American Chemical Society

Division of Medicinal Chemistry
ABSTRACTS

229th ACS National Meeting

San Diego, CA
March 13-17, 2005 **D. L. Flynn, Program Chair**

SUNDAY MORNING

- **Cannabinoid Receptors**
D. G. Brown, Organizer Papers 1-6

- **General Oral Session I**
D. L. Flynn, Organizer; B. S. J. Blagg, Presiding Papers 7-16

- **MEDI Business meeting**
D. L. Flynn, Presiding Paper 17

SUNDAY AFTERNOON

- **First Time Disclosure of Clinical Candidates: Sponsored by Biotage**
B. Balasubramanian, Organizer Papers 18-22

SUNDAY EVENING

- **Poster Session I and Social Hour**
D. L. Flynn, Organizer Papers 23-256

MONDAY MORNING

- **Biological Tools in Drug Discovery: Sponsored by Bentham Science Publishers Ltd**
L. McQuire, Organizer, Presiding Papers 257-261

- **P-glycoprotein - Structure and Function**
G. F. Ecker, Organizer, Presiding; K. A. Jacobson, Presiding Papers 262-266

MONDAY AFTERNOON

- **Gene Expression and Medicinal Chemistry: Sponsored by Elsevier**
S. Pikul, Organizer Papers 267-270

- **Druggable Targets in Functional Lipidomics**
L. Feng, Organizer Papers 271-275

MONDAY EVENING

- **Sci-Mix**
D. L. Flynn, Presiding Papers 23, 25, 31, 37, 41, 55, 63, 86, 103-104, 121, 123, 127, 133, 141, 149, 160, 172, 185, 189, 195, 208, 219, 232, 238-239, 251, 319, 327-328, 335, 350, 354, 366, 370, 373, 377, 380, 383, 403, 407, 417, 429, 431, 464, 472, 474, 485, 487, 499, 503, 517, 521, 523, 534

TUESDAY MORNING

- **E. B. Hershberg Award for Important Discoveries in Medicinally Active Substances**
K. A. Jacobson, Organizer Papers 276-280

TUESDAY AFTERNOON

- **5HT2C and Higher Order Serotonin Receptors: Sponsored by Arena Pharmaceuticals, Inc**
J. Gross, Organizer Papers 281-285

DIVISION OF MEDICINAL CHEMISTRY

1.

CANNABINOID RECEPTORS AS THERAPEUTIC TARGETS. ***Francis Barth**, Sanofi-aventis, 371 rue du Professeur Joseph Blayac, 34184 Montpellier, France, francis.barth@sanofi-aventis.com*

Cannabis has been used for centuries as a therapeutic agent and its analgesic, anti-emetic and appetite-stimulating properties are well known. The molecular targets of D9-THC, the active principle of cannabis consist of two G-protein-coupled receptors, the CB1 and CB2 cannabinoid receptors. CB1 receptors (cloned in 1990) are mostly expressed in central and peripheral neurons, while CB2 receptors (cloned in 1993) are expressed mostly in immune cells. The recent availability of selective CB1 and CB2 agonists and antagonists has allowed the discovery of novel therapeutic uses for cannabinoid ligands. Among these activities, the potential role of CB1 antagonists in the treatment of obesity and related metabolic disorders has attracted considerable attention. Since its discovery in 1994, the CB1 cannabinoid receptor antagonist SR141716 (rimonabant) has been shown to significatively decrease food intake and body weight in several animal obesity models. In humans, rimonabant was tested in obese patients, in a double blind phase III study (one year treatment). The study showed a very significant reduction in body weight which is maintained throughout the 52 weeks with a concomitant reduction in the waist circumference in the rimonabant (20 mg /day) group. Over 72% of the patients at 1 year showed a weight loss >5% with over 44% showing a weight loss of > 10%. Several associated important cardiovascular risk factors were also significantly improved. The general tolerance of the compound was excellent, and the product was well tolerated.In addition, the compound is being evaluated in smoking cessation, and preliminary results from phase III studies have shown an increased abstinence with a prevention of the post cessation weight gain generally observed.

2.

ROLE OF ENDOCANNABINOID SYSTEM IN BRAIN REWARD. ***Billy R. Martin**, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia, P.O. Box 980613, 760 Smith Building, Richmond, VA 23298-0613, Fax: 804-827-0377, martinb@hsc.vcu.edu*

Tne endocannabinoid system is involved in a wide range of physiological processes including appetite,cognition, pain perception, and reward. It is well known that dependence develops to continued marijuana use and that this dependence involves the limbic brain reward system. Appropriate animal models have been developed to demonstrate that the psychoactive constituent of marijuana, THC, is self-administered in squirrel monkeys and a physical withdrawal syndrome can be precipitated with the cannabinoid receptor antagonist rimonabant. Evidence is now emerging that the role of the endocannabinoid system in reward mechanisms may extend to regulation of appetite. CB1 cannabinoid receptor agonists stimulate appetite whereas antagonists decrease appetite. Additionally, the endocannabinoid system also contributes to the development of drug dependence to substances other than marijuana. Rimonabant has been found to be effective in altering alcohol intake as well as reinstatement of self-administration of cocaine and heroin in laboratory animals.The convergence of these findings indicate that the endocannabinoid system plays a fundamental role in natural reward mechanisms.

3.

IDENTIFICATION OF POTENT CANNABINOID-1 RECEPTOR INVERSE AGONISTS. ***William K. Hagmann**, Department of Medicinal Chemistry, Merck Research Laboratories, RY121E-105, P.O. Box 2000, Rahway, NJ 07065, Fax: 732-594-5966, william_hagmann@merck.com*

Blockade of the cannabinoid-1 receptor (CB1R) has emerged as an attractive target for numerous CNS as well as peripheral indications, including cognitive disorders, obesity, addictive behaviors, and GI disorders. Several inverse agonists are reported to be in the clinic and one has demonstrated positive effects on weight loss and smoking cessation. Considering that the endogenous ligands of the CB1R are highly hydrophobic derivatives of arachidonic acid, it is not surprising that most reported small molecular weight inverse agonists are hydrophobic compounds with less than ideal pharmaceutical properties, including poor aqueous solubility, very long plasma half-lives, and high plasma protein binding. On the other hand, the hydrophobic nature of these inverse agonists does offer good oral bioavailability and CNS penetration. This presentation will describe our efforts on two classes of CB1R inverse agonists that illustrate some of the deficiencies and attributes of working with these highly hydrophobic CB1R ligands.

4.

IDENTIFICATION OF TRIARYL BIS-SULFONES AS NOVEL, ORALLY ACTIVE CANNABINOID-2 (CB2) RECEPTOR INVERSE AGONISTS. ***Brian J. Lavey**[1], Guowei Zhou[1], James Spitler[1], Jie Wu[1], Bandarpalle Shankar[1], Razia Rizvi[1], De-Yi Yang[1], Joseph Kozlowski[1], R. William Hipkin[2], Waldemar Gonsiorek[2], Loretta Bober[2], Jay Fine[2], Alberto Rojas-Triana[2], James V. Jackson[2], James Fossetta[2], Larry Heimark[1], Nigel Clarke[3], Ronald Wolin[1], Daniel Lundell[2], Neng-Yang Shih[1], John J. Piwinski[1], Satwant Narula[2], and Charles A. Lunn[2]. (1) Department of Chemistry, Schering-Plough Research Institute, 2015 Galloping Hill Rd, K-15-1-1545, Kenilworth, NJ 07033-0539, Fax: 908-740-7152, brian.lavey@spcorp.com, (2) Department of Inflammation and Infectious Diseases, Schering-Plough Research Institute, (3) Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute*

Triaryl Bis-Sulfones have been identified as a new class of Cannabinoid-2 (CB2) inverse agonists. Compounds in the class are shown to have nanomolar potency at CB2, high selectivity for CB2 in preference to CB1, and good plasma levels after oral dosing.

5.

NOVEL PYRIMIDINE CB2 RECEPTOR AGONISTS FOR INFLAMMATORY PAIN. ***Gerard M.P. Giblin**[1], Celestine O'Shaughnessy[2], Alan Naylor[1], William L Mitchell[1], Andrew Eatherton[1], Karamjit Jandu[1], Tony Rawlings[1], Brian Slingsby[1], Jennifer Sweeting[1], Ian Wall[3], Paul Goldsmith[1], Andrew J Brown[3], Carl Haslam[3], Alex Wilson[2], Nick Clayton[2], Andrew Whittington[3], and Richard Green (Late)[1]. (1) Department of Medicinal Chemistry and DMPK, Neurology and GI CEDD, GlaxoSmithKline R & D, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, United Kingdom, Fax: 44-0-1279-622550, ged_m_giblin@gsk.com, (2) Department of Biology, GlaxoSmithKline R & D, (3) Discovery Research, GlaxoSmithKline R & D*

The CB1 and CB2 receptor are 7-transmembrane G-protein coupled receptors responsible for the therapeutic effects of cannabis and cannabinoids. The CB1 receptor is widely distributed, including in the CNS, and is associated with the undesirable therapeutic effects of cannabis. The CB2 receptor is expressed mainly in the periphery on a range of inflammatory cells. Recent studies have shown that activation of either CB1 or CB2 receptor can result in analgesic effects in pre-clinical models of pain. Our strategy is to focus on selective CB2 agonists as new therapeutic agents for inflammatory pain. A high-throughput screen identified novel pyrimidine esters as weak, partial CB2 agonists. Lead optimisation will be described that led to full CB2 agonists with high selectivity against CB1. Subsequent iterations optimised pharmacokinetic and in vivo properties. We report for the first time SAR for CB2 potency and the in vivo profile of optimised compounds.

6.

ENZYMATIC REGULATION OF ENDOGENOUS CANNABINOID SIGNALING. ***Benjamin F. Cravatt**, Departments of Cell Biology and Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Rd., la jolla, CA 92037, Fax: 858-784-8023, cravatt@scripps.edu*

Endogenous cannabinoids (endocannabinoids) constitute an emerging class of signaling lipids that act on both central and peripheral cannabinoid receptors,

which also mediate the effects of delta9-tetrahydrocannabinol, the active component of marijuana. The magnitude and duration of endocannabinoid signaling are tightly controlled in vivo by the integral membrane enzyme, fatty acid amide hydrolase (FAAH). Our group has embarked on a multidisciplinary research program aimed at understanding the molecular, cellular, and physiological functions of FAAH and its fatty acid amide substrates. These studies include: 1) the recombinant expression, characterization, and structural determination of FAAH, 2) the generation and analysis of FAAH-knockout mice, and 3) the creation and pharmacological application of potent and specific FAAH inhibitors. Here, our key findings will be reviewed and their therapeutic implications for the treatment of nervous system disorders discussed. Additionally, more recent proteomic and metabolomic studies will be presented that aim to integrate the fatty acid amide-FAAH pathway into the global metabolic and signaling networks of the nervous system.

7.

ALLOSTERIC ENHANCERS OF A1 ADENOSINE RECEPTORS. ***Mahendra D. Chordia**, Department of Chemistry, University of Virginia, Charlottesville, VA 22901, mdc3x@virginia.edu, Heidi Figler, Department of Pharmacology, University of Virginia, Ray A. Olsson, Department of Internal Medicine, University of South Florida, and Joel Linden, Department of Medicine and Molecular Physiology, University of Virginia*

Allosteric enhancers (AEs) of the A1 adenosine receptor (A1R) have potential to be clinical candidates in renal, cardiovascular and neurological diseases including pain and seizure management. We have previously reported that 2-aminothiazoles are a new class of compounds possessing A1R AE activity distinct from previously characterized 2-amino-3-aroyl thiophenes. This paper describes a detailed synthesis and biological evaluation of 2-amino-indeno-thiazoles. A few of these new 2-amino-indeno-thiazoles demonstrated higher potency (based on EC50 values) and efficacy (maximal allosteric activity) than 2-aminothiophenes, particularly when compared with PD 81, 723. These compounds are selective for A1 over A2A or A3-adenosine receptors based on AE assays performed on membranes isolated from mammalian cells stably expressing adenosine receptors. 2-Aminothiazoles are more stable than 2-aminothiophenes which are prone to oxidation in DMSO. Also, as aromatic amines, the aminothiophenes may be carcinogenic. In conclusion, the 2-amino-thiazoles are imporved A1R AEs that have clinical potential.

8.

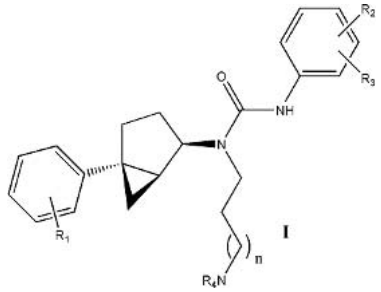
DISCOVERY AND EFFICACY OF THE FIRST ORALLY ADMINISTERED DERIVATIVES OF B-TYPE NATRIURETIC PEPTIDE. ***Kenneth D. James**[1], Mark A. Miller[1], Navdeep B. Malkar[1], Diana Severynse-Stevens[1], Kevin G. Yarbrough[1], Karen Polowy[1], Mark J. Bednarcik[1], Robert E. Dugdell[1], Alessandro Cataliotti[2], John A. Schirger[2], Radha Krishnan[1], Monica E. Puskas[1], David Surguladze[1], Jenn L. Boyer[1], Nnochiri N. Ekwuribe[1], and John C. Burnett Jr.[2]. (1) Nobex Corporation, PO Box 13940, Research Triangle Park, NC 27709, Fax: 919-474-9407, kjames@nobexcorp.com, (2) Mayo Clinic*

Human brain-type natriuretic peptide (hBNP) is an endogenous hormone that exhibits favorable hemodynamic, renal, neurohormonal, and lusitropic properties. Since 2001, exogenous hBNP has been increasingly utilized for treating patients hospitalized for acutely decompensated heart failure characterized by dyspnea at rest or with minimal activity. Because hBNP itself is not suitable for oral delivery, its use has been limited to dosing by continuous infusion. A derivative of hBNP that is orally available, however, has great potential for the chronic treatment of patients with heart failure. Through site-specific modification of hBNP with rationally designed amphiphilic oligomers, we sought to enable oral delivery, alter the pharmacokinetic and pharmacodynamic properties of the compound, and retain its natural activity. We now report the design, in vitro activity, and oral absorption in rat of hBNP conjugates. We also describe the selection and in vivo pharmacology of the first orally active derivatives of hBNP in dog.

9.

DESIGN AND SYNTHESIS OF ORALLY EFFICACIOUS MELANIN CONCENTRATING HORMONE (MCH) RECEPTOR ANTAGONISTS AS ANTIOBESITY THERAPEUTICS. ***Mark D. McBriar**[1], Henry Guzik[1], Ruo Xu[1], Jaroslava Paruchova[1], Shengjian Li[2], Anandan Palani[1], Sherry Shapiro[1], John W. Clader[1], William J. Greenlee[1], Brian E. Hawes[3], Timothy J. Kowalski[3], Kim O'Neill[3], Brian Spar[3], and Blair Weig[3]. (1) CV/CNS Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-0539, Fax: 908-740-7152, mark.mcbriar@spcorp.com, (2) Johnson and Johnson Pharmaceutical Research Institute, (3) CV/Metabolic Diseases Research, Schering-Plough Research Institute*

Melanin Concentrating Hormone (MCH) is a cyclic, nonadecapeptide found in the CNS of all vertebrates which regulates feeding behavior and energy homeostasis via interaction with the central melanocortin system. Recent studies have demonstrated that elevated levels of MCH in mice have been found to stimulate food intake and promote fat storage, while mice null for MCH or MCH-R1 exhibit a lean phenotype and are resistant to diet induced obesity. The receptor for MCH belongs to the GPCR superfamily, identified by 7-transmembrane regions embedded in the cellular wall. Antagonists of the MCH-1 receptor are expected to decrease food intake and weight gain in rodents, making MCH-R1 an attractive target for obesity therapeutics. Herein, we report the discovery and SAR development of a novel, orally active series of potent MCHR-1 antagonists of general structure I. Optimization of potency, selectivity and pharmacokinetic properties of these bicycloalkyl ureas will be addressed, along with in vivo efficacy in relevant rodent feeding models.



I

10.

OVER 100 PEPTIDE-ACTIVATED GPCRS RECOGNIZE TURN MOTIFS IN LIGANDS. ***David P. Fairlie**, Joel D. A. Tyndall, Giovanni Abbenante, and Bernhard Pfeiffer, Centre for Drug Design and Development, Institute for Molecular Bioscience, University of Queensland, Brisbane Q4072, Australia, Fax: 61-7-3346-2101, d.fairlie@imb.uq.edu.au*

G protein-coupled receptors (GPCRs) are the largest family of cellular receptors involved in signal transduction and already represent a US$30 billion annual market. Pharmaceuticals typically bind in transmembrane regions of GPCRs, whereas peptide/protein hormones mostly interact with the extracellular N-terminus or loops of GPCRs. Relatively few peptide-activated GPCRs have been successfully targeted by pharmaceuticals. Towards a better understanding of ligand-GPCR interactions, we have assembled structure/activity data on peptide/protein ligands for ~120 GPCRs. Ligand structures for (1) native peptide/protein hormone ligands, (2) bioactive peptide fragments, (3) cyclic peptide analogues, and (4) ligands with turn-inducing conformational constraints support the idea that ligand 'turns' are widely recognized by peptide-binding GPCRs. This contrasts with almost universal recognition of the beta strand by peptidases, and of the alpha helix by transcriptional receptors. Such pattern recognition may be valuable in generic drug design, and in understanding signal transduction across cell membranes.

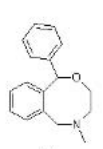
11.

NOVEL BENZOXAZOCINE ANALOGUES AS POTENT ANALGESIC AGENTS. ***Andrew D. Baxter**[1], Michael Lyne[1], and Stuart Brown[2]. (1) Arakis Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden CB10 1XL, United Kingdom, andybaxter@arakis.com, (2) SAFC Pharma, Synergy House*

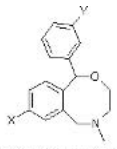
Nefopam is a modestly potent but non-selective inhibitor of serotonin and noradrenaline reuptake (S+NRI). In addition the molecule possesses significant central anti-histamine (H1 antagonist) activity. Nefopam (**1**) suffers from extensive first pass metabolism to an inactive *N*-demethyl metabolite, lowering

bioavailability and limiting efficacy in animal models. The discovery of novel anti-emetic pharmacology in nefopam (**1**) has led Arakis to undertake a property driven medicinal chemistry programme aimed at improving the potency and selectivity of the template while enhancing pharmacokinetics.

ARAK0029 (**2**) is a potent selective serotonin reuptake inhibitor (SSRI), devoid of anti-histamine activity. The compound is primarily metabolized by *O*-demethylation to an 'active' metabolite increasing the pharmacodynamics of the template. A second series based on ARAK0051 (**3**) are representative of a potent class of S+NRI's. Strategies to improve pharmacokinetics and eliminate CYP2D6 activity will be presented and exemplified by derivatives from a second wave of medicinal chemistry. Lead compounds are now devoid of both H1 antagonist activity and the CYP2D6 inhibition present in the parent molecule.



(1)



(2) X = CN; Y = OMe
(3) X = cyclopropyl; Y = OMe

Compound	IC_{50} SRI (nM)	IC_{50} NRI (nM)	IC_{50} H1 (nM)
Nefopam (1)	93	105	460
ARAK0029 (2)	15	1100	30% @ 1μM
ARAK0051 (3)	8.3	46	34

12.
WITHDRAWN.

13.
CHEMICAL GENETICS APPROACH FOR THE DISCOVERY OF NOVEL ANTI-CANCER AGENTS AND TARGETS: IDENTIFICATION OF TRANSFERRIN RECEPTOR AS THE MOLECULAR TARGET OF GAMBOGIC ACID, A RAPID AND POTENT APOPTOSIS-INDUCER. ***Sui Xiong Cai****, Han-Zhong Zhang, Katayoun Jessen, Sergei Maliartchouk, Jean Yu Wang, Nicki English, Ling Qiu, Nilantha Sirisoma, Songchun Jiang, Jared Kuemerle, John Drewe, Kurt Gehlsen, Ben Tseng, and Shailaja Kasibhatla, Maxim Pharmaceuticals, 6650 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-202-4000, scai@maxim.com*

Apoptosis (programmed cell death) is a normal process of development and tissue homeostasis. While excessive apoptosis can result in organ failure and neurodegenerative diseases, insufficient apoptosis can lead to cancer. Therefore, the discovery of novel compounds and molecular targets that modulate the apoptotic pathway could lead to the development of new anticancer agents. Herein, we report the development of a chemical genetics approach for the discovery of a novel apoptosis inducer and its molecular target. Using a cell- and caspase-based HTS assay, gambogic acid was discovered as a rapid and potent apoptosis inducer. Through SAR studies, derivatives of gambogic acid were identified with improved pharmaceutical properties and showed potent in vivo anti-cancer activity. Novel reagents were then designed and synthesized based on the SAR information, and used for the identification of the transferrin receptor as the molecular target. We will report in detail the chemistry, in vivo activity, and the identification and validation of the transferrin receptor as the molecular target for gambogic acid.

14.
CHEMOGENOMIC APPROACH FOR ION CHANNEL AND TRANSPORT MODULATORS. ***Holger Heitsch****, Medicinal Chemistry, Sanofi-Aventis, Aventis Pharma Deutschland GmbH, D-65926 Frankfurt/Main, Germany, Fax: +49 69 331399*

Our chemogenomic attempt to encode structural and functional commonalities of the target gene family of ion channels and transporters into a target family-biased lead-like compound library for optimization of lead finding and target validation capabilities within inhouse ion channel and transporter projects will be described. Design and establishment of this biased library, consisting of 16.000 compounds from 80 different chemotypes, was the result of an iterative process of in-depth literature search, combined efforts of 2-D fingerprint similarity searches, 3-D-pharmacophore generation and virtual screening on our corporate and commercial compound collections and accompanying automated solution phase synthesis of small focused libraries. These efforts have been guided by long-standing in-house knowledge on ion channel and transporter modulator projects to guarantee an optimum match between the biological space of the target protein family and the chemical space of the selected ion channel and transport modulators avoiding interference with prohibitive mechanisms (e.g. hERG blockade).

15.
CLOFIBRATE-INDUCED CHANGES IN THE LIPID METABOLOME. ***Craig E. Wheelock***[1]***, John W. Newman***[1]***, Steven M. Watkins***[2]***, and Bruce D. Hammock***[3]*. *(1) Department of Entomology and Cancer Reseach Center, University of California Davis, Briggs Hall, One Shields Ave, Davis, CA 95616, and Kyoto University, Ugi, Kyoto 611-0011, Japan, Fax: 530-752-1537, craig@kuicr.kyoto-u.ac.jp, (2) Lipomics Technologies, (3) Department of Entomology and Cancer Resarch Center, University of California Davis*

The use of metabolomics to study metabolome-wide effects of drug treatment upon an individual is increasing. A current limitation of these methods is the inability to simultaneously analyze every metabolite. However, the metabolome can be broken into sub-domains for focused analysis. One such sub-domain is lipid metabolism, or the lipidome. This study assessed the effects of the PPARα agonist clofibrate on lipid metabolism in the liver, adipose, heart and brain of clofibrate- and vehicle-treated Swiss-Webster mice. Lipid analyses were divided into polar and non-polar analyses consisting of >450 individual metabolites. Clofibrate treatment induced several substantial changes in the lipid class composition of liver, brain and heart, but not adipose. Results demonstrated that PPARα agonists influence lipid metabolism in extra-hepatic tissues in a tissue-specific manner. These data provide us with important information on the effects of clofibrate treatment on the lipidome and are another step towards full scale metabolomics.

16.
RATIONAL DESIGN OF MULTIPLE LIGANDS - RISKS AND BENEFITS. ***J. Richard Morphy****, Department of Medicinal Chemistry, Organon Laboratories Ltd, Motherwell Road, Newhouse ML1 5SH, United Kingdom, r.morphy@organon.co.uk*

There is an increasing awareness within the medicinal chemistry community that a balanced modulation of multiple targets can often provide a superior therapeutic effect and side effect profile compared to the modulation of a single target. Furthermore, it is now possible to rationally design multiple ligands that span diverse targets. This combination of the desirability of discovering multiple ligands on the one hand, and the increasing tractability of a rational approach on the other, has lead to a strengthening of interest in this area in recent years. An early evaluation of the likelihood of being able to obtain a "Designed Multiple (DM) ligand" for a particular target combination is essential. A key challenge is

achieving a balanced activity for each target of interest, whilst obtaining wider selectivity and a suitable pharmacokinetic profile. Exciting new strategies that will help medicinal chemists discover a new generation of DM ligands will be described.

17.
WITHDRAWN.

18.
DESIGN AND SYNTHESIS OF THROMBIN RECEPTOR (PAR-1) ANTAGONISTS - ALFRED BURGER AWARD ADDRESS. ***William J. Greenlee***, *Chemical Research, Schering-Plough, 2015 Galloping Hill Road, Kenilworth, NJ 07033, Fax: 908-740-7164, william.greenlee@spcorp.com*

Cardiovascular disease, especially heart attack and stroke, remains a major cause of mortality in the United States and Western Europe. In most cases, the cause of death is the presence of a thrombus in a major artery, a result of inappropriate activation of the coagulation pathway. The enzyme thrombin plays a central role in this process by cleaving fibrinogen to fibrin, and by activating platelets, which contribute to arterial thrombus formation. In a process unique to the protease-activated receptor (PAR) family, thrombin cleaves the N-terminus of the thrombin receptor (PAR-1) present on these cells, creating a tethered ligand which activates the receptor. Antagonists of PAR-1 are of high interest as potential agents for the prevention of arterial thrombosis, especially since they may lack the bleeding liability of other antithrombotic drugs (e.g thrombin and Factor Xa inhibitors). Starting from a modestly-potent lead derived from the natural product himbacine, we have discovered potent orally bioavailable PAR-1 receptor antagonists which block thrombin-induced activation of platelets and are active in a primate model of thrombosis. The design, synthesis and structure-activity relationships of this series of antagonists will be discussed.

19.
DISCOVERY OF POTENT, SELECTIVE AND ORALLY ACTIVE 5-HT1A AGONIST, PRX-00023, FOR THE TREATMENT OF ANXIETY, DEPRESSION AND ATTENTION DEFICIT HYPERACTIVITY DISORDER. ***Dale S. Dhanoa***, *Predix Pharmaceuticals Holding Inc, 10 K Gill Street, Woburn, MA 01801, Fax: 781-376-0822, ddhanoa@predixpharm.com*

5-HT1A receptor of the serotonin family has been of considerable interest for the development of CNS therapeutic agents. We will describe the discovery and development of our Phase II clinical candidate, PRX-00023, for the treatment of anxiety, depression and Attention Deficit Hyperactivity Disorder (ADHD).

20.
DESIGN, SYNTHESIS AND EVALUATION OF NOVEL PHOSPHONATES AS POTENT AND SELECTIVE FBPASE INHIBITORS WITH ORAL EFFICACY IN RODENT MODELS OF TYPE 2 DIABETES. ***Qun Dang***[1], *Mark D. Erion*[2], *K. Raja Reddy*[3], *Srinivas R. Kasibhatla*[1], *M. Rami Reddy*[2], *and Paul D. van Poelje*[3]. *(1) Medicinal Chemistry, Metabasis Therapeutics, Inc, 9390 Towne Centre Drive, San Diego, CA 92121, Fax: 858-622-5573, dang@mbasis.com, (2) Metabasis Therapeutics Inc, (3) Departments of Chemistry and Biochemistry, Metabasis Therapeutics, Inc*

Hepatic glucose output is often upregulated in type 2 diabetes (T2DM) and is a significant contributor to both postprandial and fasting hyperglycemia. Increased gluconeogenesis (GNG) accounts for this increased hepatic glucose output, suggesting that inhibitors of the GNG pathway might be potential drug candidates for T2DM. Fructose-1,6-bisphosphatase (FBPase) is a key rate-limiting enzyme of GNG and is a well known target for T2DM. Previous efforts targeting FBPase, however, were unable to find potent, specific and cell-permeating inhibitors. Herein we present the discovery of a series of low molecular weight heterocyclic phosphonates that mimic AMP and are potent inhibitors of FBPase. The initial series of compounds was identified using structure-based drug design. Key pharmacophores were identified by SAR analysis of several series of heterocyclic FBPase inhibitors (FBPaseIs) and were used to discover a series of inhibitors with low nanomolar inhibitory potency, high FBPase specificity and potent oral glucose lowering activity in rodent models of type 2 diabetes. Recently, a compound discovered from this program successfully completed a second Phase IIA clinical trial. The design, synthesis, SAR and in vivo efficacy of FBPaseIs will be presented for the first time.

21.
BMS-599626: A NOVEL DUAL INHIBITOR OF HER1 AND HER2 PROTEIN TYROSINE KINASES. ***Ashvinikumar V. Gavai***[1], *Brian E. Fink*[1], *John S. Tokarski*[2], *David Fairfax*[3], *Gregory Martin*[3], *Lana Grubb*[3], *Zice Fu*[3], *Soong-Hoon Kim*[1], *Kenneth Leavitt*[1], *Harold Mastalerz*[1], *Toomas Mitt*[1], *John T. Hunt*[4], *John F. Kadow*[1], *Karen Du*[1], *Wen-C. Han*[1], *Derek Norris*[1], *Bindu Goyal*[1], *Dolatrai M. Vyas*[1], *Chiang Yu*[4], *Simone Oppenheimer*[4], *Hongjian Zhang*[5], *Francis Y. Lee*[4], *Tai W. Wong*[4], *and Gregory D. Vite*[1]. *(1) Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, P. O. Box 4000, Princeton, NJ 08543-4000, Fax: 609-252-6601, ashvinikumar.gavai@bms.com, (2) Department of Structural Biology and Modeling, Bristol-Myers Squibb Pharmaceutical Research Institute, (3) Albany Molecular Research, (4) Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, (5) PCO, Bristol-Myers Squibb Pharmaceutical Research Institute*

Receptor tyrosine kinases play a crucial role as signal transducers in the regulation of cell proliferation, survival, and differentiation. Two members of the EGF receptor family, HER1 and HER2, have been clinically validated as rational targets for cancer therapy. Frequent co-expression of HER1 and HER2 in a variety of tumor types and their capacity to form heterodimers with other members of the EGFR family, provide a strong rationale for simultaneous targeting of the two receptors. Extensive homology of the catalytic sequences of the two receptors supports the feasibility of designing inhibitors that occupy the ATP binding pockets of both receptor kinases. This presentation will summarize efforts at Bristol-Myers Squibb on a novel series of pyrrolotriazine-based HER1/HER2 dual inhibitors that culminated in identification of BMS-599626 as the clinical candidate. Structure-activity relationships will be described along with in vivo evaluation of the clinical candidate in relevant tumor xenograft models.

22.
DISCOVERY OF IMIDAZO[1,2-B][1,2,4]TRIAZINES AS GABA-AA2/3 BINDING SITE AGONISTS FOR THE TREATMENT OF ANXIETY. ***Leslie J. Street***, *Neuroscience Research Center, Merck Sharp and Dohme, Terlings Park, Harlow, Essex CM20 2QR, United Kingdom, Leslie_Street@Merck.com*

Inhibitory neurotransmission in the mammalian central nervous system is mediated predominantly through GABAA receptors which open in response to the binding of g-aminobutyric acid (GABA), resulting in chloride ion flux into the cell and inhibition of neuronal activity. These ligand-gated ion channels, in addition to binding GABA, are the site of action of a number of pharmacologically important allosteric modulators including barbiturates, neurosteroids, loreclezole, anaesthetics, ethanol, and benzodiazepines (BZs). Non-selective benzodiazepine agonists such as diazepam are used therapeutically as anxiolytics and anticonvulsants. However, these agents also have sedative, muscle relaxant and amnesic properties. To date, 16 GABAA receptor subunits have been identified (a1-a6, b1-b3, g1-g3, d, e,O, pi) using molecular cloning techniques and GABAA receptors which bind BZs are comprised of a pentameric assembly of proteins made up of a, b and g-subunits in a 2:2:1 stoichiometry. The major BZ-sensitive GABAA receptor subtypes in the mammalian brain are a1b g2, a2b g2, a3b g2 and a5b g2. It has been demonstrated that GABAA receptors containing an a1 subunit mediate the sedative/ataxic effects of benzodiazepines, whereas those containing an a2 or a3 subunit mediate the anxiolytic effects. Work in our laboratory has focused on the identification of BZ binding site agonists which have functional selectivity for the*f*nGABAAa2 and a3 subtypes over the a1-containing GABAA subtype. It was proposed that such compounds would have anxiolytic properties with a reduced side-effect liability compared with non-selective GABAA modulation. This presentation will describe the medicinal chemistry leading to the identification of a series of imidazo[1,2-b][1,2,4]triazines as functionally selective GABAAa2/3 agonists which were shown to be non sedating anxiolytics in animal models. The preclinical properties and human pharmacokinetics of the clinical candidate will be presented.

23.

PROBING THE LIGAND BINDING POCKET OF THE CANNABINOID RECEPTORS - SYNTHESIS, BIOLOGICAL EVALUATION AND STRUCTURE ACTIVITY RELATIONSHIP STUDIES OF NOVEL CLASSICAL CANNABINOID ANALOGS.

***Mathangi Krishnamurthy**, Department of Pharmaceutical Sciences, University of Tennessee, Memphis, 847 Monroe Avenue, Rm 327, Memphis, TN 38163, Fax: 901-448-6828, mkrishna@utmem.edu*

Cannabinoids are a group of tricyclic benzopyran compounds that act on the CB-1 and CB-2 subtypes of cannabinoid receptors. The prototypical members of this class of compounds are δ9-tetrahydrocannabinol (δ9-THC) (1) and its isomer δ8-THC (2), both of which have a pentyl side chain. We had previously reported a series of phenyl substituted side chain analogs of δ8-THC with dimethyl (3), dithiolane (4), methylene (5) and ketone (6) substituents at the C-1′ position of the side chain. Compounds (3) (CB-1 (Ki) – 0.91 nM, CB-2 (Ki) – 12.3 nM) and (6) (CB-1 (Ki) – 23.6 nM, CB-2 (Ki) – 297 nM) showed 13-fold selectivity for the CB-2 receptor in contrast to δ8-THC (CB-2 selectivity – 0.88) Here we report our efforts in probing potential hydrophobic, electrostatic and hydrogen bonding interactions between this class of ligands and the ligand binding pocket of the cannabinoid receptors to further explore the SAR of this class of compounds.

24.

QSAR MODELS FOR POTENT CB1/CB2 SELECTIVE CANNABINOIDS.

***Himanshu Bhattacharjee**, Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, 847 Monroe Ave, Suite 327, Memphis, TN 38163, hbhattac@utmem.edu, and Bob M. Moore, Department of Pharmaceutical Sciences, University of Tennessee, Health Science Center*

Δ-9 Tetrahydrocannabinol (Δ-9 THC), the active constituent of Cannabis sativa, has been shown to have a variety of pharmacological effects. Modifications on the common tricyclic benzopyran core with various substitutions at the 3`side chain of THC with alkyl, cycloalkyl and aromatic substitutions have been evaluated for cannabinoid activity. Other diverse structural modifications on the tricyclic benzopyran core also have been employed in order to decipher structure activity relationship of this class of compounds. In order to elucidate the basic structural requirements for the cannabinoid class of compounds, various naturally occurring cannabinoids and synthetic molecules were employed to devise a Quantitative Structure Activity Relationship (QSAR). Both Comparative Molecular Field Analysis (CoMFA) and Comparative Similarity Index Analysis (CoMSIA) models were developed. The results of these studies give new insights about the electronic and steric factors to be considered for development of more potent and selective cannabinoids.

25.

SYNTHESIS AND SAR OF CB1 SELECTIVE CLASSICAL/NON-CLASSICAL HYBRID CANNABINOIDS.

***Ganesh A. Thakur** and Alexandros Makriyannis, Center for Drug Discovery, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, gathakur@yahoo.com*

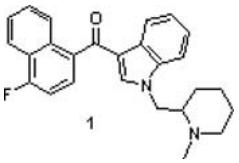
Prior to its discovery the CB1 receptor had been targeted for the development of novel analgesic agents. However, our improved understanding of the extensive physiological roles of this very interesting GPCR has opened the door for additional therapeutic indications such as neurodegeneration, appetite modulation as well as pathologies of the cardiovascular and reproductive systems. To further elucidate the physiological roles of this receptor and for the development of tissue specific CB1-medications there is an increased demand for ligands exhibiting high CB1 affinity and selectivity. Hybrid cannabinoids, which were generated by combining the structural features of classical and non-classical cannabinoids exhibit high affinity and modest selectivity for CB1 receptor. CB1 receptor affinities of these ligands were further improved by replacing the dimethylheptyl side chain with 1-adamantyl moiety at the C-3 position. The convergent synthesis of these ligands makes use of a stereoselective cyclization reaction as the key step for the assembly of the tricyclic ring system. Supported by grants DA 3801, DA7215 to Alexandros Makriyannis.

26.

SYNTHESIS AND TESTING OF A NEW SERIES OF CANNABINOID LIGANDS FOR POSITRON EMISSION TOMOGRAPHY (PET).

***Peter G. Willis**[1], Andrew G. Horti[2], Alexey G. Mukhin[1], Olga A. Pavlova[1], and Svetlana I. Chefer[1]. (1) Neuroimaging Research Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, 5500 Nathan Shock Dr, Baltimore, MD 21206, Fax: 410-550-1441, pwillis@intra.nida.nih.gov, (2) Department of Radiology, Johns Hopkins University School of Medicine*

This study varied substitution on 1-[(N-methyl-piperidin-2-yl)methyl]-3-naphthoylindole, a CB1 agonist giving 14 new ligands. Substitutions to the lead at the 4 position on the naphthyl ring gave Ki values in the range of 0.7 to 2.3 nM, allowing for variation of the lipophilicity of the ligand, giving cLogD values in the range of 2.7 to 4.5. Compound 1 gave the best Ki with a value of 0.7 nM, and a LogD of 2.6.A 4-nitro precursor for nucleophic radio-fluorination of this compound was used giving 2400-3200 mCi/μM specific activity, with enantiomers separated by HPLC. The racemic tracer was used in an ex-vivo study on mice giving target to non-target ratios of 1.6 for radioactivity measurements of hippocampus over brain stem. When separated only one enantiomer was active.



1

27.

DISCOVERY AND OPTIMIZATION OF NOVEL 4,4-DISUBSTITUTED PIPERIDINE DERIVATIVES AS POTENT AND SELECTIVE MELANOCORTIN-4 RECEPTOR ANTAGONISTS FOR THE TREATMENT OF CANCER CACHEXIA.

***Michael Soeberdt**[1], Reto Bolliger[1], Patrick Dunant[2], Marco Henneböhle[1], Karl Hofbauer[2], Sandra Leuzinger[3], Josef Magyar[3], Janet Nicholson[2], Florian Schärer[3], Fredy Schnüriger[3], Michael Stebler[3], Andreas von Sprecher[1], and Philipp Weyermann[1]. (1) Medicinal Chemistry Department, Santhera Pharmaceuticals (Schweiz) GmbH, Hammerstrasse 25, CH-4410 Liestal, Switzerland, Fax: +41-61-9068988, michael.soeberdt@santhera.com, (2) Biozentrum, University of Basel, (3) Biology Department, Santhera Pharmaceuticals (Schweiz) GmbH*

The importance of the MC-4 receptor in feeding behavior and energy homeostasis has been shown in various rodent feeding models. Therefore MC-4 receptor antagonists may be useful in the treatment of cachexia, a cytokine-driven depletion of fat and skeletal muscle mass in the context of a chronic inflammatory response which is caused by an underlying disease. The loss in muscle mass cannot be compensated for by increased nutrient intake. In this presentation we will discuss the design and evaluation of potent, selective Melanocortin-4 receptor antagonists for the treatment of cancer cachexia. The SAR of a series of 4,4-disubstituted piperidine derivatives bearing a chromone-2-carboxamide group as well as in vivo evaluation of a selected compound will be presented.

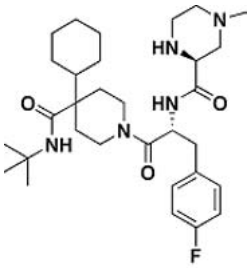
28.

DISCOVERY OF (2S)-N-[(1R)-2-[4-CYCLOHEXYL-4-[[(1,1-DIMETHYLETHYL)-AMINO]CARBONYL]1-PIPERIDINYL]-1-[(4-FLUOROPHENYL)METHYL]-2-OXOETHYL]-4-METHYL-2-PIPERAZINECARBOXAMIDE (MB243), A POTENT AND SELECTIVE MELANOCORTIN SUBTYPE-4 RECEPTOR AGONIST.

***Brenda L. Palucki**[1], Min K. Park[1], Ravi P. Nargund[1], Zhixiong Ye[1], Iyassu K. Sebhat[1], Patrick G. Pollard[1], Rubana N. Kalyani[2], Rui Tang[2], Tanya MacNeil[2], David H. Weinberg[2], Aurawan Vongs[2], Charles I. Rosenblum[2], George A. Doss[3], Randall R. Miller[3], Ralph A. Stearns[3], Qianping Peng[3], Constantin Tamvakopoulos[3], Erin McGowan[4], William J. Martin[4], Joseph M. Metzger[4], Cherrie A. Shepherd[4], Alison M. Strack[4], D. Euan MacIntyre[4], Lex H. T. Van der Ploeg[2], and Arthur A. Patchett[1]. (1) Department of Medicinal Chemistry, Merck & Co., Inc, PO Box 2000, RY123-134, Rahway, NJ 07065, Fax: 732-594-5966, brenda_palucki@merck.com, (2) Department of Obesity Research, Merck & Co., Inc, (3) Department of Drug Metabolism, Merck & Co., Inc, (4) Department of Pharmacology, Merck & Co., Inc*

We report the discovery and optimization of substituted 2-piperazinecarboxamides as potent and selective agonists of the melanocortin subtype-4 receptor. Further in vivo development of lead agonist, MB243, is disclosed. In addition,

drug metabolism studies on MB243 and the 5- and 6-alkylated piperazine derivatives reveal a potential pathway for bioactivation as measured by covalent binding in microsome preparations.



MB243

29.

NOVEL AND HIGHLY SELECTIVE ANTAGONIST SCAFFOLD FOR HUMAN MELANOCORTIN 3 RECEPTOR: COMPUTER-AIDED DESIGN AND BIOLOGICAL EVALUATION. ***Alexander V. Mayorov**, Minying Cai, April R. Van Scoy, Zerui Yu, Kevin B. Chandler, Ravil R. Petrov, Dev Trivedi, and Victor J. Hruby, Department of Chemistry, University of Arizona, 1306 E. University, Tucson, AZ 85721, Fax: 520-621-8407, amayorov@email.arizona.edu*

The melanocortin receptors (hMCR) and their ligands control a surprisingly large number of multifaceted biological actions including skin pigmentation, erectile function, blood pressure and heart rate, control of feeding behavior, and effects on memory and learning processes. To date, five melanocortin receptor subtypes with different patterns of tissue expression in the brain and in the periphery have been cloned and characterized. Of particular interest for pharmaceutical research are the hMC3R and the hMC4R, which have been implicated to play complementary roles in weight control. Selective ligands at hMC3R can provide important analytical tools for investigating the physiological functions of this receptor, as well as a novel approach to treatment of obesity, anorexia, weight loss, and related disorders. Computer-aided design of a novel cyclic lactam peptide scaffold, which exhibits exceptional (up to 1,700-fold) hMC3R selectivity vs. hMC1R and hMC4R, and biological activities of the new potent hMC3R antagonists will be presented.

30.

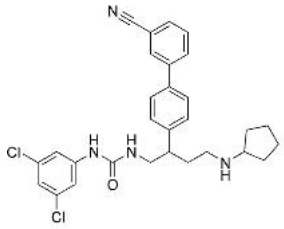
SYNTHESIS AND SAR OF NOVEL 4-PHENYLPIPERIDINE DERIVATIVES AS POTENT AND SELECTIVE MELANOCORTIN SUBTYPE-4 RECEPTOR (MC4-R) ANTAGONISTS. ***Philipp Weyermann**[1], Reto Bolliger[1], Marco Henneböhle[1], Sandra Leuzinger[2], Josef Magyar[2], Florian Schärer[2], Fredy Schnüriger[2], Michael Soeberdt[1], Michael Stebler[2], and Andreas von Sprecher[1]. (1) Medicinal Chemistry Department, Santhera Pharmaceuticals (Schweiz) GmbH, Hammerstrasse 25, CH-4410 Liestal, Switzerland, Fax: +41-61-9068988, philipp.weyermann@santhera.com, (2) Biology Department, Santhera Pharmaceuticals (Schweiz) GmbH*

Chronic diseases such as malignant tumors or infections are frequently associated with cachexia resulting from a combination of a decrease in appetite and a loss of lean body mass. Experimental evidence in tumor bearing mice suggests that cancer cachexia can be prevented or reversed by genetic MC4-R knockout or pharmacological MC4-R blockade with antagonists. We will present our efforts in the design, synthesis, and evaluation of novel MC4-R selective antagonists for the treatment of cachexia. In particular, we will focus on the synthesis and SAR of 4-phenylpiperidine derivatives, comprising a chromone-2-carboxamide moiety.

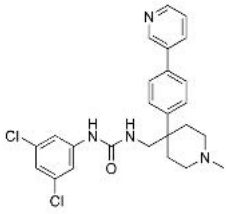
31.

DISCOVERY OF 2-BIARYL-1,4-DIAMINOBUTANE AND 4-BIARYL-4-AMINOMETHYLPIPERIDINE DERIVATIVES AS MELANIN CONCENTRATING HORMONE RECEPTOR 1 ANTAGONISTS THROUGH COMBINATORIAL CHEMISTRY. ***Doug W. Hobbs**[1], Tao Guo[1], Rachael C. Hunter[1], Huizhong Gu[1], Yuefei Shao[1], Gang Qian[1], Suresh D. Babu[1], Laura L. Rokosz[2], and Tara M. Stauffer[2]. (1) Department of Chemistry, Pharmacopeia Drug Discovery, Inc, P.O. Box 5350, Princeton, NJ 08543-5350, Fax: 609-452-3699, dhobbs@pharmacop.com, (2) Department of Biology, Pharmacopeia Drug Discovery, Inc*

Melanin concentrating hormone (MCH), a cyclic 19-amino-acid hypothalamic neuropeptide found in all mammals, plays an important role in the central regulation of food intake and energy homeostasis. For example, central administration of MCH in mice potently stimulates food intake, whereas MCH knockout mice are hypophagic and leaner than wild-type mice. MCH interacts with two distinct G protein-coupled receptors (GPCRs) in the brain, MCH1R and MCH2R, of which MCH1R has been implicated in the control of feeding behavior and energy balance. For example, MCH1R knockout mice are lean, hyperphagic but hyperactive and resistant to diet-induced obesity. Thus, MCH1R antagonists represents an attractive therapeutic approach for the treatment of obesity and related disorders. In this presentation, we will describe the discovery of novel 2-biaryl-1,4-diaminobutane and 4-biaryl-4-aminomethylpiperidine derivatives, such as 1 and 2 as MCH1R antagonists through combinatorial chemistry. Library design considerations and implementation strategies will also be discussed in the talk.



1
MCH1R K_i = 2.0 nM

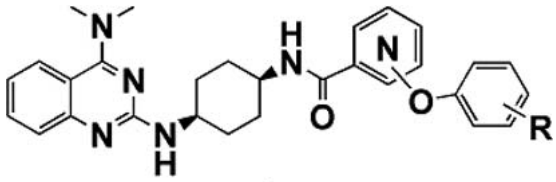


2
MCH1R Ki = 3.1 nM

32.

MCHR-1 ANTAGONISTS INCORPORATING THE PHENOXYPYRIDINE MOTIF SHOWING SUBNANOMOLAR FUNCTIONAL ACTIVITY. *Juyi Choi, ***Thuy-Anh Tran**, ***Sangdon Han**, Bryan Kramer, Debbie Hsu, Martin Casper, Ning Zou, Pureza Vallar, Jerry Xu, Bill Thomsen, Christine Testa, and Graeme Semple, Medicinal Chemistry, Arena Pharmaceuticals, 6166 Nancy Ridge, San Diego, CA 92122, ttran@arenapharm.com*

We have previously disclosed the potent and orally effective non-peptide antagonist for the MCHR1 receptor, a 4-(dimethylamino)quinazoline derivative, ATC0075. We describe herein the design and synthesis of 4-(dimethylamino) quinazoline (1) derivatives containing phenoxypyridine moieties. Several compounds showed in vitro MCHR1 antagonistic activity (IC50) values in the picomolar range. The results demonstrated that phenoxypyridine moiety constitute a pharmacophore of highly potent and selective MCHR1 antagonists.

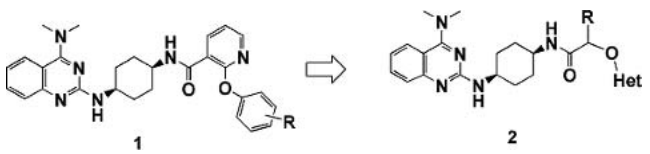


1

33.

NOVEL SERIES OF 4-(DIMETHYLAMINO)QUINAZOLINE CONTAINING HETEROCYLIC ETHERS AS POTENT ANTAGONISTS OF HMCH-R1. *Juyi Choi, Thuy-Anh Tran, ***Bryan Kramer**, Sangdon Han, Debbie Hsu, Martin Casper, Ning Zou, Pureza Vallar, Jerry Xu, Bill Thomsen, Christine Testa, and Graeme Semple, Medicinal Chemistry, Arena Pharmaceuticals, 6166 Nancy Ridge, San Diego, CA 92122*

In an effort to optimize our 4-(dimethylamino)quinazoline (1) derivatives containing phenoxypyridine moiety, we focused our attention on reducing molecular weight of (1). From these efforts we identified series of highly potent heterocylic ether derivatives (2). In this poster we will highlight the synthesis and SAR of the heterocylic ether MCHR1 antagonists.

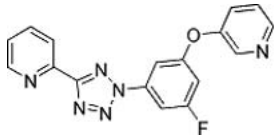


1 **2**

34.

2-{2-[3-(PYRIDIN-3-YLOXY)PHENYL]-2H-TETRAZOL-5-YL}PYRIDINE: A HIGHLY POTENT, ORALLY ACTIVE, METABOTROPIC GLUTAMATE SUBTYPE 5 (MGLU5) RECEPTOR ANTAGONIST. ***Dehua Huang**, Steve F. Poon, Deborah F. Chapman, Janice Chung, Merryl Cramer, Thomas S. Reger, Jeffrey Roppe, Lida Tehrani, Nicholas D. P. Cosford, and Nicholas D. Smith, Merck Research Laboratories, San Diego, 3535 General Atomics Court, San Diego, CA 92121-1140, Fax: 858-202-5752*

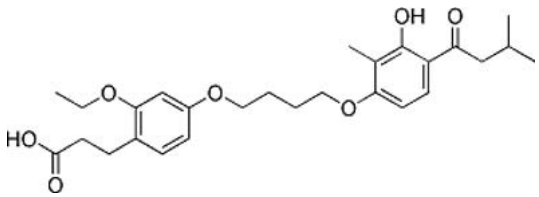
Structure-activity relationship studies on 3-(5-pyridin-2-yl-2H-tetrazol-2-yl)benzonitrile led to the discovery of 2-{2-[3-(pyridin-3-yloxy)phenyl]-2H-tetrazol-5-yl}pyridine - a highly potent and selective mGlu5 receptor antagonist with good brain penetration and in vivo receptor occupancy in rat and cross species oral bioavailability.



35.

3-(2-ETHOXY-4-{4-[3-HYDROXY-2-METHYL-4-(3-METHYLBUTANOYL)PHENOXY]BUTOXY}PHENYL)PROPANOIC ACID: A BRAIN PENETRANT ALLOSTERIC POTENTIATOR AT THE METABOTROPIC GLUTAMATE RECEPTOR 2 (MGLUR2). ***Rowena V. Cube**[1], Jean-Michel Vernier[2], John H. Hutchinson[1], Michael F. Gardner[2], Joyce K. James[2], Blake A. Rowe[2], Herve Schaffhauser[2], Lorrie Daggett[2], and Anthony B. Pinkerton[1]. (1) Department of Medicinal Chemistry, Merck Research Laboratories, 3535 General Atomics Court, San Diego, CA 92121, Fax: 858-202-5752, rowena_cube@merck.com, (2) Merck Research Laboratories-San Diego*

Glutamate agonists targeting the metabotropic glutamate receptors may have utility in a variety of diseases including epilepsy, anxiety and schizophrenia. Although, rigid glutamate analogs such as (1S,2S,5R,6S-2-aminobicyclo-[3.1.0]hexane 2,6-dicarboxylic acid have shown efficacy, these compounds are non selective mGlu2/3 receptor agonists. Selective agonists for mGlu2 over mGlu3 have not, as yet, been discovered. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding site. This poster details the discovery and SAR of a class of selective mGlu2 receptor potentiators. We have identified and synthesized a brain penetrant allosteric potentiator of the metabotropic glutamate receptor 2. SAR studies directed toward improving the potency, level of potentiation and brain penetration led to the discovery of 3-(2-ethoxy-4-{4-[3-hydroxy-2-methyl-4-(3-methylbutanoyl)phenoxy]butoxy} phenyl)propanoic acid (EC50 = 1200 nM, 77% potentiation, 119% brain/plasma in rat, 20 mpk ip, brain level of 5700 nM).



36.

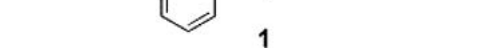
BENZAZOLES AS ALLOSTERIC POTENTIATORS OF METABATROPIC GLUTAMATE RECEPTOR 2. ***Steven P. Govek**, Celine Bonnefous, Una C Campbell, Lorrie Daggett, Michael F. Gardner, John H. Hutchinson, Joyce K. James, Jeffrey McQuiston, Richard Pracitto, Dana E. Rodriguez, Blake A. Rowe, Herve Schaffhauser, Jean-Michel Vernier, Xiumin Zhao, and Theodore Kamenecka, Merck Research Laboratories-San Diego, 3535 General Atomics Court, San Diego, CA 92129, steven_govek@merck.com*

Glutamate is the primary excitatory neurotransmitter in the central nervous system. One class of receptors at which glutamate functions is the metabotropic glutamate receptors (mGluR). These G-protein-coupled receptors, of which eight subtypes have been identified, are categorized into three groups based on primary structure, second messenger coupling and pharmacology: group I (mGluR1 & 5); group II (mGluR2 & 3); and group III (mGluR4, 6, 7, & 8). The group II mGluRs have been implicated in a variety of disease states including anxiety and schizophrenia. Moreover, group II mGluR agonists have shown efficacy in animal models of schizophrenia. It is believed that this functional activity comes specifically from agonism of mGluR2 because of this receptor's role in regulating glutamate release into the synapse. Screening of our compound collection revealed an allosteric potentiator which was selective for mGluR2. Described herein is the elaboration of this lead into a series of mGluR2-selective benzazoles which have shown to be efficacious in an animal model of schizophrenia.

37.

PHENYL-CARBOXYLIC INDANONES: DISCOVERY OF POSITIVE ALLOSTERIC POTENTIATIORS OF THE METABOTROPIC GLUTAMATE SUBTYPE 2 (MGLU2) RECEPTOR. ***Celine Bonnefous**[1], Jean-Michel Vernier[1], John H. Hutchinson[2], Michael F. Gardner[1], Blake A. Rowe[1], Herve Schaffhauser[1], Una C Campbell[1], Dana E. Rodriguez[1], Joyce K. James[1], Linda J. Bristow[3], Lorrie Daggett[1], and Theodore Kamenecka[1]. (1) Merck Research Laboratories-San Diego, 3535 General Atomics Court, San Diego, CA 92121, Fax: 858-202-5752, celine_bonnefous@merck.com, (2) Department of Medicinal Chemistry, Merck Research Laboratories, (3) Department of Pharmacology, Merck Research Laboratories*

Glutamate is the transmitter of the large majority of fast excitatory synapses in the CNS and plays an important role in a wide variety of CNS functions. It activates both ionotropic glutamate receptors, which are glutamate-gated ion channels, as well as the metabotropic glutamate (mGlu) receptors which are a family of G-protein coupled receptors. Eight subtypes of the mGlu receptors have been identified which fall into three main groups. Group I consists of mGlu1 and 5 receptors, which have mainly been shown to be stimulatory. Groups II (mGlu2 and 3) receptors and group III (mGlu4, 6, 7, 8) receptors, primarily localized presynaptically, generally inhibit neurotransmission. Agents targeting the mGlu receptors may have utility in a variety of clinical conditions including epilepsy, anxiety, and schizophrenia. Due to the high degree of sequence homology between group II mGlu receptors, especially at the glutamate binding site, selective agonists for mGlu2 receptor over mGlu3 receptor have not, as yet, been discovered. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding site. To pursue this hypothesis, a screening campaign was initiated using an assay designed to detect compounds acting at allosteric sites able to confer a positive functional modulation of the receptor. The work reported herein describes the synthesis of a new class of selective positive allosteric modulators of human mGlu2 receptors and in particular the synthesis and pharmacological characterization of 3′-{[(2-cyclopentyl-6,7-dimethyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy]methyl}biphenyl-4-carboxylic acid 1.



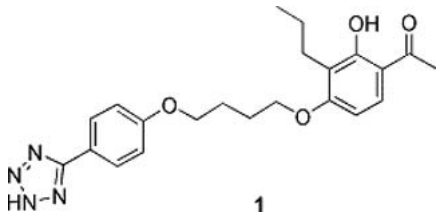
1

38.

SUBSTITUTED ACETOPHENONES AS SELECTIVE AND POTENT ALLOSTERIC POTENTIATORS OF THE METABOTROPIC GLUTAMATE RECEPTOR 2 (MGLUR2). ***Anthony B. Pinkerton**[1], Rowena V. Cube[1], John H. Hutchinson[2], Michael F. Gardner[2], Joyce K. James[2], Blake A. Rowe[2], Herve Schaffhauser[2], Dana E. Rodriguez[2], Una C Campbell[2], Chris S Baccei[3], Daniel S Lorrain[3], Lorrie Daggett[2], and Jean-Michel Vernier[2]. (1) Department of Medicinal Chemistry, Merck Research Laboratories, 3535 General Atomics Court, San Diego, CA 92121, Fax: 858-202-5752, anthony_pinkerton@merck.com, (2) Merck Research Laboratories-San Diego, (3) Department of Pharmacology, Merck Research Laboratories*

Metabotropic glutamate receptors (mGluRs) have been implicated in a number of CNS disorders including schizophrenia and anxiety. Eight subtypes of mGluRs have been identified, which fall into three main groups. Group II consists of two subtypes, mGluR2 and mGluR3, for which a number of non selective agonists and antagonists have been developed. Herein we disclose the discovery of a new class of positive allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2), substituted acetophenones, e.g. 1-(2-hydroxy-3-propyl-4-{4-[4-(2H-tetrazol-5-yl)phenoxy]butoxy}phenyl) ethanone (1). The SAR around this

scaffold will be discussed including the replacement of the tetrazole of the lead structure.

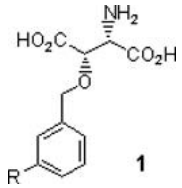


1

39.

SYNTHESIS AND EVALUATION OF 3-BENZYLOXYASPARTATE DERIVATIVES AS EAAT2 INHIBITOR. ***Akira Hiratate**, Madoka Kawamura, Mariko Nishiguchi, Masato Nakamura, and Naoya Kawashima, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403, Yoshino-cho, Kita-ku, Saitama-shi 331-9530, Japan, Fax: +81-48-652-7254*

L-glutamate is the excitatory neurotransmitter in the central nervous system. Excitatory amino acid transporter 2 (EAAT2, GLT-1), mainly expressed in astrocytes, takes a role in control of the extracellular glutamate concentration. EAAT2 inhibitors are expected as a drug for use the treatment of diseases relating to the abnormality of glutamatergic neurotransmission. A series of 3-benzyloxyaspartate derivatives is one of the most potent EAAT2 inhibitors. We focused attention on the substituents at 3-position on benzene ring, synthesized and evaluated these derivatives **1**. Among them, 3-urea bond linker derivative was found to show high EAAT2 inhibitory activity and brain drug concentrations after intravenous injection to rats. Synthesis and biological data will be presented.



1

40.

OCCURRENCE OF D-ASPARTATE AND N-METHYL-D-ASPARTATE IN THE SERUM, LIVER AND BRAIN OF CHICKEN (*GALLUS DOMESTICUS*). ***Jean-Joseph Poisson**[1], R.A. Mirza[1], G. Ferrandino[2], P. Spinelli[2], Antimo D'Aniello[2], and George Fisher[3]. (1) School of Natural and Health Sciences, Barry University, 11300 NE 2nd Ave., Miami Shores, FL 33161, poissonj@bucmail.barry.edu, (2) Laboratory of Neurobiology, Stazione Zoologica, (3) Department of Chemistry, Barry University*

In 1990, investigators Neidle and Dunlop found free D-aspartic acid (D-Asp) in the chicken. Recently, D'Aniello, et al. have demonstrated that D-Asp and N-methyl-D-aspartate (NMDA) are endogenously present in the nervous system and endocrine tissues of mammals where these amino acids play a role in the synthesis and release of pituitary hormones. Given that chickens are widely consumed, we sought to quantitatively investigate if chicken tissues indeed possess D-Asp and NMDA and assess the health impacts that a chicken diet may have on the consumer. In this research, tissues (serum, brain and liver) of chickens were homogenized in 70% methanol, purified by anion exchange and passed through a C18 Sep-Pak. D-Asp and NMDA were then determined by high pressure liquid chromatography (HPLC) and enzymatic colorimetric method assays. We found that D-Asp is present at a concentration ~400 nmole/g and NMDA is present at a concentration ~1 nmol/g tissue (about 400 times less than D-Asp). These data indicate that the concentrations of both D-Asp and NMDA in chicken tissues are negligible and therefore would not have an appreciable impact on the health of those who enjoy dieting on poultry.

41.

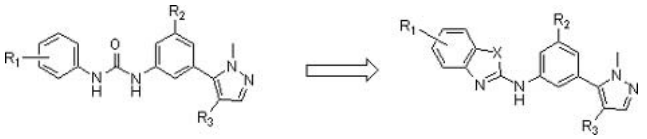
AMINOALKOXY INDOLES: POTENT AND SELECTIVE 5HT6 RECEPTOR LIGANDS. ***Ramakrishna Venkata Satya Nirogi**, Anand V Daulatabad, Sarika A Daulatabad, Sandeep B Bhosale, Narendra Varma Gaddiraju, Rajat Dwivedi, Mili A Deshpande, Rama Sastry Kambhampati, and Vikas Shreekrishna Shirsath, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, nvsrk@suven.com*

The usefulness of 5ht6 antagonists in the treatment of alzheimers, parkinsons disease and other cognitive, neurodegenerative disorders has been well demonstrated. Their application in the treatment of feeding disorders is also being pursued. However, in spite of availability of numerous potent and selective ligands, the full characterization of functional and physiological usefulness of these molecules is limited due to the lack of desirable pharmacokinetic properties required for the CNS agent. Our continuing efforts towards design and discovery of selective 5ht6 antagonists have lead to the identification of a new class of compounds. Unlike the compounds known so far, these aminoalkyl indolyl ethers are conformationally highly flexible molecules. Our effective lead generation and optimization methods have resulted in a series of potent 5ht6 receptor ligands with Ki in the range of 1 to 5 nanomoles, when tested by the in-vitro radio-ligand binding techniques. These ligands have more than 100-fold selectivity when tested in about fifteen related GPCRs. The synthesis, physicochemical properties and the in-vitro binding data along with the SAR will be discussed.

42.

BIOISOSTERIC MODIFICATIONS OF UREA DERIVATIVES AS 5-HT$_{2A}$ INVERSE-AGONISTS. ***Sonja Strah-Pleynet**, Bradley R. Teegarden, Susan D. Selaya, Honnappa Jayakumar, Robert R. Webb, Nigel R. A. Beeley, William Thomsen, and Hazel Reyes, Arena Pharmaceuticals, Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, sstrah@arenapharm.com*

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. Previously we reported the discovery of a new class of urea containing 5-HT$_{2A}$ inverse-agonists. Bioisosteric modification of the urea moiety led to the identification of a series of bicyclic diarylamines such as aminobenzoxazoles as novel 5-HT$_{2A}$ inverse-agonists. The synthesis and structure-activity relationships of a new series will be presented.



43.

CONFORMATIONALLY RESTRICTED AMINOALKYL INDOLES : A NEW CHEMICAL CLASS OF SELECTIVE 5HT6 RECEPTOR LIGANDS WITH DRUG LIKE PROPERTIES. ***Rama Sastry Kambhampati**, Prabhakar Kothmirkar, Jagadish Babu Konda, Reshma F Kurangi, Trinath Reddy Bandyala, Srinivasulu Kota, Suchitra P Chinthapalli, Vikas Shreekrishna Shirsath, and Ramakrishna Venkata Satya Nirogi, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, krsastri@suven.com*

Various research groups have demonstrated the usefulness of 5ht6 antagonists in the treatment of various cognitive disorders. Their application in the treatment of feeding disorders is also being pursued. However, in spite of availability of numerous potent and selective ligands, the full characterization of functional and physiological usefulness of these molecules is limited due to the lack of desirable pharmacokinetic properties required for the CNS agent. As a part of ongoing program for the synthesis of selective 5ht6 ligands, we have designed some novel 5ht6 ligands on a chemically novel skeleton. Attempts have been made to optimize the drug-like properties of these compounds by suitably modifying their physicochemical properties. Our primary hit was found to have the Ki in the micromolar range. Our effective lead optimization efforts have resulted in the lead molecules with Ki in the range of 1 to 5 nanomols at the 5ht6 receptor. Lead molecules have been established to be more than 100-fold

selective over a range closely related GPCRs. The synthesis, physicochemical properties and the in-vitro binding data along with the SAR will be discussed.

44.

CONSTRUCTION OF INDOLE LIBRARY FOR SEROTONIN RELATED DRUGS IN SOLID-PHASE REACTION. *Han-Seo Mun, Kyung-Tae Lee, and **Jin-Hyun Jeong**, College of Pharmacy, Kyung Hee University, #1 Hoeki-Dong, Dongdaemoon-ku, Seoul 130-701, South Korea, Fax: 82-2-961-0357, hsmun@hanmail.net, jeongjh@khu.ac.kr*

Hetero chain compounds have high possibilities of being good medicinal candidate because of their well-known medicinal activity and relatively low subtitled carbon. Among them, indole compounds are well known as headache medicine with an infinite applications even in various physiological activities. By constructing the method of making this compound library, this research has the purpose to create a new medicinal candidate materials based on an easy medicinal search.

45.

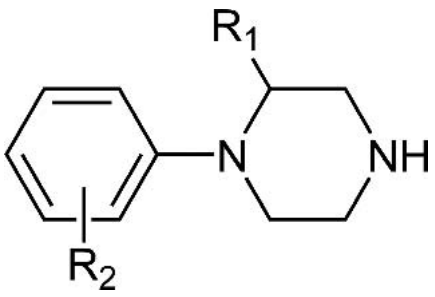
DIALKYL PIPERAZINES: A NEW CHEMICAL CLASS OF SELECTIVE 5HT6 RECEPTOR LIGANDS. ***Vikas Shreekrishna Shirsath**, Amol D Deshpande, Adi Reddy Dwarampudi, Shailesh M Patel, Rajesh Kumar Badange, Venugopala Rao Bhatta, Dharmaraju Chinthapalli, Rama Sastry Kambhampati, and Ramakrishna Venkata Satya Nirogi, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, svikas@suven.com*

The usefulness of 5ht6 antagonists in the treatment of alzheimers, parkinsons disease and other neurodegenerative disorders has been well demonstrated. However, lack of desirable pharmacokinetic properties required for these CNS agents, has greatly hampered the full characterization of functional and physiological usefulness of these molecules. A new chemical class of 5ht6 antagonists have been designed, keeping in mind the physicochemical properties required for achieving the desired pharmacokinetic and CNS penetration properties. Our effective lead generation and optimization methods have resulted in a series of potent 5ht6 receptor ligands with Ki in the range of 1 to 5 nanomoles, when tested by the in-vitro radio-ligand binding techniques. These ligands have more than 100-fold selectivity when tested in related GPCRs. The synthesis, physicochemical properties and the in-vitro binding data along with the SAR will be discussed.

46.

EVALUATION OF SELECTIVE 5-HT_{2C} PHENYLPIPERAZINE AGONISTS FOR THE TREATMENT OF OBESITY. ***James H. Tsai**[1], Brian M. Smith[1], Rita Chen[1], Emily Prieto[1], Douglas Park[1], Jeffrey Smith[1], Jeffrey A. Schultz[1], Charlemagne Gallardo[1], Scott Estrada[1], Sherry Fang[1], Charles Gilson[1], William Thomsen[1], Hazel Saldana[1], Christina Bjenning[1], Kevin Creehan[1], Lena Gonzalez[1], Kevin Whelan[1], Robert R. Webb[2], and Nigel Beeley[1]. (1) R&D, Arena Pharmaceuticals Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, (2) Arena Pharmaceuticals, Inc*

Nonselective 5-HT_{2C} receptor agonists such as *meta*-chlorophenylpiperazine (*m*-CPP) have been shown to cause weight loss though the reduction of food intake in humans and rodents. However, the activation of other targets with nonselective agonists especially the 5-HT_{2A} and 5-HT_{2B} receptors could present liabilities towards psychostimulation and valvular heart disease respectively. Here we describe the synthesis and evaluation of a modified phenylpiperazine class of potent 5-HT_{2C} agonists with substantial selectivity over the 5-HT_{2A} and 5-HT_{2B} subtype receptors.



47.

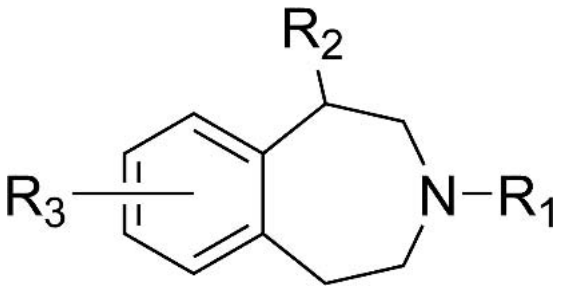
MOLECULAR MODELLING OF THE BINDING OF [18F]-MPPF, A PET RADIOTRACER, ON THE 5-HT1A CEREBRAL RECEPTORS IN THE RAT. *L Montreuil[1], **R Terreux**[1], M Domard[1], and L Zimmer[2]. (1) LCMP2, ISPB, University Claude Bernard Lyon1, 8 av. Rockefeller, lyon 69373, France, raphael.terreux@univ-lyon1.fr, (2) CERMEP Biomedical Cyclotron & INSERM U512, ISPB, University Claude Bernard Lyon1*

Serotonin is a cerebral neurotransmitter involved in several psychiatric pathologies, like depression. The antidepressant treatment using fluoxetine (Prozac®) increases the serotonin neurotransmission and implies the internalization of the 5-HT1A receptors in serotoninergic neurons. According in vivo studies, this subcellular phenomenom could be revealed by a 5-HT1A PET radiotracer, [18F]-MPPF, which binds specifically to the externalised receptors. In order to modelize this specific binding we constructed a model of the 5-HT1A receptor by homology using several X-ray structures. Long molecular dynamics simulations of the model were computed in intra- and extracellular medium. The two simulations were analysed and the cluster analysis of conformation of coils were performed. Based of these conformations docking, these simulations revealed that the specific binding of [18F]-MPPF can be explain by a conformational change.

48.

SYNTHESIS AND SAR OF SUBSTITUTED BENZAZEPINES AS SELECTIVE 5-HT_{2C} AGONISTS. ***Jeffrey A. Schultz**[1], Brian M. Smith[1], Jeffrey Smith[1], James H. Tsai[1], Charles Gilson[1], Scott Estrada[1], Rita Chen[1], Douglas Park[1], Emily Prieto[1], Charlemagne Gallardo[1], Dipanjan Sengupta[1], William Thomsen[1], Hazel Saldana[1], Christina Bjenning[1], Kevin Creehan[1], Lena Gonzalez[1], Kevin Whelan[1], Robert R. Webb[2], and Nigel Beeley[1]. (1) R&D, Arena Pharmaceuticals Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, (2) Arena Pharmaceuticals, Inc*

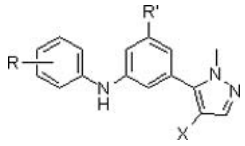
The 5-HT_{2C} receptor has been reported to be involved in the regulation of feeding and satiety. It has been demonstrated that the nonselective 5-HT_{2C} agonists *m*-CPP and nordexfenfluramine cause weight loss by reduction of food intake. However problems in these compounds associated with the closely related 5-HT_{2B} (valvular heart disease) and 5-HT_{2A} (psychoactive properties) receptors have lead to highly selective 5-HT_{2C} agonists being actively pursued as treatments for obesity. We have discovered a series of substituted benzazepines that are potent and selective 5-HT_{2C} agonists. The SAR and receptor selectivity of this series will be described.



49.

SYNTHESIS AND SAR OF SUBSTITUTED DIPHENYLAMINES AS 5-HT_{2A} INVERSE-AGONISTS. ***Honnappa Jayakumar**, Bradley R. Teegarden, Sonja Strah-Pleynet, Susan D. Selaya, Naomi Kato, Katie Elwell, Jarrod Davidson, Young-Jun Shin, Robert R. Webb, Nigel R. A. Beeley, William Thomsen, Hazel Reyes, Frederique Menzaghi, and Kevin Whelan, Arena Pharmaceuticals, Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, hjayakumar@arenapharm.com*

Previously we reported on novel, urea based 5-HT_{2A} inverse-agonists. Subsequent replacement of the urea moiety with heterocyclic bioisosteres (aminobenzoxazole etc.) led us to reduce the linker to an amino functionality. Further modification of heterocyclic derivatives resulted in a series of substituted diphenylamines. Herein we present the synthesis and SAR of diphenylamines as selective 5-HT_{2A} inverse-agonists.



50.

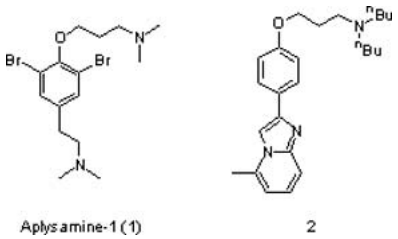
SYNTHESIS OF (*E*)-4, 3, 2-[^{11}C]METHOXY-*N*-(4-(4-(2-METHOXYPHENYL)-PIPERAZIN-1-YL)BUTYL)CINNAMOYLAMIDES AND (*E*)-4, 3, 2-[^{18}F]FLUORO-*N*-(4-(4-(2-METHOXYPHENYL)PIPERAZIN-1-YL)BUTYL)-CINNAMOYLAMIDES AS NEW POTENTIAL PET DOPAMINE D_2 AND D_3 RECEPTOR LIGANDS. ***Mingzhang Gao**, Ji-Quan Wang, and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, Room L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, migao@iupui.edu*

The neurotransmitter dopamine is implicated in various physiological and pathophysiological processes. The dopamine D_2 and D_3 receptors are recognized as potential therapeutic targets for the treatment of various neurological and psychiatric disorders. *In vivo* biomedical imaging technique positron emission tomography (PET) coupled with appropriate receptor radioligands has become a clinically valuable and accepted diagnostic tool to image brain diseases. (*E*)-4, 3, 2-[^{11}C]Methoxy-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-cinnamoyl-amides and (*E*)-4, 3, 2-[^{18}F]fluoro-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-cinnamoylamides were synthesized for evaluation as new potential PET imaging agents for brain D_2 and D_3 receptors. The carbon-11 tracers were prepared by *O*-[^{11}C]methylation of hydroxy-precursors (*E*)-4, 3, 2-hydroxy-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-cinnamoylamides using [^{11}C]methyl triflate and isolated by solid-phase extraction (SPE) purification procedure. The fluorine-18 tracers were prepared by [^{18}F]fluorination of the nitro-precursors (*E*)-4, 3, 2-nitro-*N*-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-cinnamoyl-amides with K^{18}F/Kryptofix$_{2.2.2}$ through nucleophilic substitution and purification with the HPLC method.

51.

APLYSAMINE-1 AND RELATED ANALOGS AS HISTAMINE H_3 RECEPTOR ANTAGONISTS. ***Devin M. Swanson**, Sandy J. Wilson, Jamin D. Boggs, Ann J. Barbier, Wei Xiao, Richard Apodaca, Timothy W. Lovenberg, and Nicholas I. Carruthers, Neuroscience, Johnson & Johnson Pharmaceutical Research and Development, LLC, 3210 Merryfield Row, San Diego, CA 92121, Fax: 858-450-2049, dswanso1@prdus.jnj.com*

The histamine H_3 receptor is a G-protein coupled receptor belonging to the family of histamine receptor subtypes (H_1, H_2, H_3, and H_4). The H_3 receptor is located presynaptically in the peripheral and central nervous systems, on both histaminergic neurons, as an autoreceptor, and other neuronal systems, as a heteroreceptor. In this capacity it functions as a negative modulator, inhibiting the release of histamine and other neurotransmitters such as acetylcholine, GABA, norepinephrine, and serotonin. Histamine H_3 antagonists enhance levels of cerebral histamine and, therefore, may be useful in the treatment of neurological disorders affecting memory, appetite, and sleep. Aplysamine-1 (**1**) is a marine natural product isolated in 1989 from an Australian sponge, *Aplysina sp.* The natural product is a bromotyramine derived metabolite consisting of two alkyl tertiary amines connected by a dibromo-phenol and has been previously synthesized. Aplysamine-1 (**1**) was reported to have weak H_3 binding affinity in guinea pig brain and to behave as an H_3 functional antagonist in an in vitro guinea pig tissue strip assay prior to the cloning of the H_3 receptor cDNA. Aplysamine-1 (**1**) contains a structural motif similar to non-imidazole human H_3 ligands which emerged from high thoughput screening of our corporate compound collection (**2**). Thus, we chose to explore the structure activity relationship (SAR) of aplysamine-1 based H_3 ligands. Herein the synthesis and biological activity of aplysamine-1 (**1**) and related analogs are reported.



Aplysamine-1 (1) 2

52.

DEVELOPMENT OF BENZIMIDAZOLES AS LIGANDS FOR THE H4 RECEPTOR. ***Alice Lee**[1], Kristen L. Arienti[1], James G. Breitenbucher[1], Daniel J. Buzard[1], Paku Desai[2], James P. Edwards[1], Michael D. Hack[1], Lars Karlsson[2], Haripada Khatuya[1], David E. Kindrachuk[1], Robin L. Thurmond[2], and Jennifer D. Venable[1]. (1) Department of Chemistry, Johnson and Johnson Pharmaceutical Research and Development, LLC, 3210 Merryfield Row, San Diego, CA 92121, Fax: 858-450-2089, alee7@prdus.jnj.com, (2) Department of Biology, Johnson and Johnson Pharmaceutical Research and Development, LLC*

The histamine H4 receptor is a 390-amino acid, seven-transmembrane, G protein-coupled receptor with approximately 40% homology to the histamine H3 receptor. In contrast to the H3 receptor, which is primarily located in the brain, the H4 receptor is mainly expressed in eosinophils and mast cells. The preferential expression of the H4 receptor in immune cells suggests that this receptor is involved in the regulatory functions of histamine during the immune response. Recent studies have shown that the H4 receptor controls the release of inflammatory mediators and facilitates leukocyte chemotaxis. Modulation provides the ability for treating H4-mediated diseases and conditions, including the deleterious effects of allergic responses. A novel series of benzimidizoles as ligands for the H4 receptor was discovered. Their synthesis and resultant SAR will be discussed.

53.

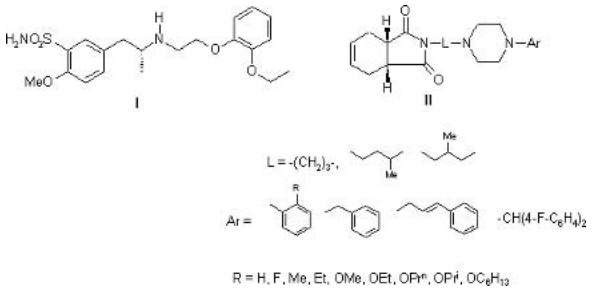
PREPARATION OF BENZIMIDAZOLE CARBOXAMIDES AS POTENT HUMAN HISTAMINE H4 ANTAGONISTS. ***Jennifer D. Venable**, Barb Pio, Curt A. Dvorak, Cheryl A. Grice, Kiev S. Ly, Chandravadan R. Shah, Jianmei Wei, Pragnya J. Desai, Wen Jiang, Steven Nguyen, Sandy J. Wilson, Paul J. Dunford, Robin L. Thurmond, Timothy W. Lovenberg, Lars Karlsson, Nicholas I. Carruthers, and James P. Edwards, Johnson and Johnson Pharmaceutical Research and Development, LLC, 3210 Merryfield Row, San Diego, CA 92121, jvenable@prdus.jnj.com*

The human histamine H_4 receptor was recently discovered and cloned by several groups. The expression profile includes eosinophils, mast cells, dendritic cells, and other leukocytes, implicating H_4 in inflammation and regulation of the immune system. A significant medicinal chemistry effort has been undertaken to discover and develop potent antagonists of the histamine H_4 receptor. During the course of this effort, the synthesis of benzimidazole-2-carboxamides via benzimidazole-2-carboxylic esters was examined. A single literature disclosure reported that condensation of a phenylenediamine with alkyl trialkoxyacetate forms the desired benzimidazole carboxylic ester. In our hands, treatment of phenylenediamines with methyl trimethoxyacetate did not yield the desired product. However, addition of a Lewis acid catalyst, such as Yb(OTf)$_3$, unexpectedly led to the formation of 3-methoxy-quinoxalin-2-ones in good yields. Ultimately, a general, two-step route was developed in order to obtain the desired carboxamides via variously substituted 2,2,2-trichloromethylbenzimidazoles. The synthesis and structure activity relationships (SAR), of the benzimidazole carboxamides will be discussed.

54.

