muscle cells and neurons, where they regulate contractility and Ca^{2+} -dependent neuronal functions, such as neurotransmission, respectively. In neurons, maxi-K channels contribute to fast membrane repolarization and may provide resistance to hypoxia-dependent toxicity related to high intracellular Ca^{2+} concentrations. Neuronal KCNQ channels are widely distributed, and as KCNQ2/3 and KCNQ5/3 heteromultimers, they constitute the slowly-activating, non-inactivating M-channels, which are inhibited by muscarinic cholinergic reception. They are a major component of basal neuronal excitability. Mutations have been linked to a number of channelopathies, notably benign neonatal familial convulsions (BFNC's) and neurogenetic myokymia (KCNQ2 and KCNQ3), and congenital deafness (KCNQ4). Small molecule modulators have been discovered and characterized for maxi-K channels and neuronal KCNQ channels. There is considerable overlap in modulator libraries for these channels. We will discuss possible mechanisms of modulation that may underlie this pharmacological intersection.

11.

DISCOVERY AND PRECLINICAL PROFILE OF A HIGHLY POTENT AND MUSCLE SELECTIVE ANDROGEN RECEPTOR MODULATOR (SARM). *Lawrence G. Hamann, Discovery Chemistry, Bristol-Myers Squibb Company, P. O. Box 5400, Princeton, NJ 08543-5400, Lawrence.hamann@bms.com*

The Androgen Receptor (AR) is a member of the Nuclear Hormone Receptor (NHR) superfamily of intracellular ligand-dependent transcription factors which mediate gene-transcription to regulate protein synthesis and cellular processes in various tissues. Traditional definitions of agonist and antagonist action associated with the natural steroid ligands testosterone and dihydrotestosterone give way to a continuum of pharmacological response achievable with non-steroidal ligand scaffolds. By analogy to the gains in understanding of the endocrinology mediated by the Selective Estrogen Receptor Modulators (SERMs), which has evolved over the past twenty years, Selective Modulators of the transcriptional activity of the AR (SARMs) show great promise in achieving the beneficial effects of classical pure agonist compounds without the associated side-effects. Emerging SARM drug candidates have the potential to provide safer treatments with fewer side-effects for a wide range of diseases and conditions, including muscle wasting from HIV, cancer chemotherapy, or chronic renal failure, male hypogonadism, and benign prostatic hyperplasia. Additionally, SARMs have the potential to provide novel treatments for male andropause, also known as androgen decline in the aging male (ADAM), and both osteoporosis and sexual dysfunction in both men and women. We have discovered a novel series of SARMs which have the potential to provide clinical benefit with reduced risk of side-effects. The preclinical profile of select compounds from this series and a discussion of selectivity mechanisms will be presented.

12.

DISCOVERY OF A PDE5 INHIBITOR FOR THE TREATMENT OF MALE ED. *Craig D. Boyle¹, Samuel Chackalamannil¹, Claire M. Larkin¹, Yuguang Wang¹, Zhiyong Hu¹, Theodoros Asberom¹, John W. Clader¹, William J. Greenlee¹, Henry Guzik¹, Dmitri Pissarnitski¹, Andrew W. Stamford¹, Ruo Xu¹, Jeffrey Skell², Stanley Kurowski³, Subbarao Vemulapalli³, Jairam Palamanda⁴, Mahdu Chintala³, Ping Wu³, Joyce Myers³, and Peng Wang³. (1) CV/CNS Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, Fax: 908-740-7152, craig.boyle@spcorp.com, (2) Pharmaceutical Development, Schering-Plough Research Institute, (3) Biological Research, Schering-Plough Research Institute, (4) Department of DMPK, Schering-Plough Research Institute*

Using a stepwise approach to improve upon the physical and pharmacological properties of a xanthine lead structure, we discovered a PDE5 inhibitor for the

treatment of male ED. This compound improves upon the PDE isozyme selectivity, enzyme inhibition, and PK profile of the leading drug on the market, sildenafil (Viagra). This paper will summarize the medicinal chemistry effort toward the discovery of potent and selective PDE5 inhibitors.

13.

DISCOVERY OF UK-383,367, A POTENT AND SELECTIVE NON-PEPTIDIC INHIBITOR OF PROCOLLAGEN C-PROTEINASE FOR THE TREATMENT OF DERMAL SCARRING. *Simon Bailey, Paul V. Fish, Kim James, and Andrew McElroy, Discovery Chemistry, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, CT13 9NJ, United Kingdom, simon.bailey@pfizer.com*

This presentation describes the discovery of a novel series of inhibitors of procollagen C-proteinase (PCP) which may have potential as anti-fibrotic agents. A member of this class, UK-383,367, is shown to be highly selective against matrix metalloproteinases involved in wound healing processes and to inhibit collagen deposition in a human fibroblast fibroplasia model. Furthermore, UK-383,367 is shown to permeate human skin, which may be advantageous in the treatment and prophylaxis of dermal scars.

14.

TIPLAXTININ: A NOVEL ORALLY EFFICACIOUS INHIBITOR OF PAI-1 FOR USE IN TREATMENT OF DISEASES OF FIBRINOLYTIC DYSFUNCTION. *Hassan*

Elokda¹, Geraldine R. McFarlane², David Z. Li¹, John A. Butera¹, Magid Abou-Gharbia¹, Girija Krishnamurthy³, James Hennan⁴, Gregory Friedrichs⁴, and David L. Crandall⁴. (1) Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, elokdah@wyeth.com, (2) Chemical Sciences, Wyeth-Ayerst Research, (3) Screening Sciences, Wyeth Research, (4) Cardiovascular and Metabolic Diseases Research, Wyeth Research

The serine protease inhibitor plasminogen activator inhibitor-1 (PAI-1) regulates fibrinolysis through its modulation of plasmin, and increased plasma PAI-1 is associated with diseases of fibrinolytic impairment. PAI-1 is the physiologic inhibitor of both urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), and its elevation is associated with clot stabilization in acute thrombosis as well as tissue remodeling occurring during atherosclerosis and cancer. The central role of plasmin in these diverse diseases suggests that inhibition of PAI-1 has potential therapeutic benefit, yet an orally active PAI-1 inhibitor has not yet been described. We present the discovery of Tiplaxtinin, a novel indole-oxoacetic acid derivative that both binds PAI-1 with high affinity ($K_d=480$ nM) and exhibits oral efficacy in preclinical models of arterial and venous thrombosis. We also describe the synthesis and structure-activity relationship studies leading to the discovery of Tiplaxtinin, the biological data predictive of its utility, and the preclinical safety assessment leading to its selection as a clinical candidate.

15.

DESIGNING AN ENRICHED SCREENING LIBRARY FOR THE DISCOVERY OF NON-NUCLEOSIDE INHIBITORS OF THE HCV NS5B RNA POLYMERASE. *Uli Schmitz, Martin Kirk, Kevin Fung, Samantha Koo McCoy, Derek Latour, Emil Michelotti, Jeff Pouliot, Kevin Dunlop, Christopher Roberts, Lillian Lou, and Ronald Griffith, Genelabs Technologies, Inc, 505 Penobscot Drive, Redwood City, CA 94063, Fax: 650-368-0709, ulis@ganelabs.com*

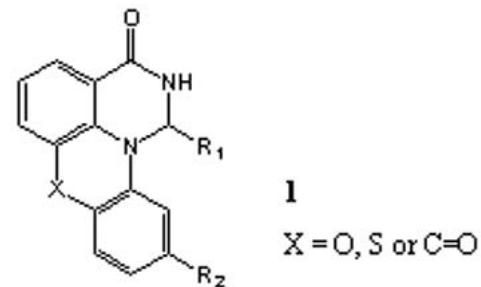
Hepatitis C is considered a major public health threat and current therapies still call for major improvements. The virus causing Hepatitis C (HCV) is a single-stranded RNA virus, whose replication in liver cells relies on several virally-encoded nonstructural proteins, including the NS5b RNA-dependent RNA polymerase. To date, a few non-nucleoside inhibitors have been published, but the wide range of inhibitors for HIV reverse transcriptase, a functionally and structurally closely related polymerase, are largely inactive against HCV NS5b. Recognizing that kinases and RNA polymerases have a common substrate, ATP, one would expect that a compound collection enriched with the chemotypes found among a plethora of kinase inhibitors should have a higher hit-rate against the NS5b polymerase compared to a diverse random library of the same size. Based on chemotypes found in ~50 diverse kinase and ATPase inhibitors found in the literature, we selected over 30,000 compounds from one particular commercial source and subjected them to a rigorous diversity pruning step. Our final HTS library indeed produced a hit rate of 0.88 % (>50% inhibition at 5 μ M)

in our primary NS5b polymerase assay. Details of the selection process and the screening results will be discussed.

16.

SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,2-DIHYDRO-2,11B-DIAZA-BENZO[DE]ANTHRACEN-ONE DERIVATIVES AS POTENT POLY(ADP-RIBOSE) POLYMERASE (PARP-1) INHIBITORS. *Weizheng Xu, Joan Chen, Brian Grella, Yao-Sen Ko, Shirley Huang, Susan Lauter, Jia-he Li, Qun Liu, Larry Williams, Qi Yi Wu, Jie Zhang, and Vincent Kalish, Guilford Pharmaceuticals Inc, 6611 Tributary Street, Baltimore, MD 21224, Fax: 410-631-6797, xuw@guilfordpharm.com*

Poly(ADP-ribose) polymerase (PARP-1) is an abundant eukaryotic nuclear enzyme which is involved in DNA repair. PARP-1 inhibition has been shown to protect normal tissues from free radical-induced necrosis and to enhance killing of tumors by radiation or chemotherapeutic agents. PARP-1 inhibitors are being developed for treating ischemia-reperfusion injuries and as adjunct treatment for cancer. Utilizing substrate-based knowledge and crystal structure information, a novel series of PARP-1 inhibitors (1) were designed and synthesized. This series of PARP-1 inhibitors are potent in vitro enzyme inhibitors and offer protection against hydrogen peroxide induced cell death. Preliminary results have demonstrated that these PARP-1 inhibitors reduce ischemic tissue damage in vivo.



17.

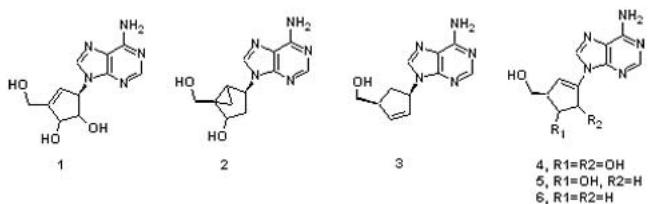
UNDERSTANDING THE INTEGRASE INHIBITORY ACTIVITY OF AZIDO CONTAINING HIV-1 INTEGRASE INHIBITORS. *Rajeshri G. Karki, and Marc C Nicklaus, Laboratory of Medicinal Chemistry, National Cancer Institute, National Institute of Health, Building 376, Boyles Street, Frederick, MD 21702, Fax: 301-846-6033, rajeshri@helix.nih.gov*

HIV-1 integrase (IN) catalyzes the integration of viral DNA into human DNA and has no direct analog in humans, thereby making it an additional attractive target for treatment of acquired immunodeficiency syndrome (AIDS). Aryl β -diketo acids (ADK) comprise a general class of potent IN inhibitors, with abilities to selectively inhibit the strand transfer reaction in extracellular recombinant IN assays. Also, azido-containing ADK's were found to be potent inhibitors of IN providing antiviral protection in HIV-infected cells. These results have rendered the azido group of potential value in the further development of ADK-based IN inhibitors. In an attempt to understand the role of the azido group toward IN inhibition we have carried out a systematic study using molecular modeling approaches. High-throughput docking of commercially available compound databases followed by screening of some selected compounds has helped in answering some of the questions. The results obtained so far will be presented here.

18.

NEW CLASS OF RING CONSTRAINED CARBOCYCLIC NUCLEOSIDES BASED ON NEPLANOCIN A. *Xueqiang Yin, and Stewart W Schneller, Department of Chemistry, Auburn University, Chemistry building, auburn, AL 36849*

Considerable evidence exists that introduction of a rigid structural element into the cyclopentane of carbocyclic nucleoside can lead to effective antiviral agents. In this regard, the presence of a double bond in neplanocin A (1) and in 3, and a cyclopropane ring in 2 is a structural feature that is important for their potent antiviral or antitumor effects. Following this lead, a new class of ring constricted carbocyclic nucleosides (4, 5, 6), which feature a 1',6' double bond were designed. The synthesis, including a highly efficient method of forming the 1' and 6' olefin, and the antiviral activity of 4, 5, 6 will be reported. This research is supported by the Department of Health and Human Services (AI 48495 and AI56540).

**19.**

SYNTHESIS OF 5-(1-PROPYNYL)-2'-DEOXYURIDINE 5'-(α -P-BORANO)TRIPHOSPHATE AND ITS INCORPORATION INTO BORANOPHOSPHATE OLIGONUCLEOTIDES. Joyce Xin Wang, Mikhail I. Dobrikov, and Barbara Ramsay Shaw, Department of Chemistry, Duke University, Box 90348, Durham, NC 27708

Oligodeoxyribonucleoside boranophosphate (BP-ODN), in which a borano (-BH₃) group replaces one non-bridging oxygen of the phosphodiester backbone, is one of only a few oligonucleotide analogues being able to induce RNase H-mediated hydrolysis of the complementary RNA strand. On the other hand, the C5-(1-propynyl) substitution on pyrimidines was shown to increase melting temperatures of DNA:RNA hybrids, while retaining the DNA strands' RNase H activity. We purpose that introducing C5-(1-propynyl) pyrimidines into boranophosphate ODNs would increase the BP-ODNs' binding affinity with complementary RNA, improve their RNase H activity, and increase their nuclease resistance. 5-(1-Propynyl)-2'-deoxyuridine 5'-(α -P-borano)triphosphate was thus synthesized. The two diastereomers were separated by reverse phase HPLC, their substrate properties for different DNA polymerases and viral reverse transcriptases, and the physico-chemical properties of the synthesized C5-propynyl BP-ODN were investigated.

20.

DESIGN AND SYNTHESIS OF THE FLUORINATED CYCLOPROPANOID NUCLEOSIDE. Joohyun Kim, Mijung Jang, Hee-Doo Kim, and Ju-Hyun Park, School of Pharmacy, Sookmyung Women's University, Chungpa-dong, Yongsan-ku, Seoul 140-742, South Korea, Fax: 2-703-0736, hdkim@sookmyung.ac.kr

In an effort to search for the chemically and enzymatically stable carbonucleoside, we designed a series of the fluorinated cyclopropanoid nucleosides. The underlying concept for our design is to seek relatively conformationally-locked compound with minimal structural disturbance from acyclic carbonucleoside such as acyclovir or penciclovir. To meet such a requirement, we need to introduce cyclopropane and fluorine moiety. Due to its hybrid character of cyclic and acyclic molecules, cyclopropyl group could render the conformational rigidity to the target molecule. It has also been suggested that a fluoromethylene is a better isostere of oxygen than is methylene. Therefore, cyclopropanoid derivatives substituted by fluorine at the oxygen position in natural nucleoside are also attractive targets. Herein, we report on the design and syntheses of the fluorinated cyclopropanoid nucleosides. The synthesized nucleosides were evaluated for their activity against the polio virus, HSV-1, HSV-2, HBV and HIV.

21.**DEVELOPMENT OF 3D-QSAR COMFA MODELS FOR ANTI-AIDS**

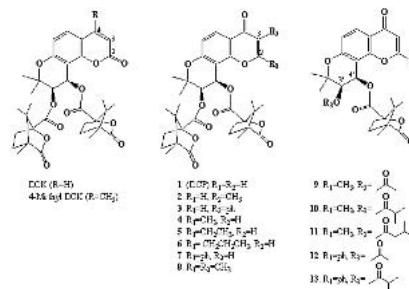
DIARYLSULFONES. Geetha T. Mukundan, and Murray Zanger, Department of Chemistry and Biochemistry, University of the Sciences in Philadelphia, 600 S 43rd Street, Philadelphia, PA 19104, Fax: 215-596-8543, gmukundan@hotmail.com

Three-dimensional quantitative structure-activity relationship (3D-QSAR) models are derived using comparative molecular field analysis (CoMFA) to correlate biological activities of a series of diarylsulfones, a structurally different chemical class of non-nucleoside reverse transcriptase inhibitors (NNRTI). A large set of 100 diarylsulfones was employed for this study. All modeling work was performed with SYBYL molecular modeling software. The best comfa model ($q^2_{cv}=0.682$) is statistically significant and demonstrated modest predictive ability. Several models were developed to derive the best model and all the models derived thus far in this study indicated that steric interactions (64-70%) contribute more towards biological activity than the electrostatic interactions. The best comfa model was used to design and predict more potent drug molecules.

22.

SUBSTITUTED 3'R,4'R-DI-O-(-)-CAMPHANOYL-2',2'-DIMETHYLDIHYDROPYRANO[2,3-F]CHROMONE (DCP) DERIVATIVES AS POTENT ANTI-HIV AGENTS. Donglei Yu¹, Chin-Ho Chen², Arnold Brossi¹, Nicole Kilgore³, and Kuo-Hsiung Lee¹. (1) Division of Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, Fax: 919-966-3893, dyu@unc.edu, (2) Medical Center, Duke University Medical Center, (3) Panacos Pharmaceuticals, Inc

In our continuing structure-activity relationship study on both 3'R, 4'R-di-O-(-)-camphanoyl-(+)-cis-khellactone (DCK) and 3'R, 4'R-di-O-(-)-camphanoyl-2',2'-dimethylidihydropyrano[2,3-f]chromone (DCP, **1**) series and to increase the anti-HIV activity in drug resistant strains, 12 new DCP analogues with substituted chromone rings were designed and synthesized. They were tested *in vitro* for suppression of HIV-1 replication in H9 lymphocytes (non drug resistant strain), as well as against the HIV-1 RTMDR1 multi-RT inhibitor resistant viral strain in MT4 cells. Compounds **1** (DCP) and **2** exhibited extremely high anti-HIV activity in the non drug resistant strain assay, with EC₅₀ values of 0.0013 μ M and 0.00099 μ M and remarkable therapeutic indexes (TI) of 1.11 \times 10⁴ and 1.48 \times 10⁴, respectively. Other methyl DCPs also showed very potent anti-HIV activity. However, the most significant compounds in the multi-RT inhibitor resistant HIV-1 strain were **5** (EC₅₀ 0.06 μ M, TI 718) and **6** (EC₅₀ 0.139 μ M, TI 272). The unsubstituted parent compound (**1**) was inactive, as were DCK, 4-methyl DCK, and AZT. Several DCP derivatives with different substitution on the 3'-position (**9-13**) were synthesized and evaluated in both assays. In the non drug resistant strain, **11** (3'-isovaleryl) was most potent (EC₅₀ 0.0057 μ M, TI >5600), while in the multi-RT inhibitor resistant HIV-1 strain, **10** and **11** (EC₅₀ 0.30 and 0.59 μ M, respectively) showed similar activities with corresponding di-camphanoyl compound (**4**). This research is aided by NIH grant No. AI 33066 (K.H. Lee).

**23.**

2'-DEOXY-2'-GEMDIFLUORO AND 3'-DEOXY-3'-GEMDIFLUORO-5'-NORASTEROMYCIN AND THEIR ANTIVIRAL ACTIVITIES. Atanu Roy, and Stewart W. Schneller, Department of Chemistry, Auburn University, Auburn, AL 36849, Fax: 334 844 0239, royatana@auburn.edu

Inhibitors of S-adenosyl-L-homocysteine (AdoHcy) hydrolase have provided a wealth of structural entities with potentially significant antiviral properties. Included in that group is 5'-norasteromycin (**1**)¹ (Figure 1). In seeking to improve upon these properties the gem-difluoro moiety² as a group that renders increased lipophilicity and physicochemical properties to a molecule attracted our attention. In that direction, the 5'-nor gem-difluoro analogs **2** and **3** were prepared and evaluated. The results of this investigation will be reported. This research has been supported by DHHS (AI 48495 and AI 56540).

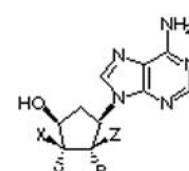


Figure 1

24.

8-FLUORO-5'-NORARISTEROMYCIN AND ITS ANTIVIRAL PROPERTIES. Atanu Roy, and Stewart W. Schneller, Department of Chemistry, Auburn University, Auburn, AL 36849, Fax: 334 844 0239, royatan@auburn.edu

Due to its apparent inhibition of S-adenosyl-L-homocysteine (AdoHcy) hydro-lase,¹ 5'-noraristeromycin (**1**)² and its enantiomer (**3**)³ (Figure 1) display significant biological activity. In seeking new nucleoside analogs endowed with potent antiviral activity, modification of the base of **1** and **3** have been sought. As a part of our ongoing drug discovery research program, we became interested in the 8-fluoro substituted compounds of 5'-noraristeromycin, (**2** and **4**). These compounds have been prepared from a common precursor. The synthesis and antiviral properties of **2** and **4** will be described. This research has been supported by DHHS (AI 48495 and AI 56540).

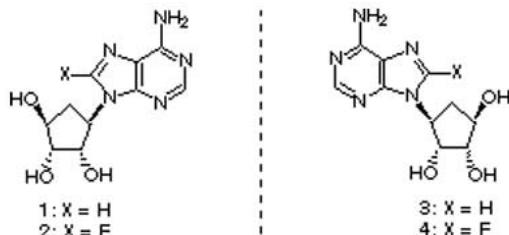
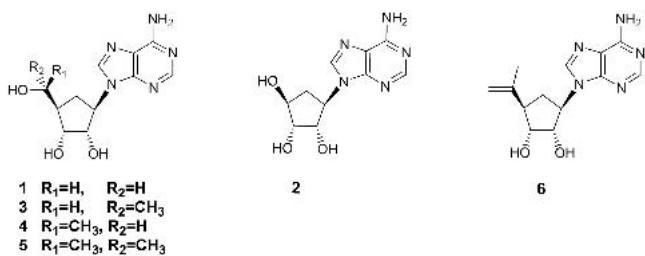


Figure 1

25.

SYNTHESIS AND BIOLOGICAL PROPERTIES OF 5'-METHYL MODIFIED ARISTEROMYCIN ANALOGUES. Wei Ye, and Stewart W. Schneller, Department of Chemistry, Auburn University, 179 Chemistry Building, Auburn University, Auburn, AL 36849, yewei01@auburn.edu

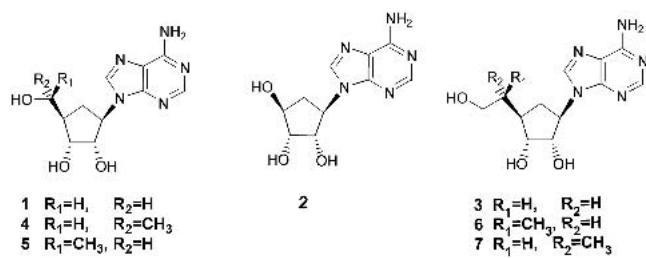
The antiviral potential of aristeromycin (**1**) is limited by its toxicity as a result of its metabolism to the 5'-nucleotide derivatives. In seeking ways around these undesirable transformations, 5'-noraristeromycin (**2**) was synthesized and found to have broad antiviral activity with reduced toxicity, likely due to its inability to undergo phosphorylation. The 5'-modified analogue, 5'-methylaristeromycin, was considered another derivative that, because of steric hindrance at the 5'-center, might not be susceptible to nucleotide formation but yet retain the biological properties of aristeromycin. The synthesis and antiviral activities of enantiopure 5'-methylaristeromycin (**3**, **4**) and other 5'-methylmodified aristeromycin analogues (**5**, **6**) will be described. This research was supported by funds from the Department of Health and Human Services (AI48495 and AI56540).



26.

SYNTHESIS AND BIOLOGICAL PROPERTIES OF 5'-METHYL HOMOARISTEROMYCIN. Wei Ye, and Stewart W. Schneller, Department of Chemistry, Auburn University, 179 Chemistry Building, Auburn University, Auburn, AL 36849, yewei01@auburn.edu

The broad-spectrum antiviral activity of aristeromycin (**1**) as an *S*-adenosylhomocysteine hydrolase inhibitor is limited by its toxicity due to 5'-nucleotide formation. 5'-Noraristeromycin (**2**) and 5'-homoaristeromycin (**3**) are two analogues of **1** from our labs that show significant antiviral activity without associated toxicity. 5'-Methylaristeromycin (**4/5**) has shown similar improved properties. Combining the features of **4/5** and 5'-homoaristeromycin (**3**) led to the desire to prepare and evaluate 5'-methylhomoaristeromycin (**6** and **7**). The results of this effort will be reported. This research was supported by funds from the Department of Health & Human Sciences (AI48495 and AI56540).



27.

HEPDIRECT PRODRUGS OF ADEFOVIR: DESIGN, SYNTHESIS AND OPTIMIZATION. K. Raja Reddy, Michael C. Matelich, Bheemrao G. Ugarkar, Jorge E. Gomez-Galeno, Joesph J. Kopcho, Serge H. Boyer, Jay S. DaRe, Zhili Sun, William A. Craig, Kristin Ollis, Timothy J. Colby, Paul D. van Poelje, and Mark D. Erion, Departments of Chemistry and Biochemistry, Metabasis Therapeutics, Inc, 9390 Towne Centre Dr, San Diego, CA 92121, Fax: 858-622-5573, rajar@mbasis.com

Adefovir dipivoxil, a prodrug of the nucleotide adefovir, has demonstrated efficacy in naïve and lamivudine-resistant Hepatitis-B (HBV) patients with minimal development of drug resistance. However, its maximal efficacy is limited by renal toxicity associated with high systemic exposure of adefovir. In an effort to enhance efficacy without increasing the risk of renal toxicity, Metabasis applied its HepDirect™ prodrug technology, which targets the active form of certain drugs to the liver, to adefovir. HepDirect prodrug analogs of adefovir were designed to optimize the activation and the byproduct properties. Synthesis of HepDirect prodrug analogs was achieved via coupling of adefovir with both enantiopure and racemic substituted 1, 3-propanediols. These HepDirect prodrugs of adefovir were evaluated in *in vitro* microsomal activation assays. Synthesis and SAR optimization of HepDirect prodrugs of adefovir resulted in the identification of MB06866, which is currently undergoing clinical development.

28.

MB06866 (HEPAVIR B), A HEPDIRECT PRODRUG OF ADEFOVIR: MECHANISM OF ACTIVATION AND LIVER TARGETING. K. Raja Reddy, Jorge E. Gomez-Galeno, Robert H. Lemus, Timothy J. Colby, Paul D. van Poelje, and Mark D. Erion, Departments of Chemistry and Biochemistry, Metabasis Therapeutics, Inc, 9390 Towne Centre Dr, San Diego, CA 92121, Fax: 858-622-5573, rajar@mbasis.com

MB06866 (Hepavir B) is a HepDirect™ prodrug of adefovir designed to enhance the therapeutic index of adefovir by targeting it specifically to the liver and thereby avoiding the renal safety issues associated with maximally efficacious doses of adefovir dipivoxil (Hepsara) therapy. The mechanism of activation of MB06866 was evaluated in microsomal, cellular, and *in vivo* metabolism studies. The studies indicated that MB06866 is activated via a hydroxylation reaction catalyzed by the cytochrome P450 isozyme, CYP3A4. The abundant and predominant expression of this isozyme in the liver forms the basis of its 12-fold improved liver/kidney AUC ratio relative to adefovir dipivoxil (Hepsara). The byproduct of prodrug activation was found to undergo rapid conjugation with glutathione and to be eliminated in this form. In hepatitis B patients, MB06866 is expected to show improved safety and antiviral activity relative to adefovir dipivoxil therapy.

29.

SYNTHESIS OF A NEW POTENTIAL PET GENE REPORTER PROBE 2-AMINO-6-(4-[¹¹C]METHOXYPHENYLTHIO)-9-[2-(PHOSPHONOMETHOXY)ETHYL]PURINE BIS(2,2,2-TRIFLUOROETHYL) ESTER FOR IMAGING HEPATITIS B VIRUS IN LIVER CANCER. Ji-Quan Wang, Qi-Huang Zheng, Xiangshu Fei, and Gary D. Hutchins, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L-3 Room 202, Indianapolis, IN 46202-2111, Fax: 317-278-9711, jiqwang@iupui.edu, xfei@iupui.edu

Hepatitis B virus (HBV) infection is responsible for both acute and chronic hepatitis. Chronic HBV infection dramatically increases risks for development of liver cancer and cirrhosis. WHO estimates about 400 million chronic carriers worldwide, with roughly 4 million deaths annually from the resulting cirrhosis and hepatocellular carcinoma. Treatment of HBV infection constitutes one of the therapeutic challenges in virology, and only a few drugs are currently available

for the clinical treatment of HBV. 2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (ABE) showed potent HBV-specific antiviral activity *in vitro*, and its active metabolite was highly detected in the liver. Compound ABE might be suitable for hepatitis B chemotherapy. Carbon-11 labeled nucleoside analogue [¹¹C]ABE was synthesized as a potential positron emission tomography (PET) gene reporter probe for imaging hepatitis B virus in liver cancer.

30.

DAG-LACTONES AS HIGH-AFFINITY PROTEIN KINASE C LIGANDS AND

ALPHA-SECRETASE ACTIVATORS. Jeewoo Lee, Ji-Hye Kang, Sung-Eun Kim, and Yerim Kim, Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea, Fax: 82-2-888-0649 jeewoo@snu.ac.kr

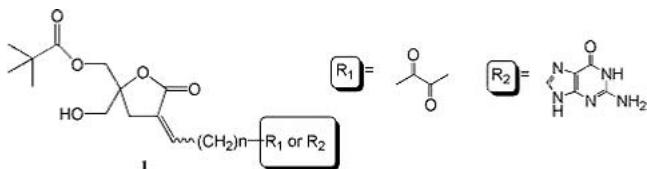
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31

NEW DESIGN APPROACHES AIMED AT TARGETING ATYPICAL PKC ISOFORMS

ZETA WITH DIACYLGLYCEROL-LACTONES (DAG-LACTONES). Ji-Hye Kang¹, Megan L. Peach¹, P. M. Blumberg², and Victor E. Marquez¹. (1) Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, NIH, Bldg. 376, Boyles Street, Frederick, MD 21702, jhkang@helix.nih.gov, (2) Laboratories of Medicinal Chemistry and of Cellular Carcinogenesis and Tumor Promotion, Division of Basic Sciences, National Cancer Institute, NIH

A constrained DAG-Lactone scaffold (1) common to ligands displaying high binding affinity toward conventional and novel PKC isozyme was modified with specific R1 and R2 groups to target the atypical PKC isoform, PKC-zeta. Since arginine residues serving as anion recognition sites are found in the binding region of PKC-zeta. The alpha-dicarbonyl group and the guanine base were employed to either form a reversible dihydroxyimidazole adduct or a strong hydrogen-bonding network with the target arginines. The distance of R1 and R2 was adjusted by the value of "n" of the carbon tether. The design and synthesis and preliminary biological data will be presented.



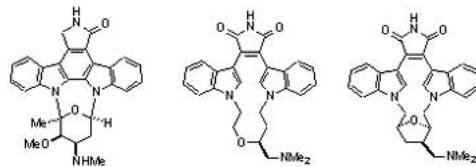
32.

SYNTHESIS OF STAUROSPORINE ANALOG AS POTENTIAL SELECTIVE

INHIBITOR OF PROTEIN KINASE C. *Yongzhong (Pete) Zhao, and Xiangjun Yue, ChemALong Laboratories, LLC, 12305 New Avenue, Suite K, Lemont, IL 60439, Fax: 630-257-9684, petezhao@chemalong.com*

Protein kinase C (PKC) is a family of serine/threonine specific protein kinases that regulates various physiological processes such as cellular proliferation, transformation and apoptosis and has been closely linked with the regulation of contractile, secretory and proliferative processes. Staurosporine (**1**), a potent while nonselective PKC inhibitor discovered in 1977, has in part stimulated the efforts in the search for more selective PKC inhibitors with therapeutic potential. The macrocyclic bis(indolyl)maleimides represent a class of isoform-selective protein kinase C (PKC) inhibitors. The inhibitor **2**, which was discovered by Eli Lilly, is more than 1000-fold selective for PKC- β over other protein kinases, and at least 60-fold selective for the β -isoform of PKC over other isoforms. This presents a significant improvement over the naturally occurring indolocarbazole, such as staurosporine (**1**).

We have carried out synthetic efforts toward this series of molecules, in an attempt to increase selectivity. This paper will describe the synthetic work toward a staurosporine analog, **3**, which has an amine function group as in **2** and a tetrahydrofuran ring like in **1**.

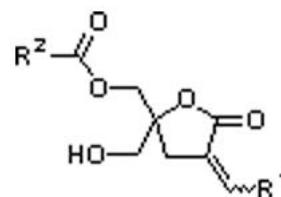


Staurosporine LY333531 CAL100603

33

COMBINATORIAL SYNTHESIS, QUALITY CONTROL AND BIOLOGICAL EVALUATION OF DIACYLGLYCEROL (DAG)-LACTONE LIBRARIES - PROTEIN KINASE C (PKC)-ISOZYME-SPECIFIC LIGANDS. *Dehui Duan*, Laboratory of Medicinal Chemistry, NCI-Frederick, NIH, 376 Boyles St., Bldg. 376, Rm 218, Frederick, MD 21702, Fax: 301-846-6033, duand@ncifcrf.gov, Christopher C. Lai, Laboratory of Medicinal Chemistry, Center for Cancer Research, NCI-Frederick, James A. Kelley, Laboratory of Medicinal Chemistry, National Cancer Institute, Nancy E. Lewin, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Institutes of Health, Peter M. Blumberg, Laboratory of Cellular Carcinogenesis and Tumor Promotion, Division of Basic Sciences, National Cancer Institute, National Institute of Health, and Victor E. Marquez, Laboratory of Medicinal Chemistry

We have developed DAG-lactones 1 to target the C1 domain of PKC and explored different combinations of R1 and R2 to produce more specific ligands. To optimize this approach, a RF-tag encoded combinatorial method was developed starting with the loading of monoprotected p-methoxyphenyl-4,4'-bis(hydroxymethyl)-gamma-butyrolactone to a PL-DHP resin packed in 96 IRORI MicroKans. These Kans were first sorted into 12 vessels to react with 12 aldehydes. Then, they were pooled to undergo a DBU-mediated elimination of the triflated aldol products followed by a removal of the aryl protection. The second sorting operation directed them into 8 vessels to react with 8 acid chlorides. TFA-assisted cleavage in AccuCleave-96 station afforded 96 final E/Z-mixtures of DAG-lactones at the same time. More than 80% compounds in these libraries have reliable mass ID peaks. The resulting libraries are routinely assayed for PKC binding affinity and a full structure-activity analysis will be presented.



1

34.

DAG-LACTONES AS HIGH-AFFINITY PROTEIN KINASE C LIGANDS AND

ALPHA-SECRETASE ACTIVATORS. *Jeewoo Lee¹, Ji-Hye Kang¹, Yerim Kim¹, Sung Eun Kim¹, Young-Ho Kim², Hee Kim², and Peter M. Blumberg³. (1) Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea, Fax: 82-2-888-0649, jeewoo@snu.ac.kr, (2) Digital Biotech, (3) Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health*

Beta-Amyloid peptide (A-beta), a 39-43 amino acid peptide, has been implicated in the pathogenesis of Alzheimer's disease (AD) and is generated from amyloid precursor protein (APP) by stepwise proteolytic processing by beta- and gamma-secretases. APP is also cleaved by alpha-secretase within the A-beta sequence (between residues K and L) to generate a large secreted fragment termed sAPPalpha and a smaller intracellular fragment, P3, which are of no pathological significance. Therefore, the activation of alpha-secretase activity would be as promising an approach for lowering the amount of A-beta as is the inhibition of beta- and gamma-secretases. PKC activators have been shown to dramatically enhance alpha-secretase activity, thus leading to enhanced secretion

of sAPP-alpha. Since numerous studies associated with defective PKC in brain and peripheral tissues of AD patients have been reported, PKC activators would be anti-AD drug candidates along with beta- and gamma-secretase inhibitors. This presentation will discuss the design, syntheses and alpha-secretase activation of DAG-lactones as high-affinity PKC ligands.

35.

STUDIES TOWARD THE SYNTHESIS OF 3-(HYDROXYMETHYL)-BEARING PHOSPHATIDYLINOSITOL ETHER LIPID ANALOG, A POTENT AKT INHIBITOR.

*Feng Zhou, EMD Biosciences, Inc, San Diego, CA 92121,
fzhou20002000@yahoo.com*

Akt [also known as RAC-PK or protein kinase B (PKB)] is a proto-oncogene that inhibits apoptosis by phosphorylating a number of downstream targets. Due to its Akt inhibitory activities, the phosphatidylinositol (PI) analog **1** proved to be a good inhibitor of the growth of various cancer cell lines with an IC₅₀ value of 5 μ M. A key step in the synthesis of **1** was the hydroboration and oxidation of olefin **2**. Studies were conducted to improve the yield of the desired product **3** by asymmetric hydroboration of **2** as well as by epimerization of the side product **4**. Details of the studies will be presented.

36.

USING CELECOXIB AS A MOLECULAR PLATFORM FOR DEVELOPING A NEW CLASS OF AKT-TARGETED ANTITUMOR AGENTS. *Jiuxiang Zhu¹, Jui-Wen Huang², Joseph Fowble², Samuel K Kulp², and Ching-Shih Chen². (1) Dept. of medicinal chemistry, ohio state university, 500 west 12th ave, columbus, OH 43210, (2) dept. of medicinal chemistry, ohio state university*

Recent epidemiological and animal-model studies have indicated the clinical application of nonsteroidal anti-inflammatory drugs (NSAIDs) as chemopreventive agents, especially in colon cancer. In early 2001 FDA approved COX-2 inhibitor celecoxib (Celebrex) for prevention of colon cancer in patients with familial polyposis coli although the underlying molecular mechanism is elusive. Lots of debates evolve around whether its antitumor effect is COX-2 dependent or not. Among all those reported COX-2 independent signaling pathways PDK-1/Akt is especially noteworthy. This pathway represents the convergence of a plethora of receptor tyrosine kinase and cytokine-mediated pathways that regulate cell proliferation, survival and apoptosis. Dysregulation of this pathway due to constitutive growth factor-receptor activation and PTEN mutation results in Akt up-regulation, which promotes tumor invasiveness, angiogenesis and progression. Thus PDK-1/Akt inhibitors are of translational relevance for development into useful chemotherapeutic or chemopreventive agents. In our previous paper we described how we modified structure of celecoxib to separate the COX-2 inhibitory effect from PDK1/Akt inhibitory effect. So in pursue of finding more potent compounds targeting PDK1/Akt signaling pathway we synthesized a number of derivatives with larger hydrophobic moiety at 5-position, which was based on the working model gotten from previous study. Among all those derivatives OSU02067 showed the highest potency not only in inhibiting PDK-1 (IC₅₀=9 μ M) but also in inhibiting PC-3 cell growth (IC₅₀=5 μ M). It's about 5-fold increase over celecoxib. Docking of OSU02067 to PDK1 showed that this compound mimicked the ATP binding mode although the detailed interactions were different. In vitro assay indicated that this compound induced apoptosis in PC-3 cells at 5 μ M, which were assessed by enzyme-linked immunosorbent assay (ELISA) and western blot detection of poly(ADP-ribose) polymerase cleavage. In vivo study showed that nude mice treated with 200mg/kg OSU02067 for 30 days had tumors only half size as that of those treated with vehicle.

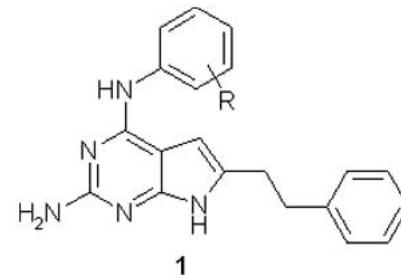
37.

DESIGN AND SYNTHESIS OF 2-AMINO-4-ANILINO SUBSTITUTED-6-PHENYLETHYLPYRROL[2,3-D]PYRIMIDINES AS RECEPTOR TYROSINE

KINASE INHIBITORS. *Aleem Gangjee¹, Ojas A. Namjoshi¹, Michael Ihnat², and Shekhar Kamat². (1) Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, Fax: 412-396-5593, gangjee@duq.edu, ojasnamjoshi@hotmail.com, (2) Department of Cell Biology, The University of Oklahoma Health Science Center*

Several tumors have dysfunctional receptor tyrosine kinases (RTKs) that are often over expressed and promote inappropriate signaling that has implications in tumor growth and metastasis. Thus inhibition of RTKs has provided a new paradigm for cancer chemotherapy and several RTK inhibitors are in clinical use

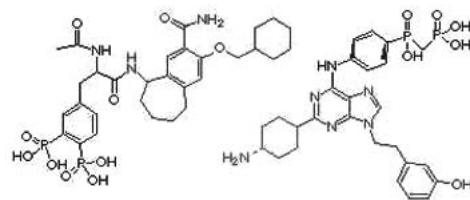
and in trials as antitumor agents. Gangjee et al. discovered a series of pyrrolo[2,3-d]pyrimidines of general structure **1** with 4-m-Br-aniline and an aryl group attached to the 6-position via an ethyl bridge. The nature and position of the substituents on the aryl moiety of **1** were found to control the selectivity and potency against a variety of RTKs. This report will discuss the design, synthesis and RTK inhibitory activities of compounds **1** in which the substituent R, on the 4-anilino moiety, was varied to determine its effect on selectivity and/or potency against a variety of RTKs implicated in tumor growth and metastasis.



38.

DESIGN AND SYNTHESIS OF NOVEL BONE-TARGETED SRC TYROSINE KINASE INHIBITOR DRUG DISCOVERY. *Raji Sundaramoorthi, William C Shakespeare, Chester A Metcalf III, Yihan Wang, Shuangying Liu, Regine S Bohacek, Surinder S Narula, Jeffery A Keats, Scott Wardwell, John Iuliucci, Manfred Weigele, David C Dalgarno, and Tomi K Sawyer, Chemistry, ARIAD Pharmaceuticals, Inc, 26 Landsdowne St., Cambridge, MA 02139, Fax: 617-494-8144, rajisundaramoorthi@ariad.com*

Bone-targeted Src tyrosine kinase (STK) inhibitors have recently been developed for the treatment of osteoporosis and cancer related disorders. Bisphosphonates were first identified as inhibitors of calcium carbonate precipitation by virtue of their affinity to chelate calcium. Drug discovery efforts led the successful clinical development of breakthrough medicines such as Merck's Fosamax and Novartis' Zometa as novel, potent and effective small-molecule antiresorptives relative to their inhibition of farnesyl diphosphate synthase in osteoclasts (bone-resorbing cells). Our work has exploited bone-targeting chemistry in the structure-based design of novel STK inhibitors in two strategies: 1. Lead optimization of Src SH2 inhibitors incorporating non-hydrolyzable phosphotyrosine mimics exhibiting molecular recognition and bone-targeting properties as exemplified by the in vivo effective nonpeptide AP22408; and, 2. Lead optimization of ATP-based Src kinase inhibitors incorporating bone-targeted moieties as exemplified by the in vivo effective compound AP23451. In summary these STK inhibitors illustrate novel bone-targeting chemistry successfully incorporated to achieve key proof-of-concept and the framework for next generation molecules (varying ligand templates and therapeutic targets) of potential application to cancer and bone diseases, including osteolytic bone metastasis, osteoporosis, hypercalcemia of malignancy, and bone cancer.



Src SH2:
AP 22408
IC 50 : 200 nM

Src Kinase:
AP 23451
IC 50 : 67 nM

39.

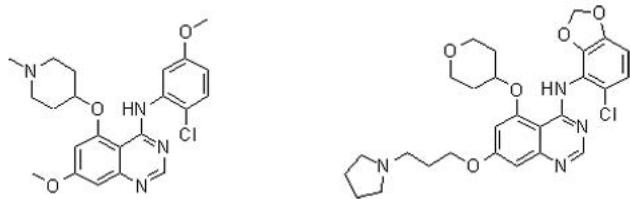
NOVEL SERIES OF C5-SUBSTITUTED ANILINOQUINAZOLINES WITH HIGHLY POTENT AND SELECTIVE INHIBITION OF C-SRC TYROSINE KINASE ACTIVITY.

A. Olivier¹, G.F. Costello², M. Fennell², T.P. Green², L.F. Hennequin¹, V. Jacobs², C. Lambert-Van der Brempt¹, R. Morgentin¹, and P.A. Plé¹. (1) Centre de Recherches AstraZeneca, Z.I.S.E. La Pompelle B.P.1050, Reims Cedex 2 51689, France, Annie.Olivier@astrazeneca.com, (2) AstraZeneca Pharmaceuticals

De-regulated activity of the non-receptor tyrosine kinase c-Src, previously described in several human tumor types, is believed to be linked to signal

transduction, cytoskeletal and adhesion changes, ultimately promoting a tumor invasive phenotype. Among the different approaches to kinase inhibition, competitive inhibition by reversible binding at the ATP binding domain of the tyrosine kinase has already proved a valuable approach. The anilinoquinazoline skeleton is a good template as an adenine mimic but fine tuning of the substituents is necessary to attain high affinity and selectivity versus other kinases. We previously reported data on a series of 6,7-disubstituted anilino-quinazolines with potent activity against c-Src kinase. Modelling of an anilino-quinazoline in a kinase active site suggested that the 'sugar pocket' of the kinase could be accessible by C5 substitution of the quinazoline for additional interactions. We report here the discovery of a novel series of C5 substituted anilinoquinazolines as potent nanomolar inhibitors of tyrosine phosphorylation by the c-Src kinase as exemplified below.

A rapid and efficient synthesis of this series of compounds that allows for modifications at C4, C5 and C7 positions of the quinazoline will be described. Conversion of a symmetrical di-O-substituted aniline leads in 3 steps to a 5,7-disubstituted quinazolone. A regioselective deprotection of the C5 ether is the key step for the differentiation between C5 and C7 positions of the quinazolone. The sequential introduction of C5 and C7 substituents is allowed using protection-deprotection steps followed by introduction of the aniline. In vitro activity of selected examples in this series will be reported from a wide range of substituted derivatives prepared using this route.



40.

DESIGN, SYNTHESIS AND UTILIZATION OF BIOTINYLATED MACROCYCLIC GRB2 SH2 DOMAIN-BINDING LIGANDS. *Zhen-Dan Shi¹, Kyeong Lee¹, Hongpeng Liu², Manchao Zhang², Lindsey R. Roberts³, Robert J. Fisher³, Dajun Yang², Donald Bottaro⁴, Marston Linehan⁴, and Terrence R. Burke Jr.¹. (1) Laboratory of Medicinal Chemistry, CCR, NCI, NIH, Frederick, MD 21702, Fax: 301-846-6033, shiz@ncifcrf.gov, (2) Department of Hematology/Oncology, University of Michigan Medical School, (3) Protein Chemistry Laboratory, SAIC-Frederick, SAIC-Frederick, (4) Urology Oncology Branch, CCR, NCI, NIH*

The growth factor receptor-bound protein 2 (Grb2) provides critical connectivity between growth factor receptor PTKs and Ras signaling involved in the etiology of several cancers. Based on structural considerations intended to take advantage of the preferred interaction of Grb2 SH2 domains with pTyr-containing peptides in beta-bend conformations, we have recently reported a number of macrocyclic peptide mimetics that exhibit from low nanomolar to low picomolar-binding affinity. In order to study the interaction of these ligands with physiological targets, biotin was conjugated onto the macrocycle structure. The design, synthesis and preliminary utilization of these analogues will be presented.

41.

EXAMINATION OF THE ROLE OF PHOSPHORYL-MIMICKING FUNCTIONALITY IN THE BINDING OF PICOMOLAR-AFFINITY GRB2 SH2 DOMAIN-BINDING MACROCYCLES. *SangUk Kang¹, Zhen-Dan Shi¹, Kyeong Lee¹, Karen M. Worthy², Robert J. Fisher², and Terrence R. Burke Jr.¹. (1) Laboratory of Medicinal Chemistry, Center for Cancer Research, National Cancer Institute, NIH, Frederick, MD 21702-1201, Fax: 301-846-6033, serforchr@hanmail.net, (2) Protein Chemistry Laboratory, SAIC-Frederick*

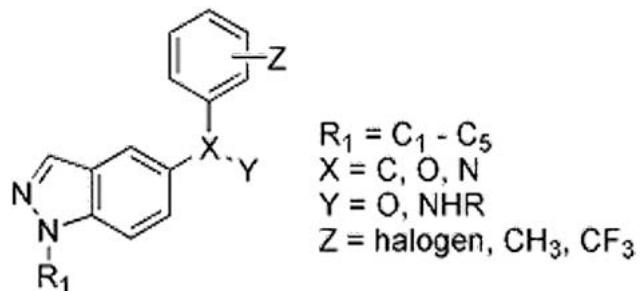
Phosphoryl or phosphoryl-mimicking functionality is typically required for high affinity binding to SH2 domains. Although the structural basis for this requirement is derived from the interaction of ligand anionic charge with conserved Arg residues within the SH2 domain phosphotyrosyl (pTyr)-binding pocket, the functional role this plays in the overall binding process is not unambiguous. For example, initial high affinity binding of a single residue within the pTyr-binding pocket could serve to "capture" the larger ligand and allow secondary conformational orientation onto the SH2 domain surface, where further interactions would determine overall binding affinity. Our recently reported low picomolar-KD

Grb2 SH2 domain-binding macrocycle, which exhibits a diffusion-limited on-rate, may represent a unique example where solution conformation is in little need of secondary pTyr-dependent orienting effects. The role of phosphoryl-mimicking functionality could therefore be significantly reduced for this macrocycle relative to conformationally more flexible ligands. In order to examine this possibility, variants of the lead macrocycle were prepared that contained minimal or no phosphoryl-mimicking functionality. The design, synthesis and Grb2 SH2 domain-binding affinity of these analogues will be presented.

42.

DESIGN, SYNTHESIS, AND EVALUATION OF 1,5-DISUBSTITUTED-1-H-INDAZOLES AS INHIBITORS OF P38 MAP KINASE. *Ganghyeok Kim¹, Mark Munson¹, Mariel Rodriguez¹, Jim Rizzi², Gary Hingorani³, Suzy Brown³, Jennifer Otten³, Darin Smith³, Guy Vigers³, Barb Brandhuber³, Jianhong Wang⁴, Michelle Goyette⁴, and Laurence E. Burgess¹. (1) Department of Lead Optimization, Array BioPharma Inc, 3200 Walnut Street, Boulder, CO 80301, Fax: 303-386-1130, gkim@arraybiopharma.com, (2) Department of Computational Technology, Array BioPharma Inc, (3) Department of Biology, Array BioPharma Inc, (4) Department of Analytical Chemistry, Array BioPharma Inc*

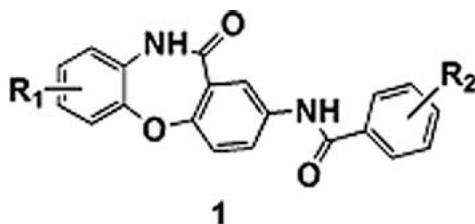
P38 MAP kinase is a key downstream signaling protein of the mitogen activated protein kinase family that triggers the biosynthesis of pro-inflammatory cytokines such as tumor necrosis factor and interleukin-1. Inhibition of p38 MAP kinase has been one of the key approaches toward the development of treatments for chronic inflammation such as rheumatoid arthritis, inflammatory bowel disease and psoriasis. The design, synthesis, x-ray co-crystal structure, and in vitro activities of 1,5-disubstituted-1-H-indazoles as inhibitors of novel p38 MAP kinase will be presented.



43.

NOVEL BENZOAZEPINONE INHIBITORS OF C-JUN N-TERMINAL KINASE 3 (JNK3) AND UTILITY AS STROKE THERAPEUTICS :- SAR AND LIGAND CO-CRYSTALLIZATION STUDIES. *Liping Pettus, Vu Ma, Paul Tempest, Janan Jona, Charles Henley, Gary Zajic, Weiya Wang, Mark Michaels, Rob Kurzeja, Harvey Yamane, Sekhar Surapaneni, Ella Magal, David Powers, Yan-yan Xie, Chris Mohr, Yax Sun, Steve Jordan, Bill Harte, Andrew Tasker, Randall Hungate, and Christopher Hulme, Medicinal Chemistry Technologies, Chemistry Research & Development, Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, chulme@amgen.com*

Stroke is the third leading cause of death and the leading cause of long-term disability in the U.S. Estimates indicate incidence of ~ 750,000 strokes per year in the U.S. Stroke represents a large unmet medical need since treatment is limited to thrombolytics, which must be administered shortly after onset. Severely ischemic brain tissue (core of infarct) may not be salvaged; however, adjacent regions (penumbra) may be rescued. Neuronal cell death in the spreading penumbra is presumed to be the result of apoptotic events. This poster reveals a novel series of biochemically potent, blood brain barrier penetrating JNK3 small molecule inhibitors. Key issues for chemistry were 1) potency in the enzymatic assay, 2) brain penetration and 3) aqueous solubility. Results from ligand-enzyme co-crystallization studies and subsequently developed SAR will be presented. The generic structure of the series is shown below, 1.

**44.****SYNTHESIS AND SAR OF DITHIAZOLE HER KINASE INHIBITORS.** *Xiaopeng Sang¹, Karen Du², John F. Kadow³, David R. Langley⁴, Gregory D. Vite¹, Dolatrai M. Vyas¹, Mark D. Wittman³, and Tai W. Wong⁵. (1) Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, P.O. Box 5100, Wallingford, CT 06492, xiaopeng.sang@bms.com, (2) Department of Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, (3) Pharmaceutical Research Institute, Bristol-Myers Squibb Co, (4) Computer-Assisted Drug Design, Bristol-Myers Squibb Co, (5) Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute*

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. The human epidermal growth factor receptor (HER) family consists of four distinct receptor tyrosine kinases referred to as HER1,2, 3, and 4. RTKs such as HER1 and HER2 are involved in cell proliferation and are overexpressed in many cancers. Disruption of signal transduction by inhibition of these kinases would have an antiproliferative and therapeutic effect. Deck screening identified a class of substituted Nitro-1*I*-benzo[1,3,2]dithiazole 3,3-dioxides as small molecule inhibitors of HER2 kinases. Kinetic analyses & molecular modeling confirmed that this class functioned as an ATP competitor. To our knowledge, this represents a new class of HER-2 inhibitors. SAR studies and the effects of chirality at sulfur on potency will be described. Isosteric replacement of the nitro group provided aza-benzodithiazoles with improved potency.

45.**HIGHLY SELECTIVE INHIBITION OF CDK4 WITH PYRIDO[2,3-D]PYRIMIDIN-7-ONES.** *Scott N. VanderWeij¹, Patricia J. Harvey², Derek J. Sheehan¹, Joseph Repine¹, Paul R. Keller², R. John Booth¹, John Quin III¹, Dennis J. McNamara³, Hairong Zhou¹, Ellen M. Dobrusin¹, Alexander J. Bridges¹, Wilbur R. Leopold², David W. Fry², Joanne Brodfuehrer⁴, Tong Zhu⁴, and Peter L. Toogood¹. (1) Chemistry Department, Pfizer Global Research & Development, 2800 Plymouth Rd., Ann Arbor, MI 48105, Fax: 734-622-5165, scott.vanderwel@pfizer.com, (2) Cancer Research, Pfizer Global Research & Development, (3) Chemistry Department, Pfizer Global Research and Development, (4) Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research & Development*

Cyclin-dependent kinases (Cdks) are essential regulators of cell proliferation. Inhibition of Cdks is anticipated to provide therapeutic benefit in the treatment of cancer. Cdk4 is implicated by biochemical and genetic data as an important modulator of cell cycle entry and progression in response to growth signals. Inhibition of Cdk4 specifically is anticipated to provide a relatively non-toxic approach to tumor growth inhibition. We have been investigating a series of pyrido[2,3-d]pyrimidin-7-ones as potential Cdk4 inhibitors. Initial SAR studies demonstrated that excellent potency against Cdk4 was attainable, however, early inhibitors also exhibited significant potency against a variety of other kinases including other Cdks as well as less-closely related kinases. Through an extensive SAR analysis, assisted by structural studies, we now have identified highly selective inhibitors of Cdk4. The best inhibitors inhibit Cdk4 with nanomolar potency and have no effect on a panel of more than 30 additional kinases at 100-1000-fold higher concentrations, with the exception of Cdk6, which is functionally equivalent to Cdk4. Representative selective Cdk4 inhibitors inhibit cell proliferation and cause a G1 block in Rb-positive but not Rb-negative cell lines. Excellent physical and pharmacokinetic properties suggest that these compounds should be active *in vivo*. In conclusion, highly Cdk4 selective inhibitors have been prepared that also display good drug-like properties. Representative compounds should provide useful probes for studying the function of Cdk4 in cell-based and *in vivo* experiments.

46.**SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL MACROCYCLIC BIS-7-AZAINDOLYLMALEIMIDES AS POTENT AND HIGHLY SELECTIVE GLYCOGEN SYNTHASE KINASE-3B (GSK-3B) INHIBITORS.** *Lan Shen, Catherine Prouty, Bruce R. Conway, Lori Westover, Jun Z Xu, Richard Look, Xin Chen, Mary Pat Beavers, Jerry Roberts, William V. Murray, Keith Demarest, and Gee-Hong Kuo, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC, 1000 Route 202, P. O. Box 300, Raritan, NJ 08869, Fax: 908-203-8109, Ishen4@prdus.jnj.com*

Abstract Palladium catalyzed cross-coupling reactions were used to synthesize two key bis-7-azaindolylmaleimide intermediates that resulted in the synthesis of novel series of macrocycles. Among the three series of macrocycles, the oxygen atom and thiophene containing linkers yielded molecules with higher inhibitory potency at GSK-3b ($K_i=0.011\text{ mM}$ - 0.079 mM) while the nitrogen atom containing linkers yielded molecules with lower potency ($K_i=0.150\text{ mM}$ - $>1\text{ mM}$). Compounds with $-(\text{CH}_2)_4\text{C}(\text{O})(\text{CH}_2)_4-$ and $(\text{CH}_2)_4\text{CH}(\text{OH})(\text{CH}_2)_4-$ linkers displayed 1-2 orders of magnitude selectivity at GSK-3b against CDK2, PKC β II, Rsk3 and little or no inhibitions to the other 62 protein kinases. Compound containing $-(\text{CH}_2)_3\text{-thiophene-}(\text{CH}_2)_3-$ linker was at least 100-fold more selective towards GSK-3b than PKC β II, and it had little or no activity against a panel of 65 protein kinases, almost behaved as a GSK-3b "specific inhibitor". All three compounds showed good potency in GS assay. Molecular docking studies were conducted in an attempt to rationalize the GSK-3b selectivity of azaindolylmaleimides. The high selectivity, inhibitory potency and cellular activities of these non-crown-ether typed molecules may provide them as a valuable pharmacological tools in elucidating the complex roles of GSK-3b in cell signaling pathways and the potential usage for the treatment of elevated level of GSK-3b involved diseases.

47.**SYNTHESIS AND DISCOVERY OF MACROCYCLIC POLYOXYGENATED BIS-7-AZAINDOLYLMALEIMIDES AS A NOVEL SERIES OF POTENT AND HIGHLY SELECTIVE GLYCOGEN SYNTHASE KINASE-3 β INHIBITORS.** *Gee-Hong Kuo, Catherine Prouty, Alan DeAngelis, Lan Shen, David O'Neill, Chandra Shah, Peter J. Connolly, William V. Murray, Bruce R. Conway, Peter Cheung, Lori Westover, Jun Z Xu, Richard Look, Keith Demarest, Stuart Emanuel, Steven A. Middleton, Linda Jolliffe, Mary Pat Beavers, and Xin Chen, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, Fax: 908-526-6469, gkuo@prius.jnj.com, cprouty@prdus.jnj.com*

Attempts to design the macrocyclic maleimides as a selective PKC γ inhibitors led to the unexpected discovery of a novel series of potent and highly selective GSK-3 β inhibitors. Palladium catalyzed cross-coupling reactions were used to synthesize the key intermediates 17 and 22 that resulted in the synthesis of novel series of macrocycles. All three macrocyclic series (bisindolyl-, mixed-7-azaindole-indolyl- and bis-7-azaindolyl-maleimides) were found to have submicromolar inhibitory potency at GSK-3 β with various degrees of selectivity toward other protein kinases. To gain the inhibitory potency at GSK-3 β , the ring sizes of these macrocycles may play a major role. To achieve the selectivity at GSK-3 β , the additional nitrogen atoms in the indole rings may contribute to a significant degree. Overall, the bis-7-azaindolylmaleimides 28 and 29 exhibited little or no inhibitions to a panel of 50 protein kinases. Compound 29 almost behaved as a GSK-3 β specific inhibitor. Both 28 and 29 displayed good potency in GS cell-based assay. Molecular docking studies were conducted in an attempt to rationalize the GSK-3 β selectivity of azaindolylmaleimides.

48.**FLUORESCENCE-BASED HIGH THROUGHPUT BIOCHEMICAL ASSAYS FOR KINASE INHIBITORS A COMPARISON OF ASSAY FORMATS.** *Richard L. Somberg, Kristin G. Huwiler, David A. Lasky, Steven R. Duff, Hildegard C. Eliason, and Mohammed Saleh Shekhani, Invitrogen Corporation, 501 Charmany Drive, Madison, WI 53719, Richard.Somberg@Invitrogen.com, Hildegard.Eliason@Invitrogen.com, Mohammed.Shekhan@Invitrogen.com*

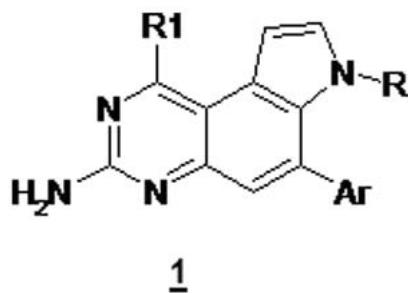
Fluorescence-based assays for discovery of kinase inhibitors are becoming increasingly popular due to practical difficulties with radioactivity-based assays. Within the fluorescence assay format, many variations exist, each with their own set of advantages and disadvantages. Invitrogen offers proprietary kinase assays using the three most common fluorescence-based formats, namely: fluorescence

polarization (FP), fluorescence resonance energy transfer (FRET), and time-resolved fluorescence resonance energy transfer (TR-FRET). The FP assay is based on competitive binding between a phosphorylated peptide (or protein) and a fluorescent tracer for an antibody. The same approach is used in the Lanthascreen TR-FRET assay except that the antibody is labeled with a proprietary terbium chelate. The Z'-LYTE FRET assay uses a dual-labeled peptide as a kinase substrate that when phosphorylated, has altered proteolytic sensitivity. A diverse library of pharmacologically active compounds was screened against a cyclic AMP-dependent protein kinase (PKA) in all three assay formats and common hits were identified. The results of these experiments will be presented and the three assay formats will be compared.

49.

DIAMINOPYRROLQUINAZOLINES 2: NOVEL AND HIGHLY SELECTIVE PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORS. *Kshitij C. Thakkar¹, Steven Berthel¹, Jolly Bose², Bruce Banner¹, Mark Dvorozniak², Joseph Grimsby², Garry Mackie², Andrée R. Olivier², Lucja Orzechowski¹, Cheryl Spence², and Joseph A. Sergi².* (1) *Discovery Chemistry, Roche Research Center, Hoffmann-La Roche Inc, 340 Kingsland Street, Nutley, NJ 07110, Fax: 973-235-2448, Kshitij_C.thakkar@roche.com*, (2) *Discovery of Metabolic Diseases, Roche Research Center, Hoffmann-La Roche Inc*

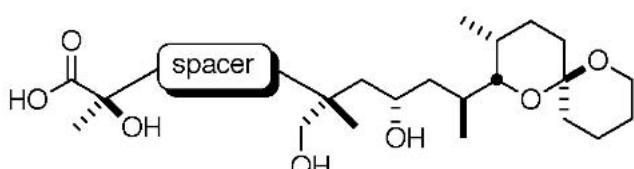
PTP1B is a clearly validated target for Type 2 diabetes and obesity. Mice lacking PTP1B have been shown to be lean and insulin sensitive. Further characterization of these mice revealed that PTP1B also plays a role in leptin signaling. Inhibitors of PTP1B would therefore not only potentiate the effects of insulin but also those of leptin supporting the rationale that these compounds would have considerable therapeutic potential for the treatment of both obesity and Type 2 diabetes. High throughput screening of our compound libraries has led to the identification of a new class of PTP1B inhibitors. Systematic structural modification yielded potent PTP1B inhibitors. The chemistry, in vitro and in vivo studies of these compounds will be presented.



50.

DESIGN AND SYNTHETIC STUDIES OF NOVEL ANALOGUES OF THE PROTEIN PHOSPHATASE 1 AND 2A INHIBITOR OKADAIC ACID. *David A. Colby, and A. Richard Chamberlin, Department of Chemistry, University of California, Irvine, Irvine, CA 92697, dcolby@uci.edu*

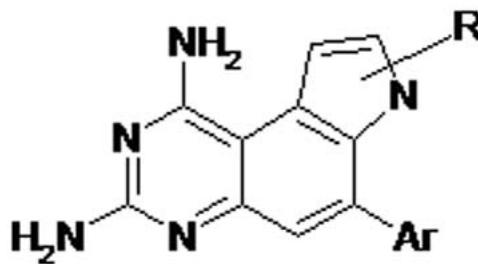
Protein phosphatase 1 and 2A are two key types of phosphatases that are involved in a variety of cell-signaling cascades. We have recently reported a minimum pharmacophore for protein phosphatase inhibition. While investigating the binding of the phosphatase inhibitor, okadaic acid, with this pharmacophore, we have discovered how to exploit key conformational data in order to develop new simplified analogues of okadaic acid. These modeling studies and a synthetic approach to the novel okadaic acid analogues will be presented.



51.