DERIVATIVES OF CIS-1,2,3,6-TETRAHYDROPHTHALIMIDE AS ALPHA1A – SELECTIVE ADRENERGIC RECEPTOR ANTAGONISTS. ***P.K.S. Sarma**[1], Sanjay Jain[1], Neelima Sinha[1], Laxminarayan G Hegde[2], Kamna Nanda[2], Anita Chugh[2], J.B Gupta[2], and Nitya Anand[1]. (1) Department of Medicinal Chemistry, Ranbaxy Research Laboratories, Sector 18, Plot No.20, Gurgaon, Haryana, 122 001, India, Fax: 91-124-2343545, pakala.sarma@ranbaxy.com, (2) Department of Pharmacology, Ranbaxy Research Laboratories*

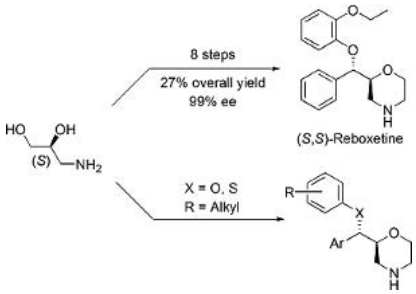
alpha1A – selective adrenergic receptor antagonists have therapeutic potential for the treatment of Benign prostatic hyperplasia (BPH). Tamsulosin (I), the first alpha1A – selective adrenergic receptor antagonist has modest selectivity for alpha1A- AR over alpha1B-AR and shows side effects such as abnormal ejaculation and dizziness.With a view to develop highly selective alpha1A - adrenergic receptor antagonists, we designed a series of cis-1,2,3,6-tetrahydrophthalimide derivatives with the general structure II. Synthesis and biological activities of these derivatives will be discussed.



55.

CHIRAL SYNTHESIS OF (+)-(S,S)-REBOXETINE VIA A NEW (S)-2-(HYDROXYMETHYL)MORPHOLINE PREPARATION: APPLICATION FOR THE DEVELOPMENT OF NEW NOREPINEPHRINE REUPTAKE INHIBITORS. *Eric Brenner[1], Ronald M Baldwin[1], frank tarazi[2], Ross J. Baldessarini[3], and* ***Gilles D Tamagnan****[4]. (1) VA CT HCS (116A2), Yale University School of Medicine, 950 Campbell Ave, West Haven, CT 06516, (2) Neuropharmacology Laboratory, McLean Hospital, Harvard Medical School, (3) Neuropharmacology Laboratory, McLean Hospital, Harvard Medical School, (4) Institute for Neurodegenerative Disorders, 60 temple street, New Haven, CT 06510, gtamagnan@indd.org*

Reboxetine is the name given to the racemic mixture of (2R,3R)- and (2S,3S)-2-[*f*Ñ-(2-ethoxyphenoxy)phenylmethyl]-morpholine, known to be a potent selective norepinephrine reuptake inhibitor (NRI). Commercially sold as an antidepressant, reboxetine has comparable efficacy to that of imipramine, desipramine and fluoxetine. Among reboxetine optical isomers, (S,S)-reboxetine presents the best affinity and selectivity for norepinephrine transporter (NET), making it an attractive lead compound for our research program, which is oriented towards drug development for depression and attention deficit hyperactivity disorder (ADHD), as well as radiotracer synthesis for in vivo imaging of NET. We present the enantioselective synthesis of (S,S)-reboxetine without the need for optical resolution, starting from commercially available (S)-3-amino-1,2-propanediol. The route is applicable to the synthesis of other novel analogs with improved pharmacological activity.



56.

DEVELOPMENT OF POLYFLUOROTROPANES FOR 19F MAGNETIC RESONANCE IMAGING (MRI) AND SPECTROSCOPIC ANALYSIS (MRS) AT THE DOPAMINE TRANSPORTER. ***Ao Zhang****[1], John L. Neumeyer[1], Nora S. Kula[2], Kehong Zhang[2], and Ross J. Baldessarini[2]. (1) Medicinal Chemistry Laboratory, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, Fax: 617-855-2519, azhang@mclean.harvard.edu, (2) Neuropharmacology Laboratory, McLean Hospital, Harvard Medical School*

Recently, we developed a novel class of nonradiolabeled, polyfluorinated tropanes which may serve as MRI- or MRS-detectable index molecules for the cerebral dopamine transporter protein (DAT). These ligands contain at least three 19F atoms/molecule in an MR-equivalent chemical environment to increase coherent MR signal characteristics. The binding assay of these compounds indicated that they have good affinity at dopamine transporter (DAT) and serotonin transporter (SERT) (see Table). Preliminary locomotor stimulant assay on compound MCL-314 showed that at a dose of 1 mg/kg, it did not reduce locomotion in rats. Thus, this compound is being developed for further imaging studies. (Supported by Adam Corneel Fellowship 040663).

Table: Binding Affinity to Monoamine Transporter Receptors

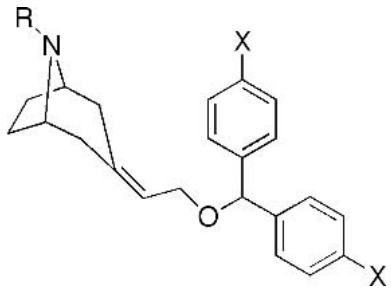
Compound		K_i (nM)	
		rDAT	rSERT
β-CIT	R = Me, X= CO_2Me, Y = H, Ar = 4-I-C_6H_4	1.33 ± 0.15	0.46 ± 0.06
MCL-313	R = Me, X= H, Y = CO_2Me, Ar = 4-CF_3-C_6H_4	1091 ± 321	166 ± 18
MCL-314	R = Me, X= CO_2Me, Y = H, Ar = 4-CF_3-C_6H_4	31.5 ± 3.0	2.39 ± 0.03
MCL-315	R = Me, X= CO_2Me, Y = H, Ar = 4-(3'-CF_3-5'-CF_3-C_6H_3)-C_6H_4	520 ± 105	463 ± 32
MCL-316	R = $FCH_2CH_2CH_2$, X= CO_2Me, Y = H, Ar = 4-(3'-CF_3-5'-CF_3-C_6H_3)-C_6H_4	1852 ± 143	>5000
MCL-317	R = Me, X= CO_2Me, Y = H, Ar = 4-(4'-CF_3-C_6H_4)-C_6H_4	107 ± 3.9	226 ± 14
MCL-318	R = Me, X= 4-CF_3-C_6H_4-CO_2CH_2, Y = H, Ar = C_6H_5	27.8 ± 2.5	5.1 ± 0.4
MCL-319	R = H, X= CO_2Me, Y = H, Ar = 4-(3'-CF_3-5'-CF_3-C_6H_3)-C_6H_4	546 ± 77	313 ± 40
MCL-321	R = Me, X= $CO_2CH_2CF_3$, Y = H, Ar = 4-I-C_6H_4	1.28 ± 0.17	2.2 ± 0.1

Assay conditions: Rat striatal (rDAT) and cortical (rSERT, rNET) homogenates were incubated with ligands [^{3}H]-CIT, [^{3}H]-mazindol and [^{3}H]-nisoxetine with rDAT, rSERT and rNET receptors

57.

SYNTHESIS AND BIOLOGICAL ACTIVITY AT MONOAMINE TRANSPORTERS OF 3-{2-(DIARYLMETHOXY-ETHYLIDENE)}-8-SUBSTITUTED-8-AZABICYCLO[3.2.1]-OCTANE ANALOGUES. ***Shaine A Cararas****[1], Sari Izenwasser[2], and Mark L. Trudell[1]. (1) Department of Chemistry, University of New Orleans, New Orleans, LA 70148, Fax: 504-280-6860, sacarara@uno.edu, (2) Department of Psychiarty and Behavioral Sciences, University of Miami School of Medicine*

A series of 3-{2-(diarylmethoxy-ethylidene)}-8-substituted-8-azabicyclo-[3.2.1]octanes were synthesized and the binding affinities of the compounds were determined at the dopamine (DAT), serotonin (SERT) and norepinephrine(NET) transporters in rat brain tissue preparations and human cell membranes. In addition, monoamine uptake inhibition was determined. In general, the N-alkylaryl derivatives exhibited high DAT affinity as well as high DAT selectivity over SERT and NET. Resolution of the alkylidene (R = Bn, X = H) into the corresponding enantiomers demonstrated a 10-fold stereoselective preference for the (+)-isomer over the (–)-isomer at the DAT. The synthesis, resolution and structure-activity data for these novel tropane analogues will be presented.



58.

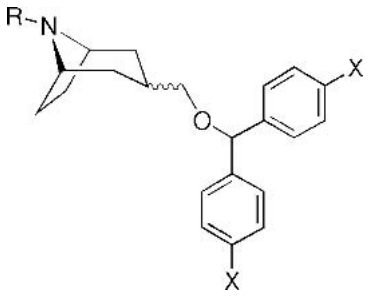
SYNTHESIS AND DISCOVERY OF NOVEL SMALL MOLECULE INHIBITORS OF THE NOREPINEPHRINE RE-UPTAKE TRANSPORTER. *Manuel Cases[1], Gordon Campbell[1], Louise Haughton[1],* ***John J. Masters****[2], Magnus W. Walter[1], Peter T. Gallagher[1], David R. Dobson[1], Terry Finn[1], Benjamin Bonnier[3], Craig White[1], Jeremy D. Findlay[1], Lorna Hayhurst[1], Ann Helene Kluge[1], Sivi Mahadevan[3], Francoise J. Brunelle[3], Claude L. Delatour[3], Annie A. Lavis[3], Nancy A. Dezutter[3], Virginie N. Vervaeke[3], Joël Y. Liénard[3], and John R. Boot[1]. (1) Eli Lilly and Company Ltd, Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey GU206PH, United Kingdom, (2) Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, jjm@lilly.com, (3) Lilly Development Centre*

Significant advances in the treatment of neurological disorders have been realized by the clinical development and use of small molecule inhibitors of neurotransmitter re-uptake transporters. Small molecule inhibitors that are selective for the norepinephrine transporter (NET) have been shown to be effective for the treatment of both depression and attention deficit hyperactivity disorders (ADHD). These laboratories have ongoing research efforts directed at the identification of selective and orally bioavailable antagonists of the NET. In this poster, we present the structure activity relationships for a select group of novel and selective inhibitors of the NET. The synthetic routes, NET binding affinity, selectivity versus other biogenic amine transporters, and physicochemical properties of these molecules will be disclosed.

59.

SYNTHESIS AND MONOAMINE TRANSPORTER AFFINITY OF GBR12909-BENZTROPINE HYBRID ANALOGUES. ***Suhong Zhang***[1], *Sari Izenwasser*[2], *and Mark L. Trudell*[1]. *(1) Department of Chemistry, University of New Orleans, New Orleans, LA 70148, Fax: 504-280-6860, szhang1@uno.edu, (2) Department of Psychiarty and Behavioral Sciences, University of Miami School of Medicine*

The search for selective dopamine transporter ligands has focused upon the synthesis of tropane hybrid analogues of GBR 12909. A series of 3-alpha- and 3-beta-(diarylmethoxymethyl)-8-alkylaryl-8-azabicyclo[3.2.1]octanes were synthesized in stereoselective fashion. The binding affinities of the compounds were determined at the dopamine and serotonin transporters in rat brain tissue preparations. The most potent compounds of this series exhibited high affinity and high selectivity for the dopamine transporter and were approximately two-fold more potent than GBR12909. The synthesis and structure-activity data for these novel tropane analogues will be presented.



60.

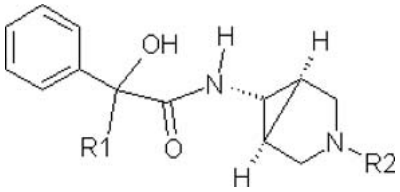
AB INITIO CONFORMATIONAL STUDIES OF IMINE AND KETONE ANALOGS: IMPLICATIONS FOR CHAT INHIBITORS. ***J. Phillip Bowen***[1], *Haizhen Zhong*[1], *Eugene L. Stewart*[2], *and Maria Kontoyianni*[3]. *(1) Center for Drug Design, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, 401 New Science Building, PO Box 26170, Greensboro, NC 27402-6170, Fax: 336-334-5402, jpbowen@uncg.edu, (2) Computational Center for Molecular Structure and Design, Department of Chemistry, University of Georgia, (3) Laboratory for Molecular Modeling, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina at Chapel Hill*

The enzyme choline acetyltransferase (ChAT) has known, inferred, and unknown functions. From a human health perspective some diseases affecting peripheral and central acetylcholine-mediated systems have been implicated or inferred to be related with the performance of this enzyme. It has been suggested that ChAT inhibitors, alone or coupled with other agents, might be used as potential prophylactic protecting agents for those who might be exposed to nerve gases that block acetylcholine esterase as their mechanism of action. Analogs of trans N-methyl-4-(1-naphthylvinyl)pyridine (NVP) have been shown to inhibit ChAT. Interestingly, replacing the CH=CH linkage with -N =CH is favorable while a -CH=N- is not. Whether or not this has conformational implications for ChAT inhibition remains to be answered. The potential energy surfaces (PES) for imines, ketones, and aldehydes have important differences. The PES of 2-butanone, 2-butanimine, 1-butenamine, propanal, and propanimine have been explored with ab initio calculations at the RHF/6-311G** and MP2/6-311G** levels of theory. Our calculations suggest that for 2-butanone and propanal, the steric and the bond dipole interactions are primarily responsible for the conformational preferences of these compounds. Additional charge-charge interaction might also play an important role in determining the imine conformations. For enamines, however, steric interactions play a critical role, with bond dipole interaction exerting some influence. The calculations and implications for ChAT inhibitors are presented.

61.

SYNTHESIS OF SOME NOVEL COMPOUNDS AS MUSCARINIC RECEPTOR ANTAGONISTS. ***Naresh Kumar***, *Kirandeep Kaur, Anita Mehta, SV Arundutt, Shelly Aeron, S Dharamarajan, Suman Gupta, Anita Chugh, and JB Gupta, Department of Medicinal chemistry, Ranbaxy Research Laboratories, Udyog Vihar Industrial Area, Sector-18, Gurgaon, Haryana-122001, India, Fax: 91-124-2343545, n.kumar@ranbaxy.com*

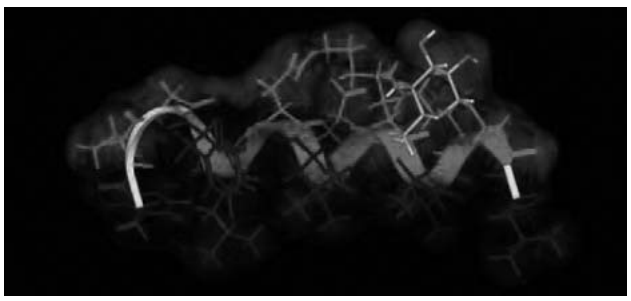
Antimuscarinic drugs for treatment of overactive bladder have common drawback of severe side effects. It arises as these subtype non selective molecules acting on all organs having muscarinic receptors. Much research is being done to improve the organ selectivity by moving to different chemotypes to get subtype selectivity which may leads to possible organ selectivity. Our efforts have been toward synthesizing compounds (type **1**) with M3 Vs M2 selectivity. The synthesis and activities of some of such compounds would be presented at meeting.



62.

ENDORPHINS: BIOUSIAN GLYCOPEPTIDES CROSS THE BBB DUE TO SURFACTANT PROPERTIES. ***Dr. Robin Polt***[1], *Muthu Dhanasekaran*[2], *Richard D. Egleton*[3], *Edward J. Bilsky*[4], *Henry I. Yamamura*[3], *Frank Porreca*[5], *Isabel Alves*[1], *and Gordon Tollin*[6]. *(1) Department of Chemistry, Univeristy of Arizona, 1306 E. Univerisity Blvd, Tucson, AZ 85721, polt@u.arizona.edu, (2) Department of Chemistry, The University of Arizona, (3) Department of Pharmacology, College of Medicine, University of Arizona, (4) Department of Pharmacology, University of New England College of Medicine, (5) Department of Pharmacology, University of Arizona, (6) Department of Biochemistry and Molecular Biophysics, University of Arizona*

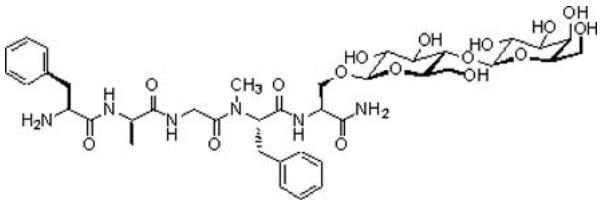
A series of glycopeptides based on the Leu-enkephalin analogue YtGFL~ leads to greatly enhanced stability *in vivo* and effective penetration of the BBB. BBB transport hinges on the *biousian nature* of the glycopeptides. The amphipathic glycopeptides possess two conflicting solubility states; one state that is completely water soluble, and another at water-membrane phase boundaries. The biousian design was applied to larger glycopeptides (16-17 residues) related to beta-endorphin. Transport and opioid activity is described. CD and NMR in the presence of membrane mimics show the amphipathic nature of the bound glycopeptides. Plasmon waveguide resonance (PWR) studies show that the amphipathic helices bind to membranes with micromolar to low nanomolar K_D's.



63.

MU-SELECTIVE OPIOID GLYCOPEPTIDE THAT CROSSES THE BLOOD-BRAIN BARRIER. ***Larisa Yeomans***[1], *Dhanasekaran Muthu*[2], *Charles M. Keyari*[2], *Neil E. Jacobsen*[1], *Peg Davis*[3], *Frank Porreca*[3], *Jean M. Bidlack*[4], *Edward J. Bilsky*[5], *and Robin L. Polt*[2]. *(1) Departament of Chemistry, University of Arizona, 1306 E. University Blvd., Tucson, AZ 85721-0041, Fax: 520-621-8407, yeomans@email.arizona.edu, (2) Department of Chemistry, University of Arizona, (3) Department of Pharmacology, University of Arizona, (4) Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, (5) Department of Pharmacology, University of New England College of Medicine*

Opioid peptides do not typically cross the blood-brain barrier (BBB). Their therapeutic use has been severely limited due to pharmacokinetic issues such as serum stability as well as their limited BBB permeability. Previous work with delta-opioid glycopeptide agonists has shown that these compounds have extended serum lifetimes, and cross the BBB to produce potent analgesia in mice. A mu-selective (0.66 nM) glyocopeptide [H-Tyr-D-Ala-Gly-MePhe-Ser(beta-D-Glc)-amide] shows much greater potency, and a different side-effect profile than the delta-selective drugs studied previously. CD and NMR studies in water and in the presence of SDS micelles are reported, as well as antinociception and open-field locomotor studies. The A(50) values are 3 pmol per mouse (icv) and 1.3 mmol/Kg (iv). The research was supported by grants form NIDA (K05 DA00360), and ONR (N00014-02-1-0471).



64.

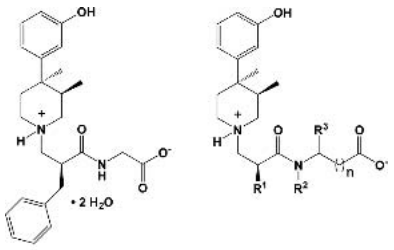
NEW BENZHYDRYLPIPERAZINES AS POTENT KAPPA OPIOID RECEPTOR AGONISTS. ***Juerg Lehmann***, *Amy Wong, Charles Xing, Hubert Otlik, David Unett, Andrew Grottick, Joel Gatlin, Graeme Semple, and Robert M Jones, Arena Pharmaceuticals, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, jlehmann@arenapharm.com*

Kappa opioid receptor (KOR) agonists are particularly effective analgesics in experimental models of visceral pain. The molecular targets involved include peripherally located KORs and possibly, at least for some nonpeptidic KOR agonists, additional non-opioid molecular targets such as Nav channels located on primary sensory afferents. These properties are expected to be of therapeutic interest in various visceral pain conditions, including abdominal surgery associated with postoperative pain and ileus, pancreatitis pain, dysmenorrhea, labor pain and functional disorders such as IBS or dyspepsia. Development of the first generation of KOR agonists was discontinued due to CNS side effects such as sedation, diuresis and dysphoria attributable to activation of KORs located behind the blood-brain barrier. In an effort to identify new KOR agonists, we have successfully switched the selectivity of known benzyhdrylpiperazine delta opioid receptor agonists using regiospecific basic functional moieties. Subsequent modifications led to the discovery of potent kappa selective and mixed kappa/mu opioid receptor ligands exhibiting picomolar affinity for the KOR.

65.

NOVEL N-SUBSTITUTED TRANS-3,4-DIMETHYL-4-(3-HYDROXYPHENYL)-PIPERIDINES AS MU SELECTIVE OPIOID ANTAGONISTS. ***Bertrand Le Bourdonnec***[1], *William M. Barker*[1], *Serge Belanger*[2], *Joel A. Cassel*[2], *Robert N. DeHaven*[2], *and Roland E. Dolle*[1]. *(1) Department of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341-1127, Fax: 484-595-1551, blebourdonnec@adolor.com, (2) Department of Pharmacology, Adolor Corporation*

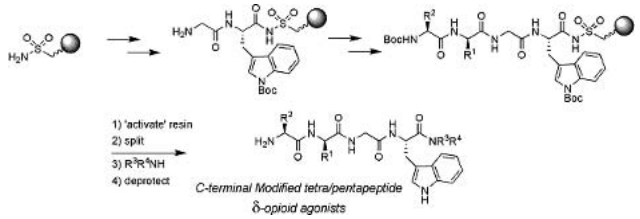
Alvimopan **1** is a peripherally-restricted mu-opioid receptor antagonist which is currently in Phase III clinical trials for the management of post-operative ileus and opioid-induced bowel dysfunction. The size and polarity of the *N*-substituent limits penetration across the blood-brain barrier and yields a peripherally restricted compound. Analogs of **1** of general formula **A** were prepared using solid phase methodology and purified by high throughput preparative HPLC. The synthesis and *in vitro* pharmacological profile of these new analogs will be presented.



66.

RAPID OPTIMIZATION OF DELTA SELECTIVE PEPTIDES. ***Thomas O. Schrader***[1], *P. Douglas Boatman*[1], *Pureza Vallar*[1], *David Unett*[2], *Bill Thomsen*[2], *John Frazer*[2], *John Adams*[3], *Daniel Connolly*[3], *and Graeme Semple*[1]. *(1) Department of Chemistry, Arena Pharmaceuticals, Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, tschrader@arenapharm.com, (2) Screening Department, Arena Pharmaceuticals, Inc, (3) Department of Biology, Arena Pharmaceuticals, Inc*

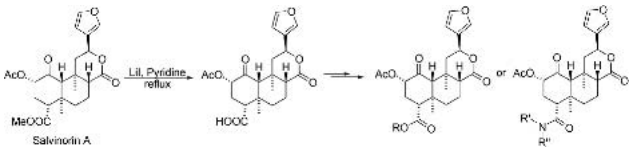
The role of the delta-opioid receptor in cardioprotection and ischemic preconditioning (IPC) has received considerable attention. Modulators of this receptor may function as novel therapeutics against myocardial infarction and related ischemic conditions. To this end, a series of small peptide agonists targeting the delta-receptor have been developed. Employment of Kenner's "Safety-Catch" linker for solid-phase peptide synthesis (SPPS) enabled the rapid production of a small library of C-terminal modified tetra- and pentapeptides. High throughput screening (HTS) using Arena's proprietary Melanophore technology identified a number of low nanomolar delta-opioid receptor agonists with high selectivity over the mu and kappa opioid receptor subtypes.



67.

SYNTHESES OF KAPPA-OPIOID RECEPTOR LIGANDS: SALVINORIN A ANALOGS WITH MODIFICATION AT C-4 POSITION. ***Minsheng He***[1], *David Y.W. Lee*[2], *Lee-Yuan Liu-Chen*[3], *Zhongze Ma*[1], *Yulin Wang*[3], *Yong Chen*[3], *William A. Carlezon Jr.*[4], *Cecile Beguin*[5], *and Bruce M. Cohen*[5]. *(1) Bio-Organic & Natural Products Laboratory, McLean Hospital, Harvard Medical School, 115 Mill street, Belmont, MA 02478, mhe@mclean.harvard.edu, (2) Bio-Organic & Natural Products Laboratory, McLean Hospital, HMS, (3) Department of Pharmacology, School of Medicine, Temple University, (4) Behavioral Genetics Laboratory, McLean Hospital, Harvard Medical School, (5) Molecular Pharmacology Laboratory, McLean Hospital, Harvard Medical School*

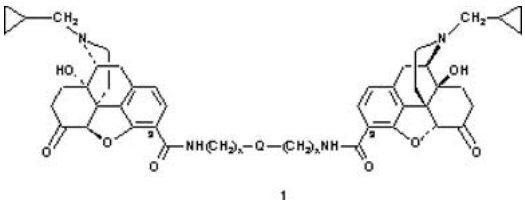
Salvinorin A is the most potent naturally occurring opioid agonist with a high selectivity and affinity for kappa-opioid receptor. In order to explore the structure and activity relationship, modifications at C-4 position have been studied. The methyl ester at C-4 position was selectively cleaved by applying LiI as the reagent in pyridine. The corresponding acid as well as the epimer obtained can be separated further coupled to alcohols or amines to give corresponding ester and amide derivatives. Several chiral amino acid derivatives were also synthesized to expand the functionalities and structural motifs. These salvinorin A derivatives were screened for binding and functional activities at the human kappa-opioid receptor.



68.

SYNTHESIS AND BINDING ASSAY OF NEW DIMERIC LIGANDS CONTAINING THE NALTREXONE PHARMACOPHORE FOR MU AND KAPPA OPIOID RECEPTORS. ***Yigong Bu***[1], *Mark P. Wentland*[1], *Qun Lu*[1], *and Jean M. Bidlack*[2]. *(1) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, buy@rpi.edu, (2) Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester*

A novel series of bivalent ligands (1) for opioid receptors have been prepared where two naltrexone molecules are connected through the 3-position with bis-carboxamide linkers. Target compounds were synthesized by direct carboxamidation of naltrexone triflate or through a N-hydroxysuccinimido ester of naltrexone, which was prepared via Pd-catalyzed carbonylation of naltrexone triflate. The binding affinities of these bivalent ligands at mu, delta and kappa opioid receptors were evaluated in a CHO membrane binding assay. The details of the syntheses and SAR will be presented.



1

69.

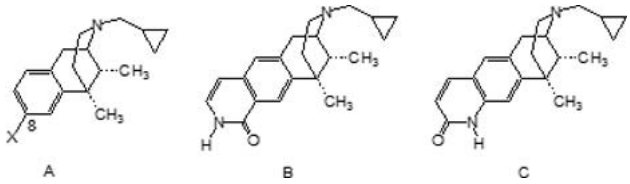
SYNTHESIS AND EVALUATION OF SALVINORIN A ANALOGUES AS KAPPA-OPIOID RECEPTOR LIGANDS FOR THE TREATMENT OF MOOD DISORDERS. ***Cecile Beguin***[1], *Michele R. Richards*[1], *Lee-Yuan Liu-Chen*[2], *David Y.W. Lee*[3], *William A. Carlezon Jr.*[4], *and Bruce M. Cohen*[1]. *(1) Molecular Pharmacology Laboratory, McLean Hospital, Harvard Medical School, 115 Mill street, Belmont, MA 02478, cbeguin@mclean.harvard.edu, (2) Department of Pharmacology, School of Medicine, Temple University, (3) Bio-Organic & Natural Products Laboratory, McLean Hospital, HMS, (4) Behavioral Genetics Laboratory, McLean Hospital, Harvard Medical School*

Current treatments for depression and bipolar disorder require several weeks to become effective and often produce undesirable side effects. New drugs with improved efficacy, faster onset of action, and fewer side effects would revolutionize the treatment of these mood disorders. We recently reported that selective kappa-opioid receptor agonists produce depressive-like symptoms in rats, whereas selective kappa-antagonists produce antidepressant-like effects. These findings raise the possibility that selective kappa-ligands could be used to regulate mood in humans. Specifically, kappa-antagonists may have utility as antidepressants, and partial kappa-agonists may be useful as mood stabilizers. Salvinorin A derived from the plant Salvia divinorum, which is used as an hallucinogen, has a unique chemical structure that potently and selectively activates kappa-receptors. Salvinorin A produced depressive-like effects in rats in models often used to study depression (the forced swim test and the intracranial self-stimulation test) but displayed a short duration of action. A recent report identified salvinorin B as the major metabolite of salvinorin A. The production of salvinorin B, which is inactive at kappa-receptors, may explain, in part, the short duration of action of salvinorin A. We synthesized novel salvinorin derivatives and evaluated their effect in in vitro binding and functional assays. Select compounds were found to be potent kappa-agonists that may be more stable towards metabolic deacetylation. In addition, our structure-activity relationship studies led to the discovery of a potent partial kappa-agonist. We will present the synthesis and biological evaluation of these novel kappa-agents. Evaluation of select salvinorin derivatives in in vivo models of depression is underway. These experiments will characterize the effects of kappa-ligands on behaviors that reflect mood states and may identify potential drug candidates for the treatment of depression and bipolar disorder.

70.

SYNTHESIS AND OPIOID RECEPTOR BINDING PROPERTIES OF CONFORMATION-RIGIDIFIED ANALOGUES OF 8-CARBOXAMIDOCYCLAZOCINE AND 8-FORMAMIDOCYCLAZOCINE. ***Xufeng Sun***[1], *Mark P. Wentland*[1], *Robert J. Kucejko*[1], *and Jean M. Bidlack*[2]. *(1) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, Fax: 518-276-4887, sunxufeng@alum.rpi.edu, (2) Department of Pharmacology and Physiology, University of Rochester*

8-carboxamidocyclazocine (A: X = CONH2) and 8-formamidocyclazocine (A: X = NHCHO) have high binding affinity for mu and kappa opioid receptors. To probe the bioactive conformation of these opiates, rigidified analogues were synthesized. Isoquinolinone B, a ring-closed analogue of 8-carboxamidocyclazocine, showed high affinity for kappa (0.74 nM), which is comparable to 8-carboxamidocyclazocine (0.53 nM); however, it was less potent for the mu receptor (5.5 vs 0.41 nM). Quinolinone C, a ring-closed analogue of 8-formamidocyclazocine, showed very low affinity for both receptors compared to 8-formamidocyclazocine (200 vs 1.9 nM for mu; 100 vs 0.85 nM for kappa). These results indicate: (1) The bioactive conformation of 8-carboxamidocyclazocine for kappa is similar to the structure of isoquinolinone B. (2) The bioactive conformation of 8-carboxamidocyclazocine for mu is different from the structure of isoquinolinone B. (3) The bioactive conformation of 8-formamidocyclazocine for both receptors is different from the structure of quinolinone C.

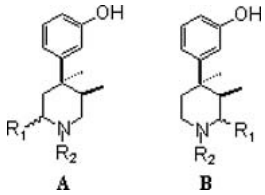


A B C

71.

SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIP OF A SERIES OF 2- AND 6-SUBSTITUTED TRANS-3,4-DIMETHYL-4-(3-HYDROXYPHENYL)-PIPERIDINE OPIOID ANTAGONISTS. ***Allan J. Goodman***[1], *Bertrand Le Bourdonnec*[1], *Mathieu Michaut*[1], *Hai Fen Ye*[1], *Serge Belanger*[2], *Joel A. Cassel*[2], *Robert N. DeHaven*[2], *and Roland E. Dolle*[1]. *(1) Department of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, agoodman@adolor.com, (2) Department of Pharmacology, Adolor Corporation*

The series of trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines have been widely investigated as opioid receptor antagonists. Structure activity relationships (SAR) in this series has focused largely on substitution of the piperidine nitrogen and modification of the phenolic moiety. To investigate the effects of substitution at the 2- or 6- position of the piperidine ring on opioid receptor binding, novel analogs (A, B) were prepared. Synthesis, SAR and in vitro profile of these novel derivatives will be presented.

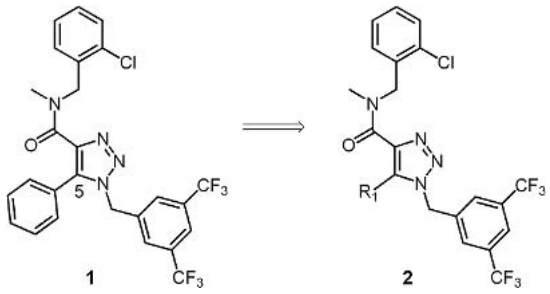


A B

72.

SYNTHESIS AND EVALUATION OF ORALLY ACTIVE NK-1 ANTAGONISTS: C-5 POSITION SAR OF THE 1,2,3-TRIAZOLE CORE. ***Albert K. Amegadzie***, *Kevin M. Gardinier, Jeffrey W Cramer, Donald A. Gehlert, Erik J. Hembre, Smriti Iyengar, Louis N. Jungheim, Dominic L. Li, Kenneth A. Savin, and Douglas A. Schober, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, amegadzie_albert@lilly.com*

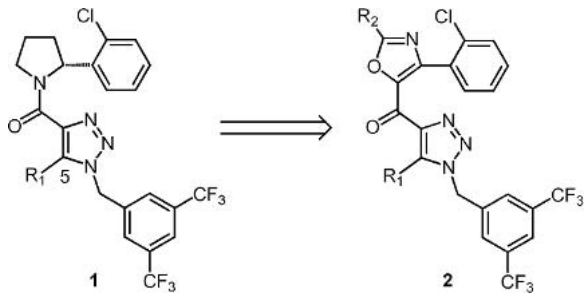
The discovery of **1** in our group as a potent NK1 antagonist led to the SAR exploration around the C-5 position of the 1,2,3-triazole core. These C-5 analogs (**2**) were obtained by the regioselective synthesis of the 1,2,3-triazole core from active methylene units. This poster will describe the synthesis and the biological data of these analogs.



73.

SYNTHESIS AND EVALUATION OF ORALLY ACTIVE NK1 ANTAGONISTS: REPLACEMENT OF PYRROLIDINE WITH OXAZOLE. ***Kevin M. Gardinier**, Albert K. Amegadzie, Jeffrey W Cramer, Donald A. Gehlert, Erik J. Hembre, Smriti Iyengar, Louis N. Jungheim, Dominic L. Li, Kenneth A. Savin, and Douglas A. Schober, Lilly Research Laboratories, Eli Lilly and Company, Discovery Chemistry Research and Technologies, Lilly Corporate Center, DC4816, Indianapolis, IN 46285, kmgardinier@lilly.com*

A series of oxazoles analogs (**2**) based on the pyrrolidine (**1**) were synthesized and evaluated for NK1 antagonist activity. The SAR at the C-5 position (R1) of the 1,2,3-triazole ring was directed by earlier studies. These efforts led to the identification of potent compounds with good in vivo efficacy and improved metabolic stability. This poster will focus on the design, synthesis, and biological evaluations of these oxazoles analogs.



74.

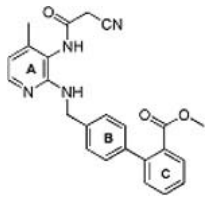
SYNTHESIS OF 1-[2-(3,5-BIS-TRIFLUOROMETHYL-BENZYLOXY)-1-PHENYL-ETHYL]-4-[^{11}C]METHYL-PIPERAZINE AND {4-[2-(3,5-BIS-TRIFLUOROMETHYL-BENZYLOXY)-1-PHENYL-ETHYL]-PIPERAZINE-1-YL}-ACETIC ACID [^{11}C]METHYL ESTER AS NEW POTENTIAL PET NK$_1$ RECEPTOR LIGANDS. ***Mingzhang Gao**, Ji-Quan Wang, and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, Room L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, migao@iupui.edu*

The NK$_1$ receptor antagonists are potentially useful for the treatment of a wide range of clinical diseases such as asthma, chronic pain, emesis, anxiety and depression. *In vivo* biomedical imaging technique positron emission tomography (PET) coupled with appropriate receptor radioligands has become a clinically valuable and accepted diagnostic tool to image brain diseases. The NK$_1$ receptor antagonist radiotracers 1-[2-(3,5-bis-trifluoromethyl-benzyloxy)-1-phenyl-ethyl]-4-[^{11}C]methyl-piperazine (**1**) and {4-[2-(3,5-bis-trifluoromethyl-benzyloxy)-1-phenyl-ethyl]-piperazine-1-yl}-acetic acid [^{11}C]methyl ester (**2**) were synthesized for evaluation as new potential PET imaging agents for brain NK$_1$ receptor. The tracer **1** was prepared by *N*-[^{11}C]methylation of precursor 1-[2-(3,5-bis-trifluoromethyl-benzyloxy)-1-phenyl-ethyl]-piperazine using [^{11}C]methyl triflate and isolated by solid-phase extraction (SPE) purification procedure. The tracer **2** was prepared by *O*-[^{11}C]methylation of precursor {4-[2-(3,5-bis-trifluoromethyl-benzyloxy)-1-phenyl-ethyl]-piperazine-1-yl}-acetic acid using [^{11}C]methyl triflate and isolated by solid-phase extraction (SPE) purification procedure.

75.

DESIGN AND SYNTHESIS OF BRADYKININ B1 RECEPTOR ANTAGONISTS: ARYL PIPERIDINES AS POTENT AND SELECTIVE REPLACEMENTS FOR BIPHENYL SCAFFOLDS. ***Christina Ng**[1], Scott D. Kuduk[1], Ronald K. Chang[1], Kathy L. Murphy[2], Richard W. Ransom[2], Cuyue Tang[3], Thomayant Prueksaritanont[3], Roger M. Freidinger[1], Douglas J. Pettibone[2], and Mark G. Bock[1]. (1) Department of Medicinal Chemistry, Merck & Co., Inc, WP14-3, Sumneytown Pike, Post Office Box 4, West Point, PA 19486, Fax: 215-652-3971, christina_ng@merck.com, (2) Department of Neuroscience, Merck & Co., Inc, (3) Drug Metabolism, Merck and Co., Inc*

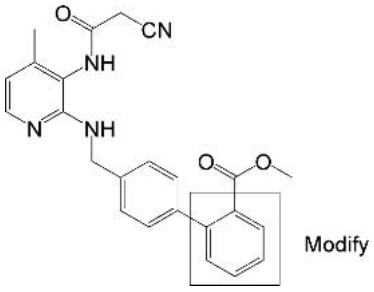
Systematic substitution of the central phenyl ring in a class of bradykinin B1 antagonists presenting the biphenyl 'privileged structure' was investigated. Several non-aromatic carbocyclic and heterocyclic rings replacements were examined to enhance receptor affinity and to expand the structural diversity of the parent compound. A piperidine ring was found to be a good replacement for the phenyl B-ring leading to compounds with equivalent potency and an improved pharmacokinetic profile relative to the lead structure.



76.

DESIGN AND SYNTHESIS OF BRADYKININ B1 RECEPTOR ANTAGONISTS: DEVELOPMENT OF POTENT AND SELECTIVE LIGANDS WITH MODIFIED BIPHENYL MOTIFS. ***Ronald K. Chang**[1], Scott D. Kuduk[1], Christina Ng[1], Kathy L. Murphy[2], Richard W. Ransom[2], Cuyue Tang[3], Thomayant Prueksaritanont[3], Roger M. Freidinger[1], Douglas J. Pettibone[2], and Mark G. Bock[1]. (1) Department of Medicinal Chemistry, Merck & Co., Inc, WP14-3, Sumneytown Pike, Post Office Box 4, West Point, PA 19486, ronald_chang@merck.com, (2) Department of Neuroscience, Merck & Co., Inc, (3) Department of Drug Metabolism, Merck & Co., Inc*

Systematic substitutions of the distal phenyl ring were investigated in a class of bradykinin B_1 antagonists, which contain the biphenyl 'privileged structure' motif. Various aromatic and non-aromatic ring replacements were examined in an effort to enhance the receptor affinity, as well as, to expand the structural diversity of the parent lead compound. Replacement of the distal phenyl ring with a cyclohexyl ring provided the optimal compounds in this investigation. These non-aromatic carbocycles were accessed *via* a key thermal Diels-Alder reaction.



77.

FOCUSED LIBRARY SETS IN DISCOVERY OF DUAL AT$_1$/ET$_A$ ANTAGONISTS. ***Alexander Kiselyov**, Chemistry, ChemDiv, Inc, 11558 Sorrento Valley Rd, San Diego, CA 92121, Fax: 858-794-4931*

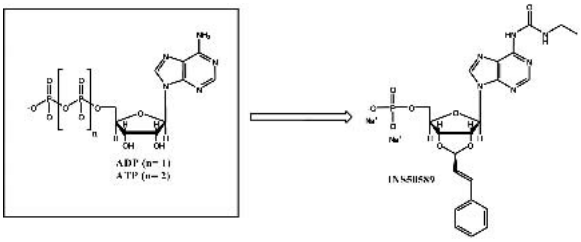
At ChemDiv, we have assembled a set of biased compound libraries designed to jump-start medicinal chemistry effort around specific targets or pathways. In this presentation, we will comment on our success in utilizing several of these targeted selections, namely kinase-, GPCR-, recognition motif- and Rule-of-Three sets in identifying potent ET_A and AT_1 hits. This initial insight allowed us to successfully complete medicinal chemistry effort that yielded three new chemical series of dual AT_1/ET_A antagonists with K_i < 250 nM against both receptors, good cellular activity, as well as *in vitro* PK and early *in vivo* profile. In addition,

identified compounds were selective against panel of 35 additional GPCRs and show no significant binding in CYP450 panel and hERG1 assay.

78.

INS50589, A POTENT, SELECTIVE, AND REVERSIBLE INHIBITOR OF $P2Y_{12}$ MEDIATED PLATELET AGGREGATION. ***James G. Douglass***[1], *Roshni I. Patel*[2], *Matthew C. Cowlen*[3], *Benjamin R. Yerxa*[1], *Sammy R. Shaver*[1], *Sanjoy Mahanty*[2], *Paul Watson*[1], *and José L. Boyer*[2]. *(1) Department of Chemistry, Inspire Pharmaceuticals, Inc, 4222 Emperor Blvd. Suite 200, Durham, NC 27703, Fax: 919-941-9177, jdouglass@inspirepharm.com, (2) Department of Molecular Pharmacology, Inspire Pharmaceuticals, Inc, (3) Department of Preclinical Studies, Inspire Pharmaceuticals, Inc*

ADP induces platelet aggregation via the simultaneous activation of two G-protein coupled receptors ($P2Y_1$ and $P2Y_{12}$). Antagonism of the action of ADP at either receptor leads to inhibition of platelet aggregation, making them attractive targets for the development of medicines to treat thrombotic diseases. ATP functions as a modestly potent $P2Y_{12}$ antagonist *in vivo*, but undergoes rapid metabolism to other nucleotides, including ADP. We studied the structure activity relationships (SAR) of modified mono and dinucleotides at $P2Y_{12}$, which led to the identification of lipophilic modifications to the ribose and base moieties that impart potent, selective, and reversible antagonist properties at this receptor. This work culminated in the discovery of INS50589, having an IC_{50} of approximately 4 nM in a washed platelet assay. INS50589 has been nominated as a clinical candidate for intravenous use. The synthetic rationale leading to this molecule and the outcome of *vitro* and *in vivo* studies will be presented.



79.

MOLECULAR RECOGNITION OF 3′-MODIFIED ADENOSINE NEOLIGANDS TO THE HUMAN A3 ADENOSINE RECEPTOR AND ITS NEOCEPTOR. ***Soo-Kyung Kim***[1], *Zhan-Guo Gao*[1], *Heng T. Duong*[1], *Lak Shin Jeong*[2], *and Kenneth A. Jacobson*[1]. *(1) Molecular Recognition Section, NIDDK, NIH, Bldg. 8A, Rm. 1A20, Bethesda, MD 20892-0810, Fax: 301-402-0008, SooKyungK@intra.niddk.nih.gov, (2) Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University*

To overcome the inherent non-selectivity of activating adenosine receptors (ARs) and other native G protein-coupled receptors (GPCRs) we have introduced neoceptors, in which the receptor is selectively mutated for stimulation by tailored small molecules (neoligands). These neoligand-neoceptor pairs are unique tools for pharmacological characterization and for therapeutic application through gene therapy. From docking analysis, the 3′-hydroxyl was predicted to interact with the H272 side chain. Based on reduced binding at native ARs of 3′-analogues, modified for H-bonding and charge, the putative hA_3AR binding site was reengineered, strategically introducing acidic residues in TMs 3,7. FlexX/FlexiDock docking programs suggested at least two energetically favorable binding modes of a 3′-modified analogue, unlike the docked selective A_3 agonist Cl-IB-MECA. It gained H-bonding stabilization at the binding site of the 3′-position in direct contact with either E272 (7.43) or T94 (3.36) side chains, explaining its selective affinity enhancement at T94D and H272E receptors.

80.

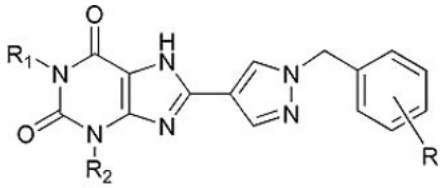
NOVEL PYRAZOLIDINE-3,5-DIONE DERIVATIVES ARE P2Y12 RECEPTOR ANTAGONISTS AND INHIBIT ADP-TRIGGERED BLOOD PLATELET AGGREGATION. ***Heinz Fretz***, *Olivier Houille, Kurt Hilpert, Oliver Peter, Volker Breu, Thomas Giller, Olivier Valdenaire, and Markus Riederer, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, Allschwil 4125, Switzerland, Fax: +41-61-487-7600, heinz.fretz@actelion.com*

G protein-coupled receptor (GPCR) P2Y12, a clinically relevant platelet receptor for adenosine diphosphate (ADP), is involved in the ADP-induced blood platelet aggregation. Adenosine triphosphate (ATP) serves as an endogenous antagonist of platelet aggregation. It has been shown that blocking ADPs action via ATP analogues, or via irreversible P2Y12 antagonists reduces the incidence of myocardial infarction, stroke. Screening of our in-house compound library by means of an in vitro fluorescent imaging plate reader (FLIPR) assay delivered a pyrazolidine-3,5-dione derivative as hit compound. Optimization of this hit led to a series of potent and selective P2Y12 antagonists inhibiting platelet aggregation induced by the agonists ADP or 2-MeSADP in a reversible and concentration dependent manner.

81.

SELECTIVE A2B ADENOSINE RECEPTOR ANTAGONISTS: NEW MONO-N-1 ALKYL 8-(PYRAZOL-4-YL) XANTHINES. ***Rao Kalla***[1], *Elfatih Elzein*[1], *Thao Perry*[1], *Xiaofen Li*[1], *Tenning Maa*[2], *Arthur Gimbel*[2], *Dewan Zeng*[2], *and Jeff Zablocki*[1]. *(1) Bioorganic Chemistry, CV Therapeutics Inc, 3172 Porter Drive, Palo Alto, CA 94304, Fax: 650-858-0390, rao.kalla@cvt.com, (2) Drug Research and Pharmacological Sciences, CV Therapeutics Inc*

A2B adenosine receptor (AdoR) antagonists may have a potential use in the treatment of asthma, diabetic retinopathy, cancer, and Alzheimer's disease. Although several high affinity A2B antagonists are known to date, there are very few A2B AdoR antagonists known so far with both high affinity and selectivity. Previously, we reported the SAR of 1,3-symmetrically disubstituted 8-pyrazolyl xanthine derivatives 1 that displayed high affinity and good selectivity for the A2B AdoR. Herein, we describe our efforts to enhance the A2B AdoR selectivity of 8-pyrazolyl xanthines by exploring the effects of mono-substitution at the N-1 and N-3 positions of the xanthine. The N-1 substituted 8-pyrazolyl xanthines 2 displayed higher selectivity compared to their corresponding disubstituted derivatives 1, whereas the mono alkyl N-3 substituted xanthines 3 lost their affinity for the A2B AdoR. The synthesis of mono substituted 8-pyrazolyl xanthines and their SAR will be discussed in detail.



1 $R_1 = R_2 =$ propyl
2 $R_1 =$ propyl, $R_2 = H$
3 $R_1 = H$; $R_2 =$ propyl

82.

SYNTHESIS AND BINDING AFFINITY OF 3′-UREIDOADENOSINE ANALOGUES AT THE A3 ADENOSINE RECEPTOR. ***Lak Shin Jeong***[1], *Myoung Jung Kim*[2], *Ae Yil Kim*[1], *Jeong A Lee*[1], *Kenneth A. Jacobson*[3], *Zhan-Guo Gao*[4], *Soo-Kyung Kim*[4], *and Moon Woo Chun*[2]. *(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 Pharmacy, Seoul National University, (3) Molecular Recognition Section, NIDDK, NIH, Bethesda, MD 20892-0810, (4) Molecular Recognition Section, NIDDK, NIH*

A number of N^6- and/or 2-substituted adenosine derivatives have been synthesized and evaluated for A_3 receptor agonistic activity. Among these compounds, N^6-(3-iodobenzyl)-5-N-methylcarbamoyladenosine (IB-MECA) and 2-chloro-N^6-(3-iodobenzyl)-5-N-methylcarbamoyladenosine (Cl-IB-MECA) were found to be highly selective full agonists with high binding affinities (Ki = 1.8 nM, 1.4 nM, respectively) at the human A_3 adenosine receptor. Recently, on the basis of high binding affinity and selectivity of Cl-IB-MECA, we have reported the synthesis of the 3′-fluoro analogue of Cl-IB-MECA, in which 3′-hydroxyl group of Cl-IB-MECA might act as hydrogen bonding donor, not as hydrogen bonding acceptor. Similarly, 3′-aminoadenosine analogues, CP-608039 was reported to be the highly selective agonist to the human adenosine A_3 receptor, indicating that 3′-amino group might play a key role as hydrogen bonding donor. Thus, on the basis of these results, it was very interesting to design 3′-ureidoadenosine analogues since 3′-ureido moiety may form stronger hydrogen bond in the binding site than the corresponding 3′-amino- or 3′- hydroxy-substituted nucleosides. It is also of interest to find out whether bigger substituent, 3′-ureido group can be tolerated in the binding site in comparison with smaller

substituent, 3′-hydroxy or 3′-amino group. The key 3′-urea moiety was introduced from reacting 3′-amino derivative with chloroacetylurea followed by treating with sodium methoxide. The synthesized 3′-ureidoadenosine analogues were totally devoid of binding affinity at the A_3 adenosine receptor, indicating that 3′-urea moiety might cause steric repulsion at the active site of adenosine A_3 receptor, leading to conformational distortion. However, surprisingly 3′-ureidoadenosine analogues exhibited highly potent binding affinity at the mutant A_3 adenosine receptor, indicating that a favorable interaction between 3′-ureido group and binding site of the mutant receptor might exist. Synthesis and binding affinities of the 3′-ureidoadenosine derivatives at the natural as well as mutant A_3 adenosine receptors will be presented in detail.

83.

DISCOVERY OF NOVEL HUMAN GLUCAGON RECEPTOR ANTAGONISTS.

Dong-Ming Shen[1], *Fengqi Zhang*[1], *Edward J. Brady*[2], *Mari Rios Candelore*[3], *Victor D.-H. Ding*[3], *Guoqiang Jiang*[3], *Steve Mock*[1], *Sajjad A. Qureshi*[3], *Richard Saperstein*[2], *Constantin Tamvakopoulos*[1], *Xinchun Tong*[1], *Laurie M. Tota*[3], *Michael Wright*[3], *Song Zheng*[1], *Kevin T. Chapman*[1], *Bei B. Zhang*[3], *James R. Tata*[1], *and Emma Parmee*[1]. *(1) Department of Basic Chemistry, Merck Research Laboratories, P O Box 2000, RY50G-146, Rahway, NJ 07065, Fax: 732-594-8080, dongming_shen@merck.com, (2) Department of Pharmacology, Merck Research Laboratories, (3) Department of Metabolic Disorders and Molecular Endocrinology, Merck Research Laboratories*

Glucagon, a 29 AA peptide hormone, plays a critical role in the regulation of glucose homeostasis by activating the glucagon receptor in the liver to synthesize glucose and mobilize hepatic glucose stores. Multiple proof of concept experiments have shown that inhibition of the glucagon receptor by small molecule antagonists is a promising target for the treatment of type II diabetes. We will discuss the discovery and SAR studies of a class of novel human glucagon receptor antagonists which contain a cyclic urea moiety as the central core. Some of these novel compounds showed good in vivo activity in blocking glucagon-induced hyperglycemia in transgenic mice that express a functioning human glucagon receptor following oral dosing.

84.

PEPTIDE-DIRECTED DELIVERY OF SMALL MOLECULE AGONISTS OF GLP-1R.

Meghan B. Scobee[1], *David R. Haines*[1], *Christine I. Worrall*[1], *and Martin Beinborn*[2]. *(1) Department of Chemistry, Wellesley College, 106 Central Street, Wellesley, MA 02481, mscobee@wellesley.edu, (2) Department of Medicine, New England Medical Center*

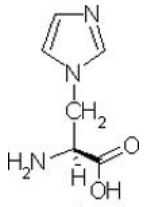
Glucagon-like peptide-1 (GLP-1) is a 30 residue peptide involved in glucose-mediated insulin production. Exendin-4 (Ex-4), a 39 residue peptide found in Gila monster venom, was found to be an agonist of the glucagon-like peptide-1 receptor (GLP-1R). Data from binding and activation assays of GLP-1 point mutants suggests that GLP-1 and Ex-4 have distinct and discrete receptor activating regions and receptor binding regions. We are examining the separability of these functions by truncating Ex-4 at the N-terminus and using a non-peptide attachment to connect model small agonists to the binding region. The first agonists studied use the natural N-terminal tripeptide molecule to show that we can preserve activation with such an attachment. Synthetic strategy and binding and activity data on the model compounds will be presented.

85.

STRUCTURE-ACTIVITY STUDIES OF THE INTERACTION OF GLP-1 WITH GLP-1R.

Kristin A. Moy[1], *David R. Haines*[1], *Alisha Weight*[1], *Christine I. Worrall*[1], *and Martin Beinborn*[2]. *(1) Department of Chemistry, Wellesley College, 106 Central St., Wellesley, MA 02481, kmoy@wellesley.edu, (2) Department of Medicine, New England Medical Center*

The N-terminal histidine of glucagon-like peptide (GLP-1) and of exendin-4 have been shown to be critical for the activation of the pancreatic receptor (GLP-1R). Previous amino acid substitutions of this histidine with lysine and with alanine have been interpreted to indicate the requirement for a positively charged residue in the N-terminal position. To further develop the structure-activity relationship for the interaction of the N-terminal amino acid of GLP-1 with GLP-1R, histidine analogs such as 1-imidazolyl-alanine **(1)**, have been synthesized via Michael addition to an acrylic acid ester. Enantiomeric resolution was achieved enzymatically. The results of binding and activity studies for the incorporation of 1 and other such analogs into GLP-1 will be reported.



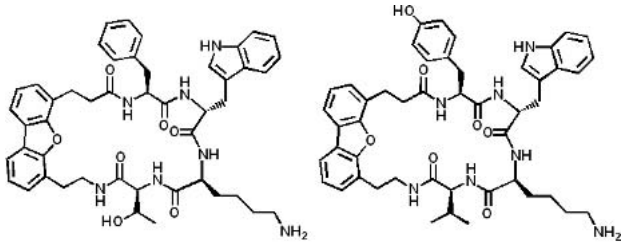
1

86.

DESIGN AND SYNTHESIS OF TYPE-II′ î²-TURN CONSTRAINED SOMATOSTATIN ANALOGUES SELECTIVE FOR RECEPTOR SUBTYPE HSST2.

Audrey Kelleman[1], *Hao Huang*[2], *Giuseppe Melacini*[2], *Michael Grant*[3], *Ujendra Kumar*[3], *Michael S. VanNieuwenhze*[1], *and Murray Goodman*[1]. *(1) Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Dr, Mail Box 0343, La Jolla, CA 92093-0343, akellema@chem.ucsd.edu, (2) Department of Biochemistry and Chemistry, McMaster University, (3) Departments of Medicine and Pharmacology and Therapeutics, McGill University and Royal Victoria Hospital*

Cyclic peptidomimetics **1** and **2** were designed and synthesized in an effort to develop selective and potent analogues for human somatostatin receptor subtype 2. Human somatostatin receptor subtype 2 (hsst2) selective analogues of somatostatin are known to display a type-II′ β-turn topology about the D-Trp-Lys residues of the pharmacophore and typically posses hydrophobic residues at the back of the turn. A dibenzofuran-based scaffold was incorporated into the tetrapeptide pharmacophore of somatostatin to induce a rigid type-II′ β-turn topology and provide the required hydrophobicity. NMR studies of compounds **1** and **2** indicate that the scaffold induces the desired conformational constraints. Both compounds display increased binding and selectivity for hsst2 over hsst1 and hsst3-5 relative to the native peptide. These constrained cyclic peptidomimetics should provide new insight into the conformational requirements for the hsst2 receptor and the physiological role of the receptor.



1 **2**

87.

DARMSTOFF ANALOGUES: SUBTYPE-SELECTIVE AGONISTS AND ANTAGONISTS OF LPA RECEPTORS.

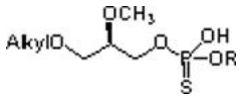
Veeresa Gududuru[1], *Michelle D. Walker*[2], *Ryoko Tsukahara*[2], *Yuko Fujiwara*[2], *Satoshi Yasuda*[2], *Gabor Tigyi*[2], *and Duane D. Miller*[1]. *(1) Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 847 Monroe Ave, Memphis, TN 38163, (2) Department of Physiology, University of Tennessee Health Science Center*

Lysophosphatidic acid (LPA) is a bioactive lysophospholipid mediator that acts through three types of GPCRs, LPA1 (EDG-2), LPA2 (EDG-4), and LPA3 (EDG-7) and recently an orphan GPCR, p2y9/GPR23 has been identified as the fourth LPA receptor (LPA4). LPA induces cell proliferation, morphological changes and has been shown to be involved in many physiological and pathological processes including neurogenesis, myelination, angiogenesis, wound healing and cancer invasion. LPA can be produced by a number of cell types including platelets, adipocytes, fibroblasts, and ovarian cancer cells. Most cells express a combination of these receptors, making it difficult to dissect the biological effects mediated by an individual receptor subtype. The need to understand the biological function of LPA receptors and the desire to pharmacologically exploit the differences in their ligand recognition requires the development of receptor subtype-selective agonists and antagonists. In our continued efforts to develop subtype-selective LPA agonists and antagonists, we identified Darmstoff analogues as a new type of ligands for LPA. The details of synthesis and pharmacological characterization of this new series of ligands will be discussed in this presentation.

88.

SYNTHESIS OF NOVEL ALKYL THIOPHOSPHATE LYSOPHOSPHATIDIC ACID (LPA) ANALOGUES AS RECEPTOR (ANT)AGONISTS. *Yong Xu, Lian Qian, Ted Simper, and **Glenn D. Prestwich**, Department of Medicinal Chemistry, University of Utah, 419 Wakara Way, STE 205, Salt Lake City, UT 84108, Fax: 801-585-6354, gprestwich@pharm.utah.edu*

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. The metabolically-stabilized LPA analog 1-oleoyl-2-O-methyl-rac-glycerophosphothioate (OMPT) was recently shown to be a potent agonist for the seven transmembrane domain G-protein coupled LPA_3 receptor. A new synthetic route for the enantiospecific synthesis of a series of alkyl thiophosphate LPA analogues, including ether OMPT and alkyl thiophosphoric diester, is described. The alkyl thiophosphoric monoesters induced cell proliferation at all concentrations and were identified as selective LPA_3 receptor agonists. In contrast, alkyl thiophosphoric diester inhibited cell viability at 1 mM. In addition, alkyl thiophosphoric diester analogues inhibited natural LPA and several synthetic LPA receptor agonists induced cell proliferation, especially cells with LPA_2 and LPA_3.



89.

DISCOVERY OF A HIGHLY POTENT AND ORALLY ACTIVE CCR5 ANTAGONIST TAK-652 AS AN ANTI-HIV-1 AGENT: SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 1-BENZAZOCINE DERIVATIVES CONTAINING A SULFOXIDE MOIETY. ***Masaki Seto*[1], *Katsuji Aikawa*[1], *Yoshio Aramaki*[1], *Naoki Miyamoto*[1], *Naoyuki Kanzaki*[1], *Yoji kuze*[1], *Katsunori Takashima*[1], *Masanori Baba*[2], *and Mitsuru Shiraishi*[1]. *(1) Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan, Fax: +81-6-6300-6306, seto_masaki@takeda.co.jp, (2) Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University*

Since CCR5 was identified as a coreceptor for entry of macrophage-tropic (R5) HIV-1 in 1996, CCR5 antagonists have received a great deal of attention as novel and attractive anti-HIV-1 agents. We previously reported a non-peptide, small molecule CCR5 antagonist TAK-779 as an anti-HIV-1 agent for injection. In order to develop orally active CCR5 antagonists, we replaced the quaternary ammonium moiety with a sulfoxide moiety of various polar substituents and performed chemical modification, mainly focused on the [6,7]-fused nucleus, which led to the discovery of a series of 1-benzazocine derivatives with potent inhibitory activity in a receptor binding assay. Among these compounds, TAK-652 exhibited highly potent CCR5 antagonistic activity and strongly inhibited replication of 6 strains of R5 HIV-1 clinical isolates in peripheral blood mononuclear cells, with less than nanomolar concentrations. TAK-652 also showed good pharmacokinetic properties after oral administration to rats and dogs, and was selected as a clinical candidate. The design, synthesis, SAR and biological properties of the sulfoxide compounds will be discussed.

90.

DISCOVERY OF THE PIPERIDINE-4-CARBOXAMIDE DERIVATIVE TAK-220, A HIGHLY POTENT CCR5 ANTAGONIST ANTI-HIV-1 AGENT. ***Shinichi Imamura*[1], *Takashi Ichikawa*[1], *Youichi Nishikawa*[1], *Shohei Hashiguchi*[1], *Naoyuki Kanzaki*[1], *Katsunori Takashima*[1], *Shinichi Niwa*[1], *Yoshio Yamamoto*[1], *Masanori Baba*[2], *and Yoshihiro Sugihara*[1]. *(1) Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan, Fax: +81-6-6300-6306, imamura_shin-ichi@takeda.co.jp, (2) Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University*

CCR5 has been identified as a major co-receptor for HIV-1 entry into host cells, and blocking this receptor with an antagonist may offer a new mechanism for inhibiting HIV-1 infection. Our laboratories have previously reported that benzocycloheptene derivative TAK-779 is an injectable CCR5 antagonist. In order to develop an orally bioavailable CCR5 antagonist, further high throughput screening was carried out, which led to the discovery of a novel lead, 5-oxopyrrolidine-3-carboxamide derivative (IC_{50} = 1900 nM), from an in-house synthesized chemical library. Subsequent optimization of the lead compound culminated in the identification of the piperidine-4-carboxamide derivative TAK-220, which showed high CCR5 binding affinity (IC_{50} = 3.5 nM) and excellent antiviral activity against CCR5-using HIV-1 in human peripheral blood mononuclear cells. TAK-220 also exhibited a good pharmacokinetic profile in monkeys and was selected as a clinical candidate for further evaluation. TAK-220 represents a promising new class of CCR5 antagonist anti-HIV-1 agent, and details of the synthesis and structure-activity relationships of this series will be presented.

91.

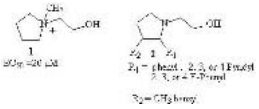
ISO-1, AN INHIBITOR OF MIF, PROTECTS IN ENDOTOXEMIA AND SEPSIS. ***Yousef Al-Abed*[1], *Kai Fan Cheng*[1], *Darrin Dabideen*[1], *Mahendra Ochani*[2], *Bayan Aljabari*[1], *Valentin Pavlov*[2], *Edmund Miller*[1], *and Kevin Tracey*[3]. *(1) Laboratory of Medicinal Chemistry, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, Fax: 1-516-365-5090, yalabed@nshs.edu, (2) Laboratory of Biomedical Sciences, North Shore-LIJ Research Institute, (3) Laboratory of Biomedical Sciences, North Shore Long Island Jewish Research Institute*

Sepsis, a potentially lethal systemic inflammatory reaction to infection, affects approximately 700,000 individuals in the US alone and kills more than 215,000 annually. No small molecule therapeutic agent is currently approved by the FDA for its clinical management. Sepsis is mediated, at least in part, by soluble factors and among these, MIF, has been shown to play an important role in the pathogenesis of this condition. In vitro, MIF expression abrogates the anti-inflammatory and immunosuppressive effect of glucocorticoids on pro-inflammatory cytokines production. In addition, in vivo administration of neutralizing MIF-antibody protects mice from: a) LPS-induced lethality; b) lethal peritonitis and septic shock induced by E. coli and c) lethal sepsis induced by cecal ligation and puncture (CLP) in TNF-a deficient mice. Furthermore, the severity of injury or infection in trauma patients and MIF levels in the serum are correlated with increased circulating levels of MIF in patients with severe sepsis (6-fold) and in patients with septic shock (15-fold) compared to normal individuals. We recently have designed (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) as an inhibitor of MIF activity. The crystal structure of MIF complexed to ISO-1 revealed that the inhibitor binds to a hydrophobic pocket. In vitro, ISO-1 inhibits 60% of TNF release by LPS-treated macrophages. In vivo, intraperitoneal administration of ISO-1 at 35 mg/kg increased the survival rate in endotoxemia and CLP-induced sepsis by 40% and 35% respectively, and decreased plasma TNF levels in LPS-treated mice. ISO-1 treatment given 24-hours post CLP increased the survival rate to 80% compared to 40% in control mice. These data suggest that MIF is mid to late mediator of sepsis and that ISO-1 may be a novel therapeutic intervention in human sepsis.

92.

DESIGN, SYNTHESIS AND EVALUATION OF ALKYL AND ARYL SUBSTITUTED N-HYDROXYETHYL PYRROLIDINES TARGETING α-7 NICOTINIC ACETYLCHOLINE RECEPTOR. ***Mallikarjun G. Puppali*[1], *J. J. Buccafusco*[2], *and J. Warren Beach*[1]. *(1) Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA 30602, mpuppali@rx.uga.edu, (2) Alzheimers Research Center, Alzheimers Research Center, Medical College of Georgia, Augusta, GA 30912*

The α-7 nicotinic Acetylcholine receptor(nAchR) subtype is currently recognized as a novel target for Alzheimer's Disease drug design. The α-7 nAchR subtype is widely distributed throughout the central nervous system and is involved in neuroprotection. We have previously shown that compound **1**, N-Methyl-N-Hydroxyethyl pyrrolidine possesses cytoprotective activity (EC50 = 20μM) in NGF deprived PC12 cells. Based on this lead compound, we have synthesized a number of analogs (**2**) in order to explore the structure-activity relationships of this series. The synthesis of these analogs as well as their evaluation as cytoprotective agents will be presented.



93.

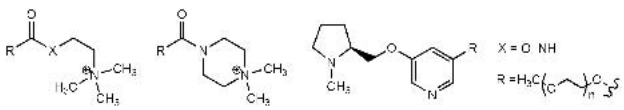
DOCKING STUDIES INVOLVING A HOMOLOGY MODEL OF AN ALPHA-7 HUMAN NEURONAL NICOTINIC RECEPTOR. ***Mee Shelley***[1], *Philip S. Hammond*[2], *Todd Minehardt*[3], *and Jeffrey D. Schmitt*[2]. *(1) Application Science, Schrodinger, 1500 SW First Ave, Portland, OR 97201, mshelley@schrodinger.com, (2) Molecular Design Group, Targacept, Inc, (3) Department of Chemistry, University of Texas at Austin*

Neuronal nicotinic acetylcholine receptors are ligand-gated ion channels that influence a variety of biological functions and have shown considerable promise in the treatment of various neurological and mental disorders. These receptors gate the flow of ions across membranes in response to the binding of subtype-selective agonists, antagonists and modulators. Using a homopentameric homology model based on an acetylcholine-binding protein from snail, we have conducted docking studies on compounds with affinity (covering 6-orders of magnitude) for the alpha-7 nicotinic acetylcholine receptor. We discuss conformations of Loop C (a flexible, key feature in the ligand binding domain), pi-cation interactions, and predicted binding modes for these protonated ligands. We also present results on ligand-induced conformational changes that occur within the ligand-binding domain.

94.

HOMOLOGATED MONOVALENT LIGANDS FOR NICOTINIC RECEPTORS BASED ON POLYETHYLENE GLYCOL TETHERS. ***Richard W. Fitch***[1], *Benjamin C. Chastain*[1], *Amanda M. Clay*[1], *Fernand M. Bedi*[2], *and Brandon T. Elliott*[1]. *(1) Department of Chemistry, Indiana State University, 600 Chestnut Street, Science Building, Room S35E, Terre Haute, IN 47809, Fax: 812-237-2232, rfitch@carbon.indstate.edu, (2) Deparartment of Chemistry, Indiana State University*

As part of our efforts toward the development of multivalent ligands for nicotinic acetylcholine receptors (nAChRs), we have prepared a series of homologated ligands based on known binding motifs, including cholines, piperaziniums and pyridyl ethers. Each of these motifs is homologated by attachment of a polyethylene glycol (PEG) chain. PEGs are biocompatible hydrophilic polymers which will ultimately serve as tethers for the preparation of high-affinity multivalent ligands. Our initial synthetic efforts and biological evaluation of these ligands will be described.



95.

HUMAN ALPHA-7 NEURONAL NICOTINIC RECEPTOR DOCKING STUDIES AS AN ALIGNMENT METHOD FOR COMPARATIVE MOLECULAR FIELD ANALYSIS (COMFA). ***Philip S. Hammond***[1], *Mee Y. Shelley*[2], *Todd Minehardt*[3], *Yun-De Xiao*[1], *Josef Klucik*[1], *and Jeffrey D. Schmitt*[1]. *(1) Molecular Design Group, Targacept, Inc, 200 East First Street, Suite 300, Winston-Salem, NC 27101-4165, Fax: 336-480-2107, phil.hammond@targacept.com, (2) Schrodinger Inc, (3) Department of Chemistry, University of Texas at Austin*

Neuronal nicotinic acetylcholine receptors gate the flow of ions across membranes in response to the binding of subtype-selective agonists, antagonists and modulators. Using a homology model based on an acetylcholine-binding protein from snail, we have explored docking of a chemically diverse set of ligands to the 5-different ligand binding sites of the homopentameric alpha-7 neuronal nicotinic acetylcholine receptor. Using Glide sampling and scoring functions, various docked poses for each compound were generated. Sets of these poses selected based on the Glide scoring function were evaluated using Comparative Molecular Field Analysis (CoMFA) to develop models for binding affinity. Models with high cross-validated correlation were developed, with good predictive ability for a diverse set of test molecules. Further, the steric and electrostatic fields derived from these models provide useful insights for identification of pharmacophoric features when overlaid on the ligand-binding domain.

96.

NICOTINE ANALOGS PROTECT IN SEPTIC SHOCK AND SEPSIS. ***Yousef Al-Abed***[1], *hong wang*[1], *Luis Ulloa*[1], *Carol Ann Amella*[1], *Mahira Tanovic*[1], *Mahendra Ochani*[1], *and Kevin Tracey*[2]. *(1) Laboratory of Medicinal Chemistry, North Shore-LIJ Research Institute, 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 nicotine or acetylcholine 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 & Nature Med. 10:1216-21). 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 endotoxemia and sepsis. 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 nicotinc 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.

97.

STRUCTURE-ACTIVITY RELATIONSHIPS FOR CYTOTOXICITY OF NICOTINIC ACID ESTER PRODRUGS FOR PULMONARY DELIVERY. ***Ling Xu***, *Sandhya M. Vyas, Hans-Joachim Lehmler, and Gabriele Ludewig, Department of Occupational and Environmental Health, University of Iowa, 100 Oakdale Campus, #124 IREH, Iowa City, IA 52242, ling-xu@uiowa.edu*

A series of nicotinic acid ester prodrugs for pulmonary delivery with varying degree of fluorination and chain length of the alcohol moiety was synthesized. Cytotoxicity experiments with prodrugs and their building moieties in five cell-lines, representing different pulmonary cell types, showed low toxicities with IC20s in the millimolar range with all compounds tested. The IC20 of nicotinic acid was close to those of the least toxic esters, but strong toxicity was seen at high concentration due to acidification of the medium. The toxicity of prodrugs and corresponding alcohols increased with increasing length of the alcohol moiety and with fluorination. The cytotoxicity ranking of the esters and alcohols correlated with the order of their octanol-water partition coefficients, suggesting that the toxicity of these compounds may be related to their lipophilicity, therefore most likely reflecting the likelihood of the prodrugs and alcohols to partition from the medium into the cells.

98.

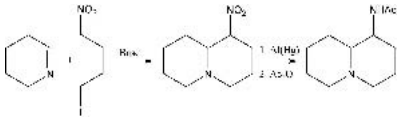
SYNTHESIS OF *N*-[^{11}C]METHYL-3-[[(DIMETHYLAMINO)CARBONYL]OXY]-2-(2′,2′-DIPHENYLPROPIONOXYMETHYL)PYRIDINIUM AS A NEW POTENTIAL PET TRACER FOR IMAGING HEART ACETYLCHOLINESTERASE. *Ji-Quan Wang,* ***Mingzhang Gao****, and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, Room L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, jiqwang@iupui.edu, migao@iupui.edu*

The acetylcholinesterase (AChE) inhibitors have been used to treat a number of heart conditions in human due to their 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. The AChE inhibitor radiotracer *N*-[^{11}C]methyl-3-[[(dimethylamino)carbonyl]oxy]-2-(2′,2′-diphenylpropionoxymethyl)pyridinium ([^{11}C]MDDP) was synthesized for evaluation as a new potential PET imaging agent for heart AChE. The quaternary amine tracer [^{11}C]MDDP was prepared by *N*-[^{11}C]methylation of the tertiary pyridine precursor 3-[[(dimethylamino)carbonyl]oxy]-2-(2′,2′-diphenylpropionoxymethyl) pyridine using [^{11}C]methyl triflate and isolated by solid phase extraction (SPE) purification procedure in 40-65% radiochemical yields.

99.

SYNTHETIC STUDIES TOWARD EPIQUINAMIDE AND ANALOGS. ***Richard W. Fitch***[1], *Shaun R. Patel*[2], *and Karen J. Lipscomb*[1]. *(1) Department of Chemistry, Indiana State University, 600 Chestnut Street, Science Building, Room S35E, Terre Haute, IN 47809, Fax: 812-237-2232, rfitch@carbon.indstate.edu, (2) Department of Chemistry, Wabash College*

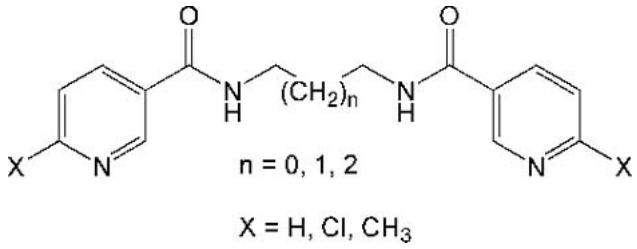
Epiquinamide, a quinolizidine alkaloid recently isolated from an Ecuadoran poison frog, is a nicotinic acetylcholine receptor (nAChR) agonist. Our lab has embarked on a program to synthesize epiquinamide and analogs in order to confirm the structure and activity of this alkaloid and evaluate preliminary structure-activitiy relationships. Our initial approach to the racemic compound is via nitroaldol reaction of nitrobutyl iodide with 2,3,4,5-tetrahydropyridine to produce the quinolizidine skeleton, followed by aluminum amalgam reduction and acylation to produce the title compound. Examination of the stereochemistry showed the epimer (epi-Epiquinamide) to be formed preferentially. Our approach to the solution of the relative and absolute stereochemistry as well as the biological activities will be discussed.



100.

UNEXPECTED NICOTINIC EFFECTS OF NICOTINIC ACID AMIDES. ***Patricio Iturriaga-Vásquez***[1], *José L. Ulloa*[2], *Bruce K. Cassels*[1], *Sévérine Peraccio*[3], *and Isabel Bermúdez*[3]. *(1) Millennium Institute for Advanced Studies in Cell Biology and Biotechnology, Las Encinas 3370, Ñuñoa, Santiago, Chile, Fax: 56-2-271-3888, piturria@uchile.cl, (2) Department of Chemistry, University of Chile, Faculty of Sciences, (3) Oxford Brookes University, School of Biological and Molecular Sciences*

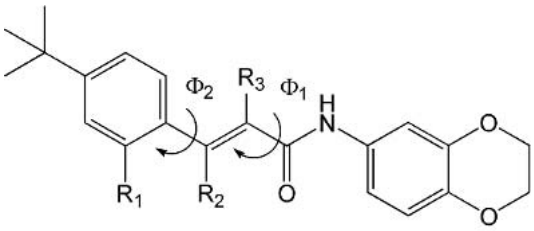
The discovery of pharmacologically active molecules is often serendipitous. While attempting to prepare a simple 2-nicotinoyl-1,4,5,6-tetrahydropyrimidine as an aza-analogue of anabaseine (a natural alkaloid with nicotinic acetylcholine receptor – NAChR - agonist activity) we isolated a side product which proved to be a nicotinoyl-bis-amide. This compound was isolated, chemically characterized and assessed in binding and functional studies in which it behaved as a NAChR agonist with a potency approximately 130% of that of nicotine. This unexpected result motivated us to prepare a new series of nicotinoyl-bis-amides. In this work, we discuss the preliminary studies of a series of bis-amides where the length of the carbon chain between both amide groups was modulated and the pyridine unit was substituted at the 6 position. These modifications we have allowed us to identify the minimal requirements for these molecules to show good affinity and potency as nicotinic agonists.



101.

CONFORMATIONAL ANALYSES OF N-ARYL CINNAMIDES AS TRPV1 ANTAGONISTS. ***Jiawang Zhu***[1], *Vellarkad Viswanadhan*[1], *Vassil Ognyanov*[1], *Yunxin Bo*[1], *Ning Chen*[1], *Partha P. Chakrabarti*[1], *Elizabeth Doherty*[1], *Christopher Fotsch*[1], *Narender Gavva*[2], *Nianhe Han*[1], *Lana Klionski*[2], *Qingyian Liu*[1], *Rami Tamir*[2], *Xianghong Wang*[1], *Yaxiong Sun*[1], *James J. S. Treanor*[2], *and Mark H. Norman*[1]. *(1) Department of Chemistry Research & Discovery, Amgen, Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-480-3015, jzhu@amgen.com, (2) Department of Neuroscience, Amgen, Inc*

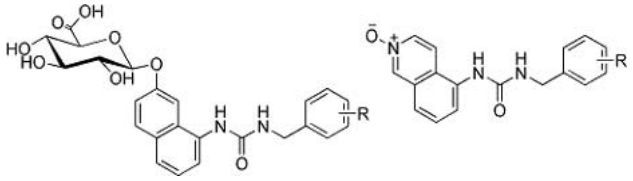
The vanilloid receptor-1 (VR1 or TRPV1) belongs to a family of transient receptor potential (TRP) cation channels and is activated by heat, low pH and capsaicin. The TRPV1 is predominantly expressed in sensory neurons, and is involved in the signal transmission of noxious pain stimuli. Blockade of the TRPV1 channel with antagonists represents a promising strategy for the development of novel analgesics.Recently, we discovered and reported a series of N-aryl cinnamides as potent and orally available TRPV1 antagonists. Herein, we propose a bioactive conformer of the cinnamides, based on alignment of the minimum energy conformations of several derivatives substituted at R1, R2, or R3. The conformational analyses of the N-aryl cinnamides was carried out with Monte Carlo searching and ab initio quantum calculations at 6-31G* level. A brief summary of synthesis and SAR (structure-activity relationship) of the N-aryl cinnamides will also be presented.



102.

EFFORTS TO CIRCUMVENT MAJOR METABOLIC PATHWAYS OF OXIDATION AND GLUCURONIDATION IN ARYLUREA-BASED VANILLOID (TRPV1) SERIES. ***Mark E. McDonnell***[1], *James J. McNally*[1], *Sui-Po Zhang*[1], *Adrienne Dubin*[2], *and Scott L. Dax*[1]. *(1) Johnson & Johnson Pharmaceutical Research & Development, LLC, Welsh and McKean Roads, P.O. Box 776, Spring House, PA 19477, (2) Neurosciences, La Jolla CA, Johnson & Johnson Pharmaceutical Research and Development, LLC*

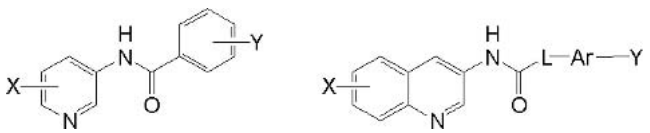
During the optimization of a series of naphthol-derived urea TRPV1 antagonists, glucuronidation was discovered to be the main route of metabolism. In a related isoquinolinylurea series, N-oxide formation predominated. In both series, attempts to thwart metabolism focused initially on the introduction of substituents ortho to the site of metabolism. The synthesis of analogs designed to test this approach as well as the impact of these modifications on both TRPV1 activity and metabolism are presented.



103.

N-PYRIDIN-3-YL- AND N-QUINOLIN-3-YL- BENZAMIDES: MODULATORS OF HUMAN VANILLOID RECEPTOR 1 (VR1). ***James J. McNally****, Adrienne Dubin, Mark A. Youngman, Michele C. Jetter, Sui-Po Zhang, Mark E. McDonnell, Ellen E. Codd, Raymond W. Colburn, Dennis J. Stone, Nadia Nasser, Christopher M. Flores, and Scott L. Dax, Johnson & Johnson Pharmaceutical Research & Development, LLC, Welsh & McKean Roads, P.O. Box 776, Spring House, PA 19477, jmcnally@prdus.jnj.com*

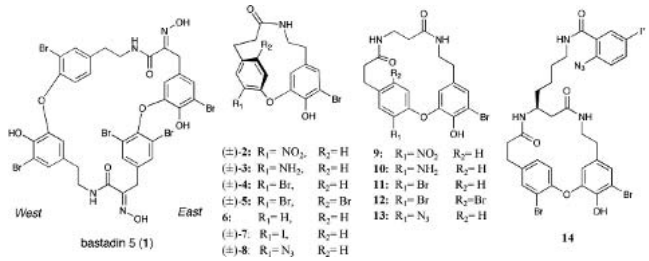
The human vanilloid receptor (VR1) is a neuronal cation-selective channel that is gated by stimuli associated with pain, including low pH, heat and naturally occurring ligands such as capsaicin (the pungent component of chili peppers). The implication of the VR1 channel in pain and hyperalgesia make it an attractive therapeutic target for drug discovery. Our laboratories developed a high throughput functional assay for the VR1 receptor, which can identify both agonists and antagonist of the VR1 receptor. Broad screening of our compound libraries, led to the identification of a series of N-pyridin-3-ylbenzamides, which act as VR1 agonists. This paper highlights the structure activity relationships of this series of compounds, which led to the identification of the closely related N-quinolin-3-ylbenzamides and analogs, which are potent VR1 antagonists.



104.

ANALOGS OF BASTADIN 5 AND STRUCTURE ACTIVITY RELATIONSHIPS OF MODULATION OF THE RYR-1 CALCIUM CHANNEL. ***Makoto N Masuno***[1], *Isaac N. Pessah*[2], *and Tadeusz F. Molinski*[1]. *(1) Department of Chemistry, University of California, Davis, 1 Shields Ave, Davis, CA 95616, Fax: 530-752-8995, mnmasuno@ucdavis.edu, (2) Veterinary Medicine, UC Davis*

Bastadin 5 (**1**) is a bromotyrosine-derived macrolactam described in 1981 by Kazlauskas *et al.* from the Verongid sponge *Ianthella basta* (Pallas). Over 20 bastadins have been reported to date. Bastadins stimulates Ca^{2+} release from stores in the junctional sarcoplasmic reticulum (JSR) by binding to the RyR-1/FKBP12 channel in the skeletal muscle. Bastadins also synergize's FK506-induced release of FKBP12 from RyR-1. The mechanism of action and locus of binding on the RyR-1 are unknown. The structure of **1** is comprised of four brominated, modified tyrosines and tyramines arrayed within a 28-membered macrolactam. Biological evaluation of simple cyclic analogs of **1** (e.g. **2**-**14**), which embody the substituted diaryl ethers of the 'western' hemisphere of **1** within smaller macrocycles have shed light on the minimum pharmacophore of **1**. Aryl azides **8**, **13** and **14** may be useful photoaffinity agents to probe the binding site of **1** on the JSR. The analogs **2**-**13** were prepared from common intermediates and evaluated in a [^{3}H]-ryanodine-binding assay that monitors the open state of the Ca^{2+} channel. We will present studies on the synthesis and evaluation of atropisomers of **4** and the potential photoaffinity reagent **14**.



105.

DESIGN, SYNTHESIS AND SAR OF NOVEL AND SELECTIVE T-TYPE CALCIUM CHANNEL ANTAGONISTS CONTAINING A BIARYL SULFONAMIDE CORE. *Jon J. Hangeland*[1], ***Todd J. Friends***[1], *Daniel L. Cheney*[2], *Paul C. Levesque*[3], *Adam J. Rich*[3], *Lucy Sun*[3], *Terry R. Bridal*[3], *Leonard P. Adam*[3], *and Diane E. Normandin*[3]. *(1) Cardiovascular Discovery Chemistry, Bristol-Myers Squibb, P.O. Box 5400, Princeton, NJ 08543, Fax: 609-818-3450, jon.hangeland@bms.com, todd.friends@bms.com, (2) Department of Macromolecular Structure, Bristol Myers Squibb Pharmaceutical Research Institute, (3) Cardiovascular Diseases, Bristol-Myers Squibb Pharmaceutical Research Institute*

Selective blockade of the T-type calcium channel has been shown to be an effective treatment for hypertension and stable angina, without the side effects common to L-type channel blockers such as increased heart rate and edema. Design of novel T-type channel blockers was carried out using an iterative design, synthesis, in vitro evaluation paradigm. Molecules were designed with the program SPROUT using constraints provided by a ComFA pharmacophore model. Scaffolds generated by SPROUT were evaluated based on frequency of occurrence and their ability to be translated into structures that were synthetically tractable. From this exercise, a novel series of potent and selective T-type channel antagonists were discovered.

106.

BENZOFUROINDOLE ANALOGUES AS POTENT CA^{+2} INDEPENDENT BK_{CA} CHANNEL OPENERS. *Ahmet Erkam Gormemis, Tal Soo Ha, Chul-Seung Park, and* ***Yong-Chul Kim****, Department of Life Science, Gwangju Institute of Science and Technology, 1 Oryong-dong, Buk-gu, Gwangju 500-712, South Korea, Fax: +82-62-970-2484, yongchul@gist.ac.kr*

Large-conductance Ca^{2+} activated potassium channels (BK_{Ca}) are widely distributed and play key roles in various cell functions. In nerve cells, BK_{Ca} channels shorten the duration of action potentials and block Ca^{2+} entry thereby repolarizing excitable cells after excitation. BK_{Ca} channel opening has been postulated to confer neuroprotection during stroke and has attracted attention as a means for therapeutic intervention in asthma, hypertension, convulsion, and traumatic brain injury. Benzofuroindole skeleton was compared with a known BK_{Ca} channel opener, BMS-204352, to optimize pharmacophore groups to be incorporated. Being evaluated on the cloned BKCa channels expressed in *Xenopus laevis oocytes* by utilizing electrophysiological methods, several derivatives were identified as the potent and effective BK_{Ca} channel openers with intracellular calcium independent manner.

107.

DESIGN OF A SELECTIVE SMALL MOLECULE KV1.3 BLOCKER. ***Ananthakrishnan Sankaranarayanan***[1], *Alexander Schmitz*[2], *Kristina Schmidt-Lassen*[2], *Daniel Homerick*[1], *Wolfram Hänsel*[2], *and Heike Wulff*[1]. *(1) Medical Pharmacology and Toxicology, University of California Davis, Genome and Biomedical Sciences Facility Room 3502, 451 East Health Sciences Drive, Davis, CA 95616, Fax: 530-752-3200, asandaranarayanan@ucdavis.edu, (2) Pharmaceutical Institute, Christian Albrechts University*

The voltage-gated K+ channel Kv1.3 in terminally differentiated T lymphocytes is an attractive novel therapeutic target for autoimmune diseases such as multiple sclerosis. The known Kv1.3 blockers correolide, PAC and Psora-4 show poor selectivity for Kv1.3 over the cardiac K+ channel Kv1.5. Using Psora-4 as a template we recently identified a series of 5-phenoxyalkoxypsoralens (PAPs) through a combination of classical medicinal chemistry and whole-cell patch-clamp. Compounds of the PAP series with one nitro group, no substituents or a phenoxy moiety display 17-50-fold selectivity for Kv1.3 over Kv1.5. The most potent and selective Kv1.3 blocker in this series, PAP-1 blocks Kv1.3 with a Hill coefficient of 2 and a Kd of 2 nM. PAP-1 is 20-125 fold selective over other voltage-gated K+ channels and 1000 fold selective over HERG and calcium-activated K+ channels. PAP-1 and its derivatives constitute excellent new tools to explore Kv1.3 as a target for immunosuppression.

108.

NOVEL CYCLOPENTANE DICARBOXAMIDE SODIUM CHANNEL BLOCKERS AS A POTENTIAL TREATMENT FOR CHRONIC PAIN. ***Pengcheng Shao***[1], *Michael H. Fisher*[1], *Maria L Garcia*[2], *Gregory J. kaczorowski*[2], *Kathryn Lyons*[3], *William J. Martin*[4], *Peter T. Meinke*[1], *Birgit T. Priest*[2], *McHardy M. Smith*[2], *Matthew J. Wyvratt*[1], *Feng Ye*[1], *and William H. Parsons*[1]. *(1) Department of Medicinal Chemistry, Merck Research Labs, RY123-232, Merck Research Labs, Rahway, NJ 07023, pengcheng_shao@merck.com, (2) Department of Ion Channels, Merck Research Labs, (3) Department of Drug Metabolism, Merck Research Laboratories, (4) Department of Pharmacology, Merck & Co., Inc*

Voltage-gated sodium channels (VGSC) have attracted considerable attention recently as a target for treatment of neuropathic pain. Our initial medicinal chemistry effort started from screening lead compound A, a potent VGSC inhibitor with poor PK profile. Conformational analysis of compound A has led us to design and synthesize conformationally restrained analogs. Among them, compound B showed great improved PK profile and in vivo efficacy in rat inflammatory and neuropathic pain models.

109.

PYRAZOLES AS MODULATORS OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR. ***Mark T. Miller****, Fred Chambers III, Caroline Decker, Adrianna Galue, Peter D.J. Grootenhuis, Sabine S. Hadida, Licong Jiang, Yahua Liu, Lewis R. Makings, Paul Negulescu, Eric Olson, James Rader, Ashvani K. Singh, Roger D. Tung, and Fredrick Van Goor, Vertex Pharmaceuticals Incorporated, 11010 Torreyana Road, San Diego, CA 92121, Fax: 858-404-6726, mark_miller@sd.vrtx.com*

To identify novel compounds capable of improving the gating function of the ΔF508 mutation of the cystic fibrosis transmembrane conductance regulator (ΔF508-CFTR), we utilized a fluorescence-based assay of membrane potential in 3T3 cells expressing recombinant ΔF508-CFTR. Approximately 200,000 compounds were screened to find small molecules which increased ΔF508-CFTR ion flux in the presence of protein kinase A (PKA) stimulation. After confirmation of the initial screening activity, hits which exhibited activity and

efficacy equal to or greater than genistein where selected for chemical optimization. Pyrazoles were found to modulate the activity of ΔF508-CFTR. Here we present the preliminary SAR around the pyrazole scaffold, and the corresponding pharmacokinetic data for selected members of the series.

110.

SCREENING FOR ION CHANNEL ACTIVITY IN HUMAN T LYMPHOCYTES FOR DIAGNOSIS AND THERAPEUTIC MONITORING OF MULTIPLE SCLEROSIS.

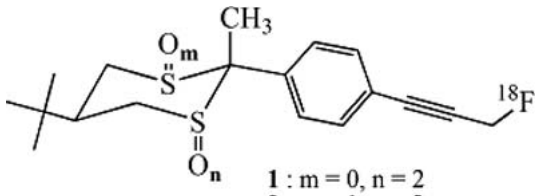
***Michael Mayer**, Department of Biomedical Engineering and Chemical Engineering, University of Michigan, Gerstacker Bldg., Room 1107, 2200 Bonisteel Blvd, Ann Arbor, MI 48109-2099, Fax: 734-763-4371, mimayer@umich.edu, and Sohiel Memarsadeghi, Department of Biomedical Engineering, University of Michigan*

Multiple sclerosis (MS), a demyelinating disease of the central nervous system with autoimmune pathogenesis, is the most common neuropathological disorder in young adults and affects more than 2 million people worldwide. An automated blood-based test for the diagnosis of MS is not available; current diagnosis is based on symptoms and on imaging techniques such as magnetic resonance imaging (MRI). Despite its enormous benefits, MRI-based diagnosis of MS is expensive and can lead to erroneous diagnoses, mostly because of the difficulty to distinguish between brain lesions originating from MS versus disseminated encephalomyelitis (DEM). Recent reports indicate that patients with MS overexpress a specific ion channel in a subclass of their white blood cells. These so-called myelin-reactive effector memory T cells express a high population of the voltage-gated potassium channel (Kv1.3) in MS patients and have been implicated in the pathogenesis of the disease. In control subjects, the expression of this ion channel in the same type of cells is several fold lower. Here we present a novel approach to clinical diagnostics that utilizes recent breakthroughs in functional and automated ion channel screening technology. We use a high-throughput screening platform that performs 384 electrophysiological whole-cell recordings in parallel. We demonstrate that crude preparations of white blood cells from MS patients contain significantly more cells with increased Kv1.3 activity than preparations of the same cells from control subjects. We believe that high-throughput screening of human cells may provide both an automated diagnostic assay for MS and an entirely new strategy for diagnosis and therapeutic monitoring of other autoimmune diseases including type 1 diabetes mellitus, rheumatoid arthritis, Crohn's disease, and psoriasis.

111.

SYNTHESIS AND EVALUATION OF 5-TERT-BUTYL-TRANS-2-[F-18]-FLUOROPROPYNYLPHENYL-2-METHYL-1,1-DIOXO-1,3-DITHIANE (FPMDD) AND 5-TERT-BUTYL-TRANS-2-[F-18]FLUOROPROPYNYLPHENYL-2-METHYL-1,1′,3-TRIOXO-1,3-DITHIANE (FPMTRD) AS GABAA-GATED CHLORIDE ION CHANNEL LIGANDS. ***Xuehe Li**, Yong-Woon Jung, Scott E. Snyder, Phillip S. Sherman, and Michael R. Kilbourn, Department of Radiology, University of Michigan Medical School, 3480 Kresge III, 204 Zina Pitcher Pl, Ann Arbor, MI 48109, xueheli@umich.edu*

The GABA system is essential for the overall balance between neuronal excitation and inhibition. Dysfunction of this inhibitory system has been proposed in a wide variety of neurological and psychiatric diseases. In our continuing efforts on developing candidates for in vivo imaging of the GABAA-gated chloride ion channel, we report here the multi-step synthesis of two new radioligands [F-18]FPMDD 1 and [F-18]FPMTrD 2. Biodistribution study on mice showed high initial uptake into brain, followed by a continuous washout and a clear heterogeneous regional distribution with highest retention in cortex and cerebellum and lowest in striatum and pons. More preliminary results from in vitro and in vivo studies of the ligands will be discussed.

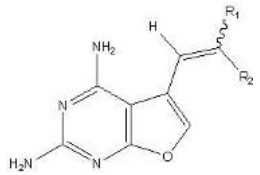


1 : m = 0, n = 2
2 : m = 1, n = 2

112.

NOVEL 5-SUBSTITUTED, 2,4-DIAMINOFURO[2,3-*D*]PYRIMIDINES AS POTENTIAL MULTI–RECEPTORS TYROSINE KINASE AND DIHYDROFOLATE REDUCTASE INHIBITORS. ***Aleem Gangjee**[1], **Wei Li**[1], Michael Ihnat[2], Dixy Green[2], W. Todd Miller[3], and Roy L. Kisliuk[4]. (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, liwei7054@hotmail.com, (2) Department of Cell Biology, The University of Oklahoma Health Science Center, (3) State University of New York at Stony Brook, (4) Department of Biochemistry, Tufts University School of Medicine*

Among the many factors that trigger angiogenesis, activation of receptor tyrosine kinases (RTKs) is most important. Inhibition of RTKs thus provides a new paradigm for cancer therapy. Single RTK targeting by small molecules is established as a mechanism of cancer therapy. Combination of the cytostatic effect of RTKs with other cytotoxic agents has also shown promising results in clinical cancer chemotherapy. In our continuing efforts to develop novel multi-acting antitumor agents, we have incorporated both RTK inhibitory activity and DHFR inhibitory activity in single molecules. Based on the reported X-ray crystal structures of RTKs and DHFR, we designed dual DHFR/RTK inhibitors using the furo[2,3-*d*]pyrimidine scaffold with a 2,4-diamino moiety. Using the most potent analogs of our previous series of general structure **1** as the lead compound, we have explored different alkyl groups (R_1) at C-9 with a 2-naphthyl or 2-methoxylphenyl as the R_2 group and studied their influence on potency and specificity of inhibition of DHFR and RTK. The design and synthesis of these compounds will be presented and discussed.



1

113.

IMMOBILIZATION OF SMALL-MOLECULE PROBES FOR KINASE PROFILING ASSAYS. *Hitesh K. Patel, **Robert M. Grotzfeld**, Zdravko V. Milanov, Shamal A Mehta, Andiliy G. Lai, Miles A. Fabian, Todd A. Carter, Philip T. Edeen, Anne Marie Velasco, Julia M. Ford, Mark Floyd, Pietro Ciceri, Darren E. Insko, Sanna Herrgard, Corey E. Atteridge, Lisa M. Wodicka, William H. Biggs III, Daniel K. Treiber, Patrick P. Zarrinkar, and David J. Lockhart, Ambit Biosciences Corp, 4215 Sorrento Valley Blvd, San Diego, CA 92121, Fax: 858-334-2199, rgrotzfeld@ambitbio.com*

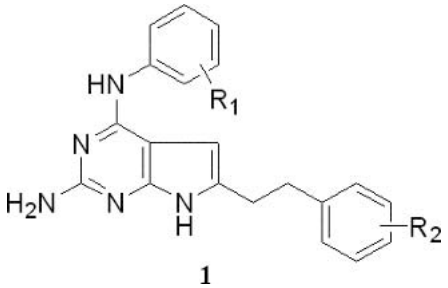
Protein kinases are critical components of cellular signaling cascades and have in recent years emerged as important and promising targets for drug development. The kinase inhibitors Imatinib (Gleevec) and Gefitinib (Iressa) have been approved, and there are now more than 30 kinase inhibitors in clinical development. Ambit Biosciences has developed a technology that allows rapid *in vitro* screening of compounds for kinase activity and specificity by directly and quantitatively measuring binding to the kinase ATP site. We have developed quantitative assays for more than 170 kinases, including many clinically relevant mutant forms of kinases such as ABL, EGFR, FLT3, and KIT. For assay development we linked a variety of ATP site binding compounds to a solid phase in such a way as to maintain their kinase binding properties. The small molecule linker, immobilization techniques, and validation of the method are presented here.

114.

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 2-AMINO-4-ANILINO SUBSTITUTED-6-ARYLETHYL PYRROLO[2,3-*D*]PYRIMIDINES AS RECEPTOR TYROSINE KINASE INHIBITORS AND ANTIANGIOGENIC AGENTS. *Aleem Gangjee[1], **Ojas A. Namjoshi**[1], Michael Ihnat[2], Dixy Green[2], and W. Todd Miller[3]. (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, namjosh205@duq.edu, (2) Department of Cell Biology, The University of Oklahoma Health Science Center, (3) State University of New York at Stony Brook*

Several tumors have dysfunctional receptor tyrosine kinases (RTKs) that are often over expressed and promote inappropriate signaling that have 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 reported a series of pyrrolo[2,3-*d*]pyrimidines of general structure **1** with 4-m-Br-aniline and a 6-arylethyl group. The nature and position of the substituent on the aryl moiety of **1** were found to dictate the selectivity and potency against a variety of RTKs. This report will discuss the design, synthesis and RTK inhibitory activities of **1** in which the R1 substituent 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.

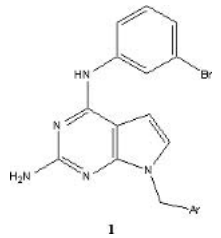


1

115.

NOVEL 2-AMINO-4-ANILINO SUBSTITUTED -7-ARYLMETHYL PYRROLO[2,3-*D*]PYRIMIDINES AS RECEPTOR TYROSINE KINASE INHIBITORS AND ANTIANGIOGENIC AGENTS. *Aleem Gangjee[1], **Nilesh Zaware**[1], Michael Ihnat[2], Dixy Green[2], and W. Todd Miller[3]. (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, nileshpharm@yahoo.com, (2) Department of Cell Biology, The University of Oklahoma Health Science Center, (3) State University of New York at Stony Brook*

Angiogenesis plays a pivotal role in the growth, invasion and metastasis of solid tumors. The most pronounced factor that triggers angiogenesis is activation of receptor tyrosine kinases (RTKs). Inhibition of RTKs thus provides an attractive target for cancer chemotherapy. Several small molecule inhibitors of RTKs have been recently approved for clinical use in combination with conventional chemotherapeutic agents or are currently in clinical trials. On the basis of known pharmacophores and X-ray crystal structures of RTKs, we have designed synthesized and evaluated compounds of structure **1** as inhibitors of RTKs. The synthesis, RTK inhibitory activities and specificities of compounds **1** with a variety of substituted aryl groups will be discussed in this report.

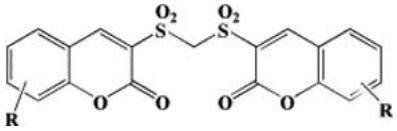


1

116.

SYNTHESIS AND BIOLOGICAL EVALUATION OF POTENTIAL DUAL ERBB-2/EGFR TYROSINE KINASE INHIBITORS: 3,3-BIS(COUMARINSULFONYL) METHANES. ***Venkat R Pallela**[1], Muralidhar R Mallireddigari[1], Kiranmai Gumireddy[1], Stephen C Cosenza[1], Stanley C Bell[2], E. Premkumar Reddy[1], and M.V. Ramana Reddy[1]. (1) Fels Institute for Cancer Research, Temple University School of Medicine, 3307, North Broad Street, Philadelphia, PA 19140-5101, pallela@temple.edu, (2) Department of Medicinal Chemistry, Onconova Therapeutics Inc*

Epidermal growth factor receptor (EGFR) tyrosine kinase family consists of four members EGFR, c-erbB-2, c-erbB-3 and c-erbB-4. All share structural homology consisting of an extracellular ligand binding domain, a transmembrane domain and intracytoplasmic tyrosine kinase domain. Over-expression of these receptors is found in a number of cancers (e.g., breast, ovarian, colon, prostate) and has been associated with poor prognosis in patients. Therefore, inhibition of EGFR and ErbB-2 kinase activity has emerged as a promising new approach to cancer therapy. This was validated by antibody-based therapy using Herceptin, which is shown to increase mean survival time in metastatic breast cancer patients overexpressing EGFR and ErbB-2, respectively. Small molecule tyrosine kinase inhibitors are another class of promising new anticancer drugs and several chemical series such as 4-anilinoquinazolines, 4-anilinopyrido[d]pyrimidines, 4-anilinopyrazolo[3,4-d]pyrimidines and dianilinophthalimides have been reported as novel tyrosine kinase inhibitors by competing with ATP at the binding site. Recent success in the clinical evaluation of TK inhibitors, Gleevec and Iressa, strongly suggests that these targets represent drug intervention opportunities. Here we wish to present the synthesis and biological activity of a series of dual EGFR and ErbB-2 tyrosine kinase inhibitors, 3,3′-bis(coumarinsulfonyl) methanes. The in vitro EGFR and ErbB-2 enzyme inhibition assay, tumor cell cytotoxic activity and structure-activity relationship will be discussed.



117.

SYNTHESIS OF 5-[5-[^{18}F]FLUORO-2-OXO-1,2-DIHYDROINDOL-(3*Z*)-YLIDENEMETHYL]-2,4-DIMETHYL-1*H*-PYRROLE-3-CARBOXYLIC ACID (2-DIETHYLAMINOETHYL)AMIDE AS A NEW POTENTIAL PET TRACER FOR IMAGING CANCER TYROSINE KINASE. *Ji-Quan Wang, **Mingzhang Gao**, and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, Room L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, jiqwang@iupui.edu, migao@iupui.edu*

Vascular endothelial growth factor (VEGF) and plated-derived growth factor (PDGF) receptors have been well validated as targets for the treatment of cancers because of their critical roles in tumor growth and survival via autocrine and paracrine loops. In this regard, both receptor tyrosine kinases (RTKs) have been found to be expressed on the tumor celss and to directly affect tumor cell proliferation. *In vivo* biomedical imaging technique positron emission tomography (PET) coupled with appropriate radiopharmaceuticals has become a clinically valuable and accepted diagnostic tool to image cancer diseases. The tyrosine kinase inhibitor radiotracer 5-[5-[^{18}F]fluoro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide ([^{18}F]SU11248) was synthesized for evaluation as a new potential PET imaging agent for cancer RTKs. The [^{18}F]SU11248 was prepared by [^{18}F]fluorination of the nitro-precursor 5-[5-nitro-2-oxo-1,2-dihydroindol-(3*Z*)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-diethylaminoethyl-)amide with K^{18}F/Kryptofix$_{2.2.2}$ through nucleophilic substitution and purification with the HPLC method.

118.

DIHYDROPYRROLO PYRAZOLE TGF-BETA RI INHIBITORS: A NOVEL BENZIMIDAZOLE SERIES AND THEIR SELECTIVITY VERSUS TGF-BETA R II AND MLK7. ***Yan Wang**[1], hongyu li[1], Sreenivasa R Mundla[2], Lei Yan[3], Robert M Campbell[4], Bryan D. Anderson[4], Jill R. Wagner[4], and Jonathan M. Yingling Yingling[3]. (1) Department of discoversy chemistry research, Eli lilly & company, Lilly Research Laboratory, A Division of Eli Lilly and Company, Lilly Corporate Center, indianapolis, IN 46285, wang_yan@lilly.com, (2) Department of Process Chemistry Research, Eli Lilly & company, (3) Department of Cancer Research, Eli Lilly & Company, (4) Department of lead optimization Biology, Eli Lilly & company*

The multifunctional cytokine transforming growth factor- β (TGF- β) is a member of a large family of growth factors involved in the regulation of a diverse array of biological processes. As a result, the TGF- β signaling pathway may play a role in a number of disease states such as angiogenesis, cancer, and inflammation. Recently, we have reported the amino-quinoline as the "warhead" portion of the pharmacophore of the TGF- β kinase binding site (H. Li et al, Bioorg. Med. Chem. Lett., 2004, 14, 3585). During our continuing efforts of examining other heterocyclic warheads we have discovered that incorporation of benzimidazole provides a series of novel, potent, and selective

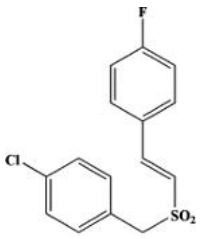
TGF-*f*'RI inhibitors. In this presentation, their synthesis, TGF- βRI enzyme, TGF-βRII enzyme, MLK7 enzyme, and TGF- βRI-dependent cellular activity will be discussed. In addition, selectivity data versus TGF- βRII and MLK7 will be presented.

119.

DOWN REGULATION OF ERK 1/2 AND STAT 3/5 PHOSPHORYLATION IN TUMOR CELLS BY NOVEL STYRYL BENZYLSULFONES. ***M.V. Ramana Reddy***[1], *Muralidhar R Mallireddigari*[1], *Venkat R Pallela*[1], *Stephen C Cosenza*[1], *Kimberly A Robell*[1], *Kiranmai Gumireddy*[1], *Stanley C Bell*[2], *and E. Premkumar Reddy*[1]. *(1) Fels Institute for Cancer Research, Temple University School of Medicine, 3307, North Broad Street, Philadelphia, PA 19140, Fax: 215-707-1454, rreddy@temple.edu, (2) Department of Medicinal Chemistry, Onconova Therapeutics Inc*

Extra cellular signals received at transmembrane receptors are relayed into cells by the signal transduction pathways that have been implicated in the induction of cell proliferation, differentiation and apoptosis. The mitogen activated protein kinases (MAPK) and signal transducer and activator of transcription (STAT) proteins are important mediators of major signaling pathways and many steps in these systems are well conserved. The best-studied members are ERK1/2 and JNK1/2 in MAPK family and STAT3 and STAT5 in cytokine activated family of proteins. Constitutively activated forms of ERK1/2, STAT3 and STAT5 have been noted in a proportion of breast cancers and other malignancies.

In an attempt to identify potent inhibitors of MAPKs and STATs, a series of novel styryl benzyl sulfones were synthesized and evaluated their activity by in vitro kinase inhibition and in vivo tumor cell cytotoxicity assay. This led to the identification of ON 01060, as a potent inhibitor of ERK1/2, STAT3 and STAT5 phosphorylation. Further modification of ON 01060 led to analogs with enhanced potency. The stereospecific synthesis, structure-activity relationships and biological activity of this series of compounds will be discussed.



ON 01060

120.

SAR OF A SERIES OF 3-[6-(4-SUBSTITUTED-PIPERAZIN-1-YL)-4-METHYL-1H-BENZIMIDAZOL-2-YL]-1H-PYRIDIN-2-ONE INHIBITORS OF THE IGF-1 RECEPTOR KINASE WITH *IN VIVO* ANTITUMOR ACTIVITY. ***Mark G. Saulnier***[1], *Upender Velaparthi*[1], *David B. Frennesson*[1], *Peiying Liu*[1], *Kurt Zimmermann*[1], *Xiaopeng Sang*[1], *Jeffrey T. Eummer*[1], *Karen Bedingfield*[1], *Francis Y. Lee*[2], *Joan Carboni*[2], *Dolatrai M. Vyas*[1], *Paul Haluska Jr.*[3], *David A Loegering*[4], *Scott H. Kaufmann*[4], *Charles Erlichman*[3], *and Mark D. Wittman*[1]. *(1) Discovery Chemistry, Bristol Myers Squibb Co, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, Fax: 203-677-7702, mark.saulnier@bms.com, (2) Oncology Drug Discovery, Bristol Myers Squibb Co, Pharmaceutical Research Institute, (3) Division of Medical Oncology, Mayo Clinic College of Medicine, (4) Oncology Research, Mayo Clinic College of Medicine*

Signaling through the insulin-like growth factor I receptor (IGF-1R) pathway mediates events which promote the malignant phenotype such as mitogenesis (via stimulation of signaling cascades through RAS/Raf/MAP kinase) and cell survival (anti-apoptosis via activation of the IRS-1/PI-3 kinase pathway). The genesis of the signal is the activation of IGF-1R via extracellular ligand (IGF) binding to the alpha subunit which likely initiates conformational changes that propagate through the transmembrane domain. Such events then promote ATP binding in the cytoplasmic tyrosine kinase domain resulting in trans-beta subunit autophosphorylation. Epidemiological studies have shown that elevated IGF-I levels correlate with increased risk of developing colon, breast, prostate, and lung tumors.

We have identified a series of 3-[6-(4-substituted-piperazin-1-yl)-4-methyl-1H-benzimidazol-2-yl]-1H-pyridin-2-ones from which several analogs emerged as a novel IGF-1R kinase inhibitors with in vivo antitumor activity in an IGF-1R-dependent tumor model (IGF-1R Sal). Drug "developability parameters" of the lead analogs within this series and related SAR will also be presented.

121.

DEVELOPMENT OF 3-(1H-INDOL-2-YL)-1H-INDAZOLE AS NOVEL KDR INHIBITORS. ***Barbara A. Hanney***, *Medicinal Chemistry Department, Merck & Co., Inc, 770 Sumneytown Pike, P.O. Box 4, West Point, PA 19486-0004, Fax: 215-652-7310, Yuntae Kim, Department of Medicinal Chemistry, Merck & Co., Inc, and George Hartman, Merck Research Laboratories*

Angiogenesis is the sprouting of new blood vessels and is required for solid tumor growth. Vascular endothelial growth factor (VEGF) is a key promoter of tumor-induced angiogenesis. Our research has focused on the kinase insert domain (KDR) receptor tyrosine kinase, the primary receptor through which VEGF exerts its biological effects. We describe our SAR studies towards identifying potent KDR kinase inhibitors from the novel 3-(1H-indol-2-yl)-1H-indazole series as well as the synthesis of these compounds.

122.

SUCCESSFUL TREATMENT OF DIABETIC PERIPHERAL FACIAL PARALYSIS BY INJECTION OF BATROXOBIN: A REPORT OF FOUR CASES. ***Qun Liu***, *Jia Fan, Hanqiu Jiang, and Jin Liu, Department of Neurology, The First Hospital of Jilin University, No.1 Xinmin Street, Changchun, Jilin, P.R. China 130021, China, Fax: 011-86-431-8526281, hanqiu_j@email.jlu.edu.cn*

Batroxobin is the single component of Serine Protease induced by bioengineering technique. It will evoke endothelial cell releasing tissue type plasminogen activator(t-PA) which can active plasminogen into plasmin thus thrombolysis realization. At the same time, Batroxobin can decrease the viscosity of the blood, inhibit the conglomeration and increase the disfiguration ability of the erythrocyte. So microcirculation can be ameliorated and the conduction ability of the nervous recoveried. The mechanism of diabetic peripheral neuropathy include proliferation of vascular endothelial cells, hyaline degeneration, constriction of the blood vessel and so on. All microvessel pathological changes may result in hypoperfusion in peripheral nerve, thus it is necessary to improve microcirculation effectively. According to the above, we have applied Batroxobin in four diabetic peripheral facial paralysis patients, the effect is significant. This shows that Batroxobin can facilitate blood circulation of the facial nerve by ameliorating microcirculation. It can also provide us a better prospect in treatment of diabetic peripheral neuropathy.

123.

DISCOVERY OF POTENT AND SPECIFIC FLT3 KINASE INHIBITORS. *Hitesh K. Patel, Zdravko V. Milanov,* ***Shamal A Mehta***, *Robert M. Grotzfeld, Andiliy G. Lai, Miles A. Fabian, Todd A. Carter, Philip T. Edeen, Anne Marie Velasco, Julia M. Ford, Pietro Ciceri, Darren E. Insko, Mark Floyd, Sanna Herrgard, Corey E. Atteridge, Lisa M. Wodicka, William H. Biggs III, Daniel K. Treiber, Patrick P. Zarrinkar, and David J. Lockhart, Ambit Biosciences Corp, 4215 Sorrento Valley Blvd, San Diego, CA 92121, Fax: 858-334-2199, smehta@ambitbio.com*

Acute myeloid leukemia (AML) is the most common variant of acute leukemia in adults. Patients with AML usually are seen with complications resulting from a decrease in the production of all types of blood cells. While chemotherapy can result in complete remissions, the long term disease-free survival rate for AML is about 19%, with about 7,400 deaths from AML each year in the US. The single most commonly mutated gene in AML is FLT3 kinase. Two classes of FLT3 activating mutations have been identified in blasts from AML patients: (A) internal tandem duplication (ITD) mutations in the juxtamembrane (JM) domain coding sequence (25-30% of patients), and (B) point mutations in the kinase

domain activation loop (7-8% of patients). Activating mutations in FLT3 gene are found in 30-40% of AML patients and are associated with a poor prognosis. Therefore, the selective inhibition of FLT3 kinase offers a promising therapeutic approach for AML. Ambit Biosciences has developed a class of highly specific, high-affinity FLT3 inhibitors.

124.

STRUCTURE-ACTIVITY RELATIONSHIP OF C4-SUBSTITUTED PYRIMIDOPYRIMIDINES; DUAL KDR/FGFR TYROSINE KINASE INHIBITORS. ***P. Rossman***[1], *K. Luk*[1], *Y. Chen*[1], *L. Garafalo*[2], *B. Graves*[1], *N. Jackson*[1], *M. Kabat*[2], *F. Konzelmann*[1], *J.-J. Liu*[1], *C. Lukacs*[1], *L. McDermott*[1], *C. Michoud*[1], *L. Portland*[1], *J. Roberts*[1], *A. Schutt*[3], *M. Simcox*[3], *S.-S. So*[1], *B. Tamborini*[3], *and H. Yang*[3]. *(1) Discovery Chemistry, Hoffmann-La Roche Inc, 340 Kingsland Street, Nutley, NJ 07110, Fax: 973-235-7239, pamela.rossman@roche.com, (2) Chemical Synthesis, Hoffmann-La Roche Inc, (3) Discovery Oncology, Hoffmann-La Roche Inc*

The pyrimidopyrimidine moiety represents a core structure that is a useful template for the design of a variety of tyrosine kinase inhibitors. From high throughput screening, a pyrimidopyrimidine analog was identified as a dual inhibitor of the growth factor receptors KDR and FGFR-1. The crystal structure of the src-family tyrosine kinase LCK with a closely related analog bound was determined, elucidating the binding mode of the pyrimidopyrimidines. Modeling of the pyrimidopyrimidine into the ATP binding pocket of KDR led to a simplified binding model which guided the investigation of the structure activity relationships at three positions (N1, N3 and C7). Modeling also revealed an additional small pocket accessible from C4 of the pyrimidopyrimidine core. A series of analogs were synthesized to study the structure activity relationship of substituents at this site. The size limitation of the pocket as well as the required configuration of the substituent at C4, as defined by activity in the in vitro kinase assays and in the growth-factor stimulated HUVEC proliferation assays, will be presented.

125.

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRAZINE-PYRIDINE BIHETEROARYLS AS NOVEL, POTENT AND SELECTIVE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 (VEGFR-2) INHIBITORS. *Gee Hong Kuo*[1], ***Catherine Prouty***[2], *Aihua Wang*[1], *Stuart Emanuel*[2], *Alan DeAngelis*[2], *Yan Zhang*[2], *Fengbin Song*[2], *Peter J. Connolly*[2], *P Karnachi*[3], *Xin Chen*[2], *Robert H. Gruninger*[1], *Jan Sechler*[1], *Fuentes-Pesquera Angel*[2], *Steven A. Middleton*[2], *Linda Jolliffe*[2], *and William V. Murray*[2]. *(1) Drug Discovery Division, Johnson and Johnson Pharmaceutical Research, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, Fax: 908-526-6469, gkuo@prius.jnj.com, (2) Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, LLC, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, Fax: 908 203-8109, cprouty@prdus.jnj.com, (3) J&J Pharmaceutical Research Development*

Pathological angiogenesis is associated with disease states such as cancer, diabetic retinopathy, rheumatoid arthritis, endometriosis and psoriasis. There is much evidence that direct inhibition of the kinase activity of VEGFR-2 will result in the reduction of angiogenesis and the suppression of tumor growth. Palladium-catalyzed C-C bond, C-N bond formation reactions were used to assemble various pyrazine-pyridine biheteroaryls as potent VEGFR-2 inhibitors. Among them, compounds 39 and 41 exhibited the highest kinase selectivity while compound 7 and compounds 56-58 displayed modest selectivity against FGFR-2, PDGF-R and GSK-3. All of these compounds showed excellent cellular potency to inhibit VEGF-stimulated proliferation of HUVEC. Meanwhile, they all displayed modest effects on the un-stimulated growth of HUVEC and very minimal inhibition of proliferation of two normal human cell types, HASMC and MRC5. The low inhibition of these compounds to the growth of tumor cell lines, such as HeLa, HCT-116 and A375 further confirms that these VEGFR-2 inhibitors are not cytotoxic agents. The in vivo antitumor activity of compound 41 was demonstrated in the A375 human melanoma xenograft nude mice model. The high VEGFR-2 inhibitory potency, good kinase selectivity profile, high cellular potency and selectivity, demonstrated antitumor activity, may render these compounds as valuable pharmacological tools in elucidating the complex roles of VEGF signaling pathways and the potential utility for anti-angiogenesis therapy.

126.

PROTEIN-LIGAND BINDING FREE ENERGY ESTIMATION USING LINEAR INTERACTION ENERGY METHOD: APPLICATION TO GRB2 SH2 DOMAIN BINDING LIGANDS. ***Rajeshri Karki***[1], *Shinya Oishi*[1], *Zhen-Dan Shi*[1], *Sang-Uk Kang*[1], *Kyeong Lee*[1], *Chang-Qing Wei*[1], *Karen M. Worthy*[2], *Lakshman Bindu*[2], *Robert J. Fisher*[2], *Terrence R. Burke Jr.*[1], *and Marc C. Nicklaus*[1]. *(1) Laboratory of Medicinal Chemistry, National Cancer Institute, National Institute of Health, Frederick, MD 21702, Fax: 301-846-6033, rajeshri@helix.nih.gov, (2) Protein Chemistry Laboratory, SAIC-Frederick*

The growth factor receptor bound protein 2 (Grb2) is an SH2 domain-containing non-catalytic module that provides important connectivity in protein-tyrosine kinase (PTK)-dependent signaling associated with a variety of cancers. Accordingly, significant effort has been expended in developing Grb2 SH2 domain-binding antagonists as potential therapeutics. Based on the preferred binding of Grb2 SH2 domains to phosphotyrosyl (pTyr)-containing sites of the sequence "pY-X-N', we have earlier synthesized and reported numerous ligands with high Grb2 SH2 domain- binding affinity. In an attempt to understand structure-activity relationships of the synthetic ligands and to derive a predictive binding model, we have made use of a linear interaction energy approach as implemented in the LIAISON program from Schrödinger, Inc. A model scoring function was built correlating the experimental binding affinity of a series of Grb2 SH2 binding ligands to calculated protein-ligand binding free energies. This model has been used for predicting *a priori* the binding affinity of newly designed ligands.

127.

SYNTHESIS AND SAR OF 3-(QUINOLIN-2-YL)INDOLIN-2-ONES AS KINASE INHIBITORS: CRYSTALLOGRAPHIC EVIDENCE FOR AN UNIQUE BINDING CONFORMATION. ***Anthony R. Gangloff***[1], *Kelie Williams*[1], *Bheema R. Paraselli*[1], *Hasanthi Wijesekera*[1], *Jerome C. Bressi*[1], *Jason W. Brown*[1], *Phong H. Vu*[1], *Andy J. Jennings*[1], *Michael Tennant*[2], *Jacek Nowakowski*[3], *Daniel Vaughn*[4], *Christopher Caster*[4], *and Jeffrey A. Stafford*[1]. *(1) Department of Chemistry, Syrrx, Inc, 10410 Science Center Drive, San Diego, CA 92121, Fax: 858-550-0526, agangloff@syrrx.com, (2) Department of Computational Sciences, Syrrx, Inc, (3) Department of Structural Chemistry, Syrrx, Inc, (4) Department of Leads Discovery, Syrrx, Inc*

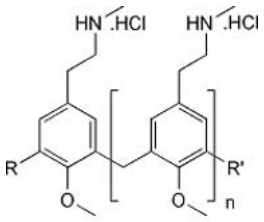
Kinases are a large family of enzymes that transfer phosphorous-containing groups from one substrate to another. Kinase proteins are important drug targets for treating or modulating diseases, including human cancer, inflammation and metabolic diseases . We have prepared a series of substituted 3-(quinolin-2-yl)indolin-2-ones which demonstrate nanomolar activity against a variety of kinases including AIK, cKIT and FAK. Using Syrrx's state-of-the-art Nanovolume Crystallization® technology, a unique binding conformation was elucidated involving the enolic form of the inhibitor, allowing for optimal interaction with the kinase hinge region. Characterization of the keto-enol tautomerism was supplied by 1H NMR experiments and multiple co-crystal structures. Structure-based drug design was used to optimize the indolinones for enzymatic potency. The indolinones were prepared by condensation of quinoline N-oxide with substituted indolinones. The resulting structure-activity relationships will be discussed, including enzymatic and cellular activity data.

128.

SYNTHETIC APPROACH TO COMPOUND 48/80 AND ITS ANALOGUES. ***Macba G. Numbere***[1], *Helen C. Hailes*[1], *Erika Rosivatz*[2], *Richard Byrne*[2], *and Rudiger Woscholski*[2]. *(1) Department of Chemistry, University College London, Christopher Ingold Building, 20 Gordon Street, London WC1H OAJ, United Kingdom, mnumbere@ucl.ac.uk, (2) Department of Biological Sciences, Imperial College*

Phosphoinositide-3-kinase (PI-3K) is of utmost importance for cellular function, and human diseases such as diabetes or cancer. It catalyses the phosphorylation of phosphoinositides, which act as secondary messengers to regulate the location and activity of an array of downstream effector molecules. Compound 48/80 (C48/80) is a polymeric mixture, which has been found to activate PI-3K. It is comprised of a number of linear p-methoxyphenethylamine oligomers of different chain lengths. We are currently identifying and synthesising C48/80 oligomers (1) and its analogues via solution and solid phase chemistry. The initial phase was to develop the methodology for the synthesis of ether-based oligomers using suitable monomeric units. After testing the methodology in

solution, the use of a solid support was investigated. The synthesis of these ether-based oligomers, is a preliminary phase to the synthesis of 1. Alternatively, we have investigated the use of the Suzuki coupling methodology to incorporate the methylene linker.

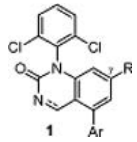


1 R = R' = H or CH_2OH

129.

C7-SUBSTITUTED QUINAZOLINONE AND DIHYDROQUINAZOLINONE INHIBITORS OF P38 MAP KINASE. ***Jianming Bao***[1], *Shouwu Miao*[1], *Kathleen M. Rupprech*[1], *James V. Pivnichny*[1], *Dennis M. Zaller*[2], *Wesley L. Shoop*[2], *Edward A. O'Neill*[2], *Stephen J. O'Keefe*[2], *Chris M. Thompson*[2], *Rose M. Cubbon*[3], *Ruixiu Wang*[3], *Wen Xiao Zhang*[3], *James E. Thompson*[3], *and James B. Doherty*[1]. *(1) Medicinal Chemistry, Merck & Co., Inc, PO Box 2000, Rahway, NJ 07065, Fax: 732-594-5350, bao_jianming@merck.com, (2) Departments of Medicinal Chemistry and Inflammation/Rheumatology, Merck and Co., Inc, (3) Departments of Medicinal Chemistry, and Inflammation/Rheumatology, and Immunology, Merck & Co., Inc*

The p38 mitogen-activated protein (MAP) kinase plays a key role in the release of proinflammatory cytokines TNF-α and IL-1β from monocytes. An excess level of these cytokines is associated with a number of inflammatory diseases, including rheumatoid arthritis (RA). Potent and selective p38 inhibitors may serve as potential therapy for treatment of RA. A series of C7-substituted quinazolinones and dihydroquinazolinones (1) are synthesized. They are highly potent inhibitors of p38 MAP kinase activity and of TNF-α*f*n release in human whole blood. The structure-activity relationship and the inhibitory activities of these compounds for p38 MAP kinase and TNF-α release in human whole blood will be presented.



130.

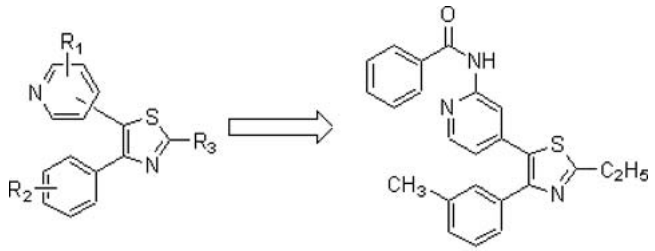
HIGHLY SELECTIVE TRIAZOLE-BASED INHIBITORS OF P38-ALPHA MAP KINASE: PROGRESS TOWARDS A CLINICAL CANDIDATE. ***Ioana Popa-Burke***, *Lynn Cheatham, John Dickson, Jennifer Clark, Scott Galasinski, Ajit Jadhav, William P. Janzen, Jose Mendoza, Jennifer L. Miller, Robert P. Mohney, Jacqueline L. Norris, Paul steed, gretchen van de carr, Kevin Williams, and C. Nicholas Hodge, Amphora Discovery Corp, 800 Capitola Drive, Durham, NC 27713, Fax: 919-806-3477, Ioana.Popa-Burke@amphoracorp.com*

p38 inhibitors have recently entered clinical development for various inflammatory disorders, but the drug candidates are not reported to be highly selective for the alpha-isozyme of p38, and several of them have encountered safety problems in preclinical and/or clinical studies. Our program is focused on identifying highly alpha-selective inhibitors with properties suitable for rapid entry into clinical studies. As described previously (ACS abstract #758048, August 2004), using our method of broad, accurate screening, substituted 3-alkylthio-1,2,4-triazoles were identified as selective p38-alpha inhibitors. Subsequent analoging resulted in highly potent mixed ATP-competitive p38-alpha inhibitors (Ki = 14 nM) with >475-fold selectivity for all three known p38 isozymes (beta, delta and gamma), as well as for more than sixty other kinases, proteases and phosphatases. We report here on the structure-activity relationships for p38-alpha activity and p38-beta selectivity of our lead series. We also describe the effects of these selective inhibitors in cellular assays (TNF-alpha release in THP-1 cells and human whole blood; measurement of MAPKAPK2 phosphorylation by p38-alpha in HeLa cells), and in an animal efficacy model (murine LPS-induced TNF-alpha production).

131.

NOVEL INHIBITOR OF P38 MAP KINASE AS AN ANTI-TNF-&ALPHA DRUG: DISCOVERY OF *N*-[4-[2-ETHYL-4-(3-METHYLPHENYL)-1,3-THIAZOL-5-YL]-2-PYRIDYL]BENZAMIDE (TAK-715) AS A POTENT AND ORALLY ACTIVE ANTI-RHEUMATIC AGENT. ***Seiji Miwatashi***, *Yasuyoshi Arikawa, Etsuo Kotani, Maki Miyamoto, Ken-ich Naruo, Hiroyuki Kimura, Toshimasa Tanaka, Satoru Asahi, and Shigenori Ohkawa, Pharmaceutical Research Division, Takeda Pharmaceutical Company, LTD, 17-85 Jusohonmachi 2-chome, Yodogawaku, Osaka 532-8686, Japan, Fax: +81-6-6300-6306, Miwatashi_Seiji@takeda.co.jp*

The p38 mitogen-activated protein kinase (MAPK) regulates the release of IL-1 and TNF-α and its inhibitors are potentially useful for the treatment of chronic inflammatory diseases such as rheumatoid arthritis. An intensive program of research exploring a new lead scaffold led to the discovery of 4-phenyl-5-pyridyl-1,3-thiazoles that showed p38 MAPK inhibitory activity. However these compounds were then found to display inhibitory effects on human cytochrome P450 (CYP) enzymes, due to interaction of the pyridyl nitrogen atom with heme iron. Modeling studies suggested that the unfavorable interaction might be overcome by introducing an appropriate substituent at the 2-position of the pyridyl moiety. Among the compounds synthesized, the most promising candidate, TAK-715, demonstrated significant *in vitro* and *in vivo* activities and was advanced into phase II clinical trials. The rational analogue design, synthesis and structure-activity relationship of this series will be discussed.

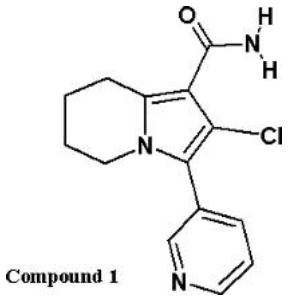


132.

DISCOVERY OF PYRROLO[1,2-A]PYRAZINE ANALOGUES AS JNK INHIBITORS. ***Sukanthini Thurairatnam***[1], *Chris Adams*[2], *David J Aldous*[1], *Shelley Amendola*[1], *Devnandan Chatterjee*[2], *Nick Hopkins*[2], *Sue King*[2], *Jean-Philippe Letallec*[1], *Tahir Majid*[1], *Neil Moorcroft*[1], *Andrew Ratcliffe*[2], *Robert Petheram*[2], *John Souness*[3], *Andreas Timm*[1], *Rachel Walsh*[2], *and Roger Walsh*[2]. *(1) Medicinal Chemistry, Sanofi-aventis, Route 202-206, PO Box 6800, Bridgewater, NJ 08807-0800, Fax: 908-231-2202, sukanthini.thurairatnam@aventis.com, (2) NA, (3) RA Pharmacology, Sanofi-aventis, Route 202-206, PO Box 6800, Bridgewater, NJ 08807-0800*

Jun N terminal Kinase (JNK) is a Serine-Threonine protein kinase that phosphorylates c-jun. In addition to inflammatory agents such as IL-1 and TNF-α, a number of cellular stresses (heat, U.V. light, osmotic stress etc) activate JNK, hence the alternative name of this enzyme family, Stress-Activated Protein Kinase (SAPK). JNK is phosphorylated and activated following the triggering of a kinase cascade in which the final step is catalyzed by upstream MAP Kinase Kinases (MKK)4 and/or 7. Activated JNK migrates to the nucleus where it phosphorylates c-Jun, a component of the transcription factor activator protein-1 (AP-1). AP-1 regulates the transcription of many genes including cytokines, growth factors, immunoglobins, inflammatory enzymes and matrix metalloproteinases (eg. MMP13). The stimuli that increase c-Jun phosphorylation cause an increase in the transcriptional activity of AP-1, leading to the expression of the inflammatory genes (COX-2, IL-2, IFN-γ, TNF-α). Thus, inhibition of JNK activity and suppression of cJun phosphorylation is a very attractive target to inhibit release of, and responses to, pro-inflammatory cytokines and production of metalloproteinases. Compounds which inhibit JNK will be useful for the treatment of inflammatory diseases in which TNF plays a major pathological role as well as diseases in which tissue destruction occurs as a consequence of excessive metalloproteinase release and apoptosis.

Screening the internal compound collection identified the Indolizine, Compound1 as a JNK inhibitor. Further optimization led to the identification of a novel class of Pyrrolo[1,2-a]pyrazine analogues as potent and selective JNK inhibitors, which could be used as in vivo candidates for target validation. The rationale behind their discovery, SAR for JNK activity, cellular activity, pharmacokinetic profile and chemical synthesis will be presented.



Compound 1

133.

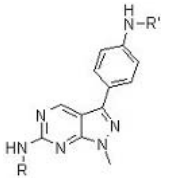
DEVELOPMENT OF POTENT INHIBITORS FOR IMATINIB (GLEEVEC) RESISTANT ABL AND KIT KINASE MUTANTS. ***Robert M. Grotzfeld****, Hitesh K. Patel, Shamal A Mehta, Zdravko V. Milanov, Andiliy G. Lai, Miles A. Fabian, Todd A. Carter, Philip T. Edeen, Anne Marie Velasco, Julia M. Ford, Mark Floyd, Pietro Ciceri, Darren E. Insko, Sanna Herrgard, Corey E. Atteridge, Lisa M. Wodicka, William H. Biggs III, Daniel K. Treiber, Patrick P. Zarrinkar, and David J. Lockhart, Ambit Biosciences Corp, 4215 Sorrento Valley Boulevard, San Diego, CA 92121, Fax: 858-334-2199, rgrotzfeld@ambitbio.com*

The fusion protein $p210^{BCR\text{-}ABL}$ is expressed in hematopoietic stem cells in 95% of chronic myelogenous leukemia (CML) patients. The leukemogenic potential of this oncoprotein is due to the constitutive activation of ABL protein kinase by the juxtaposition of BCR sequences resulting in deregulated cellular proliferation, adherence, and apoptosis. Imatinib (Gleevec, STI-571), an inhibitor of ABL, PDGFR, and KIT, induces apoptosis in CML and has been the treatment of choice since its launch in 2001. Over time, drug resistance develops in the majority of patients treated with Imatinib, with a Threonine to Isoleucine change at position 315 (T315I) being the most common Imatinib resistant mutation. To date, no high affinity inhibitors of this particular ABL mutant have been reported. Ambit Biosciences has developed a small molecule compound that inhibits this and other Imatinib resistant mutations of ABL and KIT with high affinity.

134.

DESIGN AND SYNTHESIS OF AMINO-1H-PYRAZOLO[3,4-D]PYRIMIDINES AS NOVEL AND SELECTIVE INHIBITORS OF TIE2 KINASE. ***Jiri Kasparec****, Neil W. Johnson, Cathy Yuan, Jeffrey H. Murray, and Jerry L. Adams, Medicinal Chemistry, GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, jiri_2_kasparec@gsk.com*

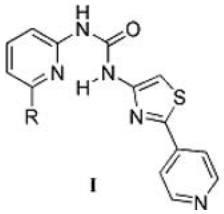
The Tie family of tyrosine kinase receptors are expressed predominantly in endothelial cells and are essential for vessel formation, where they are required for the later stages of angiogenesis and vessel maintenance. Because solid tumor growth requires vascularization in order to maintain and supply essential nutrients, angiogenesis plays an essential role in the pathogenesis of malignant tumors. Therefore, small molecule inhibitors of Tie-2 kinase are attractive for evaluation as cancer therapeutic agents. In an effort to develop a novel chemical series, we designed the 6-amino-1H-pyrazolo[3,4-d]pyrimidine template as a new scaffold for Tie-2 inhibition. This poster details the SAR of this series which led to the discovery of a potent and selective Tie-2 inhibitor. Compounds from this series showed efficacy in an in vivo model of angiogenesis.



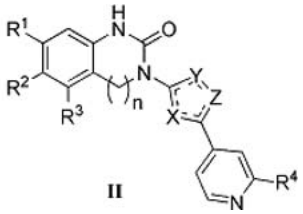
135.

DESIGN AND SYNTHESIS OF 3,4-DIHYDROQUINAZOLIN-2(1H)-ONES AS CDK5 INHIBITORS. ***Robert M. Rzasa***[1]*, Matthew R. Kaller*[1]*, Ella Magal*[2]*, Gang Liu*[1]*, Thomas T. Nguyen*[1]*, Timothy Osslund*[3]*, David Powers*[4]*, Hui-Ling Wang*[1]*, Xiaoling Xiong*[4]*, Jiandong Zhang*[3]*, Wenge Zhong*[1]*, and Mark H. Norman*[5]*. (1) Department of Chemistry Research & Discovery, Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, (2) Department of Neuroscience, Amgen Inc, (3) Department of Molecular Structure, Amgen Inc, (4) Department of HTS and Molecular Pharmacology, Amgen Inc, (5) Department of Chemistry Research & Discovery, Amgen, Inc*

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that plays a critical role in the early development of the nervous system. Deregulation of CDK5 is believed to contribute to the abnormal phosphorylation of various cellular substrates associated with neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis and ischemic stroke. Our objective is to identify soluble CDK5 inhibitors as therapeutic agents for the treatment of stroke. Previously we identified a series of 2-pyridyl ureas I, containing an intramolecular hydrogen bond, as potent CDK5 inhibitors. In this poster we present our synthetic studies toward a novel series of 3,4-dihydro-quinazolin-2(1H)-ones II and discuss their biological relevance as CDK5 inhibitors.



I

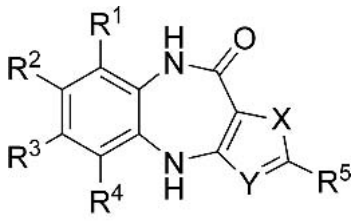


II

136.

DESIGN AND SYNTHESIS OF BENZODIAZEPINONE DERIVATIVES AS CDK5 INHIBITORS. ***Hui-Ling Wang***[1]*, Xiaoling Xiong*[2]*, David Powers*[2]*, Ella Magal*[3]*, and Mark H. Norman*[4]*. (1) Department of Chemistry Research & Discovery, Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, huiw@amgen.com, (2) Department of HTS and Molecular Pharmacology, Amgen Inc, (3) Department of Neuroscience, Amgen Inc, (4) Department of Chemistry Research & Discovery, Amgen, Inc*

The complex of cyclin-dependent kinase 5 (CDK5) and its neuronal activator p25 (an N-terminal truncated form of its precursor protein, p35) is a proline-directed Ser/Thr kinase that plays an important role in various neuronal functions. Recent findings indicate that CDK5 deregulation may be involved in neuronal death, characteristic of neurodegenerative diseases such as Alzheimer's and neurological injuries such as stroke. Our program goal is to discover small molecule CDK5 inhibitors as therapeutic agents for stroke. In this poster we present our synthetic studies toward a novel series of benzodiazepinone derivatives I and discuss their biological activity as CDK5 inhibitors.

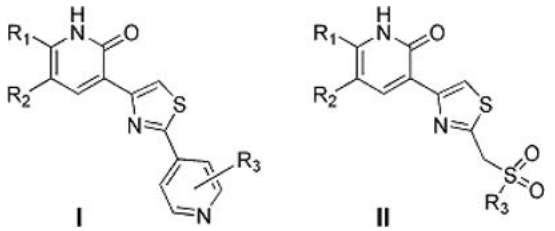


I

137.

DESIGN AND SYNTHESIS OF PYRIDONES AS CDK5 INHIBITORS. ***Matthew R. Kaller***[1], *Wenge Zhong*[1], *Thomas T. Nguyen*[1], *Robert M. Rzasa*[1], *Hui-Ling Wang*[1], *Mark H. Norman*[2], *Xiaoling Xiong*[3], *David Powers*[3], *Weiya Wang*[4], *Charles Henley*[4], *Ella Magal*[4], *Jiandong Zhang*[5], *and Timothy Osslund*[5]. *(1) Department of Chemistry Research & Discovery, Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-480-3016, mkaller@amgen.com, (2) Department of Chemistry Research & Discovery, Amgen, Inc, (3) Department of HTS and Molecular Pharmacology, Amgen Inc, (4) Department of Neuroscience, Amgen Inc, (5) Department of Molecular Structure, Amgen Inc*

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine protein kinase. It plays a significant role in neuronal development, however, when associated with cyclin p25, CDK5 abnormally phosphorylates a number of cellular targets including Tau and Rb. This pathway is suggested to be involved in neuronal apoptosis in the brain's penumbra, a continuing degenerative process after stroke attack and reperfusion injuries. Our goal is to develop soluble small molecule CDK5 inhibitors as therapeutic agents for treatment of stroke. Based on the high level of homology between CDK2 and CDK5, a structure based design approach was previously used to identify quinolin-2(1*H*)-one derivatives as potent CDK5 inhibitors. In this poster, the synthesis and structure-activity relationship of a pyridone series of inhibitors, **I** and **II**, will be presented. General properties of these compounds and important issues such as cellular activity and brain exposure will also be discussed.



138.

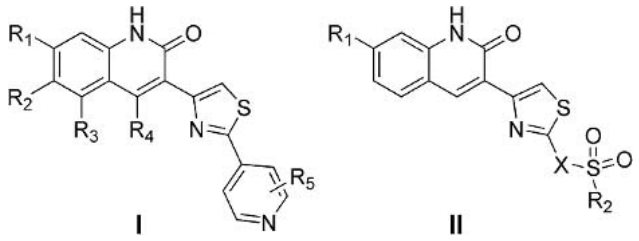
DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF AMINOTHIAZOLES AS SELECTIVE INHIBITORS OF CYCLIN-DEPENDENT KINASE 4 (CDK4). ***Allen Lovey***[1], *Wanda Depinto*[2], *Qingjie Ding*[1], *Nan Jiang*[1], *Kyungjin Kim*[1], *Xuefeng Yin*[2], *Xin-Jie Chu*[1], *Dave Bartkovitz*[1], *Bhupesh Desai*[2], *Melissa Smith*[2], *John Mullin*[1], *Warren Mccomas*[1], *Bradford Graves*[1], *Christine Lukacs*[1], *Sung-Sau So*[1], *Yingsi Chen*[3], *and Qing Xiang*[3]. *(1) Discovery Chemistry Department, Hoffmann-La Roche Inc, 340 Kingsland Street, Bldg. 123, Nutley, NJ 07110, Fax: 973-235-2448, allen.lovey@roche.com, (2) Pre-clinical Oncology, Hoffmann-La Roche Inc, (3) Discovery Technologies, Hoffmann-La Roche Inc*

The discovery of compounds that modulate selectivity toward a particular kinase family has been an ongoing activity in the search for less toxic drugs that can contribute toward cancer chemotherapy. We have investigated the so-called "Buried Region" of the ATP pocket of the cyclin dependent kinases (CDKs) using a diaminothiazole as a template that binds to the hinge region and varying substitutents to explore that region more thoroughly. The SAR of this region is limited due to the hydrophobic nature and restricted size of that portion of the kinase pocket. A series of compounds has been investigated that extends our knowledge of that region and led to a series of diaminothiazoles with low nanomolar activity toward CDK4 as well as up to 100-fold selectivity against CDK1, CDK2. Here in, we report the synthesis and biological activity of these compounds.

139.

QUINOLIN-2(1H)-ONE DERIVATIVES AS CDK5 INHIBITORS. ***Wenge Zhong***[1], *Hu Liu*[2], *Matthew R. Kaller*[1], *Thomas T. Nguyen*[1], *Robert M. Rzasa*[1], *Hui-Ling Wang*[1], *Mark H. Norman*[3], *Xiaoling Xiong*[4], *David Powers*[4], *Weiya Wang*[5], *Charles Henley*[5], *Ella Magal*[5], *Jiandong Zhang*[6], *and Timothy Osslund*[6]. *(1) Department of Chemistry Research & Discovery, Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA 91320, Fax: 805-480-3016, zwenge@amgen.com, (2) Department of Chemistry, ImClone Systems Inc, (3) Department of Chemistry Research & Discovery, Amgen, Inc, (4) Department of HTS and Molecular Pharmacology, Amgen Inc, (5) Department of Neuroscience, Amgen Inc, (6) Department of Molecular Structure, Amgen Inc*

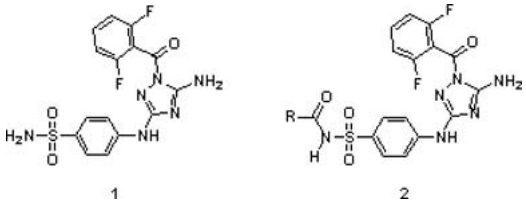
Cyclin-dependent kinase 5 (CDK5) is a serine/threonine protein kinase. Its deregulation is implicated in neuronal apoptosis in the brain's penumbra, a continuing degenerative process after stroke attack and reperfusion injuries. Our program goal is to discover soluble small molecule CDK5 inhibitors as therapeutic agents for treatment of stroke. We took advantage of the high level of active site homology between CDK2 and CDK5 and crystallographic data available for inhibitor-CDK2 complexes and explored a broad spectrum of inhibitors of different chemotypes using a structure-based drug design approach. We found that quinolin-2(1H)-one derivatives I and II are potent CDK5 inhibitors. In this poster, the synthesis and structure-activity relationship studies with the quinolinone series of inhibitors will be presented. General properties of these compounds and important issues such as cellular activity and brain exposure will also be discussed.



140.

SYNTHESIS OF N-ACYL SULFONAMIDES AS PRODRUGS OF A CYCLIN-DEPENDENT KINASE INHIBITOR. ***Shenlin Huang***, *Ronghui Lin, Peter J. Connolly, Stuart Emanuel, Steven A. Middleton, and Steven K. Wetter, Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development L.L.C, 1000 Route 202, Raritan, NJ 08869, Fax: 908-526-6469, shuang6@prdus.jnj.com*

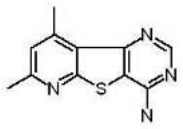
Cyclin-dependent kinases (CDKs) play a key role in regulating the cell cycle process. Deregulation of CDK function occurs with high frequency in many tumors. Small molecule inhibitors of the CDK family could serve as an efficient approach to cancer therapy. In the course of our CDK drug discovery program, compound 1 was discovered to be a potent CDK1 inhibitor. However, its poor aqueous solubility and low oral bioavailability in animals complicate its development as an anti-tumor agent. Efficient chemistry was developed to synthesize many N-acylsulfonamide prodrugs 2 in an attempt to improve these parameters. Several of these prodrugs exhibited improved solubility and were processed to the active form of the compound following intravenous administration to rats or primates.



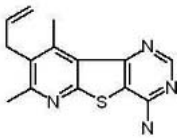
141.

SYNTHESIS AND EVALUATION OF PYRIDOTHIENOPYRRIMIDINE AS POTENT AND SELECTIVE CDC7 INHIBITOR. ***Chunlin Zhao***[1], *Li Chen*[1], *Qui Xu*[2], *Christian Tovar*[2], *and Lyubomir T. Vassilev*[2]. *(1) Discovery Chemistry, Roche Research Center, Hoffmann-La Roche, 340 kingsland street, Nutley, NJ 07110, chunlin.zhao@roche.com, (2) Oncolgy Department, Roche Research Center, Hoffmann-La Roche, Nutley, USA*

Cdc7 kinase has been shown to play a critical role in the initiation step of DNA replication in the cell cycle through phosphorylation of the MCM protein family members. Cdc7 is activated at the G1/S phase transition by forming a complex with the cyclin-like protein Dbf4. Inhibition of Cdc7 will block cell cycle progression and provide a novel approach to cancer therapy. Through HTS of large library, we identified a class Cdc7/Dbf4 inhibitor which contains pyridothienopyrrimidine (I) core structure. Focused library synthesis of pyridothienopyrrimidine leads analog II which is a potent and selective Cdc7 inhibitor with Ki of 2 nM. Microwave assisted library synthesis of Cdc2/Dbf4 inhibitors and their SAR study will be presented.



I

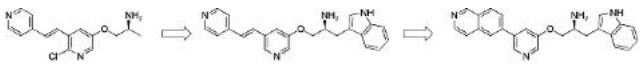


II

142.

PROTEIN KINASE B/AKT ANTAGONISTS AS ANTITUMOR AGENTS PART 1: DISCOVERY OF NOVEL, POTENT AND HIGHLY SELECTIVE PYRIDINE-ISOQUINOLINE AKT INHIBITORS. ***Jianchun Gong***[1], *Keith W. Woods*[1], *Tongmei Li*[1], *John Fisher*[1], *Garrick Packard*[1], *Viraj B. Gandhi*[1], *Akiyo Claibone*[1], *Yan Luo*[1], *Yan Shi*[1], *Xuesong Liu*[1], *Vered Klinghofer*[1], *Jennifer Bouska*[1], *Alexander Shoemaker*[1], *Anatol Oleksijew*[1], *Ken Jarvis*[1], *Vincent S. Stoll*[2], *Charles Hutchins*[2], *Ron De Jong*[3], *Tilman Oltersdorf*[3], *Qun Li*[1], *Saul H. Rosenberg*[1], *Vincent L. Giranda*[1], *and Gui-Dong Zhu*[1]. *(1) Cancer Research, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064, (2) Structural Biology, Abbott Laboratories, (3) IDUN Pharmaceuticals Inc*

Protein kinase Bs/Akts are key elements in a number of signal transduction pathways critical for cellular transformation and tumor progression. Constitutive activation of Akt has been observed in a large proportion of human malignancies and is a direct result of inactivating mutations of the *PTEN* tumor suppressor gene. Inhibition of the enhanced Akt activity would induce apoptosis and suppress the tumor progression. Here we present the development of a novel series of small molecule inhibitors of the Akt kinase. High throughput screening of the Abbott compound collection identified a pyridine-stilbene lead **1** as a micromolar inhibitor of Akt. Computer modeling of **1** bound to the structurally related protein kinase A (PKA) suggested a possible hydrogen bond between the central pyridyl nitrogen and L-72 and a critical interaction between the terminal pyridyl nitrogen and the backbone of V-123. Initial optimization directed toward enhancing hydrophobic interactions with the glycine rich loop led to the double-digit nanomolar inhibitor **2**. Conformational restriction that locked the two pyridines of **2** into different relative orientations provided the first low nanomolar Akt inhibitor **3** (IC_{50}= 1.5 nM). Compound **3** is a reversible, ATP competitive inhibitor that is highly selective for Akt over more than 20 protein kinases screened to date. Compound **3** causes dose-dependent inhibition of the phosphorylation of several downstream targets of Akt such as GSK3. Despite a relatively poor pharmacokinetic profile, compound **3** has shown statistically significant tumor growth delay in a mouse xenograft model.



1 2 3

143.

PROTEIN KINASE B/AKT ANTAGONISTS AS ANTITUMOR AGENTS PART 2: RATIONAL APPROACH TO THE IDENTIFICATION OF OXYINDOLE-PYRIDINE BASED AKT INHIBITORS. ***Viraj B. Gandhi***[1], *Jianchun Gong*[1], *Tongmei Li*[1], *Keith W. Woods*[1], *John Fisher*[1], *Garrick Packard*[1], *Xiaohong Song*[1], *Yan Luo*[1], *Yan Shi*[1], *Xuesong Liu*[1], *Vered Klinghofer*[1], *Jennifer Bouska*[1], *Alexander Shoemaker*[1], *Anatol Oleksijew*[1], *Ken Jarvis*[1], *Vincent S. Stoll*[2], *Chang Park*[2], *Ron De Jong*[3], *Tilman Oltersdorf*[3], *Qun Li*[1], *Saul H. Rosenberg*[1], *Vincent Giranda*[1], *and Gui-Dong Zhu*[1]. *(1) Cancer Research, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064, (2) Structural Biology, Abbott Laboratories, (3) IDUN Pharmaceuticals Inc*

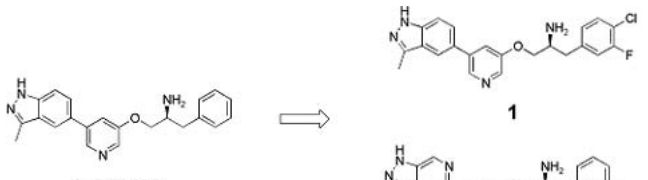
Compound **1** is a potent (IC_{50}= 1.5 nM), highly selective protein kinase B/Akt antagonist that shows significant tumor growth delay in a mouse xenograft model. One major drawback of this inhibitor is its short half-life across several species (mouse, rat and monkey). The C-1 position of the isoquinoline was identified as a major site of metabolism, however direct modification of this site failed to produce potent Akt inhibitors. To improve its pharmacokinetic profile, as well as potency against Akt , an extensive structure-activity relationship has been explored guided by X-ray crystallography of analogues bound to the structurally-related protein kinase A (PKA) and checkpoint kinase-1 (Chk-1). These efforts led to the identification of a series of very potent and highly selective oxyindole-pyridine based Akt inhibiors exemplified by **2** (IC_{50}= 170 pM). Several compounds in this series have demonstrated modest efficacy in mouse xenograft models.

1 2

144.

PROTEIN KINASE B/AKT ANTAGONISTS AS ANTITUMOR AGENTS PART 4: SYNTHESES OF POTENT, HIGHLY SELECTIVE AND ORALLY BIOAVAILABLE AKT INHIBITORS WITH REDUCED TOXICITY. ***Jianchun Gong***[1], *Viraj B. Gandhi*[1], *Tongmei Li*[1], *Yan Luo*[1], *Yan Shi*[1], *Xuesong Liu*[1], *Vered Klinghofer*[1], *Jennifer Bouska*[1], *Amanda Olson*[1], *Alexander Shoemaker*[1], *Vincent S. Stoll*[2], *Nathan L. Lubbers*[3], *James Polakowski*[3], *Silvia Ballaron*[3], *Thomas J. Campbell*[3], *Ron De Jong*[4], *Tilman Oltersdorf*[4], *Qun Li*[1], *Saul H. Rosenberg*[1], *Vincent Giranda*[1], *and Gui-Dong Zhu*[1]. *(1) Cancer Research, Abbott Laboratories, 100 Abbott Park Rd, Abbott Park, IL 60064, (2) Structural Biology, Abbott Laboratories, (3) Integrative Pharmacology, Abbott Laboratories, (4) IDUN Pharmaceuticals Inc*

A-674563 was identified as a potent (IC_{50}= 14 nM), selective and orally bioavailable (F=70% in mouse) inhibitor of protein kinase B/Akt (Giranda, V. *et al Cancer Research*). While promising efficacy was observed *in vivo*, this compound showed prominent effects on depolarization of purkinje fiber in an *in vitro* assay and severe CV toxicity (e.g. hypotension) *in vivo*. An X-ray structure of A-674563 bound to protein kinase A, which has 80% homology with Akt in the kinase domain, indicated the phenyl group is not tightly bound in the ligand-protein complex. In order to access a large number of diversely-substituted phenyl analogues, a novel and very efficient synthetic route was developed utilizing a copper-mediated aziridine ring-opening reaction as the key step. Biological evaluation of the resulting analogues led to the identification of the more potent (IC_{50}= 3 nM *vs* Akt), selective and bioavailable (F=84% in mouse) inhibitor **1**. No significant CV toxicity was observed for this compound when dosed orally up to 150 mg/kg. In addition, introduction of a nitrogen atom at the 6-position of the methyl indazole hinge binder (e.g. **2**) significantly improved selectivity against PKA and other protein kinases. The structure-activity relationships, pharmacokinetic profile and CV toxicity of these Akt inhibitors will be presented.



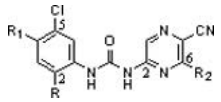
A-674563 1 2

145.

PYRAZYL PHENYL UREAS AS POTENT AND SELECTIVE CHK 1 INHIBITORS: THE EXPLORATION OF C6-POSITION OF PYRAZYL RING AND SAR STUDIES AT C4-POSITION OF PHENYL RING. ***Zhi-Fu Tao***, *Gaoquan Li, Gary T. Wang, Peter Kovar, Haiying Zhang, Chang Park, Kent Stewart, Hing L. Sham, Thomas Sowin, Saul H. Rosenberg, and Nan-Horng Lin, Cancer Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064, Zhi-Fu.Tao@abbott.com*

In response to DNA damage, normal cells are arrested in G1 phase mediated by p53 tumor suppressor and p53-deficient cancer cells arrested in S or G2 phase to repair their damaged DNA before progressing into mitosis. Checkpoint kinase 1 (Chk 1) is a serine / threonine protein kinase and plays important role in DNA damage-induced cellular response. When G2 or S checkpoint is abrogated by the inhibition of Chk1, cancer cells undergo mitotic catastrophe and eventually apoptosis, whereas normal cells are still arrested in G1 phase. Thus, Chk1 inhibitors can preferentially potentiate the efficacy of DNA damaging agents in

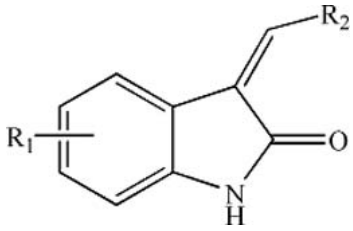
cancer cells and have emerged as a novel class of anticancer agents. We have disclosed 1-(5-chlorophenyl)-3-(5-cyano-pyrazin-2-yl)-ureas as a new class of Chk1 inhibitors. We herein present the SAR studies at C6-position of pyrazyl ring and C4-position of the phenyl ring.



146.

SYNTHESIS AND BIOLOGICAL EVALUATION OF 3-ETHYLIDENE-1,3-DIHYDRO-INDOL-2-ONE AS NOVEL CHECKPOINT 1 KINASE INHIBITORS. ***Nan-Horng Lin***[1], *Ping Xia*[1], *Peter Kovar*[1], *Chang Park*[1], *Zehan Chen*[1], *Haiying Zhang*[1], *Saul Rosenberg*[1], *and Hing L. Sham*[2]. *(1) R47B, Cancer Research, Global Pharmaceutical R & D, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-3500, nanhorng.lin@abbott.com, (2) Director, Metabolic Disease Research, Abbott Laboratories*

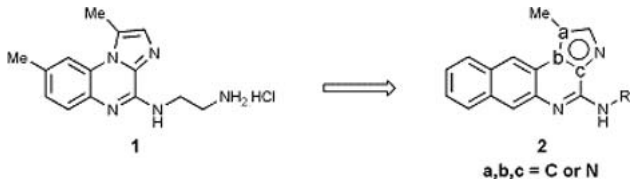
Mammalian cells undergo cell cycle arrest in response to DNA damage due to the existence of multiple checkpoints. Upon DNA damage, cancer cells often arrest at G2 phase due to mutation of the p53 suppressor gene. The Ser/Thr kinase, checkpoint 1(Chk1), is a key regulator of S and G2 checkpoints. Inhibition of Chk1 may selectively sensitize p53-deficient tumor cells to radiation or chemotherapy treatment. Chk1 inhibitors have emerged as a novel class of neoplastic agents for abrogating the G2 DNA damage checkpoint arrest. Analogs of the Chk1 inhibitor, 3-ethylidene-1,3-dihydro-indol-2-one, were synthesized and tested *in vitro* for their inhibitory activities. The synthesis and detailed biological data of this series of analogs will be presented.



147.

SYNTHESIS AND BIOLOGICAL EVALUATION OF 4-AMINO DERIVATIVES OF BENZIMIDAZOQUINOXALINE, BENZIMIDAZOQUINOLINE AND BENZOPYRAZOLOQUINAZOLINE AS POTENT IKK INHIBITORS. ***Carl Ouellet****, Francis Beaulieu, Edward H. Ruediger, Makonen Belema, Jacques Banville, James R. Burke, Kurt R. Gregor, John F. MacMaster, Alain Martel, Kim W. McIntyre, Mark A. Pattoli, Yuping Qiu, Dolatria Vyas, Xuejie Yang, and F. Christopher Zusi, Bristol-Myers Squibb Co Pharmaceutical Research Institute, 100 Industrial Blvd, Candiac, QC J5R 1J1, Canada, carl.ouellet@bms.com*

The nuclear transcription factor NF-κB plays a key role in regulating the expression of many pro-inflammatory genes and exists in the cytoplasm as an inactive form associated with the IκB inhibitory proteins. Extracellular pro-inflammatory agents (IL-1, TNF-α) initiate a signaling cascade, leading to activation of IκB kinases (IKK), which phosphorylate IκB at specific residues. Phosphorylated IκB is then selectively ubiquinated and degraded, which allows free NF-κB to translocate to the nucleus and initiate the transcription of target genes. Therefore, inhibitors of IKK may potentially be used in the treatment of inflammatory and related disorders. We have recently identified BMS-345541 (**1**) as a highly selective and potent inhibitor of IKK-2 (IC_{50} = 0.3 μM), but with considerably less potency against IKK-1 (IC_{50} = 4.0 μM). In order to further explore the SAR around the imidazoquinoxaline tricyclic structure **1**, we prepared a series of tetracyclic analogues **2**. The synthesis and biological activities of these potent IKK inhibitors will be described.



148.

UNPRECEDENTED OLEFIN-DEPENDENT HISTIDINE-KINASE INHIBITORY OF ZERUMBONE RING OPENING MATERIAL. ***Takashi Kitayama***[1], *Risa Iwabuchi*[1], *Shu Minagawa*[1], *Fumihiro Shiomi*[2], *John Cappiello*[3], *Seiji Sawada*[2], *Ryutaro Utsumi*[1], *and Tadashi Okamoto*[1]. *(1) Advanced Life Science, Graduate School of Agriculture, Kinki University, 3327-204, Naka-machi, Nara 631-8505, Japan, Fax: 81-742-43-1445, kitayama@nara.kindai.ac.jp, (2) Kyoto University of Education, (3) Allergan, Inc*

Zerumbone ring-opening derivative, (2*E*, 6*E*, 10*EZ*)-11-Bromo-4,4,7-trimethyl-2,6,10-dodecatrienoic acid (10*E*/10*Z* = 3/2) **1**, inhibited autophosphorylation of the essential Histidine Kinase YycG existing in *Bacillus subtilis* constituting a two-component system (TCS). Generation of **1***E*-form could be regulated chemically using the difference from the ring-opening reactivity of the precursor forming of **1** and pure **1E** was isolated. The stereoisomer, **1E**, showed main inhibition activity of autophosphorylation of YycG (IC_{50} = 63.5 mM).

149.

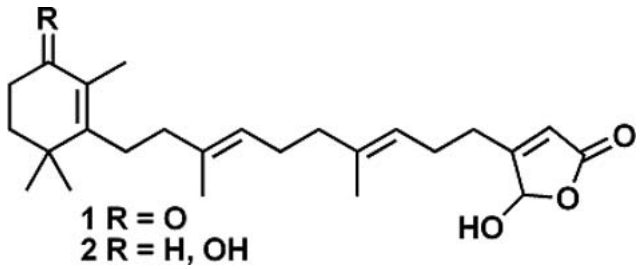
DISCOVERY OF A NOVEL LEAD FOR PROTEIN TYROSINE PHOSPHATASE 1B INHIBITION. *Yan-Ling Zhang*[1], ***Eva Binnun***[1], *Steven Kirincich*[2], *Weixin Xu*[3], *Diane Joseph-McCarthy*[2], *Michelle Markus*[3], *May Tam*[1], *Dave Erbe*[1], *Douglas P. Wilson*[2], *Zhao-Kui Wan*[2], *Bruce Follows*[2], *Junjun Wu*[4], *Alessandro Moretto*[2], *Rajeev Hotchandani*[4], *Steve Tam*[2], *James Tobin*[5], *and Jinbo Lee*[2]. *(1) Department of Cardiovascular and Metabolic Diseases, Wyeth, 200 Cambridge Park Drive, Cambridge, MA 02140, ebinnun@wyeth.com, (2) Department of Chemical and Screening Sciences, Wyeth, (3) Structural Biology, Wyeth, (4) Chemical and Screening Sciences, Wyeth, (5) Cardiovascular & Metabolic Diseases, Wyeth*

Protein Tyrosine Phosphatase-1b (PTP1b) has been shown to negatively regulate both insulin and leptin receptor signal transduction. PTP1b knockout mice demonstrate decreased insulin levels, improved insulin sensitivity, and resistance to weight gain on a high-fat diet, without any significant negative side effects. Thus, PTP1b could serve as an excellent therapeutic target for Type II diabetes as well as obesity. Two parallel approaches have been explored in an effort to identify novel leads of PTP1b inhibitors: high-throughput screening, and rational screening of phosphotyrosine mimetics at high micromolar concentrations. Considering the highly sensitive nature of the enzyme active site to reactive impurities, all hits from these two approaches have been evaluated extensively using detailed enzymology, NMR, and X-ray crystallography. Through these efforts, a novel lead compound with a Ki of 230 μM has been identified. This weak but well-characterized lead has served as an excellent starting point for developing low nanomolar inhibitors of PTP1b. This poster will describe our careful work in evaluating hit compounds and identification of a novel lead for PTP1b inhibition.

150.