DIAMINOPYRROLQUINAZOLINES 1: NOVEL AND HIGHLY SELECTIVE PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORS. *Kshitij C. Thakkar¹, Jolly Bose², Steven Berthel¹, Hong Dong¹, Donald Emerson¹, David Fry¹, Paul Gillespie¹, Shirley Li³, Andrée R. Olivier², Lucja Orzechowski¹, Philippe Pflieger⁴, Joseph A. Sergi², and Weiya Yun¹.* (1) *Discovery Chemistry, Roche Research Center, Hoffmann-La Roche Inc, 340 Kingsland Street, Nutley, NJ 07110, Fax: 973-235-2448, Kshitij_C.thakkar@roche.com*, (2) *Discovery of Metabolic Diseases, Roche Research Center, Hoffmann-La Roche Inc*, (3) *Discovery Technology, Roche Research Center, Hoffmann-La Roche Inc*, (4) *Discovery Chemistry, F. Hoffmann-La Roche Ltd*

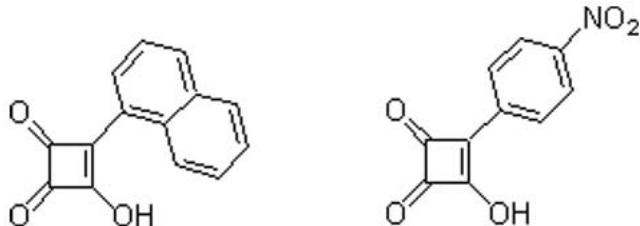
Protein Tyrosine Phosphatase 1B is an intracellular enzyme shown to be involved in the regulation of insulin and leptin signaling. Inhibitors of PTP1B would potentiate the effects of both the insulin and the leptin signaling pathways, thereby, supporting the rationale that these compounds would have considerable therapeutic potential for the treatment of both obesity and Type 2 diabetes. Through high throughput screening, we have identified a new series of diaminopyrrolquinazolines as novel and selective PTP1B inhibitors. The binding of these compounds was verified by NMR experiments. The synthesis and SAR of these compounds will be presented.



52.

SQUARIC ACID DERIVATIVES: A NEW CLASS OF PROTEIN TYROSINE PHOSPHATASES INHIBITORS. *Jian Xie, Anthony B. Comeau, and Christopher T. Seto, Department of Chemistry, Brown University, 324 Brook Street, Providence, RI 02912, Jian_Xie@Brown.Edu*

Protein tryosine phosphatases (PTPases) play an important role in cell functions. Overexpression of PTPases is involved in a number of human diseases such as type II diabetes and infection by *Yersinia pestis*, the causative agent of bubonic plague. Derivatives of squaric acids, such as 2-aryl-1-hydroxycyclobut-1-ene-3,4-diones, has been found to be a new class of mono-anionic inhibitors for PTPases.

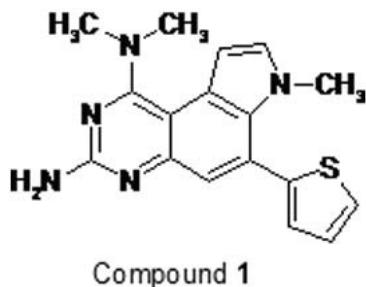


53.

DIAMINOPYRROLQUINAZOLINES 3: NOVEL AND HIGHLY SELECTIVE PROTEIN TYROSINE PHOSPHATASE 1B INHIBITORS. *Kshitij C. Thakkar¹, Jolly Bose², Bruce Banner¹, Mark Dvorozniak², David Fry¹, Joseph Grimsby², Garry Mackie², Andrée R. Olivier², Cheryl Spence², and Joseph A. Sergi².* (1) *Discovery Chemistry, Roche Research Center, Hoffmann-La Roche Inc, 340 Kingsland Street, Nutley, NJ 07110, Fax: 973-235-2448, Kshitij_C.thakkar@roche.com*, (2) *Discovery of Metabolic Diseases, Roche Research Center, Hoffmann-La Roche Inc*

Protein Tyrosine Phosphatase 1B (PTP1B), an intracellular protein tyrosine phosphatase(PTPase), has been implicated in negative regulation of insulin and leptin signal transduction pathways. Systematic structural modification on initially discovered compounds yielded the potent PTP1B inhibitors 1. The location of binding of compound 1 in comparison to known inhibitors of PTP1B was evaluated by NMR using ¹⁵N-labeled PTP1B.

We report here the synthesis, in vitro and in vivo studies that led to identification of Compound 1 as a potential treatment for Type 2 diabetes.



54.

PIPERAZINE-LINKED BISBENZAMIDINES: A NOVEL CLASS OF

ANTILEISHMANIAL AGENTS. *Annie Mayence¹, Jean J. Vanden Eynde¹, Larry A. Walker², Babu L. Tekwani³, and Tien L. Huang¹.* (1) Department of Basic and Pharmaceutical Sciences, Xavier University of Louisiana, College of Pharmacy, 1, Drexel drive, New Orleans, LA 70125, Fax: 504-520-7954, *thuang@xula.edu*, (2) National Center for Natural Products Research, School of Pharmacy, University of Mississippi, (3) National Center for Natural Products Research, School of Pharmacy, The University of Mississippi

A highly diverse library of 1,4-diaryl piperazines was synthesized and evaluated for *in vitro* inhibitory activity against the Leishmania parasite. The antileishmanial effect was dramatically dependent on the nature of the substituents attached at the para position of the aryl groups. The amidine moiety was essential and introduction of substituents on the nitrogen atoms influenced the inhibitory profile of the tested compounds. SAR studies of this series of derivatives led us to identify 1,4-bis[4-(1H-benzimidazol-2-yl)phenyl]piperazine as a promising lead (IC_{50} : 0.22 μ g/ml) because it was more potent than pentamidine (IC_{50} : 1.7 μ g/ml) and equipotent to amphotericin B (IC_{50} : 0.13 μ g/ml). The DNA binding affinity of the derivatives was also measured but no correlation with the antileishmanial activity was observed.



55.

IN VITRO INHIBITION OF MALARIA PIGMENT FORMATION BY

BISBENZAMIDINES IN CELL-FREE SYSTEMS. *Annie Mayence, Jean J. Vanden Eynde, and Tien L. Huang, Department of Basic and Pharmaceutical Sciences, Xavier University of Louisiana, College of Pharmacy, 1, Drexel drive, New Orleans, LA 70125, Fax: 504-520-7954, *thuang@xula.edu**

Recently we have designed and prepared a series of previously unknown bisbenzimidines structurally related to pentamidine, a drug clinically used in the treatment of several fungal and parasitic diseases. Evaluation of the biological properties of the synthesized compounds revealed that they were highly efficient against *Plasmodium falciparum*. The antiplasmoidal effect could be due, as in the case of chloroquine (CQ) and other antimalarials, to their ability to prevent the appearance of hemozoin (malaria pigment), which is formed from heme, the toxic residue remaining following the digestion of hemoglobin by the parasite. Evidence supporting this proposition is based on spectroscopic data (IR and colorimetry) collected using cell-free experiments and this evidence is correlated with the *in vitro* activity (IC_{50} values) determined against both CQ-susceptible and CQ-resistant strains of *P. falciparum*.

56.

DISCOVERY OF ACHIRAL, POTENT, AND ORALLY ACTIVE 1,2,4-TRIOXOLANE ANTIMALARIAL PROTOTYPES. *Yuxiang Dong¹, Hugues Matile², Jacques Chollet³, and Jonathan L. Vernerstrom¹.* (1) Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198-6025, Fax: 402-559-9543, *ydong@unmc.edu*, (2) Pharma Division, Preclinical Research, F. Hoffmann-LaRoche Ltd, (3) Swiss Tropical Institute

Nine dispiro-1,2,4-trioxolanes (ozonides) were designed to test whether they have superior antimarial properties to the structurally related dispiro-1,2,4,5-tetraoxanes. From our initial series of nine dispiro-1,2,4-trioxolanes, two achiral lead compounds with excellent *in vitro* and *in vivo* antimarial activities were identified. No toxicity was evident for either compound at the 640 mg/kg dose in the Rane test. Using iron(II) model reactions, a possible mode of activation was investigated. Results from these experiments suggest that 1,2,4-trioxolanes and artemisinins may share parallel activation mechanisms. Details of these studies will be presented.

	OZ03	OZ05
IC_{50} (nM) K1/NF54	4.5 / 6.1	0.68 / 1.3
Single dose (po) ED_{50} / ED_{90} (mg/kg)	5.0 / 8.8	3.5 / 7.0

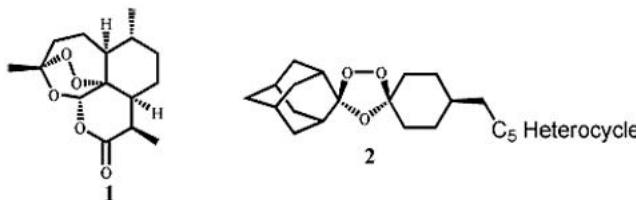
57.

SYNTHESIS OF NOVEL ORALLY ACTIVE ANTIMALARIALS:

8-HETEROCYCLEMETHYL SUBSTITUTED DISPIRO 1,2,4-TRIOXOLANES.

Yuanqing Tang¹, Yuxiang Dong¹, Jacques Chollet², Bernard Scoreaux², William N. Charman³, Sergio Wittlin², Reto Brun², and Jonathan L. Vernerstrom¹. (1) Department of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE 68198-6025, Fax: 402-559-9543, *ytang@unmc.edu*, (2) Swiss Tropical Institute, (3) Victorian College of Pharmacy, Monash University

The discovery of artemisinin (**1**), a naturally occurring endoperoxide active against chloroquine-resistant *Plasmodium falciparum*, initiated a new era in the chemotherapy of malaria. Many synthetic 1,2,4-trioxanes, 1,2,4,5-tetraoxanes, and other endoperoxides have been prepared; a fair number are quite active *in vitro*, but most suffer from low oral activity. Several N-heterocyclic dispiro-1,2,4-trioxolanes (**2**) with good oral activity in the *Plasmodium berghei* mouse model were identified. Their synthesis, physicochemical properties, antimarial activities, and SAR will be presented.



58.

EFFECTS OF PORPHYRIN DERIVATIVES ON LEISHMANIA TARENTOLAE CELL GROWTH.

Erin M Kamowski¹, Jennifer Passini¹, Timothy D. Lash², and Marjorie A. Jones¹. (1) Department of Chemistry, Illinois State University, Normal, IL 61790-4160, Fax: 309-438-5538, *ekamowski@hotmail.com*, (2) Department of Chemistry, Illinois State University

Leishmania are pathogenic parasites that infect about 1.5 million people a year. These organisms do not carry out heme synthesis and therefore must get their porphyrins from a host. This requirement is thus a possible target for chemotherapy. Using *Leishmania tarentolae* as an experimental model, several unique porphyrin derivatives were added to the cell culture. Cell growth was then evaluated spectrophotometrically over four days. Protein, using the Bio-Rad reagent, was also measured. Data for a concentration dependence study showed that a carbaporphyrin derivative is a particularly potent inhibitor (LD_{50} 10nM) of the cell viability. We are now working to evaluate the mechanism for this growth inhibition.

59.

INHIBITING EFFECTS OF *N*-(2-NAPHTHYLMETHYL) SPERMINE AND SPERMIDINE DERIVATIVES ON TRYPANOThIONE REDUCTASE. Mary O'Sullivan¹, Joseph DeLuca¹, Muris Kobaslija¹, Michael W. Fennie¹, and Cyrus Bacchi². (1) Department of Chemistry and Biochemistry, Canisius College, 2001 Main Street, Buffalo, NY 14208, Fax: 716-888-3112, osulliv1@canisius.edu, (2) Haskins Laboratories and Department of Biology, Pace University

Trypanothione reductase (TR) is an enzyme found solely in trypanosomes and leishmania. These parasitic protozoa are the etiological agents of many diseases including Chagas' disease and African sleeping sickness. TR catalyzes the reduction of the disulfide moiety of trypanothione (*N*¹,*N*⁸-bis(glutathionyl)spermidine) and plays a vital role in the antioxidant defenses of trypanosomes.

Consequently, inhibitors of TR have potential as novel antitrypanosomal agents. Here we report the results of our investigations of the inhibiting activities of a series of spermidine and spermine derivatives containing *N*-(2-naphthylmethyl) substituents. Several of the novel polyamine derivatives that we prepared are effective competitive inhibitors of recombinant *Trypanosoma cruzi* TR, and four of the compounds described have *K_i* values < 4 μM. The most effective inhibitor in this study was *N*¹,*N*⁴,*N*⁸,*N*¹²-tetra(2-naphthylmethyl)spermine which also displayed trypanocidal activity against *T. brucei* ssp. *in vitro* (with IC₅₀ values ranging from 1.5 to 3.3 μM).

60.

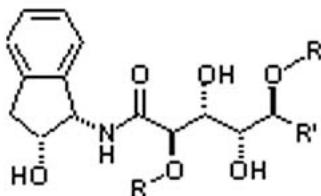
NEW UNSYMMETRICAL MANNITOL DERIVED INHIBITORS OF THE *P.*

FALCIPARUM ENZYMES PLASMEPSIN I AND II. Karolina Ersmark, Department of Medicinal Chemistry, Uppsala University, BMC, Box 574, Uppsala 751 23, Sweden, Fax: +46-18-471-44-74, karolina@orgfarm.uu.se, Elizabeth Hamelink, Medivir AB, and Anders Hallberg, Department of Medicinal Chemistry, Uppsala University

The rapid development of malaria parasite drug resistance has resulted in an urgent need for new, effective therapies. Two aspartic proteases, plasmepsin I and II, are acknowledged as potential targets for development of new anti-malarial drugs.

We have previously reported several inhibitors of plasmepsin I and II based upon a C₂-symmetric mannitol derived scaffold. This scaffold has now been modified to give unsymmetrical compounds encompassing diacylhydrazines or 1,3,4-oxadiazoles as amide bioisosters in the R' position.

In this new class of compounds highly potent inhibitors of both plasmepsin I and II have been identified. These inhibitors were also found to be selective and did not inhibit the human aspartic protease cathepsin D.

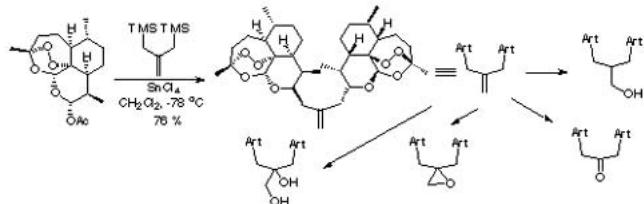


61.

ORALLY ACTIVE, ANTIMALARIAL, ANTICANCER, ARTEMISININ-DERIVED TRIOXANE DIMERS WITH HIGH STABILITY AND THERAPEUTIC INDEX IN RODENTS. Gary H. Posner¹, Ik-Hyeon Paik¹, Andrew J. McRiner¹, Surojit Sur¹, Kristina Borstnik¹, Suji Xie², Theresa A. Shapiro³, Adebusola Alagbala⁴, and Barbara Foster⁴. (1) Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218, Fax: 410-516-8420, (2) Malaria Institute, Johns Hopkins University, (3) Division of Clinical Pharmacology, Department of Medicine, School of Medicine, Johns Hopkins University, (4) Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute

In only two steps and in 70% overall yield, naturally occurring trioxane artemisinin was converted on gram scale into C-10-carba trioxane dimer. This new, very stable dimer was then transformed easily in one additional step into four different dimers. An alcohol and a diol dimer and a ketone dimer are 10 times more antimalarially potent *in vitro* than artemisinin. Also, the alcohol and diol dimers are strongly growth inhibitory but not cytotoxic toward several human cancer cell lines. An isobutyric acid dimer and an isonicotinate N-oxide dimer (structure not shown) were easily prepared in one additional step from alcohol dimer. The N-oxide dimer and the isobutyric acid dimer have a six-fold

better preliminary therapeutic index (maximum tolerated dose/ED50) than the antimalarial drug sodium artesunate. In the transgenic adenocarcinoma of mouse prostate (TRAMP) model, some of the trioxane dimers had potent anticancer activity.



62.

IN VITRO ASSAY OF THE ANTI-MALARIAL EFFECTS OF SELECTED PHILIPPINE MEDICINAL PLANTS. Kerry Jane Femia B. Ruadil, Department of Chemistry, Angeles University Foundation, 43-17 Aranda Ave. Holy Angel Village 4, Telabastagan, San Fernando, 2000, Philippines, kbhallug@yahoo.com

Anti-malarial effect of the plant extracts was determined by a colorimetric assay of the Lactate Dehydrogenase (LDH) activity of the malarial parasites using MALSTAT reagent and NBT/PES dye. The percentage survival of the parasites was computed based on the optical densities at 620 nm. The results were correlated with the anti-malarial effect of the chloroquine drug, aralen ®.

MATERIALS AND METHODS

The plants were air dried and grounded. Weighed and extracted in dichloromethane and sequentially with methanol and separately with water, it was dried under vacuum. Water extracts were freeze-dried. The stock solution was serially diluted using half dilutions. From these dilutions, 25 ml was dispensed into 80 wells of a 96-well microtitre plate. The parasitemia of the trophozoite stage was counted and set to 2% along with a hematocrit of 2%. 160 ml of parasitized red blood cells and 240 ml of normal red blood cells (rbc) were mixed with 20 ml of complete culture medium. 200 ml of this mixture was then dispensed into the 80 wells containing the plant extract and into 8 more wells to serve as the parasite control. Remaining 8 wells served as rbc control. The plate was incubated at 37 oC for 48 hours. After 48 hours, another clean 96-well microtitre plate was prepared. 100 ml of the MALSTAT reagent was placed into the wells, then 15 ml of the harvested parasites were added. Finally 25 ml of NBT/PES dye was added. After roughly 10 minutes, the plates were read at 620 nm and the survival rate computed.

RESULTS AND DISCUSSION The results showed that the plant extracts effectively inhibited malarial LDH activity up to the 1:8 dilution and the survival rate was less than 25%. However, the plant extracts had varying effects on the two species—Plasmodium falciparum and Plasmodium vivax. The plant extracts closely rivaled the anti-malarial effects of a known anti-malarial drug in the Philippines—aralen ®. **RECOMMENDATIONS** Isolation and extraction of the bio-active constituents responsible for the anti-malarial effects of the test plants should be undertaken and toxicity testing should also be done.

63.

DESIGN, SYNTHESIS, MODELLING AND ACTIVITY OF NOVEL ANTI-TUBERCULAR COMPOUNDS. Sudershan K Arora¹, NEELIMA Sinha², RAKESH Sinha³, RAMAN Bateja⁴, SHARAD Sharma⁵, and RAM S Upadhyaya². (1) PRESIDENT, NEW CHEMICAL ENTITY RESEARCH, LUPIN RESEARCH PARK, LUPIN LIMITED (RESEARCH PARK), PUNE INDIA, SITE # 46A/47A, VILLAGE: NANDE, TALUQA: MULSHI, Pune 411021, India, Fax: 91-20-5126175, sudershanarora@lupinpharma.com, (2) MEDICINAL CHEMISTRY, NCER, LUPIN LIMITED (RESEARCH PARK), PUNE INDIA, (3) MICROBIOLOGY, NCER, LUPIN LIMITED (RESEARCH PARK), PUNE INDIA, (4) ANALYTICAL RESEARCH, NCER, LUPIN LIMITED (RESEARCH PARK), PUNE INDIA, (5) TOXICOLOGY, NCER, LUPIN LIMITED (RESEARCH PARK), PUNE INDIA

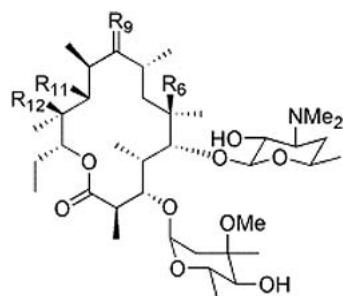
As a paradigm chronic infectious disease, tuberculosis exhibits a variety of clinical presentations ranging from primary tuberculosis to reactivation tuberculosis. In recent years there has been a worldwide upsurge in incidence of tuberculosis especially those caused by multi drug resistant strains of *M. tuberculosis*. Pyrolyles are a new class of molecules that exhibit anti microbial activity. We have synthesized ~500 novel compounds and tested these for activity against *M. tuberculosis* isolates. Several of these compounds have shown activity against *M. tuberculosis*. The MIC₅₀ and MIC₉₀ values of two compound viz. LL3522 and LL3858 were (MIC₅₀ 0.12 & 0.06 μg/ml; MIC₉₀ 0.25 & 0.25 μg/ml).

$\mu\text{g/ml}$ respectively). LL3858 demonstrated dose dependent mycobacterial activity and synergy with Rifampicin in the *in vitro* assays. When the mice were infected with a sub-lethal dose of *M. tuberculosis* and then treated with LL3858 at 12.5 mg/kg or 25 mg/kg per day for 4 weeks, the \log_{10} CFU counts in the organs of treated mice were significantly lower than the control group. Further, treatment with LL3858 for 8 and 12 weeks reduced the \log_{10} CFU counts in the target organs of treated animals and a complete absence of growth was seen in two of six mice treated with LL3858 for 12 weeks. The effectiveness of LL3858 mono-therapy was better than Isoniazid. Combination therapy with LL3858 plus anti-TB drugs (Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol) was superior to that with LL3858 or any anti-TB drug alone. Combination treatment for eight weeks with LL3858 plus Isoniazid and Rifampicin or LL3858 plus Isoniazid, Rifampicin and Pyrazinamide sterilized the lungs and spleens of three of six and four of six mice respectively. Prolonged treatment for 12 weeks with LL3858 plus IR or LL3858 plus IRZ sterilized the target organs in six of six mice infected with sensitive / resistant strains. The pilot Pharmacokinetics experiments of LL3858 conducted in mice and dogs indicated that the compound is bioavailable, levels over MIC in serum for increased time and better half life and C_{max} than Isoniazid. In the acute toxicity experiments in mice by oral route LL3858 showed LD₅₀ value of 700 mg/kg. These results indicate that LL3858 is effective in *in vitro* and *in vivo* against *M. tuberculosis* and is also effective when given in combination.

64.

PARALLEL SYNTHESIS OF MACROLIDES FOR ANTI-TUBERCULOSIS LEAD OPTIMIZATION. *Zhaohai Zhu¹, Kanakeshwari Falzari¹, Dahua Pan², and Scott G. Franzblau¹. (1) Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612, Fax: 312-355-2693, zhaohai@uic.edu, (2) Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago*

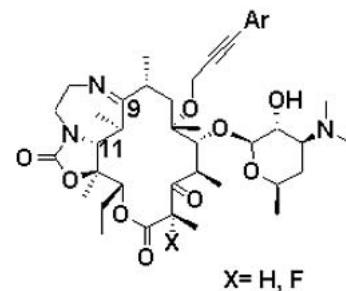
Among infectious diseases, tuberculosis (TB) is the number one killer with over two-million casualties annually worldwide. In our screening program, we have discovered anti-TB macrolides with sub-micromolar MIC *in vitro*, and with activity in TB-infected mice. The purpose of this study is lead optimization through parallel synthesis of analogues of the lead compounds and screening for anti-TB activity.



65.

DIAZALIDE DERIVATIVES OF 6-O-HOMOPROPARGYL MODIFIED KETOLIDES AS POTENT ANTIBACTERIAL AGENTS. *Hong Yong¹, Yu Gui Gu¹, Richard Clark¹, Thomas Marron¹, Zhenkun Ma², Niru Soni³, Greg Stone³, Angela Nilius¹, and Stevan Djuric¹. (1) R47H, Metabolic Diseases Research, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6112, Fax: 847-938-1674, hong.yong@abbott.com, (2) Cumbre, (3) Pfizer*

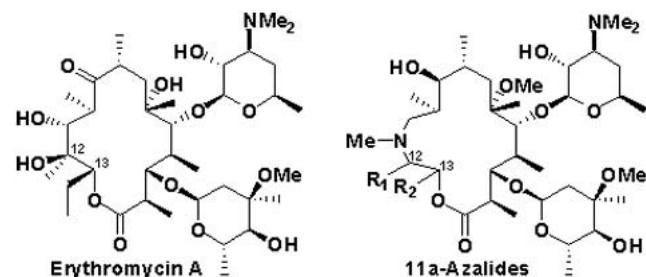
Antibiotics of the macrolide class are safe and effective agents for the treatment of respiratory tract infections. However, over the last few years bacterial resistance to macrolides has become a serious clinical problem. The ketolide family of macrolides with potent activity against all major respiratory pathogens has been developed in our group to counter this emerging resistance problem. More recently, a new structural class of macrolides, known as diazalides, has received attention due to its unique pharmacokinetic properties as well as high potency. The diazalide class is characterized by an ethylene bridge that links the C-9 imine and the C-11 carbamate nitrogen units of the macrolide. The synthesis and structure-activity relationships of members of this class will be discussed in detail.



66.

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 11a-AZALIDES: A NOVEL CLASS OF 15-MEMBERED MACROLIDE ANTIBIOTICS. *Tomohiro Sugimoto, Tetsuya Tanikawa, Akira Manaka, Youichi Shimazaki, Keiko Suzuki, Yukiko Yamasaki, Hiroyuki Sugiyama, and Toshifumi Asaka, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Saitama-shi, Saitama 331-9530, Japan, Fax: +81-48-652-7254, tomohiro.sugimoto@po.rd.taisho.co.jp*

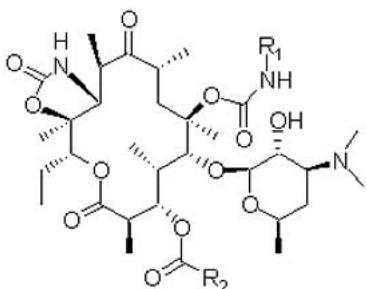
The prevalence of resistance to multiple antimicrobials in community-acquired respiratory tract infections represents a public health threat. To address this therapeutic problem, numerous chemical modifications of erythromycin A have been investigated over the past decade. In particular, modification of the aglycon skeleton is considered to be a promising approach to improve its antibacterial activity. In the course of our research, we have identified 11a-azalides (11a-aza-11a-homoerythromycin derivatives), which show improved antibacterial activity against resistant respiratory pathogens. 11a-Azalides were prepared from erythromycin A via three key chemical conversions: removal of the C12-C13 portion, introduction of an adequate counterpart and reconstruction of the aglycon ring. In this presentation, the synthesis and SAR of this novel series of macrolides will be discussed.



67.

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 3-O-ACYL-6-O-CARBAMOYL ERYTHROMYCIN A DERIVATIVES. *Bin Zhu, Brett Marinelli, Darren Abbanat, Barbara Foleno, Todd Henninger, Ellyn Wira, Karen Bush, and Mark Macielag, Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, LLC, 1000 Route 202, P.O.Box 300, Raritan, NJ 08869, Fax: 908-203-8109, bzhu@prdus.jnj.com*

The macrolide antibiotic erythromycin A and its semi-synthetic derivatives, such as clarithromycin and azithromycin, have been widely prescribed to treat respiratory tract infections. However, macrolide-resistant infections have been observed with increasing frequency in recent years. As a result, there is a clear need for new macrolide antibiotics with activity against the resistant bacteria. Herein we report a new series of erythromycin A derivatives, the 3-O-acyl-6-O-carbamoyl macrolides, which have activity against macrolide-resistant *streptococci*. Structurally, these new macrolide compounds have an acyl group at the C3 position and a heteroaryl sidechain attached to the macrolide core through a carbamate linkage at the C6 position. The synthesis and antibacterial activity of this new series of macrolide compounds will be discussed.



68.

EVALUATION OF NOVEL BISBENZAMIDINES AS POTENTIAL DRUG CANDIDATES FOR *PNEUMOCYSTIS CARINII* PNEUMONIA. Melanie T. Cushion¹, Peter D. Walzer², Jean J. Vanden Eynde³, Annie Mayence³, and Tien L. Huang³. (1) Division of Infectious Diseases, Department of Internal Medicine, University of Cincinnati, Cincinnati, OH 45267-0560, melanie.cushion@uc.edu, (2) Research Service, Veterans Affairs Medical Center, (3) Department of Basic and Pharmaceutical Sciences, Xavier University of Louisiana, College of Pharmacy, 1, Drexel drive, New Orleans, LA 70125, Fax: 504-520-7954, thuang@xula.edu

Bisbenzamidines, e.g. pentamidine, are used to combat against a number of fungal and protozoal infections despite their toxicity and poor bioavailability. As part of our goal in the development of more effective antimicrobials, we have designed and synthesized a series of novel bisbenzamidines linked by a rigid (e.g. phenylenediacetamide) or a flexible (e.g. pentanediamide) core. *In vitro* evaluation of these compounds using a *P. carinii* ATP detection assay indicated that the bisbenzamidines with flexible alkylidicarboxamides as linkers were remarkably potent (IC_{50} : 0.87 to 1.3 ng/ml) anti-*P. carinii* agents. These compounds demonstrated very low cytotoxicity in a human lung epithelial cell line (A549) and were effective against *P. carinii* in the mouse model.



69.

PARALLEL SYNTHESIS OF ANTIBACTERIAL ACPS INHIBITORS. Matthew A Kiritsis, Elizabeth Dushin, and Adam Gilbert, Exploratory Chemistry, Wyeth, 401 N Middletown Rd, Pearl River, NY 10960

Synthesis of Antibacterial AcpS Inhibitors

Acyl Carrier Protein Synthase (AcpS) is a bacterial enzyme involved in several biosynthetic pathways. It is essential and conserved among gram positive and gram negative organisms, and not homologous to mammalian organisms. AcpS was targeted with the goal of making novel antibacterials. An HTS screen revealed ML-47766 as a compound possessing inhibitory activity at AcpS, but weak MIC activity. Analogs of this compound were made with the goal of improving enzyme inhibition and MIC activity. Computational chemistry was used to direct the synthetic targets. Several analogs were made which improved upon the potency of the lead, but MIC activity was variable and a consistent SAR did not emerge.

70.

IDENTIFICATION AND CHARACTERIZATION OF POTENT MURA INHIBITORS.

Melanie A. Priestman, Martha Lyn Healy, and Ernst Schönbrunn, Department of Medicinal Chemistry, University of Kansas, 4038 Malott Hall, Lawrence, KS 66045, map2442@ku.edu

UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) belongs to the small enzyme family of enolpyruvyl transferases; it catalyzes the first committed step in the biosynthesis of the bacterial cell wall. Since this enzyme is absent from mammals but essential for bacterial growth, MurA is a prime target for the development of novel antibacterial agents effective against a broad range of pathogenic bacteria. By high-throughput screening (HTS) using a 50,000 compound library, we have recently identified five new MurA inhibitors with unique scaffolds with IC_{50} values ranged from 2 μ M to 10 μ M. Characterization of these five inhibitors is being carried out using kinetic and fluorescence

assays as well as co-crystallization with MurA. This information will be used in future work to aid in the design and synthesis of compounds with antibacterial properties and higher potency against MurA.

71.

INHIBITORS OF NAD SYNTHETASE: IDENTIFICATION OF THE OPTIMUM LINKER LENGTH FOR THE TETHERED DIMERS. Wayne J. Brouillette¹, Yong-Chul Kim², Sadanandan E. Velu¹, Christie G. Brouillette², Chi-Hao Luan², and Lawrence J. DeLucas². (1) Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294, (2) The Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham

NAD synthetase catalyzes the transformation of nicotinic acid adenine dinucleotide to the amide product NAD via a two-step process. NAD is a coenzyme that plays an important role in the biochemical transformations such as DNA repair and energy production. The protein crystal structure of *Bacillus subtilis* NAD synthetase provides an attractive target for the structure-based design of compounds that are potential antibacterial agents. Earlier combinatorial synthesis and high throughput screening identified a group of tethered dimers as lead inhibitors. They contained a benzyloxy substituted phenoxy group at one end and a positively charged 4-(*N,N,N*-trimethylammonio)phenyl acetate group at the other end of a polymethylene linker. In order to optimize the length of the linker between the two end groups, a series of new compounds were synthesized with linker lengths ranging from 2 to 12 carbons. Optimum length of the linker is identified from this series. The design, synthesis and biological data of these compounds will be presented.

72.

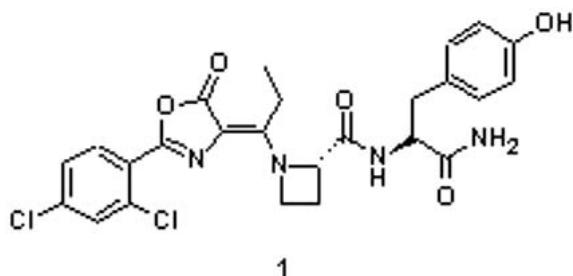
SYNTHESIS AND SAR FOR ARYL SUBSTITUENTS ON TETHERED DIMER INHIBITORS OF NAD SYNTHETASE. Wayne J. Brouillette¹, Liyuan Mou¹, Sadanandan E. Velu², Christie G. Brouillette², Chi-Hao Luan², and Lawrence J. DeLucas². (1) Department of Chemistry/Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham, 1025 18th Street South, Birmingham, AL 35294 - 1240, (2) Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham

Nicotinamide adenine dinucleotide (NAD⁺) and its reduced form NADH are essential cofactors in cellular metabolism and in energy production. NAD⁺ synthetase catalyzes the final step in the biosynthesis of NAD⁺, namely the transformation of NaAD (nicotinic acid adenine dinucleotide) into NAD⁺. Inhibitors of this enzyme may provide effective antibacterial agents. The x-ray crystal structure of NAD⁺ synthetase from *Bacillus subtilis* provided for the structure-based design of potential antibacterial agents. Earlier combinatorial synthesis and high-throughput screening identified a group of tethered dimers as lead inhibitors, which contain two end groups joined by a 8- carbon linker. One favorable end group is a substituted aromatic ring and the other is an *N,N,N*-trimethyl ammonium quaternary salt. A series of new inhibitors were designed to investigate SAR for different substituents on the terminal aromatic ring. The design, parallel solution phase synthesis, and biological data will be presented.

73.

POTENT AND SELECTIVE INHIBITORS OF BACTERIAL METHIONYL tRNA SYNTHETASE DERIVED FROM AN OXAZOLONE-DIPEPTIDE SCAFFOLD. Manish Tandon¹, David L. Coffen¹, Paul Gallant², John Finn², Dennis Keith², and Mark A. Ashwell¹. (1) Medicinal Chemistry, ArQule Inc, 19 Presidential Way, Woburn, MA 01801, Fax: 781-287-2916, mtandon@arqule.com, (2) Cubist Pharmaceuticals Inc

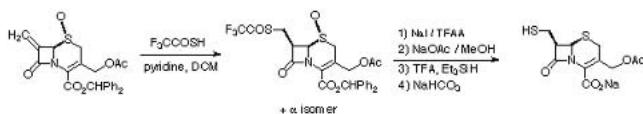
The preparation and structure-activity relationships (SARs) of potent and selective small molecule inhibitors of bacterial methionyl-tRNA synthetase (MetRS) derived from an oxazolone-dipeptide scaffold are described. Examples combine *Staphylococcus aureus* MetRS (SaMetRS) potency with selectivity over human MetRS. As a result of the SAR expansion compound **1** was identified, as a potent and selective SaMetRS inhibitor (IC_{50} =18 nM) having moderate inhibition of MetRS derived from *Enterococci faecalis* (IC_{50} =3.51 μ M) and was devoid of inhibition against human MetRS ($IC_{50} > 100$ mM).



74.

SYNTHESIS AND ACTIVITY OF CEPHALOSPORINS THAT SIMULTANEOUSLY INACTIVATE BOTH SERINE AND METALLO- β -LACTAMASES. *Hansong Chen¹, Venkat Rao Gadhachanda¹, Lakshminarayana Voleti¹, Timothy Palzkill², Robert Shaw³, and John D. Buynak¹.* (1) Department of Chemistry, Southern Methodist University, Box 0314, Dallas, TX 75275-0314, Fax: 214-768-4089, hchen@mail.smu.edu, jbuynak@mail.smu.edu, (2) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, (3) Department of Chemistry and Biochemistry, Texas Tech University

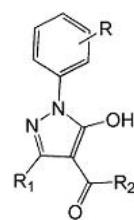
β -Lactamases represent the most important mechanism of bacterial resistance to β -lactam antibiotics. While the class A serine β -lactamases are the most clinically relevant, classes B, C, and D enzymes are a growing threat. Class B metallo- β -lactamases have an especially broad substrate profile, which includes the carbapenems. The Buynak Group has recently reported a unique penicillin-derived series of inhibitors capable of simultaneously inactivating both serine (class A and C) and metallo (class B) β -lactamases. We now report a group of cephalosporin-derived inhibitors with similar bifunctional inhibitory properties. A new reagent, trifluorothioacetic acid, was developed to introduce sulfur into the cephalosporin skeleton.



75.

4-SUBSTITUTED-5-HYDROXY-1-PHENYL-1H-PYRAZOLES AS ANTBACTERIAL AGENTS: SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS. *Syed M. Ali¹, Paul Hawkins¹, Binaya Kansakar¹, Mark A. Ashwell¹, Tim Opperman², Lucy Ling², Xiaoling Puyang², Jun Xian², and Yibin Xiang².* (1) Medicinal Chemistry, ArQuile Inc, 19 Presidential Way, Woburn, MA 01801, sali@arquile.com, (2) Genome Therapeutics Corp

A series of 4-substituted-5-hydroxy-1-phenyl-1H-pyrazoles were discovered in a cell based antibacterial high throughput screening campaign. A parallel hit expansion project was initiated combining *in silico* predictive ADME, *in vitro* ADME and parallel library based synthesis. Several new series of 4-keto-5-hydroxy-1-phenyl-1H-pyrazoles (1) and 4-amido-5-hydroxy-1-phenyl-1H-pyrazoles (2) were prepared and tested for antibacterial activity. The preparation and structure-activity relationships of the molecules will be presented and discussed.



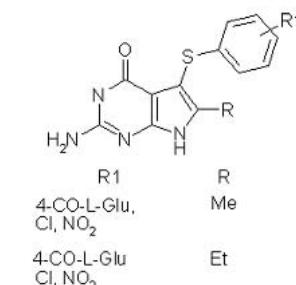
1; R₂ = alkyl and aryl
2; R₂ = N-alkyl

76.

DESIGN AND SYNTHESIS OF CLASSICAL AND NONCLASSICAL 6-ETHYL-5-ARYLTHIO-SUBSTITUTED PYRROLO[2,3-D]PYRIMIDINES AS INHIBITORS OF THYMIDYLATE SYNTHASE AND AS ANTITUMOR AGENTS.

Aleem Gangjee¹, Hiteshkumar Jain¹, John J. McGuire², Edward Chu³, and Roy L. Kisliuk⁴. (1) Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, Fax: 412-396-5593, gangjee@duq.edu, (2) Grace Cancer Drug Center, Roswell Park Cancer Institute, (3) Yale Cancer Center, Yale University School of Medicine, (4) Department of Chemistry, School of Medicine, Tufts University

Recently we reported that unlike the thymidylate synthase (TS) inhibitors **5FU**, **ZD1694** and **LY231514**, classical and nonclassical compounds of general structure **1**, inhibit TS and the inhibitor-TS complex binds to TS mRNA and prevents the synthesis of new TS protein. Increased synthesis of new TS is a mechanism for tumor resistance and chemotherapy failure. Molecular modeling using hTS crystal structures suggested that extending the C6-methyl moiety of **1** to an ethyl could enhance the hydrophobic interaction of the compounds with the Trp109 in hTS and perhaps increase the potency against TS. Thus compounds **2**, the C6-ethyl analogs of **1**, were designed. The synthesis and biological activity of compounds **2** will be presented.



77.

SYNTHESES OF HYDROXAMIC ACID BASED SIDEROPHORE ANALOGS AS

POTENTIAL INHIBITORS OF SIDEROPHORE BIOSYNTHESIS. *Xin Sha, Chemistry Department, Wake Forest University, 1834 Wake Forest Road, Winston-Salem, NC 27106, shax1@wfu.edu, and S. Bruce King, Department of Chemistry, Wake Forest University*

Siderophores are low molecular weight iron-sequestering agents produced by most microorganisms, plants and even higher organisms. Under iron-deficient conditions, microorganisms synthesize and excrete siderophores to sequester iron to maintain their regular requirement. Since iron plays an essential role in the growth and survival of these microorganisms, studies related to inhibition of iron production and assimilation provide the basis to design new anti-bacterial agents. Hydroxamic acids, one of the most conserved iron-binding functional groups in siderophores have been extensively studied. Our goal is to synthesize N-hydroxyphosphonamides as potential inhibitors of the biosynthesis of naturally occurring siderophores. The tetrahedral shape of the N-hydroxyphosphonamide group is proposed to act as a possible transition state analog for siderophore biosynthesis. The preparation of the N-hydroxyphosphonamides will be based upon the cycloaddition reactions of P-nitroso phosphine oxides followed by palladium catalyzed nucleophilic displacement.

78.

DESIGN AND SYNTHESIS OF LIGANDS TO PROBE THE ACTIVE SITE OF

LUMAZINE SYNTHASE. *Mark Cushman¹, Jinhua Chen¹, Sambaiah Thota¹, Adelbert Bacher², and Boris Illarionov².* (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, cushman@pharmacy.purdue.edu, chen28@purdue.edu, (2) Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München

Riboflavin is the precursor of both flavin mononucleotide and flavin adenine dinucleotide, which participate in many important biological reactions. A variety of human pathogens lack the capacity to absorb sufficient riboflavin from their environment and must therefore depend on biosynthesis. This makes enzymes in the riboflavin biosynthetic pathway potential new targets for the design and synthesis of antibiotics. Lumazine synthase catalyzes the penultimate step in the riboflavin biosynthetic pathway. The detailed reaction mechanism is not known. In this study, a series of stable analogs was designed and synthesized to probe

the active site of the enzyme based on a hypothetical reaction mechanism and molecular modeling. It has been found that 5-nitro-6-C-[(D-ribosyl)methyl]pyrimidine-2,4-dione is more potent as a lumazine synthase inhibitor than 5-amino-6-C-[(D-ribosyl)methyl]pyrimidine-2,4-dione. These activities can be explained by structural and electronic effects.

79.

SYNTHESIS AND EVALUATION OF TRICLOSAN ANALOGS AS ANTIBACTERIAL AGENTS.

Erin M. Skoda, and Richard R. Hark, Department of Chemistry, Juniata College, 1236 Juniata College, Huntingdon, PA 16652, skodaem@juniata.edu

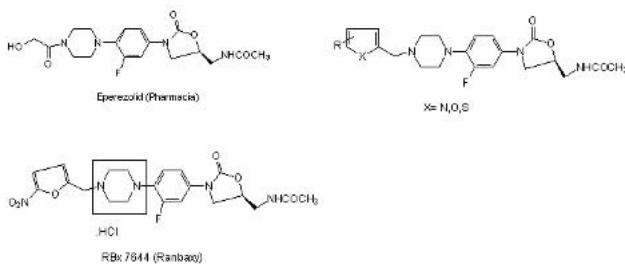
Analogs of the common antibacterial agent triclosan, 5-chloro-2-(2,4-dichlorophenoxy)-phenol, were synthesized to gain a better understanding of the how the compound binds in the active sites of enoyl reductase (ENR) and inhibits bacterial fatty acid biosynthesis. The role of the substituents of triclosan in inhibition has been suggested by crystallographic analysis, but further studies are needed to clarify the SAR. Fourteen analogs, in which H, Br, or F has replaced some or all of the halogen substituents and NH₂ or NO₂ has replaced the hydroxyl group, were prepared using a parallel synthetic protocol. Binding should be affected by the altered hydrogen bonding capabilities and molecular dimensions. Spot testing on agar plates against *E. coli*, *S. aureus*, and *P. aeruginosa* identified promising candidates which were further evaluated to determine each analog's MIC. Molecular modeling was used to attempt to rationalize these results.

80.

SYNTHESIS AND SAR OF NOVEL OXAZOLIDINONES.

Sonali Rudra¹, Ajay Yadav¹, AVS Raja Rao¹, Abhijit Ray¹, ASSV Srinivas¹, Shalini Shukla¹, Suman Saini¹, Ajay Soni¹, SK Arora¹, Ashok Rattan², Manisha Pandya², Pragya Bhateja², Sunita Malhotra², Anita Mehta¹, and Biswajit Das¹. (1) Medicinal Chemistry, NDDR, Ranbaxy, Plot-20, Sector-18, Udyog Vihar Industrial Area, Gurgaon, India, Fax: 911242342017, sonali.rudra@ranbaxy.com, (2) Microbiology, Ranbaxy

Oxazolidinones are a new class of totally synthetic antibacterials, active against gram +ve organisms. Zyvox (Pharmacia/Pfizer) is the only drug in this class approved for use in United States. Eperezolid (Pharmacia) was another compound, which went up to Phase-II clinical trial. Considering Eperezolid as the lead compound, its core structure containing the piperazinyl-phenyl-oxazolidinone ring was attached to various five-member mono-heterocycles. Synthesis of different substituted five-member heterocycles led to the identification of RBx 7644 as a clinical candidate. Further keeping the nitro-aromatic ring constant, the piperazine ring was replaced with several diamino-heterocycls and amino-oxyheterocycls. These modifications led to several compounds with potent activity against a spectrum of resistant and susceptible microorganisms.



81.

SYNTHESIS OF CLASS B SYNERGIMYCINS AND THEIR CORRESPONDING PEPTIDOMIMETICS.

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This project involves the synthesis of Class B synergimycin derivatives, which are antibiotics that bind to the peptidyltransferase center of the 50 S ribosomal subunit. This inhibits protein synthesis in bacterial cells. It is known that one mechanism of bacterial resistance to Synergimycins is the production of a lactonase by bacteria, where the opening of the lactone in Class B compounds

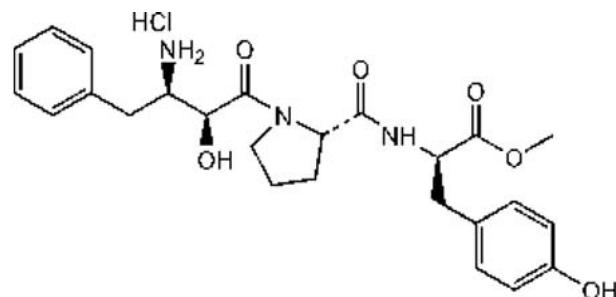
renders them useless as antibiotics. One method of preventing this mechanism is to replace the lactone with a ketone. In addition to the synthesis of class B derivatives, organozinc methodology is being utilized to synthesize Class B peptidomimetic derivatives. These derivatives no longer contain a lactone, and should therefore be immune to lactonases. This organozinc chemistry uses amino acid derivatives to maintain the integrity of the functional group positioning within the macrocycles.

82.

DESIGN AND SYNTHESIS OF BESTATIN DERIVATIVES AS POTENTIAL INHIBITORS OF BACTERIAL METHIONINE AMINOPEPTIDASE.

Shari J. Soper, Jack S. Amburgey, Sara Buhrlage, William L. Seibel, Alan W. Curnow, Jeremy M. Howard, Artem Evdokimov, and Matthew Pokross, Lead Discovery, Procter and Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45040, Fax: 513-622-0085, soper.sj.2@pg.com

Methionine aminopeptidase (MetAP), a divalent cobalt metalloprotease essential to the processing of proteins, cleaves the N-terminal methionine residue from growing polypeptides. Deletion of the gene encoding for MetAP-1 is lethal in prokaryotes, therefore inhibitors may have potential antibacterial activity. Our chemistry efforts focused on elaboration of the only known bacterial methionine aminopeptidase (bMAP) inhibitor. We report SAR regarding the potency, cell penetration, metabolic stability, and MIC values of the compounds. Several co-crystal structures from this work were obtained and used in the design process. It was found that a change in ring size clearly had an effect on the IC₅₀, with the natural proline analogs retaining highest potency. Four or six membered rings, either saturated or unsaturated, decreased the activity by 10-fold. An unsaturated proline ring retained the activity, but D-proline decreased the activity by 20-fold. We have found compounds with better cell penetration and metabolic stability. One of the most potent compounds is shown below, (IC₅₀ of 1.1mM).



83.

VIRTUAL SCREENING BASED APPROACH FOR THE DESIGN OF ERM

METHYLTRANSFERASE INHIBITORS. *Byron K. Bryant, Department of Medicinal Chemistry, The University of Mississippi, 417 Faser Hall, University, MS 38677, Fax: 662-915-5638, bkbryant@olemiss.edu, and John S. Williamson, Department of Medicinal Chemistry, University of Mississippi*

One method of resistance to macrolide-lincosamide-streptogramin type B (MLS) antibiotics seen in pathogenic bacteria is alteration of the drug target site by either posttranscriptional modifications or mutations. The former is accomplished by a base-specific methylation of bacterial 23S rRNA within the macrolide binding site, catalyzed by a methyltransferase (MTase) from the Erm family. It has been previously reported that the compounds that inhibit Erm MTases can sensitize MLS-resistant bacteria to macrolide antibiotics. We have developed a virtual screening based approach for the identification of new ErmC' MTase inhibitors. Our approach has been to first filter compounds in accessible chemical libraries based on ADME/toxicity properties. Then those compounds satisfying the criteria are subjected to a high throughput docking process, and the resulting hits are then tested for biological activity. The most active compounds are used as leads for further design and analog building to give optimized ErmC' MTase inhibitors.

84.

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL INDOLE-GUANIDINO COMPOUNDS AS POTENT ANTIFUNGAL AGENTS. Dong-Fang Shi¹, Janos Botyanszki¹, Chris Roberts¹, Mikail Gezginci¹, Sherwin Sattarzadeh¹, Corey Valdez¹, Ana Kongpachith¹, Christina Hancock¹, Kevin Fung¹, Mike Vigil¹, Konnie Pham¹, Gaurav Singh², Jackie Imai², Karl Clemons³, David Stevens³, Uli Schmitz¹, Mark Velligan¹, Lillian Lou¹, and Ronald Griffith¹. (1) Genelabs Technologies, Inc, 505 Penobscot Drive, Redwood City, CA 94063, dfshi@genelabs.com, (2) California Institute for Medical Research, (3) Stanford University; California Institute for Medical Research, San Jose, CA; Santa Clara Valley Medical Center, San Jose, CA

Serious fungal infections caused by Aspergillus species have been increasing in prevalence. Most current antifungal agents have serious limitations such as a narrow therapeutic window, inadequate spectrum of activity and rapid emergence of resistance. We describe the design, synthesis, lead optimization and structure-activity relationship of a novel class of antifungal molecules. The optimization was focused on di- and tri-heterocycles containing guanidines. The multidimensional optimization of lead GL48044, which is highly active in vitro against Candida albicans and Aspergillus fumigatus, led to the discovery of preclinical candidate GL48656. Compound GL48656 is active against A. fumigatus with an MIC₉₀ of 0.2 ug/ml. GL48656 exhibits excellent in vivo activity against A. fumigatus with efficacy equivalent to Amphotericin B and acceptable toxicity.

85.

DISCOVERY AND OPTIMIZATION OF POTENT ORALLY ACTIVE SMALL MOLECULAR THROMBIN RECEPTOR(PAR-1) ANTAGONISTS. Tetsuya Kawahara¹, Shuichi Suzuki¹, Fumiyoji matsuura¹, Richard.S.J. clark¹, Motoji kogushi¹, Hiroko kobayashi¹, Ieharu hishinuma¹, Nobuaki sato², Taro terauchi², Akiharu kaijwara², and Toshiyuki matsuoka³. (1) frontier research laboratories, Eisai Co.,Ltd, 1-3 tokodai 5-chome, tsukuba 300-2635, Japan, Fax: 81-29-847-4012, t-kawahara@hhc.eisai.co.jp, (2) discovery research laboratory I, Eisai Co.,Ltd, (3) Marketed Product Research Clinical Research Center, Eisai Co.,Ltd

Thrombin, a trypsin-like serine protease, is centrally involved in hemostasis, and also promotes diverse cellular responses such as platelet aggregation, lymphocyte mitosis, monocyte chemotaxis, and vascular smooth muscle proliferation. These actions are mediated by proteolytically-activated thrombin receptors (protease-activated receptors: PARs). A non-peptide small molecule PAR-1 antagonist (ER-97719-15) was obtained from high throughput screening using a receptor binding assay system. Through optimization of ER-97719-15, we found three types of compound with moderate PAR-1 antagonistic activity. In particular the indolin derivative ER-121958-06 inhibited human PRP aggregation by thrombin at 21nM. ER-121958-06(10 mg/kg p.o.) inhibited ex vivo aggregation induced by thrombin in the guinea pig. Furthermore ER-129614-06 (100 mg/kg, p.o.) prolonged the time to occlusion in the irradiated artery by 1.9 fold compared to control. In this PIT (photochemically-induced thrombosis) model, ER129614-06 selectively inhibited thrombin-induced PRP aggregation ex vivo. The SAR and biological evaluation of this series of compounds are described.

86.

DESIGN AND SYNTHESIS OF OXADIAZOLIDINEDIONES AS PAI-1 INHIBITORS. Ariamala Gopalsamy¹, Scott L. Kincaid², John Ellingboe¹, Thomas Groeling³, Thomas Antrilli³, Ann Aulabaugh¹, Gregory Friedrichs³, and David L. Crandall³. (1) Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, (2) Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, (3) Cardiovascular and Metabolic Diseases Research, Wyeth Research

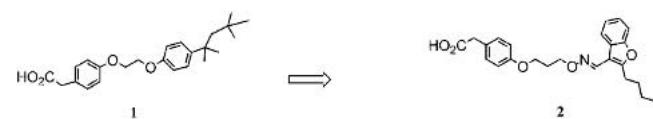
Plasminogen activator inhibitor-1 (PAI-1) is a major regulatory component of the plasminogen-plasmin system and is a member of the serine protease inhibitor family. Elevated plasma PAI-1 level is associated with decreased fibrinolysis and increased risk of thrombosis and hyper coagulation in a number of therapeutic disease states including chronic indications such as cardiovascular disease and certain types of cancer. Through high throughput screening of various libraries, substituted oxadiazolidinedione was identified as PAI-1 inhibitor. Various regions of the lead molecule were optimized using parallel synthesis. Optimization of the lead molecule retaining the oxadiazolidinedione moiety as the acid bioisostere showed flexibility in the spacer region accommodating a number of scaffolds with suitable lipophilic tailpiece. The

parallel synthesis effort, optimization approach and the SAR of this lead series will be discussed in detail.

87.

DESIGN AND SYNTHESIS OF NOVEL OXIME-BASED PAI-1 INHIBITORS. Lisa M Havran¹, John A. Butera¹, Douglas Jenkins¹, Hassan Elokdah¹, Girija Krishnamurthy², and David L. Crandall³. (1) Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, havrnl@wyeth.com, (2) Screening Sciences, Wyeth Research, (3) Cardiovascular and Metabolic Diseases Research, Wyeth Research

Plasminogen Activator Inhibitor-1 (PAI-1), a member of the Serine Protease Inhibitor (SERPIN) family, is the most important physiological inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Elevated PAI-1 activity is associated with decreased fibrinolysis and increased risk of thrombosis in many chronic and acute disease states. As part of a program to find an orally active small molecule that would normalize plasma PAI-1 activity and reduce thrombotic risk, high throughput screening was completed on the Wyeth chemical library. Several chemical leads were found including a bisphenoxy series exemplified by 1. Patent and stability issues were addressed by the development of a series of oxime based analogs. Benzofuran 2 improves the in vitro potency of previous leads and shows in vivo efficacy at 5 mpk in a clot lysis model. Recent results from this work will be presented.

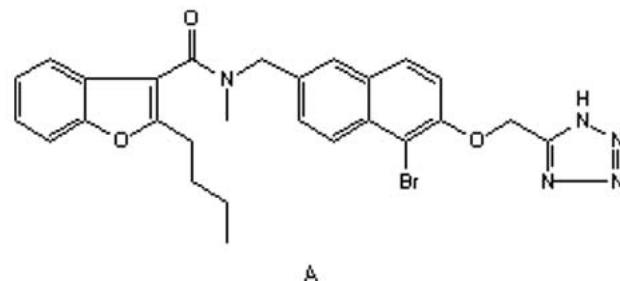


88.

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY OF A SERIES OF ARYLAMIDE-NAPHTHALEN-2-YLOXY-ACIDIC DERIVATIVES AS INHIBITORS OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1), THE MAJOR PHYSIOLOGICAL INHIBITOR OF TISSUE PLASMINOGEN ACTIVATOR (TPA).

Thomas J. Commons¹, Susan Croce¹, Richard P. Woodworth¹, Eugene J. Trybulski¹, Hassan Elokdah¹, David L. Crandall², James Hennan², Girija Krishnamurthy³, and Cheryl Mugford¹. (1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Rd, Collegeville, PA 19426, Fax: 484-865-9399, commonT@wyeth.com, (2) Cardiovascular and Metabolic Diseases Research, Wyeth Research, (3) Screening Sciences, Wyeth Research

Plasminogen activator inhibitor-1 (PAI-1) is the major physiological inhibitor of tissue plasminogen activator (tPA), a serine proteinase involved in fibrinolysis. Epidemiological studies have shown that elevated circulating levels of PAI-1 are associated with coronary heart disease and possibly atherosclerosis. These findings have generated an interest in developing a drug that specifically inhibits PAI-1. Consequently, high throughput screening (HTS) of our compound bank led to a number of leads that were grouped into eight distinct series. One such series ultimately led to the benzofuran amide A, one of five compounds selected as a Late Stage Discovery compound. The SAR leading to A, synthetic routes to various targets and the biological activity of selected compounds will be discussed.



89.

DESIGN, SYNTHESIS AND SAR OF 2-NAPHTHYL BENZOFURANS AS INHIBITORS OF PLASMINOGEN ACTIVATOR INHIBITOR-1. Hassan Elokdah¹, Geraldine R. McFarlane¹, Girija Krishnamurthy², and David L. Crandall³. (1) Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, elokdah@wyeth.com, mcfarlg@wyeth.com, (2) Screening Sciences, Wyeth Research, (3) Cardiovascular and Metabolic Diseases Research, Wyeth Research

Plasminogen activator inhibitor-1 (PAI-1) is a major regulatory component of the plasminogen-plasmin system. PAI-1 is the principal physiologic inhibitor of both tissue type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA). Elevated plasma levels of PAI-1 have been associated with thrombotic diseases. Neutralization of PAI-1 resulted in promotion of endogenous thrombolysis. Accordingly, agents that inhibit PAI-1 would be of utility in treating conditions originating from fibrinolytic disorder. High-throughput screening identified a benzoyl benzofuran hit. Subsequent substructure search and testing identified a series of naphthoyl benzofurans as more robust inhibitors of PAI-1. Synthetic efforts around the naphthoyl benzofurans led to the discovery of 2-naphthyl benzofuran series, with more potent in vitro and in vivo profiles, leading to the identification of WAY-164084 as a potent and selective PAI-1 inhibitor. This compound was subsequently advanced to pre-development status. The syntheses and SAR of these compounds as well as the binding properties and the in vivo activity of WAY-164084 in animal models of thrombosis will be presented.

90.

MECHANISTIC CHARACTERIZATION OF THE INTERACTIONS OF PLASMINOGEN ACTIVATOR INHIBITOR-1 WITH A SMALL MOLECULE INHIBITOR USING BIOPHYSICAL METHODS. Girija Krishnamurthy¹, Keith Pitts¹, Claudia Smeltzer¹, George Ellestad¹, Hassan Elokdah², and Dave Crandall³. (1) Screening Sciences, Wyeth Research, 401 North Middletown Road, Pearl River, NY 10965, Fax: 845-602-5687, krishng@wyeth.com, (2) Chemical and Screening Sciences, Wyeth Research, (3) Cardiovascular and Metabolic Diseases, Wyeth Research

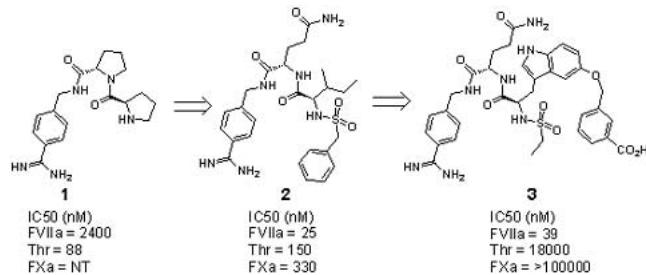
Plasminogen activator inhibitor (PAI-1) is the most important inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). PAI-1, a 50- kDa glycoprotein is a member of the serpin family of inhibitors. It plays a major role in regulating fibrinolysis by inactivating tPA and uPA. PAI-1 is a metastable protein that exists in several distinct conformational states including the loop inserted inactive latent form. We have characterized the interactions of the small molecule inhibitor, WAY-555, with PAI-1 using biophysical methods. Fluorescence binding experiments, using NBD-labeled PAI-1 show that the inhibitor binds PAI-1 with an affinity of ca.3 μ M. WAY-555 inhibits the interaction of active PAI-1 with tPA due to the formation of cleaved form of PAI-1, as evidenced by the changes in thermal unfolding transitions of PAI-1 isoforms and gel mobility assays. WAY-555 does not induce the inactive latent form of PAI-1 or other polymerized forms of PAI-1. The implications of these findings with respect to the novel mechanism of action of WAY-555 will be discussed.

91.

DESIGN AND SYNTHESIS OF PEPTIDOMIMETIC FVIIA INHIBITORS. Takuya Shiraishi, Shojiro Kadono, Masayuki Haramura, Hirofumi Kodama, Tohru Esaki, Takaki Koga, Kunihiro Hattori, Masateru Ohta, Haruhiko Sato, and Toshiro Kozono, Fuji Gotemba Research Labs, Chugai Pharmaceutical Co., Ltd, 1-135, Komakado, Gotemba 412-8513, Japan, Fax: 0550-87-5326, shiraishitky@chugai-pharm.co.jp

Factor VIIa (FVIIa), which is a trypsin-like serine protease, plays an important role in the initiation of blood coagulation. Recently, small molecule FVIIa inhibitors have been the point of much research effort because of their potential to inhibit the coagulation cascade while minimizing the risk of bleeding side effects. From Chugai compound-library, compound 1 having FVIIa inhibitory activity was identified. A systematic modification of compound 1 using structure-based drug design led to the identification of compound 2, potent Gln containing peptidomimetic FVIIa inhibitors. However, compound 2 suffered from poor selectivity over the other serine proteases in the coagulation cascade. Extensive SAR studies at P3 and sulfonamide group led to a new series of

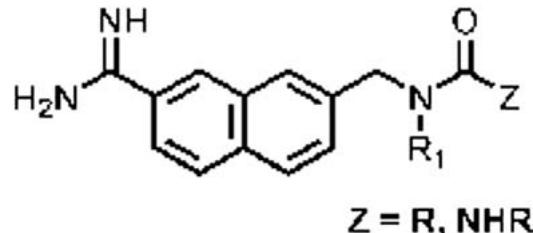
potent and highly selective FVIIa inhibitors typified by compound 3. Synthesis and structure-activity relationships of these compounds will be described.



92.

SOLID-PHASE SYNTHESIS OF NAPHTHYLAMIDES AS FACTOR VIIA/TISSUE FACTOR INHIBITORS. Brad O. Buckman, Yuo-Ling Chou, Dao Lentz, Amy Liang, David Light, Meg McCarrick, Raju Mohan, Michael M. Morrissey, Kenneth J. Shaw, and Lan Trinh, Berlex Biosciences, Richmond, CA 94804, Fax: 510-669-4310, brad_buckman@berlex.com

A strategy of reductive amination followed by acylation of polymer-linked formyl arylamides was used to generate combinatorial libraries of aryl amidines. Potent small molecule naphthylamidine inhibitors ($K_i < 100$ nM) of FVIIa/TF have been discovered and their activity against other serine proteases in the coagulation cascade is reported.



93.

X-RAY CRYSTALLOGRAPHIC STUDIES OF FVIIA/STF COMPLEXED WITH NOVEL PEPTIDOMIMETIC INHIBITORS. Shojiro Kadono, Susumu Ito, Masayoshi Oheda, Yasufumi Kikuchi, Akihisa Sakamoto, Naohiro Yabuta, Takaki Koga, Kunihiro Hattori, Takuya Shiraishi, Masayuki Haramura, Hirofumi Kodama, Tohru Esaki, Haruhiko Sato, Yoshiaki Watanabe, Masateru Ohta, and Toshiro Kozono, Fuji Gotemba Research Labs, Chugai Pharmaceutical Co., Ltd, 1-135, Komakado, Gotemba 412-8513, Japan, Fax: 0550-87-5326, kadonosuj@chugai-pharm.co.jp

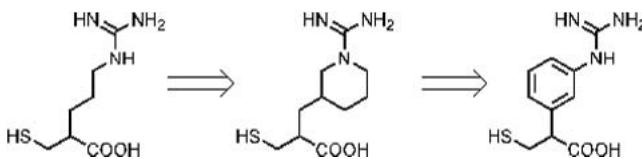
Serine protease Factor VIIa (FVIIa) initiates the blood coagulation cascade, when complexed with Tissue Factor (TF). Recent studies for the inhibition of FVIIa/TF by active-site-inhibited-FVIIa have shown promising results that the selective inhibition of FVIIa/TF may provide effective anticoagulation and the low risk of bleeding side effect. Therefore, the inhibition of serine protease activity of FVIIa/TF complex is seen as a promising target for developing anticoagulating drugs. The structures of FVIIa/soluble fragment of TF (sTF) in complex with novel, potent and highly selective peptidomimetic inhibitors developed by us have been determined by X-ray crystallography. In this poster, the structural basis for FVIIa selectivity and potency will be discussed based on the X-ray structures of the ternary complex of FVIIa/sTF/peptidomimetics inhibitor.

94.

3-MERCAPTOPROPIONIC ACIDS AS INHIBITORS OF THROMBIN ACTIVATABLE FIBRINOLYSIS INHIBITOR (TAFI). Brad O. Buckman, Marc Adler, Judi Bryant, Imadul Islam, Lorraine Kent, Brent Larson, Karen May, Raju Mohan, Mariko Nagashima, Ron Vergona, Kathy White, Marc Whitlow, Shendong Yuan, and Lei Zhao, Berlex Biosciences, Richmond, CA 94804, Fax: 510-669-4310, brad_buckman@berlex.com

TAFI is a plasma circulating zymogen that is activated by thrombin. Activated TAFI inhibits amplification of plasmin production at the clot surface by removing C-terminal lysine and arginine residues from partially degraded fibrin, thus slowing fibrinolysis. Inhibition of TAFI is a mechanism to enhance fibrinolysis without direct effects on coagulation factors or platelets. The design and synthesis of small molecule TAFI inhibitors, including data on potency, selectiv-

ity versus CPN, efficacy and X-ray structures in porcine pancreatic carboxypeptidase B will be presented.

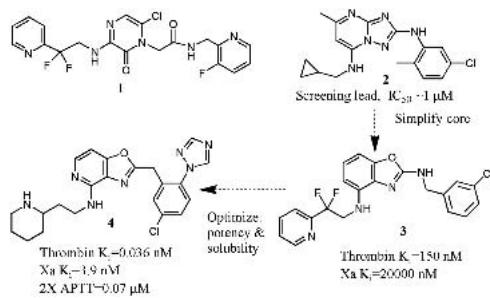


95.