SESTERTERPENOIDS AND AN ALKALOID FROM A THORECTANDRA SP. AS INHIBITORS OF THE PHOSPHATASE CDC25B. ***Shugeng Cao***[1], *John S. Lazo*[2], *Caleb Foster*[2], *and David G. I. Kingston*[1]. *(1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-3255, scao@vt.edu, (2) Department of Pharmacology, University of Pittsburgh School of Medicine*

Bioassay-directed separation of C018781, a marine organism from a *Thorectandra* sp. led to the isolation of three new sesterpenoids, 16-oxo-luffariellolide (**1**), 16-hydroxy-luffariellolide (**2**) and (2E,6E,10E)-3-formyl-7,11-dimethyl-13-(2,6,6-trimethylcyclohex-1-enyl)trideca-2,6,10-trienoic acid; two known sesterpenoids, luffariellolide and dehydroluffariellolide diacid; and one known alkaloid, fascaplysin. The structures of the new compounds were established on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. Fascaplysin showed inhibitory activity in the Cdc25B assay, with an IC_{50} value of 1.0 μg/mL.

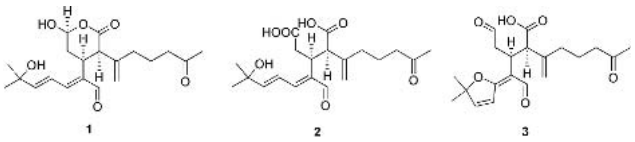


151.
SYNTHESIS AND SCREENING OF A LIBRARY OF BIDENTATE PROTEIN TYROSINE PHOSPHATASE INHIBITORS. ***Jian Xie*** *and Christopher T. Seto, Department of Chemistry, Brown University, 324 Brook St., Providence, RI 02912, Jian_Xie@Brown.Edu*

Opposing actions of protein tyrosine phosphatases (PTPases) and protein tyrosine kinase (PTKases) regulate the reversible tyrosine phosphorylation and dephosphorylation of proteins, which are essential in cell activities. PTPases such as PTP1B and Yersinia PTPase have been found playing important roles in diseases like type II diabetes and bubonic plague, respectively. A library of 67 bidentate compounds based on alpha-keto acid has been synthesized using parallel solution-phase methods. Two alpha-keto acid motif were tethered by a variety of different diamines through amide bonds. All candidates were screened as crude against Yersinia PTPase, PTP1B and TCPTP. Six compounds were then selected and evaluated against Yersinia PTPase, PTP1B, TCPTP, LAR and CD45. They all gave low micromolar IC50 values against Yersinia PTPase, PTP1B and TCPTP with good selectivity for PTP1B over LAR and modest selectivity over CD45.

152.
THREE NEW DITERPENOIDS INHIBITORS OF THE PHOSPHATASE CDC25B FROM A MARINE ORGANISM. ***Shugeng Cao***[1]*, John S. Lazo*[2]*, Caleb Foster*[2]*, and David G. I. Kingston*[1]*. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-3255, scao@vt.edu, (2) Department of Pharmacology, University of Pittsburgh School of Medicine*

Four diterpenoids have been isolated from C010505, and the structures of the new compounds **1**, **2**, and **3** were established on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. The tautomeric form of the six-membered cyclic hemiacetal of compound **1** was sensitive to solvent; it existed as the ring-opened form in basic solution (acetone/pyridine) but as the hemi-acetal in methanol. All the isolates were evaluated for their inhibition of the phosphatase Cdc25B.



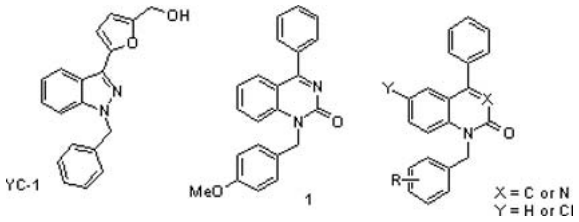
153.
TOWARDS THE SYNTHESIS OF TRUNCATED ANALOGUES OF OKADAIC ACID AND THEIR IN VITRO INFLUENCE ON PP1 ACTIVITY. ***Khuloud K Sweimeh****, Department of Chemistry, University of California, Irvine, 516 Rowland Hall, Irvine, CA 92697, ksweimeh@uci.edu, and A. R. Chamberlin, Department of Chemistry, University of California at Irvine*

Okadaic acid (OA) isolated from the marine sponge Halichondria okadai, is a tumor promoting C38 polyether fatty acid, and it inhibits protein phosphatases PP1 and PP2A, with 200–300 fold higher affinity for the latter. OA contains acidic and hydrophobic moieties and adopts a cyclic conformation. We report our strategy for synthesizing OA-based analogues that contain both moieties linked by different designed tethers. Upon completion of the synthesis, the influence of the abbreviated structures on PP1 activity will be determined in vitro.

154.
DESIGN AND SYNTHESIS OF 1-BENZYL-4-PHENYL-1H-QUINAZOLIN-2-ONE DERIVATIVES AS SELECTIVE PHOSPHODIESTERASE INHIBITORS. ***Shin-Yu Lai****, School of Pharmacy, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan, Taiwan, Fax: 886-2-23911300, darky@jwc.mc.ntu.edu.tw*

The cyclic GMP and cyclic AMP are secondary messengers responsible for transducing the signals from membrane receptors, which were stimulated by hormones, lights, and neurotransmitters. These two nucleotides play important roles in the process of signal transduction like pro-inflammatory mediator production and action, ion channel function, muscle contraction, learning, differentiation, apoptosis, lipogenesis, glycogenolysis, and gluconeogenesis. Inactivation of these two nucleotides is achieved by hydrolysis of the 3-ester bond catalyzed by the cyclic-nucleotide-dependent phosphodiesterases (PDEs). Thus, inhibition of PDEs could be potential therapeutic strategies for some diseases including erectile dysfunction, asthma, coronary artery disease, etc.

YC-1 is a soluble guanylate cyclase activator and has been shown to possess PDEs inhibitory activities and anticancer activities. Compound **1**, previously synthesized in our laboratory, demonstrated inhibitory activity against PDEs comparable to that of **YC-1**. We will present the preliminary results of our study on some 1-benzyl-4-phenyl-1*H*-quinazolin-2-one and 1-benzyl-4-phenyl-1*H*-quinolin-2-one derivatives for inhibition activities of different PDEs.



155.
FUSED PYRIMIDINE BASED INHIBITORS OF PHOSPHODIESTERASE 7 (PDE7) : SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS. ***James Kempson***[1]*, Anne Marinier*[1]*, Marco Dodier*[1]*, Claude A. Quesnelle*[1]*, Patrice Gill*[1]*, Joseph Barbosa*[1]*, Junqing Guo*[1]*, Marianne Carlsen*[1]*, Andrew Watson*[2]*, Karen Stebbins*[2]*, Deborah Lee*[2]*, Gary Starling*[2]*, Alain Martel*[1]*, William J. Pitts*[1]*, John H. Dodd*[1]*, Peter Kiener*[2]*, Joel Barrish*[1]*, and Murray McKinnon*[2]*. (1) Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, james.kempson@bms.com, (2) Immunology, Inflammation, and Pulmonary Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute*

cAMP and cGMP play pivotal roles in regulating signalling pathways for many essential cellular functions. In the immune system, cAMP is the primary regulatory cyclic nucleotide and it is believed that cAMP broadly suppresses the functions of immune and inflammatory cells. The reduction of cAMP levels is mediated principally by the action of cell-specific phosphodiesterases (PDEs) and as such, an approach to sustain cAMP levels through PDE-inhibition would provide a strategy to treat a variety of immune and inflammatory diseases. This poster will focus on the specific role of Phosphodiesterase 7 (PDE 7) and highlight the medicinal chemistry effort leading to potent and selective inhibitors of this enzyme. The implication of PDE7 in T-cell regulation will also be discussed.

156.
SUBSTITUTED PYRIMIDINES AS POTENT AND SELECTIVE INHIBITORS OF PHOSPHODIESTERASE 7 (PDE7). ***Anne Marinier***[1]*, Marco Dodier*[1]*, Claude A. Quesnelle*[1]*, Patrice Gill*[1]*, Joseph Barbosa*[2]*, Junqing Guo*[2]*, James Kempson*[2]*, Marianne Carlsen*[2]*, Andrew J. Watson*[2]*, Karen L. Stebbins*[2]*, Deborah Lee*[2]*, Gary C. Starling*[2]*, Alain Martel*[1]*, William J. Pitts*[2]*, John H. Dodd*[2]*, Peter A. Kiener*[2]*, Joel C. Barrish*[2]*, and Murry Mckinnon*[2]*. (1) Bristol-Myers Squibb Pharmaceutical Research Institute - Candiac, 100 Boul. de l'Industrie, Candiac, QC J5R 1J1, Canada, Fax: 450-444-4166, anne.marinier@bms.com, (2) Bristol-Myers Squibb Pharmaceutical Research Institute - Princeton*

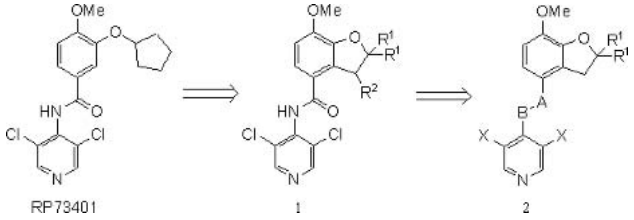
Phosphodiesterases are involved in a large number of cellular signaling pathways by hydrolyzing cAMP and cGMP, thus affecting important physiological functions. It has been shown that Phosphodiesterase 7 (PDE7) is up-regulated in activated T-cells and that abrogation of PDE7 activity by antisense nucleotides resulted in increased levels of cAMP, decreased IL2 production and

cell proliferation. A specific inhibitor of PDE7 is therefore expected to be useful as an immunosuppressant in T-cell mediated diseases. We report in this poster the identification of a new class of potent substituted pyrimidines as selective PDE7 inhibitors. The synthesis and structure-activity relationships of this series of compounds are described as well as their effects on T-cell proliferation. The implication of PDE7 in T-cell regulation will also be discussed.

157.

SYNTHESIS AND BIOLOGICAL EVALUATION OF DIHYDROBENZOFURANS AS PDE4 INHIBITORS. ***Koji Yanagawa***, *Takashi Kawakita, Haruhiko Manabe, Michio Ichimura, Ryou Hirose, and Etsuo Ohshima, Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-0943, Japan, Fax: +81-055-986-7430*

PDE4 plays significant roles on the function of inflammatory cells, such as eosinophils. The inhibition of PDE4 causes suppression of inflammatory cell activation. Therefore, selective PDE4 inhibitors have attracted considerable attention as anti-inflammatory agents for the treatment of asthma, chronic obstructive pulmonary disease (COPD) and other inflammatory diseases. Our objective was to identify PDE4 inhibitors with potent biological activities and reduced side effects. We attempted to convert the catechol moiety of selective PDE4 inhibitor RP73401 into a dihydrobenzofuran skeleton. The efforts resulted in the identification of 2-substituteddihydrobenzofurans1 as potent PDE4 inhibitors. Further modifications of the C-4 position and the pyridine ring led to compound 2 (R1=-(CH2)4-, A=CO, B=CH2, X=H), which retained potent PDE4 inhibitory activity and also showed potent inhibitory effect on ovalbumin-induced bronchoconstriction model in guinea pigs.



158.

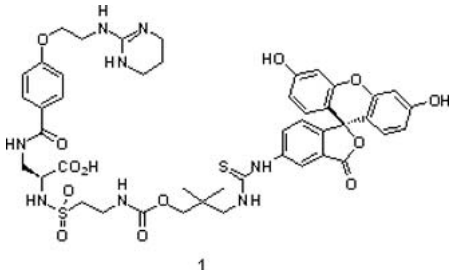
AZA-BICYCLIC AMINO ACID AMIDES AS A4B1/A4B7 INTEGRIN RECEPTOR ANTAGONISTS. ***Alexey B. Dyatkin***, *Yong Gong, Tamara A. Miskowski, Bruce E. Maryanoff, William A. Kinney, Edward S. Kimball, Rosemary Santulli, M. Carolyn Fisher, Pamela J. Hornby, and Wei He, Drug Discovery, Johnson and Johnson Pharmaceutical R&D, L.L.C, Welsh and McKean Rds, Spring House, PA 19477, Fax: 215-628-4985, adyatkin@prdus.jnj.com*

The design, synthesis, and biological activity of novel a4b1 and a4b7 integrin receptor antagonists, containing a bridged azabicyclic nucleus, will be reported. Novel improved series of N-amido, alkyl and ureido azabicyclo[2.2.2]octane carboxamides were prepared and assayed for inhibition of a4b1-VCAM-1 and a4b7-MAdCAM-1 interactions. A pro-drug approach was used to improve oral bioavailability of this series. Two compounds were selected for in vivo leukocytosis studies (3 and 30 mg/kg, subcutaneous administration) and demonstrated increasing circulating lymphocytes up to 250% over control.

159.

INTEGRIN $\alpha_v\beta_3$-TARGETED OPTICAL IMAGING PROBES. ***Christopher A. Burnett***, *Jianwu Xie, Jade Quijano, Finie Hunter, Haihao Sun, Monica Bur, King C. P. Li, and S. Narasimhan Danthi, Clinical Center, National Institutes of Health, Molecular Imaging Laboratory, 9000 Rockville Pike, Bethesda, MD 20892*

Integrin $\alpha_v\beta_3$ is a heterodimeric membrane-spanning cellular receptor protein that has been implicated in the formation of new blood vessels (angiogenesis) and tissue remodeling in major diseases such as osteoporosis, rheumatoid arthritis, macular degeneration, and cancer. Research in our laboratory has focused on targeting the integrin $\alpha_v\beta_3$ in order to develop novel molecular imaging probes that will aid in non-invasive diagnosis of these diseases. Here we report the synthesis, *in vitro*, and *in vivo* characterization of $\alpha_v\beta_3$-targeted optical imaging probes based on the centrally-constrained benzoylamino-3-propionic acid scaffold which are typified by compound **1** shown below. These probes are extremely potent nanomolar inhibitors of $\alpha_v\beta_3$ as demonstrated by ELISA assay (IC_{50} **1** 3.4±0.3 nM). In addition, *in vivo* optical imaging data in a mouse M21 melanoma model will be presented.

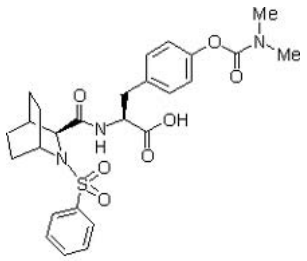


1

160.

SYNTHESIS AND EVALUATION OF 2-AZA-BICYCLO[2.2.2]OCTANE-CONTAINING $\alpha4\beta1$ INTEGRIN ANTAGONISTS IN ANIMAL MODELS OF ASTHMA. ***Edward C. Lawson***[1], *William M. Abraham*[2], *Bruce P. Damiano*[1], *Alexey B. Dyatkin*[1], *Larry De Garavilla*[1], *William A. Kinney*[1], *Bruce E. Maryanoff*[1], *Clive Page*[3], *Sandra Rudman*[3], *and Rosemary Santulli*[1]. *(1) Drug Discovery, Johnson & Johnson Pharmaceutical Research & Development, Welsh & McKean Roads, P.O. Box 0776, Spring House, PA 19477-0776, Fax: 215-628-4985, elawson@prius.jnj.com, (2) Department of Research, Mount Sinai Medical Center, (3) GKT School of Biomedical Sciences, Sackler Institute of Pulmonary Pharmacology*

The cell-surface integrin $\alpha4\beta1$ mediates cell adhesion and activation through cell-cell and cell-matrix interactions. The synthesis and biological activity of 1, a potent $\alpha4\beta1$ antagonist (Ramos cell adhesion/VCAM-1 IC_{50} = 39 nM), will be discussed. Administration of 1 to Ascaris-sensitized sheep via inhalation inhibited cell recruitment to the lung, blocked the late phase of asthma, and abolished airway hyperreactivity at 24 hours post-dosing. In ovalbumin-sensitized guinea pigs, administration of 1 (i.p.) inhibited cell recruitment to the lung and blocked airway resistance. Ester prodrugs were synthesized to improve oral bioavailability; however, the pharmacokinetic profiles in rats and dogs were not significantly changed. Interestingly, oral dosing of two different prodrugs of 1 significantly inhibited eosinophil recruitment to the lung (10 mg/kg) and ovalbumin-induced airway resistance (10, 30 mg/kg) in sensitized guinea pigs.

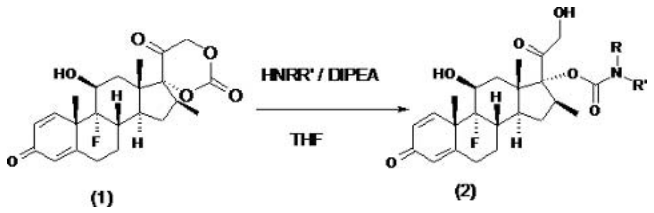


1

161.

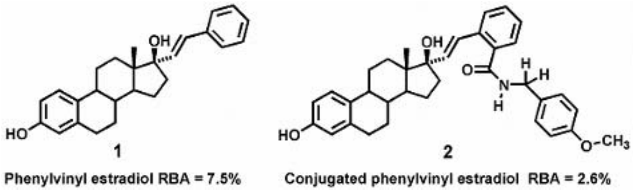
BETAMETHASONE 17α-CARBAMATES AS POTENT, DISSOCIATED GLUCOCORTICOID RECEPTOR AGONISTS. ***Gordon G Weingarten***[1], *Keith Biggadike*[1], *Torquil I Jack*[1], *Paul S Jones*[1], *Andrew J Harker*[2], *and Simon J Taylor*[2]. *(1) ri CEDD, Medicinal Chemistry 1, GlaxoSmithKline plc, Gunnels Wood Road, Stevenage, Herts SG1 2NY, United Kingdom, Fax: +44 1438 76 3616, Gordon.G.Weingarten@gsk.com, (2) ri CEDD, Stevenage DMPK, GlaxoSmithKline plc*

Secondary and tertiary 17a-carbamates (2) of betamethasone are prepared by treating the 17,21-carbonate (1) with amines. Many are more potent than betamethasone; tertiary carbamates being more potent than secondary ones. Branched and cycloalkyl carbamates show varying degrees of selectivity for transrepression over transactivation. Unlike betamethasone 17a-esters, the carbamate group does not migrate to C-21 in plasma (which in the case of selective esters leads to the formation of betamethasone, a potent and non-selective metabolite). Some of the carbamates that were poorly turned-over in an *in vitro* metabolic clearance assay, showed good plasma levels in mice dosed p.o.



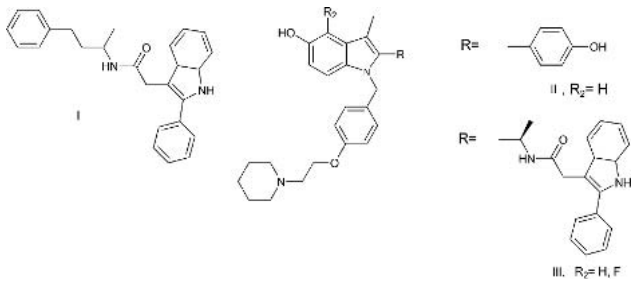
162.
CONFORMATIONAL ANALYSIS OF 17ALPHA-(PHENYLVINYL) ESTRADIOL CONJUGATES USING 1D AND 2D NMR AND COMPUTATIONAL METHODS.
Edward Y. Hua, *Emmett McCaskill, David A. Forsyth, and Robert N. Hanson, Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, hua.e@neu.edu*

We have used NMR spectrometry as part of our program to evaluate the interactions between estrogenic ligands and the estrogen receptor alpha-ligand binding domain (ERalpha-LBD). Previous reports investigated the solution conformations of simple mono-substituted phenylvinyl estradiols. We subsequently prepared a series of conjugated derivatives of phenylvinyl estradiols 1 that exhibited interesting ERalpha-LBD binding properties. For example, the ortho-substituted compound 2 expressed a relative binding affinity (RBA) about one-third that of the parent compound 1. Because the benzylic protons exhibited diamagnetic anisotropy, we undertook an intensive spectrometric analysis to understand the relationship between the solution conformation and observed biochemical properties.



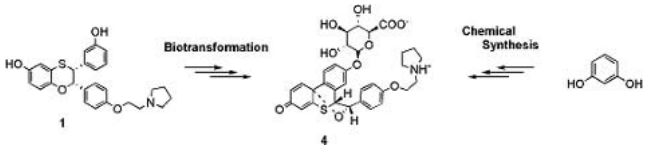
163.
DISCOVERY AND SYNTHESIS OF NOVEL SELECTIVE ESTROGEN RECEPTOR ALPHA MODULATORS (SERAMS).
Kevin D. Dykstra[1], *Liangqin Guo*[1], *Elizabeth Birzin*[2], *Wanda Chan*[2], *Yi Tien Yang*[2], *Lawrence Colwell*[1], *Ralph Mosley*[1], *Bryan Kraker*[1], *Johanna Dahllund*[3], *Frank DiNinno*[1], *Susan P. Rohrer*[2], *James M. Schaeffer*[2], *and Milton Hammond*[1]. *(1) Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co, P.O. Box 2000, Rahway, NJ 07065, Fax: 732-594-9556, kevin_dykstra@merck.com, (2) Department of Atherosclerosis and Endocrinology, Merck Research Laboratories, Merck & Co, (3) Karo Bio AB*

Raloxifene (Evista®) is a selective estrogen receptor modulator (SERM), which exhibits tissue selectivity, agonist activity on bone, is an antagonist on breast tissue and a minimal agonist on uterine tissue. Raloxifene, bazedoxifene, as well as all other third generation SERM candidates, show balanced binding to both of the estrogen receptors, Era and Erb. Although Raloxifene has a demonstrative benefit over conventional HRT therapy, it also has clinical disadvantages, such as increased hot flushes and deep vein thrombosis. The recent discovery of the Erb receptor, has initiated interest in the discovery of subtype selective ligands that may impart an "ideal" SERM profile. We have recently described the SAR of the flavanoid and dihydrobenzoxathiin classes as potent selective estrogen receptor alpha modulator (SERAM) ligands. We now report on the discovery of compounds of type I, unique as novel SERAMs, with improved selectivity for Era. In addition, combination of the chiral 2-aryl indole acetamide linkage of I, with bazedoxifene II at C-2 of the indole skeleton, produced compounds of type III, which exhibited greater than 120 fold selectivity for Era. The biological activity, SAR and molecular modeling for these novel classes of SERAMs will be described.



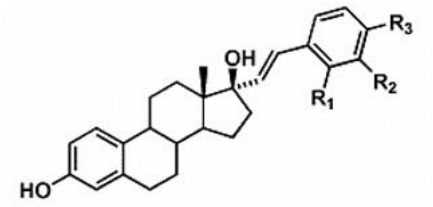
164.
IDENTIFICATION AND SYNTHESIS OF THE METABOLITES M1 AND M17 OF AN ER ALPHA-SELECTIVE ANTAGONIST FOR OSTEOPOROSIS.
Jane Y. Wu, *Seongkon Kim, Zhoupeng Zhang, Wei Tang, George Doss, Brian Dean, Frank DiNinno, and Milton L. Hammond, Merck Research Laboratories, Merck & Co., Inc, Rahway, NJ 07065*

Compound **1** was discovered to be a potent **SERAM** (**S**elective **E**strogen **R**eceptor **A**lpha **M**odulator) which exhibited sub-nanomolar binding to ERa and 40-fold selectivity. Further evaluation of **1** revealed its promise for the prevention of estrogen deficiency osteopenia in postmenopausal women, without uterotropism, and thereby qualified as a developmental candidate. During the course of drug metabolism studies, a major metabolite in rhesus monkeys was detected and designated structure **4**. The intriguing bio-transformation of compound **1** leading to the metabolite **4** was confirmed by a 19- step synthesis starting from resorcinol. The key feature of this synthesis was the construction of the oxygen bridge utilizing a phenolic oxidation and trapping reaction. The synthesis of **4** and a related metabolite **5** will be presented.



165.
MOLECULAR DYNAMICS STUDIES OF (17ALPHA,20E)-21-(2-/3-/4-SUBSTITUTED PHENYL)-19-NORPREGNA-1,3,5(10),20-TETRAENE-3,17BETA-DIOLS AS LIGANDS FOR THE ESTROGEN RECEPTOR-ALPHA-LIGAND BINDING DOMAIN (ERALPHA-LBD).
Robert N. Hanson *and Robert Dilis, Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, r.hanson@neu.edu*

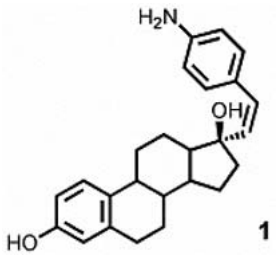
We have employed molecular modeling as part of our program to study the interactions between estrogenic ligands and the ERalpha-LBD. Previous reports, focusing on the E- and Z-(4-substituted phenyl)vinyl estradiols suggested the presence of a binding pocket to accommodate the phenylvinyl moiety. Binding studies, particularly for the 2-substituted compounds, indicated enhanced receptor interactions. Because the 2- and 3-substituted phenylvinyl estradiols can adopt multiple conformations, we undertook a more thorough evaluation to correlate their binding properties with observed in vivo activity.



166.

NEW SYNTHESIS OF POTENT APOPTOTIC STEROIDAL 17-ALPHA-[(4-AMINO)PHENYL]-(Z)-VINYL ESTRADIOL. ***Pakamas Tongcharoensirikul***[1], *James A. Mobley*[2], *James O. L'Esperance*[2], *Shuk-mei Ho*[2], *and Robert N. Hanson*[1]. *(1) Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, p.tongcharoensirikul@neu.edu, (2) Department of Urology, University of Massachusetts Medical School*

Prostate Cancer is the most common cancer in men with about 240,000 new cases being diagnosed each year. In our program related to hormone responsive prostate cancer, we synthesized a small library of estrogens that demonstrated antitumor activities in vitro. Among these compounds, 17-alpha-[(4-amino)phenyl]-(Z)-vinyl estradiol (1) induced a high level (>90%) of cell death through an apoptotic mechanism. This compound was obtained in low yield through the Stille reaction which required the vinyl stannane as one of the starting materials. In order to conduct further studies with this compound, we developed new synthetic pathway through the Suzuki reaction which utilized less toxic commercially available boronic acids. The synthesis of analogs of 1 and their biological data will be presented.



1

167.

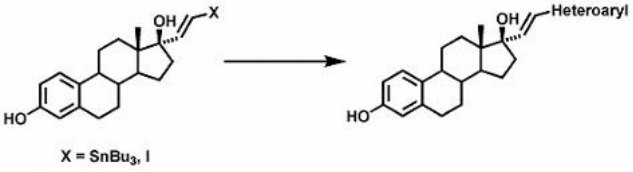
NEWLY DISCOVERED ORALLY ACTIVE PURE ANTIESTROGENS. ***Yoshitake Kanbe***[1], *Myung-Hwa Kim*[1], *Masahiro Nishimoto*[1], *Yoshihito Ohtake*[1], *Nobuaki Kato*[1], *Shin-ichi Kaiho*[1], *Iwao Ohizumi*[1], *Takaaki Yoneya*[1], *Toshiaki Tsunenari*[1], *Kenji Taniguchi*[1], *Hiroshi Araya*[1], *Setsu Kawata*[1], *Yoshiaki Nabuchi*[1], *Kazumi Morikawa*[1], *Jae-Chon Jo*[2], *Hee-An Kwon*[2], *Hyun-Suk Lim*[2], *and Hak-Yeop Kim*[2]. *(1) Fuji Gotemba Research Labs, Chugai Pharmaceutical Co.,LTD, 1-135 komakado, 412-8513 Gotemba, Japan, Fax: +81(550)87-5326, kanbeyst@chugai-pharm.co.jp, (2) C&C Research Labs*

The estrogen antagonists, or antiestrogens, have been prescribed for the treatment of breast cancer, with tamoxifen the most widely prescribed for hormone-dependent breast cancer. Recently, a pure antiestrogen, ICI182,780, attracted attention because it was found to be effective in tamoxifen-resistant breast cancer.In the course of our research directed to the generation of a pure antiestrogen, we previously found that a chroman/thiochroman derivative having a carboxy side chain functioned as a pure antiestrogen and may also be effective against tamoxifen-resistant breast cancer. In addition, it showed remarkable oral activity with good intestinal absorption and metabolic stability compared with ICI182,780. Therefore, a carboxy side chain was incorporated into a steroid scaffold, and several compounds were identified which functioned as pure antiestrogens and showed potent antiestrogenic and antitumor activities when dosed orally. The design, synthesis, and biological activity of the resulting series of derivatives will be described in detail.

168.

PREPARATION OF HETEROARYLVINYL ESTRADIOLS: COMPARISON OF SUZUKI AND STILLE COUPLING REACTIONS. ***Karla Gandiaga***, *Pakamas Tongcharoensirikul, and Robert N. Hanson, Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, r.hanson@neu.edu*

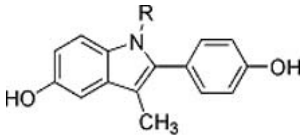
As part of our program to develop novel probes for the estrogen receptor, we undertook the preparation of heteroaryl analogs of the 17alpha-phenylvinyl estradiols. Most of our research methods utilized the Stille reaction to effect the coupling of the vinylstannane to the aryl halide. For the heteroaryl compounds the initial yields, while acceptable for generating material for bioassays, were not optimal for subsequent parallel synthesis. Therefore, we developed an undergraduate research project that would explore the alternate route via the vinyl halide and the heteroaryl boronic acid. The results suggest that the Suzuki reaction provides a potentially useful method for the parallel synthesis of these derivatives.



169.

SYNTHESIS AND APPLICATIONS OF TETHER-CONTAINING INDOLE ESTROGENS. ***Bridget G. Trogden***, *Sung Hoon Kim, and John A. Katzenellenbogen, Department of Chemistry, University of Illinois at Urbana-Champaign, RAL Box 85-5, MC 712, 600 S. Mathews Ave., Urbana, IL 61801, trogden@uiuc.edu*

We have investigated a series of N-substituted-2-phenylindole ligands (1) as non-steroidal estrogen receptor (ER) ligands that can be conjugated to other molecules to probe ER actions. We created N-alkyl, N-phenyl, and N-benzyl analogs and found that all ligands have good affinity for the ER. Notably, long-chain N-substituents are well tolerated. We applied the synthesis of these indoles to the creation of hybrid molecules, where one end is able to bind to the ER and the other to a fluorophore or dendrimer in order to investigate different ER functions, as will be presented.



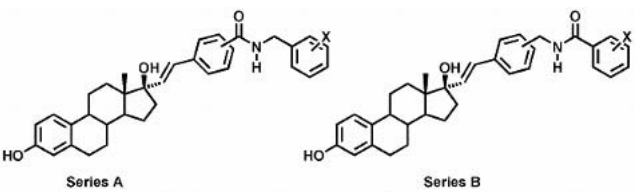
R = Ak, Bn, Ph

1

170.

SYNTHESIS AND EVALUATION OF A NEW SERIES OF 17ALPHA-(PHENYLVINYL) ESTRADIOL CONJUGATES AS PROBES FOR THE ESTROGEN RECEPTOR-ALPHA LIGAND BINDING DOMAIN (ERALPHA-LBD). ***Robert N. Hanson*** *and Emmett McCaskill, Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, r.hanson@neu.edu*

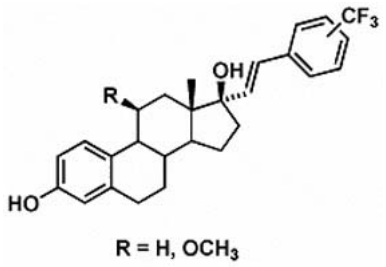
As part of our program to probe the interactions of estrogenic ligands and the ERalpha-LBD, we have prepared and evaluated a new series of 17alpha-(substituted phenylvinyl)estradiols. Previous studies with simple E- and Z-17alpha-(mono-substituted phenylvinyl)estradiols indicated the presence of a binding pocket to accommodate the phenylvinyl moiety. The substituents incorporated in this study were selected to probe some of the steric and electronic limits of that binding pocket. The initial results suggest that the ERalpha-LBD can indeed accommodate the new ligands; however, an alternate binding mode may be required to do so.



171.

SYNTHESIS AND EVALUATION OF ISOMERIC (17ALPHA,20E)-11BETA-METHOXY-21-(TRIFLUOROMETHYLPHENYL)-19-NORPREGNA-1,3,5(10),20-TETRAENE-3,17BETA-DIOLS AS ERALPHA-HORMONE BINDING DOMAIN LIGANDS: EFFECT OF THE METHOXY GROUP ON RECEPTOR BINDING AND UTEROTROPHIC GROWTH. ***Robert N. Hanson***[1]*, Pakamas Tongcharoensirikul*[1]*, Robert Dilis*[1]*, Alun Hughes*[2]*, and Eugene R. DeSombre*[2]*. (1) Department of Chemistry and Chemical Biology, Northeastern University, 102 Hurtig Hall, 360 Huntington Avenue, Boston, MA 02115, r.hanson@neu.edu, (2) Ben May Institute for Cancer Research, University of Chicago*

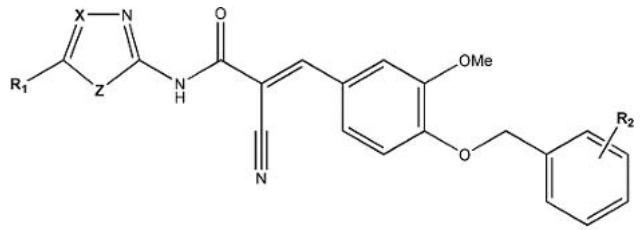
As part of our program to develop probes for the estrogen receptor we have prepared the 11beta-methoxy analogs of the isomeric (trifluoromethyl)phenylvinyl estradiols. Competitive binding assays with the ERalpha-HBD indicated little difference between the analogs; however, the in vivo uterotrophic assay demonstrated significant differences between the two series. Examination of the two series using molecular modeling suggests a basis for these observations.



172.

IDENTIFICATION OF A SELECTIVE INVERSE-AGONIST FOR THE ORPHAN NUCLEAR RECEPTOR ERRA. ***Brett B. Busch****, Department of Chemistry, Exelixis, Inc, 4757 Nexus Centre Drive, Suite 200, San Diego, CA 92121, Fax: 858-458-4501, bbusch@exelixis.com*

The estrogen-related receptor a (ERRa) is an orphan receptor belonging to the nuclear receptor superfamily. While there has been considerable progress in identifying ERRa target genes, the physiological role of ERRa has yet to be established, primarily due to lack of a natural ligand. Herein we describe the synthesis and discovery of the first potent and selective series of inverse-agonist (1) of ERRa. Through in vitro and in vivo studies, such ligands will further elucidate the biology and pharmacology of ERRa.



1

173.

SYNTHESIS AND ANDROGEN RECEPTOR AFFINITY OF SEVERAL LINKAGES OF 1,3-DISUBSTITUTED-2-HYDROXY-2-METHYLPROPIONAMIDE SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMS). ***Dong Jin Hwang***[1]*, Juhyun Kim*[2]*, James T. Dalton*[2]*, and Duane D. Miller*[1]*. (1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Memphis, TN 38163, djhwang@utmem.edu, (2) Division of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University*

Since the discovery of the natural (or endogenous) steroidal androgen testosterone (TES), in the 1930s, a variety of steroidal and nonsteroidal androgen receptors ligands (NSAR) have been synthesized and tested. The activity of bicalutamide, a NSAR antagonist, has been the most extensively studied. We discovered the first NSAR agents that were agonists by modification of the bicalutamide nucleus (Dalton et al., 1998). Proposed therapeutic uses of NSARs agonists include muscle wasting, osteoporosis, and male contraception. The NSARs synthesized here S-1 are modifications of the 1,3 disubstituted-2-hydroxy-2-methylpropionamide nucleus of bicalutamide. These are bicalutamide analogues stereospecifically synthesized as the high affinity isomers [(S) for oxy, amino, methylamino linkages and (R) for sulfide, sulfoxide, and sulfone linkages) with stereoconfigurations analogous to R-bicalutamide. These ligands differ from bicalutamide by their linkages and the 4′(para) A ring substituent is 4′-nitro (vs. sulfone linked and 4′-cyano in bicalutamide). Yin et al. proposed a metabolic scheme of acetothiolutamide in rats that the sulfur linkage underwent successive oxidations to the sulfoxide then to the sulfone. At the present study, we synthesized two possible sulfoxide structures by oxidizing reagent from sulfide linkage and separated two diastereomeric sulfoxides by column chromatography and tested the binding affinities to AR. We found one isomer had 3 times stronger binding property than the other isomer. Among the various linkages of S-1, the affinity of the methylamino linkage (Y = NO2) had relatively low binding affinity, however, the oxygen, amino and sulfide AR ligands (Y = F) showed strong binding affinities to AR (Ki = 2.3 ~ 11 nM). Supported by NIH grants R01 DK59800 and R01 DK065227 and GTx.

174.

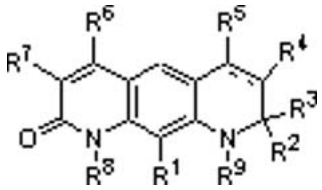
SYNTHESIS AND BIOLOGICAL EVALUATION OF N-ARYLPIPERAZINE 1-CARBOXAMIDES AS NOVEL PERIPHERALLY SELECTIVE ANDROGEN RECEPTOR ANTAGONISTS. ***Eiji Kawaminami***[1]*, Isao Kinoyama*[1]*, Eisuke Nozawa*[1]*, Takashi Kamikubo*[1]*, Masakazu Imamura*[1]*, Akira Toyoshima*[2]*, Kiyohiro Samizu*[1]*, Nobuaki Taniguchi*[1]*, Hiroshi Koutoku*[1]*, Takashi Furutani*[1]*, Minoru Okada*[1]*, and Mitsuaki Ohta*[1]*. (1) Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd, 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan, Fax: +81-29-852-2971, kawamina@yamanouchi.co.jp, (2) Drug Development Division, Yamanouchi Pharmaceutical Co., Ltd*

The androgen receptor (AR) is a member of the steroid/thyroid/retinoid/vitamin D3 nuclear receptor superfamily and acts as an androgen-dependent specific transcription factor. The development and progression of prostate cancer is known to be androgen-dependent, and AR expression is frequently observed in primary prostate tumors and metastases. Bicalutamide, one of the most efficient AR antagonist, is used as single agent therapy in the clinic for the treatment of AR dependent prostate cancer. However, it is reported that bicalutamide causes gynecomastia and breast tenderness as the major side effects probably due to its low peripheral selectivity. We have designed and synthesized a series of N-arylpiperazine 1-carboxamides, and identified YM580 as a potent and peripherally selective non-steroidal antagonist of AR. YM580 showed better efficacy both in vitro and in vivo assay than bicalutamide. The syntheses and biological activities of YM580 derivatives will be presented.

175.

SYNTHESIS AND BIOLOGICAL EVALUATION OF POTENT, EFFICACIOUS ANDROGEN ANTAGONISTS BASED ON PYRIDONO[5,6-*G*]QUINOLINES. ***Robert I. Higuchi****, Kristen L. Arienti, Lawrence G. Hamann, Francisco J. Lopez, Dale E Mais, Barbara A Pio, Todd K. Jones, Boris Risek, Keith B. Marschke, and William T. Schrader, Ligand Pharmaceuticals, 10275 Science Center Drive, San Diego, CA 92121, Fax: 858-550-7249, rhiguchi@ligand.com*

The androgen receptor (AR) is a member of the intracellular receptor superfamily of ligand-dependent transcription factors. AR antagonists are useful in the treatment of prostatic disease, alopecia, hirsutism, and acne. This work is the continuing studies of AR antagonists based on substituted 1,2-dihydro- and 1,2,3,4-tetrahydropyridono[5,6-*g*]quinolines. These compounds were synthesized and evaluated in competitive receptor binding assays and AR cell-based assays. These studies lead to the discovery of antiandrogens that demonstrate potent antagonist activity in vitro. These compounds were further evaluated in rodent models that measure androgen antagonist activity. Compounds from this series were able to fully suppress the testosterone-induced growth of the rat ventral prostate in immature rats. Furthermore, a lead compound from this series reduced flank organ weights in Syrian hamsters, a model for dermatologic indications such as acne and hirsutism. The results from these studies demonstrate that compounds from this series could have clinical utility in androgen-dependent disorders.



176.

SYNTHESIS AND BIOLOGICAL TESTING OF (2S)-MULTI-HALOGENATED B-RING 2-HYDROXY-2-METHYLPROPIONAMIDE SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMS): PROBING THE B-RING POCKET. ***Dong Jin Hwang***[1], *Jiyun Chen*[2], *Juhyun Kim*[2], *James T. Dalton*[2], *and Duane D. Miller*[1]. *(1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Memphis, TN 38163, djhwang@utmem.edu, (2) Division of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University*

New analogues of (2S)-3-(multi-halogenatedphenoxy)-2-hydroxy-2-methyl-N-(4′-nitro-3′-trifluoromethylphenyl)propionamide AR ligands S-2 were synthesized and tested in an in vitro competitive androgen receptor (AR) binding affinity assay. Marhefka et al. reported that oxy linked para halogenated 2-hydroxy-2-methyl-N-(4′-nitro-3′-trifluoromethyl-phenyl)propionamides S-1 are tissue selective androgen receptor modulators (SARMs) having anabolic agonist activity. The novel AR ligands were chiral nonsteroidal bicalutamide analogues with S-stereoconfiguration that differ from bicalutamide in that they all contain oxy linkages and the 4′-nitro in the aromatic A ring (vs. sulfone and 4′-cyano in bicalutamide). The target compounds were synthesized in an one-pot process by coupling a chiral epoxide intermediate with various commercially available multi-halogen substituted phenols. All the compounds synthesized had high binding affinity for the AR in vitro with Ki values ranging from 1.0 nM to 50.8 nM. The highest affinity SARM synthesized was the 3,4-dichlorophenoxy compound (1.0 nM) which to our knowledge is the highest affinity 2-hydroxy-2-methylpropionamide SARM reported to date. Supported by NIH grants R01 DK59800 and R01 DK065227 and GTx.

177.

SYNTHESIS OF ISOTHIOCYANATE DERIVATIVES OF IRREVERSIBLE SELECTIVE ANDROGEN RECEPTOR MODULATORS (SARMS) AND BIOLOGICAL TESTING IN PROSTATE CANCER CELL LINES. ***Dong Jin Hwang***[1], *Jiyun Chen*[2], *Huiping Xu*[2], *Suni M. Mustafa*[1], *James T. Dalton*[2], *and Duane D. Miller*[1]. *(1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Memphis, TN 38163, djhwang@utmem.edu, (2) Division of Pharmaceutics and Pharmaceutical Chemistry, The Ohio State University*

The androgen receptor in prostate cancer (CaP) therapy plays an important role not only in promoting the growth of CaP, but also in the development and maintenance of normal prostate tissue. We reported the synthesis of some electrophilic oxy linked AR ligands that were isothiocyanate, urea, isothiourea, methyl isothiourea and carboimide for the aromatic B ring para position (at 31st annual MALTO, 2004). This study extends the previous series of isothiocyanate SARMs to analogs having several different linkages (sulfide, sulfone, oxy, methylene, amino and methylamino), which bear nitro or cyano group at the 4-position and a trifluoromethyl group at the 3-position of the aromatic A ring. The isothiocyanate groups in B ring were prepared by the reaction between parent aromatic amine and thiophosgene under base condition. These novel irreversible isothiocyanate SARMs represent a new class of androgen receptor targeting agents (ARTA). These new ARTA compounds demonstrated growth inhibitory activity against CaP cell lines in vitro. The isothiocyanate derivatized electrophilic AR ligands of general structure 1 had high binding affinities for the androgen receptor and inhibited growth of CaP cell lines LNCap, DU145, PC-3, PPC-1, and TSU. Supported by NIH grants R01 DK59800 and R01 DK065227 and GTx.

178.

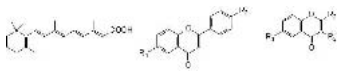
SYNTHESIS OF NEW STEROIDAL 5-ALPHA-REDUCTASE INHIBITORS. ***Eugene Bratoeff***[1], *Marisa Cabeza*[2], *Martha Ochoa*[1], *Nayeli Teran*[1], *Elena Ramirez*[1], *Victor Perez*[1], *and David Valdez*[1]. *(1) Department of Pharmacy, National University of México City, Ciudad Universitaria 04510, Mexico DF, Mexico, Fax: 525-622-5329, bratoeff@hotmail.com, (2) Biological Systems, Metropolitan University-X*

Androgen antagonists offer a potentially useful treatment for androgen mediated diseases such as: prostate cancer, benign prostatic hyperplasia, seborrhea, androgenic alopecia and precocious puberty. A recent report from the world health organization revealed that carcinoma of the prostate is the second most commonly diagnosed cancer after skin cancer in the male population in U.S.A. and the second most common cause of death after that of lung. Since testosterone is reduced by NADPH in the presence of the enzyme 5α-reductase into the more active 5α-dihydrotestosterone which interacts more effectively with the androgen receptors, this fact indicates very clearly that the logical site of the therapeutic intervention should be this last step. In this paper we describe the synthesis and pharmacological evaluation of new 17α-acyloyloxy-16β-methyl (or phenyl)-4, 6-pregnadiene-3,20-dione derivatives. These compounds were prepared from the commercially available 16-dehydropregnenolone acetate. These steroidal derivatives were evaluated as antiandrogens as well as 5α-reductase inhibitors. When the methyl group at C-16 was replaced with a phenyl group a Wagner Meerwein rearrangement took place and the 5-membered D-ring in the steroidal skeleton was expanded to a 6-membered ring. Several of these 16-methyl and 16-phenyl derivatives showed a much higher pharmacological activity than the presently used Proscar (finasteride). A mechanism for the inhibition of the enzyme 5α-reductase by these progesterone analogues is also proposed.

179.

CHROMONE AND FLAVONE BASED LIGANDS FOR RETINOIC ACID RECEPTORS. ***Shyam Desai***[1], *Safura Nantogmah*[1], *Dianne Soprano*[2], *Jerome L. Gabriel*[2], *and Daniel J. Canney*[1]. *(1) Dept of Pharmaceutical Sciences, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, (2) Department of Biochemistry, Temple University, School of Medicine*

Abstract: All-trans-retinoic acid (ATRA) and 9-cis-retinoic acid are retinoids that bind to retinoic acid receptor (RAR) and/or retinoid X receptor (RXR) subtypes (α, β and γ) and regulate cell proliferation and differentiation. The exact physiological role of the subtypes remains unclear but retinoids are used in the treatment of dermatological disorders and certain types of cancer. These agents exhibit side effects that may result from activation of the various isoforms of RARs. Accordingly, ligands that exhibit selectivity for RAR subtypes would provide valuable tools to characterize the precise role of the receptors and may be used as therapeutic agents with fewer side effects. In the present work the chromone and flavone nuclei were used in the design of novel ligands based on molecular modeling studies and SAR data. Synthetic routes to the proposed ligands were devised and the compounds were screened for the ability to inhibit the binding of [3H]-ATRA.



180.

CONFORMATIONALLY DEFINED RETINOIC ACID ANALOGS: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS FOR RING-SUBSTITUTED ANALOGS OF 9CUAB30. ***Anil M. Deshpande***[1], *Venkatram R. Atigadda*[1], *Kimberly K. Vines*[1], *Michael Xia*[2], *Xiao-kun Zhang*[3], *Donald D. Muccio*[1], *and Wayne J Brouillette*[1]. *(1) Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294, Fax: 205-934-2543, anilmd@uab.edu, (2) Department of chemistry, University of Alabama at Birmingham, (3) Burnham Institute*

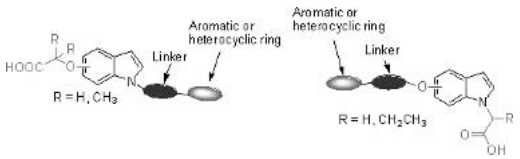
Retinoids that are selective ligands for retinoid X receptors (RXRs) offer promise as breast cancer chemopreventive agents. We have previously reported 9cUAB30, an RXR selective retinoid, as an effective breast cancer chemopreventive agent with minimal toxicity. In order to improve the RXR selectivity and chemopreventive potency we proposed the syntheses of a homologous series of ring-substituted analogs of 9cUAB30. We believed that RXR potency might be enhanced by increased hydrophobic interactions between the substituents on the

ring and the residues of the nuclear receptor's ligand binding domain. We herein present the synthesis of methyl analogs of 9cUAB30 and their structure-activity relationship studies for nuclear receptor binding and transcriptional activity.

181.

DESIGN AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL INDOLE-BASED PPAR AGONISTS AS ANTIDIABETIC AGENTS. ***Hsing Pang Hsieh**, Neeraj Mahindroo, Chiung-Chiu Wang, Chien-Fu Huang, Tzu-Wen Lien, Chia-Hua Tsai, Yi-Huei Peng, Ling-Hui Lee, Ekambaranellore Prakash, Wei-Chen Chen, Yi-Wei Chang, Tsu-An Hsu, Xin Chen, Su-Ying Wu, Chiung-Tong Chen, Shih-Jung Lan, and Yu-Sheng Chao, Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 7F, 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, Fax: 886-37-586-401, hphsieh@nhri.org.tw*

Type 2 diabetes, a chronic disease characterized by failure to respond to insulin, has assumed epidemic proportion. The single most important contributor to the pathogenesis of diabetes is obesity, which is increasing at a staggering pace with changing life styles and food habits. The peroxisome proliferators-activated receptor-g agonists, Rosiglitazone and Pioglitazone, registered an increase in sales by 73.2 and 53.1% respectively in 2001. But the mechanism based side effects including weight-gain, fluid retention and edema besides adipose tissue proliferation, fatty changes in bone marrow and significant increase in heart weight of rodents have triggered a reevaluation of the design of PPAR agonists. The addition of PPARa agonist activity could improve the profile of the PPARg agonists. Treatment with PPARd agonists retards weight gain and improves insulin resistance thus demonstrating its potential therapeutic value in diabetes and obesity. PPAR pan-agonist could represent a significant novel potential drug for diabetes and obesity with anti-hyperglycemic, lipid modulating and insulin sensitizing activity.

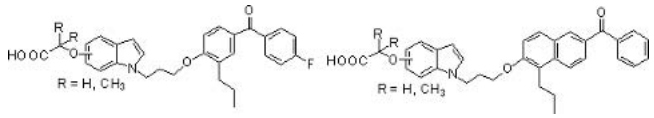


The design, synthesis, structure-activity relationships and co-crystal studies with PPARg protein of novel highly potent indole based PPAR agonists would be reported. Compounds were prepared from commercially available indole scaffolds with desired chemical groups attached at various positions on the nucleus. The synthesized compounds were evaluated for binding with PPARa, g and d receptors using binding and transactivation assays. The compounds have shown potent PPAR agonist activity in low nM range and excellent pharmacokinetic profile. The structure-activity relationships have been deduced based on the in-vitro activity and the co-crystal studies. Two of the compounds are presently undergoing in-vivo studies and have shown activity better than the reference compounds in the preliminary results.

182.

DESIGN, SYNTHESIS AND SAR OF INDOLE-BASED PPAR AGONISTS. *Hsing Pang Hsieh, Neeraj Mahindroo, **Mohane S Coumar**, Chiung-Chiu Wang, Chien-Fu Huang, Tzu-Wen Lien, Chia-Hua Tsai, Ying-Ting Lin, Ling-Hui Lee, Ekambaranellore Prakash, Tsu-An Hsu, Xin Chen, Su-Ying Wu, Chiung-Tong Chen, and Yu-Sheng Chao, Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 7F, 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, Fax: 886-37-586-401, hphsieh@nhri.org.tw, mohane@nhri.org.tw*

Type II diabetes is one of the most common chronic diseases with estimated 194 million patients worldwide and the number expected to double by 2030. The disease is largely associated with obesity, which contributes to insulin resistance. Peroxisome proliferator-activated receptors (PPAR) are members of nuclear hormone receptor superfamily. The PPARg agonists cause insulin-dependent reduction in circulating blood glucose. Successful treatment of insulin resistance with PPARg agonists such as pioglitazone and rosiglitazone has generated tremendous interest in the development of new drug candidates for this class. Moreover presence of side effects such as weight gain in the patients treated with these drugs warrants development of newer drugs with better pharmacological and safety profiles.



In an effort to identify novel PPAR agonists, we have rationally designed a series of compounds with indole as core skeleton. Compounds were prepared from commercially available indole scaffolds with desired chemical groups attached at various positions on the nucleus and a newly identified benzophenone and naphthophenone groups as the hydrophobic tail. The synthesized compounds were evaluated for binding with PPARa, g and d receptors using scintillation proximity assay and transactivation assay. The compounds have shown potent PPAR agonist activity in low nM range. From the results we were able to delineate the structural requirements for both selective PPARa and PPARa/g dual activity in this series of compounds. The most potent compound showed an EC50 of 32nm at PPARa with 80 fold selectivity over PPARg. While the most potent PPARa/g dual agonist showed EC50 ~200nm at both receptors. The molecular docking studies in the PPARg protein for these compounds were also carried out. Based on these results two of the compounds were selected for in-vivo studies.

183.

FLUORESCENCE BASED HIGH THROUGHPUT ASSAYS FOR IDENTIFICATION OF PPARγ MODULATORS. ***Mohammed Saleh Shekhani**, **Hildegard C. Eliason**, David A. Lasky, Naveeda Qadir, Steven R. Duff, Kevin L. Vedvik, Leah J. Aston, Steven Hayes, and Kurt W Vogel, Invitrogen Corporation, 501 Charmany Drive, Madison, WI 53719, Mohammed.Shekhani@Invitrogen.com, Hildegard.Eliason@Invitrogen.com*

The Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) is a ligand activated transcription factor in the nuclear receptor gene superfamily. PPARγ regulates genes involved in glucose and lipid metabolism and therefore it is a validated drug target for increasingly prominent diseases such as diabetes and obesity. Invitrogen has developed a fluorescence polarization (FP) based assay for high throughput screening of potential PPARγ modulators. The binding affinity of a test compound to the receptor is quantified by its ability to displace a fluorescent ligand from the binding pocket of PPARγ. The polarization of the fluorescent ligand is a direct measure of the fraction bound to PPARγ, and hence permits the determination of the test compound's potency at equilibrium. A red-shifted fluorescent ligand has also been developed to reduce potential compound fluorescence artifacts. Other approaches to further reduce compound fluorescence and light scattering artifacts are currently being investigated.

184.

NOVEL DUAL ACTIVATORS OF PPAR- α AND γDERIVED FROM BENZOXAZINONE CONTAINING THIAZOLIDINE DIONES HAVING ANTIDIABETIC AND HYPOLIPIDEMIC POTENTIAL. ***G. R. Madhavan**[1], Ranjan Chakrabarti[2], K. Anantha Reddy[1], P. Bheema Rao[1], V. Balraju[1], B.M. Rajesh[1], and R. Rajagopalan[2]. (1) Discovery chemistry, Metabolic disorder project group, Dr.Reddy's laboratories-Discovery Research, Bollarum Road, Miyapur, Hyderabad 500049, India, Fax: +91-40-23045438, madhavangr@drreddys.com, (2) Discovery Biology, Metabolic disorder project group, Dr.Reddy's laboratories-Discovery Research*

The Peroxisome prolifirator activated receptors (PPAR's) are the members of the nuclear hormone receptor family of transcription factors involved in regulation of lipid and glucose metabolism. The PPAR-α is reported to be primarily involved in hepatic lipid metabolism, whereas PPAR-γ plays a central role in adipogenesis and glucose homeostatis. The dual PPAR-α and γ activators are being developed to treat both hyperglycemia and hyperlipidemia associated with type-II diabetes. Antidiabetic thiazolidine diones are known to activate PPAR-γ and thereby reduces glucose levels while simultaneously reducing circulating insulin and free fatty acids. In our program while developing a potent PPAR-γ activator thiazolidine diones, we come across a series of benzoxazinone derivative of thiazolidine diones having good PPARα activity in addition to PPAR-γ. There are only few reports of thiazolidine diones having PPAR-α activity. In the SAR we have changed the substitutions on 2nd position of 1,3-benzoxazin one ring to ethyl and propyl chain and also introduced phenyl ring. We have changed the position of 'Oxygen atom' in order to form a 2,3-benzoxazinone ring. Among all the derivatives, DRF-2519 (5-{4-[2-([1,3]ben-

zoxazin-4(3H)-one-3-yl)ethoxy]benzyl} thiazolidine-2,4-dione) is the best molecule having dual PPAR-α and γ activation. DRF-2519 was normalizing plasma glucose (PG) levels in ob/ob mice (PPAR γ animal model) at a dose of 3mg/kg. In high fat fed rat (PPARα animal model) it has shown a significant reduction in Triglycerides, LDL-cholesterol (ED50= 2.8 and 6.5mg/kg respectively) and increase in HDL-cholesterol (ED50=7.4mg/kg). Synthesis of benzoxazinone containing thiazolidine diones and their in-vitro SAR and in-vivo activity will be presented.

185.

TROGLITAZONE AND ITS DERIVATIVES INDUCE DEGRADATION OF CYCLIN D1 THROUGH A PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA-INDEPENDENT MECHANISM IN BREAST CANCER CELLS. ***Jui-Wen Huang*, *Chung-Wai Shiau***, *Ya-Ting Yang, Kuen-Feng Chen, Samuel K Kulp, and Ching-Shih Chen, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Ave, Columbus, OH 43210, Fax: 614-688-8556, huang.373@osu.edu, shiau.4@osu.edu*

Cyclin D1 overexpression has been implicated in oncogene-induced mammary tumorigenesis as it is detected in over 50% of primary breast carcinomas and is correlated with poor prognosis. It has been reported that troglitazone (TG), a peroxisome proliferator-activated receptorγ(PPARγ) agonist, can induce degradation of cyclin D1 as part of its mechanism for causing cell cycle arrest and growth inhibition in breast cancer cells. In this study, we obtained evidence that the ability of high doses of TG to repress cyclin D1 is independent of PPARγ activation. First, a PPARγ-inactive TG analogue (δ2-TG) causes cyclin D1 ablation with potency similar to that of TG in MCF-7 cells. Secondly, MDA-MB-231 breast cancer cells, which exhibit higher PPARγ expression, are less sensitive to this TG-induced cyclin D1 down-regulation than MCF-7 cells. In addition, our data also indicate that TG- and δ2-TG-induced cyclin D1 repression is mediated via proteasome-facilitated proteolysis as it can be inhibited by multiple proteasome inhibitors, including MG132, lactacystin, and epoxomicin, and is preceded by increased ubiquitination. The dissociation of these two pharmacological activities, i.e., PPARγ activation and cyclin D1 ablation, provides a molecular basis to use δ2-TG as a scaffold to develop a novel class of cyclin D1-ablative agents. Accordingly, a small library of δ2-TG derivatives has been synthesized. Among derivatives in this library, δ2-TG-28 represents a structurally optimized agent with potency an-order-of-magnitude higher than that of δ2-TG in cyclin D1 repression and MCF-7 cell growth inhibition.

186.

FLUOROMETRIC AND LC-MS INVESTIGATION ON PROOXIDANT AND ANTIOXIDANT ACTIVITIES OF NO. ***Qian Li*[1]**, *Adrian C. Nicolescu[2], and Gregory R.J. Thatcher[1]. (1) Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, M/C 781, Chicago, IL 60612, Fax: 312-996-7107, qianli@uic.edu, (2) Department of Chemistry and Pharmacology & Toxicology, Queen's University*

Nitric Oxide (NO) has variously been proposed as a cytotoxic agent and as a cytoprotective agent, possessing both prooxidant and antioxidant properties. A fluorometric method based on the use of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY 581/591 C11) as an indicator, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP) as a peroxyl radical generator has been developed to evaluate the antioxidant activities of series of NO donors, including NONOates, thiolnitrites, classic organic nitrite and nitrate in 40% acetonitrile / 60% 10mM phosphate buffer (50mM NaCl), pH 7.4 at 37°C. This assay was validated by measuring the antioxidant activities of other classes of antioxidants, including well-characterized (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox C), cysteine, human plasma, human serum albumin, and serotonin. All of NO donors showed concentration-dependent inhibition on the BODIPY fluorescence decay induced by ABAP. Three semi quantitative methods have been used to quantify their inhibitory efficacy. NO itself is a potent antioxidant, whereas, peroxynitrite is a potent cytotoxic oxidant. Herein, peroxynitrite and its reliable biomimetic source, 3-morpholinosydnonimine hydrochloride (SIN-1) were used instead of ABAP to induce BODIPY fluorescence decay. Glutathione (GSH), cysteine and NO donors showed concentration-dependent antioxidant and prooxidant effects on the induction. To understand the molecular mechanism responsible for our results, the reaction products of BODIPY with ABAP, peroxynitrite, SIN-1 and of GSH with peroxynitrite at flurometric assay condition were analyzed by LC-MS. Besides the oxidation products as expected, nitration was found for peroxynitrite reaction with BODIPY and nitrosation with GSH.

187.

NON-*SECO*-STEROIDAL VITAMIN D_3 ANALOGS BEARING A DICARBA-*CLOSO*-DODECABORANE. ***Hiroyuki Kagechika*[1]**, *Kyoko Yaguchi[2], Chalermkiat Songkram[1], Aya Tanatani[3], Yoshiyuki Taoda[2], Tomohiro Yoshimi[2], Emiko Kawachi[1], and Yasuyuki Endo[4]. (1) School of Biomedical Science, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan, Fax: +81-3-5280-8127, kage.omc@tmd.ac.jp, (2) Graduate School of Pharmaceutical Sciences, The University of Tokyo, (3) Institute of Molecular and Cellular Biosciences, The University of Tokyo, (4) Tohoku Pharmaceutical University*

1α,25-Vitamin D_3 (1,25-VD_3), an active metabolite of vitamin D_3, plays significant role in cell differentiation and proliferation as well as in calcium-phosphorus homeostasis. The development of novel synthetic analogs have attracted much attention in the field of dermatology and oncology. According to our successful results that icosahedral carboranes (dicarba-*closo*-dodecaboranes) act as useful hydrophobic pharmacophore in the structures of nuclear receptor ligands, we designed and synthesized 1,25-VD_3 analogs bearing a carborane moiety. General structure composes of a *para*-carborane having two substituents on the carbon atoms, where the carborane moiety corresponds to the CD ring of the 1,25-VD_3. The biological activity of the synthesized compounds was examined in terms of the ability to induce differentiation of human promyelocytic leukemia cells HL-60 and competitive binding assay to nuclear vitamin D_3 receptor (VDR). Some carborane derivatives exhibited potent differentiation-inducing activity and high affinity to VDR. The structure-activity relationships will be discussed.

188.

PHENYLACETAMIDES AS LXR AGONISTS. ***Ginger XuQiang Yang*[1]**, *Zao Hu[2], HIroo Koyama[1], Lyndon Mitnaul[3], Jayne Chin[3], Carl Sparrow[3], Joel P. Berger[4], Taro E. Akiyama[4], and Soumya P Sahoo[2]. (1) Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co, P.O. Box 2000, Rahway, NJ 07065, Fax: 732-594-9556, ginger_yang@merck.com, (2) Department of Medicinal Chemistry, Merck, (3) Department of Atherosclerosis and Endocrinology, Merck Research Laboratories, (4) Department of Metabolic Disorder, Merck Research Laboratories*

LXR ligands have been targeted as potential HDL raising and cholesterol lowering anti-atherogenic agents. The majority of LXR target genes appear to have the beneficial biological functions of removal of excess of cholesterol through efflux, catabolism or decreased absorption. Unfortunately, activation by LXR on certain lipogenic genes is thought to cause hypertriglyceridemia. The current consensus is that the most desirable LXR modulator should be a strong inducer of ABCA1 and apoE expression, yet lacks the activity on the SREBP-1 and FAS promoters. Merck's in house screening effort identified compound 1 ({3-chloro-4-[(3-{[7-propyl-3-(trifluoromethyl)-1,2-benzisoxazol-6-yl]oxy}propyl)thio]phenyl}acetic acid) as a lead. Compound 1 is a potent PPAR pan-agonist (PPAR (spa) IC50: a = 310 nM, d = 10 nM, g = 140 nM) as described in a recent publication from our laboratory. This poster will describe the optimization of this lead from a potent PPAR agonist into a series of potent LXR ligands without the PPAR activity.

189.

SAR OF HIGHLY POTENT FULL-RANGE MODULATORS OF THE FARNESOID X RECEPTOR. ***Brenton T. Flatt*[1]**, *Jeffrey D. Kahl[1], Brett B. Busch[1], Erik Boman[1], Amy Liu[2], Peter Ordentlich[2], Grace Yan[2], Raju Mohan[1], and Richard Martin[1]. (1) Department of Chemistry, Exelixis, Inc, 4757 Nexus Centre Drive, Suite 200, San Diego, CA 92121, bflatt@exelixis.com, (2) Department of Lead Discovery, Exelixis, Inc*

The farnesoid X receptor (FXR) is a nuclear receptor expressed in tissues exposed to high concentrations of bile acids such as the liver, kidney and intestine and functions as a bile acid sensor. FXR regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of lipids, cholesterol and bile acid homeostasis. Regulation of FXR through small-molecule drugs represents a promising therapy for diseases resulting from lipid, cholesterol and bile acid abnormalities. We identified a series of novel small molecule heterocycles by high throughput screening and

optimized these leads into potent and efficacious FXR modulators that display a range of efficacies in FXR-functional cell based assays from full agonists to partial agonists and full antagonists.

190.
ELECTROSPUN POLYETHYLENIMINE DIAZENIUMDIOLATES FOR THE CONTROLLED DELIVERY OF NITRIC OXIDE. ***mahesh bhide**, Department of Chemistry, The University of Akron, OH, 2630 shoreline drive, #B3, Akron, OH 44314, maheshswara@yahoo.com, Wilmarie Flores-Santana, Department of Chemistry, University of Akron, and Dr. Daniel J. Smith, Department of Chemistry, The University of Akron*

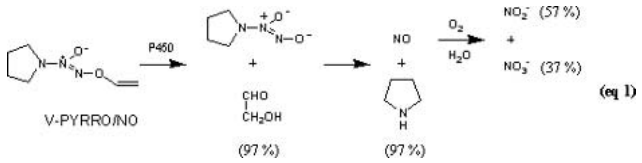
Electrospun polymeric matrices have been fabricated using entrapped linear or branched polyethylenimine diazeniumdiolates for the controlled delivery of nitric oxide (NO). Electrospinning allows entrapping or encapsulating soluble or insoluble additives within the polymeric nanofiber. Microparticles were formulated using linear or branched polyethylenimine diazeniumdiolates suspended in polymeric solution and electrospun into polymeric nanofiber matrices. Linear polyethyleneimine (M.W. 200,000) was suspended in acetonitrile and modified with NO for 4 days. Similarly branched polyethylenimine (M.W.10000), was cross linked with 1, 4, diglycidyl ether in ethanol-mineral oil emulsion at 80°C. The resulted microparticles were then suspended in a methanol-methoxide solvent and modified with NO for 4 days. The yellow microparticles from both modifications were electrospun in hydrophilic (Tecophilic®) or hydrophobic (Tecoflex®) polymers using suitable solvents. Upon hydration these matrices generate NO. Various matrices were fabricated, incorporating buffer and/or super absorbent polymer, and the effects of these additives in the release of NO was measured.

191.
MECHANISM STUDIES OF NITRIC OXIDE SYNTHASE INACTIVATION BY AMIDINES. IMPLICATIONS FOR NITRIC OXIDE SYNTHASE AND HEME OXYGENASE MECHANISMS. ***Yaoqiu Zhu** and Richard B. Silverman, Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208-3113, zhuyq@northwestern.edu*

N-(3-(Aminomethyl)benzyl)acetamidine (1400W) was reported to be a slow, tight binding and highly selective inhibitor of inducible nitric oxide synthase (iNOS) in vitro and in vivo. LC-MS analysis of the incubation mixture of iNOS with 1400W shows both loss of heme cofactor and formation of biliverdin, as was previously observed for iNOS inactivation by another amidine-containing compound, N5-(1-iminoethyl)-L-ornithine (L-NIO). Based on experimental studies of the heme degradation process of iNOS inactivation by both L-NIO and 1400W, a mechanism is proposed in which the amidine inactivators of iNOS bind as does substrate L-arginine, but because of the amidine methyl group, the heme peroxy intermediate cannot be protonated, thereby preventing its conversion to the heme oxo intermediate. This leads to a change in the enzyme mechanism to one that resembles that of heme oxygenase, an enzyme known to convert heme to biliverdin IXα. This appears to be the first example of a compound that causes irreversible inactivation of an enzyme without itself becoming modified in any way. Our mechanistic studies on iNOS inactivation also support: (1) the proton donor of the heme peroxy intermediate in NOS catalytic mechanism is the guanidine amino group of L-arginine; (2) the mechanism for heme α-meso-hydroxylation in NOS inactivation by amidines (and probably for heme oxygenase catalytic reaction) should favor a nucleophilic aromatic substitution mechanism.

192.
METABOLISM OF A LIVER-SELECTIVE NITRIC OXIDE-RELEASING AGENT, V-PYRRO/NO, BY HUMAN MICROSOMAL CYTOCHROMES P450. ***Keiko Inami**[1], Raymond W. Nims[1], Aloka Srinivasan[1], Michael L. Citro[2], Joseph E. Saavedra[2], Arthur Cederbaum[3], and Larry K. Keefer[1]. (1) Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Building 538, Frederick, MD 21702-1201, Fax: 301-846-5946, inami@ncifcrf.gov, (2) Basic Research Program, SAIC-Frederick, Inc, (3) Mount Sinai School of Medicine, The Mount Sinai Hospital*

O[2]-Vinyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO), a prodrug designed to release the versatile bioeffector nitric oxide (NO) in cytochrome P450-rich tissues, has been shown to protect liver and kidney from various toxins. To confirm and further elucidate the role of P450 (CYP) in organ protection, we probed the interaction of V-PYRRO/NO with various cDNA-expressed human CYP proteins (CYP 1A1, 1A2, 2A6, 2B6, 2E1, and 3A4). V-PYRRO/NO was metabolized preferentially by CYP2E1. In the presence of CYP2E1 and reducing equivalents, pyrrolidine, glycolaldehyde, and the NO breakdown products nitrate and nitrite were obtained in virtually quantitative yields as metabolites of V-PYRRO/NO. The results confirm the involvement of cytochromes P450 (especially CYP2E1) in the oxidative metabolism of V-PYRRO/NO as shown in eq. 1. (NO1-CO-12400)



193.
SYNTHESIS OF NEW SUGAR-NO DONOR CONJUGATES. ***Tingwei Bill Cai**[1], Xiaoping Tang[1], and Peng George Wang[2]. (1) Department of Chemistry, The Ohio State University, 100 W18th Ave, Columbus, OH 43210, (2) Departments of Biochemistry and Chemistry, The Ohio State University*

In order to achieve site specific delivery of nitric oxide (NO), a new class of glycosidase activated NO donors has been developed. Glucose, galactose and N-acetylneuraminic acid were covalently coupled to 3-morphorlinosydnonimine (SIN-1), a heterocyclic NO donor, via a carbamate linkage. The enzymatic cleavage and NO releasing ability of the new sugar-NO conjugates in the presence of glycosidases were also studied. Such NO prodrugs may be used as enzyme activated NO donors in biomedical research.

194.
STRUCTURE–BASED MODIFICATION OF INDOMETHACIN AS CYCLOOXYGENASE–2 INHIBITING NITRIC OXIDE DONOR. ***S. -J. Wey**, M. E. Augustyniak, E. D. Cochran, J. L. Ellis, X. -Q. Fang, D. S. Garvey, D. R. Janero, L. G. Letts, A. M. Martino, T. L. Melim, M. G. Murty, S. K. Richardson, J. D. Schroeder, W. M. Selig, A. M. Trocha, D. V. Young, and I. S. Zemtseva, NitroMed Inc, 125 Spring Street, Lexington, MA 02421, Fax: 781-274-8083, swey@nitromed.com*

Indomethacin, a non-selective cyclooxygenase inhibitor, was modified in three different regions to assess the cyclooxygenase-2 selectivity and to attach a nitric oxide donor to enhance drug safety profiles. Modifications on the 2-acetic acid part of indomethacin successfully increased the cycloxygenase-2 selectivity. The COX-2 selectivity from the structure modification, GI-safety profile and the benefit of the nitric oxide donor will be presented.

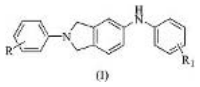
195.
DESIGN AND SYNTHESIS OF FUNCTIONALIZED CYCLODEXTRINS AS INHIBITORS OF AMYLOID-β-PEPTIDE DERIVED TOXINS. ***Zhiqiang Wang**[1], Patricia A. Fernandez[1], Lei Chang[2], William L. Klein[2], Duane L. Venton[1], and Gregory R.J. Thatcher[1]. (1) Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612-7231, Fax: 312-996-7107, wangzhi@uic.edu, (2) Department of Neurobiology and Physiology, Northwestern University*

Compelling evidences suggest that Alzheimer's disease (AD) is attributable to neuron dysfunction and death triggered by amyloid-β-peptide oligomeric assemblies (e.g. ADDLs). Clearly, a search for the drugs targeting the amyloid-β-peptide derived toxins represents a new and potentially important approach to the treatment of AD. Through a high throughput dot blot assay of libraries of β-cyclodextrin derivatives, a per-substituted-β-cyclodextrin was identified as the inhibitor of ADDLs formation. Various regions of the lead molecule were optimized using structure-based design and a series of functionalized cyclodextrins bearing amino pendent groups were synthesized. The preliminary data suggests that minor changes in substitution patterns on the primary face of the ACD molecule can alter the $A\beta_{1\text{-}42}$ self-assembly pathway. It also suggests that ACDs modified on the primary and secondary face of the molecule could be engineered to lower brain ADDLs levels as well as providing probes to better understand the pathways to $A\beta_{1\text{-}42}$ self-assembly.

196.

SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIP OF SUBSTITUTED ISOINDOLINE ANALOGS AS AMYLOID AGGREGATION INHIBITORS. ***Annette T. Sakkab-Tan***[1], *Corinne E. Augelli-Szafran*[1], *Chung Choi*[1], *Yingjie Lai*[1], *Harry Levine III*[2], *Jared Milbank*[1], *Peter Orahovats*[1], *Tomoyuki Yasunaga*[3], *and Yuyang Ye*[2]. *(1) Medicinal Chemistry, Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Annette.Sakkab-Tan@pfizer.com, (2) Medicinal Chemistry and CNS Pharmacology Departments, Pfizer Global Research and Development, (3) Tsukuba Research Center, Yamanouchi Pharmaceutical Co., Ltd*

It is believed that ß-amyloid formation is a key event in the development of Alzheimer's disease. Amyloidosis is characterized by the accumulation of fibrillar proteins with a ß-pleated sheet conformation in the tissues of a patient. Current therapies for the treatment of amyloidosis attempt to remove the source of precipitating ß-amyloid with drugs that inhibit protein synthesis. As a result of our research efforts to inhibit the aggregation of the β-amyloid protein, a series of isoindoline analogs (I) have been identified. The synthesis and structure-activity relationship of this series of compounds will be discussed.



(I)

197.

GENISTEIN, A NATURAL PRODUCT FROM SOY, IS A POTENT INHIBITOR OF TRANSTHYRETIN AMYLOIDOSIS. ***Nora S. Green***, *Department of Chemisty, Randolph-Macon College, Box 5005, Ashland, VA 23005, ngreen@rmc.edu, Ted Foss, Department of Chemistry, The Scripps Research Institute, and Jeffery W. Kelly, Department of Chemistry, Scripps Research Institute*

Rate limiting tetramer dissociation and partial monomer denaturation of transthyretin (TTR) is sufficient for TTR misassembly into amyloid associated with three diseases: senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), and familial amyloid cardiomyopathy (FAC). Small molecules can bind to one or both of the unoccupied TTR thyroid hormone binding sites and raise the kinetic barrier for tetramer dissociation. Genistein, the major isoflavone natural product in soy, works in this fashion and is an excellent inhibitor of transthyretin tetramer dissociation, reducing acid-mediated fibril-formation to less than 10% of that exhibited by TTR alone. Genistein also inhibits the amyloidogenisis of the most common FAP and FAC mutations: V30M and V122I, respectively. Isothermal titration calorimetry (ITC) shows that genistein binds to TTR with negative cooperativity (75 nM and 1.24 µM). Genistein exhibits highly selective binding to TTR in plasma over all the other plasma proteins. The benefits of employing genistein to treat the transthyretin amyloidoses include known oral bioavailability and safety data.

198.

IDENTIFICATION OF γ-SECRETASE INHIBITORS DERIVED FROM 2,4,6-TRISUBSTITUTED TRIAZINE SCAFFOLDS. ***Dean G. Brown***, *Frances M. McLaren, Reed W. Smith, Joseph Cacciola, Ashok B. Shenvi, Margaret J. Schooler, James B. Campbell, Barry D. Greenberg, Cynthia D. Sobotka-Briner, Michael P. DeMartino, and Robert T. Jacobs, CNS Discovery, AstraZeneca Pharmaceuticals, 1800 Concord Pike, Wilmington, DE 19850, Fax: 302-886-5382, dean.brown@astrazeneca.com*

γ-Secretase has been idenitifed as a trans-membrane peptidase that contributes to the processing of amyloid precursor protein (APP). Proteolytic cleavage of APP may lead to the accumulation of αβ40 and αβ42, shortened peptides prone to self-aggregation. These aggegrated peptides are found in the brain among the plaque deposits associated with Alzheimer's disease. A significant effort across the pharmaceutical industry has been placed in identifying compounds that inhibit proteolysis of APP. We will report a new class of γ-secretase inhibitors, built from a 2,4,6-trisubstitued triazine scaffold. We will present the development of the triazine series and the translation of the SAR to new scaffolds such as pyrimidine and pyridine.

199.

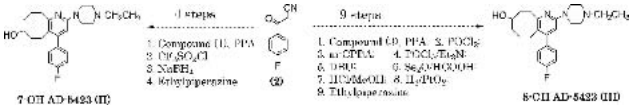
STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF NOVEL TISSUE TRANSGLUTAMINASE (TGASE 2) INHIBITORS. *Eric Duval, April Case, Ross L. Stein, and* ***Gregory D. Cuny***, *Laboratory for Drug Discovery in Neurodegeneration / Harvard Center for Neurodegeneration and Repair, Brigham and Women's Hospital and Harvard Medical School, 65 Landsdowne Street, 4th Floor, Cambridge, MA 02139, Fax: 617 768 8606, gcuny@rics.bwh.harvard.edu*

Transglutaminases (TGases) are a family of Ca^{2+}-dependent enzymes that catalyze the formation of isopeptide bonds between the carboxamide group of protein/peptide-bound glutamine residues and the ε-amino group of protein/peptide-bound lysine residues to form N $^{\varepsilon}$-(γ-L-glutamyl)-L-lysine cross links with loss of ammonia. The TGase 2 (i.e. tissue transglutaminase) isozyme is involved in several general biological functions, including apoptosis, cell adhesion and signal transduction. In addition, this particular isozyme has been most soundly linked to celiac disease, Alzheimer's and Huntington's diseases. Thieno[2,3-d]pyrimidin-4-one acylhydrazide derivatives were discovered as inhibitors (IC_{50} 0.16 µM) of TGase 2 utilizing a fluorescence-based assay that measured TGase 2 catalyzed incorporation of the dansylated Lys derivative α-N-Boc-Lys-CH_2-CH_2-dansyl into the protein substrate N,N-dimethylated-casein. This presentation will detail a structure-activity relationship (SAR) study of thieno[2,3-d]pyrimidin-4-one acylhydrazide derivatives as TGase 2 inhibitors.

200.

SYNTHESES AND PROPERTIES OF THE MAJOR HYDROXY METABOLITES IN HUMANS OF BLONANSERIN (AD-5423, A NOVEL ANTIPSYCHOTIC AGENT). ***Takeshi Ochi***, *Masato Sakamoto, Akira Minamida, Hiroshi Toda, Tomohiko Ueda, Kenji Suzuki, Teruaki Une, Kazuya Matsumoto, and Yoshiaki Terauchi, Pharmaceutical Research & Technology Center, Dainippon Pharmaceutical Co., Ltd, 5-51, Ebie, 1-chome, Fukushima-ku, Osaka 553-0001, Japan, Fax: 06-6455-8356, takeshi-ochi@dainippon-pharm.co.jp*

The two major metabolites (II and III) in human of 2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta[b]pyridine (AD-5423) (I), a novel antipsychotic agent, were synthesized. The first, 7-OH AD-5423 (II), was synthesized via a four step process starting from 1,5-cyclooctanedione (1) and 4-fluorobenzoylacetonitrile (2), and the second, 8-OH AD-5423 (III), a nine step process from cyclooctanone (3) and (2). The optical resolution, structures, and biological results of these metabolites (II and III) were also described.



201.

SYNTHESIS AND IN VIVO EVALUATION OF SELENOFLAVANONE : A NEW CLASS OF NEUROPROTECTIVE AGENT. *Dong-myung Kim*[1], *Jong-Hoon Ryu*[2], *and* ***Jin-Hyun Jeong***[1]. *(1) College of Pharmacy, Kyung Hee University, 1# Heogi-dong, Dongdaemun-ku, Seoul, South Korea, Fax: 82-2-961-0357, taataa@hanmail.net, jeongjh@khu.ac.kr, (2) College of Phamarcy, Kyung Hee University*

Selenoflavanone and Flavanone derivatives have been synthesized and evaluated for neuroprotection activity. Heterocyclic compounds with oxygen atoms are known to possess potent biological effects. The flavonoids, isoflavonoids, and coumarins which form the bulk of these compounds are quite polar and have limited use as drugs that pass through membranes. The non-polar property is increased by substituting oxygen with selenium in the heterocyclic compound. Our group focused on synthesizing selenoheterocyclic compounds with more non-polar properties. In the Selenoflavanone treated group, the anterior and medial parts of the striatum, and large areas of the cortex, remained unaffected compared with the control group. The total infarction volumes in the ipsilateral hemisphere of ischemia-reperfusion mouse were significantly reduced by Selenoflavanone treatment.

202.

SYNTHESIS AND NEUROPROTECTIVE EFFECT OF NEU2000 AGAINST RAT TRANSIENT ISCHEMIC STROKE MODELS. *Sung-Hwa Yoon[1], **HoJoon Park**[1], JaeWoo Lee[1], ByoungJoo Gwag[2], and YoungAe Lee[3]. (1) Department of Molecular Science and Technology, Ajou University, PaldalGu WonchunDong, Suwon 443-749, South Korea, Fax: 31-219-2516, shyoon@ajou.ac.kr, pkhead99@ajou.ac.kr, (2) Department of Pharmacology, College of Medicine, Ajou University, (3) Research Center, Neurotech Company*

It is known that excitotoxicity through NMDA receptor action evokes rapid neuronal death within a few hours and free radicals evoke following slowly evolving neuronal death in ischemic stroke. In an effort to develop a novel neuroprotective agent, we synthesized a series of N-substituted aminosalicylic acid. Among this series, 2-hydroxy-5-(4-trifluoromethyl-2,3,5,6-tetrafluoro-phenyl)methylaminobenzoic acid (Neu2000) which exhibits potent antioxidant action and reversible weak NMDA antagonism significantly inhibited infarction volume of rat 30 min-transient middle cerebral artery occlusion (tMCAo) model (10 mg/kg, p.o.). The synthesis and biological evaluations of Neu2000 will be presented. Supported by Frontier Program in Korea.

203.

ALPHA- LIPOIC ACID-BASED PPAR GAMMA-AGONISTS FOR TREATING TYPE II DIABETES. ***Cassia S. Mizuno**[1], Amar G. Chittiboyna[1], Meenakshi S. Venkatraman[1], Mitchell A. Avery[1], Josef Meingassner[2], Christopher Ho[3], Stephen C. Benson[3], James Varani[4], Charles N. Ellis[4], Theodore W. Kurtz[5], and Harrihar A. Pershadsingh[6]. (1) Medicinal Chemistry, University of Mississippi, Faser Hall 417, University, MS 38677, Fax: 662-915 5638, cmizuno@olemiss.edu, (2) Novarts Forschungsinstitut GmbH, (3) California State University, Hayward, (4) University of Michigan, (5) University of California, San Francisco and University of California, Irvine, (6) University of California, Irvine and Bethesda Pharmaceuticals, Inc., Mill Valley*

Development of PPAR gamma agonists is an interesting target for treatment of type II diabetes once it controls glucose homeostasis and adipocyte differentiation. Novel thiazolidinedione PPAR gamma agonists, derivatives of alpha-lipoic (thioctic, 1,2-dithiolane) acid, were prepared. The prototype: N-(2-{4-[(2,4-dioxo(1,3-thiazolidin-5-yl)methyl]phenoxy}ethyl)-5-(1,2-dithiolan-3-yl)-N-methylpentamide BP1003 showed to be potent activator of PPAR gamma (EC_{50}= 2 nM). The same compound showed EC_{50} around 5 μM towards PPAR alpha. The hydrophobic BP1003 and the water soluble derivative BP1017 inhibited the proliferation of human peripheral lymphocytes, in vitro. The synthesis of these novel thiazolidinedione derivatives and its biological assay will be presented in this work.

204.

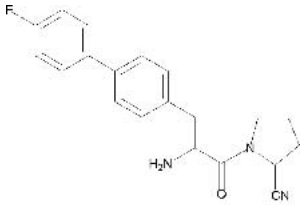
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF LISOFYLLINE (LSF) ANALOGS AS A POSSIBLE TREATMENT FOR TYPE 1 DIABETES. ***Peng Cui**[1], Timothy L. Macdonald[1], Meng Chen[2], Zandong Yang[2], and Jerry&L Nadler[2]. (1) Department of Chemistry, University of Virginia, McCormick Rd, P.O.Box 400319, Charlottesville, VA 22903, Fax: 434-982-2302, pc3n@virginia.edu, (2) Department of Medicine, University of Virginia*

Lisofylline (LSF, 1-(5-R-hydroxyhexyl)-3,7-dimethylxanthine), an anti-inflammatory agent, can protect β-cells from Th1 cytokine-induced dysfunction and reduce the onset of Type 1 diabetes in non-obese diabetic (NOD) mice. However, due to the poor oral bioavailability and weak potency, its clinical development has been limited. Our goal is to develop novel agents using LSF as a base molecule that will be more potent, selective and orally bioavailable. Our synthetic strategy is two fold. First, we held the side chain moiety (5-R-hydroxyhexyl) constant while substituting a variety of nitrogen-contained heterocyclic compounds. After investigating the core structures, we successfully identified phthalhydrazide as a lead for further optimization. The analog with this core structure showed satisfactory stability, bioavailability and safety as well as beneficial effects in human islets. Our current synthetic work is focused on optimization of the side chain structure, which will be presented.

205.

DISCOVERY OF DIPEPTIDYL PEPTIDASE IV (DPP-IV) INHIBITORS BY STRUCTURE-BASED DE NOVO DESIGN. ***Lei Qiao**, Christian Baumann, Carl Crysler, Nisha Ninan, Marta Abad, John Spurlino, Renee DesJarlais, Jukka Kervinen, Mike Neeper, Shariff Bayoumy, Robyn Williams, Ingrid Deckman, Bruce Tomczuk, and Kevin Moriarty, Johnson & Johnson, Pharmaceuticals Research and Development, L.L.C, 665 Stockton Drive, Exton, PA 19341, lqiao@prdus.jnj.com*

Inhibition of dipeptidyl peptidase IV (DPP-IV) is rapidly emerging as a novel therapeutic approach for the treatment of type 2 diabetes. DPP-IV, a serine protease, cleaves and inactivates glucagons-like peptide 1 (GLP-1). GLP-1 is an incretin hormone released from the gut during meals and serves as enhancer of glucose stimulated insulin release from the beta cells. Furthermore, GLP-1 also has been shown to stimulate insulin biosynthesis, inhibit glucagon secretion, and slow gastric emptying, each a beneficial effect in the treatment of diabetes. However, GLP-1 is very rapidly degraded in the bloodstream by DPP-IV. Therefore DPP-IV inhibition has been proposed as a new treatment of type 2 diabetes. A series of biaryl-based compounds (exemplified by structure 1, Ki = 3.1 nM) was discovered by structure-based de novo design as potent inhibitors of DPP-IV. The discovery, synthesis, structure-activity relationship, and X-ray crystallographic studies of this class of inhibitors will be presented.



1

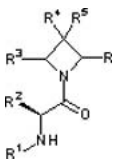
206.

DISCOVERY OF POTENT, SELECTIVE AND ORALLY BIOAVAILABLE PHENYLALANINE BASED DIPEPTIDYL PEPTIDASE IV INHIBITORS. ***Jinyou Xu**[1], Lan wei[1], Robert Mathvink[1], Jiafang He[1], You Jung Park[1], Huaibing He[1], Barbara Leiting[2], Kathryn A. Lyons[1], Frank Marsilio[2], Reshma A. Patel[2], Joseph K. Wu[2], Nancy A. Thornberry[2], and Ann E. Weber[1]. (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 glucose dependent insulinotropic 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 and GIP are rapidly degraded in plasma by the serine protease dipeptidyl peptidase IV (DPP-IV). Inhibition of DP-IV increases the levels of endogenous intact circulating GLP-1 and GIP. Therefore, inhibition of DPP-IV is rapidly emerging as a novel therapeutic approach to the treatment of type 2 diabetes. Herein, we would like to report the synthesis and biological activity of a novel series of phenylalanine based DPP-IV inhibitors. Optimized compounds exhibited excellent selectivity and good pharmacokinetic profiles.

207.

POTENT AND SELECTIVE AZETIDINE-BASED DIPEPTIDYL PEPTIDASE IV (DPP IV) INHIBITORS. ***Weixing Li**, Ed Oliver, Camilo Rojas, Susan Lauter, Bert Thomas, and Sergei Belyakov, Guilford Pharmaceuticals, Inc, 6611 Tributary Street, Baltimore, MD 21224, Fax: 410-631-6798, liw@guilfordpharm.com*



DPP IV is a serine protease that catalyzes the truncation of the N-termini of several peptides, resulting in the release of Pro- or Ala-containing dipeptides. It has become an intriguing drug target because of the potential to modulate

regulatory diseases, such as diabetes II, through inhibition of the enzyme. Over the last 20 years a wide range of DPP IV substrates has been mapped out, including important neuropeptides (e.g., neuropeptide Y, beta-casomorphin, or endomorphin). Alteration of neuropeptide depletion by DPP IV inhibition has therapeutic relevance to anxiety, depression, and psychosis. We have found a substantial inhibitory effect of peptidomimetics, derived from azetidine core, on DPP IV activity in vitro and in vivo. Discussion of SAR, stability, and relevant biological data for these inhibitors will be presented in the poster.

208.

HETEROARYL-O-GLUCOSIDES AS NOVEL SODIUM GLUCOSE CO-TRANSPORTER 2 (SGLT2) INHIBITORS. *Xiaoyan Zhang, Maud Urbanski, Mona Patel,* ***Roxanne Zeck****, Geoffrey Cox, Haiyan Bian, Bruce R. Conway, Mary Pat Beavers, Philip Rybczynski, and Keith Demarest, Johnson & Johnson Pharmaceutical Research & Development, L.L.C, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, xzhang11@prdus.jnj.com, rzeck@prdus.jnj.com*

Diabetes mellitus is a polygenic disorder characterized by chronic hyperglycemia associated with a deficiency in insulin action. In diabetic patients, one mechanism for protection against the adverse effects of high plasma glucose levels is the compensatory increase in urinary glucose excretion. In the kidney, plasma glucose is continuously filtered in the glomerulus and then reabsorbed in the proximal tubules by a class of transporters called the sodium-glucose co-transporters (SGLTs). An SGLT inhibitor in diabetic patients would be expected to aid in the normalization of plasma glucose levels by enhancing glucose excretion. Phlorizin, a natural SGLT specific inhibitor, provided proof of concept in vivo by promoting glucose excretion and lowering fasting and postprandial blood glucose without hypoglycemic side effects in several animal models. We have recently reported a series of Phlorizin analogues as selective SGLT2 inhibitors. In our search for novel structures, a series of novel benzofused heteroaryl-O-glucosides were identified as SGLT inhibitors. Synthetic methods to these compounds and SAR will be discussed.

209.

INDOLE-GLUCOSIDES AS NOVEL SODIUM GLUCOSE CO-TRANSPORTER 2 (SGLT2) INHIBITORS. *Xiaoyan Zhang, Maud Urbanski, Mona Patel,* ***Geoffrey Cox****, Haiyan Bian, Roxanne Zeck, Bruce R. Conway, Mary Pat Beavers, Philip Rybczynski, and Keith Demarest, Johnson & Johnson Pharmaceutical Research & Development, L.L.C, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, xzhang11@prdus.jnj.com, gcox3@prdus.jnj.com*

Inhibition of the sodium-glucose co-transporters (SGLTs) is a novel therapeutic approach in the treatment of type II diabetes. Efforts to further elucidate SAR within our previously disclosed series of benzofused heteroaryl-O-glucosides SGLT inhibitors led to the exploration of a series of indole-glucosides. Several potent SGLT inhibitors were identified with a range of SGLT1/SGLT2 selectivities. In vitro biological evaluation of the C-glycosides indicated the anomeric oxygen is important to the SGLT inhibitory activities. The synthesis and biological activity of these compounds will be presented.

210.

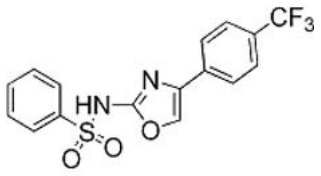
NOVEL ROUTE TO SITE-SPECIFIC CHEMICAL MODIFICATION OF INSULIN VIA PROINSULIN. ***Monica E. Puskas***[1]*, Diti Aggarwal*[2]*, and B. Radha Krishnan*[2]*. (1) Nobex Corporation, PO Box 13940, Research Triangle Park, NC 27709, (2) Chemistry Development and Manufacturing, Nobex Corporation*

The purpose of this study was to develop a site-selective modification route to make chemically modified insulin(s) using proinsulin as the precursor. Several types of proinsulins (insulin precursors) were modified by a chemical reaction with an amphiphilic oligomer. The modified proinsulin was cleaved systematically using enzyme cocktail. The major product, site-specific modified insulin, was purified by preparative RP-HPLC and clarified using tangential flow filtration. Modified insulin was then lyophilized to obtain powder and the structure was characterized by HPLC, peptide mapping and LC/MS analysis. Modification of proinsulin is a novel approach to production of site- selectively modified insulin. The C-peptide of proinsulin serves as natural protection group to block conjugation to other sites and upon enzyme cleavage provides a site-specific modified insulin without losses due to over-cleavage. The use of proinsulin as a starting material provides a facile, economical and commercially viable process to make modified insulin molecules.

211.

SYNTHESIS AND BIOLOGICAL EVALUATION OF SULFONAMIDOOXAZOLES AS SELECTIVE INHIBITORS OF THE 11B-HSD1, A POTENTIAL TREATMENT FOR DIABETES. ***Jason S. Xiang***[1]*, Ipek Manus*[1]*, Walt Massefski*[1]*, Vipin Suri*[2]*, May Tam*[3]*, Yuzhe Xin*[2]*, James Tobin*[4]*, Xin Xu*[5]*, John McKew*[1]*, and Steve Tam*[1]*. (1) Chemical & Screening Science, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, jxiang@wyeth.com, (2) Cardiovascular & Metabolic Disease, Wyeth Research, (3) Department of Cardiovascular and Metabolic Diseases, Wyeth, (4) Cardiovascular & Metabolic Diseases, Wyeth, (5) Drug Safety & Metabolism, Wyeth Research*

Glucocorticoid hormones are important chronic regulators of metabolism. Intracellular reactivation of inactive glucocorticoids has emerged as a key mechanism for regulation and amplification of glucocorticoid action. The reactivation is catalyzed by 11b-Hydroxysteroid Dehydrogenase type 1 (11b-HSD1). Mice over-expressing 11b-HSD1 in adipose or liver display a phenotype very similar to metabolic syndrome, while 11b-HSD1 knock out mice show a marked improvement in insulin sensitivity, lipid and cholesterol profiles. These data indicate that inhibitors of 11b-HSD1 could be novel therapeutics for patients with Type 2 diabetes, obesity and metabolic syndrome. Several approaches have been used in the search for 11b-HSD1 inhibitors. The approach described herein was to design compounds based on known pharmacophores from 11b-HSD1 inhibitors in the literature. The synthesis, structural characterization and biological evaluation of sulfonamidooxazoles, a novel class of 11b-HSD1 inhibitors is delineated. Compounds such as 1 show activity in the uM range in cell-based assay and >200 fold selectivity over 11b-HSD2. We are currently testing these compounds in various animal models.



1

212.

TYPE 2 DIABETES MANAGEMENT USING AEGLE MARMELOS SEEDS. ***Achyut Narayan Kesari****, Alternative Therapeutic Unit, Medicinal Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad, India, 48/12 A B.K. Banerjee Road New Katra, Allahabad, Allahabad 211002, India, achyut_nar@yahoo.co.in, Rajesh Kumar Gupta, Alternative Therapeutic Unit, Medicinal Research Laboratory,Department of Chemistry, University of Allahabad, Allahabad, India, and Geeta Watal, Alternative Therpeutic Unit, Medicinal Research Laboratory, Department of Chemistry, University of Allahabad,Allahabad, India*

The present study reveals the management of type 2 diabetes using aqueous extract of Aegle marmelose seeds. Its effect on serum lipid profile in diabetic animals is also a part of the study. Single and repeated oral administration of variable doses of the extract showed hypoglycemic and antidiabetic activity in normal as well as induced diabetic animals. The most effective dose was found to be 250-mg/kg body weight showing a significant fall of 35.15 % in six hours in the blood glucose level of normoglycemic animals. The same dose showed reduction of 60.84 % after fourteen days of regular treatment in FBG of diabetic animals. Moreover the same treatment of fourteen days in diabetic animals increases the cardio protective HDL cholesterol and decreases the levels of TC, TG and LDL. It is encouraging to get hypolipidemic effect along with hypoglycemic and antidiabetic.

213.

SPACER BASED SELECTIVITY IN THE BINDING OF TWO-PRONG LIGANDS TO RECOMBINANT HUMAN CARBONIC ANHYDRASE-I. *Abir L Banerjee*[1]*,* ***Daniel Roman Eiler***[1]*, Bidhan C Roy*[2]*, Sanku Mallik*[1]*, and D K Srivastava*[1]*. (1) Department of Chemistry and Molecular Biology, North Dakota State University, 312 IACC, NDSU, Fargo, ND 58105, Fax: 701-231-8324, (2) Department of Chemistry, North Dakota State University*

Benzenesulfonamide and iminodiacetate (IDA) conjugated Cu2+ independently interact at the active site and a peripheral site of carbonic anhydrases, respec-

tively. By attaching IDA-Cu2+ to benzenesulfonamide via different chain length spacers, we synthesized "two-prong" ligands, L1 and L2, the distance between Cu2+ and NH2 group of sulfonamide were 29 Å and 22 Å, respectively. Comparing the binding affinities of L1, L2, and parent compound, benzenesulfonamide, for recombinant human carbonic anhydrase-I (hCA-I) by performing the fluorescence titration and steady-state kinetic experiments. The experimental data revealed that the binding affinity of L1 to hCA-I was similar to that of benzenesulfonamide, the binding affinity of L2 was about 2 orders of magnitude higher, making L2 to be one of the most potent ligands/inhibitors of hCA-I. Cumulative account of the experimental data leads to the suggestion that the differential binding of L1 versus L2 to hCA-I is from the chain length of the spacer moiety.

214.

TWO-PRONG INHIBITORS FOR HUMAN CARBONIC ANHYDRASE II. *Sanku Mallik[1], D K Srivastava[2], Bidhan C Roy[1], Manas Haldar[1], **Xiao Jia**[1], Abir L Banerjee[2], and Micheal Swanson[2]. (1) Department of Chemistry, North Dakota State University, 1231 Albrecht Avenue, Fargo, ND 58105, Fax: 701-231-8831, sanku.mallik@ndsu.nodak.edu, xiao.g.jia@ndsu.nodak.edu, (2) Department of Chemistry and Molecular Biology, North Dakota State University*

The enzyme inhibitors are usually designed by taking into consideration the overall dimensions of the enzyme's active site pockets. This conventional approach often fails to produce desirable affinities of inhibitors for their cognate enzymes. To circumvent such constraints, we contemplated of enhancing the binding affinities of inhibitors by attaching tether groups, which would interact with the surface exposed amino acid residues. This strategy has been tested for the inhibition of human carbonic anhydrase II. Benzenesulfonamide serves as a weak inhibitor for the enzyme, but when it is conjugated to iminodiacetate–Cu(II) (which interacts with the surface exposed His residues) via a spacer group, its binding affinity is enhanced by about 2-orders of magnitude. This "two prong" approach is expected to serve as a general strategy for converting weak inhibitors of enzymes into tight-binding inhibitors.

215.

2-DIMENSIONAL ELECTROPHORESIS ANALYSIS SOFTWARE PACKAGES CAUSES INCREASED VARIANCE IN QUANTITATIVE PROTEOMICS STUDIES. ***Åsa M Wheelock**, University of California Davis and Kyoto University, Uji, Kyoto 611-0011, Japan, asa@kuicr.kyoto-u.ac.jp, and Alan R. Buckpitt, Departments of Molecular Biosciences, University of California Davis*

Experimental variability in two-dimensional electrophoresis (2DE) is well-documented, but little attention has been paid to variability arising from post-experimental quantitative analyses using various 2DE software packages. The performance of two 2DE-analysis software programs, Phoretix 2DE Expression v2004 and PDQuest 7.2, including all available background subtraction- and smoothing-algorithms, was evaluated in this study. Neither of them could quantify protein spots in a reproducible manner in sets of 2DE-images (n=5) produced through repeated cropping of the same 2DE-gel image. The resulting variance in quantification for PDQuest was approximately twice that for Expression. In authentic sets of replicate 2DE gels (n=5) Expression still outperformed PDQuest, where software-induced variance constituted up to 25% of the total variance. In addition, we found that the background subtraction algorithms can introduced variance. We conclude that analysis software can contribute significantly to variance in quantitative proteomics, and the use of a background subtraction algorithm can increase this variance.

216.

FLUORESCENT INTERNAL PROTEIN STANDARD FOR QUANTITATIVE 2-DIMENSIONAL ELECTROPHORESIS. ***Åsa M Wheelock**[1], Dexter Morin[2], Mathew Bartosiewicz[3], and Alan R. Buckpit[2]. (1) University of California Davis and Kyoto University, Uji, Kyoto 611-0011, Japan, asa@kuicr.kyoto-u.ac.jp, (2) Department of Molecular Biosciences, School of Veterinary Medicine, University of California Davis, (3) Department of Molecular Biosciences and Microarray Facility, School of Veterinary Medicine, University of California Davis*

2-Dimensional electrophoresis (2DE) is a powerful separation method for complex protein mixtures. However, large inter-gel variations in spot intensity limit its use for quantitative proteomics studies. To address this issue, we developed a fluorescent internal protein standard for use in 2DE analysis. Protein samples are spiked with an Alexa-labeled internal standard (ALIS) prior to separation with 2DE. Incorporation of 0.1% of total protein is sufficient to allow visualization of the internal standard, but low enough to avoid interference in subsequent quantification and identification steps. Following 2DE, total proteins are visualized with fluorescent post-electrophoresis stains spectrally separated from ALIS (e.g. Deep Purple, rutheniumII-tris(bathophenanthrolinedisulfonate) or SYPRO Ruby). This allows conservation of the proteins' natural migration pattern, and thus avoids problems associated with dual spot migration patterns observed in the well established DIGE method. Furthermore, ALIS provides significantly improved normality in the distribution of spot abundance-variance compared to normalization "total spot volume" normalization.

217.

HIGHLY EFFICIENT APPROACH FOR THE SYNTHESIS OF CATIONIC LIPID DOSPA. ***Yanhong Li**, School of Pharmacy, University of Wisconsin at Madison, 777 Highland Ave, Madison, WI 53705, yli@pharmacy.wisc.edu, and Timothy Heath, School of Pharmacy, University of Wisconsin at Madison*

The nearly completion of the human genome sequencing project offers an unparalleled opportunity to understand the genetic basis of disease. This makes gene therapy a promising strategy to cure disease in the future, however gene therapy still faces significant hurdles before it becomes an established therapeutic strategy. In other words, efficient delivery systems need be developed to deliver the therapeutic to the site of disease. Currently, cationic liposomes show particular promise for gene therapy. DOTMA analogues have achieved the most widespread use in cationic liposome formulations. The formulation of DNA with cationic lipid, 2,3-dioleoyloxy-N-[2-(spermine-carboxamido)ethyl]-N,N-dimethyli-1-propanaminum(DOSPA) (Figure. 1) has been proved to be very promising transfection system. Unfortunately, the synthesis of DOSPA has never been reported in the literature. Here we reported the synthesis of the cationic lipid DOSPA.

218.

COMPUTATIONAL AND STATISTICAL ANALYSIS OF MICROARRAY ANALYZED FAC SORTED CALVARIAL OSTEOBLAST DIFFERENTIATION. ***Rishi R. Gupta**[1], Luke E. K. Achenie[1], Ivo Kalajzic[2], and David W. Rowe[2]. (1) Department of Chemical Engineering, University of Connecticut Storrs, 191 Auditorium Road, UNIT 3222, UTEB, Storrs, CT 06269, Fax: 860-486-2959, rishi@engr.uconn.edu, (2) Department of Genetics and Developmental Biology, University of Connecticut Health Center*

The inherent heterogeneity of bone cells complicates the interpretation of microarray studies designed to identify genes highly associated with osteoblast differentiation. To overcome this problem, we have utilized Col1a1 promoter-GFP transgenic mouse lines to isolate bone cells at distinct stages of osteoprogenitor maturation. Comparison of gene expression patterns from unsorted or FACs sorted bone cell populations at days 7 and 17 of calvarial cultures revealed an increased specificity to which genes are selectively expressed in a subset of bone cell types during osteoblast differentiation. Furthermore, distinctly different pattern of gene expression associated with major signaling pathways (Igf1, Bmp and Wnt) were observed at different levels of maturation of the osteoprogenitor cells. These data contradict current models of osteoprogenitor cell differentiation and emphasize components of the pathway that were not revealed in studies based on a total cell population. Thus, applying methods to generate more homogeneous populations of cells at a defined level of cellular differentiation from a primary osteogenic culture is feasible and leads to a novel interpretation of the gene expression associated with increasing levels of osteoprogenitor maturation. A combination of SAM and SPH was used to find out the statistically significant genes out of a population of 12,442 genes from three experiments on two different cell lines (pOBCol3.6GFP and pOBCol2.3GFP) spotted in triplicates. The Adaptive Centroid Algorithm (for single clustering analysis) was used to cluster the statistically significant genes. The common gene profiles (patterns) over four cell population isolated by using FACS were considered to find the co-expressing genes. The algorithm was also applied to the complete unsorted cell population to validate the statistical tests and obtain the patterns showed in a heterogeneous cell population.

219.

NOVEL CHEMOGENOMICS APPROACH TO DESIGN SELECTIVE ENZYME INHIBITORS. ***Laszlo Urge**, Ferenc Darvas, Gyorgy Dorman, Ákos Papp, and Tamas Szommer, ComGenex Inc, 33-34 Bem rkp, Budapest, H-1027, Hungary, Fax: +361-214-2310, laszlo.urge@comgenex.hu*

In the post-genomic drug discovery large enzyme families are investigated parallel in order design selective inhibitors for many related isoforms in one combined research effort. Chemogenomics is a bioinformatics-driven approach to explore the ligand – target knowledge space based on the genetic (sequence homology) divergence of target family members. This design approach identifies the major molecular determinants of the target-family (privileged structures/ special recognition features) and virtual transformations leading selectivity within the family. Using the expert system scaffolds or robust inhibitors can be transformed into selective inhibitors ('selectivity jumping'). In the present poster we will describe our knowledge base approach to explore the ligand-target space of matrix metalloproteinases (MMPs) and its utility to design selective inhibitors of MMP-2, MMP-9, MMP-13 and MMP-1 that are implicated in a number of biological processes, e.g. in the pathogenesis of cardiovascular diseases and in tumor growth and metastasis.

220.

NEW 1,5-DIARYLHETEROCYCLES FOR SELECTIVE COX-2 INHIBITORS: SYNTHESIS OF 1,5-DIARYLHYDANTOINS BY ONE-POT REACTION. ***Myung-Sook Park**, Soon-Kyoung Kwon, Hae-Sun Park, and Eun-Hee Park, College of Pharmacy, Duksung Women's University, Ssangmundong 419, Tobonggu, Seoul 132-714, South Korea, Fax: 82-2-901-8386, mspark@duksung.ac.kr*

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used to treat pain, fever and inflammatory conditions. Common NSAIDs limits their usefulness because inhibit not only COX-2 associated with antiinflammatory activity, but also COX-1 accompanied with side effects in the stomach. On the basis of this fact, specific COX-2 inhibitors such as Celecoxib are introduced in the drug market. The distinguished feature of these drugs is that the 5-membered heterocycle ring is substituted with two aryl groups. We report on synthesis of novel 1,5-diarylhydantoin derivatives which contain phenyl (or p-halophenyl) groups at 5-position, phenyl, p-sulfonamidylphenyl, p-hydroxyphenyl and p-methoxyphenyl groups at 1-position. These compounds were prepared through esterification, bromination, α-substitution and cyclization from commercially available phenylacetic acid. Especially, N-aralkyl groups in 3-position of hydantoin ring could be introduced by one-pot reaction of methyl α-aminoacetates with aralkyl isocyanate or isothiocyanate. Some 1,5-diarylhydantoins were shown to be effective as selective COX-2 inhibitors.

221.

INHIBITION OF COX1/COX2 ACTIVITY BY SUBSTITUTED CHALCONES AND FLAVONES. ***Nelly N. Mateeva**, Chavonda J. Mills, and Kinfe K. Redda, College of Pharmacy, Florida A&M University, New Pharmacy Bld. #310, Tallahassee, FL 32307, Fax: (850)599-3243, nmateeva@chem.fsu.edu*

Cyclooxygenase (COX) enzymes mediate the synthesis of prostanoids (prostaglandins, PG and thromboxanes) from arachidonic acid. COX-1 is expressed virtually in all tissues and is responsible for the production of prostanoids critical to the maintenance of normal physiologic functions. Second isoform, COX-2, is usually absent from most normal cells and tissues but is expressed in pathologic states such as inflammated tissues and tumors. There is compelling evidence that COX-2 and its derived prostanoids play an important role in the pathogenesis and evolution of a variety of cancers. Flavonoids, such as quercetin, genistein, baicalein, etc. have been reported to have anti-cancer activity. At the same time, many of them exhibit promising anti-inflammatory properties. The current paper reports studies on the synthesis of alkylated, halogenated and nitro-substituted chalcones and flavones. The compounds will be subject of biological testing to establish their anti-inflammatory and tumor suppressing activity.

222.

SYNTHESIS AND BIOLOGICAL EVALUATION OF SALICYLIC ACID ANALOGUES OF CELECOXIB AS A NEW CLASS OF SELECTIVE CYCLOOXYGENASE-1 INHIBITOR. ***Sung-Hwa Yoon**[1], **JooYoung Lee**[1], Ju-Young Park[1], YoungAe Lee[2], and ByoungJoo Gwag[3]. (1) Department of Molecular Science and Technology, Ajou University, PaldalGu WonchunDong, Suwon 443-749, South Korea, Fax: 31-219-2516, shyoon@ajou.ac.kr, nanj02@hanmail.net, (2) Research Center, Neurotech Company, (3) Department of Pharmacology, College of Medicine, Ajou University*

It is well known that Celecoxib, the diaryl heterocyclic derivative containing a phenylsulfonamide moiety, is selective cyclooxygenase-2 (COX-2) inhibitor. While developing a new class of anti-inflammatory agent, we synthesized a series of salicylic acid analogues of Celecoxib where the phenylsulfoamide moiety in the structure of Celecoxib is replaced by salicylic acid moiety. Among the series, in vitro Cox-1 and COX-2 isozyme inhibition assay identified 5-[5-(4-chlorophenyl)-3-trifluoromethyl-pyrazol-1-yl]-2-hydroxybenzoic acid as a potent COX-1 inhibitor (IC50=0.01 uM) with a high COX-1 selectivity (SI=720). The syntheses and SAR of this series will be presented. Supported by Frontier Program in Korea.

223.

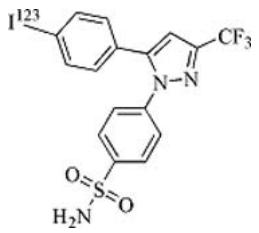
SYNTHESIS OF A HETEROARYL MODIFIED, 1, 5-DISUBSTITUTED PYRAZOLE CYCLOOXYGENASE-2 (COX-2) SELECTIVE INHIBITOR. ***Maiko Ezawa**[1], D. S. Garvey[2], D. R. Janero[2], Subhash P. Khanapure[1], L. Gordon Letts[1], Allison M. Martino[1], Ramani R. Ranatunge[1], David J. Schwalb[1], and Delano V. Young[1]. (1) NitroMed, Inc, 125 Spring Street, Lexington, MA 02421, Fax: 781-274-8002, mezawa@nitromed.com, (2) NitroMed Inc*

COX-2 selective inhibitors have proven to be effective anti-inflammatory and analgesic medicines with lower chronic gastrointestinal (GI) toxicity than traditional non-steroidal anti-inflammatory drugs (NSAIDs), which non-selectively inhibit COX-2 and COX-1. Recently, rofecoxib has been withdrawn from the market due to the increased risk of adverse cardiovascular events. For the past few years we have been interested in the design and synthesis of COX-2 selective inhibitors incorporating a nitric oxide (NO) donor moiety, on the premise that the NO bioactivity would further enhance the GI and renal safety of this drug class while additionally offering cardiovascular and tissue protection. In our efforts to synthesize NO-donor-COX-2 selective inhibitors, vicinal 1-(4-methylsulfonyl)benzene-5-(3-pyridyl) substituted pyrazole compound containing a NO-donating group at the 3-position of the pyrazole ring was synthesized and evaluated for its ability to inhibit COX isozymes in human whole blood. The synthesis of 4-{3-[(1Z)-4-(nitrooxy)but-1-enyl]-5-(3-pyridyl)pyrazolyl}-1-(4-methylsulfonyl)benzene and its COX-2 inhibitory potency will be reported.

224.

SYNTHESIS OF AN IODINE-123 LABELED COX-2 INHIBITOR AS A POTENTIAL SPECT AGENT. *George W Kabalka[1], **Arjun R. Mereddy**[1], and Hildegard Schuller[2]. (1) Departments of Chemistry & Radiology, University of Tennessee, Knoxville, TN 37996, Fax: 865-974-2997, Kabalka@utk.edu, amereddy@utk.edu, (2) Department of Pathology, Veterinary Teaching Hospital, University of Tennessee College of Veterinary Medicine*

Lung cancer is the leading cause of death in the United States. Previous reports in literature have shown an exaggerated expression of cyclooxygenase-2 (COX-2) enzyme in cancer tissue. We have developed a no carrier-added radioiodinated analogue of celecoxib for use as a single photon emission tomography (SPECT) imaging agent for potential use in the early detection of lung cancer.



225.

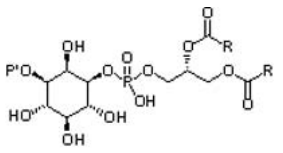
SYNTHESIS OF SUBSTITUTED FLAVONOIDS AND EVALUATION OF THEIR COX-1/COX-2 INHIBITORY ACTIVITY. ***Chavonda J. Mills**, Nelly N. Mateeva, and Kinfe K. Redda, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, Fax: 850-599-8179, chavonda_mills@hotmail.com*

Synthesis of halogenated/nitrated flavone derivatives and evaluation of their COX-1 and COX-2 inhibitory activityChavonda MillsChavonda Mills12192004-11-17T23:18:00Z2004-11-18T00:35:00Z1148938FAMU172108411.5604There has been great interest in the biological roles of naturally occurring flavonoid compounds. Previous studies have shown flavonoids to possess a wide range of pharmacological activity including anti-oxidant, anti-viral, anti-cancer, and anti-inflammatory properties. More specifically, studies have demonstrated that flavonoids inhibit cyclooxygenase activity. It is known that cyclooxygenase catalyzed synthesis of prostaglandin-E2 plays a key role in inflammation and its associated diseases, such as cancer. Therefore, the evaluation of novel flavonoid derivatives as anti-inflammatory and anti-cancer agents is of great interest. In the present study, a series of novel 8-alkylated flavonoids and related compounds (Fig. 1) were synthesized and characterized by using NMR, IR, and elemental analysis. The effects of these target compounds on cyclooxygenase in vitro using COX-1 and COX-2 assays are currently being tested.

226.

ASYMMETRIC SYNTHESIS OF ANALOGUES OF PHOSPHATIDYLINOSITOL 3-PHOSPHATE. *Yong Xu and **Glenn D. Prestwich**, Department of Medicinal Chemistry, University of Utah, 419 Wakara Way, STE 205, Salt Lake City, UT 84108, Fax: 801-585-6354, gprestwich@pharm.utah.edu*

Phosphatidylinositol polyphosphates (PtdInsPs) are key signaling molecules in cellular communication, and in particular phosphatidylinositol 3-phosphate (PtdIns(3)P) is crucial for vesicular trafficking of proteins. PtdIns(3)P is produced phosphoinositide-3-kinase (PI 3-K), an important player in the biochemistry of cell cycle progression, cell migration, and cell proliferation. In order to dissect the pleiotropic roles of PtdIns(3)P as a ligand and an enzyme substrate, we designed novel metabolically-stabilized analogs of PtdIns(3)P as reporters of cell function. Newly designed asymmetric syntheses of metabolically stabilized (phosphatase-, kinase, and phospholipase-resistant) phosphatidylinositol 3-phosphate (PtdIns(3)P) analogs will be described.

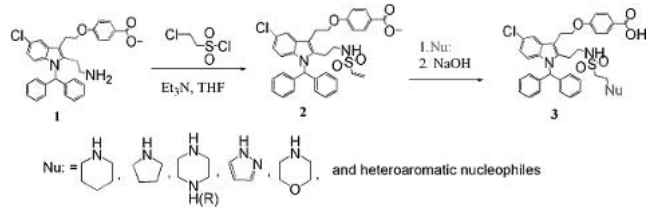


P' = $P(S)(OH)_2$, $P(O)CH_3(OH)$, $P(O)CH_2F(OH)$.

227.

EFFICIENT SYNTHESIS OF SULFONAMIDE ANALOGS OF INDOLE CPLA2A INHIBITORS: CHEMISTRY AND SAR. ***Weiheng Wang**[1], Lihren Chen[1], Katherine L. Lee[1], Marina Shen[2], Jing Lun Wu[2], Wen Zhang[2], Xin Xu[3], Steve Tam[4], James D. Clark[2], and John C. McKew[1]. (1) Department of Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5682, weihengwang@wyeth.com, (2) Department of Inflammation, Wyeth Research, (3) Drug Safety & Metabolism, Wyeth Research, (4) Department of Chemical and Screening Sciences, Wyeth*

Cytosolic Phospholipase A2a (cPLA2a) 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. The synthesis of inhibitors of cPLA2a, with drug-like properties, would lead to a novel therapeutic with applications in many disease states, such as rheumatoid arthritis, osteoarthritis, and asthma. A class of indole inhibitors of cPLA2a has been discovered that contain three important pieces to the pharmacophore, the lipophilic group at N1, a sulfonamide containing chain at C2, and a terminal carboxylic acid at C3. We undertook a study of a series of indole cPLA2a inhibitors 3 containing various heterocyclic functional groups at C2. The goals of this study were to modify physicochemical parameters by incorporating polarity while maintaining potency. These compounds were efficiently synthesized using Michael additions to vinyl sulfonamide 2. Compound 2, which was prepared from a common intermediate amine 1, was reacted with a variety of heterocyclic nucleophiles. The use of a vinyl sulfonamide as a point of diversity has limited precedence in the literature but served well to deliver the requisite compounds. The chemistry, SAR, and physicochemical characterization of this class will be presented.



228.

INDOLE-BASED INHIBITORS OF CPLA2A: PHENYLSULFONAMIDES. ***Jill Nunez**[1], Mark Behnke[1], Lihren Chen[1], Megan Foley[1], Katherine L. Lee[1], Richard Vargas[1], Weiheng Wang[1], Elizabeth Murphy[2], Marina Shen[2], Wen Zhang[2], Steve Tam[1], James D. Clark[2], and John C. McKew[1]. (1) Department of Chemical and Screening Sciences, Wyeth Research, 200 Cambridgepark Dr, Cambridge, MA 02140, jxnunez@wyeth.com, (2) Department of Inflammation, Wyeth Research*

Cytosolic Phospholipase A2a (cPLA2a), is the enzyme responsible for the specific cleavage of arachidonic acid-containing glycerophospholipds. 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. The introduction of inhibitors of cPLA2a would lead to a novel therapeutic with applications in many disease states, such as rheumatoid arthritis, asthma, and osteoarthritis. We have previously reported a class of indole-based cPLA2a inhibitors containing a benzyl sulfonamide as a key part of the pharmacophore. To improve the PK parameters of this class of inhibitors, we have focused on replacing the benzyl sulfonamide in the C2 side chain with a variety of substituted phenyl sulfonamides. The SAR leading to potent inhibition of cPLA2a will be described as well the impact these changes had on PK parameters.

229.

STRUCTURE-BASED DESIGN OF SECRETORY AND CYTOSOLIC PHOSPHOLIPASE A2 INHIBITORS. ***Brian P. Smart** and Michael H. Gelb, Department of Chemistry, University of Washington, BOX 351700, Seattle, WA 98195, bsmart@u.washington.edu*

Selective inhibitors of phospholipase A2s (PLA2s) are valuable tools that aid in elucidating the physiological functions of this broad family of enzymes. Structure-based design was used in the search for selective inhibitors for both secretory (sPLA2s) and cytosolic (cPLA2s) phospholipase A2 enzymes. Indoles, pyrrolidines, and amides were functionalized to gain selectivity between the individual human sPLA2 and cPLA2 enzymes. The synthesis of several libraries of compounds and inhibition results from fluorometric assays against the individual human enzymes will be presented.

230.

DEVELOPMENT OF A NOVEL HIGH-THROUGHPUT SCREENING ASSAY FOR RAPID DISCOVERY OF LIPOXYGENASE INHIBITORS. ***Joshua D. Deschamps**, Rachana R. Shah, 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 disease 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. In this poster we describe the development of a novel high-throughput screening assay for discovering lipoxygenase inhibitors in 384-well microplates. The assay detects and measures the amount of hydroperoxy-fatty acids produced in the lipoxygenase reaction by coupling them to the formation of ferric-xylenol orange complexes. The formation of these ferric-xylenol orange complexes is then detectable at 560 nm. The assay is quick, robust and cost-effective and has been applied to discover hLO inhibitors from our chemical libraries. The assay has also been utilized to rapidly gather IC50 values for various inhibitors. The assay can screen thousands of compounds in a few hours, which normally takes a few weeks using conventional methods. Due to the efficiency of this assay, and the increase in the amount of compounds that can be screened, lipoxygenase inhibitor discoveries in our lab have been accelerated.

231.

DEVELOPMENT OF A POTENT AND SELECTIVE INHIBITOR OF LEUKOTRIENE A4 HYDROLASE. ***Jianmei Wei**, Department of Chemistry, Johnson & Johnson Pharmaceutical Research & Development L.L.C, 3210 Merryfield Row, San Diego, CA 92121, Fax: 858-784-3116, jwei1@prdus.jnj.com, and Kevin Tays, Department of Chemistry, Jonhson & Johnson Pharmaceutical Research & Devolpment L.L.C*

Leukotriene A4 hydrolase (LTA4H), a 69 kDa zinc containing metalloenzyme, catalyzes the hydrolysis of leukotriene A4 (LTA4) to the pro-inflammatory mediator leukotriene B4 (LTB4). The downstream effect of LTB4 has been implicated in several diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and chronic obstructive pulmonary disorder (COPD). Therefore, regulating the production of LTB4 is an attractive target for drug discovery. Initial HTS/CADD leads allowed for rapid preparation of active preliminary analogs and resulted in the identification of the benzothiazole head group and amine tail group. The chemistry and the SAR detailing our efforts at discovering novel LTA4H inhibitors with reduced hERG affinity will be presented.

232.

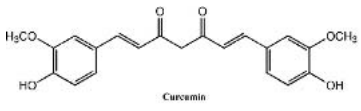
ACTIVATION OF NF-κB IS INHIBITED BY CURCUMIN AND RELATED ENONES. ***Waylon M. Weber**[1], C. Nathaniel Roybal[2], Ekaterina V. Bobrovnikova-Marjon[2], Lucy A. Hunsaker[2], Steve F. Abcouwer[2], Lorraine M. Deck[1], and David L. Vander Jagt[2]. (1) Department of Chemistry, University of New Mexico, MSC03 2060, Albuquerque, NM 87123, waylonw@unm.edu, (2) Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine*

The transcription factor NF-kappaB (NF-κB) is up-regulated in many cancer cells where it contributes to development of the pro-survival, anti-apoptotic state. The natural product curcumin is a known inhibitor of activation of NF-κB. Three series of curcumin analogues were synthesized and compared with curcumin for their abilities to inhibit the TNFα induced activation of NF-κB in cells transfected with a NF-κB-dependent reporter construct: 1) curcumin analogues that retained the 7-carbon spacer; 2) curcumin analogues with a 5-carbon spacer; and 3) curcumin analogues with a 3-carbon spacer. Inhibitors of NF-κB activation were identified in all three groups and a number of compounds were more active than curcumin.

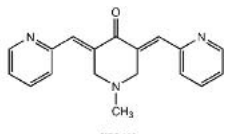
233.

DEGRADATION STUDY OF CURCUMIN ANALOG UBS 109. ***Sanna M. Malick**, Sarah Trotman-Pruett, Ustun Sunay, Marike Herold, James P. Snyder, and Dennis C. Liotta, Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322*

Curcumin is a β-diketone component of turmeric that is used as a spice to give flavor and color to curry. It exhibits anti-tumor properties in a variety of cancer cell lines including growth inhibition and induction of apoptosis. A series of novel curcumin analogs were synthesized which inhibit cancer cell growth with a good potency. Although quite active, the analog UBS 109 was found to undergo decomposition over time. Its degradation products have been analyzed by mass spectrometry and some preliminary structures have been proposed.



Curcumin



UBS 109

234.

DESIGN AND DEVELOPMENT OF HIGH AFFINITY LIGANDS THAT BIND TO HLA-DR10. ***Felice C. Lightstone**[1], Julie Perkins[2], Monique Cosman[1], Adam Zemla[3], Michele H. Corzett[1], Carlos Valdez[2], Julie L Herberg[2], Gerald L. DeNardo[4], and Rod Balhorn[1]. (1) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, 7000 East Ave., Livermore, CA 94551, felice@llnl.gov, (2) Chemistry and Materials Science, Lawrence Livermore National Laboratory, (3) Computations, Lawrence Livermore National Laboratory, (4) Department of Internal Medicine, University of California, Davis Medical Center*

The beta subunit of HLA-DR10 is an attractive protein target found on the surfaces of lymphoma cells. Traditionally, Lym-1 antibody, which binds to HLA-DR10, has been used to carry radionuclides and act as a therapeutic. In order to mimic the targeting properties of Lym-1 while eliminating the disadvantages of antibodies, multidentate selective high affinity ligands (SHALs) have been developed using a combination of computational and experimental methods to predict and confirm ligand binding. Synthetic methods are used to combine sets of ligands to form a new class of SHALs. Surface plasmon resonance was used to screen the new class of bidentate molecules, designed to bind to HLA-DR10 with high affinity and high specificity. This work was performed under the auspices of the US Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48 and NCI-CA47829.

235.

DISCOVERY OF KRP-203, A POTENT AND ORALLY ACTIVE NEW TYPE OF IMMUNOSUPPRESSANT, SPHINGOSINE-1-PHOSPHATE RECEPTOR AGONIST. ***Yasushi Kohno**, Naoki Ando, Takahiro Tanase, Takayuki Sawada, Kiyoaki Tanaka, Kazuhiko Yumoto, and Sayoko Tanioka, Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd, 2399-1 Nogi, Nogi-machi, Tochigi, Shimotsuga-gun 329-0114, Japan, Fax: +81-280-57-1293, yasuhi.kohno@mb.kyorin-pharm.co.jp*

Calcineurin inhibitors such as cyclosporine A and tacrolimus have improved grafting and patient survival after organ transplantation. However, unsolved problems remain in the long-term treatment of calcineulin inhibitors such as side effects and transplant arteriosclerosis. Therefore, a new type of immunosuppressant is desired to minimize the side effects of organ transplantation. FTY-720 has amino-1,3-propane-diol structure and possesses unique properties not shared by calcineurin inhibitors, corticosteroids, or nucleotide synthesis inhibitors such as MMF. FTY-720 is reported to be a prodrug, and its real active species is the corresponding phosphate ester metabolite, which is a potent agonist of sphingosine-1-phospate (S1P) receptors. To discover a novel immunomodulating agent, we have been independently investigating diaryl amines, diaryl ethers, and diaryl sulfides that have amino-1,3-propane-diol core structure. As a result, we have developed a potent, orally active novel type of immunosuppresant called KRP-203. The synthesis, SAR, and in vivo evaluation of these compounds are discussed.

236.

FOLATE-TARGETED IMMUNOTHERAPY TO ACTIVATED MACROPHAGES IN RHEUMATOID ARTHRITIS. ***Bindu Varghese**[1], Chrystal M Paulos[2], Gert J Breur[1], William R Widmer[1], and Philip S. Low[3]. (1) Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907, Fax: 765-494-0239, varghesb@purdue.edu, (2) Surgery Branch, National Cancer Institute/National Institute of Health, (3) Department of Chemistry, Purdue University*

We wish to report a novel therapy for rheumatoid arthritis based on the selective elimination of activated macrophages. Activated (but not resting) macrophages concentrated in the inflamed joints and organs of arthritic animals over-express a cell surface receptor for folic acid. Activated macrophages can be selectively targeted using folate-radioemitter conjugates, allowing scintigraphic imaging of arthritic joints. In an extension of this targeting capability, we demonstrate that folate-linked haptens also bind to this population of activated macrophages, decorating the cell surfaces with highly immunogenic molecules. In animals previously vaccinated against this hapten, administration of a folate-hapten conjugate leads to rapid elimination of activated macrophages without any measurable toxicity to normal tissues. Studies presented here show

that application of this folate-mediated immunotherapy to adjuvant-induced arthritic rats or collagen-induced arthritic mice greatly reduces: 1) paw swelling 2) bone and cartilage disintegration, and 3) activated macrophage accumulation in joints and other organs.

237.

HIGHLY EFFICIENT SYNTHESIS OF α O-GALACTOSYL CERAMIDES. ***Wenjun Du*** *and Jacquelyn Gervay-Hague, Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, CA 95616, wjdu@ucdavis.edu*

Galactose containing glycosphingolipids are widely found in Nature, where they play important roles in biological processes such as cellular communication. Some α O-galactosylceramides, like KRN7000 have shown potent in vivo anti-tumor activities. Synthesis of α O-galactosylceramides is a challenging endeavor due to low yields and poor α/β selectivity in the glycosylation reaction. Glycosyl fluorides and trichloacetimidates are commonly employed as glycosyl donors to produce the α O-glycosidic linkage with yields typically ranging between 30% to 70%. Here, we report a highly efficient synthesis of α O-galactosylceramides using galactosyl iodide donors and tetrabutylammonium iodide (TBAI) as a promoter. Under neutral conditions, (2S, 3S)-2-azido-3-para-methoxybenzyl sphingosine and (2S, 3S, 4R)-2-azido-3,4-para-methoxybenzyl phytosphingosine reacted with galactosyl iodide affording α O-glycosidic linkages with yields over 90%. Upon successful glycosylation reactions, converting the azido groups to amino groups, followed by fatty acid coupling and hydrogenation afforded pure KRN7000 and 4-desoxy-KRN7000. Thus, an efficient synthesis of α O-galactosylceramides is established.

238.

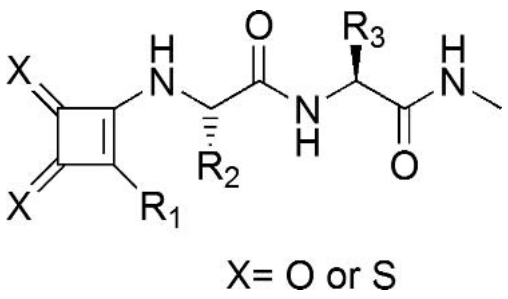
ORALLY ACTIVE SMALL MOLECULE IL-12 PRODUCTION INHIBITORS. ***Zachary Demko***[1]*, Dinesh Chimmanamada*[1]*, David James*[2]*, Elena Kostik*[1]*, Keizo Koya*[1]*, Hao Li*[1]*, Guiqing Liang*[1]*, Teresa Przewloka*[1]*, Lijun Sun*[1]*, Noriaki Tatsuta*[1]*, Yumiko Wada*[1]*, Qianfan Wang*[1]*, Yaming Wu*[1]*, Shijie Zhang*[1]*, and Dan Zhou*[1]*. (1) Chemistry, Synta Pharmaceuticals Corp, 45 Hartwell Avenue, Lexington, MA 02421, Fax: 781-274-8228, zdemko@syntapharma.com, (2) DEPARTMENT OF CHEMISTRY, Synta Pharmaceuticals Corp*

Interleukin-12 (IL-12), a p35/p40 heterodimeric cytokine, has been shown to play a critical role in a number of inflammatory disorders, including rheumatoid arthritis, Crohn's disease, and psoriasis. The possibility of using selective inhibition of IL-12 overproduction as a therapy for these diseases has been validated in a recent publication in which it was disclosed that administration of a human monoclonal antibody against IL-12 to patients suffering active Crohn's disease resulted in significant rates of remission. Herein we describe the synthesis and SAR studies of a series of novel small molecule IL-12 production inhibitors. Optimized inhibitors demonstrate potent in vitro activity against IL-12 production in human PBMC with an IC50 less than 10 nM. Lead compounds exhibit significant oral efficacy in arthritis and Crohn's disease models.

239.

SQUARATE BASED PEPTIDIC INHIBITORS OF MATRIX METALLOPROTEINASE-1 (MMP-1). ***M. Burak Onaran****, Anthony B. Comeau, and Christopher T. Seto, Department of Chemistry, Brown University, 324 Brook St. Box H, Providence, RI 02912, Mehmet_Onaran@brown.edu*

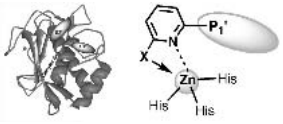
A series of squarate based peptidic inhibitors were synthesized and evaluated as inhibitors of MMP-1. In our initial studies, we observed that having an -N(alkyl)OH group at the R1 position was very important for inhibition. Changing this group to -NHOH or -OH significantly decreased the inhibition activity towards MMP-1. Comparative studies performed with monopeptidic squarates showed that -N(Me)OH at the R1 position showed the best inhibition among the various -N(alkyl)OH groups that were tested. Positional scanning with a variety of amino acids revealed that Leu-Tle-NHMe is the preferred amino acid sequence for the peptidic side chain and resulted in inhibitors with activity in the low micromolar range. Conversion of one of the carbonyl groups on the squarate moiety to a thiocarbonyl group resulted in a 3-fold increase in potency of the inhibitors.



240.

SYNTHESIS AND EVALUATION OF NOVEL PYRIDINE-BASED MMP INHIBITORS. *Gregory R. Cook and* ***Ryuji Hayashi****, Center for Protease Research, Department of Chemistry, North Dakota State University, Ladd Hall, Fargo, ND 58105, Fax: 701-231-8831, gregory.cook@ndsu.nodak.edu*

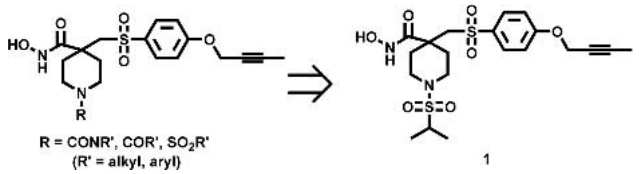
Extracellular proteolysis plays a key role in a number of biological processes. Angiogenesis, wound healing, inflammatory reactions, management of the blood brain barrier, and general maintenance of joints, to name a few, all depend on enzymes which remodel connective tissues in the extracellular matrix. The involvement of matrix metalloproteinases (MMPs) in extracellular degradation has been clearly demonstrated and the number of disease states that these enzymes impact on are many. Some of the best-known MMPIs to date combine a succinate backbone and a zinc-binding group (ZBG) and a fair amount of structure-activity data is now available on these compounds. A hydroxamic acid ZBG has resulted in optimal potencies thus far, however, they have shown little efficacy for cancer therapy in clinical trials. There are several reasons for the previous failures of the hydroxamates in clinical trials and pharmacokinetics has been a particular problem with hydroxamates. Hence, new ZBGs with better druglike properties are of current interest. Our program has focused on identifying and developing novel MMPIs based on heterocyclic ZBGs that avoid the toxicities associated with hydroxamates. Results of the synthesis and evaluation of novel pyridine-based MMPIs will be presented.



241.

SYNTHESIS OF PIPERIDINE PHENYL SULFONE HYDROXAMATES AS TACE (TNF-α CONVERTING ENZYME) INHIBITORS. ***Kaapjoo Park***[1]*, Jeremy I. Levin*[1]*, Alexis Aplasca*[1]*, Mila Du*[1]*, Frank E. Lovering*[1]*, Jeffrey S. Condon*[1]*, Yuhua Zhang*[2]*, LinHong Sun*[2]*, Yi Zhu*[2]*, and Weixin Xu*[3]*. (1) Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, Fax: 845-602-3045, Parkk3@wyeth.com, (2) Inflammation, Wyeth Research, (3) Structural Biology, Wyeth*

TNF-α is a pro-inflammatory cytokine that has been implicated in the etiology of various diseases including rheumatoid arthritis (RA), Crohn's disease, congestive heart failure and inflammatory bowel disease. TACE (TNF-α converting enzyme, ADAM-17) is a zinc metalloprotease that processes membrane-bound TNF into soluble TNF. The efficacy of anti-TNF antibodies in treating RA has led to the search for novel, orally active, and selective small molecule inhibitors of TACE for the treatment of this disease. To that end a series of piperidine sulfone hydroxamates has been prepared in which the substituent on the piperidine nitrogen has been varied. The butynyloxy group was selected as a P1′ group for this series to provide optimal potency against isolated enzyme, and in cells. Most of amide derivatives showed excellent enzyme activity in a FRET assay (low nanomolar IC_{50}) and good to moderate activity in a human whole blood (HWB) assay (2 μM < IC_{50} < 20 μM). Among sulfonamide derivatives, the sulfonamide hydroxamate **1** showed excellent enzyme activity (IC_{50} = 1.5 nM),



and good potency in HWB (IC_{50} = 1.5 μM). The sulfonamide **1** was 235-fold selective over MMP-2, 160-fold selective over MMP-13, and 3140-fold selective over MMP-14.

242.

STRUCTURE DETERMINATION OF APAZA, A SMALL, NOVEL, DIAZO MOLECULE CURRENTLY BEING DEVELOPED FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE (IBD). ***Michelle Ferro***, *Susan Donaldson, and Radha Krishnan, Chemistry Development and Manufacturing, Nobex Corporation, 617 Davis Drive Suite 100, Durham, NC 27713, Fax: 919-474-9407, mferro@nobexcorp.com*

APAZA™ is a novel, small molecule currently being developed to provide symptomatic relief for patients suffering from IBD, specifically ulcerative colitis. The unique design of APAZA™ combines two active components, 5-aminosalicylic acid (5-ASA), a gastrointestinal anti-inflammatory agent and, 4-aminophenylacetic acid (4-APAA), an immunomodulator, via an azo bond. APAZA™ was subjected to the standard analytical techniques, i.e. FT-IR, ES-MS, ^{1}H and ^{13}C NMR, to provide preliminary structural information. Additional evidence for the expected structure was provided by the 2-dimensional NMR experiments. Specifically, the use of DEPT and HMBC NMR spectra proved to be important in distinguishing the relationships of the aromatic quaternary carbons. Futhermore, the HMQC data allowed for the assignment and identification of the protonated ^{13}C resonances. Combination of all data provided the information needed to confirm the proposed structure of the APAZA™ molecule.

243.

NOVEL ORALLY BIOAVAILABLE THROMBIN INHIBITORS: CYANOFLUOROPHENYLACETIC ACID DERIVATIVES. ***Kevin D. Kreutter***[1], *Lily Lee*[1], *Tianbao Lu*[2], *Venkatraman Mohan*[1], *Sharmila Patel*[1], *Hui Huang*[1], *Guozhang Xu*[1], *Mark Fitzgerald*[1], *Carl Crysler*[1], *Mark R. Player*[1], *Edward C. Giardino*[3], *Bruce E. Maryanoff*[3], *Bruce P. Damiano*[3], *Bruce E. Tomczuk*[2], *Norman D. Huebert*[2], *Stephen Eisennagel*[2], *Malini Dasgupta*[2], *John C. Spurlino*[2], *and Martin McMillan*[2]. *(1) Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, L.L.C - Cranbury, 8 Clarke Drive, Cranbury, NJ 08512, Fax: 609-655-6930, kkreutte@prdus.jnj.com, (2) Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, L.L.C - Exton, (3) Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development - Spring House*

Thrombin, the serine protease responsible for blood clot formation through its conversion of fibrinogen to fibrin, has long been regarded as an attractive target for the prevention of thrombosis, stroke, and other undesired coagulation events. In the search for new orally bioavailable thrombin inhibitors, we have discovered and optimized a chemical series that has low nanomolar potency and good pharmacokinetics in dogs. The synthesis, SAR, and selectivity of this cyanofluorophenylacetic acid series will be presented.

244.

POTENT SMALL MOLECULE, NON-PEPTIDIC CHLOROPHENYL ACETAMIDE THROMBIN INHIBITORS. ***Lily Lee***, *Kevin D. Kreutter, Wenxi Pan, Tianbao Lu, Carl Crysler, Steven Eisennagel, Martin MacMillan, John Spurlino, Bruce Tomczuk, Mark Player, and Venkatraman Mohan, Johnson & Johnson Pharmaceutical Research and Development, L.L.C, 8 Clarke Drive, Cranbury, NJ 08512, llee6@prdus.jnj.com*

The discovery of small molecule inhibitors of thrombin, a key serine protease in the coagulation cascade, continues to be an important goal for antithrombotic therapy. Thrombin, a trypsin-like peptidase, mediates the cleavage of fibrinogen to fibrin and the activation of platelets, leading to the formation of blood clots. Inhibition of thrombin would provide an effective treatment for conditions characterized by unusually large thrombus, such as venous and arterial thrombosis, DVT and myocardial infarction. Herein, we report a series of chlorophenyl acetamides which are potent (nM) thrombin inhibitors.

245.

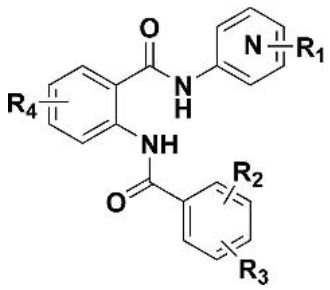
STRUCTURE-BASED DESIGN, STRUCTURE-CONFORMATION AND STRUCTURE-ACTIVITY RELATIONSHIPS OF DPHE(D/L-TIC)-PRO-DARG-P1′-CONH2 TETRAPEPTIDES WITH INHIBITORY ACTIVITY FOR THROMBIN. ***Cristina Clement***, *Chemistry Department, Lehman College, City University of New York, 250 Bedford Park BLVD West, Bronx, New York City, NY 10468, cclement_us@yahoo.com, and Manfred Philipp, Chemistry Department, Lehman College and Biochemistry Ph.D. Program, City University of New York*

A structure-based design of tetrapeptides containing the sequence space DPhe/X(P3)-L-Pro(P2)-D-Arg(P1)-P1′-CONH2 was employed to discover potential inhibitors for thrombin (X= analogs of Phe, such as constrained analogs (L)/(D)-Tic [1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid]). In order to predict if the peptides with constrained conformation are more potent inhibitors the advanced MM3 force-field was used to minimize individual tetrapeptides. The backbone dihedral angles phi and psi were predicted to favor in most cases beta turns and beta hairpin conformation, very similar with the original peptide inhibitor from which they were designed-PPACK. Circular dichroism investigations shows that the D-Arg- in i+3 position followed by D-amino acids (polar and neutral like D-Thr, D-Gln, D-Ser and D-Ala) or L-Pro in i+4 position favors beta turn and beta hairpin structures in solution at low and neutral pH. Replacement of D- with L-amino acids in i+4 position was accompanied by a significant lost in the beta turn structure. The replacement of D-Phe with L/D-Tic is accompanied by a shift toward beta-strand-like structure. SAR (structure-activity relationship) suggests that tetrapeptides which adopt beta turn or beta hairpin conformation in solution are more active toward inhibiting thrombin. The order of activity for the peptides containing analogs of Phe in the P3 position is (D)Phe>>(D)Tic(L) Tic with conserved residues at P2=Pro and P1=DArg and variable L/D- amino-acids at P1′. Lead compounds having the experimentally determined inhibitory constant (Ki) between 16.5 –0.9 uM are potential competitive inhibitors.

246.

ANTHRANILAMIDE INHIBITORS OF FACTOR XA. ***David Mendel***, *Angela L. Marquart, Sajan Joseph, Philip Waid, Ying K. Yee, Anne Louise Tebbe, David K. Herron, Theodore Goodson, John J. Masters, Jeffry B. Franciskovich, Jennifer M. Tinsley, Michael R. Wiley, Leonard C. Weir, Jeffrey A. Kyle, Valentine J. Klimkowski, Gerald F. Smith, Richard D. Towner, Larry L. Froelich, John Buben, and Trelia J. Craft, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, DC0548, Indianapolis, IN 46285, Fax: 317-433-1685, Mendel_David@Lilly.com*

Thromboembolic diseases remain one of the most significant threats to health in the developed and developing world. Because of its critical role in the coagulation cascade, factor Xa (fXa) represents a good therapeutic target for the prevention and treatment of such diseases. In our efforts to prepare orally active fXa inhibitors, we applied both traditional and parallel synthesis, computational modeling, and structure-based design methods to explore the anthranilamide scaffold shown. Starting from a micromolar screening hit, we discovered novel substituents that afforded nanomolar and picomolar fXa inhibitors having excellent selectivity vs. other proteases important to the coagulation cascade. Also described is the optimization of substituents to provide highly active anticoagulants that exhibit good oral bioavailability and plasma half lives.



247.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF PEPTIDOMIMETIC FXIA INHIBITORS. ***Jian Lin***[1], *Hongfeng Deng*[1], *Lei Jin*[1], *Pramod Pandey*[2], *Michael Rynkiewicz*[1], *Frank Bibbins*[1], *Susan Cantin*[1], *Jesse Quinn*[2], *Scott Magee*[1], *Joan Gorga*[2], *Cassandra Celatka*[1], *Pamela Nagafuji*[1], *Thomas Bannister*[1], *Harold V. Meyers*[1], *Robert Babine*[1], *Neil Hayward*[1], *Sherin S. Abdel-Meguid*[1], *and James Strickler*[1]*. (1) Daiichi Asubio Medical Research Laboratories LLC (DAIAMED), One Kendall Square, Building 700, Cambridge, MA 02139, Fax: 617-621-0555, jian.lin@daiamed.com, (2) Daiichi Asubio Medical Research Laboratories, LLC (DAIAMED)*

Human Factor XIa is a trypsin-like protease that is involved in the blood coagulation cascade. This enzyme is believed to play a major role in amplification and propagation of the thrombotic response. In order to investigate the critical involvement of FXIa in thrombosis and the potential of FXIa inhibitors as safe antithrombotic therapeutics, a series of potent, selective peptidomimetic inhibitors of FXIa have been designed and synthesized. Details of design, synthesis and the structure-activity relationships of these compounds will be discussed.

248.

SAR EXPLORATION OF ALPHA-KETOTHIAZOLE ARGININE DERIVED FACTOR XIA INHIBITORS. ***Hongfeng Deng****, Thomas D. Bannister, Lei Jin, Pamela Nagafuji, Cassandra A. Celatka, Jian Lin, Tsvetelina I. Lazarova, Michael J. Rynkiewicz, Frank Bibbins, Jesse Quinn, Joan Gorga, Pramod Pandey, James E. Strickler, Robert E. Babine, Harold V. Meyers, and Sherin S. Abdel-Meguid, Daiichi Asubio Medical Research Laboratories, LLC (DAIAMED), One Kendall Square, Bldg 700, Cambridge, MA 02139, Fax: 617-621-0555, hongfeng.deng@daiamed.com*

The development of an effective and safe orally active anticoagulant still remains as an unmet medical need. Recent studies indicate that coagulation factor XIa (FXIa), a trypsin-like serine protease, plays a major role in amplification of the thrombotic response and in maintaining clot integrity. In addition, populations with a FXIa genetic difficiency do not show severe bleeding problems. These findings suggest that FXIa may be a safe target for antithrombotic therapy. Starting with an alpha-ketothiazole arginine moiety as the P1 element, we used a structure-based drug design approach to develop FXIa inhibitors. This presentation will focus on our efforts to explore the less peptide-like FXIa inhibitors.

249.

SYNTHESIS AND BIOLOGICAL EVALUATION OF ARYL BORONIC ACIDS AS INHIBITORS OF FACTOR XIA. ***Tsvetelina I. Lazarova***[1], *Lei Jin*[2], *Michael Rynkiewicz*[2], *Joan Gorga*[3], *Frank Bibbins*[3], *Harold V. Meyers*[1], *Robert Babine*[2], *James Strickler*[3], *and Sherin S. Abdel-Meguid*[2]*. (1) Department of Medicinal Chemistry, Daiichi Asubio Medical Research Laboratories LLC, One Kendall Square, Building 700, Cambridge, MA 02139, Fax: 617-621-0555, tsvetelina.lazarova@daiamed.com, (2) Department of Structural Chemistry, Daiichi Asubio Medical Research Laboratories LLC, (3) Department of Biochemistry, Daiichi Asubio Medical Research Laboratories LLC*

Thromboembolitic diseases are a major cause of death and disability in the western world. Some of the limitations of the available antithrombotic therapies are a lack of oral bioavailability and the need to constantly monitor blood parameters. Most of the current research efforts have focused on the development of inhibitors of thrombin and factor Xa (FXa) with much progress reported in these areas, including compounds in advanced clinical development.

We have focused on a relatively unexplored target in the blood coagulation cascade, namely factor XIa (FXIa). Since FXIa plays a role in the amplification pathway in coagulation and not in initiation of clotting, an inhibitor specific for FXIa and not for other coagulation pathway proteases may be effective at reducing the risk of thrombosis without a significant risk of bleeding. Here we report the synthesis and biological evaluation of aryl boronic acid derivatives as potent and selective inhibitors of FXIa.

250.

DEVELOPMENT OF FACTOR VIIA INHIBITORS: ADDRESSING PHARMACOKINETIC PARAMETERS. *Aleksandr Kolesnikov*[1], ***Roopa Rai***[1], *Wendy B. Young*[1], *Steven Torkelson*[1], *William D. Shrader*[2], *Ellen M. Leahy*[1], *Bradley A. Katz*[1], *Paul A. Sprengeler*[1], *Liang Liu*[1], *Joyce Mordenti*[1], *Erik Gjerstad*[1], *and Jim Janc*[1]*. (1) Medicinal Chemistry, Celera, 180 Kimball Way, So. San Francisco, CA 94080, (2) Department of Medicinal Chemistry, Celera Genomics*

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. We have previously described the development of direct small-molecule inhibitors of Factor VIIa. This class of compounds suffered from short half-life and rapid clearance when dosed IV to rats. We will now present small modifications to our core structure which allow modulation of half-life and clearance, potentially making these molecules suitable candidates for drug development. 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.

251.

DEVELOPMENT OF FACTOR VIIA INHIBITORS: SELECTIVITY IN TRYPSIN FAMILY PROTEASES. ***William D. Shrader***[1], *Jennifer Costerison*[1], *John Hendrix*[1], *Huiyong Hu*[1], *Aleksandr Kolesnikov*[1], *Vijay Kumar*[1], *Ellen Leahy*[1], *Roopa Rai*[1], *Michael Shaghafi*[1], *Tony Ton*[1], *Steve Torkelson*[1], *Kieron Wesson*[1], *Wendy B. Young*[1], *Brad A. Katz*[2], *Paul A. Sprengeler*[2], *Christine Yu*[2], *Ronnel Cabuslay*[3], *Erik Gjerstad*[3], *Jim Janc*[3], *and Ellen Sanford*[3]*. (1) Department of Medicinal Chemistry, Celera Genomics, 180 Kimball Way, South San Francisco, CA 94080, Fax: 650-866-6655, bill.shrader@celera.com, (2) Department of Structrual Biology, Celera Genomics, (3) Department of Enzymology, Celera Genomics*

The development of novel antithrombotic agents for the treatment of coagulation disorders is an active area of research in the pharmaceutical industry. The enzymes (Factors IIa, Xa, VIIa, IXa, and XIa) that comprise the extrinsic and intrinsic pathways of coagulation, leading to the formation of a blood clot, are trypsin-like serine proteases. We have previously described the development of active site small-molecule inhibitors of Factor VIIa (fVIIa). Within the trypsin family of coagulation proteases, obtaining highly selective inhibitors of Factor VIIa has been a challenging. We report a series of fVIIa inhibitors based on the 2-(2-hydroxy-biphenyl-3-yl)-1H-benzoimidazole-5-amidino scaffold with potency for factor VIIa and high selectivity against factors IIa and Xa. With this scaffold class, we identified a unique hydrogen bond interaction between a hydroxyl on the distal ring of the biaryl system and the backbone carbonyl of fVIIa Lysine-195. This selective interaction is not present in other coagulation proteases, and provides for enhanced selectivity and potency for fVIIa. The structures of fVIIa and a soluble fragment of tissue factor (TF) in complex with the described potent and selective fVIIa inhibitors have been determined by X-ray crystallography, and reiterates the selectivity obtained in our enzyme activity assays. In this poster, the synthesis of the inhibitors and the structural basis for their fVIIa selectivity will be discussed based on the X-ray structures of the ternery complex of fVIIa/TF/inhibitor.

252.

DISCOVERY OF POTENT AND SELECTIVE BIARYL DERIVATIVES AS TISSUE FACTOR/FACTOR VIIA INHIBITORS THROUGH STRUCTURE-BASED DRUG DESIGN. ***Pooran Chand***[1], *Pravin L. Kotian*[1], *Ali Dehghani*[1], *Yahya El-Kattan*[1], *Tsu-Hsing Lin*[1], *Minwan Wu*[1], *R. Scott Rowland*[2], *Krishnan Raman*[1], *Shanta Bantia*[1], *Shane Arnold*[1], *and Yarlagadda S. Babu*[1]*. (1) BioCryst Pharmaceuticals, Inc, 2190 Parkway Lake Drive, Birmingham, AL 35244, Fax: 205-444-4640, pchand@biocryst.com, (2) Millennium Pharmaceuticals, Inc*

Factor VIIa is a serine protease in the coagulation cascade. Factor VIIa (FVIIa) when complexed with Tissue Factor (TF) triggers the coagulation cascade, and forms a clot. The important role of TF and Factor VIIa in both thrombotic and inflammatory processes associated with cardiovascular disease is well documented. Therefore, the inhibition of serine protease activity of TF/FVIIa complex is seen as a promising target for developing clinical candidates for various cardiovascular applications. Based upon the crystal structure of the TF/FVIIa enzyme complex, a series of 2′-[(amidinophenyl/pyridylamino)methyl/carbonyl]-

4-alkylaminocarbonyl-4′-vinyl-biphenyl-2-carboxylic acid derivatives has been synthesized. The introduction of the alkoxy group in addition to vinyl moiety in the ring resulted in increased potency of the molecule. These inhibitors demonstrate high potency against TF/FVIIa complex while maintaining substantial selectivity versus other closely-related serine proteases such as Trypsin, Thrombin, Factor Xa, Plasmin, and others.

253.
NONCOVALENT INHIBITION OF THE SERINE PROTEASES, ALPHA-CHYMOTRYPSIN AND TRYPSIN BY TRIFLUORO(ORGANO)BORATES. ***Reem Smoum**, Department of Medicinal Chemistry and Natural Products, Hebrew University in Jerusalem, Jerusalem, Israel, Jerusalem 91120, Israel, reems@md.huji.ac.il*

Fluorinated boron containing compounds were found to be reversible competitive inhibitors of alpha-chymotrypsin and trypsin. These compounds inactivate the enzymes as a result of the formation of hydrogen-bondings between fluorine atoms of the inhibitors and the serine protease.

254.
DISCOVERY OF NOVEL INHIBITORS OF KALLIKREIN. ***Mark M. Staveski**, Scott F. Sneddon, Fredric J. Vinick, Jill S. Gregory, Christopher Yee, Andrew D. Janjigian, Sharon Nahill, Andrew Napper, and Marina Leonard, Drug Discovery and Development, Genzyme Corporation, 500 Kendall Street, Cambridge, MA 02142-1108, Fax: 617-252-7550, mark.staveski@genzyme.com*

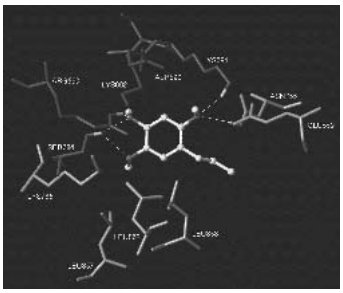
This presentation describes the discovery and optimization of novel inhibitors of the serine protease kallikrein. High-throughput screening resulted in the identification of an active minor product from a combinatorial library. The early lead compounds were characterized in several in-vivo models, and promising activity was demonstrated. The application of a genetic algorithm to the optimization of activity of combinatorial libraries is described and the "non-obvious" molecules that result.

255.
DEVELOPMENT OF NEW HEMOSTATIC DRUGS ON THE BASIS OF LABDANE DITERPENOIDS ISOLATED FROM CENTRAL ASIAN *LAGOCHILUS* PLANT SPECIES. *U. N. Zainutdinov, **M. A. Turabekova**, and Sh. I. Salikhov, Chemistry Department, National University of Uzbekistan named after Mirzo Ulugbek, Vuzgorodok, Tashkent, 700174, Uzbekistan, Fax: +998-71-1206475, malohathon@yahoo.com, malohathon@yahoo.com*

Lagochilus plant species growing in Central Asia are well-known for their hemostatic properties and have been widely applied in folk medicine. The extracts of *Lagochilus* spp have also shown a good promise when applied to treat hemophilia. The main components responsible for such activity of these herbal remedies are labdane diterpenoids, thirty of which have been isolated and extensively investigated in our research laboratory. All the compounds have demonstrated a hemostatic activity to a various extent and a new drug Lagoden ® has been developed and approved for public use. Lagoden (5% solution of lagohirisine sodium salt to be administrated intravenously) has shown to shorten considerably bleeding time, to activate fazes one and two of hemostasis and to accelerate thromboplastin formation. Here we present the results of our collaborative and multidisciplinary studies on development of new and highly effective non-toxic hemostatic agents of *Lagochilus* plant origin.

256.
MOLECULAR DOCKING OF THE HIGHLY HYPOLIPIDEMIC AGENT α-ASARONE WITH THE CATALYTIC PORTION OF HMG-COA REDUCTASE. ***Jose Luis Medina-Franco**[1], Fabián López-Vallejo[1], Sergio Rodríguez-Morales[1], Rafael Castillo[1], Germán Chamorro[2], and Joaquín Tamariz[3]. (1) Department of Pharmacy, Universidad Nacional Autonoma de Mexico, Avenida Universidad 3000, Mexico City 04510, Mexico, Fax: (+5255)-5622-5329, medinajl@correo.unam.mx, (2) Laboratorio de Toxicología Preclínica, ENCB-IPN, (3) Departamento de Química Orgánica, ENCB-IPN*

Docking experiments using a number of published crystal structures of HMG-CoA reductase with the potent hypocholesterolemic agent α-asarone are described. The results indicate that α-asarone binds in the enzyme's active site. The methoxy groups play a key role in the binding and probably also in its biological activity, as shown by extensive SAR studies reported for analogues of α-asarone. The docking results will be valuable for the structure-based design of novel hypolipidemic agents.



257.
RNA INTERFERENCE: AN ENABLING TOOL FOR TARGET IDENTIFICATION AND VALIDATION. ***Christopher P. Miller**, Department of Biological Technologies, Wyeth Research, 35 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-7519, cmiller@wyeth.com*

RNA interference (RNAi) refers to the ability of short double-stranded RNA molecules to silence expression of specific mRNAs inside cells. RNAi can be used to identify and validate candidate drug targets prior to high throughput screens for small molecule modulators or initiation of protein therapeutic development programs. An important feature of RNAi is that it enables scientists to selectively inhibit the function of any gene of interest, allowing for much more efficient target identification and validation than previously possible. The main uses to date have been in transformed cell lines, although RNAi techniques for primary cells and in vivo models are becoming available. This presentation will give an overview of how RNAi is being used by pharmaceutical companies and will include examples of how it is having a tangible impact on drug discovery.

258.
GENETIC MODELING OF CHEMICAL ANTAGONISTS: GENOME-SCALE DISCOVERY OF DRUG TARGETS BY IN VIVO FUNCTIONAL ANALYSIS. ***Brian P. Zambrowicz**, Lexicon Genetics Incorporated, 8800 Technology Forest Place, The Woodlands, TX 77381, brian@lexgen.com*

While the sequencing of the human genome has provided a list of all potential host drug targets, there is a critical need to identify the small subset of these targets useful for the development of new therapeutics. As a genetic model of a perfectly selective and potent chemical antagonist, the analysis of the phenotypes of knockout mice allows for the prediction of the mechanism-based efficacy and side effect profile of target modulation within the context of mammalian physiology. The power of this approach has been demonstrated by studies examining the targets of the best-selling drugs and current pharmaceutical pipelines. These analyses have confirmed a strong correlation between the target's knockout phenotype and the efficacy and/or side effects of drugs that modulate them. We have implemented a genome-scale target discovery and validation approach by developing systems and infrastructure to generate and comprehensively phenotype gene knockout mutant mice at a rate of about 1,000 lines per year. Gene targeting by homologous recombination and gene trapping have been successfully scaled to mutate all genes in key families encoding proteins that could be modulated by antibodies or small molecule therapeutics. The battery of tests included in our phenotypic analysis program have been specifically selected to reveal those genes that encode key control points in mammalian behavior and physiology encompassing mechanisms with direct relevance for unmet medical need. Methods for accomplishing genome-scale knockout analysis and selected examples of phenotypes demonstrating new potential mechanisms for therapeutic intervention will be discussed.

259.
PROTEASE SUBSTRATE PROFILING. ***Jennifer Harris**, Chemistry Department, Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, Fax: 858-812-1584, jharris@gnf.org*

Historically, proteases were recognized as non-selective, promiscuous enzymes that were responsible for indiscriminately degrading dietary proteins. However, it is now widely accepted that this enzyme class plays crucial roles in the initiation and regulation of biological pathways including fertilization, development,

differentiation, homeostasis, immunity, cell migration, cell activation, wound healing, and cell death. One crucial characteristic of a protease is the ability to discriminate among many potential substrates, termed the substrate specificity of the protease. The substrate specificity of a protease is determined by multiple factors that include the temporal and spatial expression of the protease, temporal and spatial expression of potential substrates, activation of the protease by post-translational modification, availability of essential co-factors or adaptor proteins, and the presence of endogenous inhibitors. Identifying the preferred substrates and substrate cleavage sequence of a protease can have a large impact on the ability to characterize the underlying biology of the protease as well as aid in the drug discovery process.

260.

BENZOPYRANS ARE SELECTIVE ESTROGEN RECEPTOR BETA AGONISTS FOR USE IN THE TREATMENT OF PROSTATIC DISEASES. ***Bryan H. Norman***[1], *Timothy I. Richardson*[2], *Venkatesh Krishnan*[2], *Jeffrey A. Dodge*[2], *Charles W. Lugar*[2], *Yong Wang*[2], *Keyue Chen*[2], *Gregory L. Durst*[2], *Robert J. Barr*[2], *Chahrzad Montrose-Rafizadeh*[2], *Harold E. Osborne*[2], *and Huaping Mo*[2]. *(1) Discovery Chemistry Research, Eli Lilly and Company, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, Fax: 317-277-3652, norman@lilly.com, (2) Eli Lilly and Company*

A novel class of selective ER beta ligands that belong to the benzopyran scaffold have been identified. These compounds bind the ER beta binding pocket with high affinity (< 1 nM Ki) and show full agonist activity for ER beta in cell based reporter assays. Additionally, these compounds show ER beta binding selectivity (>10X) and functional selectivity (>40X), relative to ER alpha. X-ray crystal structures reveal an agonist-like conformation for helix 12, when co-crystallized with the ligand binding domain of ER beta. A different binding mode has been observed when these molecules are co-crystallized with ER alpha, providing a unique platform for structure based drug design. In addition to x-ray crystallography and modeling, we have used biological tools, such as wild type and ER beta KO mice, protein NMR, TUNEL analysis and tissue biomarkers to demonstrate the potential use of ER beta agonists for the treatment of benign prostatic hyperplasia. Collectively, we report the identification, and characterization of a novel class of ER beta selective agonists that are devoid of the risk of non-selective ER agonists.