BENZOXAZOLE THROMBIN AND FACTOR XA INHIBITORS. James Zhengwu

Deng¹, Christopher S. Burgey¹, Phillippe M. A. Rabbat¹, S. Dale Lewis², Bobby J. Lucas², Julie A. Krueger², Rebecca B. White³, Bradley Wong³, Elizabeth A. Lyle⁴, Daniel R. McMasters⁵, Joseph J. Lynch⁴, Youwei Yan⁶, Zhongguo Chen⁶, Lawrence Kuo⁶, Joseph P. Vacca¹, and Terry A. Lyle¹. (1) Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, (2) Biological Chemistry, Merck Research Laboratories, (3) Drug Metabolism, Merck Research Laboratories, (4) Pharmacology, Merck Research Laboratories, (5) Molecular System, Merck Research Laboratories, (6) Structural Biology, Merck Research Laboratories

Thrombin and Factor Xa are key enzymes in the coagulation cascade that regulate the blood clotting process. Direct inhibition of thrombin and Factor Xa is a common approach for the development of treatments for thrombosis. Previous reports from our labs have detailed the development of pyrazinone based thrombin inhibitors, exemplified by 1. In our efforts to develop structurally novel thrombin inhibitors, a benzoxazole P2 scaffold was designed based on 2 (IC₅₀ ~ 1 mM for thrombin), a lead from high throughput screening. A series of potent thrombin inhibitors (e.g. 3) was developed through SAR studies on the P2 benzoxazole scaffold. Replacing the benzoxazole core with an oxazolo-pyridine resulted in thrombin inhibitors with similar potency and improved efficacy. A series of potent dual thrombin and Factor Xa inhibitors was discovered (e.g. 4), by introducing a novel P3 piperidine into the P2 oxazolo-pyridyl scaffold. Structural and SAR studies suggested that an intramolecular H-bond between the P2 pyridine nitrogen and the P3 piperidine NH maybe pivotal for the Factor Xa activity. These studies have produced some of Merck's most potent in vitro anticoagulants.



96.

PEPTIDES WITH AMINO-ACIDS ANALOGS AS INHIBITORS FOR THROMBIN. Cristina Clement

and Manfred Philipp, Department of Chemistry, Lehman College, CUNY, 250 Bedford Park BLVD, West Bronx, New York City, NY 10468, clement_us@yahoo.com

New peptides containing phenylalanine (Phe) analogs like L-2-thienylalanine ((L/D)Tic), the constrained analog 1,2,3,4- (L/D)-tetrahydroisoquinoline-3-carboxylic acid, (trans/cis)cinnamic and dihydrocinnamic acids were designed to scan the P3 position in the sequence DPhe (P3)-Pro(P2)-DArg(P1)-P1' which was already shown previously that is inhibiting activity of thrombin in an in vitro assay using the S2238 (H-D-Phe-Pip-Arg-pNA) as substrate. In addition the L-Thi was used to scan positions P2 and P1'. The P1' position contains the most conserved L-amino acids found in the natural substrates of thrombin such as Gly, Ile, Ala, Cys and Ser. The new inhibitor DPhe-Pro-DArg-(L)Thi has a K_i of 3.9 μM and is a new lead compound in the series DPhe-Pro-DArg-P1', containing an unnatural amino acid analog in the P1' position. The order of activity for the peptides containing analogs of Phe in the P3 position is (D)Phe>Transcinnamic>Dihydrocinnamic>(D)Tic>L-Thi> (L) Tic with conserved residues at P2=Pro and P1=DArg. The analysis of the docked peptides into active site of thrombin together with the K_i obtained experimentally suggests

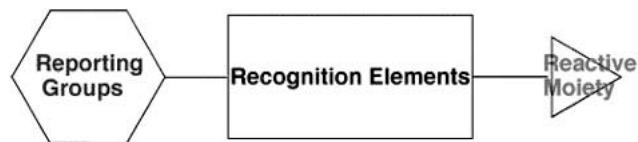
that the tetrapeptides differing in one single amino acid at P1' position are adopting different conformations into active site of thrombin, and these different conformations might be related to significant differences in their inhibitory potential.

97.

DEVELOPMENT OF ACTIVITY-BASED PROBES SELECTIVE FOR SERINE

PROTEASE SUBFAMILIES. **Zhengying Pan**¹, Amos Baruch², Kareem Chehade¹, Doug Jeffery², Matt Bogyo¹, and James M. Clark². (1) Department of Medicinal Chemistry, Celera Genomics, 180 Kimball Way, South San Francisco, CA 94080, Fax: 650-866-6654, zhengying.pan@celera.com, (2) Department of Biology, Celera Genomics

Chemical proteomics utilizes small molecule probes to profile enzymatic activity in complex proteomes and provides information on protein targets at the level of function rather than expression. Activity-based probes targeting the serine hydrolase family have been previously reported. Because this enzyme family contains more than 500 enzymes, some of which are abundant, regulatory enzymes such as serine proteases are often difficult to detect during labeling experiments. Hence it is essential to develop selective probes for a single enzyme or a subfamily of enzymes. We have developed a series of activity-based probes that selectively target the trypsin-fold family of serine proteases. After a survey of irreversible protease inhibitors, we designed small molecule probes with balanced activity, selectivity and reagent stability. Microwave-assisted synthesis provided a range of probes in good yields. In particular, we generated activity-based probes that are selective towards β and γ isoforms of human tryptase. Using these targeted probes, selective inhibition of leads was evaluated both in vitro and in situ by monitoring their ability to compete with activity-based labeling. Results including chemical synthesis and biological applications of these probes will be presented in the meeting.



98.

POTENTIAL SERINE PROTEASE INHIBITORS THAT INCORPORATE IN THEIR

STRUCTURE A FUNCTIONALIZED P2 RESIDUE. **Tzu Tshin Wong**, Liqing Wei, Christopher S. Groutas, Jiaying Zhong, Zhong Lai, Laura Stevenson, Xiangdong Gan, Bingfan Du, Kevin R. Alliston, and William C. Groutas, Department of Chemistry, Wichita State University, 1845 Fairmount, Wichita, KS 67260, Fax: 316-978-3431, ttwong@wichita.edu

The neutrophil-derived serine proteases human leukocyte elastase (HLE) and proteinase 3 (PR3) have been implicated in a wide range of diseases, including chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer metastasis. The close similarity of the active sites of the two enzymes presents a formidable challenge with respect to the design of inhibitors capable of discriminating between the two proteases. Thus, there is pressing need for the design of highly specific inhibitors of the two enzymes. Such compounds are of value as pharmacological agents, and as molecular probes in delineating the precise role that a protease plays in a particular disease. Inspection of the X-ray crystal structures of HLE and PR3 indicates that there exist subtle differences in some of the active site subsites in the two enzymes. We have sought to exploit these differences by designing reversible competitive inhibitors that incorporate in their structure a modified P2 residue. The design rationale, synthesis and results of in vitro biochemical studies will be presented.

99.

DISCOVERY OF POTENT AND SELECTIVE PROLINE AMIDE DERIVED DP-IV

INHIBITORS. **Scott D. Edmondson**¹, Anthony Mastracchio¹, Emma R. Parmee¹, Jinyou Xu¹, Maria Beconi², Lawrence F. Colwell Jr.¹, Bahau Habulihaz¹, Huaibing He¹, Barbara Leiting³, Kathryn A. Lyons¹, Frank Marsilio³, Reshma A. Patel³, Yohannes Teffera², Joseph K. Wu³, Nancy A. Thornberry³, and Ann E. Weber¹. (1) Department of Basic Chemistry, Merck & Co, PO Box 2000, RY 123-236, Rahway, NJ 07065, scott_edmondson@merck.com, (2) Preclinical Drug Metabolism, Merck & Co, Inc, (3) Department of Metabolic Disorders, Merck & Co

The increasing prevalence of type 2 diabetes mellitus has become a major focus for drug development in recent years. Glucagon-like peptide 1 (GLP-1) and

glucose-dependant insulinotropic polypeptide (GIP) are gut hormones secreted in response to increased plasma glucose levels. Both hormones stimulate insulin secretion and GLP-1 has been shown to stimulate insulin biosynthesis, inhibit glucagon secretion, slow gastric emptying, and reduce appetite. Due to rapid processing of these hormones by dipeptidyl peptidase IV (DP-IV), however, the half-lives of the active peptides in blood is extremely short. DP-IV inhibitors should therefore offer a number of potential advantages over existing diabetes therapies including a lowered risk of hypoglycemia and the potential for weight loss. Merck's in-house screening effort identified a proline-derived DP-IV inhibitor lead compound ($IC_{50}=1,700$ nM). This poster will describe the optimization of this lead to afford a potent and selective proline-derived DP-IV inhibitor ($IC_{50}=0.4$ nM).

100.

SUBSTITUTED PIPERAZINES AS NOVEL DIPEPTIDYL PEPTIDASE IV INHIBITORS.

Linda L. Brockunier¹, Emma R. Parmee¹, Jiafang He¹, Maria Beconi², Lawrence F. Colwell Jr.¹, Bahau Habulihaz¹, Huaibing He¹, Barbara Leiting³, Kathryn A. Lyons¹, Ann Mao², Frank Marsilio³, Reshma A. Patel³, Ralph Stearns¹, Yohannes Teffera², Joseph K. Wu³, Sherrie Xu², Nancy A. Thornberry³, and Ann E. Weber¹. (1) Department of Medicinal Chemistry, Merck & Co. Inc., P.O. Box 2000, Rahway, NJ 07065, Linda_Brockunier@Merck.com, (2) Preclinical Drug Metabolism, Merck & Co. Inc, (3) Department of Metabolic Disorders, Merck & Co. Inc

Inhibition of dipeptidyl peptidase IV (DP-IV) is a novel therapeutic approach to the treatment of type II diabetes. DP-IV rapidly degrades glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP) in vivo. GLP-1 and GIP are incretins which stimulate insulin secretion causing glucose uptake by cells thus decreasing serum glucose levels. Since the incretins are released by the digestive tract only in response to food consumption, inhibition of DP-IV-mediated degradation of GLP-1 and GIP can be expected to increase serum insulin without the attendant risk of hypoglycemia. SAR studies around a Merck proprietary screening lead were initiated and have resulted in the discovery of a novel series of substituted piperazines as potent inhibitors of dipeptidyl peptidase IV. The synthesis and biological evaluation of these compounds will be discussed.

101.

DISCOVERY OF POTENT, SELECTIVE BETA-HOMOPHENYLALANINE BASED DIPEPTIDYL PEPTIDASE IV INHIBITORS.

Jinyou Xu¹, Emma R. Parmee¹, Barbara Leiting², Frank Marsilio², Reshma A. Patel², Joseph K. Wu², Nancy A. Thornberry², and Ann E. Weber¹. (1) Department of Medicinal Chemistry, Merck & Co., Inc, P.O.Box 2000, Rahway, NJ 07065, Fax: 732-594-5790, Jinyou_xu@merck.com, (2) Department of Metabolic Disorders, Merck & Co., Inc

The gut hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are both incretin hormones that are released from the gut during meals, and serve as enhancers of glucose stimulated insulin release from the beta cells. GLP-1 has been proposed as a new treatment of type 2 diabetes. However, GLP-1 is rapidly degraded in plasma by the serine protease dipeptidyl peptidase IV (DP-IV). Inhibition of DP-IV increases the levels of endogenous intact circulating GLP-1. Therefore, inhibition of DP-IV is rapidly emerging as a novel therapeutic approach to the treatment of type 2 diabetes. Starting from an in-house screening lead, extensive SAR studies led to the discovery of a novel series of beta-homophenylalanine based dipeptidyl peptidase IV inhibitors. This poster will describe the synthesis and biological properties of these novel DP-IV inhibitors.

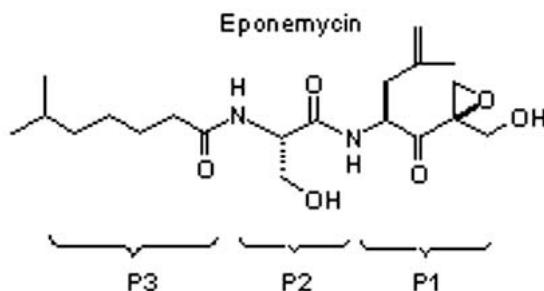
102.

NOVEL EPOXYKETONE PROTEASOME INHIBITORS-SYNTHESIS AND SAR STUDY.

Kozo Akasaka, Yimin Jiang, Xiang-Yi Li, Shawn Schiller, Bruce DeCosta, Christina Shaffer, Pamela McGuinness, Huiming Zhang, Lynn Hawkins, Jean-Christophe Harmange, Frank Fang, Charles Johannes, Jeff Zhu, Zhaiwei Lin, Thomas Zydowsky, Kenichi Nomoto, Jiayi Wu, Erin Murphy, Karen TenDyke, Timothy Chamberlain, Catherine Reardon, Mary Vermeulen, and Bruce Littlefield, Department of Lead Optimization, Eisai Research Institute, 100 Research Drive, Wilmington, MA 01887, Fax: 978-657-7715, xiangyi_li@eisai.com

The ability of natural products and other compounds to act as proteasome inhibitors has attracted significant interest because of the wide range of cellular substrates and processes controlled by the ubiquitin-proteasome pathway.

Inhibition of the proteasome by small molecules has been shown to induce apoptosis in a variety of malignant cell lines. Hence, proteasome inhibitors represent potential novel anticancer agents. In this poster, we will report the SAR of a series of epoxyketone proteasome inhibitors based on the natural product eponemycin. Our initial studies led to the proteasome inhibitor, ER-804191, which inhibited 20S proteasome chymotrypsin-like activity with a IC_{50} value at submicromolar range. ER-804191 also showed a 20-fold increase in the cell growth inhibition over dihydroeponemycin toward a variety of human cancer cell lines without much cytotoxicity toward quiescent cells. Further systematic SAR study involving the modification of the P1, P2 and P3 regions led to a series of novel compounds that significantly improved proteasome inhibition by up to 100 times over ER-804191. Our studies also showed that there is a significant correlation between inhibition of the 20S proteasome chymotrypsin-like activity and cell growth inhibition of adherent HT-29 cells by these compounds. A representative potent proteasome inhibitor, ER-807446, exhibited clear in vivo antitumor efficacy. The synthesis and a detailed SAR study of these novel proteasome inhibitors will be presented.



103.

PEPTIDE ANALOG MIMICS OF THE β -HAIRPIN OF TENDAMISTAT INHIBITOR FOR α -AMYLASE.

Steve M. Fernandes, Leena Khullar, Elizabeth J. Blaney, Dennis A. Brown, Jennifer Stephens, Horacia Opong-Owusu, M. C. Milletti, and Deborah L. Heyl, Department of Chemistry, Eastern Michigan University, 225 Mark Jefferson Building, Ypsilanti, MI 48197, Fax: 734-487-1496, mmilletti@emich.edu

Tendamistat (PDB entry 3AIT) is a proteinaceous inhibitor of α -amylase with K_i of 9pM. Triplet Trp¹⁸-Arg¹⁹-Tyr²⁰ is important in binding and forms a slightly distorted β -turn. Results of semi-empirical molecular orbital calculations were used to design the segment analogs Ac-YQ(Z)**WRY**(Z')Q-CONH₂ and Ac-YQ(Z)**WRY**-D-Pro(Z')Q-CONH₂, where Z, Z' = Cys, Pen or Ser, Ala. These peptides were synthesized and cyclized by disulfide formation between cysteine and/or penicillamine residues flanking the segment, in order to decrease flexibility. The optimized structures show that analogs have the correct configuration to fit the enzyme's active site. Total density surfaces mapped with the electrostatic potential show the region of positive potential localized on Arg¹⁹. Analogs were analyzed in a spectrophotometric assay. Although there is no expectation that small molecule mimics would attain the same affinity as Tendamistat, because of its large interaction surface with α -amylase, we are evaluating the template approach to mimic design and the effect of conformational restriction on affinity.

104.

CELL PERMEABLE PHE*-ALA BASED PEPTIDYL MIMETICS AS BACE INHIBITORS: N-TERMINI SAR.

Shu-Hui Chen¹, Jason Lamar¹, Jingdan Hu¹, Degqi Guo¹, Ana Belen Bueno¹, Todd Kohn¹, Hsiu-Chiung Yang², James McGee², Bruce Gitter², Richard Brier², Debra Laigle², Dan Czilli², Jianqing Huang², Jon Erickson¹, David Timm¹, Patrick May², and James McCarthy¹. (1) Discovery Chemistry Division and Technology, Eli Lilly and Company, Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-276-1177, Chen_Shuhui@lilly.com, (2) Neuroscience Discovery Research, Eli Lilly and Company

Since the identification of beta-secretase (BACE) as one of the enzymes involved in the production of Abeta peptide, scientists around the world are engaged in the search for BACE inhibitors as potential therapeutic agents for slowing down progression of Alzheimer's disease. We describe herein the N-termini (P2, P3 and P3 cap) SAR of a series of cell membrane permeable Phe*-Ala based BACE inhibitors. In conjunction with four fixed residues at the P1 (Phe), P1' (Ala), P2' (Val), and P2' cap (Pyridine), rather detailed SAR modifications at the P2, P3

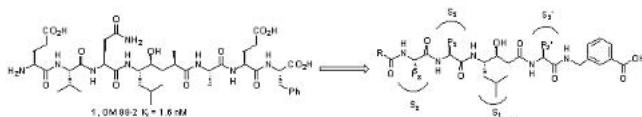
and P3 cap regions were pursued. The promising inhibitors emerging from this SAR investigation demonstrated very good enzyme potency ($IC_{50} < 50$ nM) and cellular activity ($IC_{50} < 0.5$ μ M).

105.

DESIGN, SYNTHESIS, AND SAR OF STATINE-BASED BACE INHIBITORS.

Jingdan Hu, Cynthia L. Cwi, David L. Smiley, David Timm, Jon A. Erickson, James E. McGee, Hsiu-Chiung Yang, Mike Shapiro, Patrick C. May, and James R. McCarthy, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-433-1685, hu_jingdan@lilly.com

BACE (β -amyloid precursor protein cleaving enzyme) is one of the most intriguing protease targets for the treatment of Alzheimer's disease (AD). The amyloid hypothesis suggests that inhibition of BACE may stop the production of β -amyloid, which cause plaque formation and neurodegeneration in AD patients. Based on the X-ray structure of OM 99-2, a statine-based tetrapeptide, BACE inhibitors 5 were designed and evaluated. Structure activity relationship studies at the P_3 , P_2 , and P_2' positions as well as the N-terminal capping group on scaffold 5 led to the discovery of potent inhibitors ($IC_{50} < 100$ nM). In addition, computational analysis and X-ray co-crystal structure of BACE-inhibitors are discussed.



106.

NOVEL DIARYL COMBRETASTATIN ANALOGUES: POTENTIAL AGENTS OF TUBULIN BINDING AND VASCULAR TARGETING. *Hania Wehbe, Institute of Biomedical Studies, Baylor University, P.O. Box 97224, Waco, TX 76798, Hania_Wehbe@baylor.edu, Kevin G. Pinney, Department of Chemistry and Biochemistry, Baylor University, and Christopher M. Kearney, Department of Biology, Baylor University*

Microtubules are cytoskeleton filaments intricately involved in cellular division and shape. Tubulin, the basic unit of microtubules, is a target of many anti-cancer agents. Combretastatins are tubulin binding agents isolated from the South African tree Combretum caffrum. Combretastatin A-4 (CA-4) inhibits tubulin polymerization; its prodrug, CA-4P, selectively targets endothelial cells lining the microvessels that feed tumors causing irreversible vascular shutdown within solid tumors, while leaving normal vasculature intact. The centroid to centroid distance separating the two aromatic rings of CA-4 is an important structural feature determining efficacy of tubulin binding. The design, synthesis, and preliminary biochemical evaluation of bridge-modified combretastatin analogues will be presented. CA-4 resistance to H460 lung carcinoma will be evaluated. The results of this study will clarify tubulin isotype specificity, vascular selectivity, and provide a greater understanding for the design of novel anti-tubulin agents.

107.

STEREOSPECIFIC ROUTE FOR THE PREPARATION OF COMBRETASTATIN ANALOGS: SYNTHESIS AND CYTOTOXICITY. *Zarmeem Taherhai, Andrew Staples, Lori Forrest, Martha Wicks, Suzanna Bailey, Michelle Stewart, Hari Pati, and Moses Lee, Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, Fax: 864-294-3559, Zarmeem.Taherhai@furman.edu*

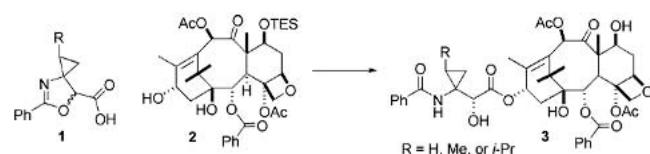
Combretastatins are cis-stilbene derivatives isolated from the African willow tree *Combretum caffrum* Kuntze (Combretaceae), and they possess potent anticancer activity. The most active of these agents is combretastatin A-4 (CA-4); it inhibits the polymerization of tubulin by binding to the colchicine site. It exhibits potent cytotoxicity against a broad spectrum of human cancer lines including those that are MDR positive. Major limitations of CA-4 are its poor solubility in biological media and poor bioavailability, which significantly impair its in-vivo activity. A water-soluble phosphate prodrug of combretastatin A-4 was subsequently developed, and it is presently undergoing phase II clinical trials. Accordingly, there is significant interest among medicinal chemists to investigate other novel combretastatin analogs, with the goal of developing more potent compounds with favorable physiochemical and pharmacokinetics properties. Our group has initiated a program to develop a series of amino analogs of combretastatins. For the project we had to develop an efficient and stereospecific route

for synthesizing the desired compounds. In this poster we will report the synthesis of a series of combretastatin analogs, that utilizes the Chugaev reaction to generate the cis double bond. Results from a cytotoxicity study on the analogs will also be presented.

108.

DESIGN, SYNTHESIS, AND BIOACTIVITY OF TAXOL ANALOGS WITH A CYCLOPROPANATED SIDE-CHAIN. *David G. I. Kingston¹, Changhui Liu¹, Armin de Meijere², Markus Tamm², Markus Nötzel², Karsten Rauch², Jennifer K. Schilling¹, James P. Snyder³, Ami Lakdawala³, Susan Bane⁴, Natasha Shanker⁴, and Rudravajhala Ravindra⁴. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-3255, dkinston@vt.edu, chliu3@vt.edu, (2) Institut für Organische Chemie, Georg-August Universität, Göttingen, Germany, (3) Department of Chemistry, Emory University, (4) Department of Chemistry, State University of New York at Binghamton*

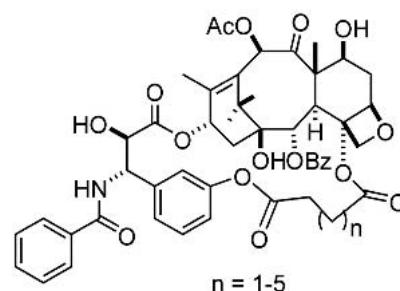
Ten Taxol analogs with a cyclopropanated side-chain (**3**) were synthesized by coupling the spirocyclopropanated oxazoline-5-carboxylic acid (**1**) with 7-TES-baccatin III (**2**), followed by hydrolytic ring opening and rearrangement. The absolute configuration of the 2'-position was determined by NMR analysis of the corresponding Mosher esters. A tubulin polymerization assay and A2780 and PC-3 mammalian cytotoxicities were used to evaluate their bioactivities. All the compounds were less active than Taxol. Molecular modeling studies in the tubulin binding pocket illustrate that an unfavorable deformation of the C-13 side chain resulting from steric compression prevents the formation of a productive ligand-tubulin complex.



109.

SYNTHESSES AND BIOACTIVITIES OF TAXOL MACROLIDES. *David G. I. Kingston¹, Changhui Liu¹, Jennifer K. Schilling¹, Susan Bane², Natasha Shanker², and Rudravajhala Ravindra². (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-7702, dkinston@vt.edu, chliu3@vt.edu, (2) Department of Chemistry, State University of New York at Binghamton*

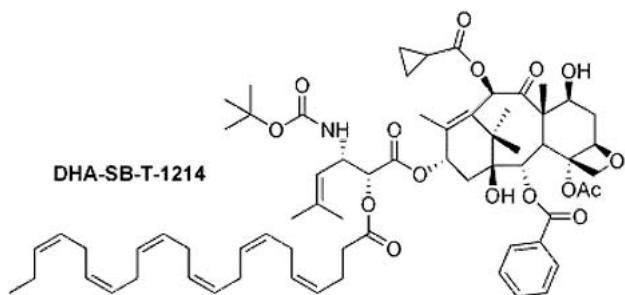
Paclitaxel (Taxol®) is one of the most important current drugs for the treatment of several cancers, including breast cancer and ovarian cancer. Its primary effect comes from its ability to bind to microtubules and stabilize them, resulting in disruption of the mitotic spindle and subsequent apoptotic cell death. In an attempt to mimic the tubulin-binding conformation of Taxol®, a number of macrocyclic diester derivatives were prepared with the general structure shown below. These compounds can isomerize to the corresponding 2'-acyl derivatives, and the relative ease of this isomerization was found to depend on the ring size. The syntheses, isomerization aptitudes, and biological activities for these Taxol® analogs will be presented.



110.

SYNTHESIS AND EVALUATION OF NOVEL FATTY ACID-2ND-GENERATION TAXOID CONJUGATES AS PROMISING ANTICANCER AGENTS. Larissa Kuznetsova¹, Xinyuan Wu¹, Jin Chen¹, Antonella Pepe¹, Liang Sun¹, Jean M. Veith², Ralph J. Bernacki², and Iwao Ojima¹. (1) Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, ikuznets@ic.sunysb.edu, (2) Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute

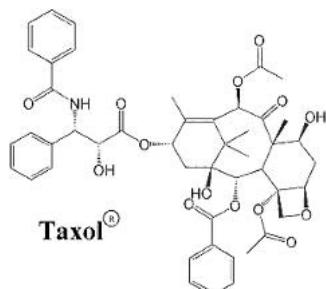
Novel fatty acid-2nd-generation taxoid conjugates were designed and synthesized, wherein the fatty acids include docosahexaenoic acid (DHA), docosanoic acid, linolenic acid (LNA) and linoleic acid (LA). These novel conjugates were evaluated in vivo against the human ovarian tumor A121 xenografts as well as the Pgp-positive human colon tumor DLD-1 xenografts in SCID mice. Some of these conjugates exhibited remarkable efficacy, e.g., DHA-SB-T-1213 brought about the complete regression (CR) of tumor in all surviving mice (4 of 5) implanted with the A121 xenografts. Even more impressive results were obtained against the Pgp-positive DLD1 xenografts, e.g., the treatment with DHA-SB-T-1214 resulted in the CR in 5 of 5 mice, while DHA-paclitaxel (Taxoprexin[®]) and paclitaxel were practically ineffective (only 4-day and 8-day tumor growth delays, respectively). SAR and a possible mechanism of action for these novel fatty acid-taxoid conjugates will also be presented.



111.

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF STA-4783: A NOVEL SMALL MOLECULE TAXOL[®] ENHANCER. Lijun Sun¹, Shoujun Chen¹, Keizo Koya¹, Zhiqian Xia¹, Noriaki Tatsuta², Timothy Korbut³, Zhenjian Du⁴, Guiqing Liang², Dan Zhou⁴, and Mitsunori Ono¹. (1) Chemistry, Synta Pharmaceuticals Corp, 45 Hartwell Ave., Lexington, MA 02421, Fax: 781-274-8228, lsun@syntapharma.com, schen@syntapharma.com, (2) Analytical Chemistry, Synta Pharmaceuticals Corp, (3) Animal Facility, Synta Pharmaceuticals Corp, (4) Biology, Synta Pharmaceuticals Corp

Nature provides the most abundant resources for treating various kinds of diseases. Isolated from Pacific yew tree *Taxus brevifolia*, Taxol[®] (generic name Paclitaxel) is clinically one of the most important chemotherapeutic agents for cancer treatment. Since its NDA was first approved by FDA in 1992 and launched by BMS in 1993, Taxol[®] has been successfully used for the treatment of various conditions including Breast, Ovarian, Testis, Melanoma, Head & Neck, Small cell lung cancers and AIDS related Kaposi Sarcomas. Despite the widely use of Taxol[®] as an anticancer agent, expanding the utility scope and increasing the therapeutic window of this natural drug has posted a great challenge. Through modification of a hit compound generated from screening of our unique compound libraries, we recently discovered a novel small molecule STA-4783 that showed potent enhancement for Taxol[®] activity. This synergistic enhancing effect efficiently increased the therapeutic index of Taxol[®]. In this presentation we will discuss and disclose the structure, synthesis and some



biological activities including HSP-70 inducing activity of STA-4783, currently a clinical candidate in Phase I human trials as a Taxol[®] enhancer.

112.

TOTAL SYNTHESIS OF 4-DEMETHYL-4-(2-HYDROXYETHYL)-EPOTHILONE-C AND ITS PHOTOAFFINITY ANALOGUES. Gunda I. Georg¹, Jun Inagaki¹, B.S.Narayan Reddy¹, and Richard H. Himes². (1) Department of Medicinal Chemistry, University of Kansas, 4001 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, KS 66045, Fax: 785-864-5836, georg@ku.edu, inaj721@yahoo.co.jp, (2) Department of Molecular Biosciences, University of Kansas

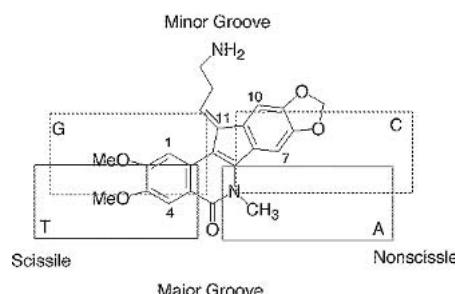
The epothilones are myxobacterial metabolites with impressive activity against multi-drug resistant cancer. They share a common mechanism of action, the hyperstabilization of microtubules, with the anticancer agent paclitaxel. In connection with our ongoing studies on the design, synthesis, and evaluation of epothilone photoaffinity probes as a tool to map the epothilone binding site, we are reporting the synthesis and anti-tubulin activity of 4-demethyl-4-(2-hydroxyethyl)-epothilone C and its photoaffinity analogues.

113.

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL CYTOTOXIC AMINOALKENYLINDENOISOQUINOLINE TOPOISOMERASE I INHIBITORS.

Xiangshu Xiao¹, Smitha Antony², G. Kohlhagen², Y. Pommier², and Mark Cushman¹. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, xsxiao@pharmacy.purdue.edu, (2) Laboratory of Molecular Pharmacology, National Cancer Institute

The cytotoxic indenoisoquinolines are a novel class of non-camptothecin topoisomerase I inhibitors with some pharmacological properties superior to the camptothecins. A new strategy was adopted to attach aminoalkenyl substituents at C-11 of the indenoisoquinoline system, which, according to molecular modeling, would orient the side chains toward the DNA minor groove. It is expected that the amino groups on these side chains will make specific hydrogen bonding interactions or nonspecific electrostatic interactions with DNA in the minor groove and thus these analogs will display increased cytotoxicity and topoisomerase I inhibitory potency. McMurry coupling between the indenoisoquinoline and appropriately protected aldehydes was employed to prepare the analogs. All of these newly synthesized compounds were more cytotoxic than the parent indenoisoquinoline. Despite an imperfect correlation between cytotoxicities and topoisomerase I inhibition results, the hypothetical model presented here provides a conceptual framework to explain the structure-activity relationships.



114.

JH-37, A HAIRPIN POLYAMIDE, INHIBITS THE BINDING OF NUCLEAR FACTOR-Y TO INVERTED CCAAT BOXES IN THE HUMAN TOPOISOMERASE II ALPHA PROMOTER: A BIOCHEMICAL AND BIOPHYSICAL STUDY. Lloyd Flores¹, James Henry¹, Suzanna Bailey¹, Zarmeen Tahberhai¹, Dorothy Harris¹, Karen Buchmueller¹, Binh Nguyen², David Wilson², Minal Koticha³, Daniel Hochhauser³, John Hartley³, and Moses Lee⁴. (1) Chemistry, Chemistry, 3300 Poinsett Highway, Greenville, SC 29613, Fax: 864-294-3559, lloyd.flores@furman.edu, (2) Department of Chemistry, Georgia State University, (3) Oncology, Royal Free & University College Medical School, (4) Chemistry, Furman University

The promoter of the topoisomerase II alpha gene contains two GC-boxes and five inverted CCAAT boxes, but it lacks a TATA element. Through deletion experiments, the second inverted CCAAT box (or ICB2) is believed to be critical for gene regulation. Using the rules of molecular recognition of DNA sequences

by stacked polyamides, in which a Py/Py pair binds to either A/T or T/A, a Py/Im prefers a C/G, and an Im/Py recognizes a G/C, we have designed a hairpin polyamide, JH-37, to target the sequence 5'-TTGGT-3'. This sequence is present within ICB2 and ICB3 of the topoisomerase II alpha promoter. Using a combination of gel shift and DNase I footprinting experiments, the ability of JH-37 to bind ICB1, 2, and 3 was observed. The results show that JH-37 is able to inhibit the binding of NF-Y to these ICB sites. The stoichiometry, sequence selectivity, as well as the thermodynamic and kinetics properties of the binding of JH-37 to an ICB site were determined using a combination of CD titrations, thermal melt, surface plasmon resonance, and isothermal titration calorimetry. The results and discussion from these studies will be presented.

115.

PREPARATION OF ANALOGS OF THE CYTOTOXIC TRYPROSTATINS A AND B. STUDY OF STRUCTURE ACTIVITY RELATIONSHIPS AS WELL AS IRREVERSIBLE INHIBITORS FOR MECHANISTIC WORK. *Chunchun Zhang, Jun Ma, and James M. Cook, Department of Chemistry, University of Wisconsin-Milwaukee, 3210 N. Cramer St., Milwaukee, WI 53211, Fax: 414-229-5530, zhangcc@uwm.edu*

Tryprostatin A and B are indole alkaloid-based fungal products that act in the G2/M phase of the cell cycle. Tryprostatin A and B as well as their two enantiomers and four diastereomers have been synthesized via a common strategy. As a measure of cytotoxicity, these eight stereoisomers were assayed for their growth inhibitory properties in human breast, prostate, and lung cancer cell lines. The ability of the tryprostatins and the tryprostatin stereoisomers to induce topoisomerase II-mediated DNA relaxation or to inhibit tubulin polymerization was also examined. Recently, a number of analogs of tryprostatin-A have been prepared and evaluated on cancer cell lines. Analogs with ring-A substituents are much less toxic and are potential anticancer drugs, because the mechanism of action differs from known clinical agents. These agents may be useful for the treatment of MDR forms of cancer. Recent studies indicate tryprostatin A does work nicely to block BCRP function in high expressing cells using flow cytometric analysis (Joel Turner and Dan Sullivan, unpublished results).

116.

SYNTHESIS OF NEW INDENOISOQUINOLINES: CYTOTOXIC AGENTS AND TOPOISOMERASE I INHIBITORS. *Alexandra S. Ioanoviciu¹, Smitha Antony², Y. Pommier², and Mark Cushman¹. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, aioanovi@purdue.edu, (2) Laboratory of Molecular Pharmacology, National Cancer Institute*

Novel indenoisoquinolines inhibit topoisomerase I and exhibit cytotoxic properties. Furthermore, these compounds overcome the limitations inherent in camptothecin therapy. The focus of our research is the design and synthesis of new indenoisoquinolines, which are potent topoisomerase I inhibitors with improved cytotoxicity. Two structural moieties that are associated with enhanced antitumor effect are the amine-containing side chains and the isoquinoline aromatic system. The amine groups located on the side chains attached to the heterocyclic nitrogen atom are expected to facilitate cell uptake, ease electrostatic binding at physiological pH to the negatively charged DNA macromolecule, and then favor the formation of cleavable ternary complexes. The planar isoquinoline moiety is likely to intercalate easily with DNA. Isoquinolinium salts are both topoisomerase I and topoisomerase II inhibitors. The biological activity of each new compound has been evaluated at the National Cancer Institute in 60 cancer cell lines and in topoisomerase I inhibition assays.

117.

INHIBITION STUDIES OF HISTONE DEACETYLASES INVOLVING TRICHOSTATIN A ANALOGS. *Jessica L. Tischler¹, Mike Baldwin¹, Ron Beaubien¹, and Ali R. Zand². (1) Department of Chemistry, University of Michigan-Flint, 303 E. Kearsly St., Flint, MI 48502, jtisch@umflint.edu, (2) Department of Science and Mathematics, Kettering University*

Histone acetylation plays an important role in regulation of transcription. Histone deacetylases (HDACs) are responsible for the removal of acetyl groups on the amino-terminal lysine residues of core nucleosomal histones. This activity is generally associated with transcriptional repression. Recently, two microbial metabolites, trichostatin A and trapoxin, were found to be potent inhibitors of

histone deacetylases. We have synthesized several analogs of trichostatin A with variations in the aromatic core, length and the nature of the side chain, and nature of the zinc-binding nucleus (hydroxamate functionality). The results of the inhibition studies of these compounds will be presented.

118.

N-(2-AMINO-PHENYL)-4-(OXO/DIOXO-HETEROARYLMETHYL)-BENZAMIDES: NOVEL HISTONE DEACETYLASE INHIBITORS. *Isabelle Paquin¹, Arkadij Vaisburg², Giliane Bouchain², Sylvie Frechette², Frédéric Gaudette², Silvana Leit², Oscar Moradei², Stéphane Raeppe², Nancy Zhihong Zhou², Soon Hyung Woo², Zhiyun Jin³, Jeff Gillespie³, James Wang³, Marielle Fournel⁴, Pu T. Yan⁴, Marie-Claude Trachy-Bourget⁴, Ann Kalita⁴, Aihua Lu⁴, Carole Beaulieu⁴, Zuomei Li⁴, Marie-France Robert⁴, Robert Macleod⁴, Jeffrey Besterman⁴, and Daniel Delorme². (1) Department of Medicinal Chemistry, MethylGene Inc, 7220 Frederick-Banting, Montreal, QC H4S2A1, Canada, Fax: 514-337-0550, paquin@methylgene.com, (2) Department of Medicinal Chemistry, MethylGene Inc, (3) Department of Analytical and PK, MethylGene Inc, (4) Department of Biology, MethylGene Inc*

Histone acetylation / deacetylation in eukaryotic cells is essential for chromatin remodeling and the regulation of gene transcription. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes that catalyze deacetylation (associated with transcriptional silencing) and acetylation (associated with transcriptional activation) of lysine residues located in the NH₂ terminal tails of core histones. Perturbations of this balance have been linked to cancer. Inhibition of HDACs is emerging as a novel therapeutic strategy against cancer. In our efforts to identify novel non-hydroxamate HDAC inhibitors with high potency and a low toxicity, we initially designed arylsulfonamido-2-aminophenyl cinnamides and 2-aminophenylamides of ω -substituted alkanoic acids. Here we present the synthesis and biological evaluation of N-(2-amino-phenyl)-4-(oxo/dioxo-heteroaryl methyl)-benzamides. These compounds were shown to inhibit partially purified human HDAC with IC 50 of low micromolar range, induce hyperacetylation of histones, expression of p21, and inhibit proliferation of human cancer cells. Certain compounds of this class were active *in vivo* against different cancer cell lines.

119.

ZN2+-CHELATING MOTIF-TETHERED SHORT-CHAIN FATTY ACIDS AS A NOVEL CLASS OF HISTONE DEACETYLASE INHIBITORS. *Qiang Lu¹, Yating Yang¹, Changshi Chen¹, Melanie Davis², John C. Byrd², Asad Umar³, and Ching-shih Chen¹. (1) Department of Medicinal Chemistry, College of Pharmacy, the Ohio State University, 500 West 12th Ave, Columbus, OH 43210, Fax: 614-688-8556, lu.159@osu.edu, (2) Division of Hematology-Oncology, The Arthur James Comprehensive Cancer Center, The Ohio State University, (3) Laboratory of Biosystems & Cancer, Center for Cancer Research, National Cancer Institute*

Among various classes of histone deacetylase (HDAC) inhibitors, short-chain fatty acids exhibit the least potency, with IC50 in the mM range. We rationalized that this weak potency was, in part, attributable to their inability to access the zinc cation in the HDAC active-site pocket, which is pivotal to the deacetylation catalysis. We thus explored the structural optimization of valproate, butyrate, phenylacetate, and phenylbutyrate by coupling them with Zn2+-chelating motifs (hydroxamic acid and phenylene diamine) through aromatic omega-amino acid linkers. This strategy has led to the development of a novel class of Zn2+-chelating motif-tethered short-chain fatty acids that exhibited varying degree of HDAC inhibitory potency. One compound, N-hydroxy-4-(4-phenylbutyrylamino)-benzamide (HTPB), display nM potency in inhibiting HDAC activity and cancer cell viability. Exposure of cancer cells to HTPB led to hyperacetylation of histones H3 and H4, and elevated levels of p21WAF/CIP1 expression, which are hallmark features associated with intracellular HDAC inhibition.

120.

APOTOSIS OF X-PHENOLS: A QSAR STUDY. *Rajeshwar P Verma¹, Sanjay Kapur¹, and Cynthia Selassie². (1) Department of Chemistry, Research Associate, 645 N College Avenue, Pomona College, Claremont, CA 91711, Fax: 909-607-7726, rverma@pomona.edu, (2) Professor*

Recent work from our laboratory on the cytotoxicity of X-phenols in murine leukemia cells has been represented by the equation: Log 1/ID50 = -0.19 BDE + 0.21 log P + 3.11; n=52, r²=0.920, q²=0.909, s=0.202. BDE values are directly related to the homolytic cleavage of the O-H bond and subsequently the

formation of phenoxyl radical. The low coefficient with log P suggests that the phenoxy radical may be directly interacting with a receptor such as DNA. These results led us a study of the induction of caspase mediated apoptosis in L1210 cells by X-phenols. We also wish to assess the role, if any, of the phenoxy-radical in controlling apoptosis. In a preliminary study of the apoptosis inducing effect of 18 X-phenols in a murine leukemia cell line (L1210), we developed the following QSAR model: Log 1/C=-0.58 Clog P + 2.18 B13 + 0.67 MR4-1.70; n=18, r²=0.840, q²=0.727, s=0.254. Log 1/C represents the 50% induction of caspase activity. This QSAR model suggests that the induction of caspases, particularly caspase 3, mediates the apoptotic action of X-phenols. The steric term suggest that bulky, hydrophilic phenols can be used as a way to therapeutically delete errant cells in proliferative diseases like cancer.

121.

DISCOVERY OF SUBSTITUTED 2-ARYL-4-ARYLAMINOPYRIMIDINES AND ANALOGS AS POTENT APOPTOSIS INDUCERS AND ACTIVATORS OF

CASPASES. *Nilantha S. Sirisoma*¹, Azra Pervin¹, Bao Nguyen¹, John Drewe¹, Ben Tseng², Shailaja Kasibhatla², and Sui Xiong Cai³. (1) Department of Chemistry, Maxim Pharmaceuticals Inc, 6650 Nancy Ridge Drive, San Diego, CA 92121, nsirisoma@maxim.com, (2) Biology, Maxim Pharmaceuticals, Inc, (3) Chemistry, Maxim Pharmaceuticals, Inc

Apoptosis is a physiological cell suicide mechanism involved in developing embryo, immune system, and in adult animal during tissue turnover. Many anti-cancer drugs are known to kill cancer cells through the induction of apoptosis. As part of our ongoing effort to discover and develop novel inducers of apoptosis as potential anticancer agents, we have discovered a series of substituted 2-aryl-4-arylamino-pyrimidines as a new class of potent inducers of apoptosis through our caspase and cell based HTS assay. These 2-aryl-4-arylamino-pyrimidines have been found to induce apoptosis in cancer cells derived from a range of human solid tumors including breast, prostate, and colon. EC50 values as measured by caspase activation, range from 0.05 μ M to 1.5 μ M for different cells. Several analogs showed sub-micromolar or better potency against most cell lines tested in the growth inhibition assay. We will report in detail the chemistry and SAR of substituted 2-aryl-4-arylamino-pyrimidines as inducer of apoptosis.

122.

AZA-PEPTIDE EPOXIDES - A NOVEL CLASS OF SELECTIVE AND POTENT

CASPASE INHIBITORS. *Juliana L. Asgian*¹, Karen E. James¹, Zhao Zhao Li¹, Ozlem D. Ekici¹, Jowita Mikolajczyk², Guy S. Salvesen², and James C. Powers¹. (1) School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, james.powers@chemistry.gatech.edu, (2) Program in Apoptosis and Cell Death Research, Burnham Institute

Aza-peptide epoxides, a novel class of irreversible protease inhibitors, are specific for the clan CD cysteine proteases. Aza-peptide epoxides with an aza-Asp residue at P1 are excellent irreversible inhibitors of caspases-1, -3, -6, and -8 with second order inhibition rates up to 1,900,000 M-1s-1. In general, the order of reactivity of aza-peptide epoxides is S,S > R,R > trans > cis. Some of the R,R epoxides, while being less potent, are actually more selective than the S,S epoxides. Aza-peptide epoxides designed for caspases are stable, potent, and specific inhibitors, as they show little to no inhibition of other proteases such as aspartyl proteases, serine proteases (granzyme B) and the cysteine proteases cathepsin B and papain (clan CA), and legumain (clan CD).

123.

DISCOVERY, SAR AND IN VIVO ACTIVITY OF 4-ARYL-4H-CHROMENES AS A

NEW SERIES OF APOPTOSIS INDUCERS USING A CELL- AND CASPASE-BASED HIGH THROUGHPUT SCREENING ASSAY. *William Kemnitzer*¹, Shailaja Kasibhatla¹, Songchun Jiang¹, Hong Zhang¹, Yan Wang¹, Jianghong Zhao¹, Shaojuan Jia¹, John Herich¹, Denis Labreque², Richard Storer², Karen Meerovitch², David Bouffard², Rabindra Rej², Real Denis², Charles Blais², Serge Lamothe², Giorgio Attardo², Henriette Gourdeau², Ben Tseng¹, John Drewe¹, and Sui Xiong Cai¹. (1) Maxim Pharmaceuticals, 6650 Nancy Ridge Dr, San Diego, CA 92121, bkemnitzer@maxim.com, (2) Shire Biochem Inc

By applying a novel cell- and caspase-based HTS assay, 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4H-chromene (**1a**) has been identified as a potent apoptosis inducer. Compound **1a** was found to induce nuclear fragmentation in Jurkat cells, and to arrest T47D cells at the

G2/M stage, and induce apoptosis as measured by the flow cytometry analysis assay. Compounds **1a** and its analogs are potent inhibitors of tubulin polymerization. These substituted chromenes were found to be active in the growth inhibition MTT assay with GI50 values in the low nanomolar range. Significantly, these compounds were highly active in the paclitaxel resistant, *p*-glycoprotein overexpressed, MES-SA/DX5 tumor cells. More importantly, these chromenes also were found to be active in the HUVEC tube formation assay, suggesting that this series of compounds may have antivascular activity. Analogs of **1a** have been found to be highly active in anticancer animal models. We will report in detail the SAR, *in vitro* and *in vivo* characterization of **1a** and its analogs.

124.

SUBSTITUTED PYRAZOLES AS SELECTIVE GGTASE-I INHIBITORS. *Jon-Paul*

Strachan, Patrick R. Kinley, Jeron H. Eaves, and Kenneth S. Rehder, Chemistry, PPD Discovery, 3500 Paramount Parkway, Morrisville, NC 27560, Fax: 919-462-5610, JonPaul.Strachan@rtp.ppd.com

In normal cells, Ras proteins cycle between their GTP (active) and GDP (inactive) bound states. Single amino acid substitutions can render Ras GTPases deficient and thus locked in the active state that results in uncontrolled cell growth. These mutations are found in over 30% of human cancers. To fulfill its signaling function Ras must associate with the plasma membrane, a process that is facilitated by prenylation of the protein. Geranylgeranyl transferases (GGTase) and farnesyl transferases (FTase) catalyze the prenylation of proteins with a carboxyl terminal sequence CAAX. It is no surprise then that GGTase-I inhibitors have emerged as a novel class of anti-cancer agents. Through a lead optimization program we have improved the potency and selectivity of our leads against GGTase-I and now have compounds with low nanomolar activity. We will discuss the synthesis of our lead, a CAAX mimetic, and the relevant structure activity relationship.

125.

COMBINATORIAL MODULATION OF PROTEIN PRENYLATION. *Sarah A.*

*Reigard*¹, Diwan S. Rawat¹, Katherine A. Hicks², Carol A. Fierke³, and Richard A. Gibbs¹. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, Fax: 765-494-1414, sreigard@pharmacy.purdue.edu, (2) Department of Biological Chemistry, University of Michigan, (3) Department of Chemistry, University of Michigan

The oncogene product Ras is farnesylated at the cysteine residue in a carboxyl terminal "CaaX" box by protein farnesyltransferase (FTase). The development of farnesyltransferase inhibitors (FTIs) as anticancer agents was based on a straightforward paradigm targeting mutant Ras proteins. However, it is unclear if Ras is the sole or most important target of FTIs; note that there are >300 prenylated proteins in mammalian cells. Chemical tools that could selectively modulate protein farnesylation would shed light on the relative roles of various farnesylated proteins in the growth of tumor cells. We have demonstrated that FPP substrate analogues can alter the peptide substrate specificity of FTase. This phenomenon is being examined in a systematic manner, through the synthesis of prenyl analogues and focused -CaaX peptide libraries combined with *in vitro* screening, and these results will be presented.

126.

SAR STUDIES OF AFC-BASED ICMT INHIBITORS. *Brian S. Henriksen*¹, Jessica

*L. Anderson*², Christine A. Hrycyna², and Richard A. Gibbs¹. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, Fax: 765-494-1414, brian@pharmacy.purdue.edu, (2) Department of Chemistry, Purdue University

Isoprenyl cysteine methyltransferase (Icmt) is a membrane bound enzyme catalyzing the methyl esterification of Ras. This methyl esterification is important in the proper localization of Ras to the plasma membrane. Ras is implicated in 30% human cancers, and 90% of pancreatic cancer. Therefore small molecules that mislocalize Ras are an attractive therapeutic approach to cancers of this kind. Recent structure activity relationship studies on the isoprenoid chain of N-Acetyl farnesyl cysteine (AFC), the minimal synthetic substrate for Icmt, lead to the discovery of the competitive N-Acetyl (3-isobutenyfarnesyl) Cysteine (Ki=13.1 μ M). To further elaborate the SAR of AFC and development potent Icmt inhibitors, modifications to the cysteine amine are being carried out. The lead amine and isoprenoid modifications will be utilized to design the next generation of Icmt inhibitors.

127.

DIETARY CONSTITUENTS AFFECT RECEPTOR ACTIVATION AND CELL PROLIFERATION IN HUMAN BREAST CANCER CELLS. Brian J. Bennion¹, Kristen S. Kulp², Jennifer L. Montgomery², Felice C. Lightstone³, Mark G. Knize², James S. Felton², and L. Michelle Bennett⁴. (1) Computational and Systems Biology, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, L-448 POBox 808, 7000 East Ave., Livermore, CA 94551, bennion1@llnl.gov, (2) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, (3) Computational and Systems Biology Division, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, (4) National Cancer Institute

We are investigating the effects of dietary carcinogens on cell proliferation in breast cancer cell lines. PhIP, a genotoxic heterocyclic amine, is a naturally occurring mutagen formed in well-cooked muscle meats. Estrogen receptor activation and cell proliferation assays show that PhIP increases estrogenic activity up to 1.8-fold compared to untreated cells and increases cell number 20-30% greater than control. Three-methyl-PhIP is not able to activate the estrogen receptor. N-OH-PhIP demonstrates anti-estrogenic activity comparable to the pure anti-estrogen ICI 182,780 (Faslodex®). To understand our experimental results, computational docking studies were done with each of the PhIP compounds and ICI 182,780 with the ligand-binding domain of the human estrogen-receptor (hERα). Molecular dynamics simulations were then performed to explore ligand-protein interactions. Demonstrating that food carcinogens modulate the estrogen receptor could have implications for women at risk for breast cancer who eat cooked meats with high concentrations of these compounds.

128.

INVESTIGATION OF POLYAMINE ANALOGS ON THE GROWTH OF MCF-7 BREAST CANCER CELL LINES. James Cuff, Julie Glover, Jacquelyn R. Lageman, and Francis Charles Mayville Jr., Natural Science Department, DeSales University, 2755 Station Avenue, Center Valley, PA 18034, Fax: 610-282-0525, immycc@aol.com, francis.mayville@desales.edu, jrl0@desales.edu

In this study, we are synthesizing new polyamine analogs using spermine as the template. The new polyamine derivatives will contain three, four or five carbon links between amine groups and two or three carbon primers at each end. It has been previously determined that the polyamine systems can bind to the minor grooves of DNA molecules and inhibit cell growth. In other previous work, the inhibition of cell growth has been studied using several synthesized polyamine derivatives, and it was found that these artificial systems had more inhibition ability than natural polyamines. In our study, the new polyamine systems will be compared with current polyamine analogs to determine their efficacy for inhibition of cell growth in MCF-7 breast cancer cell lines. The methods of analysis will include HPLC analysis of nuclear DNA and gross cell counting.

129.

NOVEL CLASS OF DUOCARMYCIN ANALOGS: DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF ACHIRAL ANALOGS OF DUOCARMYCIN SA. Michelle Stewart¹, Kristen Daniell¹, Erik Madsen¹, Minh Le¹, Heather Handl¹, Natalie Brooks², Konstantinos Kiakos², John Hartley², and Moses Lee¹, (1) Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, Fax: 864-294-3559, michelle.stewart@furman.edu, (2) Oncology, Royal Free & University College Medical School

CC-1065 and the duocarmycins belong to an extremely potent class of naturally occurring cytotoxic compounds. They have IC₅₀ values in the picoMolar range, against the growth of cancer cells in culture. Since their discovery, numerous structure-activity relationship studies have been conducted, and as a result four analogs have entered clinical evaluation. Presently, one of the four compounds, bizelesin, remains in clinical trial. One of the severe dose limiting toxicities of these compounds is bone marrow suppression. Consequently there is a strong interest in the design, synthesis, and testing of novel analogs that have comparable cytotoxicity, but with reduced systemic toxicity. In our laboratory, we have initiated a project to address the question of whether the chiral center present in the duocarmycins and CC-1065 is critically needed for DNA interaction and cytotoxicity. Accordingly, we have designed and synthesized several

analogs of duocarmycin SA, in which the chiral center has been removed. We have found the novel achiral duocarmycin compounds to exhibit potent DNA alkylating and cytotoxic properties. The results and discussion from these experiments will be presented.

130.

DESIGN, SYNTHESIS AND EVALUATION OF A NEW CLASS OF FLAVONOID AS ANTICANCER AGENTS. Gunda I. Georg¹, Lakshminarayna Vogeti¹, Yu Mi Ahn¹, Richard H. Himes², Lester A. Mitscher¹, Chun Jing Liu¹, Paul Hanson³, and Katherine F. Roby⁴. (1) Department of Medicinal Chemistry, University of Kansas, 4001 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, KS 66045, Fax: 785-864-5836, georg@ku.edu, lvogeti@ku.edu, (2) Department of Molecular Biosciences, University of Kansas, (3) Department of Chemistry, University of Kansas, (4) Department of Anatomy and Cell Biology, Kansas University Medical School

Flavopiridol, a novel semisynthetic flavone derivative of rohitukine that was first isolated from the Indian plant *Dysoxylum binectariferum*, was the first cdk inhibitor to be evaluated in phase III clinical trials as an anticancer agent. Toward the development of compounds with improved selectivity and higher binding affinity for the substrate kinase, we designed and prepared novel 8-substituted flavopiridol analogs. Their synthesis and testing for cytotoxic activity will be presented.

131.

DISCOVERY OF 2-ARYLTHIAZOLIDINE-4-CARBOXYLIC ACID AMIDES AS A NEW CLASS OF CYTOTOXIC AGENTS FOR PROSTATE CANCER. Veeresa Gududuru¹, Eunju Hurh², James T. Dalton², and Duane D. Miller¹. (1) Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, 847 Monroe Ave, Memphis, TN 38163, (2) Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, OH 43210

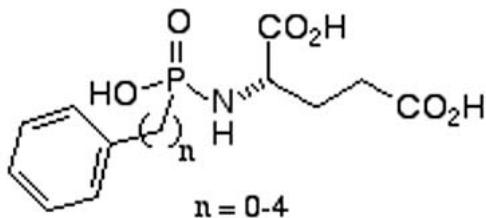
Prostate cancer is the single most common cancer in American males and is the second leading cause of cancer death in men in the United States. None of the conventional approaches to cancer therapy have proven successful for prostate cancer. It is also known that certain treatments-radiation therapy, radical prostatectomy or hormonal therapies can have detrimental effects on urinary, bowel, and sexual functions. Thus, development of a safe chemotherapeutic agent without any side effects would be an attractive proposal for the treatment of prostate cancer. We recently identified a novel series of cytotoxic serine amide phosphates (SAPs) for prostate cancer. These compounds are very effective in killing specific prostate cancer cell lines with IC₅₀ values ranging from about 2 mM to 50 mM. However, in spite of their high cytotoxicity they are not selective in differentiating cancer cells and healthy cells. It is also evident that phosphate group in this class of compounds is susceptible for hydrolysis by lipid phosphatases. To improve the selectivity, cytotoxicity and metabolic stability, we made some closely related structural changes on SAPs, which led us to discovery of a new class of 2-arylthiazolidine-4-carboxylic acid amides with IC₅₀ values at low micromolar range and enhanced selectivity. This new series of compounds were evaluated in PC-3, DU 145, LNCaP, PPC-1, and TSU-Pr1 human prostate cancer cell lines for their cytotoxicity. The details of synthesis, structure-activity relationship (SAR), and results of biological studies will be presented in this presentation.

132.

PROBING INHIBITORS OF PROSTATE SPECIFIC MEMBRANE ANTIGEN. Jack Maung¹, Tammy Campbell¹, Teri A. Girtsman¹, Nicholas Santiago¹, Bo Tan¹, David Wone², Darin Hildebrandt¹, Saeed Khazaie¹, Jun-Yong Kang¹, and Clifford E. Berkman¹. (1) Department of Chemistry and Biochemistry, San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132, bowofcupid@yahoo.com, (2) Chemistry, Elan Pharmaceuticals

Prostate-specific membrane antigen (PSMA) is a 750-amino acid type II membrane glycoprotein that is strongly expressed in prostate cancer cells, including those of the metastatic disease state. Although PSMA has attracted a great deal of attention as an antigenic target for immunotherapy, its glutamate carboxypeptidase activity has been relatively under explored. Although the known substrates for PSMA seem limited to acidic dipeptides such as gamma-glutamyl derivatives of folic acid and the neuropeptide N-acetylaspartylglutamate,

we hypothesized that auxiliary binding sites proximal to the active site of PSMA exist and could be consequently exploited by competitive inhibitors that possess complementary structural moieties. Indeed, we found that phosphonamidate derivatives of glutamic acid possessing a phenyl ring tethered 2-4 carbon atoms from the central phosphorus exhibited remarkably enhanced inhibitory potency over the benzyl and phenyl analogs. The synthesis and inhibition data for these hydrophobic-probing PSMA inhibitors along with various analogs will be presented.



133.

AMINO ACID ESTER PRODRUGS OF ANTICANCER AGENT GEMCITABINE AS SUBSTRATES OF HPEPT1 TRANSPORTER. *Xueqin Song, Philip Lorenzi,*

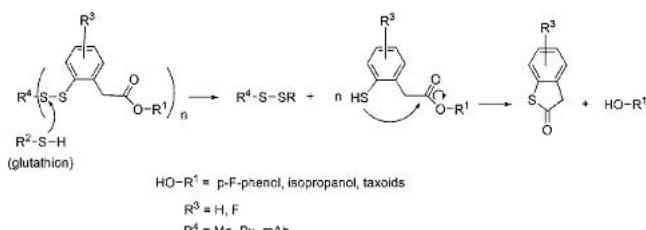
Christopher P. Landowski, Balvinder S. Vig, and Gordon L. Amidon, College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109, songx@umich.edu

Oligopeptide transporters such as hPEPT1 are highly expressed in gastrointestinal tract where they are involved in transport of di-/tri-peptides and peptidomimetic drugs. HPEPT1 is also highly expressed in a variety of cancer cells where they can be utilized to deliver peptidomimetic anticancer agents. The objective was to synthesize prodrugs of gemcitabine a well known anticancer agent to target cells over expressing hPEPT1. A series of amino acid ester prodrugs of gemcitabine were synthesized and evaluated for their affinity for hPEPT1, transport by hPEPT1, and activation by cellular enzymes. Aliphatic (Val and D-Val) and aromatic (Phe and D-Phe) amino acids were selected as promoieties. Results from the studies indicated that parent drug gemcitabine is not a hPEPT1 transporter substrate, while all prodrugs exhibited enhanced affinity for hPEPT1 transporter. Some prodrugs also showed higher uptake by cells overexpressing hPEPT1. Further, these prodrugs were also activated enzymatically into parent drug. Results from these studies will be discussed.

134.

DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF A NEW CLASS OF DISULFIDE-CONTAINING LINKERS FOR EFFICIENT INTRACELLULAR RELEASE OF ANTICANCER AGENTS. *Ioana M. Ungureanu, Jin Chen, Xinyuan Wu, Larissa Kuznetsova, and Iwao Ojima, Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, ioanau@yahoo.com, jincheng2@ic.sunysb.edu*

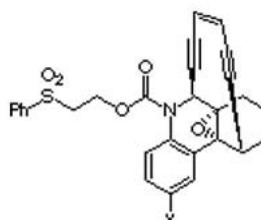
The design and synthesis of a new class of disulfide-containing linkers for novel taxoid-mAb conjugates (mAb=monoclonal antibody) and their use as target-activated prodrug is reported. A series of model experiments provided the proof of concept, i.e., S-S bond cleavage by a thiol, fast intramolecular thiolactonization, and the release of a free alcohol or taxoid. The progress of this process was followed by ¹⁹F NMR and HPLC. The key factors involved in this process and their influence on the kinetics of the process in the model systems as well as a real system will be discussed.



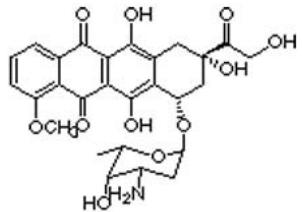
135.

NEW PRODRUGS OF DYNEMICIN ANALOGS AND DOXORUBICIN ANALOGS FOR SELECTIVE CHEMOTHERAPY MEDIATED BY AN ALDOLASE CATALYTIC ANTIBODY. *Lian-Sheng Li, Shantanu Dutta, Christoph Rader, Shin-ichi Watanabe, Eiton Kaltgrad Kaltgrad, Richard A. Lerner, Carlos F. Barbas III, and Subhash C. Sinha Sinha, The Skaggs Institute for Chemical Biology and Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, Fax: 858-784-8735, Ishli@scripps.edu*

The specific elimination of cancer cells by potent chemotherapeutic agents is limited due to their nonspecific toxicity to normal cells. In order to overcome this limitation, new methods, including antibody-directed enzyme prodrug therapy (ADEPT) approach, are emerging. In ADEPT approach, a prodrug of an anticancer agent is selectively activated using an enzyme that has been pre-directed to tumors with the help of a tumor-targeting antibody. We are using monoclonal aldolase catalytic antibody 38C2 as enzyme counterpart of the ADEPT approach. We have synthesized and evaluated prodrugs of dynemicin A analogs (1, 2) and doxorubicin (3) analogs in the presence and absence of aldolase mAbs. The prodrugs contain an aldol motif that is cleaved by an aldolase antibody-catalyzed reaction to provide a labile intermediate, which undergoes further manipulation under physiologic conditions to afford the corresponding drug. In vitro activity of the prodrugs in the presence/absence of aldolase antibody has been evaluated using cell growth assay.



1. X = H
2. X = OCH₂CH₂OH



3. Doxorubicin

136.

PURPURIN-LACTOSE CONJUGATES AS TARGET-SPECIFIC PHOTOSENSITIZERS FOR PDT. *Suresh K. Pandey¹, Xiang Zheng¹, Andrew Graham², Mahabeer P. Dobhal¹, Joseph R. Missert¹, Sue Camacho¹, Kate Rittenhouse-Olson³, Masayuki Shibata⁴, David A. Bellnier¹, Allan R. Oseroff², and Ravindra K. Pandey¹. (1) Photodynamic Therapy Center, Roswell Park Cancer Institute, Buffalo, NY 14263, suresh.pandey@roswellpark.org, (2) Department of Dermatology, Roswell Park Cancer Institute, (3) Department of Clinical Laboratory Science, School of Health Related Professions, The State University of New York at Buffalo, (4) Department of Health Informatics, Biomedical Informatics, UMDNJ-School of Health Related Professions*

The major challenge in cancer therapy is the destruction of malignant cells while sparing the normal tissues. While the demonstrated clinical usefulness of photodynamic therapy (PDT) has spawned the development of a large number of new photosensitizers, Photofrin®, a porphyrin-derivative is still the only photosensitizing agent approved worldwide for the treatment of variety of cancers. PDT is now a well-established modality for cancer treatment in which malignant cells are destroyed using the combination of a photosensitizer (PS), light (typically in 630-800 nm range) and tissue oxygen. Although PDT achieves a certain degree of selectivity through moderate therapeutic differentials of most photosensitizers and targeted light delivery to the tumor, the parameters currently chosen for optimized patient treatment are still limited by reactions of the normal tissue within the light field. Therefore, the design and development of PS targeted to the tumor has been the main goals of various investigators. In recent years, one of the objectives of our laboratory has been to develop certain porphyrin-carbohydrate conjugates, which could target those proteins (e.g. galectins) known for their high expression in a variety of malignant tissues. A few years ago, we reported the synthesis and biological significance of certain b-galactose-conjugated purpurinimides (a class of chlorins containing a six-member fused imide ring system). The preliminary in vitro and in vivo results obtained from these compounds were quite promising. In our attempt to investigate the effect of the position of lactose moiety in biological activity, the b-galactoside moiety was regioselectively introduced at various positions of the purpurinimide system. Under similar treatment conditions, the resulting novel

structures produced a remarkable difference in *in vivo* efficacy. In an *in vitro* experiment, the cells incubated with free lactose (known to bind to b-galactoside recognized proteins) prior to the addition of the photosensitizers containing b-galactose moiety produced 100% decrease in photosensitizing efficacy. Under similar experimental conditions, the related non-conjugated purpurinimides did not show any inhibition in cell kill. These results in combination with the galectin-binding data indicate a possible b-galactoside recognized protein specificity of the conjugates. The synthesis and biological efficacy of these novel structures with and without the lactose moiety will be presented.

137.

CONFORMATIONAL CHANGES IN DNA DUE TO MODIFIED MINOR GROOVE BINDERS.

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A number of clinically interesting DNA binding agents has been shown to affect the conformation of DNA. We are interested in designing sequence selective polyamide agents that have the capability to induce a bend in DNA. We hypothesize that the addition of bulky agents to the polyamide scaffold will result in bending. In order to test the hypothesis that bulky groups in the minor groove DNA can affect transcription through conformational changes, a combined computational and experimental effort has been undertaken. Molecular dynamics simulations have been carried out using CHARMM. Oligonucleotides ranging in size from 8 bp to 18 bp containing the AATT drug-binding site have been simulated both with and without drug. Through our analysis, key drug-induced conformational changes were determined.

138.

ENERGETIC BASIS FOR DRUG - DNA MINOR GROOVE INTERACTIONS.

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DNA minor groove binding compounds provide excellent probes of the groove as well as having significant therapeutic properties. However, very few detailed studies of their binding thermodynamics have been conducted, and such information is critical for rational drug design. Experimental and computational studies indicate that the DNA minor groove structure and hydration vary significantly with changes in sequence and solution conditions. Biosensor-surface plasmon resonance (free energy) and isothermal titration calorimetry (enthalpy) experiments were conducted with minor groove binding drugs and a systematic set of DNA sequences. The results reveal interesting thermodynamic linkages between DNA sequences and compound structures. The binding enthalpies and calculated entropies vary remarkably, but due to enthalpy-entropy compensation the binding free energies change much less. Negative heat capacity changes were observed and the changes varied linearly over the temperature range from 278 to 318 K. Changes in heat capacity on complex formation are generally correlated with changes in accessible surface area. Molecular dynamics simulations predict significant differences in hydration and minor groove width for the different DNA sequences. In general, smaller minor groove widths are associated with structured hydration and higher binding affinity. The thermodynamic parameters for formation of each complex depend on DNA sequence, hydration, and compound structures.

139.

POLYAMIDE DNA BENDING AGENTS BY STERIC FORCES IN THE MINOR GROOVE.

Anne Loccisano, Center for Computational Sciences, Duquesne University, Department of Chemistry and Biochemistry, 600 Forbes Avenue, Pittsburgh, PA 15282, Fax: 412-396-5683, loccsa780@duq.edu, Sarah A. Mueller-Stein, Department of Chemistry and Biochemistry, Center for Computational Sciences, Duquesne University, Steven M. Firestone, Department of Chemistry and Biochemistry, Duquesne University, and Jeffrey D. Evanseck, Department of Chemistry & Biochemistry and Center for Computational Sciences, Duquesne University

DNA bending can activate or inhibit gene expression depending on the phase of the bend. Bending by noncovalent external agents has been attributed to groove

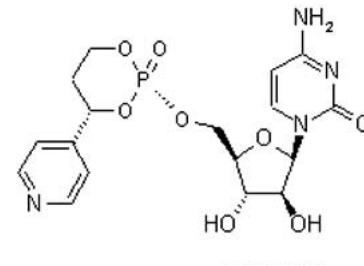
widening or contraction by steric or ionic interactions. We have adapted the CHARMM force field for sequence-specific agents that are designed to bind and bend DNA. These agents are polyamide-based drugs which bind to DNA in a 1:1 or 2:1 complex. The drugs have bulky groups, strategically placed, which contact the walls of the minor groove to bend DNA. Molecular dynamics simulations of the DNA bending agent complexes have been carried out to understand how these new agents interact with and bend DNA.

140.

DISCOVERY OF MB07133: A HEPDIRECT™ PRODRUG OF CYTARABINE MONOPHOSPHATE FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA.

Serge H. Boyer, Deidre MacKenna, Zhili Sun, Hongjian Jiang, Julie Esterbrook, K. Raja Reddy, Jorge E. Gómez-Galeno, William A. Craig, Bheemarao G. Ugarkar, Minghua Liu, Michael C. Matelich, Annika Montag, David Linemeyer, and Mark D. Erion, Metabasis Therapeutics, Inc, 9390 Towne Centre Dr, San Diego, CA 92121, Fax: 858-622-5573, boyer@mbasis.com

Hepatocellular carcinoma (HCC) remains a poorly treated cancer afflicting >500,000 people worldwide. Cytotoxic nucleosides have proven to be ineffective for treatment of HCC due in part to low levels of nucleoside kinases (NK) in hepatomas that are necessary for the conversion of nucleosides to their active nucleoside triphosphates (NTP), while other tissues have high levels of NKs giving rise to dose limiting extrahepatic toxicities. To circumvent both limitations, Metabasis has developed a prodrug technology (HepDirect™) that selectively releases the monophosphate of a nucleoside (NMP) into CYP3A4-expressing cells (hepatocytes) while leaving the prodrug inactivated in plasma and extrahepatic tissues. Cytarabine (araC) is a cytotoxic nucleoside currently used in the clinic for the treatment of myelocytic leukemia. Application of the HepDirect™ prodrug technology to araC led to the discovery of MB07133 which results in >10-fold araCTP levels in the liver vs. plasma and bone marrow. The synthesis of HepDirect™ prodrugs of araC, their enzyme and cell activation, and their *in-vivo* tissue distribution will be presented.



141.

COMPLEXATION OF RUBITECAN (9-NC) AND CYCLODEXTRINS.

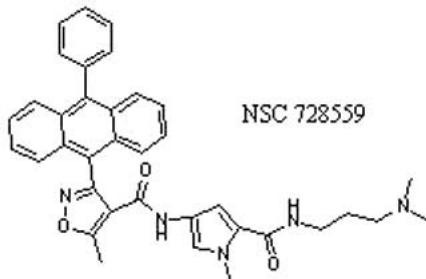
Chunlin Tang, and Rajashree Joshi-Hangal, SUPERGEN, INC, 1059 Serpentine Lane, Pleasanton, CA 94566, Fax: 925-461-1734, ctang@supergen.com

Rubitecan (9-NC), an experimental anti-cancer drug for pancreatic cancer, is a water insoluble 9-nitro derivative of Camptothecin containing a five-ring system with a lactone moiety in the E-ring that is susceptible to ring opening in pH greater than 6.5. Major challenges in formulating this drug for clinical application include, solubility enhancement and improved stability in biological fluids. Cyclodextrins are cyclic oligosaccharides that can interact with a variety of drug molecules to form inclusion complexes resulting in improved solubility and stability. Using spectral techniques of UV-Visible spectrometry and Circular Dichroism, it is demonstrated that a variety of cyclodextrins including hydroxypropyl beta cyclodextrin can complex with 9-NC in aqueous solutions. The formation constant *K_f* between 9-NC and hydroxypropyl beta cyclodextrin is estimated to be about 100/M. As a result, an improved solubility of 9-NC and stability of its lactone ring in biological fluids is expected.

142.

SYNTHESIS AND ACTIVITY OF ANTI-CANCER COMPOUNDS THAT TARGET G-QUADRUPLEX DNA. *Chun Li¹, Kevin Rider¹, William Fusco², Nicholas R. Natale¹, and Ronald Crawford².* (1) Department of Chemistry, University of Idaho, Moscow, ID 83844, (2) Environmental Research Institute, University of Idaho

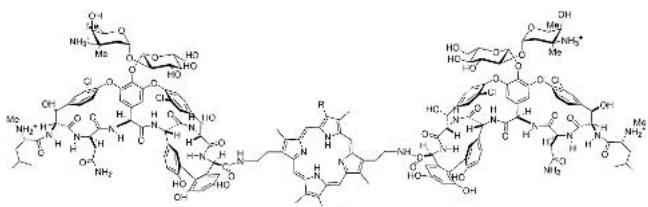
Guanine-rich sequences of DNA are not only found primarily at the ends of chromosomes as telomeric protein complexes, but also are present in a number of human genes, such as the *c-myc* oncogene and immunoglobulin switch regions. Such sequences are able to form higher-order DNA structures, e.g. G-quadruplex. Stabilization of G-quadruplex can either inhibit telomerase activity, which has been found in 85-90% of human tumors but not in adjacent normal cells, or lead to repression of c-MYC, which is associated with many human and animal malignancies. Combilexin isoxazoles prepared in our laboratories exhibited promising anti-tumor activity in the 60 cell line screening protocol. A leading analog (NSC 728559) shows high inhibition against Colon cancer cell line HT29 and Non-small cell lung cancer cell lines NCI H460, HOP-92 and HOP-62 cells with GI₅₀ values as 7.08E-07, 6.04E-07, 6.3E-08 and 1.05E-08, respectively. Both computational modeling and spectroscopic data are consistent with an external stacking mode with G-quadruplex. Nitrile oxide cycloaddition, Suzuki coupling, lateral metalation and Weinreb amidation were used for structural construction and modification. The TRAP assay was used to test the compound for telomerase inhibition.



143.

SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF MULTIVALENCY VANCOMYCINS BASED ON PORPHYRIN PLATFORMS. *Lihua Li¹, Pak Leung Ho², Chris C. K. Chang¹, and Bing Xu¹.* (1) Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, Fax: 852-2358-1594, chbingxu@ust.hk, (2) Department of Microbiology, School of Medicine, University of Hong Kong

Bacterial infections have emerged as public health threats, and it is important and urgent to develop an effective and rapid approach to counter the drug resistance of bacteria. One promising approach is multi/polyvalency—multiple simultaneous bindings between two biological entities. Here we report our work on using multivalency approach to against vancomycin resistant bacteria (VRE). We will report the synthesis and characterization of dimers of vancomycin (1) constructed from porphyrin platform. We will also report evaluation of their activities against VRE. This porphyrin based multivalent systems promise new scaffolds to explore the multi/polyvalency and develop potent antibiotics based on existing clinical drugs.



144.

SYNTHESIS AND ANTITUMOR-PROMOTING EFFECTS OF FURANOCOUMARINS AND ANALOGS ON EPSTEIN-BARR VIRUS ACTIVATION. *Xihong Wang¹, Mutsuo Kozuka¹, Harukuni Tokuda², Hoyoku Nishino², and Kuo-Hsiung Lee¹.* (1) School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, (2) Department of Biochemistry, Kyoto Prefectural University of Medicine

Twenty furanocoumarins and analogs were synthesized and screened as potential antitumor promoters by examining their ability to inhibit Epstein-Barr virus early antigen activation (EBV-EA) induced by 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. Compounds **1**, **5**, and **7** showed potent inhibitory activity, and compounds **10** and **14** showed moderate inhibitory activity without increasing cytotoxicity. The two most active compounds **1** and **7** are being tested for antitumor-promoting effects on two-stage mouse skin carcinogenesis initiated by DMBA and promoted by TPA. The synthesis and inhibitory activity as well as the structure-activity relationships of these compounds will be discussed.(Aided in part by grant CA 17625 from NCI and NIH, awarded to K. H. Lee).

145.

SYNTHESIS AND BIOLOGICAL EVALUATION OF HYDROPHYLICALLY ENHANCED N-3 CARBORANYL THYMIDINE ANALOGUES FOR BNCT.

Jayaseharan Johns Samuel¹, Youngjoo Byun¹, Ashraf S. Al-Madhoui², Nisha Lakhia¹, Junhua Yan¹, Staffan Eriksson², and Werner Tjarks¹. (1) Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, 500 W. 12th Avenue, 416 Parks Hall, Columbus, OH 43210, Fax: 614-292-4745, johnsamuel.1@osu.edu, byun.12@osu.edu, (2) Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences

Boron neutron capture therapy (BNCT) is a chemo-radio therapeutic technique for the treatment of cancer. For successful BNCT, a minimum of 20-30mg of boron-10 per gram of tumor tissue is required. The boron-10 loaded tumors are irradiated with low energy (thermal) neutrons, which converts non-radioactive boron-10 to cytotoxic alpha-particles and lithium nuclei capable of destroying tumor cells. Boronated thymidine (Thd) analogues are good candidates for BNCT because of their potential metabolic pathways. Elevated levels of cytosolic thymidine kinase 1 (TK1) are present in proliferating tumor cells, which may convert boronated Thds to the corresponding 5'-monophosphates, thereby entrapping them intracellularly. In phosphoryl transfer assays with recombinant TK1, phosphorylation rates for carboranyl Thds were up to 75% with respect to Thd depending upon the type of modifications. Among Thd analogues modified at the 3'-and N-3 positions with carboranyl groups, only the latter showed good TK1 substrate activities. Also, the degree of aqueous solubility of the N-3 modified Thds improved the relative phosphorylation rates. The synthesis of a series of N-3 substituted carboranyl Thds with additional hydrophilic moieties and the results of their evaluation in phosphoryl transfer assays with recombinant TK1 and TK2 will be presented.

146.

SYNTHESIS AND CYTOTOXICITIES OF RUTACEARPINE ANALOGUES.

Yurngdong Jahng, Seung Ill Kim, and Eung-Seok Lee, College of Pharmacy, Yeungnam University, Kyongsan 712-749, South Korea, Fax: 825-3811-3871, ydjahng@yu.ac.kr

Rutaecarpine is one of the indoloquinazoline alkaloids of Rutaceous plants such as *Evodia rutaecarpa* which has long been utilized for the treatment of inflammation-related disorders in the traditional oriental medicinal practice. Additionally, rutaecarpine, has a variety of biological such as vasorelaxing, analgesic, and antiplatelet activities. Several years ago, Lee et al prepared three derivatives, 10-methoxy, 11-methoxy, and 10,11-methylenedioxy rutaecarpine, to show high potency and selectivity against some human tumor cell lines. Synthesis and their in vitro cytotoxicities of a series of rutacarpine derivatives will be presented.

147.

SYNTHESIS AND QSAR STUDY OF THE ANTICANCER ACTIVITY MEDIATED BY PARTIAL DEPLETION OF INTRACELLULAR CALCIUM STORES OF SOME ARYLSULFONAMIDE ANALOGS. *Amarnath Natarajan, Yuhong Guo, Yun-Hua Fan, Han Chen, Fred Harbinski, and Jose A. Halperin, Laboratory for Translational Research, Harvard Medical School, One Kendall Square, Bldg. 600 3rd Floor, Cambridge, MA 02139, Fax: 617-432-0933, anatarajan@hms.harvard.edu*

A series of substituted arylsulfonamides were synthesized and assayed for 1) Depletion of intracellular calcium stores in the absence of external calcium in dye-loaded cells, and 2) Cell growth inhibition in lung cancer cells (A549). The compounds show promising anti-proliferative activity based on the IC_{50} values. The growth inhibition of A549 cells correlates well with the depletion of intracellular calcium stores. Multiple linear regression analysis was then applied to build up a preliminary QSAR model based on the retention times of the compounds on a C₁₈ column and semi-empirical derived molecular descriptors. The derived model correlates well with the experimental data $r^2 \geq 0.99$ with a modest predictive power of $rCV^2 \geq 0.66$. The final QSAR model includes the retention time on C₁₈ column and two molecular descriptors: the Log P and the solvent accessible-hydrophobic surface area.

148.

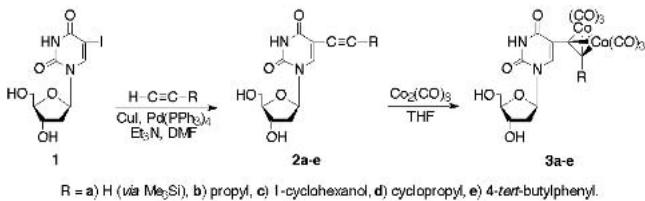
SYNTHESIS OF 5-AMINO-6-METHOXY-1,2-DIARYL-3,4-DIHYDRONAPHTHALENES: POTENTIAL ANTICANCER AND VASCULAR TARGETING AGENTS. *Christopher J. Jelinek, Kevin G. Pinney, and Andrea Tzeng, Department of Chemistry and Biochemistry, Baylor University, P.O. Box 97348, Waco, TX 76798, Christopher_Jelinek@baylor.edu*

A new and rapidly emerging approach to cancer chemotherapy centers on the development of vascular targeting agents (VTAs). By binding to the protein tubulin, VTAs can selectively breakdown the vasculature that feeds a tumor and essentially starve it to death. With the discovery of the anti-tumor activity of a group of natural products called combretastatins, many compounds have been developed to include pertinent structural features from these naturally occurring agents, especially combretastatin A-4 (CA-4). CA-4 has shown marked cytotoxicity as well as inhibition of tubulin polymerization. In this study, a variety of dihydronaphthalene analogs containing amine, methoxy, and diaryl moieties have been prepared. Not only was the structure chosen to mimic the aryl-aryl distance of CA-4, but also to determine if functionalization of the double bond would provide improved efficacy. Preliminary biochemical and biological results will be presented.

149.

SYNTHESIS OF HEXACARBONYL DICOBALT 5-ALKYNYL-2'-DEOXYURIDINES AND THEIR ACTIVITY AGAINST MCF-7 AND MDA-MB-231 HUMAN BREAST CANCER CELLS. *Craig D. Sergeant¹, Ingo Ott², Ronald Gust², and Roman Dembinski¹, (1) Department of Chemistry, Oakland University, 2200 N. Squier Rd., Rochester, MI 48309-4477, (2) Institute of Pharmacy, Free University of Berlin*

Starting with 5-iodo-2'-deoxyuridine (**1**), a series of 5-alkynyl-2'-deoxyuridines (**2a-e**) has been synthesized via the Sonogashira (palladium catalyzed) coupling reaction. Reactions of **2a-e** with Co₂(CO)₈ in THF gave dicobalt hexacarbonyl complexes (**3a-e**). The complexes have been examined for their antiproliferative activity *in vitro* against MCF-7 and MDA-MB-231 human breast cancer cell lines.



150.

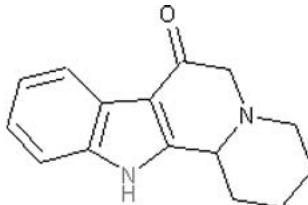
ELEMENTAL ANALYSIS AND NANOPARTICLES IN THE SYNTHESIS OF BRYOSTATIN. IS THERE A CONNECTION? *Thomas J. Manning¹, Michael Land¹, Emily Rhodes¹, Jack Rudloe², Dennis Phillips³, TuKiet T. Lam⁴, Jerry Purcell⁴, Helen Cooper⁴, Mark R. Emmett⁴, and Alan G. Marshall⁴, (1) Department of Chemistry, Valdosta State University, Valdosta, GA 31698, manning@valdosta.edu, (2) Gulf Specimen Marine Lab, (3) Mass Spectrometer Facility, University of Georgia, (4) National High Magnetic Field Lab*

The bryozoan species Bugula neritina contains the anticancer agent bryostatin. Bryostatin has been extracted from these sessile marine invertebrates since the late 1960's from the Gulf of California, Gulf of Mexico, as well as various locations on the eastern and western rims of the Pacific Ocean. While there are over twenty structures of bryostatin, only Bryostatin 1 has achieved FDA approval. In this work we are focusing on animals harvested in the Gulf of Mexico near Alligator Point (Florida). Using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) we measure the concentrations of seventy (70) elements in Bugula neritina, a sea squirt, and the sediment from the point of harvesting. This data has helped us generate an extraction process for marine natural products. It also raises a question if the mineral content of certain marine species plays a role in the synthesis of the natural products. For example, we identified relatively high levels of Ti in Bug. nert, which is mostly likely found as TiO₂. This mineral is widely studied in processing involving the oxygenation of hydrocarbons. Combining UV/VIS absorbance measurements with Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometer (MALDI-TOF-MS), we demonstrate that the specific form of bryostatin extracted is a function of the solvent. A 9.4 Tesla Fourier Transform-Ion Cyclotron Resonance (FT-ICR) mass spectrometer, whose sensitivity, mass accuracy, and resolving power allow the exact empirical formulas of potential precursors and structures of bryostatin to be identified, was employed. Specifically we propose a structure that mass spectral features and was found in both sediment and bryozoans extracts. Finally we examine extracts of fourteen Gulf of Mexico marine species, from sand trout (*Cynoscion arenarius*) to chicken liver sponge (*Chondrilla nucula*), all recently collected, that showed medicinal activity thirty years ago in a National Cancer Institute study. We identify, via MALDI-TOF-MS, mass spectral features that correspond to different variations of the basic bryostatin structure, which raises the question if bryozoans are the original source of bryostatin. Certain species, such as flounder, trout and hogfish, had relatively high levels of peaks that can be correlated with bryostatins or bryostatin fragments, while other species, such as the anchovy, had no spectral features.

151.

PARALLEL SYNTHESIS OF ANALOGS OF THE ZIPA INHIBITOR 1,2,3,4,12,12B-HEXAHYDRO-INDOLO[2,3-a]QUINOLIZIN-7-ONE. *Mohani N. Sukhdeo¹, Scott L. Kincaid², Lee Jennings¹, and Alan G. Sutherland¹, (1) Department of Medicinal Chemistry, Wyeth Research, 401 N. Middletown Road, M/S 222/2109, Pearl River, NY 10965, sukhdem@wyeth.com, (2) Department of Medicinal Chemistry, Wyeth Research*

1,2,3,4,12,12b-Hexahydro-indolo[2,3-a]quinolizin-7-one (Structure I) was identified as a ligand for ZipA from screening a set of diverse compounds. Structure I was subsequently found to be an inhibitor of ZipA. A combinatorial approach was applied to explore the SAR of the different regions of Structure I. Initial work involved building extensions off the indole nitrogen of Structure I in order to investigate effects of chain length, functionality and capping groups on activity. Further work targeted compounds that were open ring analogs of the tetracyclic Structure I. The compounds made were designed to access hydrophobic sites "north" and "north-east" of a large hydrophobic pocket of ZipA.



Structure I

152.

NEW FLUORESCENT PROBES FOR MEASURING MULTI-DRUG RESISTANCE. *George G. Yi, Jinfang Liao, and Zhenjun Diwu, Molecular Devices Corporation, 1311 Orleans Drive, Sunnyvale, CA 94089, Fax: 408-747-0965, george_yi@moldev.com*

New fluorescein derivatives have been developed to measure multi-drug resistance (MDR) through the modification of 5-carboxyfluorescein with two types of tricarboxylic acid. These modified fluoresceins were evaluated as a MDR substrate in MCF7-ADR cells using bottom-reading fluorescent microplate reader, such as FlexStation™ and FLIPR, in "No-Wash" format. The new substrates have a similar sensitivity to MDR compared to Calcein-AM. The primary results indicate that these substrates could be used as a good alternative to Calcein-AM. The detailed bioassay results with different MDR inhibitors will be presented.

153.

FLUORESCENCE TECHNIQUES IN NON-VIRAL GENE THERAPY: USING SPERMINE AND LIPOPOLYAMINES AS DNA DELIVERY AGENTS. *Ian S. Blagbrough¹, Noppadon Adjimatera¹, Adrian P. Neal¹, and Ian S. Haworth².* ⁽¹⁾ Department of Pharmacy and Pharmacology, University of Bath, Bath BA 2 7AY, United Kingdom, Fax: 44-1225-386114, prsib@bath.ac.uk, ⁽²⁾ Department of Pharmaceutical Sciences, University of Southern California

Spermine is a natural polyamine which plays important roles in DNA condensation. We are studying lipospermines to develop more efficient non-viral gene delivery systems for effective and safe non-viral gene therapy (NVGT). The formation of nanoparticles, i.e. DNA condensation, was monitored by the fluorescence quenching of ethidium bromide (EthBr). The fluorescence yield of intercalated EthBr gradually decreased when DNA phosphate anions were neutralised by adding polyamine, thus increasing the N/P charge ratio. Computer models of the polyamine-DNA association have been built to estimate the possible binding sites and bound orientations of the polyamine-steroid conjugates. Using a newly developed algorithm, we have also assessed the probable curvature of the DNA duplex that is induced by one or more polyamine conjugates. Particle formation was confirmed by measuring UV light scattering (LS) (increased absorption at $\lambda=320$ nm). The optimal N/P charge ratio for both EthBr and LS assays was 1.2. A charge ratio of 1.2 for N^2,N^3 -dioleoyl-spermine to plasmid encoding enhanced green fluorescent protein (CMV promoter p-EGFP) was used in a primary human skin fibroblast cell line. Cells transfected with fluorescent imidazolidinone-labelled protein were analysed by FACS. The transfection efficiency (62%) is significantly higher than in the control cells transfected with cationic lipid (DOTMA/DOPE) (10-40%). A novel lipopolypolyamine-fluorescent tagging strategy is being used to study the major intracellular barriers to efficient NVGT, e.g. endosome escape, nuclear localisation. We thank the EPSRC (for a PhD studentship to A.P.N.) and Universities UK (for an ORS award to NA).

154.

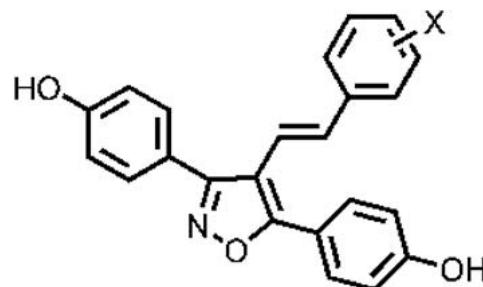
SYNTHESIS OF 1,4-DIBENZO[*B,E*]DIAZEPINES AS POTENTIAL SELECTIVE ESTROGEN RECEPTOR MODULATORS. *Dennis R. Compton, Department of Chemistry, University of Illinois, 600 S. Mathews Ave., Urbana, IL 61801, compton@scs.uiuc.edu, Kathryn E. Carlson, Department of Chemistry, University of Illinois at Urbana-Champaign, and John A. Katzenellenbogen, Department of Chemistry, University of Illinois, Urbana-Champaign*

The estrogen receptor (ER) is a ligand-activated nuclear hormone receptor that is widely distributed throughout the tissues of both men and women. The ER is a target of pharmaceutical interest for the treatment of breast cancer and osteoporosis. There are two known subtypes, ER α and ER β and each one appears to have a unique function within the body. Estrogen receptor ligands that are able to differentiate the subtypes are known as selective estrogen receptor modulators (SERMs). We are exploring the 1,4-dibenzo[*b,e*]diazepine system as a heterocyclic core for the development of a novel SERM. This is a versatile core that is readily modified to produce a small library of ligands that can be used to study structure activity relationships of the system and determine the requirements for a good ER ligand utilizing this scaffold. Several variations are currently being studied for their potential estrogenic activity and subtype selectivity.

155.

PROBES FOR THE ESTROGEN RECEPTOR-LIGAND BINDING DOMAIN (ER-LBD): DIRECTED LIBRARY SYNTHESIS OF 4-(SUBSTITUTED PHENYLVINYL)-3,5-BIS(4-HYDROXYPHENYL)-ISOXAZOLES. *Terra Haddad¹, Richard B. Hochberg², and Robert N. Hanson¹.* ⁽¹⁾ Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-8795, r.hanson@neu.edu, ⁽²⁾ Department of Obstetrics and Gynecology, Yale University School of Medicine

As part of our program to probe the ER-LBD we have used nonsteroidal as well as steroid ligands. Based upon the literature indicating estrogenic activity in the 3,5-bis(4-hydroxyphenyl)isoxazoles, we prepared and evaluated a small preliminary series. The results confirmed the activity and we undertook the preparation of a larger variety of ligands. In this report we will describe the approach to prepare a directed library of compounds using a combination of Sonogashira and Suzuki coupling reactions as well as the results of the biological evaluations. This work has been supported in part by PHS RO1 CA 81049 and DAMD17-00-1-0384.



156.

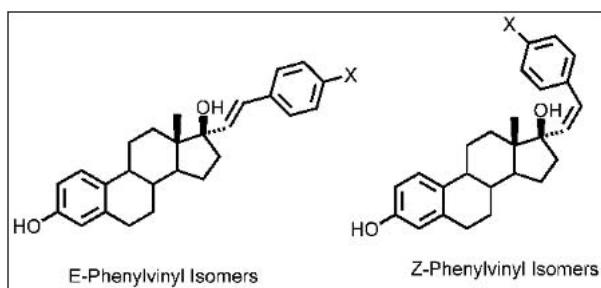
2-PHENYL-NAPHTHALENES AS SELECTIVE ESTROGEN RECEPTOR-BETA LIGANDS: SYNTHESIS AND SAR. *Richard J. Edsall Jr.¹, Eric S. Manas¹, Cuijian Yang¹, Heather A. Harris², and Richard E. Mewshaw¹.* ⁽¹⁾ Department of Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, edsallr@wyeth.com, ⁽²⁾ Women's Health Research Institute, Wyeth Research

In our quest towards understanding the therapeutic role of ER β , we sought to design selective ligands having some of the common structural features of genistein. Towards this end, we chose the 2-phenyl-naphthalene moiety as a simplified version of genistein and exploited this scaffold to produce new ER β selective ligands. We will discuss the design, synthesis and optimization of the 2-phenyl-naphthalene template that led to the discovery of ERB-196, a potential clinical candidate in development.

157.

PROBES FOR THE ESTROGEN RECEPTOR-LIGAND BINDING DOMAIN (ER-LBD): COMPUTATIONAL STUDIES WITH THE E- AND Z-17ALPHA-(4-SUBSTITUTED-PHENYL) VINYL ESTRADIOLS. *Robert Dilis, and Robert N. Hanson, Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-8795, r.hanson@neu.edu, r.hanson@neu.edu*

Previous work from our laboratory demonstrated that the E-17 α -(4-substituted-phenyl) vinyl estradiols possess significant receptor affinity and behave as agonists *in vivo*. Molecular modeling studies identified a binding mode consistent with the observed results. The Z-isomers, while retaining estrogenic activity, demonstrate a different SAR from the E-counterparts, suggesting a different binding mode. In this presentation we describe our approach to determine the binding mode for the Z-isomers and to compare it to that of the E-isomers. This work has been supported in part by PHS RO1 CA81049.



158.

PROBING THE EFFECT OF SPONGE-DERIVED AND SYNTHETIC BROMINATED ARYL COMPOUNDS ON THE ACTIVITY OF SOYBEAN, HUMAN 12-, AND HUMAN 15-LIPOXYGENASES. *Erika N. Segraves¹, Rachana R. Shah¹, Nathaniel L. Segraves², Tyler A. Johnson², Stephanie Whitman¹, Jennifer K. Sui¹, Victor A. Kenyon¹, Robert H. Cichewicz², Phillip Crews², and Theodore R. Holman¹.*

(1) Department of Chemistry and Biochemistry, UC Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, Fax: 831-459-2935, erika@chemistry.ucsc.edu, (2) Department of Chemistry and Institute of Marine Sciences, UC Santa Cruz

Lipoxygenases (LOs) are non-heme iron enzymes that contribute to the eicosanoid pathway via mediation of arachidonic acid hydroperoxidation. LO products are implicated in human diseases, such as: asthma, immune disorders, atherosclerosis, and cancer. Due to these broad biological implications, there is great interest in understanding the effects of natural and synthetic brominated compounds against LOs. Previously, we determined that marine-derived brominated diphenol ethers were potent inhibitors of LO. We initiated a SAR study of 21 brominated aryl compounds including simple monocyclic brominated phenols, dicyclic fire-retardants (PBDEs and brominated bisphenol A (BBPAs)), marine-derived dioxins, and bastadin macrocycles. In general, phenolic marine-derived compounds and BBPAs were more potent inhibitors than non-phenolic PBDEs. The most selective compounds for human 15-lipoxygenase were spongiodioxins A and C. In addition, fluorescence and EPR data of the iron active site were obtained, indicating reductive inhibition was not the main mechanism of action for these compounds.

159.

KINETIC STUDIES OF NATURAL PRODUCT INHIBITORS TO 12- AND 15-HUMAN LIPOXYGENASES. *Joshua D. Deschamps, Jeffrey T. Gautsch, Stephanie Whitman, Tyler A. Johnson, Phillip Crews, and Theodore R. Holman, Department of Chemistry and Biochemistry, UC Santa Cruz, 1156 High St., Santa Cruz, CA 95064, Fax: 831-459-2935, joshua@chemistry.ucsc.edu*

Human lipoxygenases (hLOs) are non-heme, iron-containing enzymes that catalyze the dioxygenation of polyunsaturated fatty acids to hydroperoxy-fatty acids. This is the first step in the biosynthetic pathway leading to leukotrienes and lipoxins, which have been implicated in several diseases involving inflammation, immunity and various types of cancers. Regulation of hLO activity, via small molecule inhibitors, could potentially play a key role in the prevention and treatment of such diseases. We have previously shown that plants and marine sponges are rich in bioactive compounds and are good sources for hLO inhibitors. In the current poster, extensive kinetic studies were performed on four compounds: two known hLO inhibitors from terrestrial plants (apigenin and baicalein) and two unknown hLO inhibitors from the marine sponge *Dysidea herbacea*. Results of these studies reveal mechanistic insights of inhibition and indicate structural motifs which are needed to promote selective inhibition against 12-hLO vs. 15-hLO.

160.

COMFA AND COMSIA 3D QSAR STUDIES ON PIMARANE CYCLOOXYGENASE-2 (COX-2) INHIBITORS. *Young-Ger Suh¹, Hyun-Ju Park², Young-Ho Kim³, Kwang-Ok Lee¹, Seung-Mann Paek¹, and Sung-Hyun Moon¹.* (1) Department of Pharmaceutical Chemistry, College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea, Fax: 82-2-888-0649, ygsuh@snu.ac.kr, lkosmr@hanmir.com, (2) College of Pharmacy, Sungkyunkwan University, (3) College of Pharmacy, Chungnam University

A novel pimarane diterpene, acanthoic acid was isolated from the Korean medicinal plant which has been traditionally used for treating rheumatism.

Previously, we have reported acanthoic acid analogues as novel COX-2 inhibitors and their Structure-Activity Relationships (SAR) suggesting that the substitutions at C4 and C16 lead to the variations in COX-2 inhibition activity.

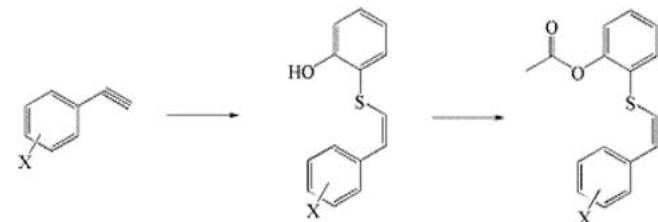
In this work, we have conducted 3D-QSAR studies on a series of acanthoic acid derivatives that act as COX-2 inhibitors, using two different methods: comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA).

CoMFA and CoMSIA analysis of twenty five pimarane analogues produced good models with high predictive abilities. The CoMSIA model showed slightly improved prediction abilities in comparison with the CoMFA model. It is well revealed that the COX-2 inhibitory activity is influenced by the character of steric, electrostatic, hydrophobic, hydrogen bonding donor, and hydrogen bonding acceptor at C4 linker and C16 of pimarane analogues. These results are consistent with our SAR studies of previous work and provide crucial information in the design and development of new pimarane analogues as anti-inflammatory agents.

161.

SYNTHESIS OF STYRYL ACETOXYPHENYLSULFIDES: NOVEL CYCLOOXYGENASE INHIBITORS. *Muralidhar Reddy Mallireddigari, Venkat R Pallela, Rengasamy Boominathan, Padmavathi Venkatapuram, E. Premkumar Reddy, and M.V. Ramana Reddy, Fels Institute for Cancer Research, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140-5101, mmreddy67@yahoo.com*

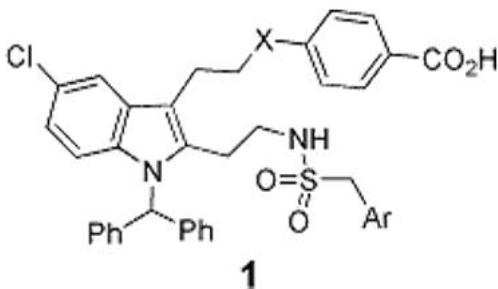
Cyclooxygenase-2 (COX-2), a key enzyme in prostaglandin synthesis has been shown to be responsible for inflammatory conditions such as rheumatoid arthritis and osteoarthritis. It is also known that COX-2 protein play a role in colorectal cancers and neurodegenerative disorders such as Alzheimer's disease. Designing and developing of new selective COX-2 inhibitors may help in prevention and progression of these conditions. We have recently designed, synthesized and studied the biological activity of novel COX-2 inhibitors. These compounds specifically inhibit COX-2 enzyme and we assume that the inactivation may be due to irreversible binding of the inhibitor to the catalytic domain of the enzyme. The synthesis, structure activity relationship and biological activity of these molecules will be described.



162.

IN SEARCH FOR CYTOSOLIC PHOSPHOLIPASE A2 INHIBITORS WITH REDUCED MOLECULAR WEIGHT. *Yonghan Hu¹, Jennifer Thomason¹, John C McKew¹, Katherine Lee¹, Frank E. Lovering¹, Baihua Hu¹, Fuk-Wah Sum¹, James D. Clark², Marina Shen², Wen Zhang², and Steve Tam¹.* (1) Department of Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5682, fhu@wyeth.com, (2) Department of Inflammation, Wyeth Research

Cytosolic Phospholipase A2 (cPLA2), is the enzyme responsible for the specific cleavage of arachidonic acid-containing glycerophospholipids. The liberated arachidonic acid is metabolized to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can then be acetylated to form yet another inflammatory mediator, platelet activating factor, PAF. Inhibitors of cPLA2 would lead to a novel therapeutic with applications in many disease states, such as rheumatoid arthritis, asthma, and osteoarthritis. We designed and synthesized Cytosolic phospholipase A2 inhibitors by pharmacophore repackaging and template substitution of the indole-based cPLA2a inhibitors inhibitor (1) previously synthesized in our group. Important SAR information is obtained.



163.

SYNTHESIS OF APIO ANALOGUES OF NEPLANOCIN A AND THEIR INHIBITORY ACTIVITY AGAINST S-ADENOSYLHOMOCYSTEINE HYDROLASE. Jeong A Lee¹, Hyung Ryong Moon², Kang Man Lee¹, Young Mi Ko¹, Hea Jin Kim¹, Mikyung Yun¹, and Lak Shin Jeong¹. (1) Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, 11-1 Seodaemun-ku, Daehyun-dong, Seoul 120-750, South Korea, smartja@freechal.com, (2) College of Pharmacy, Pusan National University

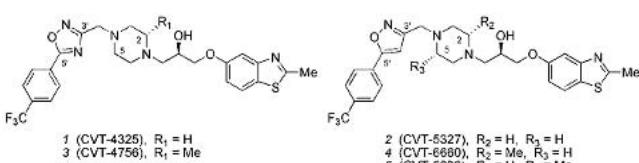
Neplanocin A is a natural carbocyclic nucleoside and one of the most potent inhibitors of S-adenosylhomocysteine hydrolase (SAH). This nucleoside also exhibits strong antiviral activity against DNA viruses as well as RNA viruses. On the other hand, apio-ddA, in which 4^o-hydroxymethyl group of normal sugar is moved to the C3^o-position has been reported to show potent anti-HIV activity comparable to the parent 2^o,3^o-dideoxyadenosine (ddA) and better stability against glycosidic bond hydrolysis than ddA. Therefore, based on these findings, it was of great interest to synthesize novel apio-neplanocin A and its related purine nucleosides, which combine the properties of neplanocin A and apio-ddA and evaluate their inhibitory effect on the SAH. Stereoselective synthesis of apio-neplanocin A and its related purine nucleosides was achieved, starting from D-ribose via regioselective hydroxymethylation and ring-closing metathesis (RCM) as key steps. However, all synthesized compounds did not show significant inhibitory activity against S-adenosylhomocysteine hydrolase, unlike neplanocin A. This lack of enzyme inhibitory activity might be due to the presence of the tertiary hydroxyl group at the C3^o-position, indicating that oxidation of 3^o-hydroxyl group to the ketone by enzyme-bound cofactor, NAD⁺ is required to be SAH inhibitor.

164.

POTENT FATTY ACID OXIDATION INHIBITORS: ISOXAZOLE BASED ANALOGS

OF CVT-4325. Robert H. Jiang¹, Timothy Marquart¹, Xiaofen Li¹, Elfatih Elzein¹, Dmitry O. Koltun¹, Prabha N. Ibrahim¹, Nancy Chu², Yuan Li³, Marie Nguyen³, Dewan Zeng³, Daniel Soohoo², Jia Hao², and Jeff A. Zablocki¹. (1) Department of Bio-Organic Chemistry, CV Therapeutics, Inc, 3172 Porter Drive, Palo Alto, CA 94304, Fax: 650-858-0390, bob.jiang@cvtherapeutics.com, (2) Department of Pre-Clinical Development, CV Therapeutics, Inc, (3) Department of Pharmacological Sciences, CV Therapeutics, Inc

CVT-4325 (**1**, IC₅₀=380 nM), a potent inhibitor of 1-¹⁴[C]-palmitoyl-CoA oxidation in cardiac mitochondria, has been demonstrated to decrease the rate of fatty acid oxidation and subsequently increase the rate of glucose oxidation (metabolic shift) in rat isolated working hearts. In an effort to optimize inhibitory activity toward palmitoyl-CoA oxidation, we discovered a novel class of potent 5'-arylisoxazole based analogs of **1** (**2**, **4**, **5**). Introduction of a (2*S*)- or (5*S*)-methyl group on the piperazine ring generally enhanced potency in isoxazole analogs (**4** or **5** vs. **2**), a finding consistent with oxadiazole series (**3**, IC₅₀=180 nM vs. **1**). 3'-Arylisoxazole regioisomers (not shown) were moderately less potent. The synthesis of these and other related analogs will be presented, along with detailed discussion of SAR with respect to 3'-/5'-aryl groups on the isoxazole ring.



165.

PARP-1 INHIBITORS AS NEUROPROTECTIVE AGENTS. *Dana Ferraris*¹

Yao-Sen Ko¹, Rica Pargas Ficco², Christina Alemu¹, Chadwick Bradford¹, Randall Hoover¹, Susan Lautar¹, Shi Liang¹, Qian Lin¹, May Lu¹, Maria Mooney¹, Lisa Morgan¹, Yongzhen Qian¹, Larry Williams¹, Qi Yi Wu¹, Weizheng Xu¹, Yinong Zou¹, Jie Zhang¹, and Vincent Kalish¹. (1) Department of Research, Guilford Pharmaceuticals Inc, 6611 Tributary Street, Baltimore, MD 21224, Fax: 410-631-6797, (2) Medicinal Chemistry, Guilford Pharmaceuticals Inc

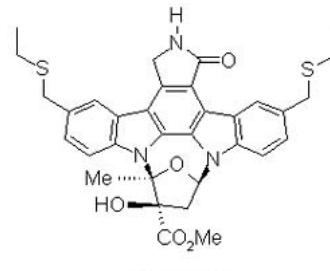
Poly(ADP-ribose) polymerase-1 (PARP-1, EC 2.4.2.30) is an abundant nuclear enzyme with an important role in the cellular life cycle including cellular differentiation, DNA base excision repair, and regulation of apoptosis. The enzyme has been implicated in the pathogenesis of several diseases as wide ranging as stroke, heart ischemia and cancer. In this talk, we present the aza-5[H]phenanthridin-6-ones as a potent class cell permeable, aqueous soluble PARP-1 inhibitors. Derivatives of this structural class were found to achieve brain levels well above the IC₅₀ after i.v. administration. Furthermore, compounds from this series demonstrated neuroprotective effects in animal models of brain ischemia.

166.

MLK1 SAR AND STRUCTURAL STUDIES OF CEP-1347. Robert L. Hudkins,

Medicinal Chemistry, Cephalon, Inc, 145 Brandywine Parkway, West Chester, PA 19380, Fax: 610-738-6558, rhudkins@cephalon.com, and Sheryl L Meyer, Protein Expression, Cephalon, Inc

Our research has focused on developing inhibitors of mixed lineage kinases (MLKs) for the treatment of neurodegenerative diseases. The MLKs function at the MAPKKK level of the stress-activated protein kinase-signaling cascade regulating JNK activation and subsequent cJun phosphorylation leading to neuronal cell death. CEP-1347, active in Parkinson's disease preclinical models and currently in Phase III clinical trials, is an inhibitor of the JNK pathway via MLK inhibition and displays a broad neuroprotective profile. Discussed will be MLK1 SAR and structural studies of CEP-1347.



CEP-1347

167.

MAXI-K OPENERS AS NEUROPROTECTIVE AGENTS. John E. Starrett Jr., Kevin

W. Gillman, Danielle M. Bocchino, Jeffrey Bien, Christopher G. Boissard, Michael Bozik, Vincenzo Calandra, Chung-Pin H. Chen, Yan Chen, Charles Dangler, Douglas Dischino, Qi Gao, Steven Hansel, Piyasena Hewawasam, Stella Huang, Sing-Hong Kang, Kimberley Lentz, Omar Lopez, Lorraine Pajor, Yadagiri Pendri, Gary Pilcher, Posmantur Rand, David Procencal, Venkatramana Rao, Elizabeth Sarsfield, William Schmitz, Sing-Yuen Sit, Hua Wang, Dedong Wu, Kai Xie, Lei Zhang, and Valentin K. Gribkoff, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, john.starrett@bms.com

Stroke and traumatic brain injury (TBI) are potentially devastating disorders for which, despite an enormous amount of research, there is a large unmet medical need. We have taken the approach to acutely treat these conditions by targeting the opening of large-conductance, calcium-activated potassium (maxi-K) channels. Opening these channels following a neuronal insult such as stroke or TBI indirectly limits calcium entry and has the potential to protect neurons at risk of damage or death. Maxipost (BMS-204352) is a potent and efficacious maxi-K opener in animal models which was recently withdrawn from human clinical stroke trials due to an inability of the drug to meet the desired clinical endpoints. We have identified analogs of MaxiPost which address some of the shortcomings identified in the clinical trials. The rationale, SAR, chemistry, and activity in animal models of neurodegeneration of these new MaxiPost analogs will be discussed.

168.

STUDIES TO ELUCIDATE THE MECHANISM OF ACTION OF PREGABALIN.

James A. Bristol, Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Fax: 734-622-7879, james.bristol@pfizer.com

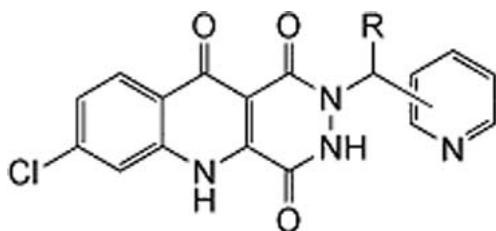
Pregabalin has robust activity in preclinical models of anxiety, pain and epilepsy and is in late-stage clinical development for these indications. Biochemical and protein purification studies have revealed that pregabalin has high affinity for the a2d subunit of calcium channels. In addition it is a substrate for the system L amino acid transporter of cell membranes. The role that pregabalin's interactions with these proteins plays in its mechanism of action has been investigated by functional biochemical techniques, structure activity relationship studies, and genetically modified mice. These data add to the understanding of a2d ligands as an important new class of CNS drugs with potentially broad applications.

169.

7-CHLORO-2,3-DIHYDRO-2-[1-(PYRIDINYL)ALKYL]-PYRIDAZINO[4,5-**B]QUINOLINE-1,4,10(5H)-TRIONES AS NMDA GLYCINE-SITE ANTAGONISTS**

WITH ANTINOCICEPTIVE ACTIVITY. *Dean G. Brown, Thomas M. Bare, Rebecca A. Urbanek, Frances M. McLaren, Carey L. Horchler, Megan Murphy, Gary B. Steelman, James R. Empfield, Janet M. Forst, Keith J. Herzog, Wenhua Xiao, Martin C. Dyroff, Chi-Ming C. Lee, Shephali Trivedi, Kathy L. Neilson, and Richard A. Keith, CNS Discovery, AstraZeneca Pharmaceuticals, 1800 Concord Pike, Wilmington, DE 19850, Fax: 302-886-5382, dean.brown@astrazeneca.com*

Potent NMDA-glycine site antagonists (**1**) have been identified with improved physical properties (e.g. solubility and oral absorption) over the initial lead structure. These compounds were screened in both a rat formalin pain model and rat CCI pain model. The lead compound [R=(S-2-Me)] was found to be orally bioavailable in rats and subsequently gave oral activity in both pain models. The synthesis and evaluation of compounds similar to type **1** will be discussed in this presentation.



1, R = alkyl, phenyl

170.

DISCOVERY PROCESS AND PHARMACOLOGICAL PROFILE OF NEW GLYCINE**ANTAGONISTS.** *Romano Di Fabio, Department of Medicinal Chemistry,*

Medicines Research Centre, GlaxoSmithKline S.p.A, Via A. Fleming, 4, Verona 37135, Italy, Fax: 00390459218196, rdf26781@gsk.com

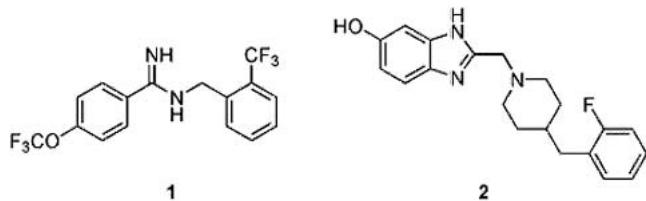
The role of glutamate in the physiology and pathophysiology of the central nervous system (CNS) has been widely investigated. Glutamatergic transmission seems to be critically involved in several CNS disorders including acute and chronic neurodegeneration after the onset of stroke. In addition, NMDA receptors play a pivotal role in the development and maintenance of pain hypersensitivity, an event associated to a high unmet need in current medicine. Antagonists of the glycine site associated to the NMDA receptor, restoring the physiological conditions, have been proven to be efficacious both in animal models of cerebral ischemia and chronic pain. The extensive exploration performed by our group in this field, gave rise to the identification of new series of tetrahydroquinoline derivatives as *in vitro* and *in vivo* potent glycine antagonists. The discovery process and the pharmacological profile of these compounds will be presented in detail.

171.

NR2B SUBTYPE SELECTIVE NMDA RECEPTOR ANTAGONISTS. *John A.*

McCauley¹, C.F. Claiborne¹, C.R. Theberge¹, J.J. Romano¹, S.B. Billings¹, B.E. Libby¹, C.J. McIntyre¹, K.D. Anderson¹, S.R. Michelson¹, D.A. Claremon¹, R.M. Freidinger¹, R.A. Bednar², S.D. Mosser², S.L. Gaul², T.M. Connolly², C.L. Condra², M. Xia², M.E. Cunningham², L. Kiss², B. Bednar², G.L. Stump³, J.J. Lynch³, N.R. Curtis⁴, H.J. Diggle⁴, J.J. Kulagowski⁴, A. Macaulay⁵, K.A. Wafford⁵, K.S. Koblan², and N.J. Liverton¹. (1) Department of Medicinal Chemistry, Merck Research Laboratories, WP14-3, West Point, PA 19486, Fax: 215-652-3971, john_mccauley@merck.com, (2) Department of Molecular Pharmacology, MRL West Point, (3) Department of Pharmacology, MRL West Point, (4) Department of Medicinal Chemistry, MRL Terlings Park, UK, (5) Department of Pharmacology, MRL Terlings Park, UK

The *N*-methyl-D-aspartate (NMDA) receptor is a ligand gated ion channel that is present throughout the mammalian CNS and is activated by the excitatory amino acid, glutamate. This receptor plays a fundamental role in a number of physiological processes and may be an avenue for the treatment of such disease states as stroke, Parkinson's disease and neuropathic pain. Agents that block the NMDA channel, such as ketamine, have been shown to alleviate pain in human clinical trials, however the low therapeutic index over side effects such as hallucinations, dysphoria and loss of motor coordination make such compounds unattractive for chronic pain therapy. A novel series of aryl benzamidines (**1**) and a novel series of benzimidazoles (**2**) are potent antagonists of the NR2B subtype of the NMDA receptor. *In vitro*, selected compounds show very good selectivity for NR2B versus the NR2A, NR2C and NR2D subtypes of NMDA receptors as well as selectivity over hERG-channel activity and α 1-adrenoceptor binding. These compounds show activity *in vivo* in the rat carrageenan-induced hyperalgesia model and show no evidence of motor coordination side effects.



172.

PARADIGM SHIFT IN NMDA RECEPTOR ANTAGONIST DRUG DEVELOPMENT:**THE STORY OF LOW-AFFINITY MEMANTINE AND ITS MECHANISM OF ACTION**

TO ACHIEVE CLINICALLY-TOLERATED NEUROPROTECTION. *Stuart A. Lipton, Burnham/Salk/Scripps Research Institutes and UC San Diego, La Jolla, CA 92037, slipton@burnham.org*

Excitotoxicity, defined as overstimulation of glutamate receptors, has been implicated in a final common pathway contributing to neuronal injury and death in a wide range of acute and chronic neurologic disorders. Excitotoxic cell death is due, at least in part, to excessive activation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, leading to excessive Ca^{2+} influx through the receptor's associated ion channel and subsequent free radical production. Our group was the first to demonstrate that gentle blockade of NMDA receptors by Memantine, via a mechanism of uncompetitive open-channel block, can prevent this type of damage in a clinically efficacious manner without substantial side effects. NMDA receptor activity leading to neurotoxic pathways can be blocked with a variety of potent drugs. However, physiological NMDA receptor activity is essential for normal neuronal function. This means that neuroprotective agents that potently block all NMDA receptor activity will have unacceptable clinical side effects. For this reason, many NMDA receptor antagonists have disappointingly failed in advanced clinical trials for a number of disorders, including stroke and Huntington's disease. In contrast, studies in our laboratory have shown that the adamantane derivative, Memantine, blocks excessive NMDA receptor activity without disrupting normal activity. Memantine does this through its action as an uncompetitive, open-channel blocker; it preferentially enters the receptor-associated ion channel when it is excessively open, and, most importantly, its off-rate from the channel is relatively fast so that it does not accumulate to interfere with normal synaptic transmission. Hence, Memantine is well tolerated, has passed multiple phase 3 trials for dementia, and was recently approved in Europe and the US for the treatment of moderate-to-severe Alzheimer's disease. Clinical studies of Memantine for additional neurologic disorders, including other

dementias, neuropathic pain, and glaucoma, are underway. We have also developed a series of second-generation that display greater neuroprotective properties than Memantine. These second-generation drugs take advantage of the fact that the NMDA receptor has other modulatory sites, including critical thiol groups that are S-nitrosylated, which can also be used for safe but effective clinical intervention.

173.

4'-THIO-SIRNA: SYNTHESIS AND IN VITRO RNA INTERFERENCE BY

4'-THIO-MODIFIED RNA. *Prasad Dande¹, Thazha P. Prakash², Brenda Baker³, Timothy Vickers⁴, Sioufi Namir⁵, Russell Jarres⁶, Richard H. Griffey⁷, Eric E. Swayze⁸, and Balkrishen Bhat¹.* (1) Department of Medicinal Chemistry & Antisense Core Research, Isis Pharmaceuticals, Inc, 2292 Faraday Avenue, Carlsbad, CA 92008, Fax: 760-603-4654, pdande@isisph.com, (2) Department of Medicinal Chemistry, ISIS Pharmaceuticals, (3) Department of Antisense Core Technology, Isis Pharmaceuticals, Inc, (4) Department of Functional Genomics, Isis Pharmaceuticals, Inc, (5) Department of Antisense Lead Identification, Isis Pharmaceuticals, Inc, (6) Department of Antisense Lead Identification, Isis Pharmaceuticals, Inc, (7) Department of Research Chemistry, Isis Pharmaceuticals, Inc, (8) Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc

RNA interference by double-stranded siRNAs selectively reduces levels of targeted mRNA. Although these siRNAs have significant potential in antisense therapeutics, their metabolic instability renders them unsuitable for therapeutic applications. In our search for stable siRNAs, we investigated the ability of 4'-thio-siRNAs to induce RNA interference. All four (A, C, U & G) 4'-thio nucleosides were synthesized following published procedures with some modifications at key steps. These nucleosides were converted into corresponding phosphoramidites and incorporated into 20mer oligonucleotides. 4'-thio-siRNAs made from these modified oligonucleotides were tested in an *in vitro* assay against a PTEN target. A number of these chemically modified siRNAs showed potent RNA interference (indicated by reduced PTEN mRNA levels). A strong correlation was observed between the number and placement of 4'-thio residues and siRNA activity. The comparison of the data on these modified siRNAs with unmodified siRNA will be presented.

174.

CHEMICALLY-MODIFIED SIRNA MOTIFS WITH ENHANCED IN VITRO ACTIVITY AND STABILITY. *Charles R. Allerson¹, Thazha P. Prakash¹, Namir Sioufi², Russell Jarres², Timothy Vickers³, Brenda Baker⁴, Eric E. Swayze¹, Richard H. Griffey⁵, and Balkrishen Bhat¹.* (1) Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc, 2292 Faraday Avenue, Carlsbad, CA 92008, Fax: 760-603-4654, callerson@isisph.com, (2) Department of Antisense Lead Identification, Isis Pharmaceuticals, Inc, (3) Department of Functional Genomics, Isis Pharmaceuticals, Inc, (4) Department of Antisense Core Technology, Isis Pharmaceuticals, Inc, (5) Department of Research Chemistry, Isis Pharmaceuticals, Inc

RNA interference (RNAi) has emerged as a powerful mechanism by which gene-silencing can be achieved in mammalian cells. The recent discovery that small-interfering RNAs (siRNAs) can activate this pathway has led to their widespread use in functional genomics *in vitro*, and has triggered interest in their potential therapeutic utility. While unmodified siRNAs are sufficiently robust for *in vitro* applications, it appears that chemical modifications will be required to optimize the *in vivo* potency, stability, and pharmacokinetic properties of siRNAs. As part of an effort to develop siRNAs with *in vivo* potency, we examined a series of siRNA duplexes in which the 2'-hydroxyl groups were partially or entirely replaced with combinations of 2'-deoxy, 2'-O-methyl, 2'-O-(2-methoxyethyl), and 2'-fluoro modifications. This effort led to the discovery of siRNAs with specific chemical motifs that demonstrate improved target reduction activity and serum stability. The key features of these motifs will be presented in detail.

175.

POSITIONAL EFFECTS OF CHEMICAL MODIFICATION ON SIRNA ACTIVITY.

Thazha P. Prakash¹, Charles R. Allerson¹, Prasad Dande¹, Timothy Vickers², Namir Sioufi³, Russell Jarres³, Brenda Baker⁴, Eric E. Swayze¹, Richard H. Griffey⁵, and Balkrishen Bhat¹. (1) Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc, 2292 Faraday Avenue, Carlsbad, CA 92008, Fax: 760-603-4654, tprakash@isisph.com, (2) Department of Functional Genomics, Isis Pharmaceuticals, Inc, (3) Department of Lead Identification, Isis Pharmaceuticals, Inc, (4) Department of Antisense Core Technology, Isis Pharmaceuticals, Inc, (5) Department of Research Chemistry, Isis Pharmaceuticals, Inc

Short interfering nucleic acids (siRNA) is emerging as a powerful tool for gene regulation. The unmodified siRNA activate RNAi pathway through a protein complex known as RISC (RNA induced silencing complex) and leads to inhibition of gene expression. However their utility is limited as therapeutic due to their poor metabolic stability and therefore, lack of persistent activity. Chemical modifications are known to stabilize oligonucleotides against metabolic degradation, alter duplex stability and improve pharmacokinetic properties. Therefore, there is a need for a systematic SAR of placement of chemical modifications throughout siRNA that would have limited effect on RISC loading of the siRNA and would provide stability and retain biological activity. A systematic scanning of chemical modifications, such as of 2'-OMe, LNA, 2'-deoxy-2'-fluoro and 2'-O-MOE was carried out. A number of modifications, showed strong positional effects throughout the antisense strand. A detailed account of this study will be presented.

176.

DESIGNED SPIROCYCLES AS PROBES FOR DNA MICROENVIRONMENTS.

Graham B. Jones¹, Yiqing Lin¹, and Irving Goldberg². (1) Department of Chemistry, Northeastern University, 360 Huntington Ave, Boston, MA 02115, Fax: 617-373-8795, Gr.Jones@neu.edu, yiqing_lin@hotmail.com, (2) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

Nucleic acids have richly diverse structures, including hairpins, knots, pseudo-knots, triple helices, loops, helical junctions, and bulges. Bulged structures in nucleic acids have been proposed as intermediates in a multitude of biological processes, including RNA splicing, frame-shift mutagenesis, intercalator induced mutagenesis, and imperfect homologous recombination.

Despite these ramifications, few attempts have been made to prepare compounds with affinity for bulged sequences. Success has been hindered by lack of an available substrate which can effectively mimic the base pairing involved at a bulged site, which requires a unique wedge-shaped template. Based on a lead compound derived from an enediyne natural product (NCS), a family of synthetic bulge binders was recently described (Chem. Biol. 2002, 9, 925). This presentation will describe end game tactics in the assembly of differentially functionalized bulge binders, which may serve as tools for the study of these important targets.

177.

SYNTHETIC MOLECULES THAT MODULATE TRANSCRIPTION AND DIFFERENTIATION.

Motonari Uesugi, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, Fax: 713-798-1625, muesugi@bcm.tmc.edu

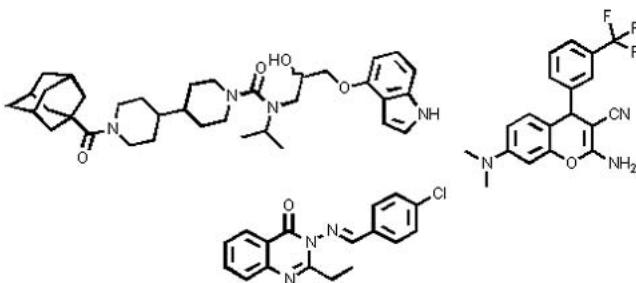
Regulation of gene transcription and cell differentiation often induces drastic phenotypes in living organisms. External and precise control over these processes through small organic molecules represents a challenge in bioorganic and medicinal chemistry. Our laboratory has been discovering small organic molecules that modulate transcription or differentiation and using them as a tool to understand biological phenomena. This presentation provides a quick overview of the chemical biology research in our group.

178.

SYNTHETIC INHIBITORS BASED ON A TERPHENYL SCAFFOLD AS ANTI-TUMOR POTENTIALS TO ANTAGONIZE P53/MDM2 INTERACTION AND INDUCE P53

ACCUMULATION IN VIVO. *Hang Yin, and Andrew D. Hamilton, Department of Chemistry, Yale University, P.O. BOX 208107, New Haven, CT 06511, Fax: 203-432-3221, hang.yin@yale.edu*

Deregulation of overexpression or hyper-activation of MDM2 plays a key role in tolerance of wild type p53, making it an attractive target for the development of

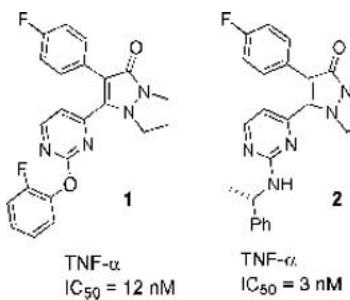


anti-tumor potentials. Recently, several approaches have been pursued to disrupt p53/MDM2 interaction. These studies provided a proof of concept for activating p53 by targeting MDM2; yet the oligonucleotides, antibodies and polypeptides used in these studies are not suitable in cancer treatment, so the development of small molecules that can perform similar functions is of great therapeutic interest. We have developed a group of low-molecule-weight inhibitors based on the terphenyl scaffold with the potential to block p53/MDM2 interaction. The terphenyl compounds were screened with an ELISA assay in vitro and good affinities were observed. p53 accumulation in HCT116 tumor cells was induced by terphenyl derivatives in cell based assays. MTT cellular studies showed the inhibitors also reduced the tumor cell survival efficiently.

179.

DEVELOPMENT OF ORALLY BIOAVAILABLE BICYCLIC PYRAZOLONES AS INHIBITORS OF TUMOR NECROSIS FACTOR-ALPHA PRODUCTION. *Michael P. Clark, Steven K. Laughlin, Matthew J. Laufersweiler, Roger G. Bookland, Todd A. Brugel, Adam Golebiowski, Mark P. Sabat, Jane F. Djung, Michael G. Natusch, Jennifer A. Townes, John C. VanRens, Biswanath De, Lily S. Hsieh, Susan C. Xu, Rick L. Walter, Marlene J. Mekel, and Michael J. Janusz, Procter and Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45040, Fax: 513-622-3681, clark.mp@pg.com*

4-Aryl-5-pyrimidinyl based tumor necrosis factor- α (TNF- α) inhibitors which contain a novel bicyclic pyrazolone core are described. Many showed low-nanomolar activity against LPS-induced TNF- α in monocytic cells. Secondary screening data are presented for the pyrimidinyl bicyclic pyrazolones. Several of these analogs showed good oral bioavailability in rat.



180.

DEVELOPMENT OF NOVEL SUBSTITUTED BICYCLIC PYRAZOLONES AS INHIBITORS OF TUMOR NECROSIS FACTOR- α (TNF- α) PRODUCTION.

Matthew J. Laufersweiler, Procter and Gamble Pharmaceuticals, 8700 Mason-Montgomery Rd, Mason, OH 45040, laufersweiler.mj@pg.com

Pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin 1- β (IL-1 β) have been implicated in a number of inflammatory diseases such as osteoarthritis, rheumatoid arthritis, and Crohn's disease. Reduction of these cytokine levels with small molecule inhibitors has been shown to reduce inflammation. Therefore small molecule inhibitors of cytokine production have become an attractive target in our efforts to discover disease-modifying treatments of inflammatory disorders. Novel spiroketal [5,5]-bicyclic pyrazolones and substituted [5,5]-bicyclic pyrazolones are presented as inhibitors of TNF- α production. Many of these compounds show low nanomolar activity against lipopolysaccharide (LPS) induced TNF- α production in THP-1 cells. Oral bioavailability and pharmacokinetic properties of these molecules will be discussed.

181.