261.

OVERCOMING IKR ISSUES IN THE SEARCH FOR A CCR5 ANTAGONIST FOR THE TREATMENT OF HIV. ***David A. Price***, *Sandwich Laboratories, Pfizer Global Research and Development, Sandwich, Kent, United Kingdom, david.a.price@pfizer.com*

Interaction of compounds with the human ether-a-go-go related gene (hERG) potassium channel has been linked to acquired long QT syndrome that predisposes individuals to lethal cardiac arrhythmias. This is recognised by the pharmaceutical industry as a key attrition risk that has had profound effects on development strategy and has led to the removal of compounds from the market. This presentation describes the challenges faced during the discovery of a potent CCR5 antagonist for the treatment of HIV. In particular it focuses on the importance of generating high selectivity over the hERG ion channel and the associated risks that are caused by inhibition of IKr. The medicinal chemistry strategy and screening methodology adopted by the project to overcome these effects is described and a review of relevant literature is also presented. The subsequent discovery and profile of UK-427,857 (currently in Phase II) is disclosed with SAR around close analogues showing the subtlety of ion channel effects.

262.

TARGETING P-GLYCOPROTEIN: A CONTINUING CHALLENGE. ***Gerhard F. Ecker***, *Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, Vienna A-1090, Austria, Fax: +431-4277-9551, gerhard.f.ecker@univie.ac.at*

Since in the early eighties P-glycoprotein (P-gp) has been discovered as target for the treatment of multidrug resistant tumors, considerable efforts have been devoted to design and development of P-gp inhibitors. Although this yielded several compounds being currently tested in clinical phase III studies, the outcome is rather disappointing. This seems to be mostly due to the physiological role of P-gp., e.g. for a proper function of the blood-brain barrier. The involvement of P-gp in the field of ADME is increasingly recognized and studies currently focus on proper prediction of substrates rather than development of new inhibitors. This needs the development of methods and virtual screening protocols which are able to handle the high promiscuity of P-gp in ligand recognition. Additionally, the molecular basis of both substrate recognition and translocation is far from being solved. With respect to these issues, P-gp undoubtedly serves as paradigm for the whole ABC-transporter family.

263.

STRUCTURAL STUDIES OF P-GLYCOPROTEIN AND MULTIDRUG RESISTANCE PROTEIN 1. ***MF Rosenberg***, *Faculty of Life Sciences, The University of Manchester, Sackville Street, Manchester M60 1QD, United Kingdom, Fax: 441612360409, mark.rosenberg@umist.ac.uk*

Resistance to chemotherapeutic agents in the clinic is an obstacle to successful treatment of cancer patients and is responsible for a large numbers of deaths. Over-expression of P-glycoprotein (P-gp) and multidrug resistance protein 1 (MRP1) are important factors in contributing to drug resistance in cancer. These membrane proteins belong to the family of ATP-Binding Cassette (ABC) transporters of which there are 48 genes in humans. Using electron microscopy of two-dimensional crystals of MRP1 and P-gp, we describe 3-D structural data of these eukaryotic ABC transporters to approximately 20 Å resolution for MRP1 and for P-gp to approximately 8Å resolution. For P-gp, each transmembrane domain consists of six long α-helical segments. Five of the α-helices from each transmembrane domain appear to be related by a pseudo-twofold symmetry, whilst the sixth breaks the symmetry. Two globular densities at the cytoplasmic side correspond to the hydrophilic, nucleotide-binding domains. Our aim is to determine their structures to the highest possible resolution with a view to improving the design of inhibitors and drugs used to treat cancer patients.

264.

FUNCTIONAL ASPECTS OF MULTIDRUG TRANSPORT. ***Peter Chiba***[1], *Gerhard F. Ecker*[2], *Karin Pleban*[2], *Stephan Kopp*[1], *and Wil N. Konings*[3]. *(1) Institute of Medical Chemistry, Medical University of Vienna, Waehringer Strasse 10, Vienna A-1090, Austria, Fax: +431 4277-60889, peter.chiba@meduniwien.ac.at, (2) Department of Pharmaceutical Chemistry, University of Vienna, (3) Department of Microbiology, University of Groningen*

Multidrug efflux transporters are found in all organisms and protect cells from diverse hydrophobic toxins. Substrate-binding domains of P-glycoprotein (P-gp) were characterized by photoaffinity labeling and detection of labeled component peptide-fragments by high resolution mass spectrometry. Photoaffinity labeling information was subsequently projected on a protein homology model of P-gp. The model indicates that drug binding occurs at the transmembrane domain (TMD) : transmembrane domain interfaces constituted by transmembrane segments 5/8 and 3/11, respectively. A recent cryo electron crystallography structure of P-glycoprotein at 8Å resolution suggested a repositioning of helices 5/6 and 11/12 during transport. This was indeed confirmed for LmrA, a bacterial homologue of P-gp. Again, initial binding occurred at the TMD:TMD interfaces. Ongoing experiments with P-gp are intended to demonstrate similar changes in human P-glycoprotein. These studies are expected to lead to a concept which links P-gp structure to the dynamics of the transport process.

265.

PHARMACOPHORE HYPOTHESIS FOR P-GLYCOPROTEIN SUBSTRATE RECOGNITION. ***Vaz Roy***[1], *Giovanni Cianchetta*[2], *Robert W Singleton*[1], *and Meng Zhang*[1]. *(1) Sanofi Aventis Pharmaceuticals, JR1-303E, 1041 Rt 202/206N, Bridgewater, NJ 08807, Fax: 908-541-5548, roy.vaz@aventis.com, (2) Dipartimento di Chimica e Tecnologia del Farmaco, Universita' di Perugia*

Trying to understand the complex interactions that substrates and inhibitors have with the efflux transporter, P-Glycoprotein has been the subject of various publications. In this work, we have confined our study to substrates by picking a diverse set of 129 based on the efflux ratios from Caco2 permeability measurements. These compounds were then evaluated for P-Glycoprotein inhibition using a Calcein AM assay. The subsequent data was used in a 3D-QSAR analysis using GRIND pharmacophore-based and physico-chemical descriptors. Pharmacophore based descriptors produced a much more robust model than the one obtained from physico-chemical based descriptors. This supports the process proposed by Seelig & co-workers previously published whereby the substrate enters the membrane as the first step and is then recognized by P-Glycoprotein. However, the strong correlation, highlighted by

PLS statistical analysis, between pharmacophoric descriptors and the inhibition values suggests that substrate interaction with perhaps the mouth of the protein or another binding site, plays a key role in the efflux process, yielding a model in which diffusion across the membrane is less important than substrate-protein interaction. One pharmacophore emerged from the analysis of the model. We pose that the recognition elements at least determined by the molecules used in this study are: 2 hydrophobic groups 11.5 Å apart, 2 hydrogen bond-acceptor groups 11.5 Å apart and the maximal dimension of the molecule is also plays a role in its recognition as a substrate.

266.
PROGRESS IN COMPUTATIONAL MODELING OF P-GLYCOPROTEIN. ***Sean Ekins**, GeneGo Inc, 500 Renaissance Drive, Suite 106, St. Joseph, MI 49085, ekinssean@yahoo.com*

The discovery and optimization of new drug candidates is becoming increasingly reliant upon the combination of experimental and computational approaches related to drug metabolism, toxicology and general biopharmaceutical properties. With the small amounts of data available for transporters a number of computational technologies can be applied including pharmacophores and statistical approaches. Examples based on recent publications for P-glycoprotein substrates and inhibitors will be provided to illustrate the insights that might be obtained using a combined in vitro and computational approach. The consideration of the various interactions with other proteins with P-glycoprotein can also be understood using systems biology software, and predictions for new inhibitors or substrates should be understood in the context of the whole cell. With the increasing identification of further drug transporters in the future it will become even more critical to understand their structure-transport-relationships if we are to predict potential drug-transporter interactions or derive potent therapeutic inhibitors.

267.
TRANSCRIPTIONAL PROFILING FOR SMALL MOLECULE DRUG DISCOVERY. ***Kyle Chan**, Celgene, 4550 Towne Center Ct, San Diego, CA 92121, KChan@CELGENE.com*

Global gene expression analysis (high-density gene array) has become a standard tool in early discovery research such as target discovery and validation in the pharmaceutical industry. This technology is also being applied further down the drug discovery process to help optimize potential small molecule drug candidates using cell-based and animal models in the preclinical setting as well as for pharmacogenomics analyses in the clinic. This presentation will focus on the application of gene expression analysis to small molecules. Case studies and examples will be presented on target-based small molecule modulators, highlighting the use of gene expression profiles to categorize compounds and to determine potential off-target properties.

268.
TUNING THE SPECIFICITY AND POTENCY OF ARTIFICIAL TRANSCRIPTIONAL ACTIVATORS. ***Anna K. Mapp**, Department of Medicinal Chemistry and Department of Chemistry, University of Michigan, 930 N University, Ann Arbor, MI 48109-1055, Fax: 734-615-8553, amapp@umich.edu*

A growing number of human diseases are characterized by aberrant gene transcription patterns linked to malfunctioning transcriptional regulators. The development of artificial transcriptional activators as tools to better characterize the relationship between altered transcription patterns and disease and in the longer term to define key characteristics of transcription-based therapeutics is thus an area of emerging importance. Among the greatest challenges is the development of artificial activators that function robustly in a cellular context and in an organism-specific or tissue-specific manner. The traditional paradigm describing activator function links affinity for transcriptional machinery targets with functional potency and ascribes specificity to the DNA binding domain of the protein. Here we show that superimposition of multiple binding interactions onto a single peptide sequence provides a mechanism for creating potent artificial activators, and further, that the potency of the activators can be tuned by altering the affinity for one of the protein binding partners. In addition, we demonstrate that by using a screening strategy, organism-specific transcriptional activators of varying potency can be generated. The application of these strategies to small molecule transcriptional activators will be briefly described.

269.
GENE EXPRESSION DRIVEN DRUG DISCOVERY. ***Stanislaw Pikul**, Avalon Pharmaceuticals, Inc, 20358 Seneca Meadows Parkway, Germantown, MD 20876, spikul@avalonrx.com*

Advances in genomics have provided many identified targets that can form the bases for variety of drug discovery programs. These new targets typically undergo an extensive validation process and fall into a limiting set of protein classes. Expression analysis enables the exploration of biological pathways or genetic networks at multiple points rather than at the level of isolated targets. This approach may allow one to identify multiple, distinct, and novel chemical entities that are 'targeting' the biological pathway of interest. We have applied this approach to create new types of primary screens, to validate and prioritize hit families, and to characterize novel molecules throughout the lead optimization process. We will discuss examples from each of these stages of the discovery process.

270.
GENOMICS APPLIED TO DRUG CANDIDATE PRIORITIZATION AND FOR IDENTIFICATION OF NOVEL THERAPEUTICALLY USEFUL PROPERTIES OF EXISTING DRUGS. ***Kurt Jarnagin**, A. Roter, A. Tolley, M. Lee, and C. G. Natsoulis, Iconix Pharmaceuticals, Inc, 325 E. Middlefield Rd., Mountain View, CA 94043, kjarnagin@iconixpharm.com*

To improve compound selection during late stage discovery and early stage drug development we have developed a large library of gene expression biomarkers, Drug Signatures(™), for important toxicities and mechanisms of action. These signatures are derived from the DrugMatrix(®) database of gene expression, clinical chemistry and molecular pharmacology data derived by treating rats with more than 600 drugs, failed drugs and standards. This comprehensive database of expression profiles was developed using ~15,000 microarrays. The Drug Signature library is composed of more than 250 diagnostic and predictive biomarker sets for toxicity and mechanism. We will discuss how critical development choices are influenced by comparison of the biological effects of candidate drugs to the reference database and by establishing the relationship of compounds to each other and to their toxicity through the use of Drug Signatures, which identify groups of genes related to the toxic process and mechanism related pathways. These findings can uncover novel properties of individual drugs or drug families. Illustrations, based on our work in-house and with pharmaceutical companies, will show applications of these techniques to prioritize drug candidates during development and to uncover interesting new leads and uses for existing drugs.

271.
HIGH-THROUGHPUT PARALLEL ANALYSIS OF MULTIPLE CELLULAR LIPIDS BY ESI MASS SPECTROMETRY. *Stephen B. Milne, Jeffery S. Forrester, and **H. Alex Brown**, AfCS Lipidomics Lab, Department of Pharmacology, Vanderbilt University Medical Center, 23rd Ave South & Pierce, Nashville, TN 37232, Fax: 615-936-6833, alex.brown@vanderbilt.edu*

A goal of our laboratory has been to understand contextual lipid changes in biological membranes resulting from disease progression or following cell signaling events (e.g., receptor activation by a hormone or drug). As the lipidomics core laboratory for the Alliance for Cellular Signaling, we focus on the analysis of a number of ligands that activate macrophages. Our multiplexed computational lipidomic approach to this complex problem couples electrospray mass spectrometric (ESI MS) analysis with a sophisticated collection of computational algorithms to facilitate the simultaneous analysis of hundreds of lipid species in mammalian cells. This lipid profiling allows us to identify temporal changes in membrane lipid species creating a better understanding of the biophysical and biochemical processes that mediate membrane signaling pathways. This top down approach allows identification of patterns of signaling and molecular diagnostics.

272.

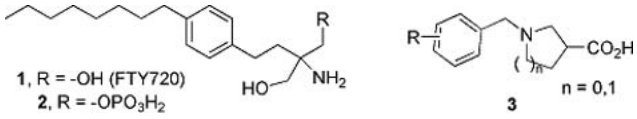
LIPID-BASED INHIBITORS OF ICMT AND OTHER PRENYLATED PROTEIN PROCESSING ENZYMES. ***Richard A Gibbs***[1], *Brian S. Henriksen*[2], *James L. Donelson*[2], *Surya K. De*[2], *Jessica L. Anderson*[3], *Sarah Hudon*[3], *and Christine A. Hrycyna*[4]. *(1) Purdue Cancer Center and Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, Fax: 1-765-494-1414, skd125@pharmacy.purdue.edu, (2) Department of Medicinal Chemistry and Molecular Pharmacology, (3) Department of Chemistry, (4) Department of Chemistry and the Purdue Cancer Center*

We report here the development of isoprenoid-based inhibitors of Isoprenylcysteine Carboxyl Methyltransferase (Icmt), through the synthesis of analogs of the minimal Icmt substrate N-acetylfarnesylcysteine (AFC). Our initial studies led to the discovery of two AFC analogs that were both low-micromolar inhibitors of yeast and human Icmt. This work was continued with the synthesis of an all-carbon AFC analog and the truncated AFC analog farnesylglycine. The all-carbon AFC analog was not a substrate and was a modest inhibitor of Icmt, while the farnesylglycine analog was unrecognized by the enzyme. Developing a pharmacophore model for Icmt inhibitors necessitated a thorough investigation of the acyl moiety of AFC. A flexible solid phase method has been developed for the synthesis of AFC analogs. A small library of acyl modified AFC analogs is being prepared via automated solid-phase synthesis. A similar approach is now being explored to develop inhibitors of the prenyl-dependent protease Rce1.

273.

DISCOVERY OF POTENT, ORALLY BIOAVAILABLE, IMMUNOSUPPRESSIVE N-BENZYL PYRROLIDINE AND AZETIDINE CARBOXYLATE $S1P_1$ RECEPTOR AGONISTS. ***Jeffrey J. Hale***, *Department of Medicinal Chemistry, Merck Research Laboratories, P. O. Box 2000, Rahway, NJ 07065*

The systemic administration of the novel immunosuppressant FTY720 (**1**) promotes a lowering of circulating T lymphocytes which prevents their infiltration into transplanted or antigen-bearing non-lymphoid tissues. The immunosuppressive efficacy of **1** is due to the formation *in vivo* of an active phosphate ester metabolite (**2**), which is a potent agonist of four of the five known sphingosine-1-phosphate receptors ($S1P_{1,2,3,4,5}$). $S1P_1$ agonism has been strongly implicated as the main driver of the observed lymphocyte changes. Elaborations of **2** afforded phosphonate-based S1P receptor agonists; structural features that independently influence pharmacokinetics and receptor-subtype selectivity have been identified. The rational incorporation of $S1P_1$ agonist high-throughput screening leads led to the identification of potent, orally bioavailable, immunosuppressive $S1P_1$ agonists of the general structure **3**. The details of this work, which will include descriptions of the structure-activity relationships of analogs of **3** and their *in vivo* characterization as potent, orally active, immunosuppressive agents, is the subject of this presentation.



274.

PHOSPHOINOSITIDE RECOGNITION DOMAINS: TARGETING OF PROTEINS TO MEMBRANES. ***Tatiana G. Kutateladze***[1], *Stephanie Lee*[1], *Matthew Cheever*[1], *Michael Overduin*[2], *and Christopher Burd*[3]. *(1) Department of Pharmacology, University of Colorado HSC, Aurora, CO 80045, Fax: 303-724-3663, Tatiana.Kutateladze@uchsc.edu, (2) Institute for Cancer Studies, University of Birmingham, (3) Department of Cell and Developmental Biology, University of Pennsylvania*

Targeting of a wide variety of cytosolic proteins to membrane surfaces involves specific recognition of phospholipid headgroups and insertion into lipid bilayers. For example, FYVE and PX domain-containing proteins are recruited through binding to phosphoinositides (PIs), and their hydrophobic elements insert into membranes. Such dual anchoring provides specificity and affinity necessary for proper protein functions. Here, the multivalent mechanism of membrane docking and insertion by these domains was characterized. The membrane insertion interface, the depth and angle of penetration, and quantitative membrane-binding parameters were determined. The residues involved in the non-specific electrostatic interactions were identified, and effects of the membrane lipid composition on the protein orientations and the depth of penetration were determined. The functional significance of hydrophobic insertion, headgroup ligation, and non-specific electrostatic interactions is established. Other components of the membrane targeting mechanism are investigated.

275.

PROBING LIPID-PROTEIN INTERACTIONS BY LIPIDOMICS APPROACHES. ***Li Feng***, *Echelon Biosciences Inc, 675 Arapeen Way, Suite 302, Salt Lake City, UT 84108, Fax: 801-588-0497, lfeng@echelon-inc.com*

Lipid signaling by phosphoinositides (PIPns) involves an array of proteins with lipid recognition, kinase, phosphatase, and phospholipase functions. The key to understanding PIPn pathway signaling in the past has been identification and characterization of PIPn-modifying enzymes and PIPn-binding proteins. Synthetic PIPns functional probes play an indispensable role in identifying macromolecular targets. These lipid probes have been widely applied in determining PIPn selectivity in vitro, characterization of PIPn-protein complexes in living cells, and establishment of non-radioactive high-throughput lipid enzyme assays. Synthetic lipid probes also provide tools to profile lipid-binding proteins in a more systematic way. Systematically analyzing the expression patterns and levels of lipid binding proteins provide opportunities for therapeutic intervention and diagnosis.

276.

PROMISCUOUS DRUGS: SUPERIOR EFFICACY OF ONE PILL ON MANY TARGETS. ***Michael D. Miller***, *Pfizer Global Research & Development, Groton, CT 06340, michael.d.miller@pfizer.com*

The emerging science of network biology and the observed robustness of phenotype as observed in the large-scale mouse knock-out studies are providing paradigm changing evidence of the necessity to hit multiple targets simultaneously for superior efficacy, in many diseases. During the last decade, the industry has followed the assumption that a single drug hitting a single target was the "rational" way to design drugs. Post-genomics biology is teaching us the fundamental limitations of the single target philosophy. Ironically many drugs on the market, discovered in "black box" phenotype screens, are observed to bind potently to multiple targets and more so, this poly-pharmacology is key to their effect. The challenge now becomes how best to tackle the poly-pharmacology paradigm. Combination therapies of single-target agents appear to be a logical evolution. However such an approach will require complex dosing-finding and safety studies necessary to find the optimal safe dose. A simpler way to derive poly-pharmacological action is the concept of promiscuous drugs. Promiscuous drugs are attractive compounds occupying discrete areas of biological space common to several targets. The challenge to the biology and chemistry communities is then to rationally design and screen for poly-pharmacology.

277.

MAPPING BIOACTIVITY SPACE FOR FRAGMENT-BASED LEAD DISCOVERY. ***Tudor I. Oprea***[1], *Marius Olah*[1], *Maria Mracec*[2], *Ramona Rad*[2], *Liliana Ostopovici*[2], *Alina Bora*[2], *Nicoleta Hadaruga*[3], *and Cristian G. Bologa*[1]. *(1) Division of Biocomputing, University of New Mexico School of Medicine, MSC 084560, 1 University of New Mexico, Albuquerque, NM 87131-0001, toprea@salud.unm.edu, (2) Coriolan Dragulescu Institute of Chemistry Timisoara, (3) Faculty of Food Technology, USAB Timisoara*

Early drug discovery imposes limitations with respect to certain molecular properties, as first noted by Lipinski et al. (1997). His 'Rule of Five' was refined to the 'Rule of Three' (Ro3) by Congreve et al. (2003) in the context of fragment-based lead discovery: Molecular weight <300, number of hydrogen-bond donors and acceptors, and rotatable bonds <=, ClogP <=, and polar surface area <=0. Ro3-compliance in WOMBAT (indexed medicinal chemistry literature) illustrates diversity in both target and scaffold space: 3634 compounds, of which 336 have 'generic' names, were reported on 531 targets with 8479 activities. Of these, 1600 activities on 177 targets are >= 10 nM. There are 450 Ro3-compliant unique 'actives', of which 75 have generic names, e.g., clonidine, diazepam, idazoxan, morphine, nicotine, ondasentron, and quipazine. After eliminating 'unwanted' chemical structures, we performed the same study on 2 commercially available collections, ChemNavigator and ChemDiv. We compare WOMBAT (confirmed chemistry/biology space) and these two collections in the context of fragment-based drug discovery.

278.

FINDING DRUGS WITHIN CHEMISTRY SPACE: IMPACT OF THE CLINICAL DEVELOPMENT PROCESS. ***James F. Blake**, Computational Technologies, Array BioPharma Inc, 3200 Walnut Street, Boulder, CO 80301, Fax: 303-386-1420, jim.blake@arraybiopharma.com*

This presentation will focus on the derivation of appropriate characteristics for small molecule lead discovery libraries with potentially enhanced survivability characteristics. From a systematic evaluation of the calculated molecular properties of compounds in clinical development we have found that the development process selects for compounds with properties that fall within certain ranges. In particular, as the stage of development progresses, compounds that are advanced have lower calculated octanol/water partition coefficient (logP), polar surface area, and molecular weight. The findings of this study provide guidance for combinatorial library design and lead selection, which may improve the chance for ultimate success in lead optimization and clinical development.

279.

DISCOVERY OF NITROIMIDAZOPYRANS AND PA824: NOVEL THERAPEUTICS FOR THE TREATMENT OF TUBERCULOSIS. ***William R Baker**, Corus Pharma, 2025 1st Avenue, Suite 800, Seattle, WA 98121, Fax: 206-728-5095, bbaker@coruspharma.com*

The Nitroimidazopyrans (NAPs) are one of the most promising anti-tuberculosis agents to be discovered in decades. NAPs possess a core chemical structure related to nitroimidazole and nitrofuran antibacterials. Structure-function studies identified NAP compounds that were highly potent and, unlike the nitroimidazoles and nitrofurans, non-mutagenic. In parallel to the medicinal chemistry effort, novel MTB testing technologies were also developed which greatly accelerated the discovery and development of the series. The most active compound that emerged from the evaluations was PA824. PA824 is a potent, narrow spectrum, anti-tubercular compound showing excellent in vitro activity against clinical isolates including multi-drug resistant MTB strains. In addition, PA824 inhibited both replicating as well as static (non-replicating) MTB in culture, thus suggesting a potential treatment for latent MTB infections. Furthermore, PA824 was shown to be effective in animal models of infection when administered orally. PA824 was licensed to the Global Alliance for TB development and human safety trials will begin in 2005.

280.

MEDICINAL CHEMISTRY AND THE INNOVATION GAP. ***Christopher A. Lipinski**, Exploratory Medicinal Sciences, Pfizer Global Research and Development, Groton Laboratories (retired), Eastern Point Road, mail stop 8200-36, Groton, CT 06340, Fax: 860-715-3149, christopher_a_lipinski@groton.pfizer.com*

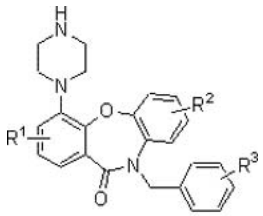
Target opportunity space is limited. Ligands for truly novel targets can be discovered but not consistently. The most innovative medicines will come from unexplored chemistry, efficacy and safety space. Where is the innovation going to come from?

Medicinal chemistry is critical to innovation; the more difficult the target the greater the importance of pristine screening libraries. HTS is not a screen but rather a process that relies on medicinal chemistry pattern recognition skills. “Tool-like” is now added to “lead-like” and “drug-like”. Drugs are discovered in the pharmaceutical industry and not in academia and government. Chemical tools to probe biology are equal opportunity; everyone can discover them. Tools enable breakthroughs in target validation. But a tool is not a drug and there is the rub. We know little about tools. Medicinal chemistry mostly exists in industry. Will there be a productive partnership between medicinal chemists and basic science expertise in academia and government? Finally, have drug-like filters and rules actually impacted on compounds described in J. Med. Chem?

281.

10-BENZYL-4-PIPERAZINYLDIBENZOXAZEPIN-11-ONE: A NEW MOLECULAR SCAFFOLD FOR REFINING THE PHARMACOPHORE CHARACTERISTICS FOR 5-HT$_6$ ANTAGONISM. ***Ralph N. Harris III**[1], David B. Repke[1], James M. Kress[1], Russell S. Stabler[1], Jacob Berger[1], Li Zhang[1], and Julie M. Brothers[2]. (1) Medicinal Chemistry, Roche Palo Alto LLC, 3431 Hillview Ave., Palo Alto, CA 94304, Fax: 650-354-2442, ralph.harris@roche.com, (2) Biochemical Pharmacology, Roche Palo Alto LLC*

The 5-HT6 receptor is a member of the trans-membrane family of G-protein coupled receptors. Although the functional role of 5-HT6 remains somewhat controversial, its exclusive localization in the central nervous system and apparent involvement in the regulation of certain neurotransmitters make it a potential target for therapy of various psychiatric disorders, particularly those involving cognition. Since its discovery, numerous high affinity ligands for 5-HT6 have been identified that share common structural features. These features suggest a pharmacophore model that is relatively simple, but incomplete. From molecular modeling and X-ray crystallographic data of known high affinity ligands, a new scaffold, the 10-benzyl-4-piperazinyl-dibenzoxazepin-11-one system I was designed and investigated as a molecular template to probe for additional points of ligand interaction at the 5-HT6 binding site. The structure-activity relationships for I will be presented and a refined pharmacophore model suggested.



I

282.

DISCOVERY AND SAR STUDIES OF 2,6-DIFLUOROBENZENESULFONIC ACID 1-METHYL-3-(1-METHYLPIPERIDIN-4-YL)-1H-INDOL-5-YL ESTER, A NOVEL AND POTENT 5-HT6 ANTAGONIST FOR THE TREATMENT OF COGNITIVE DEFICIT. ***Marta M. Piñeiro-Núñez**, Delbert D. Bauzon, Frank P. Bymaster, Zhaogen Chen, Eyassu Chernet, Michael P. Clay, Robert Crile, Neil W. DeLapp, Carl P. Denny, Julie F. Falcone, Michael E. Flaugh, Lawrence J. Heinz, Anton D. Kiefer Jr., Daniel J. Koch, Joseph H. Krushinski Jr., J. David Leander, Terry D. Lindstrom, Bin Liu, David L. McKinzie, David L. Nelson, Lee A. Phebus, Vincent P. Rocco, John M. Schaus, Mary C. Wolff, and John S. Ward, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, DC0510, Indianapolis, IN 46285, Fax: 317-276-7600, MPineiro@Lilly.com*

Cognitive deficit is a complicated and sometimes poorly-defined process that affects several domains such as short and long-term memory, attention, and executive function. Even though the only cognitive disease with an approved therapy is Alzheimer’s, cognitive deficit constitutes a major problem in other CNS disorders such as schizophrenia. Non-selective 5-HT6 antagonists such as clozapine (Ki = 9.5 nM)and olanzapine (Ki = 10 nM) have been reported to exert positive effects upon cognition, but, to date, no clinical studies with any selective 5-HT6 receptor antagonists have been reported. We describe here the discovery of 2,6-difluorobenzenesulfonic acid 1-methyl-3-(1-methylpiperidin-4-yl)-1H-indol-5-yl ester (Ki = 1.0 nM), a potent and selective 5-HT6 antagonist which was shown to improve performance in behavioral animal models for cognition. Additionally, we describe synthetic and SAR studies around this molecule, focusing our attention on a series of N(1)-alkylated derivatives and their in vitro binding and ADME properties.

283.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL, SELECTIVE 5-HT2C RECEPTOR AGONISTS FOR OBESITY. ***Taekyu Lee**[1], Albert J. Robichaud[1], Wenting Chen[1], Yimin Lu[1], Sarah Dowdell[1], Kristopher E. Boyle[1], Ian S. Mitchell[1], John M. Fevig[1], Ruth R. Wexler[1], Keith J. Miller[2], Brian L. Largent[2], Kenneth W. Rohrbach[2], James J. Devenny[2], and John F. McElroy[2]. (1) Discovery Chemistry, Bristol-Myers Squibb Company, P.O. Box 5400, Princeton, NJ 08543-5400, taekyu.lee@bms.com, (2) Discovery Biology, Bristol-Myers Squibb Company*

The 5-HT2C receptor has been consistently implicated in regulation of appetite. The role of activating the 5-HT2C receptor in the reduction of food intake and body weight has been demonstrated in a variety of animal models and short term human clinical trials. The current focus of many research groups is the development of selective 5-HT2C receptor agonists in an effort to minimize undesirable side-effects. It is especially crucial to achieve sufficient selectivity against the highly homologous 5-HT2A and 5-HT2B receptors, because they are suggested to cause hallucinogenesis and heart valve hypertrophy, respectively. Our structure-activity studies of a novel series of compounds have led to the identification of orally bioavailable, highly potent, selective ligands for the

5-HT2C receptor subtype. These functional agonists are efficacious in both acute and chronic models of feeding in rats. We will describe our recent efforts in the area of selective 5-HT2C agonists for potential use as anti-obesity agents

284.

POTENTIAL THERAPEUTICS FOR THE TREATMENT OF PSYCHIATRIC DISORDERS: DESIGN AND SYNTHESIS OF POTENT, SELECTIVE, BIOAVAILABLE 5HT2C RECEPTOR AGONISTS. ***Annmarie L. Sabb***[1], *Robert L. Vogel*[1], *Gregory S. Welmaker*[1], *Joan Sabalski*[1], *James Nelson*[1], *Gary Stack*[1], *Madeline Antane*[1], *Hossein Mazandarani*[2], *Jean Zhang*[2], *John Dunlop*[2], *Sharon Rosenzweig-Lipson*[2], *Karen Marquis*[2], *Steve Grauer*[2], *and Boyd L. Harrison*[1]. *(1) Chemical & Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, sabba@wyeth.com, (2) Wyeth Neuroscience, Wyeth Research*

"Atypical" antipsychotic agents are the most common treatment for schizophrenia. Although they have advantages over the older antipsychotic agents, such as haloperidol, some "atypical" agents, such as clozapine, possess 5HT2C antagonist affinity, which increases food intake and causes weight gain. On the contrary, 5HT2C agonists, active in animal models of schizophrenia, do not cause weight gain. Serotonin reuptake inhibitors (SSRI's) are used in the treatment of depression. Due to feedback regulation of serotonin by 5HT1A, 5HT1B and 5HT1D receptors, SSRI's increase serotonin chronically but not acutely, consistent with a 2-3 week delay before they become effective. Preclinical studies of 5HT2C receptor agonists have shown antidepressant-like effects in multiple animal models of depression. Therefore, 5HT2C agonists may provide a novel mechanism for the treatment of depression with a faster onset of action. A screen was conducted to discover potent, selective 5HT2C agonists. One lead discovered was WAY-629, a cyclohexyl[b][1,4]benzodiazepinoindole. WAY-629 (5HT2C Ki 56 nM, EC50 426 nM, Emax 90%) is a selective and efficacious 5HT2C agonist, which caused weight reduction in a rat model of obesity. In an effort to improve potency, a structure activity relationship study (SAR) was conducted. In the course of this work, a compound was prepared that contained a previously unreported heterocyclic ring structure. This compound, WAY-162545, is a potent and efficacious 5HT2C agonist (h5HT2C Ki 7 nM, Emax 90%). Resolution of WAY-162545, led to the discovery of WAY-163909, a potent, selective, bioavailable 5HT2C agonist, which is active in animal models of schizophrenia and depression.

285.

DISCOVERY, SAR AND BIOLOGY OF 5-HT2C RECEPTORS FOR THE TREATMENT OF OBESITY. ***Brian M. Smith***[1], *Jeffrey Smith*[1], *James H. Tsai*[1], *Jeffrey A. Schultz*[1], *Charles Gilson*[1], *Rita Chen*[1], *Scott Estrada*[1], *Douglas Park*[1], *Emily Prieto*[1], *Dipanjan Sengupta*[1], *Hazel Saldana*[1], *William Thomsen*[1], *Whelan Kevin*[1], *Kevin Creehan*[1], *Lena Gonzalez*[1], *Frederique Menzaghi*[1], *Christina Bjenning*[1], *Nigel Beeley*[1], *Robert R. Webb*[2], *and Dominic Behan*[1]. *(1) R&D, Arena Pharmaceuticals Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, bsmith@arenapharm.com, (2) Arena Pharmaceuticals, Inc*

Both animal and human data support a role for the 5-HT2C receptor in obesity therapeutics. The challenge has been to find compounds with appropriate safety margins, especially with regard to the valvular heart disease and pulmonary hypertension associated with the use of fenfluramine and dexfenfluramine. Although the pathogenesis of these diseases is uncertain, there is data to implicate activation of 5-HT2B and 5-HT2A receptors respectively. We have explored a number of scaffolds and scaffold subtypes, and identified structural features required for potent 5-HT2C receptor agonist activity. We have observed that certain scaffolds are inherently more selective for the 5-HT2C receptor versus the 5-HT2A and 5-HT2B receptors, and have identified key substitution patterns which enhance functional selectivity across all structural types. This selectivity can take the form of either increased spread in EC50 values, or reduction in maximal responses relative to serotonin at the 5-HT2A and 5-HT2B receptors while maintaining full response at the 5-HT2C receptor. As an example, our benzazepine series of 5-HT2C agonists generally show full agonist properties at the 5-HT2B receptor, but selectivity versus the 5-HT2C receptor can be 100-fold or greater, as measured by comparison of EC50 values in our IP3 assay. In contrast, our phenylpiperazine series of 5-HT2C agonists show a range of functional activities at the 5-HT2B receptor, from full agonism to inverse agonism, though the receptor affinity may be quite high in both cases. Our efforts in this area have led to the identification of a number of potent and selective 5-HT2C receptor agonists for the treatment of obesity, and the advancement of one of these compounds into clinical studies.

286.

DESIGN AND SYNTHESIS OF NONCOVALENT INHIBITORS OF CATHEPSIN S. ***Hong Liu***, *Phil Alper, David Tully, Rob Epple, Arnab Chatterjee, Jennifer Harris, Jun Li, Badry Bursulaya, Jennifer William, Khanhlinh Nguyen, Dan Mutnick, David Woodmansee, Michael Roberts, Ross Russo, Brian Masick, Yun He, and Donald S. Karanewsky, Medicinal Chemistry, Genomics Institute of the Novartis Research Foundation, 10675 John J. Hopkins Drive, San Diego, CA 92121, hliu@gnf.org*

Cathepsin S (CatS) is a lysosomal cysteine protease which has been shown to be critical in antigen presentation by the major histocompatibility class II complex (MHC II). Selective inhibition of CatS has been suggested as a potential therapeutic approach for the regulation of immune hyperresponsiveness, such as rheumatoid arthritis, multiple sclerosis, asthma and allergy. Secreted CatS degrades all of the major components of extracellular matrix and is culpable for the pathogenic cascade that ultimately leads to atherosclerosis, emphysema and chronic obstructive pulmonary disease. At GNF, we discovered a series of arylaminoethyl amides as noncovalent inhibitors of cathepsin S by HTS. Two issues, selectivity over other proteases and pharmacokinetic liabilities that often hinder the development of compounds against protease targets were addressed upfront. The solubility and oral bioavailability issues of the prototype peptidomimetic inhibitors were overcome by structure diversification and scaffold morphing approaches. Several chemotypes of novel noncovalent cathepsin S inhibitors were identified to possess high cathepsin S affinity (Ki < 10 nM) and excellent selectivity (>100 fold) over cathespins K, L and B. Molecular modeling, structure based drug design, synthesis and in vitro activity will be described.

287.

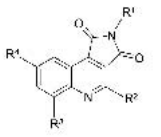
BIOAVAILABLE CATHEPSIN S INHIBITORS. ***Sukanthini Thurairatnam***[1], *David J Aldous*[1], *Joacy Aguiar*[1], *Cliff Bryant*[2], *Michael Graupe*[2], *Sue King*[3], *Justine Lai*[3], *Vincent Leroy*[1], *Jean-Philippe Letallec*[1], *John Link*[2], *Val Martichonok*[2], *John Patterson*[2], *Andreas Timm*[1], *and Sheila Zipfel*[2]. *(1) Medicinal Chemistry, Sanofi-aventis, Route 202-206, PO Box 6800, Bridgewater, NJ 08807-0800, Fax: 908-231-2202, sukanthini.thurairatnam@aventis.com, (2) Department of Medicinal Chemistry, Celera Genomics, 180 Kimball Way, South San Fransisco, CA 94080, (3) NA*

Cathepsin S (Cat S) is a 24 kD an elastolytic Cysteine protease of Papain super family. It has broad broad pH profile and active at neutral pHs. Cathepsin S has restricted tissue distribution and predominantly expressed in spleen, lymph, heart, lung and antigen presenting cells indicating its involvement in antigen presentation and T cell modulation. Cathepsin S has dual mode of action: Extracellular matrix degradation and intracellular invariant chain processing Experiments reported with the knockout mice and also using the inhibitors have indicated that Cathepsin S mediates the removal of the invariant chain from MHC class II molecules and allow the subsequent binding of antigenic peptide. MHC class II molecules then present the antigenic peptides on cell surfaces for recognition by T cells. Secreted cathepsin S has been shown to degrade all of the major components of extracellular matrix i.e. collagen, elastin, and proteoglycan. Hence, Cathepsin S inhibitors may be useful in the treatment of autoimmune diseases and tissue destructive diseases such as: COPD, Atherosclerosis, Asthma, RA. Sanofi-aventis in collaboration with Celera Genomics have identified compounds with excellent potency containing either Keto benzoxazole or nitrile moieties as Cathepsin S inhibitors. Initial lead compounds showed activity for Cathepsin S inhibition, but their profile was not optimum for a development candidate. Hence, a Lead Optimization Program was initiated with the view of improving potency, selectivity and Pharmacokinetic Profile. Variations of the P1, P2, and P3 groups have given compounds with improved potency, selectivity and Pharmacokinetic profile. Compound from the Keto benzoxazole series also demonstrated anti-inflammatory activity in the in vivo mode after oral dosing. The initial efforts leading to the identification of these analogues, their SAR, selectivity, cellular activity, eADME and PK profile along with the issues and challenges associated with their synthesis and discovery will be presented.

288.

PYRROLO[3,4-C]QUINOLINE-1,3-DIONES AS A NOVEL CHEMOTYPE OF POTENT NONPEPTIDE CASPASE-3 INHIBITORS. *Dmitri Kravchenko[1], Volodymyr Kysil[2], Alexey P Ilyin[1], Alexander Khvat[2], Sergey Tkachenko[2], Ilya Okun[2], Sergey Maliartchouk[2], and* ***Alexandre Ivachtchenko***[2]*. (1) Department of Organic Chemistry, Chemical Diversity Research Institute, Rabochaya St. 2-a, Khimki 114401, Russia, Fax: 7-095-9269780, dk@chemdiv.com, (2) ChemDiv, Inc, 11558 Sorrento Valley Rd., Suite 5, San Diego, CA 92121*

We describe synthesis, biological evaluation and structure-activity relationships for a novel class of potent caspase-3 inhibitors based on pyrrolo[3,4-c]quinoline-1,3-dione molecular scaffold. Caspase-3 inhibitory activity of the synthesized compounds is highly dependent on the substitutions on the core scaffold, especially at the 8-position. The most active compounds within the synthesized libraries inhibited caspase-3 with IC50 in the range of 3-30 nM. Evaluation against other caspases involved in apoptosis and mechanism of caspase-3 inhibitory action are discussed.



R¹ = H, alkyl, substituted alkyl, arylalkyl, heterocyclylalkyl, aryl, heterocyclyl, alkylamino, arylamino, heterocyclylamino and other.
R² = H, C₁-alkyl, substituted alkyl, aryl, heterocyclyl, substituted aminomethyl, substituted alkylamino, arylamino, substituted alkylthio, substituted alkylsulfanyl, alkylsulfinyl and alkylsulfonyl.
R³ = H, F, Cl, Br, CF₃.
R⁴ = H, F, Cl, Br, CH₃, NO₂, CN, SO₂H, SO₂NH₂, substituted sulfamoyl

289.

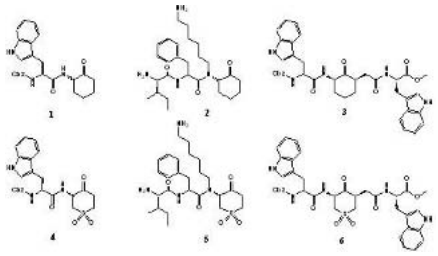
DESIGN AND SYNTHESIS OF HIGHLY POTENT AND SELECTIVE PROTEIN GERANYLGERANYLTRANSFERASE-I INHIBITORS. ***Erin E. Pusateri***[1]*, Dora Carrico[1], Hairuo Peng[1], Said Sebti[2], and Andrew D. Hamilton[1]. (1) Department of Chemistry, Yale University, 225 Prospect St., P.O. Box 208107, New Haven, CT 06520-8107, erin.pusateri@yale.edu, (2) Moffitt Cancer Center*

We have designed and synthesized a novel series of protein geranylgeranyltransferase-I (GGTase-I) inhibitors based on the c-terminal CAAL sequence of GGTase-I substrates. GGTase-I has increasingly become a target for anticancer therapies due to the recent findings that substrates of GGTase-I, including RhoA, RhoC, Rac-1, Cdc42, and R-Ras have been implicated in promoting tumorigenesis and/or metastasis. Using piperazine-2-one as a semi-rigid scaffold, we were able to develop inhibitors that selectively blocked the activity of GGTase-I over the related enzyme, protein farnesyltransferase. Our most active GGTase-I inhibitor has shown potent inhibition both *in vivo,* (IC_{50} = 9.5nM) and in whole cell assays, blocking the processing of Rap1A by GGTase-I with an IC_{50} value of 0.4 μM.

290.

4-HETEROCYCLOHEXANONE-BASED INHIBITORS OF SERINE PROTEASE PLASMIN. ***Fengtian Xue****, Department of Chemistry, Brown University, 324 Brook St., Providence, RI 02912*

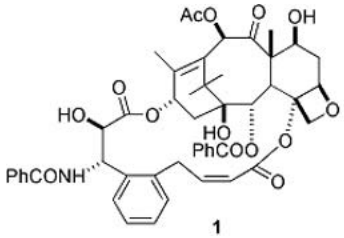
Inhibitors of plasmin have potential as cancer chemtherapeutic agents that act by blocking both angiogenesis and metastasis. We developed a new procedure for the synthesis of 4-sulfone-cyclohexaone-based inhibitors of serine protease. With this procedure, three new inhibitors were synthesized and evaluated for activities against serine protease plasmin. Though inhibitor **1** had moderate activity against plasmin, inhibitor **2** and **3**, which incorporated more enzyme binding subsites, showed good activity for plasmin. We also synthesized three other cyclohexanone-based inhibitors, **4**, **5**, and **6**, as respective counterparts of **1**, **2** and **3**. Comparative study of the three pairs proved that introducing 4-sulfone functionality into cyclohexanone nucleus dramatically enhanced activities of inhibitors.



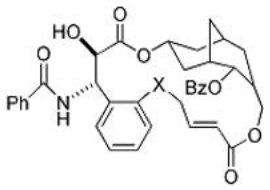
291.

DESIGN, SYNTHESIS, AND BIOACTIVITY OF SIMPLIFIED PACLITAXEL ANALOGS BASED ON THE T-TAXOL BIOACTIVE CONFORMATION. ***Thota Ganesh***[1]*, Andrew Norris[1], Shubhada Sharma[2], Susan Bane[2], Ami S. Lakdawala[3], James P. Snyder[3], and David G. I. Kingston[1]. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, tganesh@vt.edu, (2) Department of Chemistry, State University of New York, (3) Department of Chemistry, Emory University*

Paclitaxel (Taxol™) is a leading anticancer drug which promotes the assembly of tubulin to microtubules. It is a complex molecule that is difficult to synthesize in large quantities for clinical and commercial uses. It would be highly desirable if a simpler compound which retained the full activity of Taxol could be developed. The rational design of such simplified "Taxol-like" molecules is now in principle possible based on a new understanding of the conformation of Taxol in its binding site on tubulin. This understanding is based on computational and synthetic studies leading to the development of the macrocyclic taxol analog 1, which mimics the proposed T-Taxol conformation; this compound is more active than Taxol in cytotoxicity and tubulin-assembly bioassays. Based on these results, we have designed several simplified taxol analogs such as 2 and 3, in which the baccatin core of Taxol is replaced with a simple bicyclononane moiety. The synthesis and preliminary biological investigation of the model compounds 2 and 3 will be presented.



1



2 X = bond
3 X = O

292.

QUANTUM MECHANICS STUDIES ON THE DNA SEQUENCE PREFERENCE OF CAMPTOTHECIN. ***Xiangshu Xiao*** *and Mark Cushman, Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University, West lafayette, IN 47907, xsxiao@pharmacy.purdue.edu*

Camptothecin (CPT), a cytotoxic natural alkaloid isolated from Camptotheca acuminata, and its derivatives represent an important class of cancer chemotherapeutic drugs that act by inhibiting topoisomerase I (top1). The mechanism of top1 inhibition by CPT has been determined by X-ray crystallography. Biochemical studies carried out both in vitro and in vivo indicated that CPT has strict DNA sequence preference for –1 T and strong preference for +1 G at the cleavage site. However, the underlying mechanism for this sequence preference was not well understood. Here we present a quantum mechanics calculation to shed some light on the mechanism of this sequence selectivity. This ab initio calculation can not only reproduce the experimental binding orientation of CPT in the cleavage site, but also shows very good correlation between the binding energy for different sequences and the observed frequency of CPT-stabilized sites in the SV40 viral genome.

293.

SYNTHESIS AND EVALUATION OF RETINOIC ACID METABOLISM BLOCKING AGENTS (RAMBAS) AS INDIRECT DIFFERENTIATING AGENTS FOR CANCER THERAPEUTICS. ***Sook Wah Yee***[1], *Laetitia Jarno*[2], *Claire Simons*[1], *Andrea Brancale*[1], *and Robert I. Nicholson*[2]. *(1) Department of Medicinal Chemistry, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff, United Kingdom, yeesw@cardiff.ac.uk, (2) Tenovus Cancer Research, Welsh School of Pharmacy, Cardiff University*

Differentiating agents are one of the new therapeutic strategies in treating solid tumours e.g. breast and prostate cancers. All-trans retinoic acid (ATRA), derived from vitamin A, is able to inhibit cell proliferation and to restore normal differentiation of various cancer cells. However, the use of ATRA is limited by the induction of the cytochrome P-450 enzymes that are involved in the metabolism of ATRA. In addition to CYP26, which only recognises ATRA as its substrate, different P-450 isozymes, namely CYP2C8, CYP2C9, CYP3A4 are able to catalyze this reaction. A drug which can prolong the action of endogenous retinoic acid by inhibiting the P-450 retinoic acid metabolizing enzymes could have potential use as an anti-cancer agent. The synthesis of three new series of novel compounds with improved activities compared with Ketoconazole and Liarazole in two different biological assay systems will be discussed in relation to the SAR studies and molecular docking studies using homology models of CYP26.

294.

SYNTHESIS AND SAR OF NOVEL TRICYCLIC INDANOPYRAZOLES WITH DUAL ANTI-ANGIOGENESIS AND TUMOR CELL ANTI-PROLIFERATIVE ACTIVITY. ***Chih Y. Ho***, *Jay Mei, Robert Tuman, Donald Ludovici, Umar Maharoof, Eric Strobel, Laura Andraka, Jack Yen, Ann De Vine, Rose Tomminovich, Hong Lu, Judith Baker, Candace Burns, Jan Sechler, Dana Johnson, and Robert Galemmo, Oncology Research Team, Johnson & Johnson Pharmaceutical Research and Development, Welsh and McKean Roads, Spring House, PA 19477-0776, Fax: 215-628-4985, Cho@prdus.jnj.com*

JNJ-10198409 (RWJ-540973) was discovered in our laboratories to have anti-angiogenesis and broad spectrum anti-tumor cell activity both in vitro and in vivo. It has been demonstrated to be a potent PDGF receptor tyrosine kinase inhibitor and a potent inhibitor of tumor cell growth against nine of eleven human tumor cell lines. The compound has exhibited oral dose-dependent inhibition of solid tumor growth through two distinctive mechanisms by blocking PDGF-RTK activity and causing cell cycle arrest. It represents a novel new class of anti-cancer agents. The synthesis, SAR and ADME profiles of various analogs of JNJ-10198409 will be presented.

295.

WITHANOLIDES: A NEW CLASS OF ANGIOGENESIS INHIBITORS. *Paola Bargagna-Mohan, Raffaella Gambaro, and* ***Royce Mohan****, Ophthalmology & Visual Sciences and Pharmaceutical Sciences, University of Kentucky, 166 HSRB, 800 Rose Street, Lexington, KY 40536-0305, Fax: 859-257-9700, Royce.Mohan@uky.edu*

The withanolides are plant-derived natural products, many of which have anti-tumor, anti-inflammatory and neuroprotective activities. We hypothesized that inhibition of pathogenic angiogenesis, which is the blockade of disease-related new blood vessel growth, underlies the beneficial activities of certain withanolides. Using a three-dimensional endothelial cell sprouting assay to screen for bioactivity we have identified angiogenesis inhibitors from withanolide-containing medicinal plants. In an earlier study, withaferin A, the principle active agent of Withania somnifera, was shown to induce cytostatic growth arrest in vascular endothelial cells and demonstrate potent anti-angiogenic activity in vivo (Angiogenesis 7:115-122; 2004). From structure-activity relationship studies directed towards understanding the angioprotective mode of action of withanolides, we have discovered that the ubiquitin proteasome pathway-targeting activity is central to this mechanism. We will additionally present in vivo findings and DNA microarray analysis on endothelial cells that validates the cytoprotective and angiopreventive mechanisms of this class of small molecule inhibitors.

296.

DISCOVERY AND DEVELOPMENT OF ORALLY ACTIVE P38 KINASE INHIBITORS AS ANTI-TNF AGENTS. ***Rajesh V Devraj****, Global Research and Development, Pfizer, 700 Chesterfield Parkway, Mail Zone AA-2A, Chesterfield, MO 63017, Fax: 636-247-2180, rajesh.v.devraj@pfizer.com*

The role of p38 kinase in upregulating the production of pro-inflammatory cytokines (TNF and IL-1) is well documented. A series of trisubstituted pyrazoles were discovered and optimized as potent and selective p38 kinase inhibitors. Several lead analogs effectively and potently inhibited LPS-induced TNFα production in cells and in vivo, in rodents. A series of lead candidates were identified that demonstrated excellent oral bioavailability and low clearance across species. These candidates also demonstrated robust anti-inflammatory and joint protective activity in chronic disease models in rodents. A clinical candidate was identified from this effort and the Phase I PK and PD data following endotoxin challenge to healthy human volunteers will be discussed.

297.

SMALL MOLECULE MODULATORS OF NUCLEAR FACTOR-KB. ***Michael E Hepperle****, Prakash Raman, Julie Liu, Jeremy Little, Francois Soucy, Hormoz Mazdiyasni, Robert Murray, Yingchun Ye, Geraldine Harriman, Yajun Xu, Danyi Wen, Lisa Schopf, Bruce Jaffee, and Tim Ocain, Millennium Pharmaceuticals, 35-2 Landsdowne Street, Cambridge, MA 02139, Fax: 617-444-1482, Michael.Hepperle@mpi.com*

Nuclear factor-κB (NFκB) is thought to play a pivotal role in the regulation of inflammation and apoptosis. NFκB is controlled by the IκB kinase complex which consists of two catalytic subunits (IKKα, IKKβ) and one regulatory subunit IKKγ). IκB proteins are cytosolic inhibitors of NFκB. Upon phosphorylation of IκB by IKKβ, IκB is degraded and NFκB is translocated to the nucleus where it triggers the transcription of genes including pro-inflammatory and anti-apoptotic genes. There is evidence that the inhibition of IKKβ may be useful in the treatment of inflammatory diseases. We have developed substituted beta-carboline derivatives that inhibit IKKβ with good potency and selectivity. This presentation discusses the synthesis, key SAR observations and in vitro/in vivo profiling of representative beta-carboline analogs.

298.

DEVELOPMENT OF ANILINE AMIDES CONTAINING ALTERNATIVE CORES AS ORALLY ACTIVE P38 MAP KINASE INHIBITORS. ***Katerina Leftheris***[1], *John Hynes Jr*[1], *Alaric Dyckman*[1], *Tianle Li*[1], *Shuqun Lin*[1], *Stephen T Wrobleski*[1], *Hong Wu*[1], *Rosemary Zhang*[2], *Kathleen M Gillooly*[2], *Derek Loo*[2], *Kim W McIntyre*[2], *Sidney Pitt*[2], *Ding Ren Shen*[2], *David J Shuster*[2], *Arthur Doweyko*[3], *John Sack*[3], *Joel C Barrish*[1], *John Dodd*[1], *and Gary L Schieven*[2]. *(1) Discovery Chemistry, Pharmaceutical Research Institute, Bristol-Myers Squibb, P. O. Box 4000, Princeton, NJ 08543-4000, Fax: 609-252-7410, katerina.leftheris@bms.com, (2) Department of Immunology, Pharmaceutical Research Institute, Bristol-Myers Squibb, (3) Department of Macromolecular Structure, Pharmaceutical Research institute, Bristol-Myers Squibb*

Overproduction of cytokines such as TNF-α and IL-1β regulated by the p38α pathway are implicated in a wide variety of inflammatory diseases, including rheumatoid arthritis (RA). Recently, we described our initial efforts in developing potent, selective triaminotriazine and cyanopyrimidine aniline amides as inhibitors of p38α MAP kinase. Herein, we describe further development of the aniline amide class of p38 inhibitors to include alternative core structures. A description of the SAR development, in vivo activity, ADME profiling and X-ray crystallography will be presented.

299.

DISCOVERY OF JNK INHIBITORS BASED ON AN INDAZOLE TEMPLATE. ***Yoshitaka Satoh****, Steven Sakata, Adam Kois, Véronique Plantevin, Kiran Sahasrabudhe, Qi Chao, Chris Buhr, Willard Lew, Graziella Shevlin, Ron Albers, Lisa Nadolny, Neil D'Sidocky, John Sapienza, Rachel Ferri, Aparna Motiwala, Jeff Muir, Ched Grimshaw, Li Xu, Seema Pai, Oleg Khatsenko, Michael A. Shirley, Eoin O'Leary, Heather Raymon, Paul Omholt, Jim Leisten, Shripad Bhagwat, Anthony C. Manning, Jonathan Wright, and Brydon Bennett, Celgene, 4550 Towne Centre Court, San Diego, CA 92121, Fax: 858-795-4719, ysatoh@celgene.com*

It is becoming clear that Jun N-terminal kinases play major roles in human diseases such as arthritis, asthma, inflammatory bowel disease, ischemia-

reperfusion injury, CNS ailments such as epilepsy, stroke, and Parkinson's disease, and cancer. Starting from a screening hit, SP600125, we identified a series of 3,5-disubstituted indazoles as highly potent, selective inhibitors of JNK's. Structural optimization leading to the most potent series as well as efficacy of lead JNK inhibitors in several animal models will be discussed.

300.

P38 α MAP KINASE INHIBITORS: FROM DISCOVERY TO THE CLINIC. ***Sundeep Dugar***[1], *Babu Mavunkel*[1], *Sarvajit Chakravarty*[1], *John Perumattam*[1], *Greg Luedtke*[1], *Qing Lu*[1], *Zheng Chen*[1], *Yong-jing Xu*[1], *Andrew Protter*[1], *George Schreiner*[1], *Ramona Almirez*[1], *Brian Scott*[1], *Maureen Laney*[1], *Margaret Henson*[1], *John Lewicki*[1], *Adrian Moore*[2], *Sarah Lee*[3], *Earnest Brahn*[3], *and David Liu*[1]. *(1) Scios, Inc, 6500 Paseo Padre Parkway, Fremont, CA 94555, Fax: 510-739-2105, dugar@sciosinc.com, (2) William Harvey Research Institute, (3) UCLA School of Medicine*

p38α MAP kinase is an intracellular soluble serine threonine kinase which is activated in response to stress, growth factors and cytokines, such as IL-1β and TNF-α. Its activation has been shown to further activate proteins and transcription factors that lead to the production of several key pro-inflammatory and inflammatory cytokines. p38α MAP kinase has an important patho-physiological role in diseases, such as rheumatoid arthritis, where chronic inflammation is said to play a causal role. In recent years there have been several reports of efforts to find small molecule inhibitors of this enzyme as potential therapy in several disease areas. This presentation describes the SAR, in-vitro and in-vivo characterization of a class of highly specific, indole based piperidine amide inhibitors of p38α.

301.

HISTONE DEACETYLASE INHIBITORS. ***Thomas A. Miller****, Department of Chemistry, Aton Pharma Inc., a wholly-owned subsidiary of Merck & Co, 33 Avenue Louis Pasteur, Boston, MA 02115, Fax: 617-992-2405, thomas_miller3@merck.com*

Histone deacetylase (HDAC) inhibitors that target Class I and Class II HDACs are currently under clinical investigation. The results from these studies indicate that HDAC inhibitors show great promise for the treatment of cancer. Since the identification of potent naturally occurring HDAC inhibitors in the 1990's, several natural products and a multitude of synthetic inhibitors have been identified through screening and rational design. HDAC inhibitor lead structures have provided effective platforms for further optimization and created design paradigms that have afforded HDAC inhibitors with sub-nanomolar enzyme inhibitory activities. Hydroxamic acids constitute the largest chemical class of HDAC inhibitors and these agents are among the most potent HDAC inhibitors known. Additional HDAC inhibitor chemical classes include benzamides, cyclic peptides, carboxylic acids and electrophilic ketones. The discovery and development of novel HDAC inhibitors, along with relevant background information, will be discussed in detail.

302.

NOVEL HYDROXAMATE AND NON-HYDROXAMATE HISTONE DEACETYLASE INHIBITORS. ***Michael L. Curtin****, Cancer Research Area, Abbott Laboratories, Department R47J, Bldg AP10, 100 Abbott Park Road, Abbott Park, IL 60064, mike.curtin@abbott.com*

Histone deacetylases (HDACs) alter the acetylation status of chromatin and thereby affect gene expression. The inappropriate recruitment of HDACs may be one mechanism by which oncogenes can alter gene expression in favor of excessive proliferation and makes inhibition of HDACs a potential target for the development of small molecule anti-cancer agents. Characterization of several series of our hydroxamic acids indicated that while many of these analogs possessed potent enzymatic and cellular activity, in general these compounds had unacceptable pharmacokinetic profiles and modest antitumor effects. Replacement of the hydroxamic acid zinc-chelator with an alpha-ketoamide moiety provided potent HDAC inhibitors (IC50 < 10 nM) with excellent cellular activity (IC50 values < 200 nM) and dose-related anti-tumor activity in a flank tumor growth model. This presentation will outline the design, synthesis and pharmacology of these compounds as well as HDAC siRNA knockdown studies and isoform isolation work.

303.

DISCOVERY AND DEVELOPMENT OF HISTONE DEACETYLASE INHIBITORS. ***Minoru Yoshida****, Chem. Genet. Lab, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan, Fax: 81-462-4676, yoshidam@riken.jp, and Norikazu Nishino, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology*

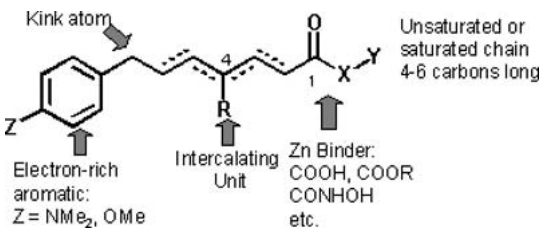
Histone deacetylase (HDAC) has emerged as an important therapeutic target for cancer and various other diseases. The first specific HDAC inhibitors trichostatin A (TSA) and trapoxin (TPX) were isolated from the natural source as inducers of cell differentiation and morphological reversion in transformed cells. These inhibitors have contributed not only to the functional analyses of histone acetylation but also the discovery of HDAC enzyme molecules. After the disclosure of the crystal structure of HDAC-like protein bound to the inhibitors, the momentum of research of the HDAC inhibitors increased and several inhibitors are currently under clinical trials. We have designed and synthesized a series of cyclic tetrapepitide inhibitors containing a variety of the zinc-interacting functional groups. We show our recent approach to develop new HDAC inhibitors with unique properties by coupling various structures of cyclic tetrapepides with diverse functional groups.

304.

COMPUTER AIDED MOLECULAR DESIGN OF HDAC INHIBITORS. ***Olaf Wiest***[1], *Di-Fei Wang*[1], *Paul Helquist*[1], *Norbert Wiech*[2], *Somdutta Roy*[3], *and Martin Tenniswood*[3]. *(1) Department of Chemistry and Biochemistry, Walther Cancer Center, University of Notre Dame, Notre Dame, IN 46556-5670, owiest@nd.edu, (2) Errant Gene Therapeutics, (3) Department of Biological Sciences, University of Notre Dame*

Structure- and ligand-based computer aided molecular design is used to understand the binding modes of known inhibitors of histone deacetylase. We have used these techniques to design new HDAC inhibitors with improved properties, which are then synthesized and tested. Analysis of the x-ray structures of histone deacetylase-like protein (HDLP) and HDAC8 using docking of known inhibitors give excellent correlation with experimentally observed binding constants and provide insights into the nature of the binding interactions. The validated scoring function is then applied to the docking of small probe molecules, revealing the function of the 14Å side pocket adjacent to the active site, and to the study of different putative HDAC inhibitors. The virtual screening of medium-sized compound libraries and *ab initio* calculations of different metal binding motifs provided new leads for non-hydroxamic acid HDAC inhibitors.

The computational results were then used to derive a general pharmacophore and design a variety of new HDAC inhibitors, which were subsequently synthesized and tested. Comparison of the results from biological testing with the computed binding modes suggests the structural origin of the selectivity the current pursued lead compound, CG1521 as well as the subtle differences between class I HDACs. Finally, an accurate QSAR equation for the activity prediction of hydroxamate HDAC inhibitors will be presented.



305.

CRYSTAL STRUCTURE OF HUMAN HDAC8 PROVIDES INSIGHTS INTO THE CLASS I HISTONE DEACETYLASES. ***John R. Somoza***[1], *Robert J. Skene*[2], *Bradley A. Katz*[1], *Clifford Mol*[2], *Joseph Ho*[1], *Andy J. Jennings*[2], *Christine Luong*[1], *Andrew Arvai*[2], *Joseph J. Buggy*[1], *Ellen Chi*[2], *Jie Tang*[1], *Bi-Ching Sang*[2], *Erik Verner*[1], *Robert Wynands*[2], *Ellen M. Leahy*[1], *Douglas R. Dougan*[2], *Gyorgy Snell*[2], *Marc Navre*[2], *Knuth Mark W*[2], *Ronald V. Swanson*[2], *Duncan E. McRee*[2], *and Leslie W. Tari*[2]. *(1) Department of Medicinal Chemistry, Celera, 180 Kimball Way, South San Francisco, CA 94080, john.somoza@celera.com, (2) Syrrx, Inc*

The modulation of the histone acetylation plays an essential part in regulating gene transcription. Two groups of enzymes affect histone acetylation: histone acetyl transferases (HATs) and histone deacetylases (HDACs), which catalyze the addition and removal, respectively, of the acetyl groups from the ε-amino groups of lysines in histones. The removal of acetyl groups by the HDACs promotes the condensation of chromatin and leads to a repression of transcription. The deregulation of the HDACs has been linked to several types of cancer, suggesting a potential use for HDAC inhibitors in oncology. In this presentation we describe the first crystal structures of a human HDAC: the structures of human HDAC8 complexed with four structurally diverse hydroxamate inhibitors. This work sheds light on the catalytic mechanism of the HDACs and suggests how phosphorylation of Ser39 affects HDAC8 activity. These structures also provide a framework for the identification of novel HDAC inhibitors.

306.

INHIBITORS OF PROTEIN KINASE SIGNALING PATHWAYS: EMERGING TARGETS AND AGENTS. ***Janet E. Dancey***, *Investigational Drug Branch, National Cancer Institute, Cancer Therapy Evaluation Program, 6130 Executive Blvd, Room 7131, Rockville, MD 20852, Fax: 301-402-0428, danceyj@ctep.nci.nih.gov*

Within the human genome are over 500 protein-tyrosine and serine/threonine kinases that organize cellular signal transduction and regulate cellular processes. Given that abnormal phosphorylation of cellular proteins is a cancer hallmark, interest in developing kinase inhibitors as drugs is considerable. Although a number of methods to inhibit kinases have been suggested, most of agents under clinical development are antibodies targeting growth factor receptors/ligands, or small molecules targeting the kinase ATP-binding site. To date, only a few protein targets and corresponding agents are validated. Considerable efforts are underway to identify new disease targets for approved agents, develop new agents to new target kinases, and to develop multi-functional agents to inhibit multiple kinases relevant to a number of cancers. Identifying alternative targets for preclinical or clinical drugs can provide new insights into their cellular modes of action, and define disease settings in which the most beneficial therapeutic effect may occur.

307.

PERSPECTIVES ON THE DISCOVERY OF VX-680, A SELECTIVE INHIBITOR OF THE AURORA KINASES. ***Julian M C Golec***, *Vertex Pharmaceuticals (Europe) Ltd, 88 Milton Park, Abingdon OX14 4RY, United Kingdom, Fax: +44 1235 820440, jgolec@vpharm.com*

The Aurora family of serine/threonine kinases is essential for the regulation of chromosome segregation and cytokinesis during mitosis. Aberrant expression of Aurora kinases occurs in a wide range of human tumors and is associated with tumor progression and poor prognosis. Over-expression of Aurora-A is oncogenic. VX-680, a clinical candidate in oncology, is a potent and selective inhibitor of the Aurora kinases. It inhibits proliferation of a diverse range of human tumor cell lines *in vitro* and induces cell death *via* apoptosis. *In vivo*, VX-680 causes profound inhibition of tumor growth in a variety of xenograft models, leading to regression of leukemia, colon and pancreatic tumors at well-tolerated doses. In addition, VX-680 dramatically increases median survival time, and induces sustained remission, in a murine leukemia model. Here we describe the biology of VX-680 and the medicinal chemistry processes that led to its discovery.

308.

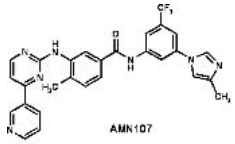
DISCOVERY OF BMS-536924, A SMALL MOLECULE INHIBITOR OF IGF-1R WITH BROAD SPECTRUM IN VIVO ACTIVITY. ***Mark D. Wittman***[1], *Joan Carboni*[2], *Francis Y. Lee*[3], *Balu Balasubramanian*[4], *Francis Beaulieu*[5], *Frennesson David*[5], *Subramaniam Krishnanathan*[4], *Liu Peiying*[5], *Carl Ouellet*[3], *Xiaopeng Sang*[5], *Mark G. Saulnier*[1], *Karen Stoffan*[5], *Upender Velaparthi*[1], *Dolatrai M. Vyas*[1], *Henry S. Wong*[4], *and Kurt Zimmermann*[1]. *(1) Discovery Chemistry, Bristol Myers Squibb Co, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492-7660, mark.wittman@bms.com, (2) Oncology Drug Discovery, Bristol Myers Squibb Co, Pharmaceutical Research Institute, (3) Oncology Drug Discovery, Bristol-Myers Squibb Co Pharmaceutical Research Institute, (4) Synthesis Group, Bristol-Myers Squibb Co Pharmaceutical Research Institute, (5) Discovery Chemistry, Bristol-Myers Squibb Co Pharmaceutical Research Institute*

Considerable attention has been focused on understanding the role of insulin-like growth factor I receptor (IGF-1R) signaling in stimulating mitogenesis, transformation to the oncogenic phenotype, and the anti-apoptotic effects observed in malignant cells. Signaling through IGF-1R results in activation two important signaling pathways for tumor growth; the RAS/Raf/MAP Kinase pathway primarily responsible for mitogensis as well as the PI-3 kinase pathway which has an anti-apototic role. Epidemiological studies have also highlighted the importance of IGF-1R in key tumor types by correlating elevated IGF-I levels with increased risk of developing colon, breast, prostate, and lung tumors. The emerging importance of this target has provided the driving force behind the search for antagonists of IGF-1R, including both biologics that target the ligand binding domain as well as small molecule kinase inhibitors. Recently, pyrrolopyrimidines, selected tryphostin analogs, and picropodophylin have been reported to inhibit the kinase activity of IGF-1R. This presentation will describe the SAR studies leading to the identification of BMS-536924, a new small molecule kinase inhibitor of IGF-1R, and the broad spectrum of anti tumor efficacy that has been observed with this compound.

309.

STRUCTURAL BIOLOGY GUIDED OPTIMIZATION OF TYROSINE KINASE INHIBITORS: AMN107 A SELECTIVE AND POTENT BCR-ABL INHIBITOR. ***Paul W. Manley***[1], *Werner Breitenstein*[1], *Josef Brüggen*[1], *Sandra W. Cowan-Jacob*[1], *Pascal Furet*[1], *James D. Griffin*[2], *Jürgen Mestan*[1], *Thomas Meyer*[1], *and Ellen Weisberg*[2]. *(1) Novartis Institutes for BioMedical Research, CH-4057 Basel, Switzerland, paul.manley@pharma.novartis.com, (2) Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115*

As an inhibitor of the tyrosine kinase activity of the Bcr-Abl oncoprotein, imatinib (Glivec) is an effective therapy of chronic myelogenous leukemia (CML). Clinical experience with imatinib suggests that the smaller the number of residual leukemia cells in peripheral blood, the better the progression-free survival among chronic phase CML patients. Consequently a more potent Bcr-Abl inhibitor could further reduce the number of Bcr-Abl positive cells and improve patient prognosis. Based upon the crystal structure of a complex between imatinib and Abl, we prepared several focused libraries of compounds designed to interact with the inactive conformation of the human Bcr-Abl kinase domain, and identified some key pharmacophore elements. Further optimization of the biopharmaceutical properties of lead compounds, afforded potent, selective Bcr-Abl kinase inhibitors, which were well tolerated and possessed good pharmacokinetic profiles following oral administration to animals. From these studies, AMN107 was selected for evaluation as a potential therapy for CML.



AMN107

310.

EVOLUTION OF THE MEK INHIBITOR PD 0325901: FROM DISCOVERY TO CLINICAL DEVELOPMENT. ***Judith S Leopold**, Department of Molecular Sciences & Technologies, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Fax: 734-622-5970, judith.leopold@pfizer.com*

An important cellular signaling pathway that is frequently altered in human cancers is the Ras/Raf/MEK signaling module. Our experience in the identification and development of highly specific MEK (MAP kinase kinase) inhibitors will be described as a number of critical questions are explored. CI-1040, the first MEK inhibitor to enter clinical evaluation, was advanced into development based on its promising preclinical activity. However, insufficient systemic exposure of CI-1040 resulted in lack of robust activity in Phase 2 trials and the inability of this agent to validate the viability of MEK as an anticancer drug target. PD0325901 has been identified as a significantly more potent analog of CI-1040 with a pharmaceutical profile encompassing improvements in potency, duration of target suppression, bioavailability, and metabolic stability. Clinical evaluation of PD0325901 is currently underway. Looking forward, key challenges impacting the future development of highly specific kinase inhibitors will be discussed.

311.

DEVELOPMENT OF NEW TUBERCULOSIS DRUG CANDIDATES. ***Barbara E. Laughon**, Therapeutics Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH, Room 5108 MSC 7624, 6700-B Rockledge Drive, Bethesda, MD 20892, Fax: 301-402-3171, blaughon@niaid.nih.gov*

The global spread of multidrug resistant tuberculosis (MDR-TB) is an eminent threat to public health worldwide, including within the United States. Tuberculosis control programs are reporting rising rates of resistance to standard therapy drugs, particularly isoniazid and rifampicin. As the spreading HIV/AIDS epidemic grows, the incidence of co-infection with HIV and TB is increasing dramatically in sub-Saharan Africa, Asia, and eastern Europe. TB has become the primary cause of death for AIDS patients in these settings. New drug development programs are urgently needed to meet the future demands for new classes of antimicrobials, both in the developing and developed worlds. The National Institutes of Health, the Global Alliance for TB Drug Development, and the the WHO STOP-TB partnerships are collaborating to support new drug discovery and development efforts. The NIAID, NIH provides resources and platform technologies to individual chemists, biologists, and pharmaceutical companies to evaluate compounds for anti-TB activity and to develop new classes, particularly for MDR-TB treatment.

312.

DEVELOPMENT OF NITROFURANYLAMIDES AS ANTITUBERCULOSIS AGENTS. ***Richard E Lee**[1], Rajendra P Tangallapally[2], Raghunandan Yendapally[2], Robin E.B. Lee[1], AnTawan J. Daniels[1], and Kirk Hevener[1]. (1) Department of Pharmaceutical Sciences, University of Tennessee HSC, 847 Monroe Ave Rm327, Memphis, TN 38163, relee@utmem.edu, (2) Department of Pharmaceutical Sciences, The University of Tennessee Health Science Center*

There is an urgent need to develop fast-acting, potent, new anti-tuberculosis agents active against multi-drug resistant strains of tuberculosis. In an effort to discover new inhibitors of mycobacterial cell wall biosynthesis, a nitrofuranyl amide was discovered with good anti-tuberculosis activity and good lead like properties. After extensive optimization and development of a detailed structure activity relationship the primary target was found to be in flavin metabolism rather than cell wall biosynthesis. The development of this nitrofuranyl amide series will be presented including: SAR developed, including a CoMFA study; the microbiological assessment against sensitive and multi-drug resistant strains; in vitro and in vivo data for lead compounds in the series. Lessons learned in the development of new anti-tuberculosis agents and strategies used to modify compounds with good in vitro activity to achieve good in vivo activity will also be highlighted.

313.

SCREENING NOVEL ANTI-TUBERCULOSIS AGENTS EFFECTIVE AGAINST MDR-TB. ***Makoto Matsumoto**, Microbiological Research Institute, Otsuka Pharmaceutical Co., Ltd, 463-10 Kawauchi-cho, Tokushima 772-0192, Japan, Fax: +81-88-665-6286, m_matsumoto@research.otsuka.co.jp*

The challenges in preventing and controlling tuberculosis are further complicated by the deadly rise of multi-drug-resistant tuberculosis (MDR-TB). Recognizing the seriousness of the situation, we initiated a program to screen new agents that would satisfy these unmet needs and have a favorable safety profile. Mycobacteria are well known for their lipid-rich properties. In Mycobacterium tuberculosis, mycolic acid in particular has been established the wall component related to the pathogenesis in the host. There are approximately 250 identified genes related to biosynthesis of the lipid turnover that contain InhA, the main target of isoniazid. Thus, the logical approach for developing a chemotherapy agent against tubercle bacilli included screening compounds that could inhibit the biosyntheses of mycolic acid and that had a novel chemical structure to ensure improved efficacy against MDR-TB. Some of the screening systems established for those purposes and some of the candidates are outlined.

314.

WHY DOES MULTIDRUG CHEMOTHERAPY STILL ALLOW THE EMERGENCE OF DRUG RESISTANCE DURING TUBERCULOSIS TREATMENT? ***Clifton E. Barry III**, Tuberculosis Research Section, NIAID, NIH, 12441 Parklawn Drive, Room 239, Rockville, MD 20852, Fax: 301-402-0993, clifton_barry@nih.gov*

Tuberculosis in humans is a complex, chronic disease responsible for taking the lives of millions every year. There are substantial scientific issues surrounding the development of new chemotherapies far more complex than typical issues surrounding development of new anti-infectives. Studies from human lung tissue removed during surgery for untreatable multi-drug resistant tuberculosis have revealed that the development of drug-resistance occurs independently within isolated lesions within a human lung. Studies in mice recapitulate the pathology of the disease to only a limited extent while examination of infected non-human primates using specific chemical probes reveal that the intracellular environment within which the bacteria reside is a unique, hypoxic environment not seen in rodents. The role of altered intracellular metabolism, exceptional DNA repair systems, and “phenotypic drug resistance” in human and primate lesions are the likely underlying factors that contribute to the development of drug resistance even in the face of combination chemotherapy.

315.

NOVEL INHIBITORS OF INHA, THE ENOYL REDUCTASE FROM MYCOBACTERIUM TUBERCULOSIS. ***Peter J. Tonge**[1], Todd J. Sullivan[1], Polina Novichenok[1], James J. Truglio[2], Caroline Kisker[2], Francis Johnson[3], and Richard A. Slayden[4]. (1) Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400, Fax: 631-632-7960, peter.tonge@sunysb.edu, (2) Department of Pharmacological Sciences, Center for Structural Biology, Stony Brook University, (3) Departments of Pharmacological Sciences and Chemistry, Stony Brook University, (4) Department of Microbiology, Immunology and Pathology, Colorado State University*

InhA, the enoyl reductase enzyme from Mycobacterium tuberculosis (MTB), catalyzes the final step in the fatty acid biosynthesis pathway (FASII). The enzyme is a target for isoniazid (INH) a front-line tuberculosis chemotherapeutic. Importantly, INH-resistance arises primarily from mutations in KatG the enzyme that activates INH, and not in InhA. Thus, compounds that inhibit InhA without requiring prior activation by KatG should be active against both sensitive and INH-resistant strains of MTB. Triclosan, a common antibacterial additive in personal care products, is a μM inhibitor of InhA and a pM inhibitor of the enoyl reductase from E. coli (FabI). Using a combination of structural and mechanistic data, we have developed a series of triclosan analogs with nM affinity for InhA and with sub-μM MIC99 values for H37Rv. X-ray crystallographic studies of enzyme-inhibitor complexes have revealed that high affinity inhibition of the enoyl reductases is coupled to ordering of a loop that closes over the active site. This detailed information on the mechanistic basis for enzyme inhibition coupled with data from an animal model of TB will direct the further elaboration of our compound library. These studies will provide critical information for the development of novel MDR-TB agents.

316.

CELL WALL OF MYCOBACTERIUM TUBERCULOSIS AND DRUG DISCOVERY. ***Patrick J. Brennan**, Department of Microbiology, Immunology and Pathology, Colorado State University, 200 Lake Street, Fort Collins, CO 80525, Fax: 970-491-1815, patrick.brennan@colostate.edu*

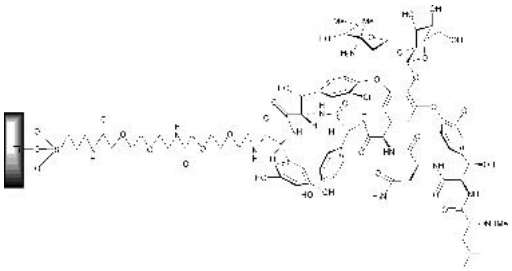
The cell envelope of *Mycobacterium tuberculosis* is made up of three major compartments, a plasma membrane, a covalently linked complex of mycolic

acid, arabinogalactan and peptidoglycan (MAPc) and a polysaccharide-rich capsule-like layer. The MAPc is made up of a highly cross-linked peptidoglycan (PG), which is covalently linked to arabinogalactan (AG). The C-6 of some of the muramic acid residues of PG form phosphodiester bonds to C-1 of a-D-GlcNAc which in turn linked to a a-L-Rha*p* residue providing the "linker unit" between the galactan of arabinogalactan (AG) and PG. Current impetus for studying biosynthesis of MAPc is the need to identify targets for the development of new drugs. In this respect we have concentrated on a few essential pathways for drug development: the non-mevalonate isopentenyl-PP pathway, central to most aspects of cell wall synthesis; galactan synthesis; and arabinan synthesis. The strategy employed for the exploitation of these pathways for new drug development and the identification of some promising lead compounds will be discussed.

317.

SOLID PHASE SYNTHESIS OF VANCOMYCIN-BIS(8-AMIDO-3,6-DIOXAOCTANOYL)-AMIDOPROPYLSILOXY-TITANIUM IMPLANTS THAT INHIBIT PROLIFERATION OF *STAPHYLOCOCCUS AUREUS*. ***Binoy Jose*[1]**, *Samuel J. King[2], Allen R. Zeiger[1], Christopher J. Adams[2], Noreen J. Hickok[2], and Eric Wickstrom[3]. (1) Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107, binoy@tesla.jci.tju.edu, (2) Department of Orthopedic Surgery, Thomas Jefferson University, (3) Department of Biochemistry & Molecular Pharmacology and Department of Microbiology & Immunology, Thomas Jefferson University*

Implant-associated infections are serious complications of medical device insertions and are difficult to treat because bacterial attachment, proliferation, and biofilm formation, i.e., bacterial encapsulation in a protective coating, occur quickly and preferentially on the inert implant surface. Current treatment usually involves aggressive, systemic antibiotic treatment, debridement, implant removal, and in joint replacements, re-implantation. Local antibiotic delivery to the site of infection has been achieved through insertion of antibiotic-impregnated bone cement, which suffers from poor material properties, and implantation of other controlled-release biodegradable materials that suffer from fragility and/or undesirable modification of the implant environment. Furthermore, these local-delivery systems are only effective during the short period of antibiotic extrusion. We hypothesized that an antibiotic covalently bonded to a metal via linkers can eradicate a nascent infection at all times post-implantation. We covalently attached a glycopeptide antibiotic, vancomycin (VAN), to titanium metal by solid phase coupling of Fmoc-8-amino-3,6-dioxaoctanoic acid linkers and VAN to aminopropylsiloxy-titanium beads and pins, yielding VAN-AEEA-AEEA-HNPrSi-O-Ti as shown below. Binding of [^{14}C]L-Lys-D-Ala-D-Ala to VAN-AEEA-AEEA-HNPrSi-O-Ti beads indicated that VAN covalently bound to Ti via linkers retained the ability to bind its natural ligand. Importantly, *S. aureus* incubated with VAN-AEEA-AEEA-HNPrSi-O-Ti beads and pins were killed. This approach is uniquely suited to the eradication of a nascent infection before biofilm formation, and thus could reduce the risk of implant-associated infections. This new paradigm could easily be extended to other agents and materials. Supported by DOD DAMD-17-03-1-0713.