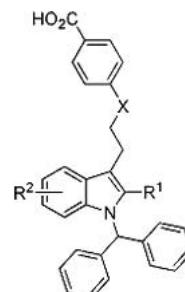
DISCOVERY OF KCNQ2 POTASSIUM CHANNEL OPENERS FOR MIGRAINE AND NEUROPATHIC PAIN. *Yong-Jin Wu, Department of Neuroscience Chemistry, Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492, Fax: 203-677-7702, yong-jin.wu@bms.com*

The synthesis and SAR of novel KCNQ2 potassium channel openers will be described. In vivo studies of KCNQ2 openers in the cortical spreading depression model and in vitro testing in hyperexcited hippocampal slices suggest that KCNQ2 openers may have potential for the treatment of CNS disorders characterized by neuronal hyperexcitability, such as migraine, epilepsy and neuropathic pain.

182.

DISCOVERY OF A NEW CLASS OF ANTI-INFLAMMATORY: INDOLE CPLA₂ α INHIBITORS. *Katherine L. Lee, John C. McKew, Mark Behnke, Lihren Chen, Megan Foley, Yonghan Hu, Paresh M. Thakker, Jennifer Thomason, Lane C. Woorder, Kun Wu, Fuk-Wah Sum, and Steve Tam, Department of Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5682, klee@wyeth.com*

Cytosolic phospholipase A₂ α (cPLA₂ α) selectively cleaves the *sn*-2 position of arachidonyl-glycerophospholipids to generate free arachidonic acid. This arachidonic acid is in turn metabolized to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can be acetylated to form yet another inflammatory mediator, platelet activating factor, PAF. Selective inhibition of cPLA₂ α would provide a novel therapeutic with applications in many disease states including osteoarthritis, rheumatoid arthritis, and asthma. The development of a class of novel and selective indole based inhibitors of cPLA₂ α will be presented.



183.

DISCOVERY AND OPTIMIZATION OF A NEW CLASS OF MC4 AGONISTS. *Kilian Conde-Frieboes¹, Henning Thøgersen², Michael Ankersen³, Ulrich Sensfuss¹, Anthony Murray³, Flemming E. Nielsen⁴, Klaus Rudolf⁵, Philipp Lustenberger⁵, Heike Wieland⁵, Marcus Schindler⁵, and Birgitte S. Wulff⁶. (1) Protein and Peptide Chemistry, Novo Nordisk, Novo Nordisk Park, DK-2760 Maaloev, Denmark, Fax: +45 44663450, kcf@novonordisk.com, (2) MedChem Research, Novo Nordisk A/S, (3) Med Chem Research, Novo Nordisk A/S, (4) Research and Development, Novo Nordisk A/S, (5) Boehringer Ingelheim Pharma GmbH & Co. KG, (6) Molecular Pharmacology, Novo Nordisk A/S*

The MC4 receptor is recognized as one of the most promising target to therapeutically approach obesity and associated insulin resistance. By activating the MC4 receptor it has been shown in several animal models, that appetite is suppressed and the metabolic rate is increased, leading to a significant weight loss. Our strategy to identify new types of compounds with MC4 agonist properties was to mimic the key residue side chain functionalities of the potent cyclic heptapeptide agonist MTII by suitable substitution on nonpeptide scaffolds. The key residues of MTII were those where an Ala replacement (or D-Ala for D-Phe7) resulted in a more than a ~100 fold reduction in the binding affinity - namely D-Phe7, Arg8 and Trp9. By this procedure several new MC4 agonist compound classes were discovered - one of which was optimized by focussed library design to provide potent MC4 agonists with an *in vitro* activity (EC50) down to 20 nM in a cAMP assay. In our assay MT-II and á-MSH have activities (EC50) of ~1 nM and ~100 nM, respectively.

184.

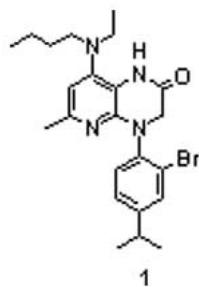
MIIF ANTAGONISTS ARE ANTI-DIABETOGENIC. Yousef Al-Abed¹, Ivana Cvetkovic², Djordje Miljkovic², Ferdinando Nicoletti³, and Stanislava Stosic-Grujicic². (1) Medicinal Chemistry, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, Fax: 516-365-5090, yalabed@nshs.edu, (2) Institute for Biological Research "Sinisa Stankovic", (3) University of Catania

The pro-inflammatory cytokine, Macrophage Migration Inhibitory Factor (MIF), plays a pivotal role in several inflammatory and autoimmune diseases. MIF mRNA expression is up-regulated in non-obese diabetic mice, yet little is known about the potential role of MIF in Type 1 diabetes. Here, we show that MIF protein was significantly elevated in islet cells during the development of experimental diabetes induced in mice by multiple low doses of streptozotocin. Attenuation of MIF activity with neutralizing antibodies against MIF, or the pharmacological MIF inhibitor (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), markedly reduced histopathological changes in pancreatic islets and suppressed the development of hyperglycemia. The observed beneficial effects could be attributed to the reduced proliferation and adhesion of autoreactive lymphocytes, down-regulation of iNOS expression, as well as NO and TNF- α secretion by islet cells and by peritoneal macrophages. This study defines a critical role for MIF in the pathogenesis of Type 1 diabetes and identifies a new therapeutic strategy to attenuate the autoimmune process at multiple levels.

185.

SYNTHESIS, STRUCTURE-ACTIVITY RELATIONSHIPS AND IN VIVO PROPERTIES OF 3,4-DIHYDRO-1H-PYRIDO[2,3-B]PYRAZIN-2-ONES AS CORTICOTROPIN-RELEASING FACTOR 1 ANTAGONISTS. Carolyn D. Dzierba¹, Amy G. Takvorian¹, Maria Rafalski¹, Padmaja Kasireddy-Polam¹, Andrew P. Combs¹, Ge Zhang², Anne Marshall², Gail K. Mattson², Thaddeus F. Molski², Nicholas J. Lodge², Shelly X. Ren³, Bitao Zhao³, Harvey Wong³, Yu-Wen Li³, Kathryn A. Ward², Snejzana Lelas², John F. McElroy², Rebecca A. Taub², Robert C. Zaczek², George L. Trainor¹, and Paul J. Gilligan¹. (1) Discovery Chemistry Department, Bristol-Myers Squibb Co, 5 Research Parkway, Wallingford, CT 06492-7660, Fax: 203-677-7702, carolyn.dzierba@bms.com, (2) Neuroscience Biology Department, Bristol-Myers Squibb Co, (3) Metabolism and Pharmacokinetics Department, Bristol-Myers Squibb Co

Corticotropin Releasing Factor (CRF) is the primary regulator of the hypothalamus-pituitary-adrenal (HPA) axis, coordinating the endocrine, behavioral and autonomic responses to stress. It has been postulated that small molecule antagonists of CRF1 may serve as a treatment for anxiety-related disorders and/or affective disorders. A series of 3,4-dihydro-1H-pyrido[2,3-b]pyrazin-2-ones, exemplified by compound 1 (IC50=0.70 nM), were found to be very potent antagonists of CRF1. The synthesis and structure-activity relationships of this series as well as the pharmacokinetic properties and efficacy in a rat behavioral model of anxiety of representative analogs will be discussed.



186.

DESIGN AND SYNTHESES OF NOVEL SMALL MOLECULE PERIPHERALLY RESTRICTED MU AGONISTS. Z. Chen, E. Davies, S. Victory, J. Huang, K. Valenzano, W. Miller, S. Shan, Y. Rotstheyn, G. Whiteside, K. Broglé, and D. Kyle, Drug Discovery, Purdue Pharma L. P., 6 Cedar Brook Drive, Cranbury, NJ 08512, zhengming.chen@pharma.com

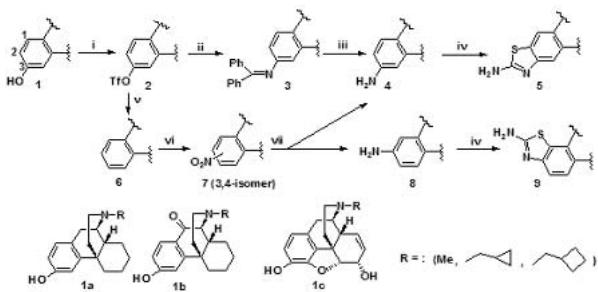
Opioid receptors exist both in the CNS and the peripheral terminals of primary afferent neurons. Recently a substantial literature has demonstrated that opioids can produce potent and clinically measurable antihyperalgesia by activation of the opioid receptors on the peripheral terminals of primary sensory neurons. It

is observed that injection of morphine directly into inflamed tissue results in potent antihyperalgesia in both animals and humans at doses that are systemically inactive. In addition, endogenous ligands of these peripheral receptors were discovered in immune cells. The discovery of opioid "peripheral analgesics" provides an opportunity to design new analgesics that produce no central side effects such as respiratory depression, dependence, dysphoria, nausea, and sedation, but retain potent analgesic actions. Such drugs should not cross the blood-brain barrier, and they would neither affect motor functions, nor have NSAID-like side effects such as gastrointestinal irritation. Some peripherally restricted mu selective peptides have already shown promising results in preclinical testing for inflammatory pain. Therefore, it was proposed that the identification of novel small molecule peripherally restricted mu agonists could lead to novel antihyperalgesic agent that lacks many of the side effects generally associated with centrally acting opiates or NSAIDs. In this presentation, we will report our efforts in the discovery of novel small molecule peripherally restricted mu agonist targeting on inflammatory pain states. We will describe the design and synthesis of a structurally novel, highly potent and peripherally-restricted mu opioid agonist, DiPOA [(8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl)-acetic acid] and its in vitro and in vivo pharmacological properties.

187.

SYNTHESIS OF AMINOTHIAZOLOMORPHINANS AS MIXED KAPPA AND MU OPIOID LIGANDS. Ao Zhang, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, Fax: 617-855-2519, azhang@mclean.harvard.edu, Jean M. Bidlack, Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, and John L. Neumeyer, Medicinal Chemistry Laboratory, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School

We have synthesized a series of aminothiazolomorphinans from the corresponding phenolic opioids (1a-c). The key step was the synthesis of amine 4 or 8 by a Pd-catalyzed amination strategy from triflate 2 or by a tandem nitration - reduction procedure from compound 6 (Zhang A. et al., Tetrahedron Lett., 2003, 44, 6459-6462). The subsequent cyclization was achieved by the treatment of the corresponding amine 4 or 8 with KSCN and Br2 in AcOH to yield the 2-aminothiazole 5 or 9 as the only product. Among this series, compound 5a (R=cyclopropylmethyl) was found to have high affinity at kappa receptor (Ki=0.049 nM), and a kappa/mu selectivity of 30. Compared to cyclorphan 1a (R=cyclopropylmethyl), compound 5 retained high affinity at kappa, but the selectivity for kappa over mu was greatly increased (10-fold). [35S]GTPgammaS binding studies showed that most of these compounds are kappa agonists with both agonist and antagonist activities at mu receptor. These novel compounds may be valuable templates for the investigation of the relative selectivity of kappa/mu opioids. Further evaluation of these compounds as long-acting analgesics and as probes for drug abuse medications is presently in progress (Supported by NIH Grants R01-DA 14251 and K05-DA00360).

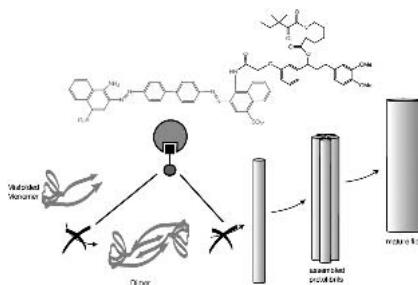


188.

BIFUNCTIONAL SMALL MOLECULES AS INHIBITORS OF AMYLOIDOGENIC PROTEIN-PROTEIN INTERACTIONS. Jason E. Gestwicki, Isabella Graef, and Gerald R Crabtree, Department of Pathology, Stanford University School of Medicine, 279 Campus Drive, B209 Beckman Center, Stanford, CA 94305, Fax: 650-723-5158, gestwick@cmgm.stanford.edu

The deposition of amyloidogenic peptide fibrils is the hallmark of numerous neurodegenerative diseases, such as Alzheimer's and Parkinson's. In Alzheimer's disease, fibrils are assembled as a result of progressive protein-protein interactions between copies of the amyloid beta (Ab) peptide. Small molecules

that effectively bind Ab have been generated, but these compounds typically have disappointing inhibition constants. It is likely that small molecules lack sufficient steric bulk to prevent interactions between relatively large peptide surfaces. Mindful of these considerations, we report a strategy that takes advantage of bifunctional molecules to recruit endogenous cellular proteins to aggregating Ab. These compounds are comprised of an Ab targeting moiety and a recruitment domain that interacts with the ubiquitous cellular protein, FKBP. In the presence of FKBP, our inhibitors are at least 5-fold more potent than equivalent small molecules. Thus, these compounds are among the most effective inhibitors of Ab aggregation reported.



189.

CHEMTECTICS - DRUG DISCOVERY IN ONE TUBE. *Alex H. Gouliaev*, Drug Discovery, Nuevolution A/S, Roennegade 8, 5. floor, DK-2100 Copenhagen, Denmark, Fax: +45-7020-0986, ahg@nuevolution.com

Nuevolution's Chemetics technology allows the synthesis of ultra-large libraries (10^{exp}8-10^{exp}14 small molecules) and the subsequent isolation of NCE's by a selection process mimicking natural evolution. As such, Chemetics promises to revolutionize drug discovery.

Chemetics is a wet chemistry approach enabling DNA-directed, one-pot synthesis and screening of billions of small drug-like molecules in weeks.

A huge library of small organic molecules is generated in one tube through the combination of Drug Fragments, wherein each Drug Fragment has been encoded by DNA. The overall outcome of the Chemetics encoding is a small organic molecule attached to the DNA that encodes it. The library of small molecule-DNA complexes generated above can be exposed to an affinity chromatography column to which a target of interest has been immobilized. Ligands bind the target; those small molecules that do not bind are washed off the column. The ligands are now eluted and the small molecule ligands can then be amplified, by first amplification of the DNA by PCR, and then Chemetics encoding. Alternatively, their structure may be revealed through the sequencing of their DNA. Overall, the Chemetics technology will allow the isolation and identification of the higher affinity, small molecule ligands in the huge library.

190.

PLEXX-BASED COMBINATORIAL DOCKING METHODS FOR DEALING WITH PROTONATION AND TAUTOMER AMBIGUITIES. *Ingo Dramburg¹*, Jens Sadowski², Holger Claußen³, Marcus Gastreich⁴, and Christian Lemmen¹. (1) BioSolveIT GmbH, An der Ziegelei 75, 53757 St. Augustin, Germany, Fax: +49-2241-9736688, dramburg@biosolveit.de, (2) Structural Chemistry Laboratory, AstraZeneca, (3) Chemoinformatics, BioSolveIT GmbH, (4) BioSolveIT GmbH

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191.

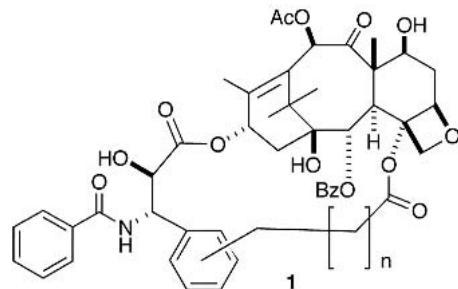
NOVEL APPROACH TO DRUG DISCOVERY - SCAFFOLD-BASED DRUG DISCOVERY TM. *Prabha N. Ibrahim*, Department of Chemistry, Plexxikon, 91, Bolivar Drive, Berkeley, CA 94710, Fax: 510-548-8014, pibrahim@plexxikon.com

Plexxikon's approach to Drug Discovery focuses on identifying novel chemical scaffolds which enable the rapid generation of optimized drug candidates for a specific target. Our discovery process utilizes high through-put co-crystallography as a part of screening paradigm. Examples of novel scaffolds discovered through Plexxikon's unique approach and their use in identifying lead candidates in kinase, phosphodiesterase, and nuclear receptor protein family targets will be presented. Using our novel approach we have identified a new class of PPAR pan agonist which is in pre-clinical development at Plexxikon.

192.

TAXOL PHARMACOPHORE: EXPERIMENTAL EVIDENCE FROM A HIGHLY CONSTRAINED ANALOG AND REDOR NMR. *David G. I. Kingston¹*, Thota Ganesh¹, Jennifer K. Schilling¹, Rebecca C. Guza¹, Susan Bane², Natasha Shanker², Rudravajhala Ravindra², James P. Snyder³, Ami Lakdawala³, Lynette Cegelski⁴, Robert D. O'Connor⁴, and Jacob Schaefer⁴. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-7702, dkingston@vt.edu, (2) Department of Chemistry, State University of New York, (3) Department of Chemistry, Emory University, (4) Department of Chemistry, Washington University

Detailed knowledge of the crucial interaction of paclitaxel and tubulin is of paramount importance in the design and development of highly potent paclitaxel analogs. In an effort to understand the mechanism of action of paclitaxel, we have combined experimental results from REDOR NMR and fluorescence spectroscopic studies with molecular modeling and electron crystallographic studies to design and build bridged paclitaxel analogs that are consistent with biophysical data for the paclitaxel pharmacophore. The general structure of these analogs includes a bridge from the C-3' phenyl group to the C-4 position, as exemplified by structure 1. Two of the resulting constrained analogs have tubulin-assembly and cytotoxic activities equal to or better than those of paclitaxel, indicating that our proposed model for the paclitaxel pharmacophore is essentially accurate. Structures, synthesis, bioactivities, and modeling studies of the constrained analogs will be presented.



193.

ANTIOXIDANT DRUG DISCOVERY: IS THERE A THERAPEUTIC ROLE FOR ANTIOXIDANTS IN CHRONIC INFLAMMATION? *Charles Q Meng*, James A Sikorski, Jayraz Luchoomun, Ki-Ling Suen, Charles Kunsch, Cynthia L Sundell, Robert A D Scott, and Martin A Wasserman, AtheroGenics, Inc, 8995 Westside Pkwy, Alpharetta, GA 30004, cmeng@atherogenics.com

Man relies on atmospheric oxygen to oxidize fuel molecules to provide energy. This process inevitably generates reactive oxygen species (ROS) that can cause damages to cells leading to inflammatory consequences. A large body of experimental work supports a role of oxidative stress in the pathophysiology of numerous diseases. As such, antioxidants have been rationally considered as a means to correct oxidative/inflammatory stress and, hence, prevent and/or treat oxidative stress-related diseases. We initiated a research program in the late 1990's aiming at the discovery of novel antioxidant/anti-inflammatory agents based on the hypothesis that properly designed mono-substituted probucol derivatives would retain the antioxidant property of probucol through the remaining phenol group and gain additional physical properties to enable them to reach their biological targets. Some of these derivatives showed similar antioxidant potencies as probucol, and inhibited the expression of vascular cell adhesion molecule-1 (VCAM-1) and LPS-induced secretion of cytokines such as TNF-alpha, IL-1beta and IL-6, further confirming the contribution of antioxidant properties to anti-inflammatory effects. At this meeting we will review the current status of antioxidant drug discovery, report findings from our research program, especially on the antioxidant/anti-inflammatory aspects of our compounds, and point out that there is a potential therapeutic role for antioxidants to play in chronic inflammation.

194.

OVERVIEW OF DRUG HEPTOTOXICITY. *Neil Kaplowitz, University of Southern California, Keck School of Medicine, USC Research Center for Liver Diseases, 2011 Zonal Avenue, HMR 101, Los Angeles, CA 90033, kaplowit@usc.edu*

Drugs and natural chemicals are a major cause of liver injury. Chemical hepatotoxicity is the leading cause of acute liver failure in the U.S. and also can induce of an injury pattern that mimicks virtually any form of acute or chronic liver disease. Drug hepatotoxicity is usually caused by toxic metabolites which exert chemical consequences, such as covalent binding, lipid peroxidation, redox changes, leading to direct necrosis (bioenergetic catastrophe), apoptosis, or indirect toxicity due to sensitization to the innate immune system and its mediators (cytokines, chemokines, eicosanoids, reactive oxygen or nitrogen species) or activation of an immunoallergic response. Individual drugs exhibit a characteristic signature which includes clinical presentation (hepatitis, cholestasis, mixed) and altency to onset of liver injury. The diagnosis of drug-induced liver disease is circumstantial and relies on the characteristic signature, response to discontinuation and exclusion of alternative causes.

195.

AVOIDING SAFETY PROBLEMS WITH DRUG CANDIDATES: ISSUES AND APPROACHES. *F. Peter Guengerich, Department of Biochemistry, Vanderbilt University School of Medicine, 638 Robinson Res. Bldg, 23rd & Pierce Avenues, Nashville, TN 37232, Fax: 615-322-3141, f.guengerich@vanderbilt.edu*

Two phenomena of particular interest in the current knowledge of toxicology are ligand/receptor-mediated gene transcription and covalent binding to macromolecules. Modern assays have been developed to screen for both *in vitro*. Receptor-based assays can be done on a rapid and large scale; deficiencies involve the limited state of knowledge of receptors and the need for co-activator molecules in many systems. Technology is also becoming available for large-scale analysis of covalent binding to proteins. Issues include the transient nature of some adducts, the question of which targets are truly critical, and the general relevance of binding to drug idiosyncrasies. A remaining need is better animal models to predict immunological and other rare events.

196.

METABOLIC ACTIVATION - A PHARMACEUTICAL INDUSTRY PERSPECTIVE.

David C. Evans, Merck & Co. Inc, PO Box 2000, Rahway, NJ 07065, david_c_evans@merck.com

It is generally accepted that there is neither a well defined nor consistent link between the formation of drug-protein adducts and organ toxicity. Since the potential does exist, however, for these processes to be causally related, the general strategy at Merck Research Laboratories has been to minimize reactive metabolite formation to the extent possible by appropriate structural modification during the lead optimization stage. This requires a flexible approach to defining bioactivation issues in a variety of metabolism vectors, and typically involves the initial use of small molecule trapping agents to define the potential for bioactivation. At some point, however, there is a requirement to synthesize a radiolabeled tracer and to undertake covalent binding studies *in vitro*, usually in liver microsomal (and sometimes hepatocyte) preparations from preclinical species and human, and also *in vivo*, typically in the rat. A perspective on how to address the issue of bioactivation from an industry viewpoint based on protocols adopted by Merck Research Laboratories will be presented. The availability of a dedicated Labeled Compound Synthesis group, coupled to a close working relationship between Drug Metabolism and Medicinal Chemistry, represents a framework within which this perspective becomes viable; the overall aim being to bring safer drugs to patients.

197.

ADDRESSING REACTIVE METABOLITE FORMATION IN EARLY DRUG DISCOVERY. *Amit S Kalgutkar, Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, Fax: 860-686-1059, amit_kalgutkar@groton.pfizer.com*

There are myriad examples of drugs that are hepatotoxic or cause idiosyncratic toxicity for which bioactivation mechanisms have been described. These examples provide guidance in that chemical substituents that are known to undergo bioactivation to a reactive intermediate should be avoided in early drug discovery efforts. However, when considering bioactivation and reactive metabolite formation within a new chemical entity, several factors must be

accounted for before making a judgment based solely on structure. For instance, not all compounds possessing "structural alerts" are bioactivated and not all compounds that are bioactivated cause toxicity. The presentation will highlight literature and in-house examples of such compounds with experimental approaches designed to assess the risk of adverse drug reactions.

198.

RESOLVING DRUG BIOACTIVATION PROBLEMS IN DRUG DISCOVERY AND DEVELOPMENT. *Gary L. Skiles, Drug Disposition & Bioanalytical Sciences, Bristol-Myers Squibb, PO Box 4000, F13-04, Princeton, NJ 08543, gary.skiles@bms.com*

An increasing awareness of the liabilities associated with the formation of reactive metabolic intermediates of drugs has lead to a more careful assessment of that liability in drug candidates. These liabilities are sometimes revealed in unexpected ways, necessitating careful vigilance at all stages of the drug discovery and development process to minimize the risks of drug toxicity. The unequivocal identification of products formed by addition of nucleophiles to electrophilic metabolic intermediates can often guide the design of molecules that are not susceptible to bioactivation. The problem can, however, be particularly intractable in some chemotypes, either because of physico-chemical constraints, or because structural changes simply shift the site of bioactivation to different parts of the molecule. In some cases the new sites of bioactivation might have been anticipated based on known bioactivation chemistry, but sometimes they represent novel bioactivation pathways. These challenges will be illustrated by several examples.

199.

CALIBRATION STRATEGIES FOR NEAR-INFRARED GLUCOSE SENSORS. *Gary W. Small, Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701, Fax: 740-593-0148, small@ohio.edu*

Near-infrared spectroscopy represents one possible strategy for implementing a noninvasive home glucose monitor. Two issues that impede the successful development of this method are the implementation of a successful and stable calibration model to relate the spectral measurements to the glucose level and the need to produce a simple, rugged instrument for the measurement that still possesses the required optical performance. These two issues are intertwined because the calibration requirements dictate the characteristics of the measurement platform in terms of parameters such as spectral range, signal-to-noise ratio, scan speed, and resolution. In this presentation, both of these issues will be addressed in the context of glucose measurements made in model systems that are designed to simulate the pertinent characteristics of the physiological measurement. Specific topics to be discussed include calibration strategies for overcoming instrumental drift and ways to use the results of calibration experiments to simplify the instrument design.

200.

HYDROGEL PREPOSITIONED, SELF ASSEMBLED SUPRAMOLECULAR COMPLEX FOR GLUCOSE RECOGNITION AND SIGNALING. *Sanford A. Asher¹, Anjal C. Sharma¹, Vladimir L. Alexeev¹, Igor K. Lednev², Craig S Wilcox¹, and David N. Finegold³.* (1) Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, Fax: 412-624-0588, asher@pitt.edu, (2) Department of Chemistry, SUNY @ Albany, (3) Department of Pediatrics, University of Pittsburgh Medical School

There is intense interest in developing glucose sensing methodologies for patients with diabetes mellitus. We report here a new glucose sensing material based on boronic acid glucose binding within a crystalline colloidal array hydrogel photonic crystal. This sensing material both responds and signals the presence of glucose. The signaling response utilizes the Bragg diffraction of light by an embedded face centered cubic (fcc) array of colloidal particles. These intelligent polymerized crystalline colloidal array materials determine glucose at the high ionic strengths of bodily fluids. These novel glucose sensing and signaling materials show a potential for *in-vivo* glucose sensing.

201.

NEW FLUORESCENT PROBES FOR MONOSACCHARIDES: TOWARDS THE NON-INVASIVE CONTINUOUS MONITORING OF PHYSIOLOGICAL GLUCOSE USING A DAILY DISPOSABLE CONTACT LENS. *Ramachandram Badugu¹, Joseph R. Lakowicz¹, and Chris D. Geddes².*

(1) Department of Biochemistry and Molecular Biology, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, 725 West Lombard St, Baltimore, MD 21201, cfs@cs.umbi.umd.edu, (2) Medical Biotechnology Center, University of Maryland Biotechnology Institute, Institute of Fluorescence and Center for Fluorescence Spectroscopy

By incorporating new glucose sensitive probes within a disposable daily contact lens we are able to continuously monitor tear glucose concentrations which are typically 10 fold lower than, but track, normal blood levels, > 50 fM. We envisage that this non-invasive optical technique will readily allow for the continuous monitoring of hypo- and hyperglycemic episodes, alleviating the requirement for painful frequent finger testing. Glucose diffusion from tears, into the lens, reversibly complexes with boronic acid fictionalized fluorescent probes, which quantifiably change their spectral characteristics upon binding. Our new probes are based on the quaternized form of the 6-methoxyquinoline heterocyclic nucleus with either ortho, meta or para phenyl boronic acid. The novelty of the new probes lies in their reduced sugar-bound pKa, which enables the probes to respond to low glucose concentrations from within the internal environment of disposable, off-the-shelf, contact lenses, chosen because the physiological compatibility of disposable plastic contact lenses has already been assessed and optimized with regard to vision correction, size and oxygen / analyte permeability etc.

Our novel approach to the long-standing problem of non-invasive and painless physiological glucose monitoring offers many modalities for fluorescence sensing, including, intensity, lifetime and polarization based sensing. We present our recent findings.

202.

STRATEGIES TOWARD THE ORAL DELIVERY OF INSULIN: USING MOLECULAR MODIFICATION TO SOLVE DRUG DELIVERY CHALLENGES. *Jennifer A.*

Riggs-Sauthier¹, Kenneth D. James¹, Navdeep Malkar¹, Mark A. Miller¹, Robert E. Dugdell¹, Krisstina S. Danek Burgess¹, Diana Severynse-Stevens², David Surguladze¹, and Nnochiri Ekwuribe¹. (1) Drug Discovery & Chemical Innovation, Nobex Corporation, P.O. Box 13940, Research Triangle Park, NC 27709, Fax: 919-474-9407, jriggs@nobexcorp.com, (2) Biochemical Pharmacology, Nobex Corporation

While the conjugation of polyethylene glycol (PEG) to protein and peptide therapeutics is well known to enhance the aqueous solubility, render proteins non-immunogenic, reduce kidney clearance rate, and increase the circulation time of the parent peptide, the high molecular weights that are commonly used preclude oral delivery of the therapeutic. Nobex Corporation has proprietary amphiphilic oligomers of PEG and alkyl combinations that have been successfully applied to several peptide therapeutics to enhance their PK/PD profiles and enable oral delivery. In an effort to understand the effects of conjugating these amphiphilic oligomers to insulin and develop structure relationship activities, a broad range of insulin conjugates varying in size, sterics, and amphiphilic balance were synthesized. The conjugates were screened by *in vitro* and *in vivo* assays to measure activity and determine oral bioefficacy. In addition, many physicochemical properties such as aqueous solubility, protease stability, circular dichroism (CD), and thermal denaturation (Tm) were evaluated.

203.

DEVELOPMENT OF NEUTRAL PHOSPHOTYROSINE MIMETICS AS PROTEIN TYROSINE PHOSPHATASE INHIBITORS. *Dehua Pei*, Department of Chemistry, Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, Fax: 614-292-1532, pei.3@osu.edu

Protein tyrosine phosphatases (PTPs) together with protein tyrosine kinases regulate the levels of protein tyrosine phosphorylation in cells, thereby playing key roles in a variety of physiological and pathological processes. Therefore, specific PTP inhibitors provide valuable tools in studying the functions of these enzymes in cellular processes as well as potential therapeutic agents. We have developed several classes of neutral phosphotyrosine mimetics as covalent PTP inhibitors. The first class of inhibitors each contains an alpha-halogenated acetophenone core, which covalently alkylates the conserved catalytic cysteine in

the PTP active site. The inhibitory effects of these alpha-haloacetophenone derivatives can be reversed by photolysis at 350 nm, providing potential on-off switches for controlling the activities of intracellular PTPs. A second class of inhibitors contain a cinnamaldehyde core structure, appended to specific peptide or peptidomimetic sequences. The aldehyde group forms a reversible, covalent enamine adduct with a conserved arginine in the PTP active site. Finally, trans-beta-nitrostyrene inhibits PTPs in a yet unknown mechanism. Compared to the existing pY mimetic inhibitors, which are generally negative charged, the neutral pY mimetics should have improved membrane permeability.

204.

SMALL MOLECULE INSULIN MIMICS. *Michael Pirrung*, Department of Chemistry, Duke University, B120 Levine Science Research Center, Durham, NC 27708, Fax: 919-660-1591, michael.pirrung@duke.edu

A novel natural product of the asterriquinone class (demethylasterriquinone B1 (DAQ)) reported in 1999 exhibits insulin mimetic activity in two mouse models of diabetes. It directly activates the tyrosine kinase of the insulin receptor β -chain, and as a small molecule, is orally active. This discovery of a small molecule that mimics a proteinaceous growth factor opens new avenues for controlling cell physiology and perhaps the development of human therapeutics. We have therefore studied synthetic and combinatorial methods for creating new compounds of the asterriquinone class. New strategies for mapping the sites of interaction of these ligands with their receptor targets have been developed, the activities of the compounds have been studied in a variety of model cell types, and novel proteomic and functional genomic methods have been applied to understanding their mode of action.

205.

PHYSICOCHEMICAL PROPERTIES OF BINDING POCKETS FOR AGONISTS VERSUS ANTAGONISTS IN 7TM RECEPTORS - BASIS FOR KNOWLEDGE-BASED DRUG DISCOVERY. *Thue W. Schwartz*, Laboratory for Molecular Pharmacology, University of Copenhagen, 7TM Pharma A/S, Horsholm, Denmark, schwartz@molpharm.dk

Exhaustive mutational mapping of binding sites performed in the NK1, AT1, k-opioid, galanin, MC1, ghrelin, LTB4, and CCR5 and CXCR4 receptors has provided an overall concept of small molecule binding in various modes in subsets of the main ligand-binding pocket of 7TM receptors. This approach lead to characterization of a rather well defined binding sites—whereas identification of actual point-to-point molecular interactions between the ligand and the receptor was defined mainly by computational chemistry guided by the mutational finding. In a few cases the insight has reached a level of detail where the main anchor-points of a binding site for a small molecule drug could be transferred to a distantly related receptor through just a few substitutions in the target receptor giving a potency within 10-fold of the original one. Based on the detailed knowledge about the basic geometry of metal-ion binding in proteins and a number of inactivating and especially activating metal-ion sites, a “toggle switch” model for activation of 7TM receptors has been established, where the top of TM-VI and VII moves inwards during activation while the bottom of these two helices moves away from TM-III and opens up for the binding of transducer molecules: G-protein and arrestin. Molecular details of this model has allowed for the definition of some general rules for the molecular mechanism of agonists versus antagonists in 7TM receptors. This insight into ligand binding and action in the main ligand-binding pocket of 7TM receptors has formed the basis for the development of a knowledge and structure-based drug discovery technology called “Site Directed Drug DiscoveryTM”.

206.

STRUCTURE-FUNCTION ANALYSIS OF THE M3 MUSCARINIC ACETYLCHOLINE RECEPTOR USING DISULFIDE CROSS-LINKING AND RECEPTOR RANDOM MUTAGENESIS APPROACHES. *Jürgen Wess*, Bo Li, Fadi F. Hamdan, Lars Brichta, Nicola Nowak, Lanh Bloodworth, Clarice Schmidt, and Sung-Jun Han, Lab. of Bioorganic Chemistry, NIH-NIDDK, 8 Center Drive MSC 0810, Bldg. 8A, Room B1A-05, Bethesda, MD 20892, Fax: 301-480-3447, jwess@helix.nih.gov

To study the molecular mechanisms involved in G protein-coupled receptor (GPCR) function, we have used the M3 muscarinic acetylcholine receptor, a prototypical class A GPCR, as a model system. To elucidate the structural changes involved in ligand-dependent M3 receptor activation, we employed a novel *in situ* disulfide cross-linking strategy. The observed cross-linking patterns

indicated that M3 receptor activation is associated with specific movements involving the endofacial segments of various transmembrane helices. We recently also established a system that allows the functional expression of the M3 muscarinic receptor in yeast (*S. cerevisiae*). Yeast strains were genetically modified in a fashion such that yeast growth was strictly dependent on productive M3 receptor/G protein coupling. Following PCR-directed random mutagenesis of the M3 muscarinic receptor, we used yeast functional screens to identify mutant M3 receptors endowed with novel/altered functional properties, including mutant receptors which are constitutively active or which regain functional activity despite the presence of a primary inactivating mutation. These studies have led to novel insights into the structural determinants governing GPCR function.

207.

FUSION PROTEINS BETWEEN G PROTEIN-COUPLED RECEPTORS AND THEIR INTERACTING PROTEINS. *Graeme Milligan, Molecular Pharmacology Group, University of Glasgow, Davidson Building, University Avenue, Glasgow G12 8QQ, United Kingdom, Fax: 44-141-330-4620, g.milligan@bio.gla.ac.uk*

Interactions between G protein-coupled receptors and a variety of other proteins, including G proteins, arrestins and Regulators of G protein Signalling have been studied following construction and expression of fusion proteins in which the interacting protein is linked in frame to the C-terminal tail of the receptor to generate single, but bifunctional, open reading frames. G protein-coupled receptor-G protein fusions have been widely used to examine the selectivity of receptor-G protein interactions, the role of post-translational acylation of both polypeptides, the importance of the G protein beta/gamma complex in information transfer between receptor and G protein alpha subunit and the basis and specificity of the dimerization G protein-coupled receptors. A range of such studies will be described.

208.

NEOCEPTOR CONCEPT APPLIED TO HUMAN A2A AND A3 ADENOSINE RECEPTORS. *Kenneth A. Jacobson¹, Soo-Kyung Kim¹, Michihiro Ohno¹, Heng T. Duong¹, Philippe Van Rompaey², Serge Van Calenbergh², and Zhan-Guo Gao¹. (1) Molecular Recognition Section, NIDDK, NIH, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810, Fax: 301-480-8422, kjacobs@helix.nih.gov, (2) Laboratorium voor Medicinale Chemie (FFW), Harelbekestraat 72 B-9000 Gent, Belgium*

The clinical use of adenosine agonists as cytoprotective agents has been limited by the widespread occurrence of adenosine receptors (ARs), thus leading to undesirable side effects of exogenously administered adenosine derivatives. In order to overcome inherent nonselectivity of activating the native receptors, we have introduced neoreceptors. By this strategy, intended for eventual use in gene therapy, the putative ligand binding site of GPCRs is reengineered for activation by synthetic agonists (neoligands) built to have a structural complementarity. The feasibility of neoreceptors was demonstrated for A2A and A3ARs, which mediate anti-inflammatory and cardioprotective functions, respectively. Certain positively-charged amine-modified nucleosides have enhanced affinity at ARs strategically-mutated with anionic residues that activate second messengers. Specifically, amino groups introduced in the ribose moiety of adenosine interact favorably with receptors modified in the putative ribose binding regions associated with TMs 3 and 7 (both subtypes). Rhodopsin-based modeling of the receptors indicated that hydrophilic Thr(A2A,3.36) and His(A3,7.43) were in proximity to ribose. The affinity of 5'-(2-aminoethyl)uronamidoadenosine (MRS 3366) was selectively enhanced at the T88D mutant A2AAR, at which the standard AR agonists were poorly recognized. At the H272E mutant A3AR, a 3'-amino-3'-deoxy derivative of adenosine displayed selective affinity enhancement. Amino groups placed at other positions did not enhance the receptor binding. In general, amine derivatization of the adenosine agonists greatly decreased potency at WT ARs. Thus, using a rational design process we have identified pairs of neoreceptor-neoligand, which are pharmacologically orthogonal with respect to the native species.

209.

DECODING HORMONAL RESPONSES WITH RASSLS, AND PATHWAY-ORIENTED GENE EXPRESSION ANALYSIS. *Bruce R. Conklin, Kimberly Scearce-Levie, Supriya Srinivasan, Whittemore G. Tingley, Peter WC Chang, and Alexander C. Zambon, Medicine and Pharmacology, Gladstone, UCSF, PO Box 419100, San Francisco, CA 94141-9100, Fax: 415-285-5632, bconklin@gladstone.ucsf.edu*

We are designing new G protein-coupled receptors (GPCRs) to allow experimental control of physiological processes. We have engineered versions of the kappa opioid receptor (KOR) to be unresponsive to the endogenous levels of natural hormones but still be activated by administration of synthetic small molecule drugs. These modified receptors are called RASSLs (Receptor Activated Solely by a Synthetic Ligand). These KOR-based RASSLs have potent biological effects when expressed in the heart, brain, bone or other tissues. We have built a series of RASSLs that activate each of the major G protein pathways. We have devised a way to insert the RASSLs into thousands of genomic loci using a single "Floxin" vector with BayGenomics ES cells (www.BayGenomics.ucsf.edu). To view RASSL activated gene expression changes in the context of biological pathways we have developed a freely distributed software package.

210.

ENGINEERED KINASES FOR UNNATURAL LIGANDS: NEW TOOLS FOR TARGET VALIDATION. *Kevan Shokat, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, 513 Parnassus, San Francisco, CA 94143-0450, Fax: 415-514-0822*

Our laboratory focuses on the development of novel chemically based tools to decipher signal transduction pathways on a genome-wide scale. We have developed a method for producing small molecules that are specific for any protein kinase of interest in a signaling cascade by combining protein design with chemical synthesis. These highly specific inhibitors of individual kinases have revealed a number of new principles of signal transduction that have complemented genetic and biochemical studies of cell signaling. Examples where new pathways and new functions can be revealed by small molecule inhibitors of protein kinases will be highlighted. A second area of interest in our laboratory is the tracing of direct kinase substrates. We have designed and synthesized unnatural ATP analogs which are substrates of our engineered kinases but are poorly accepted as substrates of wild-type kinases. This specific nucleotide substrate of any kinase of interest allows for the radiolabelling of the direct substrates of a wide variety of protein kinases including both serine/threonine and tyrosine kinases. New methods for the isolation and identification of low abundance substrates of kinases from cells will be discussed. Once a phosphoprotein substrate of a kinase is identified, the specific phosphorylation site is often difficult to identify using traditional tryptic peptide phosphorylation site mapping. Using a novel strategy based on the design of tailor made proteases which specifically cleave proteins after sites of phosphorylation, we have developed a rapid means to map protein phosphorylation patterns. Finally, a potential link between the unnatural ligands of engineered kinases and a set of plant hormones, the cytokinins, will be discussed in the context of a custom designed database created for the genome wide analysis of protein kinase catalytic domains.

211.

EVOLUTION OF SMALL-MOLECULE REGULATED INTEINS FOR MODULATING PROTEIN FUNCTION. *David R. Liu, Allen R. Buskirk, Zev J. Gartner, and Yi-Ching Ong, Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, Fax: 617-496-5688, drliu@fas.harvard.edu*

A longstanding goal in chemistry and biology is the development of tools for controlling protein activity *in vivo*. Small-molecule modulators of protein function offer powerful temporal, spatial, and dose-dependent precision but require the discovery of a different active small molecule for each protein of interest. Genetic approaches lack these important control features but offer specificity for one protein of interest. In an attempt to combine the advantages of both of these approaches, we have developed a tool for controlling protein function *in vivo* that activates a protein post-translationally but only in the presence of a small molecule. Inteins are protein-splicing elements that, when inserted into a protein of interest, usually render the target protein inactive. Upon intein-catalyzed protein splicing, the target protein's activity is restored. Certain inteins can splice out of virtually any protein context. To achieve small

molecule-mediated control of intein splicing, we replaced the endonuclease domain of the *M. tuberculosis* RecA intein with a natural ligand-binding domain known to undergo conformational changes upon binding a cell-permeable synthetic small molecule. As this simple replacement did not yield the desired ligand-dependence, we implemented a directed evolution approach in which protein splicing *in vivo* was linked to cell survival in *S. cerevisiae*. Mutagenesis and selection of the modified intein yielded evolved variants with splicing activity that was highly dependent on the presence of the synthetic small molecule. The evolved inteins' ligand dependence was further enhanced by a combination of positive and negative screening together with selection until protein splicing was not observed in the absence of the synthetic small molecule. The ability of the resulting evolved inteins to modulate protein function *in vivo* was characterized in several protein contexts by both Western blotting and functional phenotypic assays. These findings represent one of the first examples of controlling protein activity via small molecule-dependent splicing *in vivo*. Evolved allosteric inteins may represent a powerful tool for modulating the activity of virtually any protein of interest without the effort associated with small molecule discovery for each target protein.

212.

NEW STRATEGIES FOR THE RIBOSOMAL SYNTHESIS OF MODIFIED ENZYMES.

Sidney M. Hecht, Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, VA 22904-4319, Fax: 434-924-7856, sidhecht@virginia.edu

The cell free synthesis of proteins containing non-natural amino acids at specific, predetermined sites can be accomplished by the use of misacylated tRNAs. While the technology is now well established, there are significant limitations as regards the amounts of protein that can be prepared and the repertoire of amino acids that can be introduced. Presently, the modification of the bacterial ribosome to enhance the introduction of D-amino acids is described, as is a strategy to enhance the stability of the misacylated tRNAs employed for protein synthesis.

213.

CHEMICAL BIOLOGY OF PROTEIN SPLICING. *Tom W Muir*, Laboratory of Synthetic Protein Chemistry, Rockefeller University, 1230 York Avenue, New York City, NY 10021, Fax: 212-327-7358, muirt@mail.rockefeller.edu

Protein splicing is a posttranslational process in which an intervening sequence, an intein, is removed from a host protein, the extein. In protein trans-splicing the intein is split into two pieces and splicing only occurs upon reconstitution of these fragments. We have shown that thiolysis of mutant intein-fusions leads to the generation of recombinant protein-thioester derivatives that can be chemically ligated to polypeptides (synthetic or recombinant) bearing an N-terminal Cys. This semisynthetic process, generally referred to as Expressed Protein Ligation (EPL), has been used to incorporate unnatural amino acids, posttranslational modifications and isotopic probes site-specifically into proteins. EPL has been applied to numerous systems over the last few years and has allowed a variety of biological questions to be addressed. Selected examples from our own work will be discussed. A second application of protein splicing that will be discussed is in the area of chemical genetics. We have recently developed a novel system that allows protein trans-splicing to occur only in the presence of the small molecule, rapamycin. This 'conditional protein transsplicing' (CPS) technique provides a means to trigger the post-translational synthesis of a target protein from two fragments. In principle, CPS provides a level of temporal control over protein function that is difficult to achieve using standard genetic approaches. Recent progress in this area will be described.

214.

CHEMICAL AND BIOLOGICAL ENGINEERING OF ENZYMES. *Ronald T. Raines*,

Department of Biochemistry and Department of Chemistry, University of Wisconsin - Madison, 433 Babcock Dr, Madison, WI 53706-1544, Fax: 608-262-3453, raines@biochem.wisc.edu

The human genome contains 30,000 or so genes. Scientists from a broad range of disciplines are now working to reveal the structure and function of the proteins encoded by these genes. Their findings could lead to the solution of a multitude of problems in biology and medicine. In addition to structure-function analyses of extant proteins, chemical biologists are working to create new proteins with desirable properties, either by altering natural frameworks or

by de novo design. New methods for the chemical synthesis and semisynthesis of proteins is beginning to make a significant contribution to these efforts.

215.

PROGESTERONE, ESTROGEN AND ANDROGEN RECEPTOR SIGNALING

PATHWAYS ARE COMPLEX AND PROVIDE A WEALTH OF OPPORTUNITIES FOR NEW DRUG DISCOVERY. *Donald P. McDonnell*, Department of Pharmacology and Cancer Biology, Duke University Medical Center, Box 3813, C259 LSRC, Durham, NC 27710, Fax: 919-681-7139, donald.mcdonnell@duke.edu

The classical models of steroid receptor pharmacology held that agonists functioned by binding to their cognate receptors facilitating their conversion from an inactive form to one that was capable of activating transcription. By extrapolation, it was believed that antagonists functioned by competitively inhibiting agonist binding, freezing the receptor in an inactive state. However, as early as 1967 when the biological actions of the "antiestrogen" tamoxifen were first described it was clear that this simple model did not adequately describe estrogen receptor (ER) pharmacology. Tamoxifen is more appropriately classified as a Selective Estrogen Receptor Modulator (SERM), one of a group of compounds whose agonist or antagonist activity can differ between cells. Similarly, tissue selective progesterone, androgen and glucocorticoid receptor modulators have also been identified indicating that the observed complexity of ER action extends to other steroid receptors. Significant progress has been made in defining the molecular mechanism(s) by which cells distinguish between agonists and antagonists and how some receptor modulators can manifest their actions in a cell-selective manner. The most important of these are (1) differences in the relative expression level of receptor isoforms or subtypes, (2) the impact which the bound ligand has on the structure of its cognate receptor, and (3) the complement of coactivators and corepressors in a target cell which can interact with the activated receptor. This presentation will focus on the role of coactivators and corepressors in nuclear receptor pharmacology and how these proteins regulate cellular responses to agonists and antagonists and how perturbations in these regulatory mechanisms can have pathological consequences.

216.

DESIGN, SYNTHESIS, AND EVALUATION OF NAPHTHALENE-BASED ESTROGEN RECEPTOR-BETA SELECTIVE LIGANDS. *Richard E. Mewshaw¹, Richard J. Edsall Jr.¹, Cuijian Yang¹, Eric S. Manas¹, James C. Keith Jr.², Yelena Leathurby², Leo M. Albert², and Heather A. Harris³.*

(1) Department of Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, mewshar@wyeth.com, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth Research, (3) Women's Health Research Institute, Wyeth Research

The unexpected discovery of a second subtype of the estrogen receptor (ER β) in 1996 has added a new level of uncertainty to our understanding of the role of estrogen and prompted intense interest by both academic and industrial scientists. Over the last several years our laboratories have been committed toward elucidating ER β function with the use of ER β selective ligands. This has been a very challenging problem due to the similarity of the ligand binding domains of ER α and ER β , with only two conservative amino acid changes occurring between these two isoforms. However, using a structure-based design approach our company has successfully identified several series of ER β selective agonists. In this presentation we will discuss our design strategy that has led to the discovery of a series of ER β selective phenyl-naphthalenes as well as related templates that embrace other novel classes of ER β ligands. This seminar will emphasize the SAR that led to the discovery of ERB-196, a selective ER β agonist that is currently in development.

217.

ESTROGEN RECEPTOR: LIGANDS AND PROBES OF RECEPTOR CONFORMATIONS AND INTERACTIONS. *John A. Katzenellenbogen*,

Department of Chemistry, University of Illinois, Urbana-Champaign, University of Illinois, 600 South Mathews Avenue, Urbana, IL 61801, Fax: 217-333-7325, jkatzene@uiuc.edu

The activity of nuclear hormone receptors as modulators of transcription is regulated by their binding of ligands. We have developed a systematic approach to design ligands for the estrogen receptor (ER), which has led to the discovery of novel ligand classes, some of which are selective for the ER subtypes. We

can monitor ligand-induced conformation and conformational dynamics in the ER, by tagging the ligand-binding domain with fluorophores directly, in a site specific manner, and we can show that ligands function in a pharmacological class-specific manner to stabilize the dimer interface and cause regional alterations in the conformational mobility of the helix11-helix 12 loop. Furthermore, we have characterized the effect of coactivator binding on these processes by excimer formation using pyrene-labeled receptors. Finally, we have developed protein arrays of the ERs with which we can monitor subtype-specific ligand binding and coactivator recruitment activities of these receptors.

218.

IN SEARCH OF ER-BETA SELECTIVE LIGANDS. *Brad R. Henke, Department of Medicinal Chemistry, GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, Brad.R.Henke@gsk.com*

Since the discovery in 1996 of a second estrogen receptor subtype, termed ERbeta, there has been a considerable amount of research into discovering the pharmacology of this new ER and its role in estrogen signaling pathways. Much of the early information on the role of ERbeta has come from phenotypic studies of estrogen receptor subtype-selective knockout mice and double-knockout mice, which have suggested that the two ER subtypes have distinct biological roles. While these studies have provided useful insight into ERbeta pharmacology, the availability of subtype-selective ligands are needed to better understand the functions of ERbeta and its utility as a target for pharmaceutical intervention. Thus we initiated a program to identify novel small-molecule ERbeta selective agonists and antagonists in order to provide additional insight into the pharmacology associated with subtype-selective modulation of ERbeta. The details of our efforts towards this goal will be discussed.

219.

PHARMACOLOGICAL CHARACTERIZATION, SAR, AND X-RAY CRYSTAL STRUCTURE OF AN ESTROGEN RECEPTOR ALPHA SELECTIVE SERM. *Jeffrey A. Dodge¹, Henry U Bryant¹, Timothy A. Grese², Yong Wang¹, Masahiko Sato¹, and Thomas B. Burris¹. (1) Eli Lilly and Company, Indianapolis, IN 46285, Fax: 317-277-2035, dodge_jeffrey_a@lilly.com, (2) Discovery Chemistry Research, Eli Lilly and Company*

Selective estrogen receptor modulators (SERMs) are estrogen receptor (ER) ligands that exhibit both agonist and antagonist properties in a tissue-, cell type-, and promoter-dependent manner. In order to examine the effects of receptor subtype selectivity on SERM pharmacology, a novel ERα selective compound, LY315471, was identified by structure-activity optimization. LY315471 is a high affinity benzothiophene-based ligand with approximately 130-fold greater affinity for ERα ($K_i=0.29$ nM) vs. ERβ ($K_i=38$ nM). The X-ray structure of LY315471 complexed with ERα was consistent with retention of ERα binding activity with selective loss of ERβ affinity. Helix 12 positioning was similar to that found in the raloxifene/ERα structure. In contrast, the His 524 is significantly displaced. LY315471 displayed antagonist activity in the MCF-7 breast cancer cell proliferation assay ($IC_{50}=40$ nM) and was a weak partial agonist in the Ishikawa uterine adenocarcinoma line. Consistent with the partial agonist activity identified in the Ishikawa cell line, LY315471 also demonstrated low but significant uterotrophic activity in rats. However, in the presence of estradiol, LY315471 showed potent antagonist activity in vivo. Treatment of ovariectomized rats with LY315471 demonstrated beneficial effects on bone mineral density and content as well as bone strength and lipid profile. In conclusion, increased ERα selectivity does not appear to be beneficial in terms of the pharmacological profile of a SERM and may actually be detrimental for the uterine agonism profile.

220.

DISCOVERY OF POTENT, SELECTIVE, NON-STEROIDAL MINERALOCORTICOID RECEPTOR ANTAGONISTS. *David A. Neel¹, Matthew L. Brown¹, Peter A. Lander¹, Timothy A. Grese¹, Jean M. Defauw², Robert A. Doti¹, Todd Fields², Stephon Smith², Sally Ann Wiest¹, Karen M. Zimmerman¹, Mitchell I. Steinberg¹, and Prabhakar K. Jadhav¹. (1) Discovery Chemistry Research, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, neel_david_a@lilly.com, (2) Research Triangle Park Laboratories, Eli Lilly and Company*

Elevated levels of aldosterone, an endogenous ligand of the mineralocorticoid receptor (MR), have been implicated in various cardiovascular disorders

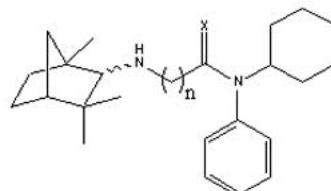
including high blood pressure, cardiac and perivascular fibrosis, and potentiation of catecholamines. Spironolactone and eplerenone are the only steroid based therapeutic agents available to antagonize the effects of aldosterone on MR. Chronic dosing with spironolactone results in undesirable side effects arising from its affinity to androgen and progesterone receptors. Eplerenone, while being more selective for human mineralocorticoid receptor (hMR), is less potent than spironolactone. Our objective was to identify novel non-steroidal ligands that act directly on hMR and thereby antagonize the adverse effects of excessive endogenous aldosterone. We have identified a novel series of potent oxindole analogs that are selective for hMR over related steroid nuclear hormone receptors such as GR, PR, AR, and ER. The synthesis and SAR of this series will be presented.

221.

LIVER X RECEPTOR (LXR) AGONISTS AS AGENTS TO UPREGULATE ABCA1 GENE EXPRESSION—A POTENTIAL THERAPY TO ELEVATE LOW HDL LEVELS.

Christopher F. Morrison¹, Robert H. Jiang¹, Prabha N. Ibrahim¹, Kevin D. Shenk¹, Kenneth S. Rehder², Sangeeta Dhamija³, Bindu Joshi³, Dewan Zeng³, Kwan Leung⁴, David Lustig⁴, and Jeff A. Zablocki¹. (1) Department of Bio-Organic Chemistry, CV Therapeutics, Inc, 3172 Porter Drive, Palo Alto, CA 94304, Fax: 650-858-0390, chris.morrison@cvt.com, (2) Chemistry, PPD Discovery, (3) Department of Pharmacological Sciences, CV Therapeutics, Inc, (4) Department of Pre-Clinical Development, CV Therapeutics, Inc

HDL levels are inversely correlated with the risk of coronary heart disease (CHD). In ABCA1 overexpressing transgenic mice, there is an increase in HDL. The nuclear receptor LXR regulates ABCA1 transcription. CVT-4681 (**1**), an LXR agonist, furnished a 5-fold induction (relative to DMSO control) of ABCA1 message in THP-1 cells ($EC_{50}=1$ uM). In this assay, the *1S,2S,4R* enantiomer (**1a**) of **1** was approximately 2-3 fold more potent than its *1R,2R,4S* counterpart (**1b**). Thioamide CVT-4928 (**2**) showed improved efficacy (6-7 fold increase in ABCA1 message) and pharmacokinetics relative to **1**. However, all of these molecules upregulated SREBP1-c in McCardle cells, a surrogate marker for lipogenesis. Analogs of **1** that illustrate the importance of the secondary amine, attempts at chain extension ($n>1$), and successful surrogates of the norbornyl amine will be presented.



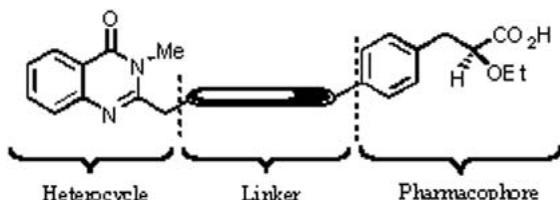
1 $X=O, n=1$
1a $X=O, n=1$
(*1S,2S,4R*)
1b $X=O, n=1$
(*1R,2R,4S*)
2 $X=S, n=1$
(6-7 fold
induction
of ABCA1)

222.

NEW 2-ETHOXY-3-(PHENYL)PROPANOIC ACID DERIVATIVES AS PPAR LIGANDS. *Debnath Bhuniya, Javed Iqbal, Ranjan Chakrabarti, Sankar Mohan, Sanju Narayanan, T. Ranjit Kumar, and Raichur Suryaprakash, Metabolic Disorder Group, Dr. Reddy's Laboratories Ltd.- Discovery Research, Miyapur, Bollaram Road, Hyderabad-500 049, India, Fax: +91-40-23045438, debnathbhuniya@drreddys.com*

Peroxisome proliferators-activated receptor (PPAR) is being recognized as versatile target for managing metabolic syndromes. Glitazones, as PPAR-γ agonist, and fibrates as PPAR-α agonists are in the market for treatment of insulin resistant type 2 diabetes and dyslipidemia respectively. In order to address diabetes and dyslipidemia with a single molecule, a PPAR-α/g dual acting compound has been conceptualized. We believe that a proper combination of α/g character may lead to a compound with such characteristics, without any significant PPAR-γ related side effect. Along that line we have been working on design, synthesis and biological evaluation of a series of 2-ethoxy-3-(phenyl)propanoic acids where phenyl ring is linked through various spacers to a heterocycle. Quinazolinone as a representative heterocycle a general structure **1** with different examples have been screened on cell based PPAR assay. Selected compounds have been tested on relevant mice and rat models for type 2 diabetes and for dyslipidemia finally to come up with a lead structure having

significant glucose and lipid lowering activity. Detailed synthesis and SAR will be presented in the form of poster.



223.

NOVEL DUAL PPAR α AND γ AGONISTS DERIVED FROM 2-ALKOXY-3-PHENYL PROPAANOIC ACID SERIES, WHICH AMELIORATES METABOLIC

ABNORMALITIES AND REDUCES BODY WEIGHT. *G. R. Madhavan, Ranjan Chakrabarti, Kalusam Anantha Reddy, B. M. Rajesh, K. V. L. Narasimha rao, P. Bheema rao, T. Ranjith kumar, and R. Rajagopalan, Metabolic Disorder Project Group, Dr. Reddy's Laboratories - Discovery Research, Bollaram Road; Miyapur, Hyderabad 500050, India, Fax: 91-40-3045438, MadhavanGR@drreddys.com*

Obesity is a disorder of fat accumulation and is associated with several risk factors known as metabolic syndrome. Present therapeutic approach to obesity is therefore also focused on overall management of metabolic syndrome.

Peroxisome proliferator activated receptor (PPAR) is a member of nuclear receptor super family. Two of its isoforms - PPAR α and PPAR γ are involved in the regulation of fat and carbohydrate metabolism and targets for hypolipidemic fibrates and antidiabetic thiazolidinediones. Considering the role of PPAR α in catabolism of fat, we have initiated a program to discover a dual PPAR α / γ -agonist with greater specificity towards PPAR α , so that it can be used for improving metabolic syndromes and body weight gain. We have investigated 1, 3-Benzoxazine-4(3H)-one derivatives of 2-alkoxy-3-phenyl propanoic acid derivatives. The SAR contains Cl, NO₂, di-isopropyl derivatives on aromatic ring of 1, 3-benzoxazinone and attachment of linker to 'N' or 'C' of the ring. Many compounds showed insulin sensitization and lipid lowering properties. DRF-2655 was selected as the lead molecule and resolved the racemic mixture in to its enantiomers (R and S). To our surprise both the enantiomers were having similar efficacy. Interestingly, DRF-2655 showed a significant body weight reduction in obese animal models along with good insulin sensitization and lipid lowering activity.

224.

DESIGN AND SYNTHESIS OF PPAR γ -INDEPENDENT SYNERGISTIC EFFECT OF TROGLITAZONE DERIVATIVE. *Chung-Wai Shiau, Kuen-Feng Chen, Chih-Cheng Yang, Chia-Yu Ku, and Ching-Shih Chen, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, shiau.4@osu.edu*

Celecoxib exhibits in vitro antiproliferative effects through both COX-2-dependent and γ V-independent mechanisms with moderate potency. Our evidence indicates that inhibition of PDK-1/Akt signaling represents a major COX-2-independent mechanism whereby celecoxib induces apoptosis and cell cycle arrest in cancer cells. We hypothesize that the antitumor effect of celecoxib can be optimized through mechanistic synergy with another therapeutic agent. Seven therapeutic agents were examined for their plausible synergy with celecoxib in inducing apoptosis in PC-3 prostate cancer and HT-29 colon cancer cells. Among them, only troglitazone acted synergistically with celecoxib in triggering apoptosis in PC-3 and HT-29 cells. Two lines of evidence show that the synergistic effect was independent of PPAR γ activation. Rosiglitazone and pioglitazone which have stronger binding affinity than troglitazone could not achieve the synergy effect with celecoxib. Moreover, we design and synthesize a troglitazone derivative, TG-8, that is devoid of PPAR γ ligand activity. TG-8 shows higher potency in inducing apoptosis than troglitazone. Therefore, TG-8 provides a proof of concept for the design of a new class of agents that optimally synergize with celecoxib in antitumor effects.

225.

DISCOVERY OF NOVEL AND EFFICACIOUS PAN PPAR AGONISTS THROUGH SCAFFOLD BASED DRUG DISCOVERY™ APPROACH. *Jack J. Lin¹, Upasana Mehra², Weiru Wang³, Heike I. Krupka³, Chao Zhang⁴, Adhirai Marimuthu⁵, Benjamin Powell⁶, Clarence R. Hurt¹, Prabha N. Ibrahim¹, Dean R. Artis⁴, and Micheal V. Milburn¹. (1) Department of Chemistry, Plexxikon Inc, 91 Bolivar Dr, Berkeley, CA 94710, Fax: 510-548-4785, jlin@plexxikon.com, (2) Department of Assay Development and Screening, Plexxikon Inc, (3) Department of Structural Biology, Plexxikon Inc, (4) Department of Informatics, Plexxikon Inc, (5) Department of Molecular Biology, Plexxikon Inc, (6) Department of Protein Chemistry, Plexxikon Inc*

PPARs (Peroxisome Proliferator-Activated Receptors- alpha, gamma, and delta), members of nuclear receptor super family, are regulators of lipid and glucose metabolism. Our approach to a pan PPAR agonist is to provide an effective therapy for treating insulin resistance (from PPAR γ) and homeostasis and catabolism of dietary lipids (through PPAR α and PPAR δ) associated with type II diabetes and metabolic syndrome. We identified novel PPAR pan agonists through our Scaffold Based Drug Discovery™ approach, within a period of six months. The lead compounds, PLX203 and PLX204, were tested in a fa/fa Zucker rat model for efficacy and the results are shown in Table 1. PLX203 was further evaluated in a db/db mice model demonstrating similar results. Currently, the lead compounds are being evaluated in a safety—efficacy study in a diet induced rat model. The presentation will include our structure-guided scaffold based drug discovery approach in improving the potency and selectivity of our lead compounds, and the results of efficacy studies. Table 1: Compound PLX203 PLX204 t1/2(h) 6.1 6.8 AUC(uMXh) 929 286 F 100% 100% Glucose* 57% inc 55% inc. Triglyceride* 63% dec. 55% dec. HDLc* 20% inc. 31% inc.

* inc.=increase; dec.=decrease

226.

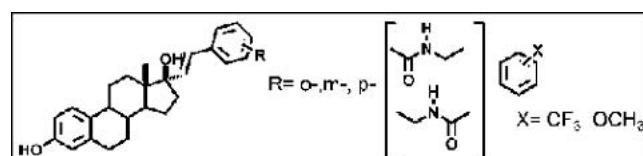
α -METHYLTRYPTAMINE SULFONAMIDE DERIVATIVES AS NOVEL GLUCOCORTICOID RECEPTOR LIGANDS. *Daniel R. Marshall, Gustavo Rodriguez, Richard Nelson, and David Thomson, Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals Inc, 900 Ridgebury Road, Ridgefield, CT 06877-0368, Fax: 203-791-6072*

α -Methyltryptamine sulfonamides were identified as glucocorticoid receptor ligands in a UHTS campaign. Described will be the Hit-to-lead activities, including parallel and single point analog synthesis to map the scaffold. Ligands were identified in the 30 nM range against GR and selectivity against other nuclear receptors will be discussed.

227.

NOVEL PROBES FOR THE ESTROGEN RECEPTOR-LIGAND BINDING DOMAIN (ER-LBD): SYNTHESIS AND EVALUATION OF 17 α -E-PHENYLVINYL ESTRADIOL AMIDES. *Emmett McCaskill¹, Richard B. Hochberg², and Robert N. Hanson¹. (1) Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-8795, r.hanson@neu.edu, r.hanson@neu.edu, (2) Department of Obstetrics and Gynecology, Yale University School of Medicine*

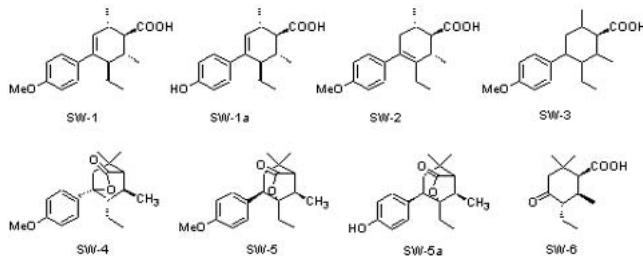
As part of our program to probe the ER-LBD we have prepared a family of ligands based on the 17 α -E-phenylvinyl estradiol scaffold. Previous studies demonstrated that the substituted phenylvinyl estradiols not only possessed significant receptor affinity but also were full estrogens in vivo. In this study we have prepared a new series of estradiol derivatives in which the phenyl substituent is further functionalized through an amide linkage to a second aromatic group. The new compounds, prepared in high yields via Stille coupling reactions were evaluated for their biological activity. This work has been supported in part by PHA R01 CA81049 and DAMD17-99-1-9333.



228.

SYNTHESSES AND RESOLUTION OF SMALL ESTROGENIC CARBOXYLIC ACIDS AND LACTONES FOR MCF-7 AND PC-3 CELL-LINE STUDIES. *Songwen Xie¹, Laura L. Murphy², Nancy Henry³, Yuqing Hou¹, and Cal Y. Meyers¹. (1) Meyers Institute for Interdisciplinary Research in Organic and Medicinal Chemistry and Department of Chemistry and Biochemistry, Southern Illinois University Carbondale, Mail code 4409, Carbondale, IL 62901, Fax: 618-453-6408, xiesw@siu.edu, (2) Department of Physiology, Southern Illinois University Carbondale, (3) Department of Animal Science, Food and Nutrition, Southern Illinois University Carbondale*

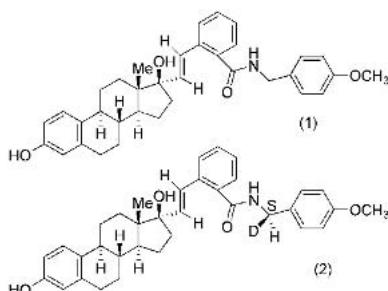
The (+) and (-) enantiomers, respectively, of cis-bisdehydrodiosynolic acid (cis-BDDA) have specific physiological effects in males and females, and we have found them to show good prospects in treating breast and prostate cancer. Some of the related carboxylic acids studied years ago by Crenshaw et al. were reported to be highly estrogenic in vivo. However, they studied the activities of mixtures of isomers and, moreover, did not undertake either binding-affinity or cell-line studies. We have now synthesized, isolated, and unequivocally characterized by X-ray analysis some of these racemic carboxylic acids and corresponding lactones, some of which displayed good MCF-7 cell-line activity, but poor estrogen-receptor binding activity. Their anti-prostate-cancer activity was established by PC-3 cell-line studies. The activities of the respective (+) and (-) enantiomers, now being isolated from the racemates by chemical as well as chiral-HPLC resolution, will be studied.



229.

SYNTHESIS AND CONFORMATIONAL STUDIES OF ENANTIOTOPIC HYDROGENS OF 17ALPHA-E-(N-(4-METHOXYBENZYL)-2-BENZAMIDE) VINYL ESTRADIOL. *Edward Y. Hua, David A. Forsyth, and Robert N. Hanson, Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, Fax: 617-373-8795, hua.e@neu.edu*

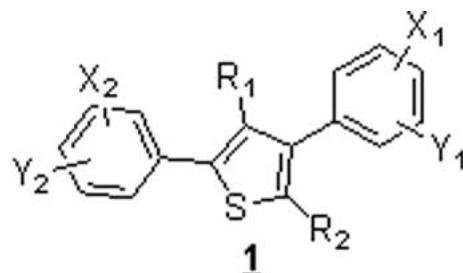
Substituted o, m, p-benzamide N-2, 3, 4-methoxybenzyl vinyl estradiol were synthesized as novel estrogen receptor probes. Characterization of the 17alpha-E-(N-(4-methoxybenzyl)-2-benzamide) vinyl estradiol (1) indicated enantiotopism. Therefore, 17alpha-E-(2-benzylamide, (1S) [1-D]-N-(4-methoxybenzyl)-2-benzamide) vinyl estradiol (2) was prepared. The conformations of the novel estrogen receptor ligands were investigated using 1D and 2D NMR spectroscopy by comparison of the 1H NMR chemical shifts and coupling constants in different solvents at various temperatures. The 13C NMR chemical shifts were also compared with calculated values. The predicted low-energy conformers of (1) and (2) were generated using MM2 force field via conformational searching. This research was supported in part by PHS R01 CA81049, DAMD 17-99-1-9333 and DAMA 17-00-1-0384.



230.

TETRA-SUBSTITUTED THIOPHENES AS SELECTIVE ESTROGEN RECEPTOR-BETA (ER- β) AGONISTS. *William F. Fobare¹, Richard D. Coghlan¹, Eugene J. Trybulski¹, Heather A Harris², and Eric S. Manas³. (1) Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08653-8000, Fax: 732-274-4505, fobarew@wyeth.com, (2) Women's Health Research Institute, Wyeth Research, (3) Department of Chemical and Screening Sciences, Wyeth Research*

In 1996 a second subtype of the estrogen receptor was identified, its tissue distribution suggested it was an attractive drug target. One approach to determining ER- β s function was to design selective ligands. As part of a program to identify potent and selective ER- β agonists, a series of tetra-substituted thiophenes 1 was identified. These tetra-substituted thiophenes were synthesized utilizing a regiospecific lithium halogen exchange and two regiospecific Suzuki couplings. Similarities between the binding pocket of ER- α and ER- β forced the differentiation of the two phenyl groups in respect to the thiophene substitution and the search for an agonist versus antagonist mandated the size constraints of the heterocyclic substituents. These manipulations resulted in molecules with no selectivity for ER- β to 30-40-fold. The SAR of these thiophenes and x-ray structures of these agonists with ER- β will be presented.



231.

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF BIPHENYL-CARBALDEHYDE OXIME DERIVATIVES. *Cuijian Yang¹, Richard E. Edsall Jr.¹, Heather A Harris², Xiaochun Zhang², Eric S. Manas¹, and Richard E. Mewshaw¹. (1) Department of Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, yangj1@wyeth.com, (2) Women's Health Research Institute, Wyeth Research*

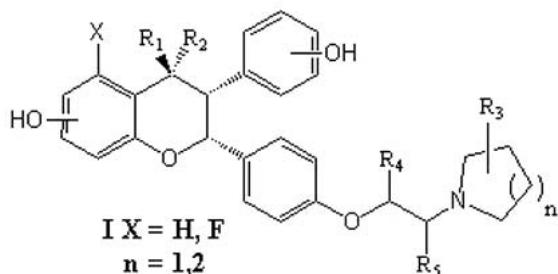
The identification of ER β selective ligands is essential in elucidating ER β function. Towards this end, we sought to design selective ligands having some of the common structural features and pharmacophoric requirements of genistein. A series of biphenyl carbaldehyde oximes was prepared and shown to have significant affinity and selectivity for ER β . The SAR and proposed binding mode within the ligand binding domain, as well as the role of the oxime moiety will be presented. Several analogs were observed to have similar selectivity to that of genistein, when the appropriate substituents were attached to the biphenyl carbaldehyde oxime template.

232.

CHROMANES: OLD PLATFORMS FOR NEW SERMS. *Qiang Tan, Timothy A.*

Blizzard, Jerry Morgan, Sharon Adamski, Elizabeth T. Birzin, Wanda Chan, Lee-Yuh Pai, Donald Kimmel, Frank DiNinno, Susan P. Rohrer, James M. Schaeffer, and Milton L. Hammond, Merck Research Laboratory, Merck & Co., Inc, RY800-B107, Merck & Co., Inc, Rahway, NJ 07065, Fax: 732-594-9556, qiang_tan@merck.com

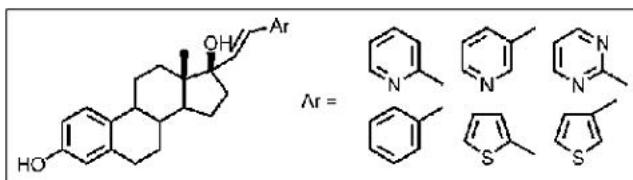
Chromanes I have been identified as Selective Estrogen Receptor Modulators (SERMs) with the potential for the treatment and prevention of osteoporosis. These compounds exhibit ER-alpha selectivity when R1=Me and R2=H, and thus have been classified as SERMs (Selective Estrogen Receptor Alpha Modulators). The stereo-controlled synthesis and biological properties of the class will be presented.



233.

SYNTHESIS OF ERBETA SELECTIVE 17-SUBSTITUTED ESTRADIOLS FOR THE TREATMENT OF PROSTATE CANCER. *Pakamas Tongcharoensirkul¹, Shuk-mei Ho², and Robert N. Hanson¹. (1) Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, Fax: 617-373-8795, ptongcha@lynx.neu.edu, (2) Department of Urology, University of Massachusetts Medical School*

Recent evidence suggests that Estrogen Receptor beta (ERbeta) is down regulated during the precancerous prostate intraepithelium neoplasia and reappears again during the metastatic prostate cancer. We have synthesized novel ERbeta agonists based on the lead structure 17beta-estradiol, the endogenous ligand for both ERalpha and ERbeta. The modification of the lead structure is based on the biological data and molecular docking studies. The biological data suggested that 17alpha-phenylvinyl estradiols were active against the prostate cancer DU-145 cell line. Molecular docking demonstrates that estradiol moiety fits in the pocket of ligand binding site while the phenyl moiety is adjacent to isoleucine 373 in ERbeta or methionine 421 in ERalpha. Therefore, the selectivity toward ERbeta could be modified by varying the aromatic moieties. The synthesis of the first generation and the biological data will be presented. The research is supported in part by DAMD17-03-1-0116 and PHS R01 CA81049.



234.

DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF AZOLE DERIVATIVES AS A SELECTIVE INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR. *Shigeo Ueda¹, Akihiro Yano², Hideo Terauchi³, Yoko Kyoya¹, Masashi Matsumoto², Motoharu Ido², Motoji Kawasaki¹, and Iwao Fujiwara¹. (1) Chemistry Research Laboratories, Dainippon Pharmaceutical Co. Ltd, Enoki-cho 33-94, Suita, Osaka 564-0053, Japan, Fax: +81-6-6338-7656, shigeo-ueda@dainippon-pharm.co.jp, (2) Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd, (3) Pharmaceutical Research & Technology Center, Dainippon Pharmaceutical Co., Ltd*

Inducible nitric oxide synthase (iNOS) is implicated in excessive production of nitric oxide (NO) which causes inflammatory diseases such as septic shock, rheumatoid arthritis and colitis, whereas NO is thought to serve in host defense mechanism. In our search for a novel class of selective iNOS inhibitor, a series of 2-amino-azole derivatives were found to be promising scaffolds as a potent and selective iNOS inhibitor. Extensive investigation of this class led to the discovery of (4R,5R)-2-amino-5-ethyl-4-methyl-1,3-dihydrothiazole (ES-1537), which exhibits remarkable inhibitory activity and selectivity against iNOS (IC₅₀ value of 6.6 nM) and excellent oral bioavailability. We will present the structure-activity relationships of 2-amino-azole derivatives and the in vivo effect of ES-1537.

235.

IONIC TRIGGERING FOR NITRIC OXIDE DELIVERY. *Wilmarie Flores-Santana, and Daniel Smith, Department of Chemistry, University of Akron, 190 East Buchtel Commons, Akron, OH 44325, wf1@uakron.edu*

Diazeniumdiolates or NONOates are one class of nitric oxide (NO) donors that have been used to treat important clinical problems such as cancer, cardiovascular diseases, and impotence, among others. Currently, the design of new diazeniumdiolates has been focused on natural nontoxic metabolites derivates such as polyamines, which can be metabolized by the body once the NO has been released. Polyamine-based diazeniumdiolates can be used in topical formulations to treat diseases related to NO deficiency, like diabetic peripheral neuropathy and some types of sexual dysfunction. The primary limitation of this approach is the instability of these compounds in aqueous solutions, as a result of their NO release being pH dependant. At higher pH values, the NONOates are stable in aqueous solutions. This pH restriction renders them useless in water-based creams or ointments. In order to stabilize the NONOate in aqueous media, the anionic group of the NONOate has been bound to a cationic ion exchange resin, thus resulting in a bound NONOate that is stable in water. This approach only requires an ion exchange process to activate the release of NO, which follows normal release kinetics. This new approach presents a novel, viable way of stabilizing these types of NO releasing drugs, making them suitable for several biomedical applications.

236.

NITRIC OXIDE REACTION NETWORKS IN THE VASCULATURE. *Tae H. Han¹, Daniel R. Hyduke¹, Jon M. Fukuto², James C. Liao³, and Mark W. Vaughn⁴. (1) Department of Chemical Engineering, University of California, 5531 Boelter Hall, 420 Westwood Plaza, Los Angeles, CA 90024, Fax: 310-206-4107, taehhhan@ucla.edu, (2) Dept. of Pharmacology/School of Medicine, UCLA, (3) Chemical Engineering, University of California, (4) Texas Tech University*

Nitric oxide (NO) is a reactive free radical that is involved in a myriad of interactions in the vasculature. As alterations in NO homeostasis is linked to a variety of disease states, elucidating the interactions that shift NO bioavailability is of high importance. Distinguishing all the viable reaction pathways is a primary challenge that arises with the vast number of interactions. We have constructed the NO reaction network for the vasculature to systematically determine and assess these viable pathways. From this network of interactions, we were able to extract new and interesting pathways that have not been previously considered. Using this method, we discovered a variety of novel pathways for the formation of iron-nitrosylhemoglobin (HbNO) and S-nitrosated hemoglobin (SNO-Hb) from the oxyhemoglobin reaction of NO. Using a combination of electronic absorbance spectroscopy, electron paramagnetic spectroscopy, and chemiluminescence, we experimentally assessed each pathway to determine which were dominant. We determined the involvement of reductive nitrosylation for both HbNO and SNO-Hb formation.

237.

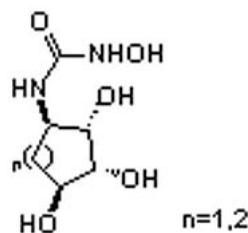
POTENTIAL PRODRUGS OF NITRIC OXIDE-RELEASING PROLI/NO. *Brett M. Showalter¹, Joseph E. Saavedra², Michael L. Citro², and Larry K. Keefer¹. (1) Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, PO Box B, Frederick, MD 21702, Fax: 301-846-5946, bshowalter@nccifcrf.gov, (2) BRP, SAIC-Frederick, Inc., National Cancer Institute at Frederick*

O²-Substituted diazeniumdiolates [compounds of structure R₂NN(O)=NOR'] are nitric oxide(NO)-releasing prodrugs that have proven effective in relevant experimental models as treatments for several different clinical disorders, including cancer. PROLI/NO [R₂N=L-proline, R'=Na] is an ionic diazeniumdiolate with many advantages (2-s half-life, favorable toxicological profile, presence of a carboxylic acid moiety which allows for further derivatization) as a caged NO prodrug, but it has proven unusually sensitive to decomposition on attempted O²-substitution. We are currently seeking means of circumventing this limitation. This presentation will compare synthetic routes we have examined and introduce a series of new drug candidates based on the PROLI/NO structure. (NO1-CO-12400)

238.

WATER-SOLUBLE N-HYDROXYUREAS AS NEW NITRIC OXIDE DONORS. Zhou Zou, Dennis A. Parrish, and S. Bruce King, Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, Fax: 336-758-4656

Hydroxyurea is a new approved treatment for sickle cell disease. Oxidation of N-hydroxyurea produces nitric oxide(NO), an important biological messenger molecule. Recent experiments also indicate that hydroxyurea acts as an NO donor *in vivo* and suggest that the biological effects of N-hydroxyurea may be mediated by NO. Our previous results indicate that heme containing proteins and enzymes oxidize N-hydroxyurea with NO release. Based upon these introductory results, we began to prepare amino-hydroxycyclohexanes and cyclopentanes as water-soluble N-hydroxyureas as potential NO donors. These compounds are prepared by cycloadditions of acyl nitroso species and cyclohexadiene or cyclopentadiene to give products that yield protected amino alcohols upon reduction. The amino group is converted to an N-hydroxyurea by condensation with p-nitrophenyl O-benzyl carbamate followed by hydrogenation. The results regarding the ability of these compounds to release NO by oxidation with chemical agents and hemoglobin will be presented.



239.

ALPHA-SUBSTITUTED N-(4-T-BUTYLBENZYL)-N'-[4-(METHYLSULFONYLAMINO)BENZYL]THIOUREA ANALOGUES AS VANILLOID RECEPTOR ANTAGONISTS. Mi-Kyung Jin¹, Hae-Seok Yoon¹, Sang-Uk Kang¹, Jeewoo Lee¹, Hee-Jin Ha², Young-Ho Kim², and Peter M. Blumberg³. (1) Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea, Fax: 82-2-888-0649, jeewoo@snu.ac.kr, (2) Digital Biotech, (3) Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health

The vanilloid receptor VR1 is a cation permeable ion channel present on polymodal nociceptors that is activated by protons, heat, and ligands such as capsaicin and resiniferatoxin (RTX). Either the receptor desensitization by VR1 agonists or the blocking of pain-producing endogenous agonists by VR1 antagonists forms a basis for the therapeutic use of vanilloid ligands. Recently, a series of N-(4-t-butylbenzyl)-N'-[4-(methylsulfonylamino)benzyl]thioureas have been reported as high affinity antagonists of the vanilloid receptor 1 (VR1) and exhibited potent analgesic profiles devoid of hypothermia and pungency observed in capsaicin. This presentation will discuss the syntheses and biological activities of a series of alpha substituted N-(4-t-butylbenzyl)-N'-[4-(methylsulfonylamino)benzyl]thiourea analogues as VR1 antagonists.

240.

CHAIN-BRANCHED ACYCLIC THIOUREAS AS VANILLOID RECEPTOR

ANTAGONISTS. Hee-Doo Kim¹, Mijung Jang¹, Joohyun Kim¹, Chonghyun Ryu¹, Young-Ho Park², and Young Hoon Jung³. (1) School of Pharmacy, Sookmyung Women's University, Chungpa-dong, Yongsan-ku, Seoul 140-742, South Korea, Fax: 2-703-0736, hdkim@sookmyung.ac.kr, (2) AmorePacific R & D Center, AmorePacific, (3) College of Pharmacy, Sungkyunkwan University

Due to the unique biological activity, vanilloid receptor (VR1) is at present one of the most attractive targets for the treatment of pain. Despite the concentrated effort on VR1 agonists, they have been exposed to the side effects such as pungency and/or hypothermia responses. Thus, development of VR1 antagonist has been a main focus in this research area. In contrast to the agonist's coplanar conformation, the antagonists are known to have an orthogonal conformation between the vanilloid aromatic ring and the amide/thiourea bond. Most of acyclic vanilloid compounds known to date are act as receptor agonists. It is anticipated that chain branching may destabilize the agonist binding mode

(conformation) of acyclic vanilloid compounds for steric reason, thereby leading to more favorable conformation for antagonist binding mode. Our basic strategy is to seek the chain-branched acyclic antagonists deviated from coplanar conformation with minimal structural disturbance from acyclic agonist such as SDZ-249482. We will present here our approach for antagonism by chain-branching method.

241.

NON-VANILLOID RESINIFERATOXIN ANALOGUES AS VANILLOID RECEPTOR 1 LIGANDS. Hyun-Kyung Choi¹, Sukhyun Cho¹, Jeewoo Lee¹, and Peter M. Blumberg². (1) Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea, Fax: 82-2-888-0649, jeewoo@snu.ac.kr, (2) Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health

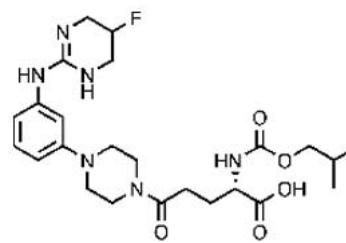
Resiniferatoxin (RTX), a tricyclic diterpene isolated from Euphorbia resinifera, is an extremely potent irritant tricyclic diterpene which is structurally related to phorbol-related diterpenes except for its homovanillyl ester group at C-20. RTX functions pharmacologically as an ultrapotent vanilloid receptor agonist with displaying 103- to 104-fold greater potency than the prototype capsaicin and is being developed as potent sensory neuron desensitizing agent for the treatment of urinary urge incontinence and the pain associated with diabetic neuropathy. Structure-activity studies on RTX analogues indicated that their binding affinities and functional activities were quite sensitive to small modification. In order to reduce high irritancy of RTX as keeping its high binding ability, we have investigated non-vanilloid RTX analogues in which 4-hydroxy-3-methoxyphenyl group, so-called A-region, was substituted with non-phenolic phenyl groups. The SAR result will be presented.

242.

BS-1417: A POTENT $\alpha_1\beta_3$ INTEGRIN ANTAGONIST WITH STRONG INHIBITORY ACTIVITY AGAINST NEOINTIMA FORMATION IN RAT BALLOON INJURY MODEL.

MODEL. Seiji Iwama¹, Tomoko Kitano², Fumiyo Fukuya², Yayoi Honda², Yuji Sato², Mitsue Notake², and Toshiya Morie¹. (1) Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd, Enoki-cho 33-94, Suita, Osaka 564-0053, Japan, Fax: +81-6-6338-7656, seiji-iwama@dainippon-pharm.co.jp, (2) Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co., Ltd

Percutaneous coronary interventions (PCIs) have been playing an increasingly important role in the management of patients with coronary artery diseases. However, long-term success of PCIs remains limited due to restenosis which occurs within six months in 20-50% of patients who undergo PCIs. Although, numerous pharmacological agents have been evaluated in an attempt to reduce the rate of restenosis, few of these agents have been shown to inhibit restenosis. Recently, we found phenyl-piperazine moiety as a constrained scaffold for $\alpha_1\beta_3$ integrin antagonists. Extensive structure-activity relationship (SAR) studies on compounds containing this moiety led to the discovery of BS-1417 which has strong inhibitory activity and high selectivity for $\alpha_1\beta_3$ integrin receptor. BS-1417 showed strong inhibition of neointima formation in rat balloon injury model. Synthesis, SARs and *in vivo* evaluation of this series of compounds will be presented.



BS-1417

243.

RELEVANCE OF PLASMA PROTEIN BINDING TO THE EFFICACY OF INHALED α -4 INTEGRIN ANTAGONISTS. *Stuart Holman¹, Peter Ward¹, Jill Coates², Jane Denyer², Rita Field¹, David Gray³, Phillip Green³, Neil Miller⁴, and Elizabeth Pickup⁴. (1) Medicinal Chemistry, GlaxoSmithKline R & D, Medicines Research Centre, Gunnels Wood Road, Stevenage SG4 9QH, United Kingdom, Stuart.X.Holman@gsk.com, (2) Respiratory Biology, GlaxoSmithKline R & D, (3) Integrin Systems Research, GlaxoSmithKline R & D, (4) Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline R & D*

The potent α -4 integrin inhibitor 559090 has previously been reported as a potential inhaled treatment for asthma and rhinitis. 559090 is greater than 99% bound to plasma proteins and it was of interest to determine the relevance of protein binding to activity in vivo for an inhaled drug. We have shown that the introduction of polar substituents into the 559090 template can result in a dramatic reduction in plasma protein binding without compromising α -4 affinity. Evaluation of compounds after dosing to the lung in the Brown Norway rat indicates that reduced PPB is associated with a significant increase in potency. These results suggest that protein binding plays a role in the efficacy of inhaled α -4 antagonists in vivo.

244.

COMPARISON OF N-TERMINAL α -AMINE MODIFICATIONS AS STRATEGIES FOR PEPTIDE DRUG DEVELOPMENT. *Kevin S. Orwig, and Thomas A. Dix, Department of Pharmaceutical Sciences, Medical University of South Carolina, 280 Calhoun Street, PO Box 250140, Charleston, SC 29425, Fax: 843-792-0759, orwigs@musc.edu*

Derivatives of NT(8-13), the active fragment of the endogenous peptide neurotensin (NT), are under development in our laboratory as potential antipsychotic agents. We have produced several generations of NT(8-13) analogues that exhibit CNS activity when administered both peripherally and orally. A common structural modification in many of these compounds is the substitution of an azido group for the N-terminal α -amine. This substitution increases peptide lipophilicity and has been shown to increase peptide serum stability. However, no direct comparison exists between the azido group and other common N-terminal modifications. To address this issue, we synthesized peptide series based on NT(8-13) and two analogues, KK13 and NT1 (the Eisai hexapeptide). Peptides in each series featured either an amino, azido, N- α -acetyl, or N- α -methyl functional group at the N-terminal α -amine. The peptides were tested in vitro for stability in human serum and in vivo for ability to partition into the CNS.

245.

DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL "SHURIKEN DERIVATIVES" AS NON-COMPETITIVE AMPA ANTAGONISTS (1). *Koki Kawano¹, Satoshi Nagato¹, Kohshi Ueno¹, Yoshihiko Norimine¹, Kouichi Itoh¹, Takahisa Hanada¹, Masataka Ueno¹, Shinji Hatakeyama¹, Makoto Ohgoh¹, Hiroyuki Amino¹, Toshihiko Yamauchi¹, Naoki Tokuhara¹, Terence Smith², Anthony Groom², Leanne Rivers², Yukio Nishizawa², and Masahiro Yonaga¹. (1) Tsukuba Research Laboratories, Eisai Co., Ltd, Tokodai 5-1-3, Tsukuba-shi, Ibaraki 300-2635, Japan, Fax: +81-298-47-2037, k-kawano@hhc.eisai.co.jp, (2) Eisai London Research Laboratories .Ltd*

Glutamate plays a significant role in excitatory neurotransmission in the central nervous system. Recently, a large number of published reports have described how that within the ionotropic class of glutamate antagonists, alpha-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid (AMPA) receptor antagonists demonstrate an ameliorative effect in the animal models of various neurodegenerative and demyelinating diseases. A series of pyridazinone derivatives were synthesized and examined for their ability to inhibit excitatory neurotransmission via the AMPA receptor. These compounds, especially 2,4,6-tri-aryl pyridazinone derivatives, showed a potent non-competitive inhibitory activity against AMPA in vitro (calcium influx in rat cortical neurons), and in vivo (mice seizure model, p.o. administration). The synthesis and structure-activity relationships of this series of compounds will be presented and discussed.

246.

DESIGN, SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF NOVEL "SHURIKEN DERIVATIVES" AS NON-COMPETITIVE AMPA ANTAGONISTS (2). *Satoshi Nagato¹, Koki Kawano¹, Kohshi Ueno¹, Yoshihiko Norimine¹, Kouichi Itoh¹, Takahisa Hanada¹, Masataka Ueno¹, Shinji Hatakeyama¹, Makoto Ohgoh¹, Hiroyuki Amino¹, Toshihiko Yamauchi¹, Naoki Tokuhara¹, Terence Smith², Anthony Groom², Leanne Rivers², Yukio Nishizawa², and Masahiro Yonaga¹. (1) Tsukuba Research Laboratories, Eisai Co., Ltd, Tokodai 5-1-3, Tsukuba-shi, Ibaraki 300-2635, Japan, Fax: +81-29-847-5306, (2) Eisai London Research Laboratories .Ltd*

Glutamate plays a significant role in excitatory neurotransmission in the central nervous system. Glutamate antagonists, and especially AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid) antagonists are expected to be one solution to various neurodegenerative and demyelinating diseases. A series of pyridone derivatives were designed and examined for their ability to inhibit excitatory neurotransmission via the AMPA receptor. We found that 1,3,5-tri-aryl pyridone derivatives have a potent non-competitive inhibitory activity against AMPA (in vitro: AMPA-induced calcium influx in rat cortical neurons, in vivo: mice AMPA-induced seizure model). The synthesis and structure-activity relationships of this series of compounds will be disclosed in this presentation.

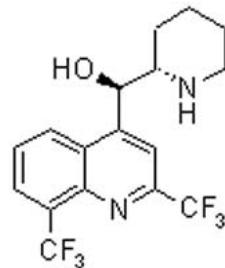
247.

EVALUATION OF ANALOGUES OF (-)-MEFLOQUINE AS ADENOSINE A_{2A} RECEPTOR ANTAGONISTS. *Roger J. Gillespie, Paul R. Giles, Joanne Lerpiniere, Simon E. Ward, Scott M. Weiss, Tony R. Knight, Anil Misra, Karen Benwell, Colin T. Dourish, and Ian A. Cliffe, Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Winnersh RG41 5UA, United Kingdom, Fax: +44(0)-118-989-9300, r.gillespie@vernalis.com*

Evaluation of analogues of (-)-mefloquine as adenosine A_{2A} receptor antagonists.

The adenosine A_{2A} receptor plays an important role in regulating smooth and well-coordinated movement, in part, by modulating the activity of dopamine sensitive neurones in the striatum. Blockade of the adenosine A_{2A} receptor has been shown to offer considerable promise as a novel treatment for the symptoms of Parkinson's disease. We have discovered that (-)-(1R,2'S)- α -2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol, the (-)-enantiomer of the antimalarial drug mefloquine, is a potent and moderately selective adenosine A_{2A} receptor antagonist. This compound has a K_i of 61 nM at human adenosine A_{2A} receptors, is moderately selective over human adenosine A₁ receptors (K_i 255 nM), and highly selective over human adenosine A_{2B} and A₃ receptors (K_i 7072 and 6941 nM, respectively). The synthesis and evaluation of a series of analogues of mefloquine as adenosine A_{2A} antagonists will be described.

(-)-mefloquine



248.

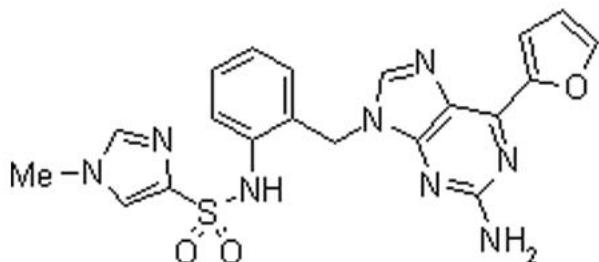
NOVEL PURINE DERIVATIVES AS SELECTIVE ADENOSINE A_{2A} RECEPTOR ANTAGONISTS FOR THE TREATMENT OF PARKINSON'S DISEASE. *Samantha J. Bamford, Joanne Lerpiniere, Gemma C. Stratton, Claire E. Dawson, Robert M. Pratt, Suneel Gaur, Scott M. Weiss, Tony R. Knight, Anil Misra, Julie Jones, Karen Benwell, Rebecca Upton, Colin T. Dourish, Ian A. Cliffe, and Roger J. Gillespie, Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Winnersh RG41 5UA, United Kingdom, Fax: +44(0)-118-989-9300, s.bamford@vernalis.com*

Novel purine derivatives as selective adenosine A_{2A} receptor antagonists for the treatment of Parkinson's disease

In the brain, adenosine A_{2A} receptors are located primarily in the striatum

where they are co-localised with dopamine D₂ receptors and play a role in regulating movement. There is now accumulating evidence that adenosine A_{2A} receptor antagonists may provide a novel therapy for the treatment of Parkinson's disease with a lower risk of dyskinesias. As part of our ongoing efforts to discover new antiparkinsonian drugs we have synthesised and evaluated a series of novel purine derivatives. Many of these compounds are potent and selective adenosine A_{2A} receptor antagonists and a number are active in animal models of Parkinson's disease. VER-8177 has a K_i of 4.0 nM at human adenosine A_{2A} receptors and is highly selective over human A₁, A_{2B}, and A₃ receptors (K_i 2268, 3036, and 4264 nM, respectively). The synthesis, biological evaluation and SAR of this series will be described.

VER-8177



249.

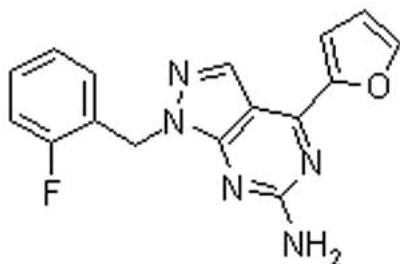
SYNTHESIS AND EVALUATION OF NOVEL PYRAZOLO[3,4-D]PYRIMIDINE DERIVATIVES AS SELECTIVE ADENOSINE A_{2A} RECEPTOR ANTAGONISTS.

Gemma C. Stratton, Joanne Lerpiniere, Suneel Gaur, Scott M. Weiss, Tony R. Knight, Anil Misra, Julie Jones, Anthony Lawrence, Karen Benwell, Rebecca Upton, Colin T. Dourish, Ian A. Cliffe, and Roger J. Gillespie, Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Winnersh RG41 5UA, United Kingdom, Fax: +44(0)-118-989-9300, g.stratton@vernalis.com

Synthesis and evaluation of novel pyrazolo[3,4-d]pyrimidine derivatives as selective adenosine A_{2A} receptor antagonists.

Four sub-types of adenosine receptors, designated A₁, A_{2A}, A_{2B} and A₃, have been cloned and characterised pharmacologically. The development of selective adenosine A_{2A} receptor antagonists is of interest as a potential new avenue towards novel therapies for Parkinson's disease. As part of our ongoing efforts to discover new antiparkinsonian drugs we have synthesised and evaluated a number of novel pyrazolo[3,4-d]pyrimidine derivatives. Many of these compounds are potent and selective adenosine A_{2A} receptor antagonists and a number are active in animal models of Parkinson's disease. VER-7866 has a K_i of 3.2 nM at human adenosine A_{2A} receptors and is selective over human A₁, A_{2B}, and A₃ receptors (K_i 251, 390, and 3260 nM, respectively). The synthesis and structure-activity relationships for these compounds will be presented.

VER-7866



250.

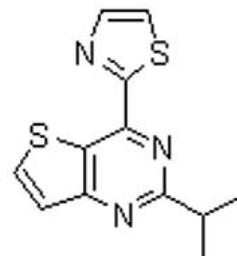
SYNTHESIS AND SAR OF NOVEL THIENO[3,2-D]PYRIMIDINE DERIVATIVES AS SELECTIVE ANTAGONISTS OF THE ADENOSINE A_{2A} RECEPTOR. Richard S.

Todd, Joanne Lerpiniere, Robert M. Pratt, Paul R. Giles, Claire E. Dawson, Suneel Gaur, Scott M. Weiss, Tony R. Knight, Anil Misra, Anthony Lawrence, Karen Benwell, Rebecca Upton, Colin T. Dourish, Ian A. Cliffe, and Roger J. Gillespie, Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Winnersh RG41 5UA, United Kingdom, Fax: +44(0)-118-989-9300, r.todd@vernalis.com

Synthesis and SAR of novel thieno[3,2-d]pyrimidine derivatives as selective antagonists of the adenosine A_{2A} receptor.

Adenosine receptors are a class of GPCRs comprising four distinct sub-types, designated A₁, A_{2A}, A_{2B} and A₃. There is compelling evidence that selective adenosine A_{2A} receptor antagonists may provide a novel treatment for Parkinson's disease with a lower risk of dyskinesias. As part of our ongoing efforts to discover new antiparkinsonian drugs we have synthesised and evaluated a series of novel thieno[3,2-d]pyrimidine derivatives. Many of these compounds are potent and selective adenosine A_{2A} receptor antagonists and a number are active in animal models of Parkinson's disease. VER-6623 has a K_i of 1.4 nM at human adenosine A_{2A} receptors and is selective over human A₁, A_{2B}, and A₃ receptors (K_i 273, 821, and 508 nM respectively). The synthesis and adenosine receptor pharmacology of this series will be described.

VER-6623

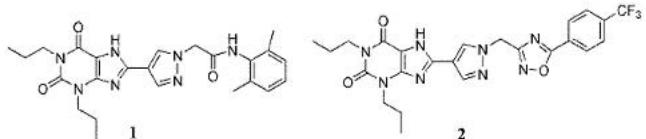


251.

NEW 1,3-DIPROPYL-8-(1-HETEROARYLMETHYL-1H-PYRAZOL-4-YL)-XANTHINE DERIVATIVES AS POTENT AND SELECTIVE A_{2B} ADENOSINE RECEPTOR

ANTAGONISTS. Elfatih Elzein¹, Xiaofen Li¹, Rao Kalla¹, Thao Perry¹, Venkata Palle¹, Vaibhav Varkhedkar¹, Tenning Maa², Marie Nguyen², Yuzhi Wu², Victoria Maydanik³, David Lustig³, Kwan Leung³, Dewan Zeng², and Jeff Zablocki¹. (1) Bioorganic Chemistry, CV Therapeutics Inc, 3172 Porter Drive, Palo Alto, CA 94304, elfatih.elzein@cvt.com, (2) Drug Research and Pharmacological Sciences, CV Therapeutics Inc, (3) Pre-clinical Development, CV Therapeutics Inc

Studies have shown that A_{2B} adenosine receptors (A_{2B}-AdoR) present in airway mast cells mediate the bronchoconstriction response to adenosine (unique to the airways of asthmatics) and also facilitate mast cell degranulation in response to allergen. Accordingly, A_{2B}-AdoR antagonists have been suggested to be useful as a potential treatment for asthma. In our search for new potent and selective A_{2B}-AdoR antagonists, we have identified compound **1** as our lead. To further enhance the A_{2B}-AdoR binding affinity, selectivity and metabolic stability of **1**, we have replaced the metabolically labile amide bond with different oxadiazoles, oxazoles and isoxazoles as amide bioisosteres. Compound **2**, that contains a 1,2,4-oxadiazole as an amide mimetic, showed excellent binding affinity for the A_{2B}-AdoR (K_i=21 nM) as well as high selectivity ratios versus A₁, A_{2A}- and A₃-AdoRs (285, 238 and 61, respectively). The synthesis of these new analogues and their SAR will be presented.



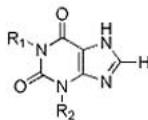
252.

NOVEL 1,3-DIPROPYL-8-(4-(PHENYLOXADIAZOLYL)METHOXY)PHENYL XANTHINES : A2B ADENOSINE RECEPTOR ANTAGONISTS WITH HIGH

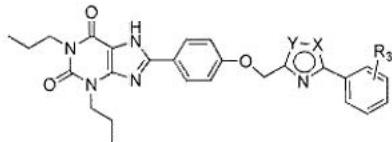
AFFINITY AND SELECTIVITY. **Jeff Zablocki**¹, Rao Kalla¹, Thao Perry¹, Venkata Palle¹, Vaibhav Varkhedkar¹, Dengming Xiao², Anthony D. Piscopio², Yuzhi Wu³, Victoria Maydanik⁴, Tenning Maa³, Marie Nguyen³, David Lustig⁴, Kwan Leung⁴, and Dewan Zeng³. (1) Bioorganic Chemistry, CV Therapeutics Inc, 3172 Porter Drive, Palo Alto, CA 94304, jeff.zablocki@cvt.com, (2) Department of Process, Array BioPharma Inc, (3) Drug Research and Pharmacological Sciences, CV Therapeutics Inc, (4) Department of Pre-Clinical Development, CV Therapeutics

Theophylline and enprofylline are PDE and non-selective adenosine receptor (AdoR) antagonists that are currently used in the treatment of asthma. It has been proposed that the therapeutic effect of these agents is due to inhibition of

the A_{2B} AdoR. We explored two series of 8-phenylxanthine derivatives that contain a 3- or 5-phenyloxadiazole ring system as illustrated by **2** and **3**, respectively, to discover selective A_{2B} AdoR antagonists (**3** A_{2B} Ki=50 nM). In the 3-phenyloxadiazole series we explored a range of phenyl substituents with 4- and 3- electron withdrawing groups providing favorable affinity. The 4-F-phenyl ring of **2** can be replaced by cyclopentyl (A_{2B} AdoR Ki=246 nM), but not cyclohexyl. In the SAR of the 5-phenyloxadiazole series, a methoxy substituent provided favorable affinity (3-OMe > 2-OMe > 4-OMe) with **3** having the best selectivity (selectivity ratio >150). Additional SAR and PK properties of selected members will be described.



1 Theophylline R₁, R₂ = Me



2 R₃ = 4-F X = N Y = O CVT-5306

2 Enprofylline R₁ = H, R₂ = n-Pr

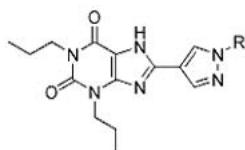
3 R₃ = 3-OMe X = O Y = N CVT-5440

253.

NOVEL 1,3-SYMMETRICALLY SUBSTITUTED 8-(1-BENZYL-1H-PYRAZOL-4-YL) XANTHINES: POTENT AND SELECTIVE A2B ADENOSINE RECEPTOR ANTAGONISTS.

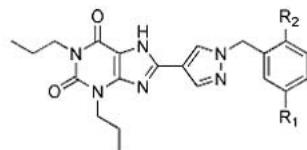
Rao V Kalla¹, Thao Perry¹, Elzein Elfaith¹, Venkata Palle¹, Xiaofen Li¹, Vaibhav Varkhedkar¹, Tenning Maa², Marie Nguyen², Yuzhi Wu², Victoria Maydanik³, David Lustig³, Kwan Leung³, Dewan Zeng², and Jeff Zablocki¹. (1) Department of Bio-Organic Chemistry, CV Therapeutics, Inc, 3172 Porter Drive, Palo Alto, CA 94304, Fax: 650-858-0390, rao.kalla@cvt.com, thao.perry@cvt.com, (2) Department of Drug Research and Pharmacological Sciences, CV Therapeutics, Inc, (3) Department of Pre-Clinical Development, CV Therapeutics, Inc

In order to develop novel potent and selective A_{2B} adenosine receptor (AdoR) antagonists that may have potential in the treatment of asthma, diabetic retinopathy, cancer, and Alzheimer's disease, we explored various 8-substituted heteroaryl xanthine derivatives. The pyrazole derivative **1**, that represents a new class of AdoR ligands, showed good binding affinity for the A_{2B} AdoR, but has poor selectivity versus the other AdoR subtypes. Introduction of a benzyl group at the N-1-pyrazole of **1**, resulted in **2**, that had moderate selectivity. The corresponding N-1-phenyl, phenethyl and phenylpropyl 8-pyrazolyl derivatives lost the A_{2B} affinity and/or the selectivity. The SAR for substitution on the phenyl suggests that an electron-withdrawing group like CF₃ at the m-position increased the selectivity as in **3**. The A_{2B} AdoR affinity of the symmetrically N-1 and N-3 substituted xanthines followed the trend ethyl=propyl >> methyl, butyl and isobutyl. Further substitution on **3** led to **4** that retained the A_{2B} receptor binding affinity and further enhanced the selectivity.



1; R = H; CVT-5450

2; R = -CH₂Ph; CVT-5299



3; R₁ = CF₃; R₂ = H; CVT-6275

4; R₁ = CF₃; R₂ = Cl; CVT-7136

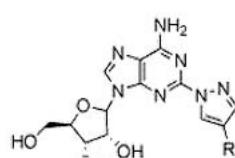
254.

2-PYRAZOLYL-N⁶-SUBSTITUTED ADENOSINE DERIVATIVES AS POTENT AND SELECTIVE A₃ ADENOSINE RECEPTOR AGONISTS.

Elfatih Elzein¹, Venkata Palle¹, Yuzhi Wu², Tenning Maa², Dewan Zeng², and Jeff Zablocki¹. (1) Bioorganic Chemistry, CV Therapeutics Inc, 3172 Porter Drive, Palo Alto, CA 94304, elfatih.elzein@cvt.com, (2) Drug Research and Pharmacological Sciences, CV Therapeutics Inc

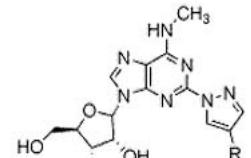
A₃ adenosine receptor (A₃-AdoR) has been linked to several diseases such as cardiac ischemia, cerebral ischemia, inflammation, cancer and hence has been a primary target for new therapeutics. Studies have shown that introducing a methyl group into the N⁶ position of the A_{2A} selective 2-alkynyl adenosine derivatives induces an increase in the affinity for the human A₃-AdoR and correspondingly decreases the affinities for the A₁ and A_{2A}-AdoRs. Accordingly, we investigated the effect of introducing substituents at the N⁶-position of the

A_{2A} selective 2-pyrazolyl adenosine analogues (e.g. **1** and **2**) on the binding affinity and selectivity for the A₃-AdoR. The N⁶-substituted analogues **3** and **4** displayed much greater binding affinity and selectivity for the A₃-AdoR relative to the unsubstituted analogues **1** and **2**. Detailed SAR of this novel series of compounds will be described.



1 R = CONHCH₃,

2 R = 4-OCH₃-Ph



3 (CVT-3641) R = CONHCH₃

4 (CVT-3866) R = 4-OCH₃-Ph

255.

STRUCTURE-ACTIVITY RELATIONSHIPS OF THIAZOLE AND THIADIAZOLE DERIVATIVES AS POTENT AND SELECTIVE HUMAN ADENOSINE A₃ RECEPTOR ANTAGONISTS.

Yong-Chul Kim¹, Kwan-Young Jung¹, Soo-Kyung Kim², Zhan-Guo Gao², Ariel S. Gross², N. Melman², and Kenneth A. Jacobson². (1) Department of Life Science, Kwangju Institute of Science and Technology, 1 Oryong-dong, Buk-gu, Gwangju 500-712, South Korea, Fax: +82-62-970-2484, yongchul@kjist.ac.kr, (2) Molecular Recognition Section, NIDDK, NIH

4-(4-Methoxyphenyl)-2-aminothiazole and 3-(4-methoxyphenyl)-5-aminothiadiazole derivatives have been synthesized and evaluated as selective antagonists for human adenosine A₃ receptors. A methoxy group in the 4-position of the phenyl ring and N-acetyl or propionyl substitutions of the aminothiazole and amino-thiadiazole templates displayed great increases of binding affinity and selectivity for human adenosine A₃ receptors. The most potent A₃ antagonist of the present series, *N*-[3-(4-methoxy-phenyl)-[1,2,4]thiadiazol-5-yl]-acetamide exhibiting a K_i value of 0.79 nM at human adenosine A₃ receptors, showed antagonistic property in a functional assay of cAMP biosynthesis involved in one of the signal transduction pathways of adenosine A₃ receptors. Molecular modeling study of conformation search and receptor docking experiments to investigate the dramatic differences of binding affinities between two regioisomers of thiadiazole analogs, *N*-[3-(4-Methoxyphenyl)-[1,2,4-thiadiazole-5-yl]-acetamide and *N*-[5-(4-Methoxyphenyl)-1,3,4-thiadiazole-2-yl]-acetamide, suggested possible binding mechanisms in the binding pockets of adenosine receptors.

256.

NEW SYNTHETIC ROUTE TO (N)METHANOCARBA NUCLEOSIDES ACTING AS POTENT A3 ADENOSINE RECEPTOR AGONISTS.

Bhalchandra V. Joshi¹, Victor E. Marquez², James C. Fettinger³, and Kenneth A. Jacobson¹. (1) Molecular Recognition Section, NIDDK, National Inst. of Health, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810, Fax: 301-480-8422, BalachandraJ@intra.niddk.nih.gov, (2) Laboratory of Medicinal Chemistry, CCR, NCI-F, NIH, (3) Department of Chemistry and Biochemistry, University of Maryland at College Park

Activation of the A₃ adenosine receptor (AR) is associated with cerebroprotective, cardioprotective, and anticancer effects. Among potent and selective A₃ AR agonists are novel methanocarba adenosine analogues in which a pseudo-ribose moiety is locked in a Northern (N) conformation. 2-Chloro-5'-N-methyluronamide derivatives such as MRS1898 and MRS2346 were found to be full agonists at the human A₃AR. There is a need for more efficient synthetic procedures for this class of compounds in order to carry out further biological studies. We have devised an improved synthetic route for this class of compounds, which is more versatile for adenine substitution, by employing intramolecular cyclopropanation of an appropriately substituted L-ribose derivative and the isomerization of the isopropylidene group as the key steps. This new synthetic route uses readily available building blocks that should allow us to prepare this class of compounds on a reasonably large scale. Synthesis and binding affinity at ARs will be presented.

257.

EXPLORING DISTAL REGIONS OF THE A₃ ADENOSINE RECEPTOR BINDING SITE: STERICALLY-CONSTRAINED N⁶-(2-PHENYLETHYL)-ADENOSINE DERIVATIVES AS POTENT LIGANDS. *Susanna Tchilibon¹, Soo-Kyung Kim², Zhan-Guo Gao², Brian A. Harris V², Joshua Blaustein², Ariel S. Gross², Heng T. Duong², Neli Melman², and Kenneth Jacobson³. (1) Molecular Recognition Section, NIDDK, National Inst. of Health, Bldg 8A, Rm B1A-15, Bethesda, MD 20892-0810, Fax: 301-480-8422, susannat@intra.niddk.nih.gov, (2) Molecular Recognition Section, NIDDK, NIH, Bethesda, MD 20892-0810, (3) Molecular recognition section, National Institutes of Health*

We have synthesized phenyl ring-substituted analogs of N6-(1S,2R)-(2-phenyl-1-cyclopropyl)adenosine, a highly potent agonist at the human A3AR (Ki 0.63 nM), and measured effects on affinity at human and rat adenosine receptors and on intrinsic efficacy at the human A3AR. A 3-nitrophenyl analog was resolved chromatographically into pure diastereomers which displayed 10-fold stereoselectivity in A3AR binding in favor of the 1S,2R isomer. A molecular model defined a hydrophobic region (Phe168) in the putative A3AR binding site around the phenyl moiety. A heteroaromatic group (3-thienyl) could substitute for the phenyl moiety with retention of high A3AR affinity. Although the N6-(2-phenyl-1-cyclopropyl) derivatives were full A3AR agonists, several structurally related derivatives had greatly reduced efficacy. N6-Cyclopropyladenosine was an A3AR antagonist, while adding either one or two phenyl rings at the 2-position of the cyclopropyl moiety restored efficacy. N6-(2,2-Diphenylethyl)adenosine was an A3AR antagonist, while N6-9-fluorenylmethyl- and N6-diphenylmethyladenosine were full agonists. Thus, a new series of high affinity A3AR nucleoside agonists/antagonists has been explored.

258.

STRUCTURE-ACTIVITY RELATIONSHIP OF N6-SUBSTITUTED D-4'-THIOADENOSINE DERIVATIVES AS POTENT AND SELECTIVE AGONISTS AT THE HUMAN A3 ADENOSINE RECEPTOR. *Lak Shin Jeong¹, Dae Hong Shin¹, Hea Ok Kim¹, Ji Young Jung¹, Hyun Ji Kim¹, Won-Ki Kim², Neli Melman³, Zhan-Guo Gao³, and Kenneth A. Jacobson³. (1) Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, 11-1 Seodaemun-ku, Daehyun-dong, Seoul, South Korea, Fax: 82-2-3277-2851, lakjeong@mm.ewha.ac.kr, (2) College of Medicine, Ewha Womans University, (3) Molecular Recognition Section, NIDDK, NIH*

The A3 adenosine receptor is G protein-coupled receptor and its activation inhibits adenylate cyclase and stimulates phospholipase C, leading to protein kinase C activation and histamine release from rat mast cells. Since the A3 adenosine receptor is closely related to several diseases such as cardiac ischemia, cerebral ischemia, and inflammation, it has been a promising target for the development of new therapeutic agents. A number of ligands have been synthesized and tested for binding affinity at the rat, sheep, and human A3 versus A1 and A2A receptors. Among these ligands, IB-MECA was found to be a highly potent rat A3 agonist (Ki=1.1 nM), which is 50-fold selective for rat brain A3 versus either A1 or A2 receptors. Introduction of chlorine at the 2-position of IB-MECA, resulting in the formation of Cl-IB-MECA dramatically increased binding affinity and selectivity. Cl-IB-MECA has been reported to display a Ki value of 1.0 nM at the human A3 adenosine receptor. It is now being used extensively as a pharmacological tool for studying A3 receptors. Therefore, based on the high binding affinity and selectivity of Cl-IB-MECA on A3 adenosine receptors, we designed and synthesized the 4'-thio analogue of Cl-IB-MECA and its related analogues, since a sulfur atom may serve as a bioisostere of an oxygen atom. A new ligand, thio-Cl-IB-MECA and its derivatives were efficiently synthesized starting from D-gulono γ -lactone via 4-thioribosyl acetate as the key intermediate. All synthesized N6-substituted 4'-thionucleoside derivatives exhibited consistently subnanomolar affinity and high selectivity for human A3 receptors versus human A1 and A2A receptors. Among them, N6-methyl analogue showed the most potent binding affinity (Ki=0.28 ± 0.09 nM). It was also selective for A3 vs human A1 and human A2A receptors by 4,800- and 36,000-fold, respectively. The finding that the 4'-thio modification is associated with high potency and selectivity significantly expands the possibilities to design additional A3 agonists, which may potentially be useful as in vivo tools.

259.

NUCLEOTIDE ANALOGUES CONTAINING 2-OXA-BICYCLO[2.2.1]HEPTANE AND L- α -THREOFURANOSYL RING SYSTEMS: INTERACTIONS WITH PURINE/PYRIMIDINE RECEPTORS. *Michihiro Ohno¹, Hak Sung Kim², Stefano Costanzo¹, Veerle Kempeneers³, Karen Vastmans³, Piet Herdewijn³, Savitri Maddileti⁴, T. Kendall Harden⁴, and Kenneth A. Jacobson¹. (1) Molecular Recognition Section, NIDDK, NIH, Bethesda, MD 20892-0810, Fax: 301-480-8422, michihiro@intra.niddk.nih.gov, (2) College of Pharmacy and Medicinal Resources Research Center, Wonkwang University, (3) Laboratory of Pharmaceutical Chemistry, Rega Institute for Medical Research, Katholieke Universiteit Leuven, (4) School of Medicine, Univ. of North Carolina*

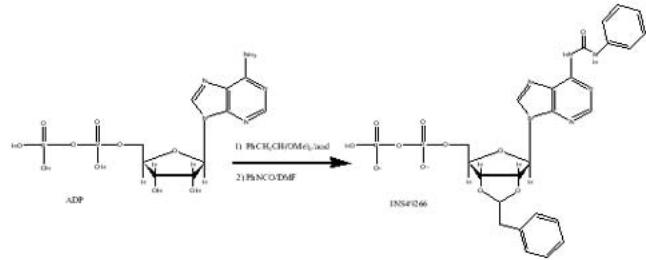
The ribose moiety of adenine nucleotide 3',5'-bisphosphate antagonists of the P2Y1 nucleotide receptor prefers the Northern (N) ring conformation, as established by using rigid methanocarba ring systems. We now have examined substitution with novel ring systems: 1) Northern locked-carbocyclic (cLNA) derivatives which contains the oxabicyclo[2.2.1]heptane ring system and 2) L- α -threofuranosyl derivatives. A cLNA bisphosphate derivative displayed a Ki value of 23 nM in binding to the human P2Y1 receptor, at which it acts as an antagonist. The parent cLNA nucleoside did not bind appreciably to adenosine receptors, thus this ring system affords some P2Y receptor selectivity. An L- α -threofuranosyl bisphosphate derivative displayed an IC50 of 1 μ M in inhibition of phospholipase C stimulated by the potent agonist 2-methylthio-ADP (30 nM). L- α -threofuranosyl-UTP was an agonist with a preference for P2Y2 (EC50 3 μ M) versus P2Y4 receptors. The P2Y1 receptor binding modes, including rotational angles, were estimated using molecular modeling and receptor docking.

260.

ADENINE MONO- AND DINUCLEOTIDES MODIFIED WITH LIPOPHILIC ACETAL AND UREA MOIETIES AS REVERSIBLE P2Y12 ANTAGONISTS. *James G. Douglass, Roshni I. Patel, Catherine C. Redick, Arthur C. Jones, Sammy R. Shaver, and Jose L. Boyer, Departments of Chemistry and Molecular Pharmacology, Inspire Pharmaceuticals, Inc, 4222 Emperor Blvd. Suite 470, Durham, NC 27703, Fax: 919-941-9177, jdouglass@inspirepharm.com*

Inhibition of platelet aggregation has been shown to be an efficacious approach to the treatment of thrombotic diseases. We have discovered that addition of a phenylacetaldehyde acetal group to the 2'/3' hydroxyls of the endogenous P2Y₁₂ receptor agonist ADP converted it into a low micromolar antagonist. Likewise, the installation of a phenylurea moiety to the 6-amino position of ADP gave the same result. The incorporation of both lipophilic modifications into ADP resulted in a compound (INS49266) displaying a dramatic increase in potency (IC₅₀=52 nM):

These compounds inhibited platelet aggregation induced by the agonists ADP or 2-MeSADP in a reversible and concentration dependent manner. Here we discuss these results and expand them to a related series of nucleotides.

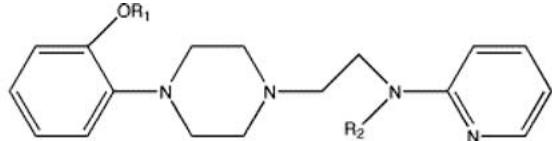


261.

ROLE OF TERTIARY AMINE IN THE CONDENSATION REACTION OF ¹¹C-WAY AND ¹¹C-DWAY SYNTHESIS. *Dilip K. Maiti, Pulak K. Chakraborty, Thomas J. Mangner, Diane C. Chugani, and Harry T. Chugani, PET Center, Children's Hospital of Michigan, Wayne State University School of Medicine, 3901 Beaubien Blvd., Detroit, MI 48201, Fax: 313-993-3845*

Radioligands **III** and **IV** are commonly used for quantitation of 5-HT_{1A} receptors in the human brain with Positron Emission Tomography (PET). ¹¹C-WAY and ¹¹C-DWAY were synthesized by the condensation of **I** or **II**, respectively, with [carbonyl-¹¹C]cyclohexanecarbonyl chloride in the presence of a tertiary amine in 1,2-dichloroethane. In the preparation of both

III and **IV**, we have detected, by radio-HPLC, a radiochemical impurity with a similar chromatographic profile to that of the target compound (for **III**, $\alpha=1.12$; for **IV**, $\alpha=1.09$). In order to minimize the formation of these impurities and to maximize the radiochemical yield of the target compounds, we have conducted a series of experiments examining the effect of the nature and relative amount of the tertiary amine used in the condensation reaction. As a result of these experiments, we were able to increase the radiochemical yield of both **III** and **IV** while decreasing the amount of radiochemical impurities in the final radioligand preparation. The results of our investigation will be presented.

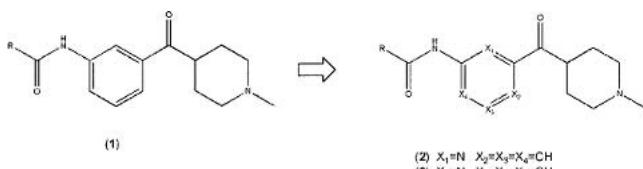


I: R₁ = CH₃, R₂ = H, WAY-100634; **II:** R₁ = H, R₂ = H, DWAY-100634;
III: R₁ = CH₃, R₂ = [carbonyl-¹¹C]cyclohexanecarbonyl, ¹¹C-WAY-100635;
IV: R₁ = H, R₂ = [carbonyl-¹¹C]cyclohexanecarbonyl, ¹¹C-DWAY-100635

262.

SAR STUDIES IN THE IDENTIFICATION OF 2-CHLORO-4-FLUORO-N-[6-(1-METHYL-PIPERIDINE-4-CARBONYL)-PYRIDIN-2-YL]-BENZAMIDE AS A POTENT AND SELECTIVE 5-HT_{1F} RECEPTOR. Yao-Chang Xu, Daniel T. Kohlman, Sidney X. Liang, Bai-Ping Ying, Michael P. Cohen, DeAnna P. Zacherl, Dana R. Benesh, María-Jesús Blanco, Sandra A. Filla, Kevin J. Hudziak, Brian M. Mathes, Frantz Victor, Deyi Zhang, Qi Chen, Wendy H. Gough, David L. Nelson, Suzanne E. Nutter, and David B. Wainscott, *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-276-7600, Xu_yao-chang@lilly.com, kohlman_dan_t@lilly.com*

Structure (1) has been recently identified by scientists at Lilly as a potent and selective 5-HT_{1F} receptor agonist. In order to explore the SAR scope and potentially to improve pharmacokinetic and pharmacodynamic properties, a nitrogen atom was introduced into the central phenyl ring at different positions (compounds 2-5). Analog (2) was the only compound to exhibit good affinity and selectivity at the 5-HT_{1F} receptor. Synthesis of these molecules, binding affinity and selectivity as well as some functional data will be presented along with computational analysis.

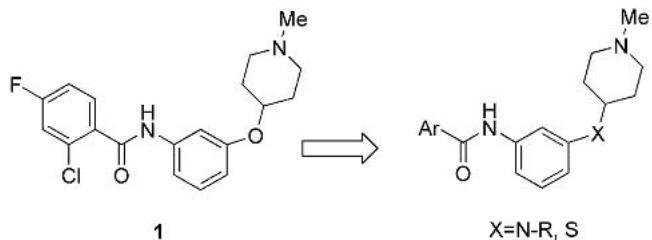


263.

PROGRESS TOWARDS AN ORALLY BIOAVAILABLE 5-HT_{1F} RECEPTOR

AGONIST. María-Jesús Blanco¹, Dana R. Benesh¹, Sivi Mahadevan², Sandra A. Filla¹, Kevin J. Hudziak¹, Daniel T. Kohlman¹, Brian M. Mathes¹, Frantz Victor¹, Yao-Chang Xu¹, Bai-Ping Ying¹, Deyi Zhang¹, Michael P. Cohen¹, DeAnna P. Zacherl¹, Donna K. Dieckman¹, Wendy H. Gough¹, Kirk W. Johnson¹, David L. Nelson¹, Suzanne E. Nutter¹, Lee A. Phebus¹, Kristel Van Belle², and David B. Wainscott¹. (1) *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, blanco_maria@lilly.com*, (2) *Lilly Development Centre S.A.*

During the course of our research efforts focusing on the identification of novel 5-HT_{1F} receptor agonists, we discovered a series of ether-linked phenyl piperidine amides such as **1**. In spite of showing high 5-HT_{1F} receptor binding affinity, selectivity and good plasma exposure in rat, they suffered from low dog bioavailability. Presented here are some structure-activity relationships designed to increase dog oral exposure of this series and thus improve dog bioavailability. We found that modifying the ether linkage led to increased dog exposure levels. Amino-linked phenyl piperidine amides provided a >10-fold increase in dog exposure and also retained excellent potency and selectivity vs. the other 5-HT receptor subtypes.

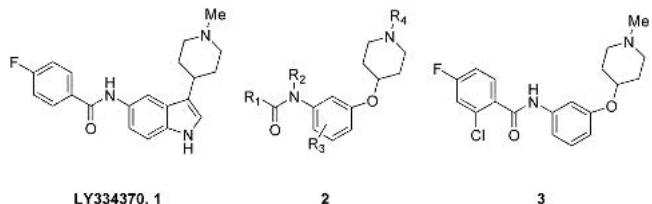


264.

NOVEL SUBSTITUTED N-[3-(1-METHYL-PIPERIDIN-4-YLOXY)-PHENYL]-AMIDES AS POTENT AND SELECTIVE 5-HT_{1F} RECEPTOR AGONISTS.

María-Jesús Blanco, Dana R. Benesh, Sandra A. Filla, Kevin J. Hudziak, Daniel T. Kohlman, Brian M. Mathes, Frantz Victor, Yao-Chang Xu, Bai-Ping Ying, Michael P. Cohen, DeAnna P. Zacherl, Deyi Zhang, Donna K. Dieckman, Wendy H. Gough, Kirk W. Johnson, David L. Nelson, Suzanne E. Nutter, Lee A. Phebus, and David B. Wainscott, *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, blanco_maria@lilly.com*

Investigators at Lilly have shown that selective 5-HT_{1F} receptor agonists inhibit neurogenic dural inflammation in the plasma protein extravasation model of migraine. LY334370 (**1**) was the first selective 5-HT_{1F} agonist to show clinical efficacy. Recently, we became interested in identifying structurally unique, potent and selective 5-HT_{1F} receptor agonists that do not incorporate an indole nucleus. Described here is the synthesis and evaluation of a series of novel ether-linked N-[3-(1-methyl-piperidin-4-yl)-phenyl]-amides (**2**). SAR analysis of the different molecular domains will be presented with an emphasis on the substitution in the amide domain and the piperidine nitrogen, which both had a profound effect on the potency and selectivity. This effort led to the discovery of **3**, a subnanomolar 5-HT_{1F} agonist with selectivity >100-fold vs. other 5-HT₁ receptor subtypes.

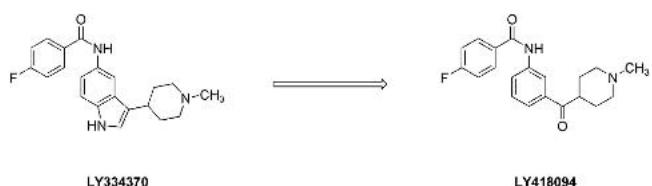


265.

DISCOVERY AND SAR OF THE 4-[(3-SUBSTITUTED)BENZOYL]PIPERIDINES AS SELECTIVE 5-HT_{1F} RECEPTOR AGONISTS.

Joseph H. Krushinski Jr., John M. Schaus, David L. Nelson, David B. Wainscott, Kirk W. Johnson, and Donna K. Dieckman, *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-276-7600, krush@lilly.com*

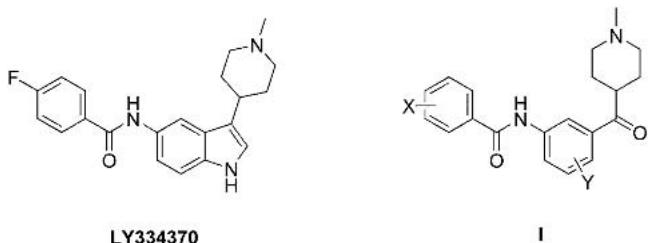
LY334370 is a selective, high affinity agonist for the 5-HT_{1F} receptor with a K_i of 1.6 nM. This 5-HT_{1F} receptor agonist has been shown to be efficacious in the treatment of acute migraine pain in Phase II clinical trials. The indole platform of LY334370 was investigated as a point for structural diversification. During this investigation, the 4-[(3-substituted)benzoyl]piperidines were found to be ligands for the 5-HT_{1F} receptor. An extensive SAR was conducted on the 3-substituent. These 4-[(3-substituted)benzoyl]piperidines, such as LY418094 (4-fluoro-N-[3-(1-methylpiperidine-4-carbonyl)-phenyl]-benzamide), have proven to be potent and selective 5-HT_{1F} receptor agonists.



266.