318.

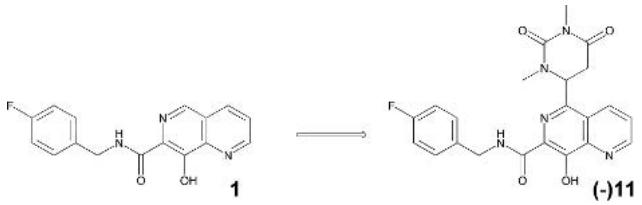
DEVELOPMENT OF GENE PROBES: SYNTHESIS OF RADIOIODO AND RADIOIODOVINYL ARABINOSYL URIDINE ANALOG. ***Chung-Shan Yu**, Li-Wu Chiang, Chien-Hung Wu, Ren-Tsong Wang, Heng-Yen Wang, and Chien-Hung Yeh, Department of Atomic Science, National Tsing-Hua university, 101 Sec. 2, Guang-Fu Rd., Hsinchu 300, Taiwan, Fax: 886-3-5718649, csyu@mx.nthu.edu.tw*

Application of virion vectors in cancer therapy has attributed an impressive milestone in clinical trials. The rationale is based on the idea that these modified and avirulent virion vectors can maintain their capability to infect specific host cell, hence replicated and expressed proteins inside infected host cell without causing any severe disease. It has been reported that nucleoside prodrugs can be transformed into monophosphates derivatives in the presence of virus protein, herpes simple virus thymidine kinase type one (HSV-1 TK). Once these monophosphate derivatives are formed, they can further interrupt the de novo DNA synthesis with their structural nature or block the function of thymidine synthase, and ceases the DNA synthesis leading to the cell death. In order to fulfill the goal for antitumor regimen, virion vectors have to be transported into target tissues effectively. A reliable and sensitive method needs to be developed for monitoring in vivo virus genes expression to pursuing this goal. Recently, positron-emitter labeled nucleosides by utilizing PET model becomes a rising star in this field. Herein, we report a facial synthesis of a stannan thymidine analog with an identical in vivo stable hydroxy group at the axial position on furanose. Hopefully, this stannan derivative could act as a novel precursor for subsequent radiolabeling with 125I. We acknowledge financial support from NSC (93-2113-M-007-038).

319.

5-(5,6)-DIHYDROURACIL SUBSTITUTED 8-HYDROXY-[1,6]NAPHTHYRIDINE-7-CARBOXYLIC ACID 4-FLUOROBENZYLAMIDE INHIBITORS OF HIV-1 INTEGRASE AND VIRAL REPLICATION IN CELLS. ***Mark W. Embrey*[1]**, *John S. Wai[1], Timothy W. Funk[1], Carl F. Homnick[1], Debbie S. Perlow[1], Steven D. Young[1], Joseph P. Vacca[1], Daria J. Hazuda[2], Peter J. Felock[2], Kara A. Stillmock[2], Marc V. Witmer[2], Gregory Moyer[3], William A. Schleif[3], Lori J. Gabryelski[3], Lixia Jin[4], I-Wu Chen[4], Joan D. Ellis[4], Bradley K. Wong[4], Jiunn H. Lin[4], Yvonne M. Leonard[1], Nancy N. Tsou[5], and Linghang Zhuang[1]. (1) Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, Fax: 215-652-3971, mark_embrey@merck.com, (2) Department of Biological Chemistry, Merck Research Laboratories, (3) Vaccine and Biologics Research, Merck Research Laboratories, (4) Drug Metabolism and Pharmaceutical Research, Merck Research Laboratories, (5) Department of Medicinal Chemistry, Merck Research Laboratories, Rahway*

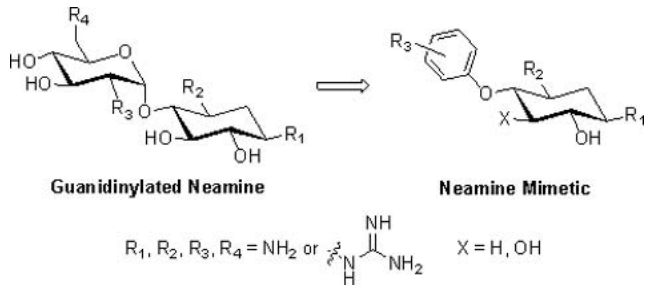
Introduction of a 5,6-dihydrouracil functionality in the 5-position of N-(4-fluorobenzyl)-8-hydroxy-[1,6]naphthyridine-7-carboxamide 1 led to a series of potent HIV-1 integrase inhibitors. These compounds displayed low nanomolar activity in inhibiting both the strand transfer process of HIV-1 integrase and viral replication in cell cultures. Compound 11 is a 150-fold more potent antiviral agent than 1, with a CIC_{95} of 40 nM in the presence of human serum in the cell assay. It displays good pharmacokinetics when dosed in rats and dogs.



320.

SYNTHESIS AND SAR ANALYSIS OF NOVEL ANTHRAX LETHAL FACTOR INHIBITORS. ***Cho Tang*[1]**, *Guan-Sheng Jiao[1], Sherri Millis[2], Lynne Cregar[2], Dominique Nguyen[2], and Mark Goldman[2]. (1) Department of Chemistry, Hawaii Biotech, Inc, 99-193 Aiea Heights Drive, #200, Aiea, HI 96701, Fax: 808-792-1348, ctang@hibiotech.com, (2) Department of Lead Discovery, Hawaii Biotech, Inc*

We have previously reported the synthesis of a library of selectively guanidinylated neamines, some of which showed strong inhibitory activity against lethal factor protease, one of the fatal components in the tripartite anthrax toxin. We now report a series of novel mimetic compounds of neamines as anthrax lethal factor inhibitors, which are simpler in structure and synthesis and retain similar activity with better lipophilicity. The design, synthesis, and SAR analysis of these compounds will be discussed.



321.
EFFICIENT SYNTHESIS OF 3′-DEOXY-3′BETA-FLUORO AND 3′-DEOXY-3′ALPHA-FLUORO -5′-NORARISTEROMYCIN AND THEIR ANTIVIRAL ACTIVITIES. ***Atanu Roy**, Tesfaye Serbessa, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Building, Auburn, AL 36849, Fax: 334 844 0239, royatan@auburn.edu*

5′-Noraristeromycin (**1**) (Figure 1) has broad-spectrum antiviral activity due to its apparent inhibition of S-adenosyl-L-homocysteine (AdoHcy) hydrolase. In building upon the therapeutic potential of **1** via analog design, we were attracted to the well documented biological correlation of a fluorine with a hydroxyl moiety. Thus, the fluoro derivatives **2** and **3** were early examples sought in this direction as surrogate compounds for **1**. The preparation and antiviral properties of **2** and **3** will be described. This research has been supported by DHHS (AI 56540).

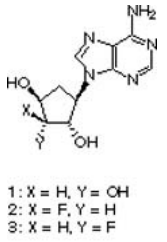


Figure 1

322.
DESIGN AND SYNTHESIS OF PYRANOBENZOTHIOPHENES AS INHIBITORS OF HCV RNA DEPENDENT RNA POLYMERASE. *Ariamala Gopalsamy[1], **Gregory M. Ciszewski**[1], Alexis Aplasca[1], John W. Ellingboe[1], Boris Feld[2], Mark Orlowski[2], and Anita Y.M. Howe[2]. (1) Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, Fax: 845-602-3045, gopalsa@wyeth.com, ciszewg@wyeth.com, (2) Infectious Diseases, Wyeth Research*

Hepatitis C virus (HCV) infection is the cause of significant long-term morbidity and mortality. While often asymptomatic, the majority of HCV infections result in chronic hepatitis that can progress to cirrhosis, end-stage liver disease, and hepatocellular carcinoma. Presently, there is no specific antiviral agent directed against HCV and no vaccine for prevention of HCV infection, making it an attractive therapeutic target. Through high throughput screening of various libraries against HCV RNA dependent RNA polymerase NS5B, a substituted pyranoindole was identified as an HCV polymerase inhibitor. During the course of optimization of this lead, pyranobenzothiophenes were designed and synthesized as novel HCV polymerase inhibitors. Modification of various substituents indicated a good correlation to the SAR obtained for the pyranoindole series. The parallel synthesis effort, optimization approach and the SAR of this lead series will be discussed in detail.

323.
DESIGN, SYNTHESIS AND METABOLIC STABILITIES OF ALKENYLDIARYLMETHANES (ADAMS) HAVING NONIDENTICAL AROMATIC SUBSTITUENTS AS NNRTIS. ***Bo-Liang Deng** and Mark Cushman, Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907, Fax: 765-494-6790, deng@purdue.edu*

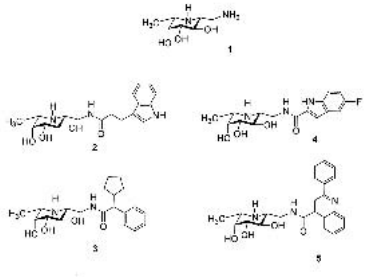
The non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) constitute a large and structurally diverse set of compounds that have potential value in the treatment of AIDS. The alkenyldiarylmethanes (ADAMs) are a new class of non-nucleoside reverse transcriptase inhibitors. Certain ADAMs have been found to inhibit the cytopathic effect of HIV-1 in cell culture at low nanomolar concentrations. Recently, benzophenones including 5-chloro-2-methoxyphenyl or 3-fluoro-5-trifluoromethylphenyl groups as NNRTIs of HIV-1 showed IC50 values 2nM against wild type HIV-1 and <10 nM against 16 mutants. Therefore, in order to discover metabolically stable ADAMs with enhanced bioavailability and improved therapeutic potential in the treatment of AIDS, several alkenyldiarylmethanes with 5-chloro-2-methoxyphenyl or 3-fluoro-5-trifluoromethylphenyl groups were designed and have been synthesized via the Sonogashira and Stille cross-coupling reactions. Design and synthesis of alkenyldiarylmethanes will be presented with accompanying the results of anti-HIV activities and metabolic stabilities in rat plasma.

324.
DESIGN, SYNTHESIS, AND EVALUATION OF DIOXANE ANTIVIRAL AGENTS TARGETED AGAINST THE HYDROPHOBIC BINDING POCKET OF SYNDBIS VIRUS CAPSID PROTEIN. ***Ha Young Kim**[1], Ranjit Warrier[2], Chinmay Patkar[2], Richard Kuhn[2], and Mark Cushman[1]. (1) Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, hykim@pharmacy.purdue.edu, (2) Department of Biological Sciences, Purdue University*

The alphaviruses are pathogenic viruses with worldwide distribution. The prototypic alphavirus genus is Sindbis virus, which is transmitted to humans through mosquito bites and can cause fever, rash, arthralgia or arthritis, lassitude, headache, and myalgia. As a group, the New World alphaviruses can cause encephalitis, and could theoretically be produced in either wet or dried form and stabilized for weaponization. The virus could be delivered in aerosol form. There is presently no treatment available. The X-ray structure of the crystalline Sindbis virus capsid protein hydrophobic pocket shows that it is occupied by one or two dioxane molecules derived from the crystallization solvent. This result led us to select two dioxane moieties connected by a three-carbon linker as the first target compound. In this research, dioxane-based antiviral agents targeted at the hydrophobic binding pocket of Sindbis virus capsid protein were designed and synthesized. These ligands are expected to block the interaction of the viral capsid protein with the N-terminal arm of an adjacent capsid protein molecule, which should inhibit capsid assembly. In addition, the occupation of the hydrophobic binding pocket is also expected to block the binding of the viral capsid to membrane-bound E2 glycoprotein spikes, thus inhibiting viral budding. Two assays are employed to evaluate antiviral activity: in vivo virus production using SIN-ires-Luc and in vitro capsid assembly.

325.
DISCOVERY OF POTENT AND SELECTIVE FUCOSIDASE INHIBITORS BY RATIONAL AND COMBINATORIAL APPROACH. ***Ching-Wen Ho**, **Chuan-Fa Chang**, **Chung-Yi Wu**, and **Chun-Hung Lin**, Institute of Biological Chemistry and the Genomics Research Center, Academia Sinica, No. 128, Academia Road Section 2, Nan-Kang, 115, Taipei, Taiwan, Fax: 886-2-2789-8670, cheeri17@yahoo.com.tw, affa@gate.sinica.edu.tw, cage.sugar@msa.hinet.net, chunhung@gate.sinica.edu.tw*

Glycosidases represent an important class of enzymes as therapeutic targets, as exemplified by Tamiflu, a well-known neuraminidase inhibitor to treat the infection of influenza virus and Miglitol, targeting the intestinal disaccharidases, prescribed for insulin-independent diabetes. We report the discovery of picomolar, slow tight-binding inhibitors (**2-5**) against the α-fucosidases from *Corynebacterium sp.* (Csfd) and *Thermotoga maritima* (Tmfd) by a rapid screening for an optimal aglycon attached to 1-aminomethyl fuconojirimycin **1**. Particularly compound **2** with a K_i^* of 0.46 pM of Csfd represents the most potent glycosidase inhibitor to date. In contrast, these molecules are reversible-type inhibitors of the human fucosidase. In order to understand if the inhibitory distinction corresponds to the different binding sites of α-fucosidases. Various substituted fuconojirimycins have been synthesized and more diversity was generated by the aforementioned methods to afford a library for fast screening. Distinctive inhibition profile was established for each α-fucosidase and the interesting results will be discussed in detail.



326.

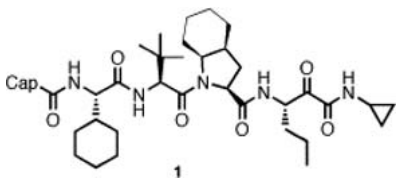
IDENTIFICATION OF CARBAZOLE AND CYCLOPENTAINDOLE DERIVATIVES AS INHIBITORS OF HCV RNA DEPENDENT RNA POLYMERASE. *Ariamala Gopalsamy[1], **Mengxiao shi**[1], Gregory M. Ciszewski[1], Kaapjoo Park[1], John W. Ellingboe[1], Boris Feld[2], Mark Orlowski[2], and Anita Y.M. Howe[2]. (1) Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, Fax: 845-602-3045, gopalsa@wyeth.com, shim2@wyeth.com, (2) Infectious Diseases, Wyeth Research*

Hepatitis C is a common viral infection that can lead to chronic hepatitis, cirrhosis, liver failure, and hepatocellular carcinoma. The HCV genome contains a number of non-structural proteins including NS5B, an RNA-dependent RNA polymerase that is essential for viral replication. Therefore, inhibition of NS5B is a suitable target for the development of therapeutic agents. Through high throughput screening of various libraries against NS5B, a substituted pyranoindole was identified as an HCV polymerase inhibitor. During the course of optimization of this lead, carbazole and cyclopentaindole derivatives were designed and synthesized as novel HCV polymerase inhibitors. Modification of various substituents indicated a distinct SAR for these scaffolds leading to sub-micromolar potency in the polymerase assay. The synthesis effort, optimization approach and the SAR of these analogs will be discussed in detail.

327.

IMPORTANCE OF BACKBONE HYDROGEN BONDS IN BINDING A TETRAPEPTIDE SCAFFOLD TO THE HCV NS3-4A PROTEASE. ***Robert B. Perni**, Kevin C. Cottrell, John J. Court, Luc J. Farmer, Cynthia A. Gates, Chao Lin, Kai Lin, Yu-Ping Luong, Janos Pitlik, B. Govinda Rao, Wayne Schairer, Yunyi Wei, and John H. Van Drie, Vertex Pharmaceuticals Inc, 130 Waverly Street, Cambridge, MA 02139, Fax: 617-444-6766, Robert_Perni@vrtx.com*

The unusual, shallow, solvent exposed active site of the HCV NS3 protease has provided formidable hurdles for medicinal chemists to investigate ways to maximize inhibitor-protein interactions while trying to maintain drug-like properties in the inhibitors. One common approach, based on the concept of product-based inhibition has been to optimally fill the hydrophobic S2 pocket with large groups while truncating the inhibitor backbone that sits in the binding groove from S6 to S3. An alternative approach is to anchor the inhibitor assembly at both ends with a serine-trap warhead and a P4 capping group that can form a hydrogen bond to an NS3 surface hydrogen-bond acceptor. The readily available octahydroindole carboxylic acid fragment was used as the constant P2 group for this study as shown in **1**. The structure-activity relationship of the resulting series of compounds reveals the contribution of key hydrogen bonds to inhibitor binding.



1

328.

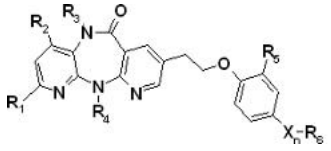
NMR-BASED DESIGN OF CD4-BINDING PEPTIDE MIMETICS AS HIV ENTRY INHIBITORS. ***Heiko M Möller**[1], Jan Wülfken[2], Britta Hünnefeld[3], Atilla Çoksezen[3], and Bernd Meyer[3]. (1) Department of Chemistry, University of Konstanz, Universitätsstrasse 10, 78457 Konstanz, Germany, Fax: +49-7531-88-3898, Heiko.Moeller@uni-konstanz.de, (2) Varian Deutschland GmbH, (3) Institute of Organic Chemistry, University of Hamburg*

The primary receptor for the human immunodeficiency virus (HIV) is the CD4 protein on T-cells and macrophages which interacts with the viral glycoprotein gp120. Inhibitors of this process could act as anti-HIV therapeutics. Here, peptide segments of gp120 that have contact to CD4 in an Xray crystal structure analysis served as lead structures for ligand development. It was possible to convert the decapeptide NMWQKVGTPL - a millimolar ligand of CD4 - into a molecule with submicromolar affinity. First, promising amino acid substitutions were identified via molecular modeling on the basis of the crystal structure and of an STD NMR group epitope mapping. The substituted peptides were then synthesized in form of a positional scanning library which was screened using Biacore and STD NMR. Successful variations were combined to yield a peptide mimetic with 300 nM affinity to CD4. STD NMR revealed strong binding contributions of the newly introduced amino acids.

329.

NOVEL 8-SUBSTITUTED DIPYRIDODIAZEPINONE DERIVATIVES AS HIV NNRTIS WITH BROAD ANTIVIRAL POTENCY. ***Serge Landry**, Pierre R. Bonneau, Josée Bordeleau, Louise Doyon, Jianmin Duan, Ingrid Guse, Eric Malenfant, Julie Naud, Jeff A. O'Meara, Bounkham Thavonekham, Christiane Yoakim, Bruno Simoneau, Michael Bös, and Michael G. Cordingley, Boehringer Ingelheim (Canada) Ltd., Research & Development, 2100 Cunard Street, Laval, QC H7S 2G5, Canada*

HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a potent component of highly active anti-retroviral therapies (HAART). More than 8 years after the introduction of nevirapine (the first NNRTI that was later joined by delavirdine and efavirenz), a new generation of NNRTI has yet to be approved for use in the clinic. In this drug class, the emergence of resistance is the major cause of treatment failure: Patients failing a first generation NNRTI are left without further NNRTI options because of cross-resistance to the entire class. There is therefore a therapeutic need for a next generation NNRTI that displays potent antiviral activity against wild-type and clinically observed NNRTI-resistant viruses associated with treatment failure. In addition, a convenient dosing regimen (once a day, low pill burden) must be achieved. The optimization of the C-8 substituent of dipyridodiazepinone derivatives led us to the identification of highly potent NNRTIs against wild-type virus, prevalent single and double mutants. The biological activities, biopharmaceutical profile and the syntheses of these inhibitors will be discussed.



330.

NOVEL C-TERMINAL FUNCTIONALITIES IN HEPATITIS C VIRUS NS3 PROTEASE INHIBITORS. ***Robert Rönn**[1], Thomas Gossas[2], Yogesh A. Sabnis[1], Eva Åkerblom[1], U. Helena Danielson[2], Bertil Samuelsson[3], Anders Hallberg[1], and Anja Johansson[1]. (1) Department of Medicinal Chemistry, Uppsala University, Husargatan 3, Uppsala SE-751 23, Sweden, Fax: +46 18 471 4474, Robert.Ronn@orgfarm.uu.se, (2) Department of Biochemistry, Uppsala University, (3) Department of Organic Chemistry, Stockholm University*

Currently, an estimated 170 million people worldwide are infected by the hepatitis C virus (HCV). Although there is an existing therapy, the need for new virus-specific drugs is urgent. The NS3 protease has during the last two decades evolved as a potential drug target for the treatment of HCV. Today, proof-of-concept for the first NS3 protease inhibitor in humans has been established. Product-based inhibitors, comprising a C-terminal carboxylic acid, have proven to be potent and selective inhibitors of the NS3 protease. We recently found that this carboxylic acid could successfully be replaced by the acyl sulfonamide functionality. In our effort to further develop the acyl sulfon-

amide inhibitors and to obtain more information about the inhibitor-enzyme interactions we have designed and synthesized inhibitors encompassing novel C-terminal functionalities, e.g. acylhydrazines and diacylhydrazines (Figure 1).

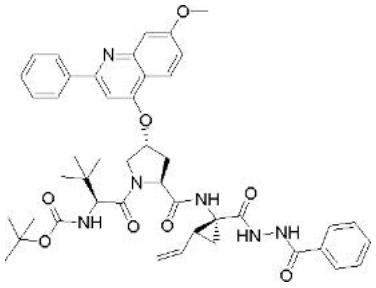


Figure 1. Structure of an NS3 protease inhibitor encompassing a novel C-terminal diacylhydrazine functionality.

331.

PHARMACOPHORE MODELING AND COMPUTATIONAL ANALYSIS OF A KEY PROTEIN-PROTEIN INTERACTION IN HERPES SIMPLEX VIRUS. ***Melanie J. Grubisha*** *and Steven M. Firestine, Mylan School of Pharmacy, Duquesne University, 600 Forbes Ave., Mellon Hall, Pittsburgh, PA 15213, Saboo23@aol.com*

In the herpes simplex virus, it is known that the interaction between two virally encoded proteins, UL30 and UL42, is essential for its virulent properties. Inhibiting this interaction could lead to the development of new treatment methods. Molecular surface modeling was done to determine the binding site of UL42 where critical residues of UL30 make key interactions. Identification of the 3 critical UL30 residues, coupled with this model, allowed for the development of a pharmacophore model to screen compounds for potential inhibitory actions. Using a bicyclic scaffold with various substitution points and a library of functional groups, a virtual combinatorial library was generated and screened. Of the 1156 compounds screened through the pharmacophore model, 71 hits were obtained. These hits were evaluated with respect to the functional groups present and their alignment with the UL30 ligand. The best fitting compounds were docked to confirm their possible actions as inhibitory compounds.

332.

SYNTHESIS AND EVALUATION OF [^{18}F]-2′-DEOXY-2′-FLUORO-1-BETA-D-ARABINOFURANOSYLURACIL DERIVATIVES AS PET PROBES FOR IMAGING HSV1-TK GENE EXPRESSION IN VIVO. ***Nagavarakishore Pillarsetty***[1]*, Shangde Cai*[1]*, Doubrovin Mikhail*[2]*, Lyudmila Ageyeva*[2]*, Ronald D. Finn*[3]*, and Ronald G. Blasberg*[4]*. (1) Radiochemistry & Cyclotron Core, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, Box 407, New York, NY 10021, Fax: 212-717-3063, pillarsn@mskcc.org, (2) Department of Neurology, Memorial Sloan Kettering Cancer Center, (3) Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, (4) Molecular Pharmacology and Chemistry, Memorial Sloan-Kettering Cancer Center*

A series of radiolabeled (^{18}F or ^{124}I) thymidine analogs are currently being developed in our laboratory as part of our program to develop PET (Positron Emission Tomography) probes for imaging Herpes Simplex Virus 1- thymidine kinase (HSV1-tk) gene expression in vivo. Recently, we reported synthesis of two analogs [^{18}F]-2′-Deoxy-2′-fluoro-5–(E-2-bromovinyl)-1-b-D-arabinofuranosyluracil ([^{18}F]-FBrVAU) and [^{18}F]-2′-Deoxy-2′-fluoro-5-Propyl-1-b-D-arabinofuranosyluracil ([^{18}F]-FPAU) (J. Nucl. Med. **2004**, 45(5), S-1392). In the current report we report the synthesis and characterization of a new probe [^{18}F]-2′-Deoxy-2′-fluoro-5-trifluoromethyl-1-b-D-arabinofuranosyluracil ([^{18}F]-FTAU) and compare the in vitro and in vivo properties with [^{18}F]-FBrVAU and [^{18}F]-FPAU.

333.

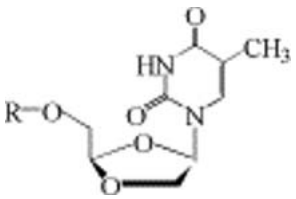
PREDICTION OF HIV-1 PROTEASE INHIBITORS THROUGH MOLECULAR MODELING AND STATISTICAL ANALYSIS. ***Catharine J. Collar****, Department of Chemistry, Central Washingron University, 400 East University Way, Ellensburg, WA 98926-7539, Fax: 509-963-1050, collarc@cwu.edu, and Levente Fabry-Asztalos, Department of Chemistry, Central Washington University*

Drugs available to treat HIV/AIDS patients have limited clinical benefits due to poor resistance profiles, low bioavailability, and undesirable side effects. Thus, the development of new HIV-1 protease inhibitors is a high priority. Previous studies which involve the synthesis of potential HIV-1 protease inhibitors provide novel structures with ideal characteristics. This information, in combination with molecular modeling and statistical analysis, has been used to create molecular mathematical models. These models aided in the prediction of novel HIV-1 protease inhibitors with greater affinity and better bioavailability profiles.

334.

PRODRUGS OF 1-(β-D-DIOXOLANE)THYMINE (DOT): SYNTHESIS, ANTI-HIV-1 ACTIVITY AND STABILITY STUDY. ***Y.Z. Liang***[1]*, V. Yadav*[1]*, R.F. Schinazi*[2]*, and C. K. Chu*[1]*. (1) The University of Georgia College of Pharmacy, Athens, GA 30602, yliang@rx.uga.edu, (2) Emory University School of Medicine/Veterans Affairs, Atlanta, GA, 30033*

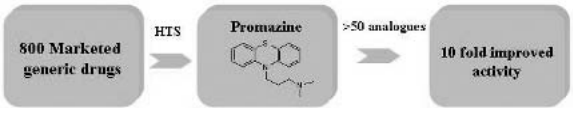
DOT exhibits significant anti-HIV-1 activity against all nucleoside-resistant mutants. We designed and synthesized various 5′-O-substituted DOT prodrugs with the objectives of increasing anti-HIV activity, by-passing the first phosphorylation step, enhancing cell penetration, improved pharmacokinetic properties to increase plasma half-life as well as to improve the drug delivery. The DOT prodrugs include: (1) 5′-O-carboxylic and amino acid ester; (2) 5′-O-phosphoramidate diester; (3) substituted cyclic 1′3-propanyl ester; (4) DOT nanoparticles; (5) pegylated DOT. Their both anti-HIV-1 activity and chemical and enzyme-catalyzed hydrolyses were investigated (Supported by NIH AI32351 and AI25899).



335.

PROMAZINE ANALOGUES AS POTENTIAL ANTI SARS-COV DRUGS. *Hsing Pang Hsieh*[1]*,* ***Yu-Shan Wu***[1]*, Ping-Hsun Lu*[1]*, Chi-Min Chen*[2]*, Yu-Chan Chao*[3]*, Jia-Tsrong Jan*[4]*, Huei-Ru Lo*[3]*, Shiou-Hwa Ma*[3]*, Yu-Sheng Chao*[1]*, and Tsu-An Hsu*[1]*. (1) Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 7F, 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, Fax: 886-37-586-401, hphsieh@nhri.org.tw, yswu@nhri.org.tw, (2) Division of Animal Medicine, Animal Technology Institute Taiwan, (3) Institute of Molecular Biology, Academia Sinica, (4) Institute of Preventive Medicine, National Defence Medical Center*

Severe Acute Respiratory Syndrome (SARS), caused by the newly discovered coronavirus SARS-CoV, is a life-threatening infectious disease. The high mortality rate necessitates the immediate development of an effective antiviral agent. A family of tricyclic antidepressant (TCA) drugs was found active against SARS coronavirus (SARS-CoV) replication in cell culture, screening from a library of 800 compounds using porcine transmissible gastroenteritis virus (TGEV) as a surrogate system. Selected TCA drugs showed full protection of SARS-CoV-infected cells at low concentration (≤gM range) by CPE-inhibition assay and in yield-reduction and immunoblot analyses, they significantly blocked SARS-CoV replication. More than fifty analogues of a TCA lead drug, promazine, were synthesized and their structure-activity relationships were established. Fifteen analogues were found to reduce the fluorescence intensity of Vero E6 cells upon the infection by spike containing baculovirus, and four analogues in particular showed a 10-fold improvement in potency comparing to promazine in the assay. The same series of compounds were also tested against the porcine transmissible gastroenteritis virus and twenty three analogues showed a better activity. The tricyclic phenothiazine/ phenoxazine core structure thus represents a promising lead series for developing more efficacious anti-SARS-CoV drugs.



336.

PYRAZOLO [1,5-A] PYRIDINE ANTIHERPETICS. ***Jason G. Weatherhead**, Brian A. Johns, Scott H. Allen, Kristjan S. Gudmundsson, F. Leslie Boyd Jr., and George A. Freeman, Department of Medicinal Chemistry, GlaxoSmithKline Research & Development, Five Moore Drive, Research Triangle Park, NC 27709, jason.g.weatherhead@gsk.com*

The synthesis and structure-activity relationships surrounding the C-7 position of a novel class of 3-pyrimidinyl-pyrazolo [1,5-a] pyridine antiherpetic compounds is described. Methodology was developed to allow late stage functionalization of the C-7 position. Coupling 7-chloro pyrazolopyridines with aryl boronic acids gave a number of 7-aryl pyrazolopyridines. In addition, a pyrazolopyridine borinate complex was generated in situ by direct lithiation of the C-7 position and coupled with a variety of aryl halides. These two complementary methods allowed for rapid and efficient access to a diverse set of 7-aryl analogs.

337.

PYRAZOLOPYRIMIDINES AS INHIBITORS OF HCV RNA POLYMERASE. ***Janeta V. Popovici-Müller**[1], Gerald W. Shipps Jr.[1], Kristin E. Rosner[1], Yongqi Deng[1], Tong Wang[1], Patrick Curran[1], Meredith A. Brown[1], Alan B Cooper[2], Mickey Cable[2], Nancy Butkiewicz[2], and Viyyoor Girijavallabhan[2]. (1) NeoGenesis Pharmaceuticals, 840 Memorial Drive, Cambridge, MA 02139, Fax: 617-868-1515, janeta@neogenesis.com, (2) Schering-Plough Research Institute*

The hepatitis C virus is recognized as a major human pathogen and is believed to infect approximately 3% of the worldwide population. HCV infection frequently results in chronic hepatitis leading to cirrhosis and, in some cases, to hepatocellular carcinoma. HCV is a single stranded RNA virus in the Flaviviridae family. Its genome encodes for a polyprotein consisting of both structural, and nonstructural proteins such as NS3 (protease and helicase) and NS5B (RNA dependent RNA polymerase (RdRp)). Existing therapies for HCV are limited and although HCV RdRp is an ideal target for antiviral drugs only a few inhibitors are known. The present paper describes a novel series of HCV RNA polymerase inhibitors based on a pyrazolopyrimidine scaffold bearing hydrophobic groups and an acidic functionality. Several compounds were optimized to low nanomolar potencies in a biochemical RdRp assay. SAR trends clearly reveal a stringent preference for cyclohexyl as one of the hydrophobes, and improved activities for carboxylic acid derivatives.

338.

STRUCTURE ACTIVITY RELATIONSHIP OF DISULFONE-CONTAINING HIV-1 INTEGRASE INHIBITORS. ***D. Christopher Meadows**[1], Jacquelyn Gervay-Hague[1], Timothy B. Matthews[2], and Thomas W. North[2]. (1) Department of Chemistry, University of California, Davis, One Shields Ave, Davis, CA 95616, Fax: 530-752-8995, dcmeadows@ucdavis.edu, (2) Department of Molecular Biosciences, University of California*

Research in the Gervay-Hague laboratories has recently focused on the identification of new HIV-1 integrase inhibitors. Considering there are no commercially-available inhibitors and that it performs a necessary step in viral replication, integrase is an ideal therapeutic target. We have developed a disulfone-containing scaffold that, depending on the flanking motifs, shows potent anti-integrase and antiviral activity. Using Autodock 3.0, the inhibitors were docked into the integrase active site to identify possible binding modes and exploit these in the design of new inhibitors. Based on structural similarity to known inhibitors and the modeling studies, we have conducted an SAR study on this new class of integrase inhibitors. Results of the SAR study and antiviral data obtained through focal infectivity assays will be presented.

339.

STRUCTURE ACTIVITY RELATIONSHIP OF SUBSTITUTED PYRANOINDOLES AS HCV RNA DEPENDENT RNA POLYMERASE INHIBITORS. *Ariamala Gopalsamy[1], ***Gregory M. Ciszewski**[1], Kitae Lim[1], Kaapjoo Park[1], Mengxiao Shi[1], Jonathan Bloom[1], Rajiv Chopra[1], Atul Agarwal[1], Girija Krishnamurthy[1], John W. Ellingboe[1], Janis Upeslacis[2], Tarek S. Mansour[1], Stephen M. Condon[3], Matthew G. LaPorte[3], Lori M. Miller[3], Christopher J. Burns[3], Anita Y.M. Howe[4], Boris Feld[4], Mark Orlowski[4], Marja van Zeijl[4], and John O'Connell[4]. (1) Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, Fax: 845-602-3045, gopalsa@wyeth.com, ciszewg@wyeth.com, (2) Director, Chemical and Screening Sciences, Wyeth Research, (3) ViroPharma Inc, (4) Infectious Diseases, Wyeth Research*

Hepatitis C virus (HCV) is emerging as one of the most significant infections in humans. The currently approved treatments are not effective in all patient populations and are associated with considerable side effects. As a result, there is an unmet need for a safe and effective antiviral agent. The HCV genome encodes the RNA-dependent RNA polymerase (NS5B) as a protein essential for viral replication. Through high throughput screening against NS5B, a substituted pyranoindole was identified. Various regions of the lead molecule were optimized using parallel synthesis. Optimization of the pyran region indicated the importance of the acid moiety and an SAR requirement for the C1 substituents along with the preference for the "R" enantiomer. A number of aromatic substituents were incorporated for the indole region to identify compounds with a good biological profile in both enzyme and cellular assays. A co-crystal structure indicates an allosteric binding for this class of molecules. Optimization approach and SAR of this lead series will be discussed in detail.

340.

SUBSTITUTED 4H-PYRAZOLO[1,5-A]PYRIMIDIN-7-ONES AS HEPATITIS C VIRUS POLYMERASE INHIBITORS. ***Yongqi Deng**[1], Janeta V. Popovici-Müller[1], Gerald W. Shipps Jr.[1], Kristin E. Rosner[1], Tong Wang[1], Patrick Curran[1], Alan B. Cooper[2], Viyyoor Girijavallabhan[2], Nancy Butkiewicz[2], and Mickey Cable[2]. (1) NeoGenesis Pharmaceuticals, 840 Memorial Drive, Cambridge, MA 02139, Fax: 617-868-1515, ydeng@neogenesis.com, (2) Schering-Plough Research Institute*

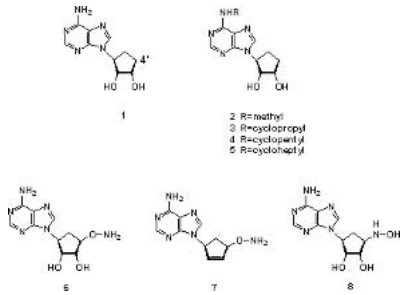
The hepatitis C virus (HCV) chronically infects approximately 3% of the world's population and is a leading cause of liver transplantation in the United States. HCV is a single stranded RNA virus in the Flaviviridae family. Its genome encodes for a polyprotein consisting of both structural, and nonstructural proteins such as NS3 (protease and helicase) and NS5B (RNA dependent RNA polymerase (RdRp)). Although HCV RdRp is considered an ideal target for antiviral drugs, only a few inhibitors are known. The present paper describes an efficient synthetic route to substituted pyrazolo[1,5-a]pyrimidin-7-ones and their biological evaluation in HCV polymerase biochemical assays. Combining the optimization results at C-6, C-5 and C-2 afforded several compounds with potent HCV polymerase inhibitory activity in biochemical RdRp assays.

341.

SYNTHESES AND ANTI-VIRAL PROPERTIES OF N-6 SUBSTITUTED DERIVATIVES OF L-LIKE 4′-DEOXY-5′-NORARISTEROMYCIN AND OXYAMINO AND HYDROXYLAMINO CARBANUCLEOSIDES. ***Tesfaye Serbessa**, Atanu Roy, Minmin Yang, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Building, Auburn, AL 36849, serbete@auburn.edu*

Analogues of naturally occurring nucleosides have become major therapeutic agents for the treatment of viral infectious diseases such as human immunodeficiency virus (HIV, AIDS), hepatitis B virus and the herpes viruses. Replacing the furan ring of the more traditional ribofuranosyl derived nucleosides by a carbocyclic system results in a class of compounds referred to as carbanucleosides. One relevant feature of these derivatives is their limited susceptibility to enzymatic degradation as a consequence of the absence of the natural N-glycosidic bond. Recently, considerable attention has focused on L-nucleosides, the enantiomers of natural D-nucleosides. Part of this of this presentation deals with the optimization of the lipophilic nature of the L-like carbanucleoside, 4′-deoxy-5′-noraristeromycin, (**1**), which possesses significant activity towards hepatitis B virus. In this direction, syntheses and biological evaluations of several analogues (compounds **2-5**) are described. Furthermore, the syntheses of L-like oxyamino compound **6** and its dideoxydidehydro analog **7** and the

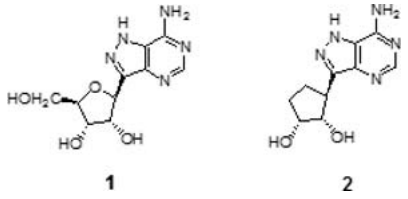
L-like hydroxylamino carbanucleoside **8** are also presented. This research was supported by NIH AI 56540.



342.

SYNTHESES AND ANTIVIRAL PROPERTIES OF CARBOCYCLIC FORMYCINS. ***Jian Zhou**, Minmin Yang, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Bldg, Auburn, AL 36849, Fax: 334 844 0239, zhoujia@auburn.edu*

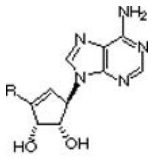
Formycin (**1**) is a naturally occurring C-glycoside or C-nucleoside that possesses antitumor, antibacterial, antifungal, and antiviral activity. A number of C-nucleosides are under active investigation by research groups throughout the world. In connection with our ongoing interest in the design and syntheses of C-nucleoside antibiotics and structurally related analogues we now report the preparation of (±)-(1S,2S,3R)-2,3-dihydroxy-1-(3-[7-aminopyrazolo[4,3-*d*]pyrimidyl])cyclopentane (**2**) starting with (±)-(1R,2R,3S)-3-(Benzyloxy)-1,2-epoxycyclopentane. Because of the structural relationship of **2** to antiviral candidates derived from aristeromycin, the antiviral data of for compound **2** will also be described. (Support AI 56540 is appreciated.)



343.

SYNTHESIS AND ANTIVIRAL ACTIVITY STUDIES OF 5′-DEOXY-5′, 5′, 5′-TRIFLUORO NEPLANOCIN A. ***Atanu Roy** and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Building, Auburn, AL 36849, Fax: 334 844 0239, royatan@auburn.edu*

The broad-spectrum antiviral activity displayed by neplanocin A (NPA, **1**) (Figure 1) is due to its apparent inhibition of S-adenosyl-L-homocysteine (AdoHcy) hydrolase. The therapeutic usefulness of **1** is limited by its cytotoxicity because of phosphorylation of its 5′-primary hydroxyl group by adenosine kinase and, subsequent, metabolism by cellular enzymes. In seeking new non-toxic carbocyclic nucleoside analogs based on **1** while retaining its potent antiviral properties, modification at C-5′-position has been considered. One target has been trifluoromethyl derivative **2**. The preparation and antiviral activity of **2** will be presented. This research has been supported by DHHS (AI 56540).



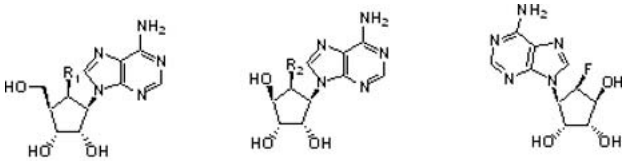
1: R = CH_2OH
2: R = CF_3

Figure 1

344.

SYNTHESIS AND ANTIVIRAL PROPERTIES OF 6′-â-F-5′-NORORISTEROMICIN. ***Xueqiang Yin**, Department of Chemistry, Auburn university, Chemistry building, auburn, AL 36849, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University*

Aristeromycin (**1**) and 5′-noraristeromycin (**3**) are two important biologically active carbocyclic nucleosides. Compared with 1, 5′-noraristeromycin exhibits broad-spectrum antiviral properties with less cytotoxity. 6′-β-F-5′-Noraristeromycin (**4**) is not only an analog of 5′-noraristeromycin, but also retains the important structural feature of 6′-b-F-aristeromycin (**2**), which, like **1** and **3**, is a potent inhibitor of S-adenosylhomocysteine hydrolase. The synthesis of **4**and the L-like **5** as well as their antiviral properties will be reported. This research was supported by funds from the Department of Heath and Human Services (AI 56540).



1. R_1=H
2. R_1=F

3. R_2=H
4. R_2=F

5

345.

SYNTHESIS AND BIOLOGICAL PROPERTIES OF 2′-C-MODIFIED ANALOGUES OF 5′-METHYL ARISTEROMYCIN ANALOGUES. ***Wei Ye**, Department of Chemistry & Biochemistry, Auburn University, 179 Chemistry Building, Auburn University, Auburn, AL 36849, yewei01@auburn.edu, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University*

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 to limit these undesirable transformations, (5′*R*)-5′-methylariseromycin (**2**) and 4′-*iso*propenyl-5′-nor-5′-deoxyaristeromycin (**3**) were synthesized in our lab and found to have great antiviral activities without associated toxicity. Also, 2′-C-methyl nucleosides have been synthesized and showed great activities against HCV. Exploiting these properties for new antiviral agents led to a series of 2′-C-modified analogues (for example, **4**-**7**), whose syntheses and antiviral profiles will be reported. This research is supported by funds from the Department of Health and Human Services (AI56540).

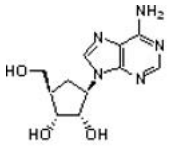
1 R_1=H, R_2=H, R_3=H, R_4=OH
2 R_1=CH_3, R_2=H, R_3=H, R_4=OH
4 R_1=CH_3, R_2=H, R_3=H, R_4=H
5 R_1=CH_3, R_2=H, R_3=CH_3, R_4=OH

3 R_3=H, R_4=OH
6 R_3=H, R_4=H
7 R_3=CH_3, R_4=OH

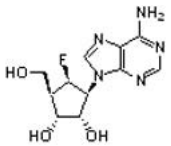
346.

VERSATILE SYNTHETIC ROUTE TO 6′-β-F-ARISTEROMYCIN, ARISTEROMYCIN, 2′,3′-DIDEOXYDIDEHYDROARISTEROMYCIN AND THE ANTIVIRAL ACTIVITY OF 6′-β-F-ARISTEROMYCIN. ***Xueqiang Yin**, Department of Chemistry, Auburn University, Auburn, AL 36849, xqyin@yahoo.com, and stewart W Schneller, Chemistry, auburn*

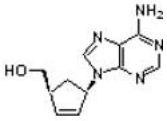
Aristeromycin (**1**) plays an important role in the search for biologically active carbocyclic nucleosides. The racemic 6′-β-F-aristeromycin (**2**) , an analog of (**1**), has been reported to be a potent S-adenosylhomocysteine hydrolase inhibitor. To investigate the antiviral potential of(**2**)that may be associated with this property, an efficient synthetic route to the pure enantiomer has been achieved. This procedure can also be utilized to synthesize 1 and 2′,3′-dideoxydidehydroaristeromycin(**3**). The results of this investigation will be reported. This research was supported by funds from the Department of Heath and Human Services (AI 56540).



1



2



3

347.

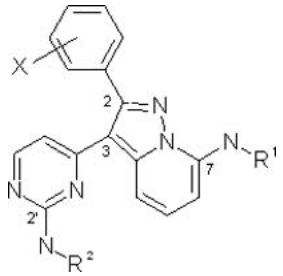
SYNTHESIS OF N3,5′-CYCLO-4-(BETA-D-RIBOFURANOSYL)-VIC-TRIAZOLO[4,5-B]PYRIDIN-5-ONE ANALOGUES WITH IMPROVED ANTI-HEPATITIS C ACTIVITY IN VITRO. ***Peiyuan Wang***[1]*, Jianfa Du*[1]*, Suguna Rachakonda*[1]*, Byoung-Kwon Chun*[1]*, Lieven J. Stuyver*[1]*, Michael J. Otto*[1]*, Raymond F. Schinazi*[2]*, and Kyoichi A. Watanabe*[1]*. (1) Department of Chemisty, Pharmasset, inc, 1860 Montreal Road, Tucker, GA 30084, Fax: 678-395-0039, pwang@pharmasset.com, (2) Department of Pediatrics, Emory University*

Recently, we discovered that N3,5′-cyclo-4-(beta-D-ribofuranosyl)-vic-triazolo[4,5-b]pyridin-5-one (I, R = H) exhibits moderate anti-HCV activity in the HCV replicon assay. In order to develop better anti-HCV agents, several 7 substituted N3,5′-Cyclo-4-(beta-D-ribofuranosyl)-vic-triazolo[4,5-b]pyridin-5-one derivatives have been synthesized and some structure-anti-HCV activity relationships were obtained. Among the compounds synthesized the 7-methylamino-, 7-amino-, and 7-methyl analogues exhibited improved anti-hepatitis C virus (HCV) activity (EC90 = 7.4, 29.7 and 15.4 microM, respectively) compared to I (R = H, EC90 = 79.8 miroM).

348.

SYNTHESIS OF NOVEL PYRAZOLOPYRIDINE ANTIHERPETICS: SAR OF THE C2 PHENYL SUBSTITUENT. ***Elizabeth M. Turner***[1]*, Kristjan S. Gudmundsson*[1]*, Brian A. Johns*[1]*, Zhicheng Wang*[1]*, Scott H. Allen*[1]*, George A. Freeman*[1]*, Connie J. Sexton*[2]*, Dean W. Selleseth*[2]*, Katrina L. Creech*[2]*, and Kelly R. Moniri*[2]*. (1) Department of Medicinal Chemistry, GlaxoSmithKline Research & Development, Five Moore Drive, Research Triangle Park, NC 27709-3398, Fax: 919-483-6053, elizabeth.m.turner@gsk.com, (2) Department of Virology, GlaxoSmithKline Research & Development*

A novel pyrazolo[1,5-*a*]pyridine scaffold which showed promising activity against HSV-1 was recently identified. The synthesis and SAR of the 2-phenyl substituent will be described. Several of the compounds described have antiherpetic activity comparable to that of acyclovir.



349.

CHEMICAL SYNTHESIS OF POLY(ETHYLENE OXIDE)-BASED BIOMIMETIC SDF-1 MATERIALS FOR ANTI-HIV-1 STUDY. ***Ming-Hsiung Chen****, Department of Research and Development, Mackay College of Medicine, Nursing, and Management, 92, Saint-Jing Road, Beitou, Taipei 11272, Taiwan, Fax: 886-2-28584183, m20774@yahoo.com, and Chie-Pein Chen, Department of Obstetrics and Gynecology, Mackay Memorial Hospital*

Stromal cell-derived factor 1 (SDF-1) was found to be the ligand for a chemokine-like receptor, and belonged to the CXC family. However its structural and functional relationship to other chemokines is unknown. Interest in SDF-1 has grown since it was identified as a co-receptor that inhibits the entry and replication of SI forms of HIV-1 in CD4 T-cells. Previous researches have found that the N-terminal residues preceding the first cysteine have been shown to be critical for both receptor binding and functional activation. The key receptor binding sites are found in the N-terminal region near the CXC portion, however, the two-disulfide bridges are also important as they help to stabilizes the active conformation. To determine the structural requirements for function, the Poly(ethylene oxide) based biomimetic N-terminal-CXC- of SDF-1 were chemically synthesized and assayed for their binding and induction ability to SDF-1 receptors.

350.

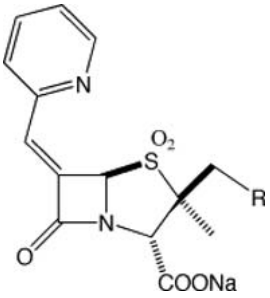
TOWARD SMALLER HCV NS3-4A PROTEASE INHIBITORS: 3-SUBSTITUTED PROLINE-BASED TRIPEPTIDE SCAFFOLDS. ***Robert B. Perni****, Kevin C. Cottrell, John J. Court, Luc J. Farmer, Cynthia A. Gates, Yu-Ping Luong, Janos Pitlik, and B. Govinda Rao, Vertex Pharmaceuticals Inc, 130 Waverly Street, Cambridge, MA 02139, Fax: 617-444-6766, Robert_Perni@vrtx.com*

The shallow substrate binding site of the HCV NS3-4A protease has generally required large peptidomimetic molecules to efficiently inhibit the enzyme, as exemplified by the two compounds that have entered clinical trials, VX-950 and BILN 2061. VX-950 is comprised of a tetrapeptide backbone while BILN 2061 is a large macrocyclic molecule based on a tripeptide scaffold. Drug-like properties are clearly at a premium for molecules such as these with high molecular weights and large hydrophobic surface areas. We will herein present the structure-activity relationship of a series of capped-tripeptide NS3-4A inhibitors based on a 3-substituted proline scaffold. Our results show that effective binding can be achieved with smaller tripeptide inhibitors.

351.

2′-SUBSTITUTED PENICILLIN-DERIVED INHIBITORS OF β-LACTAMASE. ***John D. Buynak***[1]*, Lakshminarayana Vogeti*[1]*, Venkat Rao Gadhachanda*[2]*, and Anjaneyulu Sheri*[1]*. (1) Department of Chemistry, Southern Methodist University, Box 0314, Dallas, TX 75275-0314, Fax: 214-768-4089, jbuynak@mail.smu.edu, (2) Dept. of Chemistry, Southern Methodist University*

We will present the activity of 2′-substituted β-lactamase inhibitors **1**. This will include both their inherent inhibitory activity against reprentative serine enzymes, as well as the in vitro activity of some against representative resistant strains. Modifications will include the attachment of siderophores intended to improve transport into the periplasmic space of Gram-negative bacteria via the TonB iron transport pathway. We will also present improved synthetic methodology for the preparation of these molecules.

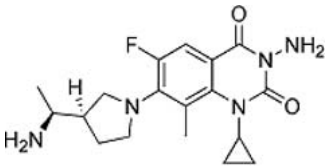


352.

3-AMINO-1H-QUINAZOLINE-2,4-DIONES: A NOVEL CLASS OF ANTIBACTERIAL AGENTS. ***Tuan P. Tran***[1]*, Edmund L. Ellsworth*[1]*, Joseph P. Sanchez*[1]*, Michael A. Stier*[1]*, Brian M. Watson*[1]*, Sharon A. Powell*[1]*, Kim M. Hutchings*[1]*, H. D. Hollis Showalter*[1]*, John M. Domagala*[1]*, Martin A. Shapiro*[2]*, Michael D. Huband*[2]*, Jeffrey W. Gage*[2]*, Themis E. Joannides*[2]*, Stephen J. Gracheck*[2]*, Paul Bird*[3]*, Dai Q. Nguyen*[3]*, Judy Yip*[3]*, Tingsheng Li*[3]*, Jyoti Tailor*[3]*, and Rajeshwar Singh*[3]*. (1) Chemistry Department, Pfizer Global Research and Development, Michigan Laboratories - Ann Arbor, 2800 Plymouth Road, Ann Arbor, MI 48105, Fax: 734-622-2265, tuan.tran@pfizer.com, (2) Antibacterial Pharmacology Department, Pfizer Global Research and Development, Michigan Laboratories - Ann Arbor, (3) Chemistry Department, NAEJA Pharmaceuticals*

The 3-amino-1H-quinazoline-2,4-diones (3-AQDs) represent a novel class of antibacterial agents that inhibit bacterial topoisomerases. Structurally, this class shows similarities to the fluoroquinolone antibiotics, yet displays different structure-activity relationships (SAR) against a range of medically important bacterial pathogens. While the 3-AQDs exhibit good activity against Gram-negative bacteria, they display more significant activity against Gram-positive pathogens. Furthermore, compounds of this class are not cross-resistant with mutants that are no longer sensitive to many fluoroquinolone antibiotics. PD 0305970, one of the most potent compounds in this series, showed in vivo

activity in a standard mouse infection model. The chemistry and SAR of selected compounds within this series will be presented.

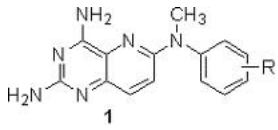


PD 0305970

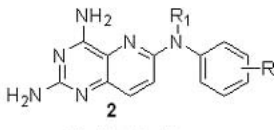
353.

SYNTHESIS AND DESIGN OF 2,4-DIAMINO-6-SUBSTITUTED-PYRIDO[3,2-*D*]PYRIMIDINES AS DIHYDROFOLATE REDUCTASE INHIBITORS FROM OPPORTUNISTIC PATHOGENS. *Aleem Gangjee, Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, Fax: 412-396-5593, gangjee@duq.edu,* ***Zhengqu Ye****, Division of Medicinal Chemistry, Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, 600 Forbes Ave., pittsburgh, PA 15282, ye_zhengqu@hotmail.com, Roy L. Kisliuk, Department of Chemistry, School of Medicine, Tufts University, and Sherry F. Queener, Department of Pharmacology and Toxicology, Indiana University*

We have previously reported the potent and moderately selective inhibition of dihydrofolate reductase (DHFR) from *T.gondii* with N9-methyl analogs of piritrexim of general structure **1**. As a logical extension of this work to explore the effect of a variety of alkyl substitution on the selectivity and potency against *T.gondii* DHFR we have synthesized N9-alkyl analogs of general structure **2**.The synthesis and inhibitory activities of compounds **2** against DHFR from pathogens and human will be presented.



1



2

R1= Et, Pr, iBu

354.

DESIGN AND SYNTHESIS OF A SER-PHE-PHE KETOMETHYLENE ISOSTERE FOR INHIBITORS OF BOTULINUM NEUROTOXIN B. ***Brian M. Bax****, Department of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, bmbax@pharmacy.wisc.edu, and Daniel H. Rich, Department of Chemistry & School of Pharmacy, University of Wisconsin-Madison*

Botulinum Neurotoxin B (BoNT B) is a member of a unique class of zinc metalloproteases. To elucidate structural information about the active site in the catalytically "competent" form of this protease, several mechanism-based inhibitors have been designed and synthesized. These inhibitors are generated by replacement of the scissile bond with a non-cleavable transition-state analogue in the minimum substrate sequence. The asymmetric synthesis of a SerPhe($COCH_2$)Phe isostere and its use to prepare inhibitors of BoNT B will be presented.

355.

DESIGN, PARALLEL SYNTHESIS AND SAR FOR TETHERED DIMER INHIBITORS OF NAD SYNTHETHASE. *Wayne J. Brouillette[1],* ***Liyuan Mou****[2], Sadanandan E. Velu[3], Christie G. Brouillette[4], Chi-Hao Luan[4], and Lawrence J. DeLucas[4]. (1) Center for Biophysical Sciences and Engineering, Department of Chemistry, The University of Alabama at Birmingham, 1025 18th streets south, Birmingham, AL 35294-4400, (2) Center for Biophysical Sciences & Engineering, University of Alabama at Birmingham, 1025 18th Street South, Birmingham, AL 35294, (3) The Center for Biophysical Sciences and Engineering, Department of Chemistry, University of Alabama at Birmingham, (4) Center for Biophysical Sciences and Engineering, University of Alabama*

In effort to develop novel antibacterial agents, we've been interested in inhibitors of nicotinamide adenine dinucleotide (NAD) synthetase. NAD is an essential cofactor in cellular metabolism and in energy production. NAD synthetase catalyzes the transformation of nicotinic acid adenine dinucleotide (NaAD) to the amide NAD via a two-step process. 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 tethered dimers as lead inhibitors, which contain two end groups joined by an 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*

356.

MOLECULAR RECOGNITION IN THE ADENINE-BINDING REGION OF AN AMINOGLYCOSIDE ANTIBIOTIC KINASE. ***David D. Boehr****[1], Adam R. Farley[2], Frank J. LaRonde[3], Tera Rica Murdock[2], Ahmad Al-Mestarihi[2], Gerard D. Wright[3], and James R. Cox[2]. (1) Department of Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N Torrey Pines Rd, San Diego, CA 92037, boehr@scripps.edu, (2) Department of Chemistry, Murray State University, (3) Department of Biochemistry, McMaster University*

The protein-based molecular recognition of the adenine ring has implications throughout chemical and biological systems. The aminoglycoside antibiotic kinase APH(3′)-IIIa can serve as a model system to study this interaction, employing a hydrogen-bonding network involving water molecules along with enzyme backbone/side-chain atoms and a pi-pi stacking interaction to recognize the adenine ring. We have used computational methods, site-directed mutagenesis, calorimetric experiments and inhibition studies with a variety of adenosine analogs to probe the interactions in the adenine-binding pocket. These studies suggest that both electronic and size factors are important in binding aromatic systems, delineating the importance of electrostatic complementarity in high-affinity adenine binding. The principles governing adenine recognition established in this study may be applied to other proteins that bind adenine-bearing ligands and used to navigate future studies directed at discovering potent and selective inhibitors of APH-type enzymes.

357.

NEW INSIGHTS INTO THE USE OF ß-CHLORO-ALANINE IN THE TRYPTOPHAN SYNTHASE KINETIC ASSAY. ***Andrea L. Looney****, Jeffrey O. Boles, and Carie Harrington, Department of Chemistry, Tennessee Technological University, 55 University Drive, Cookeville, TN 38505, Fax: 931-372-3434, andiloo1983@yahoo.com*

Bacterial tryptophan synthase is a $\alpha 2\beta 2$ heterodimer that catalyzes the last two steps in the biosynthesis of L-tryptophan. The $\alpha 2\beta 2$ complex dissociates reversibly into two monomeric a subunits (Mr 28,700) and one dimeric $\beta 2$ subunit (Mr 86,000). The α subunit catalyzes the cleavage of indole-3-glycerol phosphate to indole and 3-glyceraldehyde 3-phoshate (a reaction), while the pyridoxal phosphate dependent b2-subunit catalyzes the condensation of indole with L-serine to form L-tryptophan (b reaction). The physiologically important $\alpha\beta$ reaction is the sum of the α and β reactions. Since this enzyme is currently getting much broader attention, especially as a catalyst in synthetic organic chemistry, we sought to reinvestigate the kinetic assay design and reaction conditions. Our preliminary results, included in this poster, indicate real problems associated with the use of β-chloro-alanine in the place of serine as a co-substrate.

358.

NEW LASPARTOMYCIN-BASED SEMI-SYNTHETIC LIPOPEPTIDE ANTIBIOTICS. ***Dale R. Cameron****[1], Yuchen Chen[1], Dominique Dugourd[2], Jenny Sun[1], Lixia Wang[1], Donald B. Borders[3], William V. Curran[3], and Richard A. Leese[3]. (1) Dept. of Chemistry, MIGENIX Inc, BC Research Complex, 3650 Wesbrook Mall, Vancouver, BC V6S 2L2, Canada, Fax: 604-221-9688, dcameron@migenix.com, (2) Dept. of Microbiology, MIGENIX Inc, (3) BioSource Pharm Inc*

Laspartomycin, an acidic lipopeptide antibiotic related to Amphomycin, was used as a template for making novel semi-synthetic antibiotics. Laspartomycin, produced by fermentation with *Streptomyces viridochromogenes ssp. Komabensis*, was isolated as a complex mixture where each constituent has the same central cyclic peptide core attached to a different C_{15}-α,β-unsaturated fatty acid. The cyclic peptide core was generated by enzymatic cleavage of the complex with the deacylase from Actinoplanes utahensis, which removes the fatty acid tails leaving a free amine, either directly off the cyclic peptide or with an exocyclic aspartic acid included. These biologically inactive peptide intermediates

were the key intermediates allowing multiple Laspartomycin-based semisynthetic antibiotics to be prepared both by substitution at the free amine and/or by substitution of the aspartic acid sidechain of the peptide core. Multiple analogs had antimicrobial activity in a panel of Gram positive organisms of medical importance.

359.

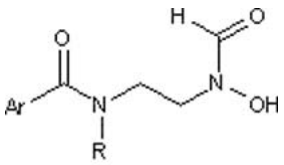
NOVEL ANTIBACTERIAL TRIPEPTIDES. ***Bengt Erik Haug*** *and John S. M. Svendsen, Department of Chemistry, University of Tromsø, N-9037 Tromsø, Norway*

Antibacterial peptides have been recognized as a novel class of antibiotics, and several candidates are currently in clinical trials. In our group we have discovered that replacing tryptophan residues by larger unnatural aromatic amino acids can enhance the antibacterial activity of 15-residues bovine lactoferricin peptides. These findings have further been employed in the preparation of short antibacterial peptides with high antibacterial activity against several Gram-positive and Gram-negative bacterial strains. These exceptionally short peptides were also found to be highly active against staphylococci strains resistant to commercial antibiotics. To further explore the pharmacophore of short cationic antibacterial peptides, we have prepared tripeptide derivatives suitable for derivatization through solid phase Suzuki coupling reactions, furnishing tripeptide derivatives encompassing novel biaryl moieties. Results from these studies will be presented.

360.

POLYPEPTIDE DEFORMYLASE INHIBITORS: DESIGN, SYNTHESIS AND EVALUATION OF A NOVEL BENZAMIDE SCAFFOLD. ***Joseph M. Karpinski***[1]*, Siegfried B. Christensen*[1]*, Kelly M. Aubart*[1]*, Glenn S. Van Aller*[2]*, Peter L. DeMarsh*[3]*, Thomas F. Lewandowski*[3]*, Stephen Rittenhouse*[3]*, Swarupa G. Kulkarni*[4]*, Timothy A. McIntyre*[4]*, Lisa C. Woods*[4]*, Michael A. Lonetto*[5]*, Stewart C. Pearson*[6]*, Kate J. Smith*[7]*, Ajita Bhat*[7]*, Maxwell Cummings*[7]*, and Kevin Saylers*[4]*. (1) Department of Medicinal Chemistry, GlaxoSmithKline, 1250 South Collegeville Road, PO Box 5089, Collegeville, PA 19426-0989, Joseph_M_Karpinski@gsk.com, (2) Department of Enzymology, GlaxoSmithKline, (3) Department of Microbiology, GlaxoSmithKline, (4) Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, (5) Department of Genetics Research, GlaxoSmithKline, (6) New Product Planning, GlaxoSmithKline, (7) Department of Computational and Structural Science, GlaxoSmithKline*

The increasing resistance to prescribed antibiotics for the treatment of respiratory tract infections has propelled research to identify new molecular targets for the discovery of novel antibacterial agents. Polypeptide deformylase (PDF), a metalloenzyme essential in prokaryotes for the removal of the formyl group on the N-terminus of nascent polypeptides during protein synthesis, has recently been of interest as a target for antibacterial drug design. We have synthesized a series of benzamide-based inhibitors that possess sub-micromolar PDF enzyme inhibitory activity for both Gram-positive and Gram-negative bacteria. Structurally (1), these compounds possess a terminal N-formyl hydroxylamine that acts as a metal chelator in the PDF active site. The SAR of this series of PDF inhibitors, with a detailed discussion of the profile of SB 660618, will be presented.



(1)

361.

POST-POLYKETIDE TAILORING OXYGENASES OF THE LANDOMYCIN BIOSYNTHESIS. ***Lili Zhu*** *and Jürgen Rohr, College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, KY 40536, Fax: 859-257-7585, lzhu3@uky.edu*

Landomycins A and E, the principle products of *Streptomyces cyanogenus* S136 and of *Streptomyces globisporus* 1912, respectively, belong to the angucycline family of antibiotics and differ only in their saccharide chain. The landomycins are potent antitumor drugs. Their biosynthetic pathway is encoded by very similar gene clusters. In both of the landomycin gene clusters, three oxygenase encoding genes were found, namely *lan/lndE*, *lan/lndM2* and *lan/lndZ5*. Inactivation of these three oxygenase and of selected adjacent reductase encoding genes/domains in *S. globisporus* 1912 led to the accumulation of various metabolites that allowed elucidating the biosynthetic oxygenation/reduction sequence of the landomycin pathway. The characterization of these genes might open up their usage for combinatorial biosynthetic derivation approaches.

362.

RAPID METHOD TO SYNTHESIS OF 2,3-DIHYDRO-2-PHENYL-4-QUINOLONE DERIVATIVES AS NEW ANTIBACTERIAL AGENTS. ***Myung-Sook Park***[1]*, Ju-Hee Kim*[1]*, and Jae-In Lee*[2]*. (1) College of Pharmacy, Duksung Women's University, Ssangmundong 419, Tobonggu, Seoul 132-714, South Korea, Fax: 82-2-901-8386, mspark@duksung.ac.kr, (2) Department of Chemistry, Duksung Women's University*

In general, quinolones are extensively used as first-line treatments of many infections, but the drugs are not entirely safe and are liable to cause adverse reactions such as nonspecific neurological effects. We developed the convenient synthetic route for the 2,3-dihydro-2-phenyl-4-quinolone was expected to retain antibacterial activity. 2,3-Dihydro-2-phenyl-4-quinolones could be converted through acid catalyzed hydrolysis from its dimer. New unsymmetric dimer, N,N-dialkyl-4-hydroxy-4-oxo-2,2,3,3- tetrahydro-2,2-diphenyl-4,4-quinolones were synthesized through the dehydration and dealcoholation of N-alkylanilines and ethyl benzoylacetate. Dimer was identified with NMR, IR and GC-MS. A series of dimer has been synthesized using acid-catalyzed one-pot reaction that involved the condensation, cyclization and dimerization. Similarly, the meta (or para)-substituted (acetoxy, methoxy, ethoxy, methyl, ethyl) dimers were prepared from N-methyl-meta(or para)-substituted anilines. Formation of dimers was undertaken with p-toluenesulfonic acid (p-TSA) at 90-110°C in toluene for 2-6 hours over the dean-stark apparatus. All synthetic process from anilines and ethyl benzoylacetate to 2,3-dihydro-2-phenyl-4-quinolones could be carried out in one-pot without isolation of intermediates.

363.

STRUCTURE GUIDED DESIGN, SYNTHESIS AND IN VITRO CHARACTERIZATION OF AQUEOUS SOLUBLE INHIBITORS OF STAPHYLOCOCCAL ENOYL-ACP REDUCTASE. ***J Berman****, Affinium Pharmaceuticals, Inc, 100 University Avenue, 12th Floor, North Tower, Toronto, ON M5J 1V6, Canada, Fax: 416-646-1553, jberman@afnm.com*

The increasing emergence of bacterial drug resistance has created an urgent need for novel antibiotics, especially those active against methicillin-resistant *S. aureus*. Bacterial fatty acid biosynthesis is carried out by a series of enzymes organized in a synthetic cycle. In certain pathogenic bacteria (e.g. the genus *Staphylococcus*), enoyl-ACP reductase is responsible for the terminal step in the synthesis and is an essential gene. A novel series of enoyl-ACP reductase inhibitors were used in a structure guided approach to optimize inhibitor properties (i.e. aqueous solubility). Inspection of an enamide inhibitor bound to *E. coli* enoyl-ACP reductase (PDB code 1LXC) indicated that the right-hand side moiety of the inhibitor was in contact with bulk solvent. Accordingly, water solubilizing functions were incorporated into this part of the molecule. Potent inhibitors with markedly improved water solubility were synthesized. An X-ray structure of a representative inhibitor in complex with *S. aureus* enoyl-ACP reductase corroborates our design hypothesis. These inhibitors displayed submicromolar inhibitory activities against *S. aureus* enoyl-ACP reductase IC50 (0.020- 0.50 microM) and had potent MICs against staphylococcal strains (<0.063 microgram/mL). Conclusions: Structure guided drug discovery can be used to design novel, potent enoyl-ACP reductase inhibitors with modified physicochemical properties.

364.

STRUCTURE-ACTIVITY RELATIONSHIP OF MANSONONE F, A POTENT ANTI-MRSA SESQUITERPENOID QUINONE: SAR STUDIES ON C6 AND C9 ANALOGUES. *Young-Ger Suh,* ***Sun Nam Kim****, Dong-Yun Shin, Soon-Sil Hyun, Kyung-Hoon Min, Yong-Sil Lee, Seok-Ho Kim, Seung-Mann Paek, and Jong-Wha Jung, Department of Manufacturing Pharmacy, 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*

Recently, we have reported isolation, synthesis, and partial SAR study of mansonone F, which is structurally unique and highly potent in anti-MRSA

activity. The structural feature of mansonone F may impose that the mode of antibiotic action can be different from that of the antibiotics currently used for anti-MRSA therapies. Our earlier partial SAR studies on mansone F have disclosed that both the quinone moiety and the tricyclic system of mansonone F are essential for anti-MRSA activities. For the detailed systematic studies on mansonone F, a series of C6 and C9 analogues of mansonone F have been designed and synthesized on the basis of our previous SAR results. Most of the analogues exhibited good anti-MRSA activities and the steric effect (lipophilicity) turned out to be more important than the electronic effect. In particular, C6 n-butylmansonone F showed much higher anti-MRSA activity compared to that of vancomycin.

365.

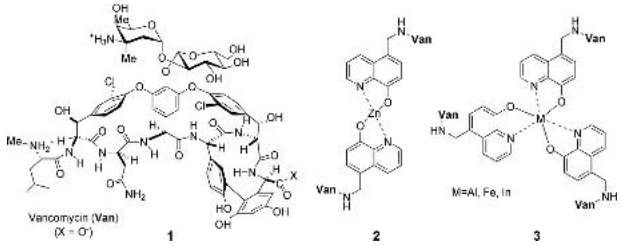
SUB-CLONING AND PURIFICATION OF THE LID SUB-DOMAIN OF DNAK. ***NC Bahr**, NK Steede, and SJ Landry, Department of Biochemistry, Tulane University Health Sciences Center, 1430 Tulane Avenue, New Orleans, LA 70112, Nathan.Bahr@loras.edu*

The Escherichia coli Hsp70 DnaK binds target proteins with the assistance a number of co-chaperones, including Hsp40 DnaJ. The DnaK-DnaJ system carries out a number of functions. Among these are: protein stabilization prior to complete folding, protein transport across membranes, and prevention of enzyme denaturation due to heat. A conformational change occurs in DnaK when it binds to the substrate; this change is linked with ATP hydrolysis. The J domain of Hsp40 DnaJ stimulates the aforementioned ATP hydrolysis in addition to substrate capture. Although the basic mechanism of this system is understood, it is not yet understood at the level of domain-wise interactions of DnaK. The DnaK protein is composed of an ATPase domain and a peptide-binding domain. The peptide-binding domain is further subdivided into a "beta-sandwich" domain and a "lid" domain. Preliminary data has suggested that the lid sub-domain of DnaK may be making a tertiary contact with the ATPase domain. In order to test this hypothesis, the lid sub-domain needed to be isolated. Therefore, the lid was sub-cloned, and the recombinant protein was purified. The recombinant lid was then titrated into purified recombinant ATPase domain in order to determine whether or not binding was occurring using Isothermal Titration Calorimetry (ITC). No excess heat was associated with mixing of the two proteins. This result is consistent with either of two possibilities, that the two domains do not bind or that they bind with no significant enthalpy. Further binding studies at different temperatures can eliminate the latter possibility. In that event, the mechanism that couples ATP hydrolysis to peptide capture must be mediated entirely by contacts between ATPase domain and the beta-sandwich.

366.

SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF MULTIVALENT VANCOMYCINS BASED ON 8-HYDROXYQUINOLINE PLATFORMS. ***Lihua Li**[1], Pak Leung Ho[2], and Bing Xu[1]. (1) Department of Chemistry, The Hong Kong University of Science & Technology, Clear Water Bay, Hong Kong, China, Fax: 852-2358-1594, (2) Department of Microbiology, Hong Kong University*

Recently, antibiotics resistant strains cause serious bacterial infections that threat public health. Particularly, vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens since 1988. Here we report our work on using multivalency approach to against VRE. We will report the synthesis and characterization of dimer (2) and trimers (3) of vancomycin (1) constructed from 8-hydroxyquinoline platform. We will also report evaluation of their activities against VRE.



367.

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES ON DAB-9 SUBSTITUTIONS OF THE LIPOPEPTIDE ANTIBIOTIC AMPHOMYCIN. ***Shirley A. Wacowich-Sgarbi**[1], Vincent A. Boyd[1], Dale R. Cameron[1], Yuchen Chen[1], Dominique Dugourd[2], Qi Jia[1], Matthew Nodwell[1], Paulo W. M. Sgarbi[1], Jenny Sun[1], Lixia Wang[1], Donald B. Borders[3], William V. Curran[3], and Richard A. Leese[3]. (1) Dept. of Chemistry, MIGENIX Inc, BC Research Complex, 3650 Wesbrook Mall, Vancouver, BC V6S 2L2, Canada, Fax: 604-221-9688, ssgarbi@migenix.com, (2) Dept. of Microbiology, MIGENIX Inc, (3) BioSource Pharm Inc*

Amphomycin (Amp), a natural product produced by *Streptomyces species*, is a cyclic 11-membered lipopeptide which is intrinsically active against aerobic and anaerobic Gram-positive bacteria. We have explored the potential of this core as a source of new antibacterial agents. While maintaining the lipophilic acyl chain constant, we have generated a focused library of Dab-9 substituted Amp analogues to study their effect on antibacterial activity. The *in vitro* MIC results revealed structural features of Dab-9 attachments that produced enhanced antimicrobial activity. Features of particular importance are length, bulk and the range of well tolerated functional group modifications.

368.

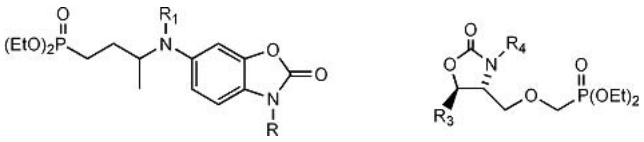
SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES ON THE LIPOPHILIC TAIL OF THE LIPOPEPTIDE ANTIBIOTIC AMPHOMYCIN. ***Paulo W. M. Sgarbi**[1], Vincent A. Boyd[1], Dale R. Cameron[1], Yuchen Chen[1], Qi Jia[1], Matthew Nodwell[1], Raymond Siu[2], Jenny Sun[1], Shirley A. Wacowich-Sgarbi[1], Lixia Wang[1], Donald B. Borders[3], William V. Curran[3], and Richard A. Leese[3]. (1) Dept. of Chemistry, MIGENIX Inc, BC Research Complex, 3650 Wesbrook Mall, Vancouver, BC V6S 2L2, Canada, Fax: 604-221-9688, psgarbi@migenix.com, (2) Dept. of Microbiology, MIGENIX Inc, (3) BioSource Pharm Inc*

Lipopeptides, the newest class of approved antibiotics, are effective against a wide variety of Gram-positive microorganisms, and are characterized by a macrocyclic peptide or depsipeptide core, flanked by an N-terminal hydrocarbon acyl chain. Amphomycin (Amp), a fermentation product of *Streptomyces griseus*, is a decapeptide lactam linked to an unsaturated fatty acid through an amino acid spacer (Asp). In developing new antibacterial agents, we prepared semisynthetic analogues of Amp, improving their *in vitro* activity through structure-activity relationship studies (SAR) on the lipophilic tail. The MIC results revealed some of the structural features of the tail required for activity, including length, bulk and a range of well tolerated functional group modifications and spacers.

369.

SYNTHESIS OF BIOLOGICALLY ACTIVE PHOSPHONATE OXAZOLIDINONE DERIVATIVES. ***Kang-Yeoun Jung**, Jae-Min Hwang, and Uk-Il Kim, Department of Environmental & Applied Chemistry, Kangnung National University, Kangnung Daehackro 120, Kangnungsi 210-702, South Korea, Fax: 82-33-641-2410, kyjung@kangnung.ac.kr*

Oxazolidinones are very important class of antibacterial agents, which are active against Gram-positive human pathogens as well as selected anaerobic organisms. Several synthesis and biological activity of novel oxazolidinones have been reported and the first oxazolidinone derivative, linezolid (Zyvox¢â) is now being marketed for the treatment of multi-drug resistant Gram-positive infections. Benzoxazolone moiety in many drugs and aminophosphonic acids which exist in many natural products, play very important role in biological system. Our idea was to combine aminophosphonate moiety with variously substituted benzoxazolones giving access to a wide array of structures having interesting biological and pharmacological properties. We, therefore, selected the aminophosphonate group as potential substituents in our designed compounds, and report here, the preliminary synthesis of benzoxazolidinone phosphonate derivatives. Several new oxazolidinone phosphonates were also prepared efficiently using aminoacids such as D-Serine and L-Threonine. Currently, the biological activity of these oxazolidinone derivatives is being investigated.



370.

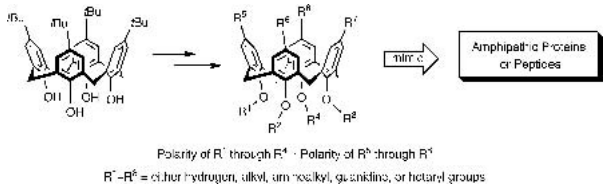
SYNTHESIS OF POTENTIAL HOLLIDAY JUNCTION INHIBITORS. ***Irene Medina***[1], *Chris Carroll*[2], *and Shelli R. McAlpine*[2]. *(1) Department of Chemistry and Biochemistry, San Diego State University, 5500 Campanile Drive, CSL 206, San Diego, CA 92182-1030, imedina_@hotmail.com, (2) Department of Chemistry, San Diego State University*

Holliday Junctions occur during site-specific recombination, which is a DNA repair mechanism found in bacteria. Trapping Holliday Junctions inhibits site-specific recombination, which shuts down this major DNA repair mechanism, thus causing bacteria death. Two generations of C-2 symmetrical macrocyclic peptides have been synthesized in our lab and tested for antibacterial activity. The assays show that many of these macrocyclic peptides are successful in binding to Holliday Junctions. However, bacteria growth inhibition assays show that although our compounds trap Holliday Junctions they do not cause bacteria cell death. Therefore, we have used a solid-phase synthetic strategy to synthesize C-2 symmetrical cyclic peptides containing hydrophilic residues. This should promote hydrogen bonding between the cyclic peptides and the Holliday Junction and therefore induce bacteria death.

371.

SYNTHESIS, ANTIBIOTIC ACTIVITY, AND ANTIANGIOGENIC ACTIVITY OF CALIXARENE DERIVATIVES THAT ARE TOPOLOGICAL MIMETICS OF AMPHIPATHIC PEPTIDES. ***Xuemei Chen***[1], *Thomas R. Hoye*[1], *and Kevin H Mayo*[2]. *(1) Department of Chemistry, University of Minnesota, 207 Pleasant St SE, Minneapolis, MN 55455-0431, xchen@chem.umn.edu, (2) Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota*

Anti-angiogenic and bactericidal proteins or peptides sometimes possess a common topology that enforces in amphipathic character. The calix[4]arene core represents a template upon which to array sets of polar (including cationic) or hydrophobic substituents with appropriate molecular dimensions to establish topological mimics of such amphipathic structures. We have prepared a set of calixarene-based amphipathic compounds. Two were found to have good bactericidal activity and two different compounds were found to display promising antiangiogenic activity in both in vitro and in vivo bioassays. The synthesis of these calixarene derivatives and some of their biological properties will be described in this presentation.



372.

VOLSURF STUDIES OF 5-NITRO-2-THIOPHYLIDENE DERIVATIVES WITH ANTIMICROBIAL ACTIVITY AGAINST MULTIDRUG-RESISTANT STRAIN OF *STAPHYLOCOCCUS AUREUS*. *Andrea Masunari*[1], *Leandro de Rezende*[2], *Antônia Tavares do Amaral*[2], *and* ***Leoberto Costa Tavares***[1]. *(1) Departamento de Tecnologia Bioquímico-Farmacêutica, Universidade de São Paulo, Avenida Professor Lineu Prestes 580, São Paulo 05508900, Brazil, Fax: 11-3815-6386, andreamasunari@yahoo.com.br, leoberto@usp.br, (2) Departamento de Química Fundamental, Universidade de São Paulo*

Infectious disease caused by MRSA (Methicillin-Resistant *S. aureus*) is currently a serious problem in hospitals because this bacteria shows a phenotype of multidrug resistance. Thus, the purpose of this study was the synthesis and Volsurf characterization of 5-nitro-2-thiophylidene derivatives with antimicrobial activity against MRSA. Minimal Inhibitory Concentration (MIC) values of a set of eighteen 5-nitro-2-thiophylidene derivatives have been correlated with the corresponding 3D molecular fields, generated by probes water, DRY, carbonyl oxygen atom and amide NH group using a GRID force field. Structures were modeled in their neutral forms and further minimized using CORINA program. Forty-eighth Volsurf descriptors were extracted from 3D maps and for seventeen compounds, significant correlation (r^2 = 0,93, q^2 = 0,87) has been observed between MIC values obtained by the model and experimentally. It was observed that the equivalent distribution of hydrophobic regions on derivatives seems to be necessary to result in lower MIC values.

373.

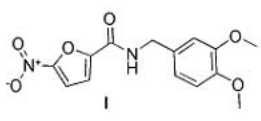
ANTI-TUBERCULOSIS STRUCTURE-ACTIVITY RELATIONSHIP OF MACROLIDES. ***Zhaohai Zhu***, *Kanakeshwari Falzari, Dahua Pan, Olga Krasnykh, and Scott G. Franzblau, Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 S. Wood St., Room 412, Chicago, IL 60612, Fax: 312-355-2693, zhaohai@uic.edu*

Among infectious diseases, tuberculosis (TB) is the number one killer with over two-million casualties annually worldwide. There has not been a new TB drug marketed in almost four decades. Resistances to existing drugs have been continuously increasing. TB-HIV co-occurrence in patients has emerged as a new threat to human beings. In our screening program, we have discovered anti-TB macrolides with sub-micromolar MIC in vitro, and with activity in TB-infected mice. The current study is lead optimization for the discovery of new anti-TB drug candidates for clinical development. We have synthesized series of macrolides and ketolides and tested their anti-TB activities and toxicities. Structure-activity relationship will be presented.