DISCOVERY OF POTENT AND SELECTIVE 5HT_{1F} RECEPTOR AGONISTS: EFFECTS OF AROMATIC SUBSTITUTIONS ON N-[3-(1-METHYL-PIPERIDINE-4-CARBONYL)-PHENYL]-BENZAMIDES SERIES. Deyi Zhang, Bai-Ping Ying, Daniel T. Kohlman, DeAnna P. Zacherl, Frantz Victor, Dana R. Benesh, María-Jesús Blanco, Qi Chen, Michael P. Cohen, Sandra A. Filla, Kevin J. Hudziak, Sidney X. Liang, Brian M. Mathes, John M. Schaus, Suzanne E. Nutter, Wendy H. Gough, and Yao-Chang Xu, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-276-5431, zhang_deyi@lilly.com, victor_frantz@lilly.com

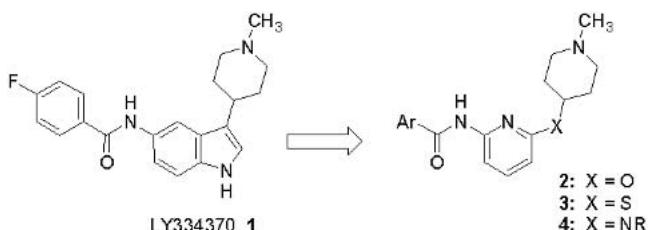
LY334370, a potent and selective 5HT_{1F} receptor agonist, has been shown to be efficacious in ending migraine attack in clinical study. LY334370 has appreciable affinity for the 5HT_{1A} receptor ($K_i=22.1$ nM), and like other "triptaN" anti-migraine drugs, it has an indole core structure. In our pursuit of structurally diversified, potent and selective 5HT_{1F} receptor agonists, a novel *N*-[3-(1-methyl-piperidine-4-carbonyl)-phenyl]-benzamide series (**I**) was discovered. Substitution of the middle aromatic ring with fluorine (Y=F) at C-2 position led to a new series of potent and selective 5HT_{1F} receptor agonists. In this presentation, our SAR efforts on aromatic substitutions will be disclosed. The introduction of different substituents on the middle aromatic ring and its effects on 5HT_{1F} receptor binding and selectivity will be highlighted. The observed SAR trend will be discussed through computational analyses of the key compounds.



267.

HETEROARYL-LINKED N-[6-(1-METHYL-PIPERIDIN-4-YL)-PYRIDIN-2-YL] BENZAMIDES AS NOVEL AND POTENT 5-HT_{1F} RECEPTOR AGONISTS. Sandra A. Filla¹, Kevin J. Hudziak¹, Dana R. Benesh¹, María-Jesús Blanco¹, Michael P. Cohen¹, Daniel T. Kohlman¹, Brian M. Mathes¹, Frantz Victor¹, Yao-Chang Xu¹, Bai-Ping Ying¹, Deanna P. Zacherl¹, Deyi Zhang¹, Donna K. Dieckman¹, Wendy H. Gough¹, Kirk W. Johnson¹, Sivi Mahadevan², David L. Nelson¹, Suzanne E. Nutter¹, Kristel Van Belle², and David B. Wainscott¹. (1) Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, (2) Lilly Development Centre SA

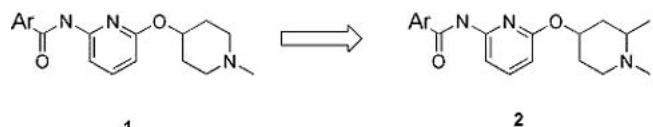
Potent and selective 5HT_{1F} receptor agonist, LY334370 (**1**), demonstrated clinical efficacy in ameliorating acute migraine. In an effort to expand the scope of the SAR to identify novel non-indole platforms, we targeted a series of structurally related oxygen, sulfur, and nitrogen-linked *N*-[6-(1-methyl-piperidin-4-yl)-pyridin-2-yl benzamides (**2-4**). Compounds from these series exhibited high 5HT_{1F} receptor binding affinity and were >100-fold selective versus the other 5HT₁ receptor subtypes. Described here is the synthesis as well as the pharmacological and pharmacokinetic evaluation of these novel series of compounds.



268.

IMPROVING DOG ORAL PHARMACOKINETICS FOR A NEW SERIES OF 5-HT_{1F} RECEPTOR AGONISTS THROUGH RATIONAL STRUCTURAL MODIFICATION. Yao-Chang Xu, Bai-Ping Ying, D. T. Kohlman, Vincent I. Mancuso, Sivi Mahadevan, Sandra A. Filla, Brian M. Mathes, Kevin J. Hudziak, María-Jesús Blanco, Dana R. Benesh, Deyi Zhang, Frantz Vicotr, Michael P. Cohen, DeAnna P. Zacherl, Suzanne E. Nutter, Wendy H. Gough, David B. Wainscott, and D. L. Nelson, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Xu_Yao_Chang@lilly.com, bpingy@lilly.com

Certain pyridyloxy-piperidine analogs such as (**1**) display good binding affinity and functional activity (GTP-γ-S) at the 5-HT_{1F} receptor, and excellent selectivity against other 5-HT receptor subtypes. Pharmacokinetics studies in the rat indicated good plasma exposure and acceptable bioavailability. However, the dog oral exposure is very poor. To improve this property, metabolite identification studies were performed for (**1**) and other related compounds. The major metabolic pathways were found to be N-demethylation and N-oxidation at the piperidine nitrogen. In order to slow down these processes, a methyl group was introduced at the C-2 position of the piperidine ring (**2**). The methyl group was anticipated to provide steric hindrance for both the demethylation and the N-oxidation processes. This approach turned out to be very successful, not only in retaining high affinity and selectivity at the 5-HT_{1F} receptor, but also in improving the dog oral exposure. Detailed chemistry of preparing four isomers and biological activities will be discussed.



269.

SYNTHESIS OF NOVEL SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIS): TARGETING SERT AND 5-HT2A RECEPTORS. M. Graciela Miranda, Michael S. Stewart, and Kevin G. Pinney, Department of Chemistry and Biochemistry, Baylor University, P.O. Box 97348, Waco, TX 76798-7348, Fax: 254-710-4272, Maria_Miranda@baylor.edu

Since the 1980's, clinical use of selective serotonin reuptake inhibitors (SSRIs) in the treatment of depression has revealed that SSRIs are also effective against the symptoms of anxiety coexistent with depression. Evidence indicates the serotonin type 2A receptors play an important role in anxiety, depression, anorexia nervosa, and other disorders. Moreover, researchers have found that blockade of the 5-HT2A receptor may restore some of the undesired side effects of SSRIs. The proposed research focuses on the development of novel bi-functional molecules that, by exhibiting an enhanced antagonism towards the 5-HT2A receptors while keeping a highly selective inhibition of serotonin reuptake activity, provide synergism in their potential efficacy over a wider variety of both depressive and anxiety disorders. In order to achieve our goal, we have designed and plan to prepare a series of molecules which judiciously combine portions of known 5-HT2A receptor antagonists with portions of SSRI's such as fluoxetine.

270.

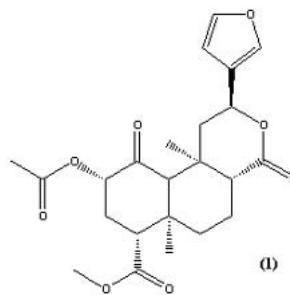
DISCOVERY AND SAR OF NOVEL 5HT_{2A} RECEPTOR INVERSE-AGONISTS. Sonja Strah-Pleynet¹, Bradley R. Teegarden¹, Keith Drouet¹, Honnappa Jayakumar¹, William Thomsen¹, Hazel Reyes¹, Jonathan Foster¹, Paul Maffuid¹, Katie Elwell¹, Konrad Feichtinger¹, Jarrod Davidson¹, Naomi Kato¹, Susan D Selaya¹, Young-Jun Shin¹, Darrick Seconi¹, Richard Foster², Julian Smith², Frederique Menzaghi¹, Kevin Whelan¹, Dominic Behan¹, Derek Chalmers¹, and Nigel R. A. Beeley¹. (1) Arena Pharmaceuticals, Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, sstrah@arenapharm.com, (2) Tripos, Inc

Modulation of serotonin (5-HT) receptors is a pharmacological target of interest. Specifically, inverse-agonists of the 5-HT_{2A} receptor subtype are known to mitigate negative symptoms in schizophrenia and are known to influence sleep patterns. Utilizing our constitutively activated receptor technology (CART) we have discovered a novel 5-HT_{2A} specific inverse-agonist lead. Herein we describe the synthesis and SAR of some of our 5-HT_{2A} inverse-agonists.

271.

DISCOVERY OF APD356: A POTENT AND SELECTIVE 5-HT2C RECEPTOR AGONIST FOR THE TREATMENT OF OBESITY. *Brian Smith, Jeffrey Smith, Rita Chen, Jon Covel, Peter Dosa, Scott Estrada, Charles Gilson, Douglas Park, Emily Prieto, Jeffrey Schultz, James Tsai, Rob Webb, Shelley Aytes, Beverly Burbbaum, John Fritch, Dipanjan Sengupta, James Henshilwood, Hazel Reyes Saldana, William Thomsen, Kevin Whelan, Frederique Menzaghi, Christina Bjenning, Michael Martin, Dennis Chapman, Paul Maffuid, Nigel Beeley, Derek Chalmers, and Dominic Behan, R&D, Arena Pharmaceuticals Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, bsmith@arenapharm.com*

Compounds acting as agonists at the 5-HT_{2C} receptor have been shown to reduce food intake and body weight in both laboratory animals and humans. However, a major hurdle in developing a safe and effective 5-HT_{2C} agonist for the treatment of obesity has been selectivity over other 5HT receptor subtypes, most notably 5-HT_{2A} and 5-HT_{2B}. APD356 is a new 5-HT_{2C} agonist that has been shown to decrease food intake and fat mass in the Levin rat model for obesity. We describe the synthesis and biological evaluation of APD356 and analogs as a new series of potent 5-HT_{2C} agonists having considerable selectivity over 5-HT_{2A} and 5-HT_{2B} receptor subtypes.



272.

EVALUATION OF THE ANTIPSYCHOTIC POTENTIAL OF NOVEL, ORALLY BIOAVAILABLE, NEUROTENSIN ANALOGUES CONTAINING NON-NATURAL

ARGININE AND LYSINE RESIDUES. *M. Kyle Hadden, and Thomas A. Dix, Department of Pharmaceutical Sciences, Medical University of South Carolina, 280 Calhoun Street, Charleston, SC 29425, Fax: 843-792-0759, haddenmk@musc.edu*

Neurotensin (NT) is a linear tridecapeptide that elicits distinct physiological responses in the central nervous system. Low levels of NT in the cerebrospinal fluid of schizophrenic patients have led to the postulation that NT acts as an endogenous neuroleptic. The active C-terminal portion of NT, NH₂-Arg(8)-Arg(9)-Pro(10)-Tyr(11)-Ile(12)-Leu(13)-COOH [NT(8-13)], is equipotent at eliciting the biological effects demonstrated for NT. Modification of the Arg(8) residue with non-natural analogues of Arg and Lys provided a set of NT(8-13) analogues with increased CNS activities. The most active compound, KK13, showed antipsychotic activity in various rat models of schizophrenia. Subsequent modifications of KK13 have yielded a set of "second generation" NT(8-13) analogues which were screened for CNS activity by measuring the induction of hypothermia after peripheral administration. A new compound, KH11, emerged from the screen showing greater maximal hypothermic effects as compared to KK13 and is currently under evaluation for activity in animal models of schizophrenia.

273.

MOLECULAR INTERACTIONS OF SALVINORIN A AT THE KAPPA OPIOID

RECEPTOR. *Brian E Kane, Department of Medicinal Chemistry, University of Minnesota, 308 Harvard St. SE, 8-101 WDH, Minneapolis, MN 55455, Fax: 612-624-0139, Christopher R. McCurdy, Department of Medicinal Chemistry and Laboratory for Applied Drug Design and Synthesis, University of Mississippi, and David M Ferguson, Medicinal Chemistry, University of Minnesota*

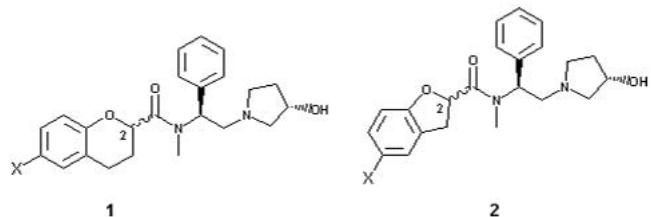
Salvinorin A (1) is a selective and potent kappa opioid agonist isolated from *Salvia divinorum*. It has been shown to be hallucinogenic and is on the DEA's list of chemicals of concern due to its increasing recreational use. Unlike most known opioid ligands, salvinorin A does not contain a protonatable nitrogen. A putative pharmacophore for this unique agonist has been proposed (Roth, et al., PNAS, 2002, 99, 11934) based on structural comparisons of existing opioid receptor/ligand models. This model suggests several key interactions between salvinorin A and transmembrane residues in the kappa opioid receptor. Herein, we report site-directed mutagenesis data at and near the pharmacophore, which helps to give us more insight on salvinorin A's binding mode.

274.

POTENT AND HIGHLY SELECTIVE KAPPA OPIOID RECEPTOR AGONISTS

INCORPORATING CHROMAN- AND 2,3-DIHYDROBENZOFURAN-BASED CONSTRAINTS. *Guo-Hua Chu¹, Minghua Gu¹, Roland E. Dolle¹, Joel A. Cassel², and Robert N. DeHaven². (1) Department of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, Fax: 484-595-1551, ghchu@adolor.com, (2) Department of Pharmacology, Adolor Corporation*

Kappa opioid receptor agonists display potent antinociceptive activity *in vivo*. As a part of our discovery research program in this area, two novel series of constrained kappa opioid agonists 1 and 2 with high affinity toward the kappa opioid receptor and high selectivity over the delta and mu opioid receptor were synthesized. The details for the synthesis, bio-activity of these novel constrained kappa opioid agonists, and the establishment of the absolute stereochemistry at the 2-position of the most active diastereomer will be presented.



275.

FLUORINATED ANALOG OF GR103545 IS A POTENT KAPPA RECEPTOR

LIGAND. *Dah-Ren Hwang, Kim Chung Ngo, Henry Huang, Diana Martinez, and Marc Laruelle, Department of Psychiatry, Columbia University, 1051 Riverside Dr., Unit 31, New York, NY 10032, Fax: 212-568-6171, hd72@columbia.edu*

Kappa opioid receptor (KOR) regulates dopamine (DA) functions in the striatum and modulates the rewarding properties of drugs of abuse. To probe the interactions between KOR and DA receptors *in vivo* a positron-labeled KOR ligand is needed. Recently, we and others have demonstrated that [¹¹C]GR103545, a potent kappa agonist with a Ki of 0.22 nM, is a potential ligand for imaging KOR using Positron Emission Tomography (PET). However, the short half-life of C-11 (20 min) limits the application of this tracer. In an effort to develop a [¹⁸F]-labeled KOR selective PET ligand, we prepared the fluorinated analog of GR103545, compound 1. *In vitro* binding assay showed 1 is a potent KOR ligand with a Ki of 0.5nM. Synthesis and *in vivo* evaluation of [¹⁸F]-1 will be presented.

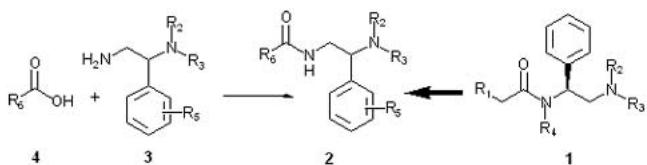
	X	#	Ki (nM)
<chem>CC1(C)CCN(C2=CC=C(Cl)C(Cl)=C2)CC1</chem>	H	GR103545	0.22
<chem>CC1(C)CCN(C(F)(F)c2ccccc2)CC1</chem>	F	1	0.55
<chem>CC1(C)CCN(C(O)c2ccccc2)CC1</chem>	OH	2	0.73
<chem>CC1(C)CCN(C(=O)c2cc(Cl)cc(Cl)c2)CC1</chem>	OMs	3	

276.

NOVEL OPIOID AGONISTS: (2-CARBONYLAMINO-1-PHENYLETHYL)-

DIMETHYLAMINES. *Blanca Martinez-Teipei¹, Baudouin Gerard¹, Hai Fen Ye¹, Joseph M. Salvino¹, Roland E. Dolle¹, Serge Belanger², Joel A. Cassel², Gabriel Stabley², and Robert N. DeHaven².* (1) Department of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19431, Fax: 484-595-1551, *bmartinez@adolor.com*; (2) Department of Pharmacology, Adolor Corporation

During the course of our structure-activity relationship (SAR) studies around arylacetamides **1** as selective kappa agonists, the effects of the substituents on the basic nitrogen and the position of the phenyl ring were investigated. It was discovered that (2-carbonylamino-1-phenylethyl)-dimethylamines **2** showed an increased affinity for other opioid receptors, including the ORL-1 receptor. The SAR of amides **2** was expanded with the goal of achieving enhanced ORL-1 selectivity. A focused library was created by reacting eight amines **3** with forty acids **4** in the presence of PS-carbodiimide to yield 320 compounds in excellent purity. Highlights of the library SAR for the ORL-1 and kappa receptors will be presented.



277.

NOVEL PERIPHERAL DUAL δ/μ OPIOID RECEPTOR AGONISTS FOR

GASTROINTESTINAL DISORDERS. *Robert W. Kavash, Henry J. Breslin, Chaozhong Cai, Craig J. Diamond, Santosh V. Coutinho, Paul R. Wade, Sandra L. McKenney, Sui-Po Zhang, Nathaniel H. Wallace, Craig R. Schneider, Pamela J. Hornby, and Wei He, Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Welsh and McKeon Roads, P. O. Box 776, Spring House, PA 19477, rkavash@prdus.jnj.com*

The use of peripherally acting opioid receptor agonists as a therapeutic strategy for gastrointestinal (GI) motility disorders, such as irritable bowel syndrome (IBS), is under investigation. Preliminary work revealed that a series of novel heterocyclic peptidomimetics, based on truncated enkephalin-like compounds, afforded non-peptide compounds with nanomolar binding affinities for both the δ and μ opioid receptors. These dual δ/μ receptor binders were generally found to be functionally full agonists. Opening the central ring of the initial leads provided acyclic analogs such as JNJ-17153656 that maintained *in vitro* potency ($K_i \delta=5.3$ nM, $K_i \mu=0.1$ nM) and comparable oral activity in an *in vivo* model to assess inhibition of mouse fecal pellet output. This promising observation was followed up with the preparation of a diverse set of analogs. The synthesis and SAR of this series of novel opioid receptor agonists will be presented.

278.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL OPIOID

RECEPTOR ACTIVE AGENTS. *Qiang zhang¹, youyi peng¹, Susan M. Keenan¹, Seong-Jae Yu², Anil C. Nair³, and William J. Welsh¹.* (1) Department of Pharmacology, Robert Wood Johnson Medicine School, University of Medicine and Dentistry, 661 hoes lane, pascataway, NJ 08854, Fax: 732-235-3475, (2) Agricultural Product Group, FMC Corporation, (3) Aventis Pharmaceuticals Inc

The major effects of the opioids are mediated by three families of receptors, designated by the Greek letters, delta, kappa, and mu, each of which exhibits a unique ligand (drug) specificity profile. These protein receptors modulate endocrine, cardiovascular, respiratory, gastrointestinal, and immune functions in humans. A large and growing body of research indicates that subtype-selective opioid receptor ligands may produce therapeutic benefits devoid of the numerous side effects (e.g., physical dependence, respiratory depression) associated with narcotics such as morphine. The delta-selective opioids thus represent extremely attractive candidates for a broad range of novel pharmaceutical applications including powerful yet safe analgesics, immunomodulatory agents for treating immune disorders, and new treatments for drug addiction. By virtue of the extraordinary medical and commercial impact of breakthroughs in this rapidly developing area, our primary goal in this research project has been the discovery and development of novel compounds that target the delta-opioid receptor. Using computer-aided design technologies, our lab has discovered a

family of substituted triazoles that bind with high affinity to the opioid receptors. These triazoles are structurally distinct from the morphine-like alkaloids and from other known opioid receptor active agents. Some of our triazole compounds exhibited high binding affinity and selectivity for the delta receptor. We report here the molecular design, chemical synthesis and biological evaluation of these novel compounds. The rational design and synthesis of the next generation of triazoles with improved binding affinity and selectivity are now underway.

279.

DESIGN AND SYNTHESIS OF NOVEL INDOLINONES AS LIGANDS FOR THE

NOCICEPTIN/ORPHANIN FQ RECEPTOR. *Faming Jiang¹, Cris M. Olsen¹, Willma E. Polgar², Lawrence Toll², and Nurulain Zaveri¹.* (1) Drug Discovery Program, Biosciences Division, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, Fax: 650-859-3153, *faming.jiang@sri.com*; (2) Neuropharmacology Program, Biosciences Division, SRI International

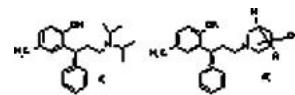
The nociceptin/orphanin FQ receptor (NOP receptor), discovered in 1994, is the fourth member of the opioid receptor family. Although it has high degree of structural homology to the opioid receptors and is also a G-protein coupled receptor, it does not appear to have affinity for opioid peptides or classical opiates. The endogenous ligand for the NOP receptor, identified in 1995, is a 17-amino acid peptide named nociceptin/orphanin FQ. The NOP receptor and nociceptin have been shown to be involved in pain, anxiety, learning and memory, diuresis, drug addiction and modulation of tolerance to opiates. Nonpeptide ligands for this receptor not only provide pharmacological tools for studying the role of this receptor but can also provide useful therapeutic candidates. We report the discovery and SAR of a novel class of indolinones as nonpeptide ligands for the NOP receptor. The relationship between structure and agonist/antagonist profiles at the receptor will be discussed.

280.

NOVEL DERIVATIVES OF TOLERODINE IN HUNT FOR M3 SELECTIVE

MUSCARINIC ANTAGONISTS. *Naresh Kumar¹, PKS Sarma¹, Kirandeep Kaur¹, Shelly Aeron¹, Arun Dutt¹, M Bruhaspathy¹, Sankar Shetty¹, Suman Gupta², Anita Chugh², JB Gupta², and Anita Mehta¹.* (1) Department of Medicinal Chemistry, Ranbaxy Laboratories, New Drug Discovery Research, Sector 18, Udyog Vihar, Gurgaon 122001, India, Fax: 91-124-2343545, *n.kumar@ranbaxy.com*, (2) Department of Pharmacology, Ranbaxy Laboratories

Tolterodine (**I**) and oxybutynine are two main drugs available for treatment of overactive bladder. These drugs have common drawback of their subtype non-selectivity, which results in many side effects. Much research had been done to improve this selectivity. Our efforts have been toward modifying amine portion of **I**. For that purpose diisopropyl amine moiety of Tolterodine was replaced with some novel azabicyclo derivatives, as shown in general structure (**II**). The synthesis and activities of some of such compounds would be presented at meeting.

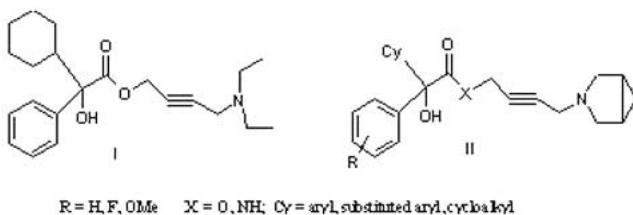


281.

ANALOGUES OF OXYBUTYNIN AS M3 SELECTIVE MUSCARINIC RECEPTOR

ANTAGONISTS. *PKS Sarma¹, Naresh Kumar¹, Kirandeep Kaur¹, Anita Chugh², Suman Gupta², Arun Dutt¹, JB Gupta², and Anita Mehta¹.* (1) Department of Medicinal Chemistry, Ranbaxy Laboratories, New Drug Discovery Research, Sector 18, Udyogvihar, Gurgaon 122001, India, Fax: 91-124-2343545, *pakala.sarma@ranbaxy.com*, (2) Department of Pharmacology, Ranbaxy Laboratories

M3 selective Muscarinic receptor antagonists have therapeutic potential for the treatment of urinary incontinence. Oxybutynin (**I**) is a muscarinic receptor antagonist and has well documented efficacy in the treatment of detrusor over activity, but its usefulness is limited by nonselectivity. With a view to develop M3 selective muscarinic receptor antagonists, we designed a series of Oxybutynin analogues with the general structure **II**. Synthesis and biological activities of these analogues will be discussed.



282.

GTS-21, AN A7-NACHR AGONIST, DOWN-REGULATES TNF-A AND PROTECTS IN SHOCK. Yousef Al-Abed¹, Hong Wang², Carol Ann Amella Amella², Mahira Tanovic², Mahendra Ochani², and Kevin Tracey². (1) Laboratory of Medicinal Chemistry, 350 Community Drive, Manhasset, NY 11030, Fax: 1-516-365-5090, yalabed@nshs.edu, (2) Laboratory of Biomedical Sciences, North Shore Long Island Jewish Research Institute

We have recently discovered that acetylcholine, the principle neurotransmitter of the vagus nerve, interacts with the nicotinic acetylcholine receptor a7 subunit expressed on macrophages, and induces intracellular signals that inhibit cytokine release (*Nature* 405:458-62 & 421:384-8). These observations suggest that pharmacologic agents that activate cholinergic signaling through a7 may be capable of rapidly and precisely modulating cytokine activity to therapeutic advantage. To test this hypothesis, we examined whether GTS-21, a partial a7 agonist and was tested in Phase I for improvement of cognition in Alzheimer Disease, could attenuate the TNF-a release from LPS-stimulated macrophages similar to acetylcholine or nicotine. To this end, we found that GTS-21 indeed down-regulates the TNF-a release and is protective in an animal model of septic shock. We also will present the results of a dozen of commercial nicotinic analogs that were tested for suppression of TNF-a release in a similar setup. These findings prompted us to design a new class of nicotinic acetylcholine receptors a7 agonist and found that our lead agonist, namely CAP2001-55, is a more potent than GTS-21 or nicotine in vivo experimental of septic shock.

283.

SYNTHESIS OF [¹¹C]PYRIDOSTIGMINE AND ITS ANALOGUES AS NEW POTENTIAL PET RADIOTRACERS FOR IMAGING HEART

ACETYLCHOLINESTERASE. Ji-Quan Wang, Qi-Huang Zheng, Xiangshu Fei, and Gary D. Hutchins, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L-3 Room 202, Indianapolis, IN 46202-2111, Fax: 317-278-9711, jqwang@iupui.edu, xfei@iupui.edu

Pyridostigmine is an acetylcholinesterase (AChE) inhibitor used to treat a number of heart conditions in human due to its high affinity to the enzyme target. AChE positive neuron fibers make up a significant portion of the heart's conduction system and sensory nerves, and AChE enzyme-based imaging agents could be useful in studying parasympathetic function in the heart. In vivo biomedical imaging technique positron emission tomography (PET) coupled with appropriate radiopharmaceuticals has become a clinically valuable and accepted diagnostic tool to image heart diseases. [¹¹C]Pyridostigmine and its analogues were synthesized for evaluation as new potential PET imaging agents for heart AChE. The quaternary amine tracers were prepared by N-[¹¹C]methylation of *meta*-, *para*- and *ortho*- substituted pyridine analogue precursors using [¹¹C]methyl triflate and isolated by solid-phase extraction (SPE) purification procedure in 50-65% radiochemical yields.

284.

NEW SYNTHETIC ROUTE TO D-TUBOCURARINE FRAGMENT MOLECULES.

Harish S. Parikh, Karen S. Kirschbaum, and Ronald A. Hill, Department of Basic Pharmaceutical Sciences, The University of Louisiana at Monroe, School of Pharmacy, Monroe, LA 71209, Fax: 318-342-1737, harishparikh@hotmail.com

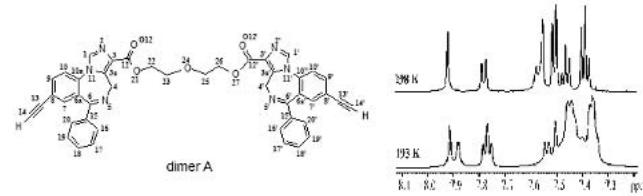
d-Tubocurarine (d-TC), the classical nicotinic neuromuscular blocker, is also a well-known 5-HT₃R antagonist. To further investigate the pharmacophoric components of d-TC binding to nAChRs and 5-HT₃Rs, we have been synthesizing abbreviated substructures (i.e., the "fragmentation" approach), in which broader variation is attainable vs. semisynthetic transformations of curare-class natural products. Our previously reported synthetic scheme incorporates late-stage boronic acid coupling. We now report an alternative method, in which requisite diaryl ethers are obtained in the first step, thus enabling a more convenient subsequent

elaboration to various secondary, tertiary, and quaternary amine target molecules. This new general approach also opens the door to a total synthesis of d-TC, and thus, a much wider range of full-structure analogs than are now available.

285.

STUDY OF THE STRUCTURE-ACTIVITY RELATIONSHIPS OF GABAA-BENZODIAZEPINE RECEPTOR BIVALENT LIGANDS BY LOW TEMPERATURE NMR SPECTROSCOPY AND X-RAY ANALYSIS. Dongmei Han¹, F. Holger Försterling¹, Xiaoyan Li¹, Jeffrey R. Deschamps², Hui Cao¹, and James M. Cook¹. (1) Department of Chemistry, University of Wisconsin-Milwaukee, 3210, N. Cramer Str, Milwaukee, WI 53211, Fax: 414-229-5530, dmhan99@uwm.edu, (2) Naval Research Laboratory

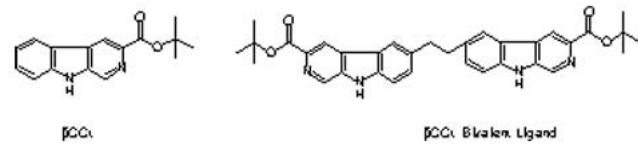
The stable conformations of GABAA-benzodiazepine receptor bivalent ligands which contained linkers of different length were determined by low temperature NMR spectroscopy and confirmed by single crystal X-ray analysis. ¹HNMR, ¹³CNMR, COSY, PECOSY, NOESY, ROESY and HSQC etc were run at variable temperatures in both protic and aprotic polar solvents. The results indicate the behavior in solution mirrors that in the solid state. The linear conformation is important for these dimers to access the BzR binding site and exhibit potent *in vitro* affinity. Bivalent ligands which folded back upon themselves did not bind to Bz receptors. Analysis of the results of this study reveals the type and length of linker play an important role in the conformation of bivalent ligands and the affinity at BzR in these series. This will help to design bivalent ligands in the future.



286.

SEARCH FOR BENZODIAZEPINE/GABA_A SUBTYPE SELECTIVE BIVALENT LIGANDS THAT REVERSE ALCOHOL SELF-ADMINISTRATION. Wenyuan Yin¹, Chunchun Zhang¹, Srirama Sarma V.V. Pvillela¹, Harry June², and James Cook¹. (1) Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, Fax: 414-229-5530, wenyin@uwm.edu, (2) Department of Psychology, IUPUI

To elucidate the role of specific GABA_A/benzodiazepine receptor subtypes in regulating alcohol reinforcement, a number of active β -carbolines has been synthesized and evaluated. Previously, a study with orally active β -carboline-3-carboxylate t-butyl ester (β CCt) examined the role of α_1 receptor subtypes within the ventral pallidum (VP) on alcohol self-administration. Currently, bivalent β CCt ligands which contain two antagonist pharmacophores within the same molecule were synthesized and tested for receptor binding affinity. Examination of the α_1 pharmacophore/receptor model with these bivalent ligands supports a linear binding conformation and supports the hypothesis that the GABA_A/BzR receptor contains a large binding pocket in the L_{D1} region. These results support a new avenue for the design of clinically safe and effective drugs against alcoholism. Recent synthetic studies as well as affinities of these ligands will be presented.



287.

DEVELOPMENT OF SELECTIVE LIGANDS FOR BENZODIAZEPINE RECEPTOR SUBTYPES BY MANIPULATING THE STEREOCHEMISTRY OF OPTICALLY ACTIVE BZR LIGANDS. Xiaoyan Li¹, John R. Atack², and James M. Cook¹. (1) Department of Chemistry, University of Wisconsin-Milwaukee, 3210 N. Cramer, Milwaukee, WI 53211, Fax: 414-229-5530, xli@uwm.edu, (2) Merck Sharpe & Dohme Research Laboratories, Harlow, Essex CM20, 2QR, U. K

The synthesis and *in Vitro* Affinities of a series of optically active (S)-enantiomers imidazobenzodiazepines at six recombinant GABA_A/BzR subtypes expressed from human cell lines are described. The binding affinities of these framework constrained (rigid) ligands provide evidence for the first time which indicates the conformational preference for BzR ligands is highly conserved at

these six recombinant receptor subsites. The configurational preference for the (S)-enantiomers in both series suggests that pharmacophoric descriptors H₁, H₂, and L₁ are the same in these six binding subsites in agreement with previous modeling studies. Recent results in this area will be reported.

288.

SYNTHESIS OF A NOVEL ISOQUINOLINONE DERIVATIVE AS HIGHLY POTENT PARP INHIBITORS. *Futoshi Shiga, Yasuo Takano, Takahiro Kanda, Tetuya Kimura, Jun Asano, Kumi Ishikawa, Jun-ichi Ishiyama, Tomoyuki Kawai, and Tsuyoshi Anraku, Discovery Research Laboratories, Kyorin Pharmaceutical CO., LTD, 2399-1, Nogi, Nogi-machi, Shimotuga-gun, Tochigi 329-0114, Japan, Fax: +81-280-57-1293, futoshi.shiga@mb.kyorin-pharm.co.jp*

Poly(ADP-ribose)polymerase (PARP), a nuclear enzyme activated by single-stranded DNA breaks, has been thought to play an important role in the development of post-ischemic neuronal damage. Excessive activation of PARP induced by cerebral ischemia results in cellular energy depletion, and subsequently neurodegeneration. Therefore, a PARP inhibitor may have a potential as a therapeutic agent for treating acute stroke. Although many research groups have reported PARP inhibitors, there have been no conclusive clinical trials showing a therapy for acute stroke. Our research efforts have focused on synthesizing novel PARP inhibitors with good neuroprotective effects *in vivo*. Recently we have designed and synthesized a novel isoquinolinone derivative based on nicotinamide that is a known PARP inhibitory compound. As a result, we found the most effective compounds, in particular 4-(4-(N,N-Dimethylamino-methyl)phenyl)-5-hydroxyisoquinolinone is highly potent, has good effects in a rat model of MCAOR, and its salts have useful water solubility for use in an injection.

289.

ENHANCEMENT OF NERVE GROWTH FACTOR'S CELLULAR EFFECTS WITH A VERBENACHALCONE DERIVATIVE. *Li-An Yeh¹, Deepa Padmanaban¹, Pei Ho¹, Xuechao Xing¹, Patricia Rowley², Lee Jae Morse², Roderick J. Jensen², and Gregory D. Cuny¹.* (1) *Laboratory for Drug Discovery in Neurodegeneration, Brigham and Women's Hospital and Harvard Medical School, 65 Landsdowne St., 4th Floor, Cambridge, MA 02139, Fax: 617-768-8606, gcuny@rics.bwh.harvard.edu*, (2) *Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School*

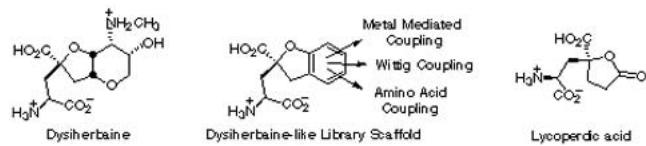
A decrease in neurotrophic support, such as nerve growth factor (NGF), is among the suspected causes of neurodegenerative diseases, such as Alzheimer's disease (AD). A considerable amount of data generated from both *in vitro* and *in vivo* experiments has demonstrated NGF's neuroprotective role. However, administration of exogenous NGF has failed to exhibit efficacy in several human clinical trials. Studies have found that NGF protein and mRNA levels remain either unchanged or are increased in certain brain regions of AD patients. However, the high affinity NGF receptor (i.e. TrkA) expression was reduced and its lower affinity receptor (i.e. p75NTR) expression was increased in AD patients. This has lead to a hypothesis that dysfunction of NGF support in neurodegenerative diseases may involve inadequate signal transduction as opposed to insufficient levels of the neurotrophin. Therefore, molecules that augment the cellular response to NGF may prove beneficial in treating Alzheimer's disease and other dementias. Recently, the natural product verbenachalcone was reported to enhance NGF's ability to stimulate neurite outgrowths from PC12D cells and to be devoid of this activity in the absence of the neurotrophin. This presentation will describe the synthesis of a verbenachalcone derivative, its NGF enhancing effects on both neurite outgrowth in PC12 cells and neuroprotection from serum starvation in N2a cells, and the results of differential gene expression profile experiments as an initial investigation into the compound's mechanism of action.

290.

SYNTHESIS OF NOVEL IONOTROPIC GLUTAMATE RECEPTOR LIGANDS BASED ON (S)-(+)–LYCOPERICID ACID AND (—)–DYSIHERBAINE. *Jamie L. Cohen, and A. R. Chamberlin, Department of Chemistry, University of California, Irvine, Irvine, CA 92612, jlcohen@uci.edu*

Due to their unique structures, glutamate-containing natural products serve as lead compounds for the design of novel glutamate receptor ligands to investigate neurotransmission in the CNS. In our continuing efforts to identify potent and selective probes of the glutamate receptors, this presentation will describe

recent progress toward the construction of a small molecule library based on the potent glutamate receptor agonist (—)–Dysiherbine. The synthesis of a 5-deoxy analogue of (S)–(+)–Lycopericid acid, a non-proteinogenic α -amino acid with structural similarities to glutamic acid, will also be presented.



291.

SYNTHESIS OF NOVEL, CONFORMATIONALLY-RESTRICTED ANALOGS OF GLUTAMATE AND PRELIMINARY ANALYSIS OF THEIR ACTIVITY AT RECEPTORS AND TRANSPORTERS. *Charles M. Thompson¹, Jason E. Mullins², Jean-Louis G. Etoga², and Richard J. Bridges¹.* (1) *Center for Structural and Functional Neuroscience, University of Montana, Dept of Biomedical and Pharmaceutical Sciences, Missoula, MT 59812, Fax: 406-243-4643, cmthomp@selway.umt.edu*, (2) *Department of Chemistry, University of Montana*

Much of the current understanding of glutamate neurotransmitter system proteins has been advanced through the strategic design and development of conformationally-restricted analogs of glutamate. The efficiency of this strategy is exemplified by the selective agonists that define the five major classes of glutamate receptors (mGluR & iGluR) and several excitatory amino acid transporters (EAAT's). Despite numerous advances in analog design, new glutamate analogs are needed that show both the desired efficacy and importantly, selectivity between receptor and transporter binding. We previously reported (*Bioorg. Med. Chem. Lett.* 2002, 12, 3209) a Diels-Alder based preparation of cyclohexenyl-trans-3,4-conformationally restricted glutamate analogs that showed selectively toward EAAT2. Using a similar synthetic approach, we now report the preparation of substituted piperidine-trans-2,3-diacids as glutamate analogs. The synthesis and preliminary biological activity (*in vitro*) will be presented.

292.

SYNTHESIS AND EVALUATION OF NOVEL SMALL-MOLECULE CGRP ANTAGONISTS. *C. Blair Zartman¹, Ian M. Bell¹, Steven N. Gallicchio¹, Samuel L. Graham¹, Stefanie A. Kane², Yvonne M. Leonard³, John Malley², Cynthia Miller-Stein⁴, Ruth Rutledge², Christopher Salvatore², Joseph P. Vacca¹, Audrey Wallace³, and Theresa Williams¹.* (1) *Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, blair_zartman@merck.com*, (2) *Molecular Pharmacology, Merck Research Laboratories*, (3) *Pharmacology, Merck Research Laboratories*, (4) *Drug Metabolism, Merck Research Laboratories*

Calcitonin Gene-Related Peptide (CGRP) is a 37-amino acid neuropeptide which is widely distributed in various tissues including the CNS. CGRP-containing nerves are closely associated with blood vessels and CGRP's most pronounced effect is vasodilation. This vasodilatory effect and the elevated levels of CGRP associated with migraine headache attacks initiated the investigation of antagonists of the CGRP receptor as a possible treatment for migraine and pain. Recently, Boehringer Ingelheim reported the first selective small molecule CGRP antagonist for the human CGRP receptor. This compound has entered human clinical trials as an iv agent for the treatment of migraine. Our project team is attempting to identify an orally bioavailable CGRP antagonist and a structurally novel non-peptide lead was identified from screening. Extensive SAR studies defined the key pharmacophoric elements and provided insight into the bioactive conformation. These compounds are competitive with CGRP but, in contrast to other non-peptide antagonists, their activity is affected by the presence of divalent cations. Ultimately, optimization of this series produced a low molecular weight CGRP receptor antagonist with excellent pharmacokinetic properties in both rat and dog.

293.

SYNTHESIS AND PHARMACOLOGY OF POTENTIAL SITE SPECIFIC COCAINE ABUSE TREATMENT AGENTS: EXPLORATION OF THE PHENYL GROUP INTERACTION AT THE DOPAMINE TRANSPORTER. *Susanna Coons¹, Howard M. Deutsch², David M. Collard², and Margaret M. Schwei³. (1) Department of Chemistry, Georgia Institute of Technology, 770 State St, School of Chemistry and Biochemistry, Atlanta, GA 30313, Fax: 404-894-7452, gt7116b@prism.gatech.edu, (2) School of Chemistry and Biochemistry, Georgia Institute of Technology, (3) School of Medicine, Mercer University*

(-)Cocaine, a major drug of abuse, binds to the dopamine transporter (DAT) blocking the reuptake of dopamine (DA) and therefore disrupting normal DA neurotransmission between neurons. It also possesses the ability to block the uptake of serotonin at the serotonin transporter (5-HTT) and norepinephrine at the norepinephrine transporter (NET). LR5182, an amine-substituted 2-phenylbicyclo[2.2.2]octane, is also a potent and somewhat selective inhibitor of WIN 35,428 binding at the DAT and [³H]DA uptake. This has led to our interest in derivatives of LR 5182 as potential therapeutic agents to combat cocaine addiction. Structure-activity studies involving modification of the rigid framework (stereochemistry, substituents on the bicyclic framework and phenyl ring, type of amine, etc.) allowed for a plethora of target molecules to be synthesized to explore binding to the DAT. A series of 2,5-disubstituted and 2,6-disubstituted bicyclo[2.2.2]alkane derivatives were synthesized and tested for inhibition of [³H](DA) uptake, [³H]WIN 35,428 binding at the DAT, and [³H]citalopram at the 5-HTT in order to establish further structure-activity relationships (SARs).

294.

SYNTHESIS AND SAR OF BIPHENYL CANNABINERGIC RECEPTOR LIGANDS.

Xinzhong Lai, Pusheng Fan, and Alexandros Makriyannis, Center for Drug Discovery, Department of Pharmaceutical Sciences, University of Connecticut, 372 Fairfield Road, U-92, Storrs, CT 06269, Fax: 860-486-4998, Xin-Zhong.Lai@huskymail.uconn.edu

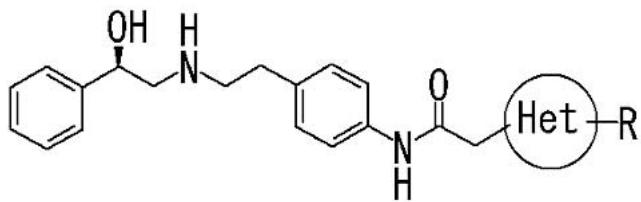
Cannabinoids are known for their therapeutic potential in various conditions, including analgesia, glaucoma, and as modulators of the immune system. These tricyclic terpenoids exert their biological effects by interacting with CB1 and CB2, two GPCRs. While the CB1 cannabinoid receptor is found in the brain and other tissues, CB2 is present in cells associated with the immune system but is absent from the brain. Hence CB2 selective ligands have excellent potential as novel antinociceptive and immunoregulatory agents without the undesired psychotropic effects of cannabinoids.

The biphenyl non-classical cannabinoids are a class of cannabinergic ligands that selectively bind to the CB2 receptor with high affinity. We used successfully the Suzuki reaction to obtain a series of these analogs with diverse substituents. This series was tested for affinities for CB1 and CB2. The results of these tests allowed us to develop a useful profile on the SAR of this class of ligands.

295.

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF HETEROARYLACETAMIDE DERIVATIVES AS A POTENTIAL HUMAN BETA 3 ADRENERGIC RECEPTOR AGONIST. *Tatsuya Maruyama, Takayuki Suzuki, Ken-ichi Onda, Masahiko Hayakawa, Tetsuo Matsui, Toshiyuki Takasu, Itsuro Nagase, Makoto Takeuchi, and Mitsuaki Ohta, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co.,Ltd, 21, Miyukigaoka, Tsukuba, Ibaraki 3058585, Japan, Fax: +81-29-852-2971, maruyat@yamanouchi.co.jp*

Activation of beta 3 adrenergic receptors has been shown to increase metabolic rate, and this stimulation of these receptors with selective agonists is an attractive approach for the treatment of obesity and type II diabetes. We have studied the structure-activity relationships of a series of phenylethanolamine derivatives possessing a substituted heteroarylacetamide pharmacophore. Among these compounds, some analogs having a nitrogen-containing heteroaromatic ring, such as imidazole, tetrazole, thiazole and pyridine ring, exhibited potent human beta 3 agonistic activity with excellent selectivity against other human beta receptor subtypes. And some of them also showed the improvement of hyperglycemia in diabetic KK mice. The synthesis and SAR of these compounds will be discussed.



296.

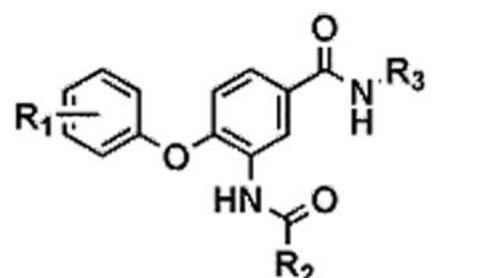
MODELING STUDIES ON THE STRUCTURE AND ACTIVITY RELATIONSHIPS OF MELATONIN ANALOGUES. *Neil C. Mitchell¹, Jerry A. Darsey¹, Cesar M. Compadre², Alice Price², and R. Lilia Compadre². (1) Department of Chemistry, University of Arkansas at Little Rock, 2801 South University Avenue, Little Rock, AR 72204, Fax: 501-569-8838, ncmttchell@ualr.edu, (2) Biomedical Visualization Center, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences*

Melatonin is a therapeutic agent in the treatment of delayed sleep-phase syndrome, seasonal depression, jet lag, shift work disturbances, and other neurodegenerative disorders. Melatonin is also a physiological hormone synthesized at night in the pineal gland from tryptophan. Unfortunately, various undesirable side effects from therapeutic doses of melatonin have been reported, including: tachycardia, altered sleep patterns, confusion, dysphoria, increased seizure activity, psychosis, confusion, sedation, and headache. To gain insight into the molecular features associated with the desired therapeutic and undesired side effects of melatonin, we applied complementary quantitative structure-activity relationships (QSAR) techniques to a database containing biological and physicochemical parameters of over one hundred melatonin analogues. Comparative molecular field analysis (CoMFA) and neural networks were used to analyze the relationships between parameters, which included various electronic indicators obtained from ab initio molecular orbital calculations.

297.

NOVEL POTENT BIARYL-ETHER CONTAINING MELANIN CONCENTRATING HORMONE (MCH) RECEPTOR ANTAGONISTS. *Vu Ma¹, Paul A. Tempest¹, Carlo van Staden², John Salon², Kirk Rorer³, Jamie Baumgartner³, Clarence Hale³, Tony Bannon³, and Christopher Hulme¹. (1) Medicinal Chemistry Technologies, Chemistry Research & Development, Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-480-1337, chulme@amgen.com, (2) Chemistry Research and Development, Amgen, (3) Department of Neuroscience, Amgen*

Obesity has reached epidemic levels worldwide. Of patients who do lose weight, 95% regain all lost weight within 5 years. Currently, 5 million patients are treated for obesity with an estimated 55 million going untreated in the US alone. Melanin Concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide that is an important regulator of energy balance in rodents. Evidence for its role as a modulator of energy balance include: 1) its location in brain areas associated with the control of feeding. 2) MCH levels regulated in fasted and obese animals. 3) Intracerebroventricular administration increases food intake. 4) MCH knockout mice are lean and hypophagic. 5) MCH over-expressing mice have an obese phenotype. This poster reveals a library-derived discovery of a novel highly potent, functionally active, small molecule series of MCH1 receptor antagonists with the generic structure shown below 1. SAR studies and preliminary pharmacokinetic data are revealed.



298.

NOVEL POTENT TETRAZOLE CONTAINING MELANIN CONCENTRATING HORMONE (MCH) RECEPTOR ANTAGONISTS—MULTI-COMPONENT REACTIONS LEAD THE WAY. *Paul A. Tempest¹, Thomas Nixey¹, Vu Ma¹, Guity Balow¹, Carlo van Staden², John Salon², Kirk Rorer³, Jamie Baumgartner³, Clarence Hale³, Tony Bannon³, Randall Hungate¹, and Christopher Hulme¹. (1) Medicinal Chemistry Technologies, Chemistry Research & Development, Amgen, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-480-1337, pttempest@amgen.com, (2) Chemistry Research and Development, Amgen, (3) Department of Neuroscience, Amgen*

Obesity has reached epidemic levels worldwide. Of patients who do lose weight, 95% regain all lost weight within 5 years. Currently, 5 million patients are treated for obesity with an estimated 55 million going untreated in the US alone. Melanin Concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide that is an important regulator of energy balance in rodents. Evidence for its role as a modulator of energy balance include: 1) its location in brain areas associated with the control of feeding. 2) MCH levels are regulated in fasted and obese animals. 3) Intracerebroventricular administration increases food intake. 4) MCH knockout mice are lean and hypophagic. This poster reveals the one step library-derived discovery of novel highly potent, functionally active tetrazole based small molecule MCH1 receptor antagonists. A rapid hit-lead transition and results from in vivo efficacy studies in fasted rats are also described.

299.

GENERATION OF A 3D INHIBITOR-BASED PHARMACOPHORE MODEL OF THE NOREPINEPHRINE TRANSPORTER (NET) EMPLOYING SUPERPOSITION ANALYSIS. *Erin S. Davis, and John M. Gerdes, Center for Structural and Functional Neuroscience, Department of Chemistry, University of Montana, Missoula, MT 59812, Fax: 406-243-5255*

Modulation of the norepinephrine transporter (NET) provides therapeutic opportunities for the treatment of select CNS disorders. In an effort to design new selective and potent NET inhibitor agents, a 3D pharmacophore model has been constructed. A training set of four structurally diverse and semi-rigid NET inhibitor ligands has been used to create three plausible multi-ligand superposition models. Challenge of the models with structurally unique test ligands has enabled the selection of a high priority NET pharmacophore construct. The computational methods utilized to produce the NET inhibitor model, the preliminary validation of the pharmacophore and the initial evaluation of its predictive qualities will be discussed.

300.

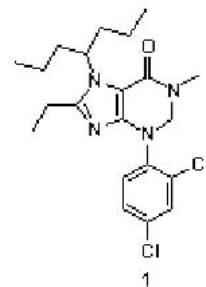
DEVELOPMENT OF VALIDATED QSAR MODELS OF P2Y12 RECEPTOR ANTAGONISTS AND THEIR APPLICATION TO DATABASE MINING. *Scott Oloff, Department of Pharmacology, University of North Carolina, Laboratory for Molecular Modeling, CB # 7360 Beard Hall, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, Fax: 919-966-0204, scott_loff@med.unc.edu, Raed Khashan, Laboratory for Molecular Modeling, Laboratory for Molecular Modeling, School of Pharmacy, University of North Carolina, Robert Plourde Jr., Department of Chemistry, Inspire Pharmaceuticals, Inc, 4222 Emperor Blvd, Suite 470, Durham, NC 27703, rplourde@inspirepharm.com, and Alexander Tropsha, Laboratory for Molecular Modeling, School of Pharmacy, University of North Carolina*

The P2Y12 receptor is a validated target for inhibiting platelet aggregation, and there is a medical need for improved platelet aggregation inhibitors to treat conditions such as myocardial infarction, stroke, and pulmonary embolism. Support Vector Machines and k-Nearest Neighbors were employed to build validated QSAR models for 49 structurally diverse P2Y12 receptor antagonists. Molecular connectivity indices calculated with the MolConnZ program were used as descriptors, and the datasets was divided into multiple training and test sets. Multiple SVM and kNN models were built for each training/test set pair; ones that could accurately predict a test set were considered valid. Validated models were used to screen virtual and available chemical libraries. Of the database compounds experimentally tested, the largest error of activity prediction was only 0.7 log orders. These results suggest that thoroughly validated models can afford accurate database screening to rapidly identify promising candidate molecules.

301.

CORTICOTROPIN RELEASING FACTOR-1 RECEPTOR ANTAGONISTS: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 1,2,3,7-TETRAHYDRO-6H-PURIN-6-ONE AND 3,7-DIHYDRO-1H-PURINE-2,6-DIONE DERIVATIVES. *Richard A. Hartz¹, Kausik K. Nanda¹, Charles L. Ingalls¹, Vijay T. Ahuja¹, Thaddeus F. Molski², Gail K. Mattson², Ge Zhang², Harvey Wong³, Robert Zaczek², George L. Trainor¹, and Paul J. Gilligan¹. (1) Discovery Chemistry, Pharmaceutical Research Institute, Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492-7660, Fax: 203-677-7702, richard.hartz@bms.com, (2) Neuroscience Biology Department, Bristol-Myers Squibb Co, (3) Metabolism and Pharmacokinetics Department, Bristol-Myers Squibb Co*

Corticotropin releasing factor (CRF) receptors have become an important target for medicinal chemists, as CRF is believed to be the primary regulator of the hypothalamic-pituitary-adrenal (HPA) axis. A growing body of evidence suggests that CRF1 receptor antagonism offers considerable therapeutic potential in the treatment of diseases resulting from elevated levels of CRF, such as anxiety and depression. A series of novel 1,2,3,7-tetrahydro-6H-purin-6-one and 3,7-dihydro-1H-purine-2,6-dione derivatives were synthesized and evaluated as corticotropin releasing factor-1 (CRF1) receptor antagonists. Compounds within this series, represented by compound 1 ($IC_{50}=5.0\text{ nM}$), were found to be highly potent CRF1 receptor antagonists. The synthesis and structure-activity relationships of this series will be presented.

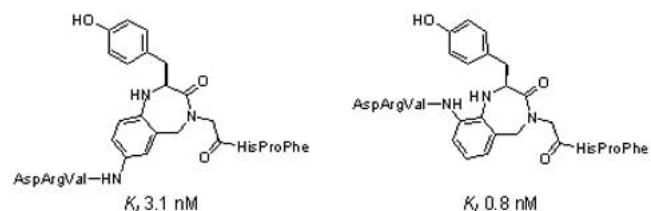


302.

NEW AT₂ SELECTIVE ANGIOTENSIN II ANALOGUES ENCOMPASSING A BENZODIAZEPINE-BASED TURN MIIMETIC. *Ulrika Rosenström¹, Christian Sköld¹, Gunnar Lindeberg¹, Milad Botros², Anders Karlén¹, and Anders Hallberg¹. (1) Department of Medicinal Chemistry, Uppsala University, BMC, Box 574, Uppsala 753 21, Sweden, Fax: +46-18-4714474, ulrika@orgfarm.uu.se, (2) Department of Biological Research on Drug Dependence, Uppsala University*

Important information on the bioactive conformation of biologically active peptides may be obtained by studies of rigid peptidomimetics. The structural requirements for the interactions of angiotensin II (Ang II) with its AT₁ and AT₂ receptors have been the subject of this study.

A turn conformation centered around Tyr in Ang II has been suggested when the peptide interacts with the AT₁ receptor. The bioactive conformation adopted in activation of/binding to the AT₂ receptor has so far been less investigated. To obtain more information about Ang II and its interaction with the AT₂ receptor we have synthesized benzodiazepin-based turn mimetics that were incorporated into Ang II. AT₂ selective pseudopeptides have been identified in the biological evaluation.



303.

SYNTHESIS AND BIOLOGICAL EVALUATION OF FATTY ALCOHOL PHOSPHATES AS LPA RECEPTOR LIGANDS. *Gangadhar G. Durgam¹, Tamas Virag², Michelle D. Walker², Gabor Tigyi², and Duane D. Miller¹. (1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Room 327, Memphis, TN 38163, (2) Department of Physiology, University of Tennessee Health Science Center*

Lysophosphatidic acid (LPA), a member of the phospholipid growth factor (PLGF) family, exerts pleiotropic biological effects, such as activating platelet aggregation and affecting cell proliferation, apoptosis, migration, and cell shape. LPA elicits its biological effects through the activation of three-EDG family G protein-coupled receptors LPA1, LPA2 and LPA3, and transcription factor PPAR g. A complete understanding of the physiological and pathological role of these receptors and the desire to pharmacologically exploit the differences in their ligands recognition requires the development of receptor subtype-specific agonists and antagonists. Here, we report the synthesis and pharmacological characterization of unsaturated C10-C18 fatty alcohol phosphates (FAPs), head group modified stable saturated C10-C18 fatty alcohol phosphonates, and saturated and unsaturated C10-C18 thiophosphate analogs. We identified oleoyl fatty alcohol thiophosphate as a novel agonist at all three receptors and several LPA1 and LPA3 selective antagonists with IC50 values in the nanomolar range. Supported by NIH-CA 92160

304.

DOWN-REGULATION OF CYP1A-1 EXPRESSION BY 4-NONYLPHENOL AND BISPHENOL A IN MOUSE HEPATOMA HEPA-1C1C7 CELLS. *Hye Gwang Jeong¹, Ji Young Kim¹, Dong Weon Shin¹, Eun Hee Han¹, Tae Cheon Jeong², and Eung-Seok Lee². (1) College of Pharmacy, Chosun University, 375 Seoseok-dong, Dong-gu, Kwangju 501-759, South Korea, Fax: +82-62-222-5414, hgjeong@chosun.ac.kr, (2) College of Pharmacy, Yeungnam University*

4-Nonylphenol (NP) is a degradation product of a widely used non-ionic surfactant group, alkylphenol polyethoxylates that are mainly found as an intermediate in the chemical manufacturing industry. 4,4'-isopropylidenediphenol (Bisphenol A; BPA) is a monomer in polycarbonate plastics and a constituent of epoxy and polystyrene resins that are used extensively in the food-packaging industry and it has been shown to possess estrogenic properties. Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either NP, BPA or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or in combination to assess the role of NP or BPA in the process of cytochrome P450 1A1 (Cyp1a-1) induction. Treatment of Hepa-1c1c7 cultures with TCDD induced Cyp1a-1, as indicated by analysis of 7-ethoxyresorufin O-deethylase (EROD) activities. NP or BPA alone did not affect the activity of Cyp1a-1-specific EROD; in contrast, TCDD-induced EROD activities were markedly reduced in the concomitant treatment of TCDD and NP or BPA in a dose dependent manner. Treatment with tamoxifen, an antiestrogen that acts through the estrogen receptor did not affect the suppressive effects of NP or BPA on TCDD-induced EROD activity. TCDD-induced Cyp1a-1 mRNA levels were markedly suppressed in the concomitant treatment of TCDD and NP or BPA consistent with EROD activity. Transient transfection assay using dioxin-response element (DRE)-linked luciferase revealed that nonylphenol reduced transformation of the aryl hydrocarbons (Ah) receptor. These results suggest the down regulation of the Cyp1a-1 gene expression by NP or BPA in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear Ah receptor but not through estradiol receptor. (Supported by the grant No. R01-2003-000-10560-0 from KOSEF, Korea).

305.

SUPPRESSIVE EFFECTS OF GENISTEIN ON TCDD-INDUCIBLE CYP1A-1 EXPRESSION IN MOUSE HEPATOMA HEPA-1C1C7 CELLS. *Hye Gwang Jeong¹, Ji Young Kim¹, Eun Hee Han¹, Tae Cheon Jeong², and Eung-Seok Lee². (1) College of Pharmacy, Chosun University, 375 Seoseok-dong, Dong-gu, Kwangju 501-759, South Korea, Fax: +82-62-222-5414, hgjeong@chosun.ac.kr, (2) College of Pharmacy, Yeungnam University*

The phytoestrogen and principal isoflavone in soy, genistein, has adverse effects on reproductive physiology in rodents. Cultured mouse hepatoma Hepa-1c1c7 cells were treated with either genistein or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or in combination to assess the role of genistein in the process of cytochrome P450 1A1 (Cyp1a-1) induction. Treatment of Hepa-1c1c7 cultures with TCDD induced Cyp1a-1, as indicated by analysis of 7-ethoxyresorufin

O-deethylase (EROD) activities. Genistein alone did not affected the activity of Cyp1a-1-specific EROD activities; in contrast, TCDD-induced EROD activities were markedly reduced in the concomitant treatment of TCDD and genistein in a dose dependent manner. Treatment with tamoxifen, an antiestrogen that acts through the estrogen receptor did not affect the suppressive effects of genistein on TCDD-induced EROD activity. TCDD-induced Cyp1a-1 mRNA levels were markedly suppressed in the concomitant treatment of TCDD and genistein consistent with EROD activity. Transient transfection assay using dioxin-response element (DRE)-linked luciferase revealed that genistein reduced transformation of the aryl hydrocarbons (Ah) receptor. These results suggest the down regulation of the Cyp1a-1 gene expression by genistein in Hepa-1c1c7 cells might be antagonism of the DRE binding potential of nuclear Ah receptor but not through estradiol receptor. (Supported by the grant No. R01-2003-000-10560-0 from KOSEF, Korea).

306.

EFFECTS OF HEPATIC CYTOCHROME P450 BY GLYCRRHIZIN AND BETA-GLYCRRHETINIC ACID IN MICE. *Hye Gwang Jeong¹, Chul Yong Choi², Young Chul Chung², Kyung Sik Jung¹, Hyung Gyun Kim¹, Tae Cheon Jeong³, and Eung-Seok Lee³. (1) College of Pharmacy, Chosun University, 375 Seoseok-dong, Dong-gu, Kwangju 501-759, South Korea, Fax: +82-62-222-5414, hgjeong@chosun.ac.kr, (2) Division of Food Science, Chinju International University, (3) College of Pharmacy, Yeungnam University*

There have been numerous reports of the antihepatotoxic activity of glycyrrhizin, a triterpenoid saponin of Glycyrrhiza glabra L. as well as its genuine aglycon, 18beta-glycyrrhetic acid, but little attention has been paid to regarding to its effects of cytochrome P450 (P450). Therefore, in this study, we investigate the effects of glycyrrhizin and 18beta-glycyrrhetic acid on the constitutive and inducible microsomal activities and expression of P450 in rat. The administration of glycyrrhizin and 18beta-glycyrrhetic acid to rats significantly decreased the activities of microsomal pentoxyresorufin O-dealkylase and aniline hydroxylase, representative activities of P4502B1/2 and P4502E1, respectively, in a dose- and time-dependent manner. However, ethoxyresorufin O-deethylase and methoxyresorufin O-demethylase, a representative activity of P4501A1 and P4501A2, respectively, was decreased to a lesser extent. Glycyrrhizin and 18 beta-glycyrrhetic acid also decreased inducible monooxygenase activities in the same manner. Suppressions of P450 isozyme expression occurred in glycyrrhizin and 18beta-glycyrrhetic acid treated hepatic microsomes, as determined by immunoblot analysis in a manner consistent with that of the enzyme activity levels. These results suggest that glycyrrhizin and 18beta-glycyrrhetic acid may act as a more specific suppressor for P4502B1/2 and P4502E1 than P4501A2. (Supported by the grant No. R01-2003-000-10560-0 from KOSEF, Korea).

307.

EFFECTS OF PENTACYCLIC TRITERPENE URSOLIC ACID ON METALLOTHIONEIN EXPRESSION. *Hye Gwang Jeong, College of Pharmacy, Chosun University, 375 Seoseok-dong, Dong-gu, Kwangju 501-759, South Korea, hgjeong@chosun.ac.kr, and Young Nan Cho, Research Center for Proteineous Materials, Chosun University*

Ursolic acid (UA) is a pentacyclic triterpene compound isolated from many kinds of medicinal plants and has anti-inflammatory and anti-proliferative properties. The expression of metallothioneins is increased in the liver of UA-treated mouse although the mechanism is not identified. In this study, the transcriptional regulation of metallothioneins by UA was investigated. The expression of mouse metallothionein (MT) 1 and 2 were increased in the Hepa1C1C7 cells after treatment of UA by the result of reverse transcription-polymerase chain reaction (RT-PCR). This increment was inhibited by addition of RU486, an antagonist of glucocorticoid receptor (GR). This means that GR could mediate the induction of MT expression by UA. UA, whose structure is similar to glucocorticoid, seems to bind with GR and activate it. Additionally, MT was induced by supplementation of the conditioned medium obtained after treatment of UA into Raw 264.7 cells. UA could induce the production of various cytokines, like as TNF- α (tumor necrosis factor α), IL-1 β and IL-6, in Raw 264.7 cells. It could be possible that the cytokines, secreted from UA-treated Raw 264.7 cells, contribute to induce the expression of MT. TNF- α was existed in the conditioned medium according to enzyme-linked immunosorbent assay (ELISA) using the antibodies against TNF- α , IL-1 β and IL-6, respectively. Therefore, the transcription of MT

could be induced by activated GR and cytokines, like as TNF α and IL-6, in the mouse liver after the treatment of UA.

308.

EFFICIENT SYNTHESIS 2-5A TETRAMER AND 2-5A-ANTISENSE MORPHANT

CHIMERA. *Longhu Zhou, Edgar R. Civitello, and Paul F. Torrence, Department of Chemistry, Northern Arizona University, Box 5698, Flagstaff, AZ 86011-5698, Fax: 928-523-8111, longhu.zhou@nau.edu*

2-5A-Antisense can target a chosen RNA sequence which is destroyed through localized activation of latent 2-5A-dependent RNase L. 2-5A-Antisense has been employed 1) to block respiratory syncytial virus (RSV) replication through targeting of RSV genomic and mRNAs and 2) to halt proliferation of t tumors by telomerase template RNA destruction. Morpholino analogues (morphants) of oligonucleotides have superior resistance to enzymatic degradation. To capitalize on this and to endow morphants with a catalytic mode of action, we prepared 2-5A-morphants, a new class of 2-5A-antisense. Thus we synthesized 2-5A tetramer from 2,3-N-tetrabenzoyladenosine by solution phase phosphoramidite chemistry. 2-5A-Morpholino antisense chimera was generated from 2-5A tetramer through NaIO₄ oxidation to the dialdehyde, followed by coupling with 5'-aminotether thymidine morphant 15-mer. Reduction of the unisolated intermediate aminal with NaBH₃CN gave 2-5A-T15-Morphant. RNase L assays show that this novel 2,5-A-antisense has biological activity comparable the earlier versions of 2-5A-antisense based upon a phosphodiester antisense domain.

309.

STRUCTURE-ACTIVITY RELATIONSHIPS IN A SERIES OF PYRIDINIUM-BASED

CATIONIC LIPIDS. *Marc A. Ilies¹, William A. Seitz¹, Betty H. Johnson², Aaron Miller², E. Brad Thompson², and Alexandru T. Balaban¹. (1) Marine Sciences Department, Texas A&M University at Galveston, 5007 Avenue U, Galveston, TX 77551, Fax: 409-740-4787, iliesm@tamug.tamu.edu, (2) Department of Biological Chemistry and Genetics, University of Texas Medical Branch*

A new approach for generating cationic lipids for gene delivery bearing pyridinium polar heads was described [1]. It is based on the reaction of pyrylium salts with primary amines thus generating the polar head and the linker of the non-viral transfection vector in a single, high yield step. Various aliphatic and aromatic linkers can be introduced this way, allowing also the facile choice of the counterion of the final positively charged species. Extensive structure-activity correlations were done for structures 1-3 at the level of the linker, hydrophobic anchor and counterion in order to identify the most effective features in terms of transfection efficiency for these compounds.

The optimization of the composition, method of generating the cationic lipids—DNA complexes (lipoplexes), and their efficiency on several tumor cell lines is also discussed.

310.

IDENTIFICATION OF GLUTATHIONE CONJUGATES OF 1,2-DIBROMOPROPANE

IN FEMALE BALB/C MICE BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY. *Tae Cheon Jeong¹, Sang Kyu Lee¹, Eung-Seok Lee¹, Jaeick Lee², Dong Hyun Kim², and Hye Gwang Jeong³. (1) College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Kyongsan 712-749, South Korea, Fax: +82-53-811-3871, taecheon@ymail.ac.kr, (2) Bioinformation and Bioanalysis Research Center, KIST, (3) College of Pharmacy, Chosun University*

In our previous studies, 1,2-dibromopropane caused hepatotoxicity and immunotoxicity in female BALB/c mice, as well as a reduction of hepatic glutathione level. In the present studies, the formation of glutathione conjugates of 1,2-dibromopropane was investigated in vivo. The following four metabolites were identified in livers 12 hr after the treatment by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI MS): M1, 2-hydroxypropyl glutathione; M2, 2-oxopropyl glutathione; M3, N-acetyl-S-(2-hydroxypropyl)-L-cysteine; and M4, N-acetyl-S-(2-oxopropyl)-L-cysteine. Ions of individual conjugates were observed at m/z 366, 364, 222 and 220, respectively. In the sera isolated from same animals, only two kinds of mercapturic acid (i.e., M3 and M4) were observed by the LC-ESI MS. Characteristic product ions for the identification of M1, M2, M3 and M4 were observed at m/z 237, 235, 204 and 202, respectively. When 1,2-dibromopropane was administered to female BALB/c mice orally at the doses of 150, 300 and 600 mg/kg once, the amounts

of glutathione and mercapturic acid conjugates were dose-dependently produced in the liver homogenates. In addition, the mercapturic acid conjugates of 1,2-dibromopropane were dose-dependently produced in the sera. When the production of metabolites from 1,2-dibromopropane was investigated in livers following an oral treatment with 600 mg/kg of 1,2-dibromopropane for 6, 12, 24 and 48 hr, all metabolites were detected maximally 6 hr after the treatment. Our present results were consistent with our previous studies that an oral administration with 1,2-dibromopropane reduced the hepatic content of glutathione. (Supported by the grant No. R01-2003-000-10560-0 from KOSEF, Korea).

311.

MECHANISM OF ACTION OF ANTIDIABETIC EFFECT OF THE AQUEOUS EXTRACT OF ANNONA SQUAMOSA LEAVES. *Achyut Narayan Kesari¹, Geeta Watal², Rajesh Kumar Gupta¹, and Vibha Tandon³. (1) Department of Chemistry, University of Allahabad, Allahabad, India, 48/12 A.B.K. Banerjee Road, New Katra, Allahabad, India, Allahabad- 211002, India, achyut_nar@yahoo.co.in, (2) Department of Chemistry, University of Allahabad, Allahabad, India, (3) Dr. B.R.Ambedkar Centre for Biomedical Research, ACBR, University of Delhi, Delhi, India*

Aqueous extract of *Annona squamosa* (Fam: Annonaceae) leaves was found to lower blood sugar level and improved Glucose tolerance in diabetic experimental animals. It also brought fall in total Cholesterol with increase in HDL cholesterol and fall in LDL cholesterol. To understand the mechanism of action rat pancreatic islets were incubated with plant extract and insulin release was measured. Isolated psoas muscle of rat was incubated with the extract to check the effect on insulin. The extract was found to enhance the insulin release from isolated pancreatic islets as well as uptake of glucose in psoas muscle. The effect of glucose absorption through small intestine was studied invitro. It was found that glucose absorption through the isolated intestinal segment was inhibited. The present study reveals that the aqueous extract of *A. squamosa* leaves seems to act by enhancing insulin level from pancreatic islets and increased utilization of glucose in muscle

312.

PHOTOPHYSICAL PROPERTIES OF PROTRPTYLINE AND DIBENZOCYCLOHEPTENE. *Dionne Hernández, Carmelo García, Rolando Oyola, and Luis E. Piñero, Department of Chemistry, University of Puerto Rico at Humacao, UPRH - Chemistry, Humacao, PR 00791-4300, j_suarez@webmail.uprh.edu*

Protriptyline hydrochloride (PTL-HCl) is a tricyclic antidepressant and a skin photosensitizing agent in humans. The mechanism of this cutaneous photosensitivity is still unknown. It has been proposed that the excited states and/or some of the short-lived intermediates of this drug are directly associated with its *in vivo* phototoxic effects. Therefore, we have measured the photophysical properties of the protonated (PTL-HCl) and unprotonated (PTL) forms of protriptyline and the parent molecule (dibenzocycloheptene, DBCH). The ground and excited singlet state properties (molar extinction coefficient, fluorescence quantum yield, fluorescence lifetime and fluorescence quenching constant) were measured in methanol and acetonitrile. The ground state properties are solvent and protonation independent. The fluorescence quantum yield and lifetime, on the other hand, strongly depends on the solvent. The quenching studies were done in acetonitrile using diethyl and triethyl amine as quencher. The Stern-Volmer quenching constants are also solvent dependent.

This work has been supported by NIH-MBRS Grant S06GM08216 and Puerto Rico - AMP

313.

PURIFICATION AND CHARACTERIZATION OF THE MAJOR CELL-ASSOCIATED HEPARAN SULFATE OF MOUSE VARIOUS TISSUES. *Wenjun Mao, Marine Drugs and Foods Institute, Ocean University of China, 5 Yushan Road, Qingdao 266003, China, wenjunmqd@hotmail.com*

Heparan sulfate is the polysaccharide portion of a ubiquitous proteoglycan, localized on cell surface membranes and in the extracellular matrix of all animal tissues. It consists primarily of unsulfated disaccharide [-4] α -D-*N*-acetyl-glucosamine (1 \rightarrow 4)- β -D-glucuronic acid- (1 \rightarrow) and [-4]- α -D-*N*-acetyl-glucosamine-6-sulfate- (1 \rightarrow 4)- β -D-glucuronic acid (or α -L-iduronic acid)-(1 \rightarrow). Virtually any cationic protein is capable of binding heparan sulfate under physiological conditions and the activity of these proteins is often modified by

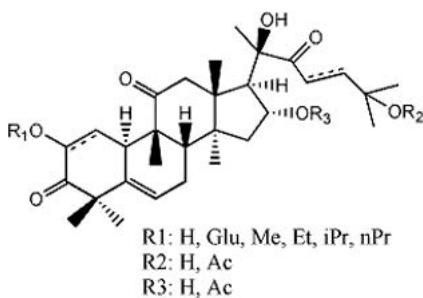
the binding. The current study investigated the structure and characterization differences of heparan sulfate isolated from mouse various tissues. A method for the isolation and purification of heparan sulfate from mouse various tissues is described. The purity and identity of the heparan sulfate was determined following treatment with heparin lyases by polyacrylamide gel electrophoresis. The disaccharide compositional analysis determined by treatment with a mixture of heparin lyases followed by high-resolution capillary electrophoresis. The structural characterization is further studied by MALDI-TOF-MS, one-dimensional and two-dimensional NMR spectroscopy.

314.

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP OF CUCURBITACIN HOMOLOGUES USING HEPG2 CELLS AND CHROMATOGRAPHIC HYDROPHOBICITY INDEXES. *Judit Bartalis*, Chemistry & Biochemistry

Department, South Dakota State University, SH 121, Box 2202, Brookings, SD 57007, Fax: 605-688-6364, judit_bartalis@sdsstate.edu, and Fathi T. Halawehi, Chemistry & Biochemistry, South Dakota State University

The water-octanol partition coefficient, log P, is the most popular quantitative scale to model partitioning processes in an interphase such as biological membranes. It is a commonly encountered descriptor in computer assisted drug design, a well-recognized and extremely productive tool in pharmaceutical chemistry. It is shown recently that chromatographic hydrophobicity index, CHI, is more accurate than log P to measure hydrophobicity of compounds. CHI is determined by fast gradient RP-HPLC using a standard mixture proposed in the literature. The conventional bulk phase shake flask method gives poor values for compounds with log P>4, such as cucurbitacins, while the chromatographic mobile phase/stationary phase resemble better the biological membrane/water interface. Cucurbitacins, which are mainly tetracyclic and highly oxygenated triterpenes from plants, have been used in many folk medicine preparations. In our search for new and less toxic cucurbitacins for hepatoprotective activity we studied cucurbitacins lipophilicity to get an indication of their biological activity and use this data for computational chemistry. A total of 18 cucurbitacins were isolated and semi synthesized for this purpose. Cucurbitacins were isolated from Cucurbita texana squash and Bitter Hawkesbury watermelon. Glucosides were hydrolyzed enzymatically and cucurbitacins E and I were further modified by etherification and/or acetylation. Their structures were identified by spectroscopic means. For CHI determination RP-HPLC system was utilized with C18 column and acetonitrile in buffered water at pH=7 as eluent. Because CHI values depend on the choice of organic modifier, the test mixture and cucurbitacins lipophilicity will be determined in methanol as well. The CHI of cucurbitacins is compared with their in vitro toxicity on HepG2 human hepatoma cell lines. Our results indicate, that CHI determination is a high-throughput alternative to log P evaluation and cucurbitacins lipophilicity directly correlate with their toxicity on HepG2 cells.



315.

RAPID ESTIMATION OF DRUG STABILITY TO OXIDATIVE DEGRADATION USING A COULOMETRIC ELECTROCHEMICAL SYSTEM. *Ian N Acworth, Darwin Asa*

Mark Bowers, David Meyer, and Paul H Gamache, ESA Inc, 22 Alpha Road, Chelmsford, MA 01824, Fax: 978-250-7065, ianacworth@esainc.com, dasa@esainc.com

Chemical oxidative degradation is a concern throughout drug discovery and development. A compound's tendency for chemical oxidative degradation is related to, and can be predicted from, its electrochemical (EC) redox potential. This study examines the utility of coulometric array EC techniques for estimation of chemical stability. Numerous compounds of differing stabilities were analyzed by FIA with a coulometric electrochemical oxidation system. Data obtained from

this method were compared to those from forced degradation studies and to data from literature. Correlation to existing oxidative stability data was excellent and allowed rapid estimation of the unknown's stability, oxidation potential and the relative rank ordering for stability within a set of compounds. This method has high throughput (less than one minute per analyte). Utilization of electrochemical oxidation facilitates easy and rapid compound stability profiling and is a simple technique that can be widely implemented in many areas of the drug discovery process.

316.

RAPID SYNTHESIS, IDENTIFICATION AND QUANTITATION OF PHARMACEUTICAL METABOLITES, USING AN ELECTROCHEMICAL SYSTEM.

David F. Meyer, Darwin Asa, Michael Granger, Ian N. Acworth, and Paul H. Gamache, ESA Inc, 22 Alpha Road, Chelmsford, MA 01824, Fax: 978-250-7065, dmeyer@esainc.com, dasa@esainc.com

In drug discovery and development, the synthesis of substances derived from investigational compounds is a major obstacle. These substances can include degradants and metabolites derived from oxidative processes. Several studies utilizing coulometric electrochemical (EC) flow cells online with mass spectrometry (MS) have demonstrated that electrochemically-derived products often correspond to biological metabolites and chemical degradants. The objective of these studies is to use defined-potential electrolysis as a means to generate small-scale synthesis of metabolites, to facilitate pharmaceutical discovery and development. Electrochemical flow cells were used on-line with MS, and initial outcome using ng quantities of material have indicated selective and semi-quantitative formation of Phase I metabolic products. Included are those obtained via S and P oxidation, N-dealkylation, aromatic hydroxylation and dehydrogenation. Phase II metabolites can be formed by the addition of a nucleophile either upstream or downstream of the EC cell. Results from optimal conditions for product yield and purity will be discussed from the perspective of metabolite and degradant structural identification and LC-MS method development.

317.

REGIO-SELECTIVE SYNTHESIS AND IN VITRO EVALUATION OF INSULIN-ALBUMIN CONJUGATES. *A. Boutros, C. Soucy, X Huang, I. Pellerin, K. Thibaudeau, M. Robitaille, R. Léger, and D. Bridon, ConjuChem Inc, 225*

President-Kennedy Ave., Suite 3950, Montreal, QC H2X 3Y8, Canada, boutros@conjuchem.com

We have demonstrated that bioconjugating peptides to Cys34 of Human Serum Albumin (HSA) prolong their presence in plasma by protecting them against enzymatic degradation and reducing elimination while retaining most of their desired biological activity. Insulin has a short half-life within the circulation due to the high clearance rate and in view of this, diabetic patients need to receive multiple daily subcutaneous injections to maintain glycemic control. The bioconjugation of insulin to HSA should increase the plasma half-life by adopting the pharmokinetic profile of albumin. Structurally, there are three available amino groups on insulin, namely the α -amino groups Gly A1 and Phe B1 and the Ne-Lys B29. We report the regio-selective synthesis, purification and analysis of three 3-maleimidopropionic acid (MPA) derivatives via the exploitation of the different amine pKas. For example Gly-(A1)-MPA-Insulin was synthesized in one step with a 37% yield and the two other isomers were synthesized through selective protection with good yields. In vitro efficacy of the corresponding Insulins-MPA:Cys34 HSA bioconjugates was assessed by receptor binding assay and induction of glucose uptake.

318.

SIMPLIFYING REACTION WORKUP IN THE COPPER-CATALYZED COUPLING OF ALKYLAMINES AND ARYL IODIDES. *Jack Liu, and Peter Rahn, Biotage AB, PO Box 8006, Charlottesville, VA 22906, jliu@biotage.com*

This paper illustrates the application of catch and release methodology to simplify sample workup and purification. High-boiling-point solvents used by synthetic chemists poses a significant problem when purifying the desired compounds because the solvents are difficult to evaporate, and in many cases they are incompatible with normal-phase chromatography. To solve the problem, it typically requires a laborious extracting procedure. As exemplified by the copper-catalyzed reactions for coupling alkylamines and aryl iodides in this study, however, the reaction crude is loaded onto a unique cartridge that enable

the compounds of interest to be retained while the high-boiling-point ethylene glycol, which is used as reaction solvent, and impurities pass through as waste, eliminating aqueous extracting procedure. A series of amines was synthesized and purified by the catch and release method. The cartridge with retained compounds can be either released directly by adding a competing solvent or coupled to column chromatography to simplify synthesis and purification.

319.

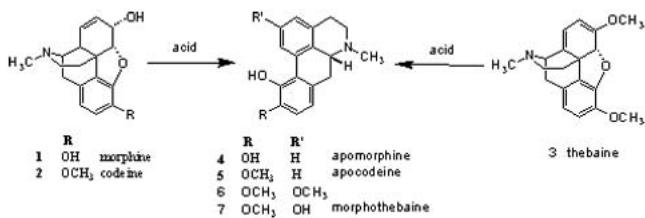
SYNTHESIS OF GAMMA-TURN MIMETICS UTILIZING FAST MICROWAVE-ASSISTED PALLADIUM COUPLINGS. *Jennie Georgsson, Mats Larhed, and Anders Hallberg, Department of Medicinal Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden, jennie@orgfarm.uu.se*

Peptides are often not suitable as drugs due to lack of oral bioavailability and insufficient metabolic stability. The use of peptidomimetics is one way to address this issue. Our approach is to convert peptides by an iterative displacement of peptide fragments for organic moieties to finally afford more drug-like molecules. We are addressing this issue by utilizing Angiotensin II (Asp¹-Arg²-Val³-Tyr⁴-Ile⁵-His⁶-Pro⁷-Phe⁸) as a model peptide. The overall aim is to develop an efficient synthetic route, synthesize turn mimetics and introduce them into Angiotensin II. We are in particular addressing the affinity for the AT2 receptor. Since a turn structure centred around the Tyr⁴ in Angiotensin II has been suggested, the target benzene scaffold of this study was designed to partly fill the criteria of an inverse γ -turn. In the synthesis, fast microwave-assisted palladium-catalyzed couplings such as carbonylation and cyanation were used in combination with more traditional methods.

320.

MICROWAVE-PROMOTED, HIGH YIELD SYNTHESIS OF R(-)-APOMORPHINE AND ITS CONGENERS. *Csaba Csutoras, John L. Neumeyer, and Xuemei Peng, Medicinal Chemistry Laboratory, Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, csutoras@mclean.harvard.edu, xpeng@mclean.harvard.edu*

It is well known, that morphine (1) and its congeners rearrange with concentrated acids to aporphines, that have pharmacological activity as dopamine agonists. The yields of such acid-catalyzed rearrangements are generally low, due to thermal sensitivity of the products, as well as the formation of side products. We recently investigated the acid-catalyzed rearrangement of morphine (1), codeine (2) and thebaine (3) using microwave promoted rearrangement, instead of the traditional techniques. In all cases a significant improvement in the yields were obtained, and the crude products had >95% purity (HPLC). (This work was supported in part by a grant from the Brantman Family Foundation.)

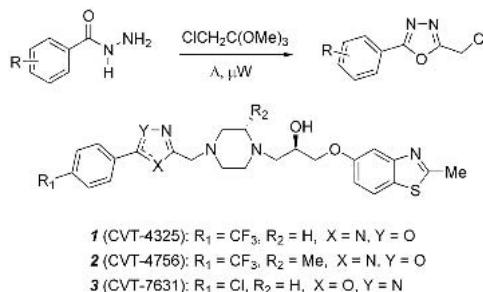


321.

MICROWAVE-ASSISTED SYNTHESIS OF 1,3,4-OXADIAZOLE ANALOGS OF CVT-4325: A METABOLIC SHIFT PHARMACOLOGICAL AGENT. *Reina Natero¹, Dmitry Koltun¹, Elfatih Elzein¹, Yuan Li², Marie Nguen², Suresh Kerwar², Dewan Zeng², Nancy Chu³, Daniel Soohoo³, Jia Hao³, Victoria Maydanik³, David Lustig³, Heather Fraser², and Jeff Zablocki¹. (1) Department of Bioorganic Chemistry, CV Therapeutics, 3172 Porter Drive, Palo Alto, CA 94304, reina.natero@cvt.com, (2) Department of Pharmacological Sciences, CV Therapeutics, (3) Department of Pre-Clinical Development, CV Therapeutics*

Compounds that inhibit palmitoyl-CoA oxidation in mitochondrial preparations, such as CVT-4325 (**1**, IC₅₀=380 nM), have been shown to decrease the rate of fatty acid oxidation and subsequently increase the rate of glucose oxidation (metabolic shift) in rat isolated working hearts. We report a new method for the synthesis of 1,3,4-oxadiazoles utilizing the microwave-assisted reaction of acyl hydrazides with 2-chloro-1,1,1-trimethoxyethane. The resulting 2-chloromethyl-1,3,4-oxadiazoles were converted into analogs of CVT-4325 and CVT-4756 (IC₅₀=180 nM), which showed good inhibitory potency in the palmitoyl-CoA

oxidation assay and favorable pharmacokinetic properties (e.g. CVT-7631, **3**, IC₅₀=690 nM, F=21%, t_{1/2}=4 h).



322.

TRACELESS SYNTHESIS OF HYDANTOIN BY FOCUSED MICROWAVE IRRADIATION.

Ming-Juan Lee, and Chung-Ming Sun, Department of Chemistry, National Dong Hwa University, 1, Sec.2, Da-Hsueh Rd, Shou-Feng, Hualien 974, Taiwan, Fax: 011-886-8630110, d9012002@em92.ndhu.edu.tw, cmsun@mail.ndhu.edu.tw

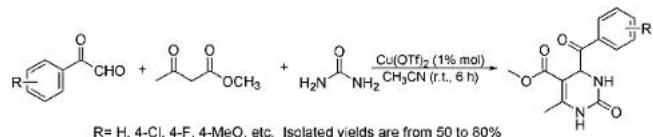
An efficient, microwave-assisted method for the liquid-phase combinatorial synthesis of 1,3-disubstituted hydantoin has been developed. Chloroacetyl chloride was directly anchored to HO-PEG-OH and subsequently reacted with various primary amines in microwave cavity. The PEG bound secondary amine coupled with isocyanates and concomitant cyclization-cleavage step occurred in mild basic condition by microwave flash heating. The desired products were then liberated from the soluble matrix in modest yield and high purity.

323.

PROCESS IMPROVEMENTS ON NOVEL DIHYDROPRIMIDINONE SCAFFOLDS.

Qiang Yu, Liangfu Huang, Shanshan Yao, Zhiqiang Fang, and Wuping Ma, SynChem, Inc, 1700 S. Mount Prospect Rd, Des Plaines, IL 60018, Fax: 847-298-2439, qyu@synchem.com

We have investigated the application of arylglyoxals as substrates in the Biginelli multi-component reactions (MCRs). The novel dihydropyrimidine products obtained possess interesting pharmacological activities. In addition, the extra ketone group which is inherited from the arylglyoxal, provides a potential site for further transformation. Herein we report a more effective and environment friendly process by employing Cu(OTf)₂ as a catalyst. This remarkable procedure that was performed under ambient temperature led to easier isolation, high yields, and an energy saving reaction.



324.

PARALLEL SOLUTION/SOLID-PHASE LIBRARY SYNTHESIS EMPLOYING NATURAL PRODUCT AND PRIVILEGED SCAFFOLDS FOR DRUG DISCOVERY.

Cy O. Ogbu, Maher Qabar, and Hwa-OK Kim, Aurigene Discovery Technologies, Inc, 99 Hayden Ave, Lexington, MA 02420, Fax: 781-541-6742, cy_o@aurigene.com

The desire to acquire small molecule drug candidates has continued to drive discovery research in many of today's drug companies. Rapid and efficient lead optimization requires enormous amounts of relevant chemical and biological data, based upon which the desired drug-like properties can be attained. At Aurigene, we offer structurally diverse molecular scaffolds, from natural

products as well as privileged and proprietary structures in the design and synthesis of focused libraries. We utilize these libraries to iteratively focus on selected chemical spaces and target classes for rapid lead generation and optimization. Our approach to this concept will be highlighted.

325.

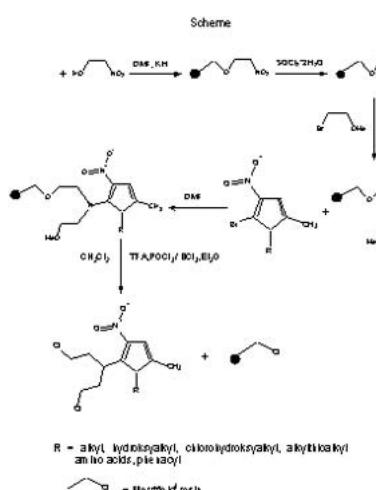
SOLID PHASE SYNTHESIS OF NITROIMIDAZOLE DERIVATIVES OF NITROGEN MUSTARD.

Iwona Weidlich, and Stanislaw Sobiak, Department of Chemical Technology of Drugs/Faculty of Pharmacy, University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland, Fax: 61-8-659-566, iweidlic@am.p.edu.pl

The SAR studies in a group of nitroimidazole derivatives of nitrogen mustard showed that the title compounds are the most important moieties for promising inhibition of the growth of HeLa cancer cell lines. We obtained some group of compounds by regular organic synthesis methods. Substitution of bromine by means of secondary amines such as bis(2-hydroxyethyl)amine and halogenation of the product resulted in formation of N-substituted derivatives of bis(2-chloroethyl)amino-2-methyl-4-nitroimidazole. We encountered a lot of problems both in synthesis and purifications. In this research we managed to synthesize a new anticancer derivatives and designed the synthesis using combinatorial method (solid Merrifield resin), which was accomplished in only 5 steps (Scheme), promises to get compounds more easily and with better yield.

Structures of obtained compound, after removing from Merrifield's resin, were confirmed by ¹H, ¹³C NMR, and MS.

Scientific research was supported by Center of Committee of Scientific Investigations (Poland)-project # 3P05D03523.



326.

SOLID STATE STRUCTURES AND DYNAMICS OF BIOLOGICALLY RELEVANT WATER CHAIN ASSEMBLIES STABILIZED BY IMIDAZOLE CHANNELS. MODELS OF WATER CHAIN STRUCTURES FOUND IN AQUAPORIN AND PROTON TRANSPORT PROTEINS. *Lionel E. Cheruzel, Maxim S. Pometun, Matthew R. Cecil, Mark S. Mashuta, Richard Wittebort, and Robert M. Buchanan, Department of Chemistry, University of Louisville, Brook Street, Louisville, KY 40292, Fax: 502-852-8149, lionel.cheruzel@louisville.edu*

One-dimensional water chains constitute an important form of water that participates in many fundamental biological processes. For example, water is transported through red blood cell membranes and renal proximal tubules by aquaporin1 (Agre et al., Am. J. Physiol. 1993, 265, 463). Other aquaporin proteins are important to the biological function of the nicotinic receptor M2α, influenza A M2 virus, and are associated with several human diseases (e.g. diabetes). Water chains also assist in proton translocation through membranes by functioning as "proton wires". Interestingly, little is known of the structure of water chains in these systems and the intrinsic properties leading to their stabilization is under intense investigation. We have discovered that certain imidazole compounds stabilize 1-D water chains relevant to the biological systems mentioned above. Crystal structures and preliminary ssNMR studies have established the waters undergo reorientation dynamics, important to the construction of functional proton wires and biomimetic aquaporin-like channels.

327.

SYNTHESIS OF NOVEL ACRYLAMIDES AS KCNQ2 POTASSIUM CHANNEL OPENERS.

Yong-Jin Wu¹, Huan He¹, Li-Qiang Sun¹, Pierre Dextraze², Jie Chen¹, John E. Starrett¹, Valentin K. Gribkoff³, Christopher G. Boissard³, Rick L. Pieschl³, Ronald J. Knox³, David G. Harden³, David D. Weaver³, Mark W. Thompson³, Qi Gao⁴, DeDong Wu⁴, Joanne T. Natale³, and Steven I. Dworetzky³. (1) Department of Neuroscience Chemistry, Bristol-Myers Squibb Company Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, Fax: 203-677-7702, yong-jin.wu@bms.com, huan.he@bms.com, (2) Bristol-Myers Squibb Co, Candiac, Canada, (3) Neuroscience Drug Discovery, Bristol-Myers Squibb Co, (4) Bristol-Myers Squibb Pharmaceutical Research Institute

A new class of acrylamides was synthesized, and the effects of these analogs were determined on mKCNQ2 currents heterologously expressed in HEK 293 cells using whole-cell patch-clamp. These acrylamides were subsequently tested on rat brain hippocampal slices. These KCNQ2 openers showed significant activity in reducing neuronal hyperexcitability. In addition, these effects were shown to be enantiomer specific. Thus, KCNQ2 openers may have therapeutic potential for the treatment of CNS disorders characterized by neuronal hyperexcitability, such as migraine, epilepsy and neuropathic pain.

328.

SUBSTITUTED SULFONAMIDES ACTING AS CARBONIC ANHYDRASE INHIBITORS.

Bruhaspathy Miriyala¹, Estela Maris F. Muri², and John S. Williamson². (1) Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, 427 FASER HALL, University, MS 38677, Fax: 662-915-5638, bru@olemiss.edu, (2) Department of Medicinal Chemistry, University of Mississippi

The carbonic anhydrases (Cas, EC 4.2.1.1) are ubiquitous zinc enzymes, present in Archaea, prokaryotes and eukaryotes. In higher vertebrates, including humans, 14 different CA isozymes or CA-related proteins (CARP) have been described with very different subcellular localization and tissue distribution. These enzymes catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion, and are thus involved in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate between metabolizing tissues and lungs, pH and CO₂ homeostasis, bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes (Supuran, C.T.; Scozzafava, A.; Casini, A. Med. Res. Rev. 2003, 23, 146-189). Two main classes of CA inhibitors (CAIs) are known: the metal complexing anions, and the unsubstituted sulfonamides, which bind to the Zn(II) ion of the enzyme. Owing to their unique properties for CA inhibition, unsubstituted sulfonamides are mainly used as antiglaucoma agents, antithyroid drugs, hypoglycemic agents and also as some novel types of anticancer agents. Here, we describe the synthesis of a novel series of substituted sulfonamide derivatives designed to act as CA inhibitors with considerable specificity to the physiologically active Type II isozyme.

329.

PYRIDINE ACETIC ACIDS: POTENT, ORALLY ACTIVE AND HIGHLY SELECTIVE ALDOSE REDUCTASE INHIBITORS (ARIS).

Leo S. Geraci¹, David E. Gunn², Al Sabetta¹, Diane Sawicki¹, Susan Cannan¹, Anne Carrington¹, Janet Sredy³, Thomas DiCioccio¹, Michael C. Van Zandt¹, Alberto Podjarny⁴, and Ossama El-Kabbani⁵. (1) Chemistry, The Institute For Diabetes Discovery, 23 Business Park Drive, Branford, CT 06405, Fax: 203-315-4002, leo.geraci@ipd-discovery.com, (2) Bayer Corporation, (3) ADSERVIO, (4) IGBMC, UPR de Biologie Structurale, (5) Montash University, Victorian College of Pharmacy

Hyperglycemia is a major contributor to diabetic complications such as neuropathy, retinopathy, nephropathy and cardiovascular disease. Aldose reductase (ALR2), present in the tissues where complications arise, is the first enzyme in the polyol pathway and reduces some of the excess glucose to sorbitol using NADPH as a cofactor. The accumulation of intracellular sorbitol and the osmotic stress induced is implicated in the progression of diabetic complications. Other factors contributing to complications related to glucose flux through the polyol pathway include reductive stress (high ratio of NADH/NAD⁺), non-enzymatic glycation and the impairment of anti-oxidant defense. Therefore, an aldose reductase inhibitor developed as a treatment should slow the progress of diabetic complications. Selectivity of ARIs relative to aldehyde reductase (ARL1), a detoxifying enzyme with 65% sequence homology to aldose reductase, is also important. A novel series of substituted pyridine acetic acids was discovered and developed as part of our aldose reductase inhibitor program. Numerous compounds exhibited low inhibitory

concentrations ($IC_{50} < 10$ nM) and enzyme selectivity greater than 25,000 (ratio of ARL1(IC_{50})/ARL2(IC_{50})). Several were extremely effective *in vivo* by lowering nerve and lens sorbitol levels and possessed favorable pharmacokinetic profiles. The discovery, synthesis, structure-activity relationships, X-ray data and biological activity of these inhibitors will be discussed.

330.

SYNTHESIS AND CHARACTERIZATION OF A PARTIALLY WATER-SOLUBLE PENTAAZADENTATE PORPHYRIN-LIKE GADOLINIUM (III) COMPLEX FOR

PHOTODYNAMIC THERAPY APPLICATION. *Paul M Barron¹, Fengqi Guo¹, and Wenfang Sun². (1) Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105, Paul.Barron@ndsu.nodak.edu, (2) Department of Chemistry, North Dakota State University*

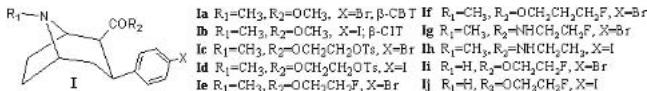
Photosensitizers with absorption in the near infrared spectral range (700–900nm) and high efficiency to generate singlet oxygen are desired for photodynamic therapy application. A partially water-soluble pentaazadentate porphyrin-like Gadolinium (III) complex is synthesized and characterized. The photophysical properties and the pH sensitive characteristics of this complex have been studied. The Q-band of this Gadolinium (III) complex appears at 880nm. The pH studies show that a blue shift (~257nm) of the Q-band occurs at pH above 6.7, possibly due to oxygen-bridged dimerization. The study of the quantum yield of singlet oxygen generation is currently undergoing. These preliminary results suggest that the Gadolinium (III) complex may be a promising candidate for Photodynamic therapy.

331.

SYNTHESIS AND DA TRANSPORTER AFFINITIES OF A SERIES OF NOVEL PHENYLTROPANE DERIVATIVES AS POSITRON EMISSION TOMOGRAPHY (PET)

IMAGING AGENTS. *Xuemei Peng¹, Ao Zhang², John L. Neumeyer², Nora S. Kula³, and Ross J. Baldessarini⁴. (1) Natural Pharmacia International, Inc, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, xpeng@mclean.harvard.edu, (2) Alcohol and Drug Abuse Research Center, McLean Hospital, Harvard Medical School, (3) Department of Psychiatry, Mailman Research Center, McLean Hospital, Harvard Medical School, (4) Department of Psychiatry, Mailman Research Center, McLean Hospital, Harvard Medical School*

A series of novel fluoroalkyl-containing tropane derivatives Ie–Ij were synthesized from cocaine. beta-CBT (Ia) and beta-CIT (Ib) were obtained according to the procedure we reported previously (Bioorg. Med. Chem. Letters 11, 3049, 2001). N-Demethylation of Ie afforded the nortropanes II. The desired esters and amides Ic–Ih were obtained by treatment of the appropriate acid chloride with the corresponding fluoroalkyl alcohols or amines. The binding affinities for DAT, SERT and NET were determined via competitive binding assays. The nortropane fluoroethyl ester Ij showed the highest SERT binding affinity ($K_i=0.18$ nM) and the highest SERT selectivity. Two tosylates (Ic and Id), prepared as precursors for the [^{18}F] labeled PET agents, also showed high affinity for DAT. (Supported by NIH Grant NS 40587).



332.

SYNTHESIS AND EVALUATION OF JNJ 17161911 AS A MODULATOR OF GASTROINTESTINAL MOTILITY AND PAIN RESPONSE.

Janet L. Ralbovsky, Joseph G. Lisko, Jeffrey M. Palmer, Paul R. Wade, Raymond W. Colburn, Sandra L. McKenney, Kristen M. Chevalier, Paul R. Saunders, Edward S. Kimball, Nathaniel H. Wallace, Craig R. Schneider, Santosh V. Coutinho, Pamela Hornby, and Wei He, Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, L.L.C, Welsh & McKean Rds, P.O. Box 776, Spring House, PA 19477, Fax: 215-628-4985, jralbovs@prdus.jnj.com

In an effort to search for new agents to treat GI disorders, JNJ 17161911 was screened in several *ex vivo* and *in vivo* models of GI motility and somatic pain. JNJ 17161911, an orally bioavailable 4-substituted piperidine, acts as a modulator of GI motility by decreasing propulsive transit and inhibiting smooth muscle contraction. It significantly delayed distal colonic propulsion in conscious mice in a dose-dependent manner following both oral and intraperitoneal administration. Studies of its actions on isolated smooth muscle strips and

intact segments from mice and guinea pigs indicated that JNJ 17161911 had an inhibitory effect on spontaneous and electrical field-stimulated colonic contractility. Its oral anti-nociceptive effect was demonstrated in mice by significant reduction of pain responses in both the Mouse Abdominal Irritant Test and the Hotplate Test. The synthesis of JNJ 17161911 and its further profiling will also be described.

333.

SYNTHESIS, IDENTIFICATION, CHARACTERIZATION AND QUANTIFICATION OF IN VITRO AND IN VIVO GLUTATHIONE (GSH) METABOLITES OF 1- AND 2-BROMOPROPANE. *Eung-Seok Lee, Arjun Basnet, Yoon-Soo Moon, Eun-kyung Kim, Long-Xuan Zhao, Dae-Ok Kim, Sang-Kyu Lee, Yurongdong Jahng, Whigun Chae, and Tae Cheon Jeong, College of Pharmacy, Yeungnam University, 214-1 Daedong, Kyongsan 712-749, South Korea, Fax: +82-53-811-3871, eslee@yu.ac.kr*

2-Bromopropane, $\text{CH}_3\text{CHBrCH}_3$, is widely utilized as a cleansing solvent in electronic factories. It has been reported that a number of female workers, exposed occupationally to 2-bromopropane, were diagnosed with amenorrhea and male workers with oligospermia in Korea. 1-Bromopropane, $\text{CH}_3\text{CH}_2\text{CH}_2\text{Br}$, known as an alternative to ozone depleting solvents, which has structural similarity to 2-bromopropane, has been reported to be neurotoxic to rats in long-term inhalation exposure. 1- and 2-Bromopropane were also reported as the causative agents for reproductive toxicity and immunotoxicity. The glutathione (GSH) metabolites resulting from *in vitro* and *in vivo* treatment of 1- and 2-bromopropane were detected, identified and characterized. For the facile identification, expected GSH metabolites formed by 1- and 2-bromopropane were chemically synthesized as reference materials (positive controls) and characterized by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, HPLC and LC/MS/MS. For the *in vitro* experiments, the treatment of GSH and S-9 fraction with 1- or 2-bromopropane at a physiological condition (pH 7.4, 37°C) for 1hr produced GSH metabolites, which were identified and quantitated by ESI LC/MS/MS analyses. In addition, time-response and dose-response effects of formation of GSH metabolites were investigated. The treatment of 1- and 2-bromopropane to mice *in vivo* resulted in detection and quantification of GSH metabolites. The present results might explain the detoxification process of 1- and 2-bromopropane.

334.

SYNTHETIC PATHWAY FOR ACANTHOSIDE-D AND ITS ANALOGS. *Haeil Park, and Ngoc Tuyen Truong, College of Pharmacy, Kangwon National University, 192-1, Hyoja-2-dong, Chunchon 200-701, South Korea, haeilp@kangwon.ac.kr*

Acanthoside-D, one of major components of Acanthopanax Cortex, is known as a ginseng-like substance. It has been known to possess diverse biological effects and the synthesis of which poses interesting and often unsolved problems of stereo-control. Although a few interesting syntheses providing this natural product have been reported, a practical route to synthesize acanthoside-D and its analogs for structure-activity relationship study has not yet been explored. We report here a short and efficient synthetic pathway for synthesis of acanthoside-D and its analogs from aryl aldehydes and methyl acrylates via Baylis-Hillman reaction, intermolecular McMurry coupling and intramolecular Mitsunobu cyclization as key reactions.

335.

SYNTHETIC ROUTES TO METABOLITES AND METABOLIC ANALOGUES OF THALIDOMIDE: REFINEMENTS OF THE SYNTHESIS OF 5'-HYDROXYTHALIDOMIDE AND ITS ACYCLIC DERIVATIVES. *Frederick A. Luzzio¹, Damien Y. Duveau¹, Alexander V. Mayorov², and William D. Figg³. (1) Department of Chemistry, University of Louisville, 2320 South Brook Street, Louisville, KY 40292, Fax: 502-852-8149, faluzz01@athena.louisville.edu, d0duve01@louisville.edu, (2) Chemistry, University of Louisville, (3) Cancer Therapeutics Branch, National Cancer Institute*

Thalidomide and its metabolites and metabolic analogues, including structures corresponding to their *in vivo* hydrolysis products, have served as leads to a

number of compounds which are active antiinflammatory, TNF α -inhibitory and antiangiogenic agents. Although it has been established that the glutarimide ring is not essential for antiangiogenic activity, there remains interest in the metabolites which are a result of hydroxylation on the glutarimide ring since there is strong evidence supporting the prodrug nature of thalidomide itself. Our present synthetic efforts in the area of thalidomide metabolites and analogues have included both stereoselective and nonstereoselective routes to 5'-hydroxythalidomide, a compound which is the only glutarimide-derived metabolite isolated from plasma after administration to healthy human male volunteers. Three major synthetic routes have evolved that should provide sufficient quantitites of material for both further derivatization and biological testing. The first route encompasses refinements of a *de novo* synthesis of 4-hydroxyglutamic acid where further derivatization yields the title compound. The second involves a direct synthesis of the derivatized title compound from cyclic precursors without passing through the hydroxylated amino acid intermediate. The third route is centered on a one-carbon homologation sequence of suitably-protected aspartic acid derivatives. All three routes, the latter two which are stereoselective schemes designed to provide each of the possible stereoisomers of the title compound, will be presented.

336.

NUCLEATION OF POLYMERS OF SICKLE CELL ANEMIA HEMOGLOBIN. Peter G. Vekilov, and Oleg Galkin, Department of Chemical Engineering, University of Houston, Houston, TX 77204-4004, Fax: 713-743-4323, vekilov@uh.edu

The primary pathogenic event of sickle cell anemia is the polymerization of the mutant hemoglobin (Hb) S within the red blood cells, occurring when HbS is in deoxy state in the venous circulation. Polymerization is known to start with nucleation of individual polymer fibers, followed by growth and branching via secondary nucleation, yet the mechanisms of nucleation of the primary fibers have never been subjected to dedicated tests. We implement a technique for direct determination of rates and delay times of primary nucleation of HbS fibers, based on direct detection of emerging HbS polymers using optical differential interference contrast microscopy after laser photolysis of CO-HbS. We show that: (i) nucleation throughout these determinations occurs homogeneously and not on foreign substrates; (ii) individual nucleation events are independent of each other; (iii) nucleation induction times agree with an *a-priori* prediction based on Zeldovich's theory; (iv) the critical nucleus contains 11 or 12 molecules. (v) the nucleation rates are of the order of 10^6 – 10^8 cm $^{-3}$ s $^{-1}$. The nucleation rate values are comparable to those leading to erythrocyte sickling *in vivo* and suggest that the mechanisms deduced from *in-vitro* experiments might provide physiologically relevant insights. The nucleation rate values are 9-10 orders of magnitude higher than those known for protein crystals. We also provide evidence that the nucleation of the sickle cell hemoglobin polymers proceeds via a precursor that is likely a metastable droplet of a dense liquid phase, evidenced in such solutions [Galkin et al, PNAS 99, 8479 (2002)], i.e., the nucleation process can be viewed as a superposition of a density and a structure fluctuations. This finding suggests that tools employed to control the dynamics of phase behavior in protein and colloid solutions may be applicable to control the pathogenic polymerization of HbS.

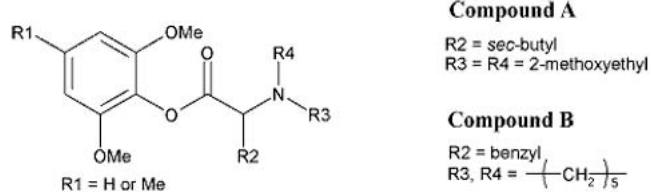
337.

NEW SAR FOR A SERIES OF α -AMINO ACID PHENOLIC ESTER DERIVATIVES WITH INTRAVENOUS ANESTHETIC ACTIVITY. Kirsteen Buchanan, Alison

Anderson, Jonathan Bennett, Alan Byford, Andrew Cooke, David Gemmell, Niall Hamilton, Ed Hutchinson, Petula McPhail, Maurice Maidment, Susan Miller, Donald Stevenson, Hardy Sundaram, and Peter Vijn, Organon Laboratories, Ltd, Newhouse, Lanarkshire, Scotland ML1 5SH, United Kingdom, Fax: 441698-736187, k.buchanan@organon.co.uk

We have previously reported on the SAR and intravenous anesthetic activity in rodents of a series of α -amino acid phenolic ester derivatives of general structure (1). 1,2,3

Here we report the preparation of a series of analogues in which R2 is alkyl, aryl, alkyl ester or, together with R3, cycloalkyl, and R3, R4 are either cycloalkyl or acyclic substituents. Good anesthetic activity was retained in this series and compounds A and B had better potency than propofol and comparable activity to ORG 25435 (R1=Me, R2=Et, R3=R4=2-methoxyethyl). 3

**Compound A**

R2 = sec-butyl
R3 = R4 = 2-methoxyethyl

Compound B

R2 = benzyl
R3, R4 = $\left(-\text{CH}_2-\right)$

338.

EFFICIENT SYNTHESIS AND BIOLOGICAL ACTIVITY OF SACCHARIDE-PORPHYRIN CONJUGATES FOR PHOTODYNAMIC THERAPY (PDT).

Xin Chen, Li Hui, David A. Foster, and Charles Michael Drain, Hunter College & Graduate Center, City University of New York, 695 Park Ave., New York, NY 10021, Fax: 212-772-5332, chenxin@hunter.cuny.edu

The uptake of exogenous molecules such as drugs into cells can arise from a variety of mechanisms that can be broadly classified as active and passive transport. The former requires that the molecules be recognized, selected, and shuttled across the cell membrane. Passive uptake involves diffusion at some point in the process and arises from non-specific cell-molecule interactions. Since the role of saccharides in cell recognition, metabolism, and cell labeling is also well established, the conjugation of saccharides to drugs is an active area of research. Thus, one goal in the use of saccharide-drugs conjugates is to impart a greater specificity toward a given cell type or other target. Tough widely used to treat some cancers and age related macular degeneration, the drugs used in Photo Dynamic Therapy (PDT) display poor chemical selectivity towards the intended targets. Instead, the specific irradiation of the target tissues and the formation of the toxic species *in situ*, are the primary factors that modulate the selectivity in PDT. We report herein a two step method to make non-hydrolyzable sccharide-porphyrin conjugates in high yields. As a demonstration of their properties the selective binding of these compounds to several cancer cell types is examined. It is found that these compounds have greater quantum yields of the triplet than simple porphyrins and that they induce cell death both by necrosis and by apoptosis. Cell migration assays indicate that low concentrations of these compounds significantly reduce cell migration, which indicates a reduction in aggressiveness of the cancer cells.

339.

FLOW CYTOMETRY CORRELATION OF ANTIGEN DISPLAY AND COBALAMIN UPTAKE IN CLINICALLY-DERIVED BLOOD AND BONE MARROW SAMPLES. Yao Shi¹, Leah Hartung², and Charles B. Grissom¹. (1) Department of Chemistry, University of Utah, 315S, 1400E, Salt Lake City, UT 84112-0850, yshi@chem.utah.edu, (2) ARUP Institution

Fluorescently labeled cobalamin was synthesized to determine the cobalamin uptake level in different subtypes of clinically derived acute myelogenous leukemia samples. It is hypothesized that the most immature myeloblasts will take up cobalamin significantly. Flow cytometry was used to characterize the immunophenotypes and to evaluate the uptake level of AML samples. The subtypes of AML with the greatest ability of cobalamin uptake will be treated selectively with targeted cobalamin based drug delivery.

340.

HOW IMPORTANT IS THE B-N INTERACTION IN REGULATING THE FLUORESCENCE INTENSITY IN THE ANTHRACENE BORONIC ACID SYSTEM?

Weijuan Ni¹, Gurpreet Kaur², Binghe Wang², and Stefan Franzen¹. (1) Department of Chemistry, North Carolina State University, Raleigh, NC 27695, weijuanni@yahoo.com, (2) Department of Chemistry, Georgia State University, 33 Gilmer St. SE Unit 8, Atlanta, GA 30303, gkaur@student.gsu.edu

An anthracene based boronic acid fluorescent reporter developed by the Shinkai group has been widely used for the preparation of fluorescent sensors for carbohydrates. However, the detailed mechanism through which the fluorescence intensity changes upon carbohydrate binding is not clear. Originally, it was proposed that the formation of a B-N bond upon carbohydrate binding was the critical contributing factor in regulating the fluorescence of this system. We have systematically examined this issue and found that the B-N bond formation cannot be the reason for the fluorescence intensity change. The detailed experimental results and a new mechanism will be presented.

341.

IDENTIFICATION OF ORALLY BIOAVAILABLE SMALL MOLECULE ACTIVATORS OF GLUCOKINASE. Darren McKerrecher, Joanne V Allen, Michael H Block, Suzanne S Bowker, Scott Boyd, Peter W R Caulkett, Gordon S Currie, Christopher D Davies, Mark L Fenwick, Harold Gaskin, Rodney B Hargreaves, Barry R Hayter, Roger James, Keith M Johnson, Craig Johnstone, Cliff D Jones, John W Rayner, and Rolf P Walker, CVGI Research, AstraZeneca UK, Mereside, Alderley Park, Macclesfield SK10 4TG, United Kingdom, Fax: (+44) 01625 516667, darren.mckerrecher@astrazeneca.com

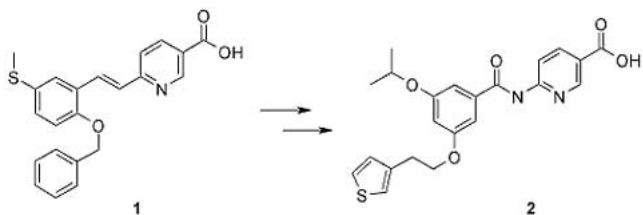
Type 2 Diabetes is a condition of increasing prevalence with significant unmet medical need. Defective hepatic glucose utilisation and pancreatic glucose dependent insulin release both contribute to the hyperglycaemia that characterises Type 2 Diabetes.

Glucokinase (GLK) acts as the rate-limiting step in hepatic glucose utilisation, and controls the glucose-sensitive release of insulin in the pancreas. Activation of GLK should therefore moderate hyperglycaemia by improving hepatic glucose uptake and decreasing the threshold for insulin secretion.

This poster describes the identification and in vitro optimisation of a series of small molecule glucokinase activators.

SAR studies are discussed highlighting progress from 2,5-substituted styryl pyridine acids HTS-leads (eg, 1) to orally bioavailable 3,5-substituted amido pyridine acids (eg, 2) with improved physical properties. Further optimisation of the series will be discussed in due course.

PCT Int. Appl. WO 0300262 (2003); PCT Int. Appl. WO 0315774 (2003); PCT Int. Appl. WO 0300267 (2003)



342.

HEPAVIR B (MB06866): A NOVEL HEPATITIS B ANTIVIRAL THERAPY USING THE HEPDIRECT PRODRUG TECHNOLOGY FOR TARGETING ADEFOVIR TO THE LIVER. K. Raja Reddy, Bheemarao G. Ugarkar, Michael C. Matelich, Jorge E. Gómez-Galeno, Timothy J. Colby, James M. Fujitaki, Paul D. van Poelje, and Mark D. Erion, Departments of Chemistry and Biochemistry, Metabasis Therapeutics, Inc, 9390 Towne Centre Dr, San Diego, CA 92121, Fax: 858-622-5573, rajar@mbasis.com

A new class of phosphate and phosphonate prodrugs, called HepDirect prodrugs, has proven useful for the delivery of drugs to the liver while simultaneously diminishing drug exposure to extra-hepatitic tissues. The technology combines liver selective cleavage with high plasma and tissue stability to achieve increased drug levels in the liver. Hepsera, the dipivoxil prodrug of adefovir, was recently approved for treating hepatitis B patients but at sub-optimal doses due to dose-limiting renal toxicity. Accordingly, the HepDirect technology was applied to adefovir for targeting to the liver. The lead HepDirect prodrug, MB06866 (Hepavir B), resulted in a 12-fold increase in the liver/kidney AUC ratio in rats relative to Hepsera. Hepavir B is currently undergoing studies in HBV patients. In this presentation, the synthesis and SAR of HepDirect prodrugs of adefovir as well as their mechanism of activation and unique liver targeting profile will be described.

343.

VITAMIN B₁₂ TARGETED DELIVERY OF A TUBULIN-POISON TO CANCER CELLS. Joshua D. Bagnato¹, Alanna L. Ellers², Robert A. Horton¹, and Charles B. Grissom¹. (1) Department of Chemistry, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112, Fax: 801-581-8433, bagnato@chem.utah.edu, (2) MantiCore Pharmaceuticals, Inc

Colchicine, a highly potent tubulin-binding drug, was modified at the C7 position with a *p*-alkoxyacetophenone linker and attached to Vitamin B₁₂ (cobalamin) via

a pH-labile hydrazone. This pH-labile linker is activated by the low intralyosomal pH to release an active form of the drug. The bioconjugate showed excellent stability in cell media and at neutral pH but hydrolyzed rapidly at slightly acidic pH as anticipated. Initial in vitro studies using three tumor models have been carried out with the colchicine bioconjugate and show toxicity in the low nanomolar range, similar to unmodified colchicine. As a control, a pH-stable colchicine-cobalamin bioconjugate was synthesized using an amide bond to attach the drug. This bioconjugate showed no toxicity in any of the three cell lines, indicating that the release of colchicine from cobalamin is required to exhibit toxicity.

344.

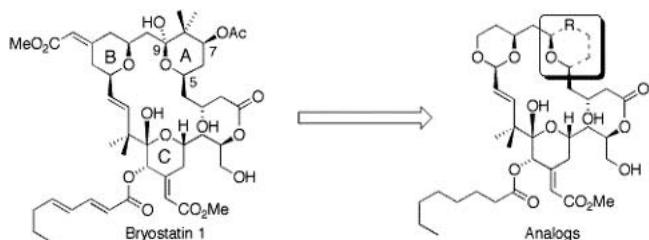
PSA ACTIVATED PRODRUGS AS PROSTATIC IMAGING AGENTS. Graham B. Jones¹, Longfei Xie¹, Glenn J. Bubley², Anthony V. D'Amico³, and Curtis Crasto¹. (1) Department of Chemistry, Northeastern University, 360 Huntington Ave, Boston, MA 02115, Fax: 617-373-8795, Gr.Jones@neu.edu, xie.l@neu.edu, (2) Genitourinary Oncology Group, Beth Israel Deaconess Medical Center, (3) Department of Radiation Oncology, Brigham and Womens Hospital

A cardinal feature of prostate cancer is its consistent secretion of prostate specific antigen (PSA). The goals of our program are to use PSA's enzymatic activity to activate a prodrug either in the prostate cell or in the metastatic microenvironment. PSA is a serine protease and continues to be a relevant tumor marker in well over 90% of late stage patients. Our strategy has involved coupling of a high affinity PSA substrate through a biodegradable linker, to a series of cytotoxins and fluorescent markers. We have demonstrated the effectiveness of this methodology with a DNA alkylating agent, and with a series of aniline dyes. Current work focuses on near-IR dyes, including the cyanine family.

345.

SELECTIVITY IN TRANSLOCATION OF PROTEIN KINASE C ISOFORMS BY NEW ANALOGS OF BRYOSTATIN. Paul A. Wender¹, Jeremy L. Barya¹, Stacey E. Brenner¹, Madeleine L. Craske², Michael O. Clarke¹, Joshua C. Horan¹, Alex V. W. Mayweg³, and Tobias Meyer². (1) Department of Chemistry, Stanford University, Rothway, Stanford, CA 94305, Fax: 650-725-0259, wenderp@leland.stanford.edu, jbarya@stanford.edu, (2) Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford University, (3) Pharma Research Basel Discovery Chemistry PRBD-CM, F. Hoffmann - La Roche AG F. Hoffmann - La Roche AG F. Hoffmann - La Roche AG

New analogs of bryostatin-1, a novel marine natural product with potent *in vivo* anti-cancer activity, have been tested for their ability to bind and translocate protein kinase C (PKC) isoforms, the protein targets believed to be at least partially responsible for the therapeutic activity of bryostatin. These analogs, designed to retain the biological function of bryostatin, are up to 50 times more potent than the natural product at inducing translocation of PKC isoforms. Additionally, variations in the A-ring (C5-C9) region of the molecule can confer varying degrees of selectivity for translocation and downregulation of different PKC isoforms. By using confocal microscopy with fluorescent PKC-GFP fusion proteins in living cells, the kinetics of translocation and the distribution patterns of individual isoforms in response to the analogs has been compared to that induced by bryostatin 1. Traditional biochemical techniques were also used to compare the translocation and downregulation response of PKC isoforms.



346.

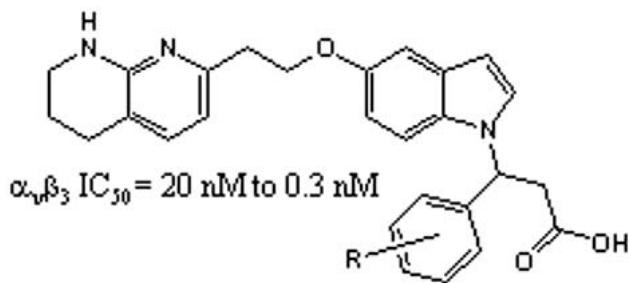
SIGNAL TRANSDUCTION PATHWAYS AND BONE DISEASES: NOVEL BONE-TARGETED SRC KINASE INHIBITORS WITH DUAL ANTIRESORPTIVE AND BONE ANABOLIC PROPERTIES. *Yihan Wang¹, Lianping Xing², Chet Metcalf¹, William Shakespeare¹, Raji Sundaramoorthi¹, Regine Bohacek¹, Surinder Narula¹, Scott Wardwell¹, John Iuliucci¹, Manfred Weigle¹, David Dalgarno¹, Brendan Boyce², and Tomi Sawyer¹. (1) Chemistry, Ariad Pharmaceuticals, Inc, 26 Lansdowne St., Cambridge, MA 02139, Fax: 617-494-8144, YIHAN.WANG@ARIAD.COM. (2) Department of Pathology and Laboratory Medicine, University of Rochester Medical Center*

A number of cellular protein kinases play important roles in signal transduction pathways involved in bone diseases such as osteoporosis and osteolytic bone metastasis. The first identified and genetically-validated oncogenic tyrosine kinase, Src, has been shown to have a prominent role in the biological activities of osteoclasts, osteoblasts, and several types of cancer cells. ARIAD has established an integrated drug discovery approach to advance proprietary, small-molecule inhibitors of Src using structure-based drug design, combinatorial chemistry, and mechanism-based cell biology. This presentation will describe the development of novel Src inhibitors which are highly potent, selective and effective *in vivo*. A series of 2,6,9-trisubstituted purines were synthesized incorporating effective bone-targeting functionalities. Key compounds showed both antiresorptive and bone anabolic effects *in vitro* and *in vivo*. Noteworthy was a lead compound, AP23588, which will be highlighted in terms of its structure-based design and structure-activity relationships.

347.

N-SUBSTITUTED INDOLES AS POTENT, SELECTIVE AND BIOAVAILABLE ALPHAVBETA3/ALPHAVBETA5 INHIBITORS. *Juan Jose Marugan, Beth Anacletio, Lou LaFrance, Tianbao Lou, Thomas Markotan, Joan Gushue, Kristi Leonard, Xihong Guo, Wenxi Pan, Pierre Raboisson, Bruce Tomczuk, and Carl Manthey, Medicinal Chemistry, 3-Dimensional Pharmaceuticals, Inc, 665 Stockton Drive, Suite 104, Exton, PA 19341, Fax: 610-458-8249, jmaruga1@prdus.jnj.com*

Integrins are a family of heterodimeric cell surface receptors responsible for the regulation of cell attachment to the extracellular matrix. Two of the integrins that have received considerable attention are avb3, the vitronectin receptor that up-regulate endothelial cells during tumor angiogenesis and smooth muscle cells mobility during proliferation, and avb5, which is also involved in angiogenesis. These receptors represent an interesting therapeutic target because of their important role in diverse pathologies such as osteoporosis, restenosis, acute renal failure, ocular diseases, tumor-induced angiogenesis, metastasis formation and sickle cell anemia disease. We would like to report the findings of a new series of compounds with good potency, selectivity and bioavailability. Synthesis, SAR and pharmacokinetics will be addressed.

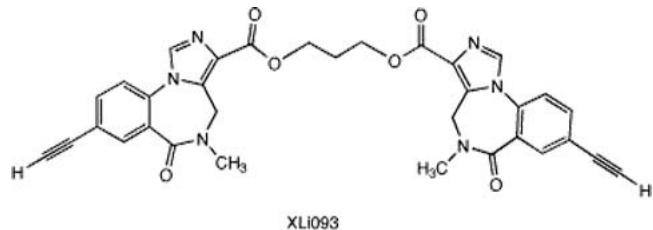


348.

SYNTHESIS, IN VITRO AFFINITY AND EFFICACY OF THE FIRST BIVALENT A5 SUBTYPE SELECTIVE BzR/GABA_A ANTAGONIST. *Xiaoyan Li¹, Werner Sieghart², Galen R. Wenger³, and James Cook¹. (1) Department of Chemistry, University of Wisconsin-Milwaukee, 3210 N. Cramer, Milwaukee, WI 53211, Fax: 414-229-5530, xli@uwm.edu, (2) Brain Research Institute, Medical University, Vienna, A-1090 Vienna, Austria, (3) Department of Pharmacology & Toxicology, University of Arkansas for Medical Sciences*

The synthesis and *in vitro* affinity of the $\alpha 5\beta 3\gamma 2$ ($\alpha 5$) subtype selective BzR/GABA_A antagonist XLi093 is described. This ligand is selective for $\alpha 5$ subtypes *in vitro* and is a potent antagonist of the effects of diazepam only at $\alpha 5\beta 3\gamma 2$ subtypes (oocytes). Ligands such as XLi093 will be important in the

determination of which physiological function(s) are subserved by this GABA_A $\alpha 5$ subtype. In addition, preliminary evidence suggests that XLi093 enhances cognition in a rodent memory model. Synthesis of a series of analogs of XLi093 has been carried out. Recent results in this area will be presented.



349.

PREPARATION OF HTLV-I PROTEASE PEPTIDE MIMETICS AND DETERMINATION OF THE C-TERMINAL CLEAVAGE JUNCTION. *Kelly J. Dennison, Bryan E. Herger, and Suzanne B. Shuker, School of Chemistry and Biochemistry, Georgia Institute of Technology, 315 Ferst Drive, Atlanta, GA 30332-0363, Fax: 404-894-2295, kelly.dennison@chemistry.gatech.edu*

Human T-cell leukemia virus (HTLV-I) is the causative agent of adult T-cell leukemia. HTLV-I requires a protease to process translated precursors into functional proteins of the mature virion. This makes the protease a suitable target for drug therapies to treat HTLV-I infection. We are preparing peptide mimetic compounds incorporating the tetrahedral intermediate of aspartyl protease catalyzed cleavage. Compounds containing statine, 4-amino-3-hydroxy-5-phenylpentanoic acid, or hydroxyethylamine are expected to be potent protease inhibitors. Additionally, HTLV-I protease has an extended C-terminus that is only found in the leukemia virus proteases. There is a potential HTLV-I cleavage site (Leu115-Pro116) within the last ten C-terminal residues. We investigated peptides with mutations in the S2, S2', and S3' positions that were incubated with the protease and analyzed by LC-MS. We also prepared protease mutants by site-directed mutagenesis. The results indicate that it is the charged Glu117 that hinders cleavage of the C-terminal tail.

350.

KINETIC STUDIES OF MUTANTS OF HTLV-I PROTEASE. *Bryan E. Herger, Kelly J. Dennison, Victoria L. Mariani, and Suzanne B. Shuker, School of Chemistry and Biochemistry, Georgia Institute of Technology, 315 Ferst Drive, Atlanta, GA 30332-0363, Fax: 404-894-2295, herger@chemistry.gatech.edu*

HTLV-I protease is essential in the life cycle of HTLV-I, a retrovirus implicated in adult T-cell leukemia. It is important to understand the properties of the protease in order to develop therapeutic agents which target the protease. In this work, we present several new observations. We have developed a sequence alignment and theoretical structure of the protease. We have assayed the effect of single mutations on residues believed to be involved in substrate recognition and catalysis. We have studied whether HTLV-I protease could be mutated to recognize an HIV-1 protease substrate.

351.

DEVELOPMENT OF FACTOR VIIA INHIBITORS AS NOVEL ANTICOAGULANTS. *Aleksandr Kolesnikov, Roopa Rai, Wendy B. Young, Steven Torkelson, William D. Shrader, Ellen M. Leahy, Bradley A. Katz, Paul A. Sprengeler, Liang Liu, and Joyce Mordenti, Celera, 180 Kimball Way, So. San Francisco, CA 94080*

The development of novel antithrombotic agents for the treatment of coagulation disorders is an active area of research in the pharmaceutical industry. The enzymes that comprise the extrinsic and intrinsic pathways of coagulation, leading to the formation of a blood clot, are trypsin-like serine proteases. At Celera, we have focused on the direct inhibition of coagulation proteases such as Factor VIIa, with small molecules. The structural basis for potency and selectivity of our small molecule Factor VIIa inhibitors will be described. The synthesis and *in vitro* and *in vivo* data of some lead compounds will be presented.

352.

HIGHLY SELECTIVE CHIRAL PDE5 INHIBITORS FOR TREATMENT OF MED.
*Mark E Bunnage¹, John P Mathias¹, Stephen D A Street¹, Anthony Wood¹,
Stephen A Ballard², and Kevin C Beaumont³. (1) Department of Discovery
Chemistry, Pfizer Global Research & Development, Ramsgate Road, Sandwich,
Kent, CT13 9NJ, United Kingdom, Mark_Bunnage@sandwich.pfizer.com, (2)
Department of Biology, Pfizer Global Research & Development, (3) Department
of Drug Metabolism, Pfizer Global Research & Development*

Viagra™ is a potent and selective inhibitor of phosphodiesterase PDE5 and, as the first safe and effective oral therapy, has revolutionised treatment for Male

Erectile Dysfunction. Recent efforts in the PDE5 arena have focussed on designing agents with greater selectivity over the related enzyme PDE6. We will describe a series of potent PDE5 inhibitors with >100-fold selectivity over PDE6 and show how an early candidate from this series was discontinued due to non-linear human pharmacokinetics. A rationale for this non-linearity will be presented which led to the design of lower MWt compounds with improved Caco-2 flux profiles. A novel series of chiral PDE5 inhibitors will be detailed which meet these criteria and maintain high selectivity over PDE6. Eudismic analysis will demonstrate the high specificity of these agents for PDE5. Clinical data for a development candidate from this series will also be disclosed which supports the hypothesis for achieving linear pharmacokinetics.