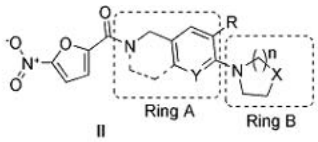
374.

SYNTHESIS AND EVALUATION OF SUBSTITUTED BENZYL NITROFURANYL AMIDES AS NOVEL ANTITUBERCULOSIS AGENTS. ***Rajendra P Tangallapally***[1], *Robin E.B. Lee*[2], *Anne J. M. Lenaerts*[3], *and Richard E Lee*[2]. *(1) Department of Pharmaceutical Sciences, The University of Tennessee Health Science Center, 847 Monroe Ave Rm327, Memphis, TN 38163, rtangallapal@utmem.edu, (2) Department of Pharmaceutical Sciences, University of Tennessee HSC, (3) Department of Microbiology, Colorado State University*

In an ongoing effort to develop new and potent anti-tuberculosis agents a new series of compounds with general structure II have been synthesized based on our initial lead compound I. Compounds produced in this series were discovered with increased potency and solubility. In the general structure II, ring A was modified with 1,2,3,4-tetrahydro-isoquinoline, 3-fluoro-benzylamine and 3-picolylamine structural moieties. The ring B was modified with 4-alkyl piperazines, morpholine, thiomorpholine and 4-alkyl diazepanes. The synthesis of these compounds and their in vivo - in vitro activities against M. tuberculosis will be presented.



I



II

2 : X = N-R, S, O; R = H or F; n = 2 or 3
3 : X = NCOOR; R = H; Y = C or N; n = 2 or 3
4 : X = NCONHR; R = H; Y = C or N; n = 2 or 3

375.

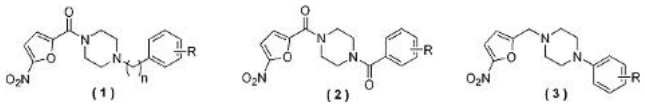
COMPETITIVE INDUCTION OF ANTI-MYCOBACTERIAL ACTIVITY IN MARINE BACTERIA. ***Jacqueline A. Trischman***[1], *Richard E. Oeffner*[2], *Ryan Nelson*[2], *Travis Cook*[3], *and Paul Rascoe*[2]. *(1) Department of Chemistry & Biochemistry, California State University San Marcos, College of Arts & Sciences, San Marcos, CA 92096, trischma@csusm.edu, (2) Department of Chemistry & Biochemistry, CSU San Marcos, (3) Deprtament of Chemistry & Biochemistry, University of California San Diego*

This study demonstrates that marine bacteria can be induced to produce anti-mycobacterial compounds. This competitive induction method may prove to be a useful tool in the development of new antibiotics against the leading cause of death from infectious disease worldwide, *Mycobacterium tuberculosis*. In this study, heterotrophic strains of marine bacteria were grown in both monoculture and challenge culture, i.e. grown to early stationary phase then challenged by addition of live mycobacterial cells. Cultures were extracted and screened for inhibitory activity against *M. marinum*, chosen for its genetic similarity to *M. tuberculosis*. Assay of extracts of 299 strains resulted in 79 monoculture extracts and an additional 59 challenge culture extracts showing significant inhibitory activity against the target strain. This represents a 75% increase over

the activity seen using exclusively monoculture methods. Several small inhibitory molecules have been identified, and structure elucidation is underway on compounds responsible for more significant anti-mycobacterial activity.

376.
DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NITROFURANYL AMIDES, DIAMIDES AND AMINES WITH ENHANCED ACTIVITY AGAINST *MYCOBACTERIUM TUBERCULOSIS*. *Raghunandan Yendapally*, *Rajendra P Tangallapally, Robin E. B. Lee, and Richard E Lee, Department of Pharmaceutical Sciences, The University of Tennessee Health Science Center, 847 Monroe Ave Rm327, Memphis, TN 38163, Fax: 901-448-6828, ryendapa@utmem.edu*

As a part of an ongoing effort to discover novel and more potent therapies for the treatment of tuberculosis we have been developing a class of nitrofuranyl amides. This study has evaluated the importance of the secondary amide functionality in nitrofuranyl amides by replacing it with tertiary amides, diamides and tertiary amines. Several substituted nitrofuranyl piperazine amides **(1)**, nitrofuranyl piperazine diamides **(2)** and nitrofuranyl phenyl piperazines **(3)** have been successfully synthesized and tested for their ability to inhibit the growth of *M. tuberculosis*. Interestingly, it has been discovered that substituted nitrofuranyl phenyl piperazines have enhanced *in vitro* activity and many of these compounds exhibited submicromolar MIC values against *M. tuberculosis*. The synthesis, structure-activity-relationship and biological activities of this series will be presented.

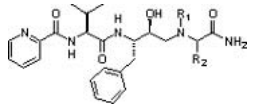


377.
DEVELOPING NOVEL INHIBITORS OF THE ENOYL REDUCTASE FROM MYCOBACTERIUM TUBERCULOSIS (INHA) : SAR STUDIES OF TRICLOSAN CONGENERS. *Todd J. Sullivan*[1], *Polina Novichenok*[1], *James J. Truglio*[2], *Caroline Kisker*[2], *Francis Johnson*[3], *Richard A. Slayden*[4], *and Peter J. Tonge*[1]. *(1) Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400, Fax: 631-632-7960, (2) Department of Pharmacological Sciences, Center for Structural Biology, Stony Brook University, (3) Departments of Pharmacological Sciences and Chemistry, Stony Brook University, (4) Department of Microbiology, Immunology and Pathology, Colorado State University*

InhA, the enoyl reductase enzyme from *Mycobacterium tuberculosis* (MTB), catalyzes the last step in the fatty acid biosynthesis pathway (FAS II). Frontline anti-tuberculosis drugs such as isoniazid (INH) target this enzyme. Drug resistance to INH results primarily from mutations in KatG, the enzyme that activates INH. Consequently, InhA inhibitors that do not require activation by KatG are attractive candidates for drug discovery. One such inhibitor is our lead compound for SAR studies triclosan, a common antibacterial additive in personal care products. Triclosan is a μM inhibitor of InhA and a pM inhibitor of the enoyl reductase from E. coli (FabI). Using structural and mechanistic data, we have developed a series of aliphatic-substituted triclosan analogs with a nM affinity for InhA and with sub-μM MIC99 values for H37Rv MTB. These compounds are currently being evaluated in an animal model of tuberculosis. Second generation analogues are now being developed to investigate and address compound bioavailability and cell membrane permeability.

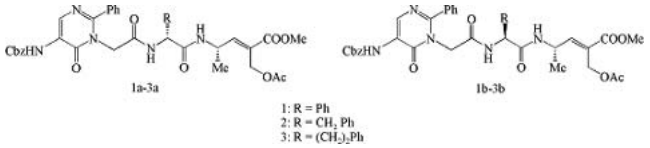
378.
DOCKING AND 3D QSAR MODELS OF HYDROXYETHYLAMINE BASED PLASMEPSIN II INHIBITORS. *Daniel Muthas*[1], *Yogesh A. Sabnis*[1], *Daniel Nöteberg*[1], *Elizabeth Hamelink*[2], *Johan Hultén*[1], *Lotta Vrang*[2], *Bertil Samuelsson*[2], *Anders Hallberg*[1], *and Anders Karlén*[1]. *(1) Department of Medicinal Chemistry, Uppsala University, Husargatan 3, SE-751 23 Uppsala, Sweden, Fax: +46 18 471 44 74, danielm@orgfarm.uu.se, (2) Medivir AB*

Malaria is the most widespread tropical disease accounting for 400 million cases and 1.2 million deaths every year. It has been shown that plasmepsin II is one of the principal hemoglobinases of the malarial parasites. Herein we present docking and 3D-QSAR studies on thirty-seven hydroxyethylamine based plasmepsin II inhibitors having varying activities, with the generic structure shown in figure 1. Interestingly, small R1 substituents or bulky R2 substituents yielded compounds with high plasmepsin II activity. Docking studies could generalize the different SAR observed for P1′ substituents attached on R1 or R2. Both series seemed to be accommodated in the S1′ pocket although binding in different regions. A Comparative Molecular Field Analysis (CoMFA), where the alignment was based upon the docking results, was performed to give a quantitative model. This model was built using a leave-one-out (LOO) cross-validation method, and had a q^2 of 0.4 and a r^2 of 0.5. Figure 1. Generic structure of the investigated series of inhibitors.



379.
DESIGN, SYNTHESIS AND ANTIMALARIAL ACTIVITY OF NOVEL PEPTIDOMIMETICS BASED ON MICHAEL ACCEPTOR CORE. *Olga V. Miroshnikova*, *Shuren Zhu, Thomas H. Hudson, Lucia Gerena, and Ai J. Lin, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910, Fax: 301-319-9449, olga.miroshnikova@na.amedd.army.mil*

Extending our design and synthesis of novel peptidomimetic antimalarials based on Michael acceptor core, efforts are directed toward lengthening of peptide chain by addition of extra amino acids, such as phenylglycine, phenylalanine and homophenylalanine, into the Michael acceptor backbone. Peptide coupling procedure of Michael acceptor with amino acids resulted in a mixture of diastereomers, which were successfully separated by column chromatography. The purified isomers were coupled with a 5-substituted aminopyrimidinyl carboxyl acid to give the final products 1a–3a and 1b-3b in high yield. Synthesis, structural characterization and in vitro antimalarial activities of the new peptidomimetics will be presented.



380.
INHIBITION OF M. TUBERCULOSIS FATTY ACID SYNTHETASE I ISOLATED FROM M. SMEGMATIS BY 5-CL-PYRAZINAMIDE AND ANALOGS. *Silvana C. Ngo*[1], *Oren Zimhony*[2], *Halimah Sayahi*[1], *William R. Jacobs Jr.*[3], *and John T. Welch*[1]. *(1) Department of Chemistry, University at Albany, SUNY, 1400 Washington Ave., Albany, NY 12222, Fax: 518-442-3462, silvana@albany.edu, (2) Unit for Infectious Diseases, Kaplan Medical Center, Hadassah Medical School, The Hebrew University, (3) Albert Einstein College of Medicine*

Currently, one third of the world's population is infected with tuberculosis, with an ongoing rate of one new infection per second. The emergence of multidrug resistant strains of M. tuberculosis makes the development of new, better therapies ever more important. The addition of pyrazinamide (PZA) to the drug regimen for the treatment of tuberculosis is essential to shorten the treatment period. While the mechanism of action of PZA is still not clearly understood, it is under intense investigation. We have previously shown that an analog of pyrazinamide (PZA), 5-chloropyrazinamide (5-Cl-PZA) inhibits fatty acid synthetase I (FASI) in M. tuberculosis. To pursue the study of FAS1 as drug target, we constructed a strain of M. smegmatis (a fast growing non pathogenic mycobacteria), mc2 2700, that has the native fas1 gene deleted and bears M. tuberculosis fas1. We present here our results on the isolation, purification and inhibition of M. tuberculosis FAS1 isolated from M. smegmatis mc2 2700.

381.
MICROBIAL TRANSFORMATION OF ARTEMISININ TO 5-HYDROXYARTEMISININ. *Igor A. Parshikov, **Bruhaspathy Miriyala, MItchell A. Avery, and John Williamson, Department of Medicinal Chemistry, University of Mississippi, University, MS 38677, Fax: 662-915-5638, bru@olemiss.edu***

Malaria is estimated to be responsible for the death of over two million people each year across the continents of Africa and Asia. Chloroquine has been the first drug of choice over a long time. Emergence of chloroquine-resistant parasite has led to the identification of Artemisinin as an alternative; however, toxicities and water insolubility limit its usefulness. Semi-synthetic derivatives of

the anti-malarial drug artemisinin hold great promise in the search for an effective and economical treatment of chloroquine-resistant forms of malaria. Synthetic functionalization of the artemisinin skeleton, particularly at positions 4, 5, 6 or 7 is often tedious and/or impractical, leading to few known derivatives. Biotransformation by microorganisms has occasionally been employed for this purpose. The ability of a locally isolated soil fungus, Aspergillus niger A-51 for bioconversion of artemisinin to its hydroxyderivatives was investigated. Fungal cultures were grown in malt/peptone medium in 750 mL flasks at 28°C with shaking at 180 rpm. After 48 hours, the cultures were dosed with artemisinin to a final concentration of 500 mg/L and incubated for further 14 days. HPLC analysis of the ethyl acetate extract of the cultures of A. niger revealed the presence of a major metabolite eluting at 14.8 min (63%), one minor metabolite eluting at 13.3 min (32%) and residual artemisinin at 19.2 min. These metabolites were then purified by flash-chromatography and characterized using high-resolution mass spectra (HR-MS) and nuclear magnetic resonance (NMR) spectra. HR-MS and NMR analysis showed the major metabolite to be 5β-hydroxyartemisinin ($[M+Na]^+ = 321.13$); and minor metabolite as 7β-hydroxyartemisinin, ($[M+Na]^+ = 321.13$). These hydroxyl derivatives of artemisinin are currently being used for the synthesis of new and effective anti-malarial agents.

382.

PREDICTING ANTIMYCOBACTERIAL ACTIVITY OF QUINOLONE DERIVATIVES USING THEORETICAL MOLECULAR DESCRIPTORS. ***Denise Mills***[1], *Manish C. Bagchi*[2], *Bhim C. Maiti*[2], *and Subhash C. Basak*[1]. *(1) Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, 5013 Miller Trunk Hwy, Duluth, MN 55811, Fax: 218-720-4328, dmills@nrri.umn.edu, (2) Indian Institute of Chemical Biology*

Several quinolones act as inhibitors of Mycobacterium tuberculosis infection as well as other mycobacterial infections. Since physicochemical data are not always available to develop predictive models, the only alternative is to utilise theoretical molecular descriptors, derived solely from chemical structure, in the development of structure-activity relationship models. The current study involves structure-activity relationship models of quinolone antibacterials against mycobacteria. Topostructural (TS), topochemical (TC) and geometrical (3D) indices associated with chemical structures of N-1 and C-7 substituted quinolone derivatives as well as 8-substituted quinolones with good anti-mycobacterial activities against M. fortuitum and M. smegmatis have been evaluated. The activities of the compounds against these two organisms are used as a measure of anti-Mycobacterium tuberculosis activity. Various linear regression methods were used for developing QSAR models and the results show that for the full set of compounds, the combination of TS and TC indices explain most of the variance in the data.

383.

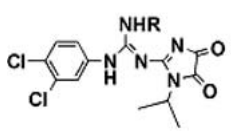
PROTEIN FARNESYLTRANSFERASE INHIBITORS EXHIBIT POTENT ANTI-MALARIAL ACTIVITY. ***Laxman Nallan***[1], *Kevin Bauer*[2], *Pravin M. Bendale*[3], *Kohei Yokayama*[3], *Oliver Hucke*[4], *Christophe L. M. J. Verlinde*[5], *David Floyd*[6], *Louis J. Lombardo*[7], *David Williams*[8], *Wesley C Van Voorhis*[9], *and Michael H Gelb*[3]. *(1) Department of chemistry, University of washington, Box; 351700, Seattle, WA 98195-1700, Fax: 206-685-8665, nallan@u.washington.edu, (2) Department of Medicine, University of Washington, (3) Department of Chemistry, University of Washington, (4) Department of Biochemistry, University of Washington, (5) Biomolecular Structure Center, University of Washington, (6) Bristol-Myers Squibb, (7) Discovery Chemistry, Bristol-Myers Squibb, (8) Discovery Chemistry, Bristol-Myers Squibb PRI, (9) Infectious Diseases, University of Washington*

Protein prenylation, a novel post translational modification involves the covalent attachment of either a 15 carbon farnesyl or 20-carbongeranyl geranyl isoprenoid to the carboxy terminal of the cystine residue via a thioether bond, and the attachment is catalyzed by protein prenyltransferases. Recent studies have shown that protein prenylation occurs in the parasites, Giardia lamblia and Schistosoma mansoni. Our lab, as well as and others have found, this process also occurs in protozoan parasites, Trypanasoma brucei (African sleeping sickness), Trypanasoma cruzi (Chagas disease), Leishmania spp. (Leishmaniasis) and Plasmodium Falciparum (malaria). Protein Farnesyltransferase inhibitors (PFTIs) show high toxicity to the parasite cells compared to mammalian cells even without selective potency against parasite PFTs over mammalian enzyme. Thus PFT has been suggested to be an ideal target for protozoan parasites. Because of their anti-cancer properties, PFTs comprise a highly developed class of compounds in the pharmaceutical industry. This provides us with a wealth of lead compounds for the rapid development of anti-malarials in a non-industrial setting. Thus we tested entire PFT inhibitor series that we synthesized the existing compounds in addition to available from the pharmaceutical industry.

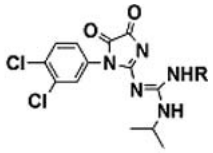
384.

SYNTHESIS AND PROPHYLACTIC ANTIMALARIAL ACTIVITIES OF 2-GUANIDINYLIMIDAZOLIDINEDIONE DERIVATIVES. ***Quan Zhang***[1], *Jian Guan*[1], *Gettayacamin Montipa*[2], *William Y Ellis*[1], *Arba Ager*[3], *Wilbur K Milhlous*[1], *Donald R Skillman*[1], *and Ai J Lin*[1]. *(1) Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Av, Silver Spring, MD 20910, Fax: 301-319-9449, quan.zhang@na.amedd.army.mil, (2) Armed Forces Research Institute of Medical Sciences (AFRIMS), (3) Center for Tropical Parasitic Diseases, University of Miami*

WR182393, a 2-guanidinoimidazolidinedione derivative with high prophylactic antimalarial activity, was a mixture of 3 closely related products. Poor solubility and impractical synthetic method have made the purification and structure identification of the mixture a highly challenging task. The problems were circumvented by pro-drug approach involving carbamate formation of the mixture. Thus, WR182393 components 1 and 2 were separated, purified and identified as carbamate derivatives by NMR and x-ray crystallography. Unambiguous synthetic procedures were developed to prepare the components 1 and 2. Structure activity relationship studies (SAR) resulted in discovery of new carbamates with 100% oral protection activity against Plasmodium yoelii sporozoites challenged mice at a dose as low as 2.5 mg/kg. Two of the new carbamates were found to possess higher intramuscular (im) efficacy than the parent compound WR182393 against P. cynomolgi in Rhesus monkey. Structure determination of the active components of WR182393, development of novel synthetic procedure and the SAR studies of the new carbamates will be presented.



1 (R=H)



2 (R=H)

385.

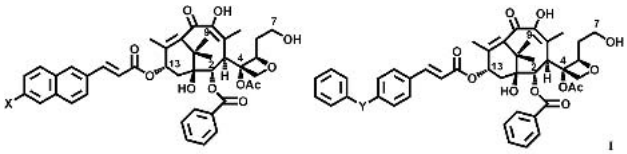
SYNTHESIS OF METHYLHEMIGOSSYPOL FOR BIOLOGICAL STUDIES. ***Jun Wei***[1], *Lucy A. Hunsaker*[2], *David L. Vander Jagt*[2], *Robert E. Royer*[2], *and Lorraine M. Deck*[1]. *(1) Department of Chemistry, University of New Mexico, MSC03 2060, Albuquerque, NM 87131, weijun@unm.edu, (2) Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine*

Gossypol and its derivatives have shown a wide range of biological activities including anticancer, antiparasitic and antiviral activity. We demonstrated that gossypol and its derivatives exhibit antimalarial activity against both chloroquine sensitive and chloroquine resistant strains of P. falciparum. We then demonstrated that gossypol analogues, dihydroxynaphthoic acids, are potent and selective inhibitors of human and parasitic lactate dehydrogenases. We now report the synthesis and kinetic inhibition studies of methylhemigossypol and related compounds. The synthesis is convenient, short and amenable to modification resulting in an array of 4, 6 and 7 substituted compounds. The key step in the synthesis involves Grignard condensation to form a lactone, which upon reaction with boron tribromide forms methyl deoxyhemigossypol. Formylation is accomplished using the Duff reaction or the Vilsmeier-Haack reaction.

386.

TARGETING FTSZ FOR ANTI-TUBERCULOSIS DRUG DISCOVERY: NON-CYTOTOXIC TAXANES AS NOVEL ANTI-TB AGENTS. ***Qing Huang***[1], *Antonella Pepe*[1], *Ilaria Zanardi*[2], *Peter J Tonge*[3], *Richard A. Slayden*[4], *Fumiko Kirikae*[5], *Teruo Kirikae*[5], *and Iwao Ojima*[3]. *(1) Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, Fax: 631-632-7942, qinhuang@ic.sunysb.edu, (2) Institute of Chemical Biology & Drug Discovery, State University of New York at Stony Brook, (3) Institute of Chemical Biology & Drug Discovery and Department of Chemistry, State University of New York at Stony Brook, (4) Department of Microbiology, Immunology and Pathology, Colorado State University, (5) Department of Infectious Diseases, Research Institute, International Medical Center of Japan*

FtsZ, the bacterial tubulin homologue, is an essential cell-division protein that polymerizes into a cytokinetic ring at the septum site. Based on the structural homology with tubulin, we hypothesized that compounds that stabilize microtubules should inhibit the (de)polymerization of FtsZ from *Mycobacterium tuberculosis* (MTB). Subsequently, screening of 120 taxanes identified a number of compounds that exhibited significant anti-tuberculosis activity. Through systematic rational drug design, we discovered that C-seco-TRAs (ι) are non-cytotoxic at the upper limit of detection (>80 μM), while maintaining MIC_{99} values of 1.25-2.5 μM against drug-resistant and drug-sensitive MTB strains. Polymerization assays demonstrated that these C-seco-TRAs inhibited MTB FtsZ polymerization in a dose dependent manner. Thus, these novel taxanes specifically target FtsZ, but not microtubules. In addition, treatment of MTB cells with taxane SB-RA-20018 at the MIC (~1 μM) caused filamentation, a phenotype indicative of FtsZ inhibition. Our results suggest that C-seco-TRAs have high potential to serve as novel anti-TB drugs against multidrug-resistant strains of MTB.



SB-RA-5001: X=H
SB-RA-5001MeO6: X=MeO

SB-RA-5011: Y=O
SB-RA-5001MeO6: Y=S

387.

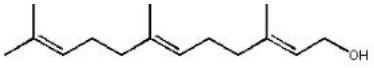
3D-QSAR STUDY OF HETEROCYCLIC QUINONE COMPOUNDS WITH ANTIFUNGAL ACTIVITY BY COMFA. ***Hea-Young Park Choo****, Su-young Choi, and Chung-Kyu Ryu, School of Pharmacy, Ewha Womans University, Seoul 120-750, South Korea, Fax: 82-2-3277-2851, hypark@ewha.ac.kr*

Recently, we reported that some heterocyclic quinone compounds such as 6-(N-arylamino)-7-chloro/ 6,7-bis[S-(aryl)thio]-5,8-quinolinedione,6-arylthio-/ 5,6-aryl amino-4,7-dioxobenzothiazoles and 2,5-disubstituted-6-arylamino-4,7-benzimidazolediones have antifungal effects. To understand the structural basis for antifungal activity and guide the design of more potent agents, we perfomed three dimensional quantitative structure activity relationship studies for these series of compounds using CoMFA. All molecular models and statistical analyses were performed with SYBYL 7.0 molecular modeling software and Silicon Graphics Indy workstation. The MIC values of heterocyclic quinone compounds on A.niger exhibited a strong correlationship with steric, electrostatic and lipophilic factors of the molecules. The statistical results of the training set with 49 compounds, cross-validated q2(0.759) and conventional r2(0.936) values gave reliability to the prediction of inhibitory activity of these compounds. The contour maps obtained by CoMFA gave an indication for favorable regions for bulkier and electropositive substituents. The contribution for the steric factor was more important (71.1%) than electrostatic factor (28.2%).

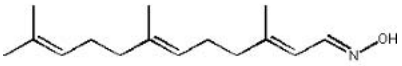
388.

EXPLORING THE MECHANISM OF QUORUM-SENSING IN *CANDIDA ALBICANS* WITH SYNTHETIC FARNESOL ANALOGS. ***Roman Shchepin***[1], *Patrick H. Dussault*[1], *Kenneth W. Nickerson*[2], *Raluca Dumitru*[2], *and Audrey Atkin*[2]. *(1) Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588, zoren111@yahoo.com, (2) School of Biological Sciences, University of Nebraska-Lincoln*

Candida albicans, normally a commensal in man, can be a life-threatening opportunistic pathogen in immunocompromised individuals. *C. albicans* is able to switch between yeast-like and mycelial forms. This interconversion, which is believed to be related to pathogenicity, is influenced by cell density, specifically by the levels of extracellular farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol) secreted as a quorum-sensing molecule. Accumulation of farnesol results in suppression of the mycelia morphology. We have been studying the mechanism of quorum-sensing in *C. albicans* using unnatural farnesol analogs. We have now developed a class of unnatural quorum-sensing molecules with potency equal to or greater than farnesol. In addition, we have synthesized a family of fluorescent probes which display good activity in quorum-sensing assays. Our presentation will focus on the preparation and properties of these molecules, their application in studies of quorum-sensing *in vitro* and *in vivo*, and efforts to identify a farnesol-binding protein.



Farnesol



A potent fanesol analog

389.

ANTIPARASITIC COMPOUNDS FROM *PSOROTHAMNUS ARBORESCENS*. ***Manar M. Salem*** *and Karl A. Werbovetz, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, the Ohio State University, 500 W. 12th ave, Columbus, OH 43210, Fax: 614-292-2435, salem.17@osu.edu*

As part of an ongoing effort to discover novel compounds with activity against kinetoplastid parasites, the genus *Psorothamnus* had been identified as a source of potential new antiparasitic agents. In a previous work, we isolated antileishmanial and antitrypanosomal compounds from *P. polydenius* that displayed selectivity for these organisms. Another member of the same genus, *P. arborescens*, exhibited significant activity against *Leishmania donovani* axenic amastigotes. Bioassay-guided fractionation of the methanol extract from *P. arborescens* resulted in the isolation of several bioactive flavonoids, some with IC_{50} values less than 10 μg/mL. Structural elucidation of these compounds was performed using NMR spectroscopy and mass spectrometry techniques. Further details regarding the isolated compounds and their antiparasitic activity and selectivity will be presented in this poster.

390.

DESIGN, SYNTHESIS AND EVALUATION OF NOVEL INHIBITORS OF T. CRUZI DUTPASE AS POTENTIAL ANTI-PARASITIC DRUGS. ***Orla K Mc Carthy***[1], *Ian H Gilbert*[1], *Dolores González Pacanowska*[2], *and Reto Brun*[3]. *(1) Welsh School of Pharmacy, University of Wales, Redwood Building, King Edward VII Avenue, Cardiff CF103XF, United Kingdom, Fax: 0044 2920 874149, mccarthyo@cf.ac.uk, (2) Instituto de Parasitología y Biomedicina "Lopez-Neyra', (3) Swiss Tropical Institute*

Chagas' disease is a debilitating disease caused by the Trypanosoma cruzi parasite. It is endemic in 18 countries in South and Central America where it is the cause of 21,000 deaths annually. Current drug treatment for this disease is unsatisfactory and the need for new drugs is urgent.dUTP nucleotidohydrolase is an enzyme which catalyses the hydrolysis of dUTP to dUMP in the presence of Mg2+ ions. dUTPase plays a vital role in maintaining a low dUTP:dTTP ratio in the cell, thereby preventing the overincorporation of dUTP into DNA which would lead to DNA fragmentation and cell death. dUTPase is therefore crucial for cell viability.The aim of this project is to design and synthesise novel inhibitors of the T. cruzi dUTPase enzyme which is a dimeric form of the enzyme, the crystal structure of which is known. Based on computational modelling and docking studies, potential inhibitors were synthesised and their biological activity evaluated.

391.

DNA BINDING PROPERTIES OF AN ANTITRYPANOSOMAL AGENT. ***Binh Nguyen***[1], *Jaroslav Stanek*[2], *Reto Brun*[3], *and W. David Wilson*[1]. *(1) Department of Chemistry, Georgia State University, 50 Decatur St, Atlanta, GA 30303, chebkn@langate.gsu.edu, (2) Novartis Pharma AG, (3) Swiss Tropical Institute*

Anti-parasitic diamidine compounds such as berenil, propamidine, pentamidine, and furamidine have been found to bind strongly to the minor groove of AT rich DNAs. These compounds have some common features such as curved shapes and hydrogen donor groups. A new antitrypanosomal agent, CGP 40215A, has been found to bind to a DNA minor groove with a high affinity, but this compound lacks curvature when compared to the drugs above. Studies by several biophysical techniques indicated that water molecules bridge the interaction between the compound and DNA bases. Calorimetric titrations support a proton-linkage in binding and are in a good agreement with spectroscopic titrations. The observed binding enthalpies as a function of temperature indicate a negative heat capacity change that is typical for DNA minor groove binders. The proton uptake and direct involvement of a water molecule in the complex can serve as a template for design of new anti-parasitic drugs.

392.

IDENTIFICATION OF NOVEL PARASITIC CYSTEINE PROTEASE INHIBITORS USING VIRTUAL SCREENING. ***Prashant V. Desai***[1], *Akshay Patny*[1], *Yogesh A Sabnis*[1], *Babu L. Tekwani*[2], *Jiri Gut*[3], *Philip J. Rosenthal*[3], *Anuradha Srivastava*[2], *and MItchell A. Avery*[1]. *(1) Department of Medicinal Chemistry, University of Mississippi, 417 Faser Hall, School of Pharmacy, University, MS 38677, pdesai@olemiss.edu, (2) National Center for Natural Products Research, University of Mississippi, (3) Department of Medicine, University of California, San Francisco*

Trypanosomiasis, leishmaniasis and malaria are major parasitic diseases in developing countries. The existing chemotherapy of these diseases suffers from lack of safe and effective drugs and/or the presence of widespread drug resistance. Cysteine proteases are exciting novel targets for antiparasitic drug design. Virtual screening was performed to scan two commercially available databases in an attempt to identify novel druglike nonpeptide inhibitors of parasitic cysteine proteases. The databases were prescreened to collect only 'drug-like' molecules which were then screened against homology models of falcipain-2 and falcipain-3. In case of the ChemBridge database, a total of 24 diverse inhibitors were identified from an initial group of 84, of which 12 compounds appeared to be dual inhibitors of falcipain-2 and falcipain-3. Similarly, more than 15 active compounds were identified from the ACD database. The hits can be further optimized to obtain potent and selective nanomolar inhibitors of protozoal cysteine proteases.

393.

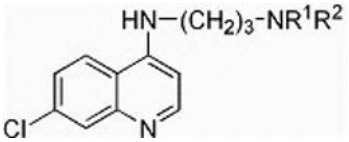
RATIONAL DESIGN OF INHIBITORS OF THE CRYPTOSPORIDIUM PARVUM ATP-BINDING CASSETTE 3 PROTEIN. ***R Terreux***, *P lawton, S radix, D Deruaz, M Lussignol, J Reynaud, C Marminon, P Nebois, Z Bouaziz, and N Walchshofer, LCMP2, ISPB, University Claude Bernard Lyon1, 8 av. Rockefeller, lyon 69373, France, raphael.terreux@univ-lyon1.fr*

Cryptosporidium parvum is an intracellular protozoan, which causes life-threatening diarrheas in immunodeficient patients. ATP-Binding Cassette protein (ABC) genes have been found in this parasite, suggesting that ABC proteins could be effective targets for chemotherapy. Our aim is to identify selective inhibitors of one of them, CpABC3. Like all ABC proteins, CpABC3 possesses two ATP binding sites: the Nucleotide Binding Domains (NBD) 1 and 2. We overexpressed and purified a recombinant N-terminal NBD1 (198 aa), in order to investigate possible interactions with different classes of compounds: natural compounds (mainly flavones, flavonols and isoflavonoids) and purine bases analogs (imidazopyridinones...). A model of the NDB1 of CpABC3 has been built by homology with other ABC proteins, and models of NBD1/Isoflavone complexes have been computed. Studies on the binding of selected molecules to NBD1 are under way.

394.

SYNTHESIS AND ANTIPLASMODIAL ACTIVITIES OF 4-AMINO-7-CHLOROQUINOLINES WITH TERMINAL N-SUBSTITUTIONS ON THE SIDE CHAIN. ***Huayin Liu***, *Frances M. Krogstad, Haiyan Deng, and Donald J. Krogstad, Department of Tropical Medicine and Center for Infectious Disease, Tulane University, 1430 Tulane Ave, SL-17, New Orleans, LA 70112*

Aminoquinolines (AQs) with diaminoalkane side chains shorter than the isopentyl side chain of chloroquine are active against both chloroquine-susceptible and -resistant Plasmodium falciparum in vitro, modification of the AQ ring (except for replacement of the chlorine at the 7-position with iodine or bromine) reduced or abolished antiplasmodial activity. Here, the synthesis and antiplasmodial activities of 4-amino-7-choroquinolines with terminal N-substitutions on the three carbon short side chain will be disscused. After either pilot synthesis or solid phase synthesis of these short side chain AQs, antiplasmodial activities in vitro of short side chain AQs with terminal aliphatic or aromatic N-substitutions were assayed. AQs with three carbon aminoalkane side chains with a terminal piperidine are active against both chloroquine-susceptible and chloroquine-resistant P. falciparum. Substitution on the terminal piperidine ring resulted in AQs which were likewise active against chloroquine-susceptible P. falciparum, and still active against chloroquine-resistant P. falciparum. More results after substitution on the terminal group such as aromatic and aliphatic substitution will be presented.



395.

AMINO ACID CONTENT OF PROPOLIS, WORKED OUT IN UZBEKISTAN. ***Burhon N. Khodjakulov***, *Pediatrics, Samarkand State Medical Institute, A.Temur str., 18, Tamhid str., 1-13, Samarkand 703024, Uzbekistan, Fax: 998-662 312199, m_nurali@hotmail.com*

Propolis is used in different fields of medicine, including in combustiology in treatment of patients with burns. Therapeutic efficacy of propolis depends on chemical content and quantity of its components. During several we used propolis in treatment of patients and studied its chemical content as well. Present work shows the results of amino acid analysis of propolis using the method of ion – exchange chromatography. As the result of qualitative and quantitative analysis we have established amino acid content of propolis: Isoleucine, Leucine, Cysteine, Valine, Glutamine, Tyrosine, Proline, Methionine, Tryptophan, Asparagine, Phenylalanine, Oxyproline, Serine, Threonine, Glycine, Alanine. Amino acid content depends greatly on the place of making honey. Propolis is characterized by high content of irreplaceable amino acids, which range from 16 to 25 % relatively of total quantity of amino acids. Correlation of amino acid content with clinical efficacy has been carried out and established that clinical efficacy of propolis has direct correlation with the content of irreplaceable amino acids.

396.

CHARACTERIZATION OF MEDICINAL PLANTS OF SOUTHWEST DESERT BY GC/MS ANALYSIS. ***Kaveh Zarrabi***[1], *Ezekiel Gebrekidane*[1], *Liliya Harizanova*[1], *Jacob Smigel*[1], *Heater Fels*[1], *Juliane Fietzke*[1], *and Patrick Leary*[2]. *(1) Department of Physical Sciences, Community College of Southern Nevada, 6375 West Charleston Blvd., Las Vegas, NV 89146-1164, Fax: 702-651-5028, kaveh_zarrabi@ccsn.edu, (2) Department of Biological Sciences, Community College of Southern Nevada*

Medicinal plants have been employed by Native Americans throughout the southwest of the United States for centuries to combat and cure various ailments. This project concentrates on the following seven commonly known plants found in the Nevada desert. Acaccia greggii (catclaw), Artemisia ludoviciana (sagebrush), Gutierrezia sarothae (snakeweed),Anemopsis californica (yerba mansa), Chilopsis linearis (desert willow), Krameria parvifolia (ratany), and Rhus trilobata (squaw bush)Dried leaves of each plant was dried followed by consecutive extractions with hexane, dichloromethane, and water/methanol. Each fraction was subjected to GC/MS analysis, and identification of each compound was confirmed by the use of second MS library or derivatization of each fraction.

This study is a part of an undergraduate research program to encourage retention and science carrier orientation for community college students.

397.

TWENTY HIGH-GRADE TRADITIONAL CHINESE MEDICINES: THEIR ANTI-HIV, ANTIBACTERIAL, AND ANTICANCER BIOLOGICAL SCREENING. ***Jin-Feng Hu***[1], *Kelli Kuhen*[2], *Doris Hafenbradl*[2], *Jun Li*[2], *Teresa Chen*[2], *Jennifer Harris*[2], *Nathanael Gray*[2], *and Peter G. Schultz*[1]. *(1) Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, (2) Genomics Institute of the Novartis Research Foundation*

The ethanol extracts of 20 high-grade Traditional Chinese Medicines were assayed for their biological activities of anti-HIV, antibacterial, and anticancer. Artemisia capilllaris (Yin-Chen-Hao), Lonicera japonica (Jin-Yin-Hua), Paeonia lactiflora (Bai-Shao) and Panax ginseng (Ren-Shen) showed anti-HIV activity at concentrations of 10 mM, 10 mM, 10 mM and 1 mM, respectively. Glycyrrhiza uralensis (Gan-Cao), Artemisia capillaries, Cassia tora (Jue-Ming-Zi), Lonicera japonica, Schizandra chinensis (Wu-Wei-Zi), Astragalus membranaceus (Huang-Qi), Coptis chinensis (Huang-Lian), Rehmannia glutinosa (Shou-Di-Huang), Zea mays (Yu-Mi-Xu), Lycium barbarum (Gou-Qi-Zi) and Aquilaria sinensis (Chen-Xiang) displayed antibacterial activity against Gram-positive and/or Gram-negative bacteria. Angelica sinensis (Dang-Gui) and Coptis chinensis exhibited 57% and 42% inhibition of Kallikrein 6 (an ovarian cancer model) at a concentration of 2 mM, respectively. 15 pure components were isolated from the EtOH extract of the very high-grade herb Glycyrrhiza uralensis. Their structures were determined by NMR and MS spectroscopic methods. Some of them were found to have unreported anti-HIV and antibacterial activities.

398.

SYNTHESIS OF SOME ALKENOL ANALOGS OF 1-OCTEN-3-OL FOR USE AS MOSQUITO ATTRACTANTS. ***C.O. Ikediobi***, *Department of Chemistry, Florida A&M University, C.O. Ikediobi, Department of Chemistry, Florida A&M University, room 219 Jones Hall, Tallahassee, FL 32307, Tallahassee, FL 32307, Fax: 850-561-2388, christopher.ikediobi@famu.edu*

Many species of mosquitoes have been implicated as vectors for many human and animal disease-causing viruses and parasites. One of the most efficient methods for the control of mosquitoes is the use of mosquito attractants, in contrast to mosquito repellants. 1-octen-3-ol, first isolated from cattle odor, has been used with success in the control of mosquitoes in Florida and other parts of the world. Unfortunately, many species of mosquitoes are not very sensitive to this alkenol, thus generating the need to discover other compounds that have broad applicability to a wide range of mosquito species. As part of an ongoing effort to make potent alkenol analogs of the lead compound, 1-octen-3-ol, we have synthesized 1-hexen-4-ol, 1-hepten-4-ol, 1-nonen-4-ol, 1-decen-4-ol, 1-undecen-4-ol, and 1-dodecen-4-ol for use as mosquito attractants under laboratory and field conditions. Synthesis of these compounds was carried out using the Grignard reaction, between organometallic Grignard reagents and appropriate aldehydes to give the desired alkenols in reasonable purity and yield. The biological activity tests using the mosquito laboratory olfactometer and the CDC light traps with natural mosquito populations are currently being conducted at the Center for Medical, Agricultural, and Veterinary Entomology, USDA/ARS, Gainesville, FL.

399.

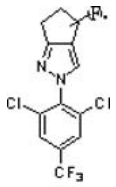
SYNTHESIS OF SOME CIS & TRANS-ALKENOL ANALOGS OF 1-OCTEN-3-OL FOR USE AS MOSQUITO ATTRACTANTS. ***Tryphon K. Mazu***[1], *Christopher O. Ikediobi*[1], *Lekan M. Latinwo*[2], *Lambert Ayuk-Takem*[1], *and James E. Cilek*[3]. *(1) Department of Chemistry, Florida A&M University, Jones Hall 219, tallahassee, FL 32307, Fax: 850 561 2388, tmazu2002fr@yahoo.fr, (2) Department of Biology, Florida A&M University, (3) Public Health Entomology Res. & Ed. Ctr, Florida A&M University*

Synthesis of some cis & trans-alkenol analogs of 1-octen-3-ol for use as mosquito attractantsMazu. K. Tryphona, Christopher O Ikediobia, Lambert Ayuk-Takema, Lekan M. Latinwob, and James Cilekc a Department of Chemistry, b Department of Biology, Florida A&M University,Tallahassee, c Public Health Entomology Research and Education Center, Florida A&M University, Panama City, Fl.Many species of mosquitoes have been implicated as vectors for many human and animal diseases caused by viruses and parasites. The use of mosquito attractants that attract mosquitoes to traps where they are killed is a modern approach to mosquito control and surveillance. In the last 10 years, 1-octen-3-ol has been used with some success in the control of mosquitoes throughout the world. Unfortunately, many mosquito species are not sufficiently responsive to this alkenol. The compelling need to discover other compounds, with broad applicability to a wide range of mosquito species led to an effort to make more potent alkenol analogs of 1-octen-3-ol. We have synthesized trans-3-penten-2-ol, trans-3-hexen-2-ol, trans-3-hepten-2-ol, trans-3-octen-2-ol, trans-3-nonen-2-ol, trans-3-decen-2-ol, trans-3-undecen-2-ol, trans-3-dodecen-2-ol, cis-5-octen-2-ol, cis-8-undecen-2-ol, and cis-9-dodecen-2-ol, using a variety of modern synthetic methods. Promising analogs will be further evaluated in field tests using standard CDC light traps with natural mosquito populations using carbon dioxide as a standard comparison.Supported by grant from USDA.

400.

SYNTHESIS AND INSECTICIDAL ACTIVITY OF FLUORINATED 2-(2,6-DICHLORO-4-TRIFLUOROMETHYL- PHENYL)-2,4,5,6-TETRAHYDROCYCLOPENTAPYRAZOLES. ***Sanath K. Meegalla***[1], *Dario Doller*[1], *Ruping Liu*[1], *Deyou Sha*[1], *YuKai Lee*[1], *Richard M. Soll*[1], *Nancy Wisnewski*[2], *Gary M. Silver*[2], *and Dale Dhanoa*[1]. *(1) Medicinal Chemistry, 3-Dimensional Pharmaceuticals Inc, 665 Stockton Drive, Exton, PA 19341, smeegalla@prdus.jnj.com, (2) Heska Coporation*

Synthesis and Insecticidal activities of fused fluorinated tetrahydro cyclopentapyrazoles (generic structure 1) will be presented.



(1)

401.

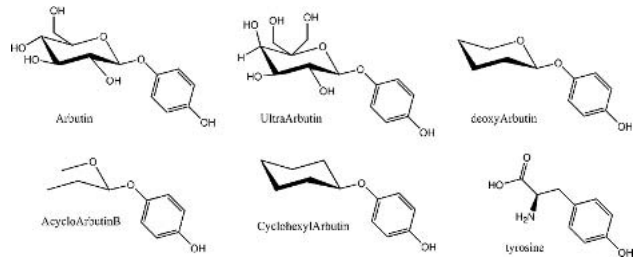
DO MARINE MOLLUSKS POSSESS APHRODISIACAL PROPERTIES? ***Raul A. Mirza***[1], *Jean-Joseph Poisson*[1], *George H. Fisher*[2], *Antimo D'Aniello*[3], *Patrizia Spinelli*[3], *and Gabrielle Ferrandino*[3]. *(1) School of Natural and Health Sciences, Barry University, 11300 NE 2nd Ave., Miami Shores, FL 33161, raulmirza@aol.com, (2) Department of Chemistry, Barry University, (3) Laboratory of Neurobiology, Stazione Zoologica, "Anton Dohrn'*

For centuries marine bivalves have been thought to possess aphrodisiacal properties. Recent evidence has demonstrated that D-aspartic acid (D-Asp) and N-methyl-D-aspartate (NMDA) are endogenously present in the endocrine tissues of rats and incur a natural role in the release of hormones that are involved in reproductive activity, e.g., luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone and testosterone. We sought to determine the concentrations of D-Asp and NMDA in the most consumed Mediterranean bivalve mollusks. These were homogenized in 70% methanol then purified by anion exchange and passed through a C18 Sep-Pak. D-Asp and NMDA were then determined by the use of two sensitive methods: (i) colorimetric methods based on the determination of oxaloacetate and hydrogen peroxide obtained from the oxidation of D-Asp and NMDA with D-aspartate oxidase (D-AspO) and (ii) the fluorometric high pressure liquid chromatography (HPLC) method in combination with D-AspO to determine D-Asp and NMDA individually. We have determined the presence of D-Asp and NMDA in the following bivalve mollusks: Mytilus galloprovincialis, Tapes decussates, Chamalea gallina and Donax trunculus. Since D-Asp and NMDA have been proven to act on the release of sexual hormones and since prior investigations have established a correlation of D-Asp and NMDA with testosterone, estradiol and sexual activity, we believe it plausible that the presence of D-Asp and NMDA in these mollusks could correlate with aphrodisiacal properties of these mollusks. Supported by NIH MIRT Grant TW00033, Barry University and Stazione Zoologica "Anton Dohrn".

402.

SYNTHESIS, SAR, IN VIVO, AND CLINICAL DATA OF THE DEOXYARBUTIN CLASS OF TYROSINASE INHIBITORS. ***Mitchell A. deLong**, Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221, mitch.delong@stanfordalumni.org, Raymond E Boissy, Department of Dermatology, University of Cincinnati College of Medicine, and Marty Visscher, Cincinnati Children's Hospital Medical Center*

The deoxyarbutins represent a new class of tyrosinase inhibitors. The lead molecule of this class, deoxyArbutin, (dA) demonstrates inhibition of mushroom tyrosinase in vitro with a Ki that is 10-fold lower then that of hydroquinone and 350-fold lower than arbutin. In a pigmented guinea pig model of human skin, dA initiates a rapid and sustained skin lightening. Remarkably, the change in pigmentation is completely reversible, with *f*xL values returning to baseline within eight weeks after halting dA's topical application. In contrast, hydroquinone induces a short but unsustained skin lightening effect while other agents such as kojic acid and arbutin fail to exhibit skin lightening. Safety tests support the establishment of deoxyarbutin as an actionable molecule. In a human clinical trial, topical treatment of deoxyarbutin for 12 weeks resulted in a significant reduction in overall skin pigmentation and improvement of solar lentigines in a population of freckled Caucasians. These data demonstrate that deoxyarbutins have significant tyrosinase inhibitory activity that can result in skin lightening and have the potential to be used to ameliorate hyperpigmentary lesions.



403.

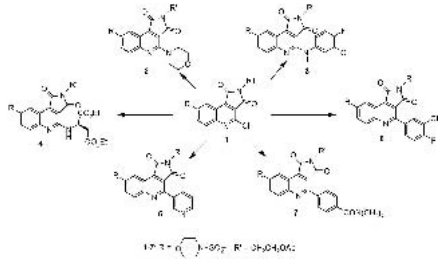
4-ARYL-4H-CHROMENES AS A NEW SERIES OF APOPTOSIS INDUCERS USING A CELL- AND CASPASE-BASED HIGH THROUGHPUT SCREENING ASSAY. STRUCTURE-ACTIVITY RELATIONSHIPS OF THE 7- AND 5-, 6-, 8-POSITIONS. ***William Kemnitzer**[1], Shailaja Kasibhatla[1], Songchun Jiang[1], Hong Zhang[1], Jianghong Zhao[1], Shaojuan Jia[1], Rabindra Rej[2], Real Denis[2], Serge Lamothe[2], Henriette Gourdeau[2], Ben Tseng[1], John Drewe[1], and Sui Xiong Cai[1]. (1) Maxim Pharmaceuticals, 6650 Nancy Ridge Dr, San Diego, CA 92121, bkemnitzer@maxim.com, (2) Shire Biochem Inc*

We recently reported the discovery of 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4*H*-chromene **(1a)** as a potent apoptosis inducer using a novel cell- and caspase-based HTS assay and the SAR of the 4-position with a dimethylamino group in the 7-position (Kemnitzer, W. *et al. J. Med. Chem.* **2004,** ASAP article). These chromenes were found to be active in the HUVEC tube formation assay, suggesting that this series of compounds may have antivascular activity. In addition, several compounds were also found to be highly active in several *in vivo* tumor models (Kasibhatla, S. *et al. Mol. Cancer Ther.* ASAP article and Gourdeau, H. *et al. Mol. Cancer Ther.* ASAP article). In this presentation we will describe the SAR of the 7-position and the 5-, 6-, and 8-positions of 4-aryl-4*H*-chromenes as inducers of apoptosis. We will report in detail the chemistry, *in vitro* and *in vivo* characterization of compounds with modifications at the 7-position, as well as at the 5-, 6-, and 8-positions.

404.

4-SUBSTITUTED 2-(2-ACETHYLOXYETHYL)-8-(MORPHOLINE-4-SULFONYL)-PYRROLO[3,4-C]QUINOLINE-1,3-DIONES AS POTENT CASPASE-3 INHIBITORS. *Dmitri Kravchenko[1], Y.A. Kuzovkova[1], Volodymyr Kysil[2], Sergey Tkachenko[2], Sergey Maliartchouk[2], Ilya Okun[2], and **Alexandre Ivachtchenko**[2]. (1) Department of Organic Chemistry, Chemical Diversity Research Institute, Rabochaya St. 2-a, Khimki 114401, Russia, Fax: 7-095-9269780, dk@chemdiv.com, (2) ChemDiv, Inc, 11558 Sorrento Valley Rd. Suite 5, San Diego, CA 92121*

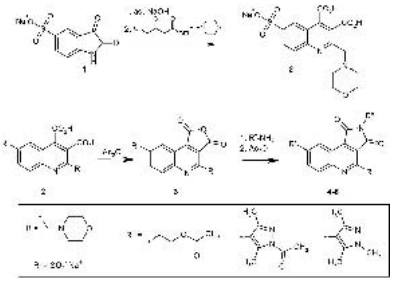
We describe synthesis, biological evaluation and structure-activity relationships for a series of novel 4-amino- (structures 2-4) and 4-aryl- (structures 5-7) substituted 2-(2-acetyloxyethyl)-8-(morpholine-4-sulfonyl)-pyrrolo[3,4-c]quinoline-1,3-diones. Key intermediate 1 was synthesized from isatin-5-sulfonate using a previously reported synthetic method based on Pfitzinger reaction. The obtained compounds 2-7 displayed high caspase-3 inhibitory activity in an in vitro assay (IC50 in a low-nanomolar range).



405.

FACILE SYNTHESIS OF 2-SUBSTITUTED 4-(MORPHOLIN-4-IUM-4-METHYL)-1,3-DIOXO-2,3-DIHYDRO-1H-PYRROLO[3,4-C]QUINOLIN-8-SULFONATES AS POTENT CASPASE-3 INHIBITORS. *Dmitri Kravchenko[1], Y.A. Kuzovkova[1], Volodymyr Kysil[2], Sergey Tkachenko[2], Sergey Maliartchouk[2], Ilya Okun[2], and **Alexandre Ivachtchenko**[2]. (1) Department of Organic Chemistry, Chemical Diversity Research Institute, Rabochaya St. 2-a, Khimki 114401, Russia, Fax: 7-095-9269780, dk@chemdiv.com, (2) ChemDiv, Inc, 11558 Sorrento Valley Rd., Suite 5, San Diego, CA 92121*

We present a convenient synthesis of new nonpeptide small molecule inhibitors of caspase-3 (compounds 4-6) based on 1,3-dihydro-pyrrolo[3,4-c]quinoline-1,3-dione molecular scaffold. The method features (a) Pfitzinger reaction of isatin-5-sulfonate 1 with ethyl chloroacetoacetate in the presence of morpholine under strong alkali conditions, and (b) formation of furan-2,5-dione intermediate 3, as key steps to assemble the target heterocyclic system. We demonstrate the usefulness and versatility of the developed approach for the synthesis of variously substituted 1H-pyrrolo[3,4-c]quinolines, and discuss the scope and limitations of the chemistry involved.



406.

DESIGN, SYNTHESIS AND BIOCHEMICAL EVALUATION OF CYSTEINE PROTEASE INHIBITORS: NOVEL COMPOUNDS FOR CHAGAS TREATMENT. ***Rogelio Siles**, Ming Zhou, Shen-En Chen, Kevin G. Pinney, and Mary L. Trawick, Department of Chemistry and Biochemistry, Center for Drug Discovery, Baylor University, one bear place, 97348, waco, TX 76798-7348, Rogelio_Siles@baylor.edu*

Leishmaniasis, trypanosomiasis and malaria are devastating parasitic diseases that are a major cause of death in developing countries due, in part, to the lack of inexpensive treatment agents. During the last decade, a group of proteolytic enzymes, called cysteine proteases, have been identified as involved in many important biological functions of these parasites including replication and metabolism. Cysteine proteases represent important molecular targets for the development of antiparasitic chemotherapy. Our research in this area is focused on the design, synthesis, and biochemical evaluation of new synthetic compounds that selectively target cruzain, the main cysteine protease of Trypanosoma cruzi, a causative agent of Chagas disease. More than 20 compounds containing alfa, and beta-tetralones, chroman-4-ones, thiochroman-4-ones, 3-bromophenylcyclopentanone and 3-bromopheny cyclohexanones were functionalized by introducing the thiosemicarbazone scaffold, a functional group that has an effective interaction with the catalytic triad of cruzain (Cys25, His159, and Asn175). In addition, we have synthesized a variety of novel compounds containing alfa,beta- unsaturated ketones and 1-oxyranylketones to

improve efficacy and selectivity in the interaction of these molecules with cruzain. Details of molecular design, synthesis, and preliminary biochemical evaluation will be reported and discussed.

407.

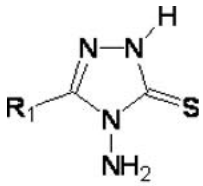
DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF SMALL-MOLECULE INHIBITORS OF XIAP. ***Jianyong Chen**, Nikolovska-Coleska Zaneta, Chao-Yie Yang, Guoping Wang, Qiu Su, Liang Xu, and Shaomeng Wang, Department of Internal Medicine, University of Michigan, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, Fax: 734-647-9647, jiachen@umich.edu*

The inhibitors of apoptosis proteins (IAPs) were recently discovered as an important class of intrinsic cellular inhibitors of apoptosis. XIAP (X-linked IAP) is the most potent inhibitor of apoptosis among all the IAP proteins and represents a promising new molecular target for anti-cancer drug design aiming at overcoming apopotosis-resistance of cancer cells. We wish to report the design, chemical synthesis, biochemical and biological evaluation of a novel class of small-molecule inhibitors of XIAP.

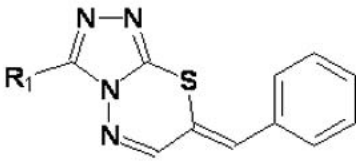
408.

3-R-7-(PHENYLMETHYLENE)-S-TRIAZOLO[3,4-B][1,3,4]-THIADIAZINES AS ANTICANCER AGENTS. ***Hasnain Malik**[1], Ned Heindel[1], Christophe Guillon[1], Lakeisha O'Keiffe[1], Peter DeMatteo[1], and Jeffrey Laskin[2]. (1) Department of Chemistry, Lehigh University, 6 East Packer Avenue, Bethlehem, PA 18015, hasnain.a.malik@Lehigh.EDU, (2) EOHSI, UMDNJ*

4-Amino-1,2,4-triazol-3-thiones (1), heterocyclic bioisosteres of N-aminoarginine, a known inhibitor of nitric oxide synthase (NOS), are i-NOS-inhibitors and anti-cancer agents. In expanding the structural diversity in this family we have uncovered a fascinating cyclization to a unique class of homologues (2) which display even more significant anti-tumor activity and a potentially useful fluorescence. 3-R-7-(phenylmethylene)-s-triazolo[3,4-b][1,3,4]-thiadiazines (2) result from the condensation of (1) with alpha-halocinnamaldehyde in yields >90%. Eight analogues of (2) were generated in yields >75%. Orteps and NMRs were obtained, and anticancer activity (IC50) in four tumor lines fell in the low microM range.



(1)



(2)

409.

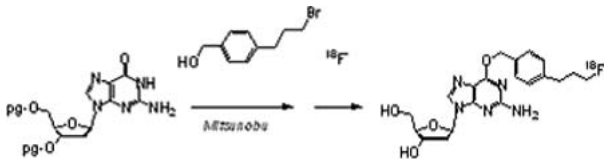
4-X-CATECHOLS-TYROSINASE INTERACTIONS: A QSAR STUDY. ***Rajeshwar P. Verma**, Ann Z. Tan, and Cynthia D. Selassie, Department of Chemistry, Pomona College, 645 North College Avenue, Claremont, CA 91711, Fax: 909-607-7726, rverma@pomona.edu*

It is well documented that simple phenols and catechols have dual mechanism of action at the molecular level that is anti-oxidative as well as carcinogenic. A recent study suggests that oxidases such as peroxidase and tyrosinase act as potential target sites for the enzyme-catalyzed autoxidation of simple phenols. Tyrosinase catalyzes the hydroxylation of monophenols to ortho-diphenols and the oxidation of ortho-diphenols to ortho-quinones via a 2-electron oxidation utilizing molecular oxygen. In this study, we examined the tyrosinase catalyzed oxidation of 4-X-catechols. QSAR models were then developed to understand the importance of substituent effects on binding and catalytic activity. Binding data indicates that these exists a bifurcation in mechanism in catechols depending on their electron densities.

410.

O^6-(4-(3-[^{18}F]FLUOROPROPYL)-BENZYL)-2′-DEOXYGUANOSINE ([^{18}F]FPBDG) – SYNTHESIS AND EVALUATION OF A POTENTIAL DNA REPAIR PROTEIN O^6-ALKYGUANINE-DNA ALKYLTRANSFERASE (AGT) IMAGING AGENT. *Ganesan Vaidyanathan, **Karel Base**, and Michael R. Zalutsky, Department of Radiology, Duke University Medical Center, Box 3808, Durham, NC 27710, ganesan.v@duke.edu, base0001@mc.duke.edu*

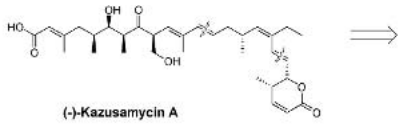
The human DNA repair protein AGT is the primary cause of drug resistance in alkylator chemotherapy. O^6-benzylguanine (BG) inactivates AGT by a process in which AGT transfers the benzyl moiety of BG to cysteine-145 in its active site. An analogue of BG containing a radiolabel on its benzyl moiety can potentially be used for the in vivo radiodetection of AGT. O^6-(4-(3-fluoropropyl)-benzyl)-2′-deoxyguanosine (FPBdG) and O^6-(4-(3-bromopropyl)-benzyl)-2′-deoxyguanosine (BPBdG) were synthesized by Mitsunobu coupling of 3′, 5′-diacetyl-2′-deoxyguanosine with the respective [4-(3-halopropyl)]-benzyl alcohol and subsequent deacetylation. FPBdG decreased the uptake of O^6-3-[^{131}I]iodobenzylguanine by DAOY cells with an IC_{50} of 16 μM (11 μM for BG). [^{18}F]FPBdG was synthesized from BPBdG in a single step in approximately 5% radiochemical yield and a preliminary study demonstrated its specific binding to AGT. Although further synthetic refinements are clearly needed, [^{18}F]FPBdG may be an useful agent for the in vivo mapping of AGT by positron emission tomography.



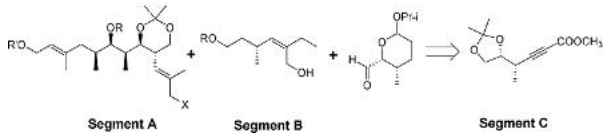
411.

EFFICIENT FORMAL TOTAL SYNTHESIS OF (–)-KAZUSAMYCIN A, A POTENTIAL ANTITUMOR AGENT. *Shengfeng Zhou[1], Huaxiang Chen[1], **Wensheng Liao**[1], Shuhui Chen[1], Ge Li[1], Ryoichi Ando[2], and Isao Kuwajima[3]. (1) WuXi Pharmatech Co., Ltd, No.1 Building, 288 FuTe ZhongLu, Shanghai 200131, China, Fax: 86-21-50461000, liao_wensheng@pharmatechs.com, (2) Mitsubishi Pharma Corporation, (3) The Kitasato Institute*

Kazusamycin A, a natural product isolated from the culture broth of an actinomycete strain, 81-484, has demonstrated potent antitumor activity as well as antimicrobial activity. It was also found that kazusamycin A exhibited profound inhibitory potency against Rev protein translation from the nucleus to the cytoplasm, suggesting its potential utility in HIV therapy. With the intention to synthesize enough quantities of kazusamycin A for additional biological testing, we devised several efficient and scalable routes for the preparation of three key building blocks, namely segments A, B, and C needed for the total synthesis of kazusamycin A.



(-)-Kazusamycin A



Segment A Segment B Segment C

412.

SAR STUDY OF CONJUGATED INDOLE-IMIDAZOLE DERIVATIVES DISPLAYING SUBSTANTIAL *IN VITRO* ANTIPROLIFERATIVE ACTIVITIES AGAINST CANCER CELL LINES. ***David James**, Hao Li, Shoujun Chen, Zhiqiang Xia, Weiwen Ying, Yaming Wu, Lijun Sun, and Keizo Koya, DEPARTMENT OF CHEMISTRY, Synta Pharmaceuticals Corp, 45 Hartwell Avenue, Lexington, MA 02421, Fax: 7812748228, djames@syntapharma.com*

The development of resistance to chemotherapy with existing anticancer drugs has challenged the pharmaceutical industry to rapidly identify and develop new chemical entities able to counteract this unmet medical needs. We report herein the detailed SAR studies of a series of indole-imidazole compounds that demonstrate substantial in vitro anti-proliferative activities against cancer cell

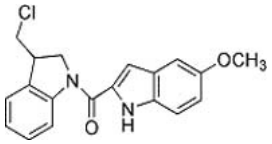
lines, including multi-drug resistance (MDR) phenotypes. The in vitro cytotoxic effects have been demonstrated across a wide array of tumor types, including hematologic and solid tumor cell lines of various origins (e.g. leukemia, breast, colon, uterine).

413.

DESIGN AND SYNTHESIS OF BIO-OXIDATIVELY ACTIVATED PRODRUGS BASED UPON THE DUOCARMYCINS: ROUTES TO BOC-CI AND CI-MI PRODRUGS.

Natalia Ortuzar Kerr[1], *Sukhwant S. Grewel*[1], *Blanca Garcia-Ochoa Martin*[1], *Kersti Karu*[1], *Laurence H. Patterson*[1], *and Mark Searcey*[2]. *(1) Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London, WC1N 1AX, England, Fax: 0044-207-753-5964, (2) Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London*

The duocarmycins are potent antitumour antibiotics that have failed to date to demonstrate clinical utility. We have identified the family of cytochrome P450 enzymes as being present in tumours and able to give an intratumoral mode of activation for prodrug analogues of the natural products. Here we describe the synthesis and activation of des-hydroxy-CI-MI (1), the simplest, potent member of this class. Compound 1 was synthesised by two complementary routes. Initial investigations of the 5-exo-trig-free radical cyclization with catalytic tributyltin hydride, using PMHS as a hydride source gave a good yield. Multigram quantities of the prodrug were obtained using an alternative route involving simple alkylation of 2-fluoronitrobenzene with dimethyl malonate, reduction, mesylation, ring closure and chlorination. This efficient synthesis allows the application of an enzyme-based resolution of des-hydroxy-Boc-CI. Deprotection and coupling to 5-methoxyindole-2-carboxylic acid gave the extended DNA binding agent in good yield. Initial biological studies will also be presented.



414.

PRODRUG ANALOGS OF THE DUOCARMYCINS ACTIVATED BY CYP-OXIDATION.

Mark Searcey[1], *Kersti Karu*[2], *Sukhwant S. Grewal*[2], *Natalia Ortuzar Kerr*[2], *William Griffith*[2], *and Laurence H. Patterson*[2]. *(1) Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom, Fax: 0044-207-753-5964, mark.searcey@ulsop.ac.uk, (2) Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London*

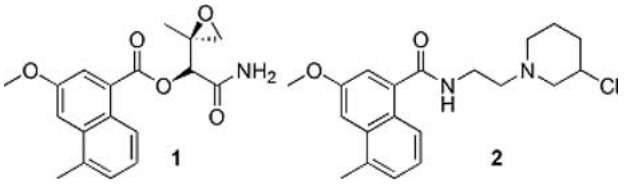
Proteomics represents a tool for the analysis of the protein profile of tumor tissues and has been suggested as a mechanism for the identification of drug targets. In our studies, we have focussed on differences in tumor expression of the cytochrome P450 enzymes and suggested that the presence of CYPs in tumors represents a potential for the biooxidative-activation of prodrugs. The duocarmycins were selected as candidate ultrapotent cytotoxins. Herein, we present the first definitive account of designed, bio-oxidatively-activated prodrugs based upon this class of natural product. Des-OH-CI is an analog of CI lacking the hydroxyl function. It was incubated with human liver microsomes and microsomes derived from hepatic metastases of a human colon carcinoma. The resulting metabolites were analysed by mass spectrometry. Extensive studies of metabolism and CYP-expression profiles revealed the involvement of the CYP1A family in activation of this prodrug. Synthetic and metabolism studies will be presented.

415.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF POTENTIAL ANTICANCER AGENTS BASED ON THE AZINOMYCINS.

Maxwell A. Casely-Hayford[1], *Klaus Pors*[1], *Laurence H. Patterson*[1], *and Mark Searcey*[2]. *(1) Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom, Fax: 0044-207-753-5964, maxwell.casely-hayford@ams1.ulsop.ac.uk, (2) Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London*

Azinomycins A and B are extremely potent antitumour antibiotics that derive their activity from the alkylation of duplex DNA at GXN. At the time of isolation, compound 1, which constitutes the left hand portion of the parent azinomycins, was also discovered and was later shown to possess significant cytotoxicity. Due to our continuing interest in potential bioreductive drugs, and in the design and synthesis of DNA-binding antitumour agents, we synthesised compound 2, an analogue of 1 that contains a piperidine mustard. The alkylating group-chromophore distance is similar to that found in the natural product and is connected to the chromophore by a more robust amide bond. Synthesis of the target molecule and a non-alkylating analogue was successfully achieved and the resulting compound shown to bind to and alkylate DNA in a similar fashion to the natural product. Analogues of 2 with differing mustard functions are also under development.



416.

DESIGN, SYNTHESIS AND CYTOTOXICITY OF DISCODERMOLIDE ANALOGUES.

Mark A. Burlingame[1], *Simon J. Shaw*[1], *Kurt F. Sundermann*[1], *Dan Zhang*[1], *Joseph Petryka*[1], *Esteban Mendoza*[1], *Fenghua Liu*[1], *David C. Myles*[1], *Matthew J LaMarche*[2], *Tomoyasu Hirose*[2], *B. Scott Freeze*[2], *and Amos B. Smith III*[2]. *(1) Department of Chemistry, Kosan Biosciences Inc, 3832 Bay Center Place, Hayward, CA 94545, Fax: 510 732-8401, burlingame@kosan.com, (2) Department of Chemistry, Monell Chemical Senses Center, and Laboratory for Research on the Structure of Matter, University of Pennsylvania*

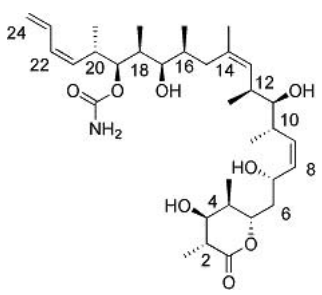
Discodermolide, a marine polyketide natural product, has potent growth inhibitory activity against human tumor cell lines. The mode of action, similar to that of paclitaxel, involves the perturbation of microtubule assembly, leading to mitotic arrest, and ultimately cell death. The synthesis of discodermolide analogues for evaluation in a drug discovery program represents a significant challenge to the medicinal chemist. The design and and synthesis of a number of significantly modified discodermolide analogues including replacement of the "C lactone" with aryl and alkyl groups and the activity of these compounds against the MCF-7, NCI/ADR, A549, and SKOV3 human tumor cell lines will be presented.

417.

SAR AND STRUCTURAL MINIMIZATION OF THE C1-C8 REGION OF (+)-DISCODERMOLIDE.

Kurt F. Sundermann[1], *Simon J. Shaw*[1], *Mark A. Burlingame*[1], *David C. Myles*[1], *B. Scott Freeze*[2], *Ming Xian*[2], *Ignacio Brouard*[2], *and Amos B. Smith III*[2]. *(1) Department of Chemistry, Kosan Biosciences Inc, 3832 Bay Center Place, Hayward, CA 94545, sundermann@kosan.com, (2) Department of Chemistry, Monell Chemical Senses Center, and Laboratory for Research on the Structure of Matter, University of Pennsylvania*

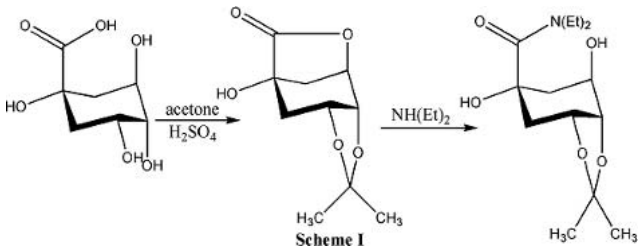
(+)-Discodermolide is a microtubule-stabilizing marine polyketide. It has undergone an early-stage human clinical trial using synthetic material and is a promising anti-tumor lead compound. Novel C(1)-C(8) analogs of (+)-discodermolide were synthesized in order to help elucidate a pharmacophore and to minimize structural complexity. A variety of synthetic strategies were exploited. Initial cellular in vitro evaluation indicated that minor modifications and simplification of the C(1)-C(8) region can substantially enhance anti-proliferative potency.



418.

FURTHER STUDIES OF ADVERSE EVENTS RELATING ECHINACEA ANGUSTIFOLIA AND CANCER CHEMOTHERAPY. ***Eric D Huntimer*** *and Fathi T. Halaweish, Chemistry & Biochemistry, South Dakota State University, Shepard hall 121, Brookings, SD 57007, Eric.Huntimer@SDSTATE.EDU*

Previous studies in our group demonstrated Echinacea angustifolia preparations could interfere with hyaluronidase-supplemented chemotherapy and doxorubicin chemotherapy. Individual compounds and fractions were reported. Caffeoyl esters represent a diverse group of phenolics. The number of different phenolic compounds makes the isolation of one specific compound extremely tedious. To accommodate a structure-activity relationship, synthesizing specific compounds seems reasonable. Chicoric acid was synthesized through a known method. The compounds were synthesized by coupling caffeoyl substituents to protected acid backbones and deprotecting the compound. Quinic acid was protected (Scheme I) and the coupling and deprotection reactions are being conducted. A structure-activity relationship would help explain the different interfering effects of the compounds. This will be established using cell culture assays and GAMESS. Selected compounds are 1,3-dicaffeoylquinic acid (cynarine), 1,5-dicaffeoylquinic acid, and chicoric acid. The compounds are being tested for energy and potential energy surfaces to determine which structural features are important.



Scheme I

419.

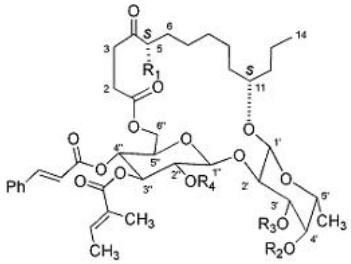
INHIBITION OF UROKINASE BY SUBSTITUTED CHLOROISOCOUMARINS: POTENTIAL THERAPEUTICS FOR BREAST CANCER METASTASIS. ***Justin J. Heynekamp***[1]*, Thomas A. Vander Jagt*[2]*, Lucy A. Hunsaker*[2]*, Lorraine M. Deck*[1]*, and David L. Vander Jagt*[2]*. (1) Department of Chemistry, University of New Mexico, Chemistry Department, MSC03 2060, Albuquerque, NM 87131, juster@unm.edu, (2) Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine*

A series of thirty two 4- and 7- substituted chloroisocoumarins was synthesized as potential inhibitors of urokinase–type plasminogen activator (uPA). uPA is implicated in cancer metastasis. Two series of uPA inhibitors were designed, one with an isothiourea and the other with a bromine tethered to the chloroisocoumarin. The structural design of the inhibitors was checked with the aid of molecular modeling. The compounds functioned as simple competitive inhibitors of substrate binding rather than as suicide substrates or active site inactivators, with dissociation constants as low as 20 nM. A select group of inhibitors was synthesized that are uncharged compounds. The significance of these compounds is their potentially improved bioavailability compared to numerous compounds developed by pharmaceutical companies that are charged compounds with poor bioavailability.

420.

IPOMOEASSINS A-E, FIVE NEW CYTOTOXIC MACROCYCLIC GLYCORESINS, FROM THE LEAVES OF IPOMOEA SQUAMOSA FROM THE SURINAME RAINFOREST. ***Shugeng Cao***[1]*, Rebecca C. Guza*[1]*, Jan H. Wisse*[2]*, Randy Evans*[3]*, James S. Miller*[3]*, and David G. I. Kingston*[1]*. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, 3111 Hahn Hall, Blacksburg, VA 24061, Fax: 540-231-3255, scao@vt.edu, (2) Bedrijf Geneesmiddelen Voorziening Suriname, (3) Missouri Botanical Garden*

In a continuing search to discover bioactive compounds from the Suriname and Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, extracts of the leaves of Ipomoea squamosa were found to be cytotoxic. Bioassay-directed fractionation led to the isolation of ipomoeassins A-E (1-5), identified as new glycoresins. The structures were elucidated by spectroscopic analyses and chemical transformations, and the absolute configurations of C-5 and C-11 were determined by analysis of the NMR spectra of the (R) and (S)-MPA Mosher esters. All the isolates were active in the A2780 human ovarian cancer cell line assay, and ipomoeassin E (5) showed significant activity with an IC50 value of 35 nM.

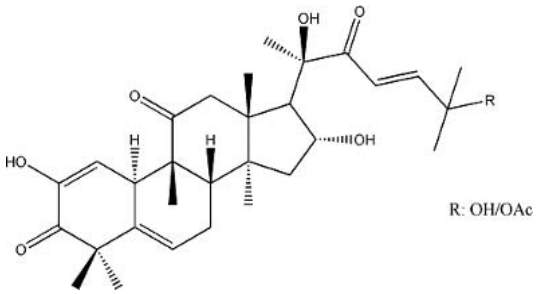


1 $R_1 = R_3 = R_4 = H$; $R_2 = Ac$;
2 $R_1 = R_2 = R_3 = R_4 = H$
3 $R_1 = OH$; $R_2 = Ac$; $R_3 = R_4 = H$
4 $R_1 = OAc$; $R_2 = Ac$; $R_3 = R_4 = H$
5 $R_1 = OAc$; $R_2 = R_3 = R_4 = H$

421.

NEW CUCURBITACIN DERIVATIVES: POTENTIAL ANTICANCER CANDIDATES. ***Andrew J. Young*** *and Fath T. Halaweish, Department of Chemistry and Biochemistry, South Dakota State University, Shepard Hall 121, Box 2202, Brookings, SD 57007, Fax: 605-688-6364, andrew.young@sdstate.edu*

Cucurbitacins are tetracyclic triterpenoid compounds which are predominantly found in the Cucurbitaceae family. A class of cucurbitacin aglycones were identified as potential anticancer compounds. Recently, it has been shown that some rare cucurbitacins have increased selectivity and differential cytotoxicity when compared to the commonly studied and available cucurbitacins. Cucurbitacins possess a number of carbonyl groups which can be reduced to alcohols. Several cucurbitacin derivatives were prepared using the selective reagents sodium borahydride and 9-borabicyclo[3,3,1]nonane. The products were analysed using HPLC, and identified using NMR and MS. Selective cytotoxicity towards cancer cell lines (prostate, cervical, and leukaemia) will be conducted. QSAR study will be assisted.



R: OH/OAc

422.

NITROIMIDAZOLE DERIVATIVES AS A NEW CLASS OF ANTICANCER COMPOUNDS. ***Iwona Weidlich***[1]*, D. Nevozhay*[2]*, A. Opolski*[2]*, and S. Sobiak*[1]*. (1) Department of Chemical Technology of Drugs/Faculty of Pharmacy, University of Medical Sciences, Grunwaldzka 6, 60-780 Poznan, Poland, iweidlic@amp.edu.pl, (2) Department of Experimental Oncology, Institute of Immunology and Experimental Therapy, Wroclaw, Poland*

The nitroimidazole derivatives can be used as new anticancer compounds. In our scientific program we synthesized some N-substituted derivatives of 5-bromo-4-nitroimidazoles which showed promising inhibition of the growth of cancer cell lines A 549 and P 388.Alkylation of 4(5)-bromo-2-methyl-5(4)-nitroimidazole gives a mixture of regioisomers. The N-substituted-4-nitroimidazole derivatives were filtered and purified by column chromatography. We used a variety of alkylating reagents: halogenoketones, halogenoacids and halogenoesters. The imidazolylcarboxylic acids were obtained by hydrolysis of appropriate esters.Synthesized nitroimidazole derivatives gave a new class, which characterized better anticancer activity than 1(4-chlorophenacyl)-5-bromo-4-nitroimidazole (IC50 HeLaV185=1.95 uM) (1).1.S.Sobiak, "Synthesis of some 5-amino-2-methyl-4-nitro-1-phenacylimidazoles", Polish J.Chem.,72, 78-83(1998).Scientific research was supported by Center of Committee of Scientific Investigations (Poland)-project # 3P05D03523.

423.

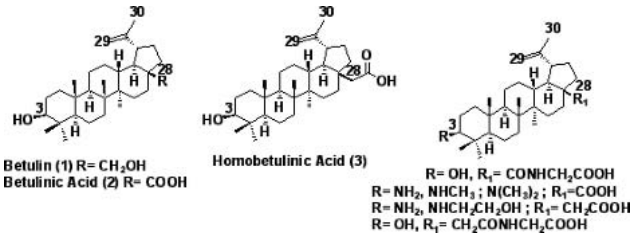
NOVEL SYNTHESIS AND ANTICANCER ACTIVITY OF GEMCITABINE-CARDIOLIPIN CONJUGATE. ***Abdul R. Khan**, Shoukath M. Ali, Moghis U. Ahmad, Paul Chen, Saifuddin Sheikh, and Imran Ahmad, Research and Development Facility, NeoPharm Inc, 1850 Lake side Drive, Waukegan, IL 60085, Fax: 847-887-9281, imran@neophrm.com*

Gemcitabine (dFdC) is a difluorinated analogue of deoxycytidine which is currently marketed as Gemzar® for the treatment of non-small cell lung and pancreatic cancer. The drug acts by inhibiting riboneucleotide reductase and DNA synthesis via conversion to its active forms, the diphosphate and triphosphate (dFdCDP, dFdCTP). Gemzar® has a narrow therapeutic index due to rapid deamination to its inactive uracil derivative (dFDU) and also due to toxicity, oral bioavailability, short-half life and extracellular drug metabolism etc. To overcome these problems, we have synthesized a novel ether analog of cardiolipin conjugated with gemcitabine. This compound showed promising anticancer activity and reduced toxicity compared to Gemzar®. Synthesis and biological data of this novel conjugate will be presented.

424.

NOVEL SYNTHETIC ANALOGS OF BETULINIC ACID AND THEIR BIOLOGICAL ACTIVITY. ***Pranab K Gupta** and Bashir Kaskar, Detroit Research Laboratories, Ash Stevens Inc, 5861 John C. Lodge Freeway, Detroit, MI 48202, Fax: 313-872-6841, guptapk@ashstevens.com*

Betulinic acid (2) has been demonstrated to possess a remarkably selective antitumor activity against human melanoma, as well as anti-HIV activity in H9 lymphocytic cells. Betulinic acid (2) can be obtained from active betulin (1) with ease, however the solubility of betulinic acid is a major challenge to overcome. It had been already demonstrated that betulin can be structurally modified in three places to improve its pharmacological profile, the C-3 hydroxy group on the A ring, the C-28 hydroxy group and the isopropylidene moiety. Betulinic acid (2) has an extremely hindered neopentyl-type carboxylic acid in the 28-position.The poor solubility of betulinic acid (2) is attributed to the hindered carboxylic acid group in the 28- position.Synthesis of homobetulinic acid (3) was undertaken thinking an extra methylene group in the 28-position should move the carboxylic acid from the hindered ring, make it more accessible towards making some salt and therefore impart better solubility over parent compound betulinic acid (2). Total synthesis of homobetulinic acid (3), synthesis of several modified analogs of homobetulinic acid and the biological activity of compound (3) will be the subject of our presentation.



425.

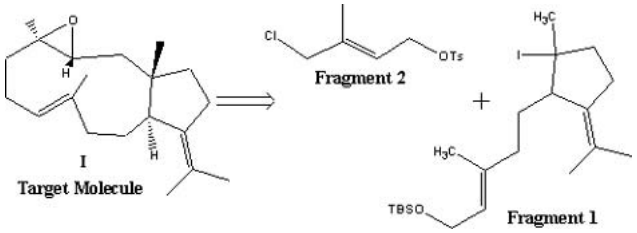
POLY(SEBACIC ACID-CO-RICINOLEIC ACID) BIODEGRADABLE CARRIER FOR ANTI -TUMOR DRUGS. ***Ariella Shikanov**, Department of Medicinal Chemistry, School of Pharmacy, Hebrew University in Jerusalem, 91120 Jerusalem, Israel, Fax: 972-2-6757629, shikaa@md.huji.ac.il, and Abraham J. Domb, Department of Medicinal Chemistry, School of Pharmacy-Faculty of Medicine, Hebrew University of Jerusalem*

The objective of this in vivo study was to develop an injectable polymeric formulation containing paclitaxel or cis-platin for intratumoral injection in C3H mice bearing subcutaneous bladder tumor. Liquid biodegradable polyesteranhydrides made of ricinoleic acid and sebacic acid that solidify in tissue after injection were used. Paclitaxel (5% and 10%w/w) or cisplatin (1%) were incorporated in the polymer matrix. C3H mice bearing bladder tumors grown subcutaneously were intratumorally injected with the formulation and changes in tumor progression were assessed by measuring tumor volume. Twenty-one days post drug administration, the tumor volume increased from 0.4 to 11cm3 for the control group treated with polymer without the drug. However, mice treated with a single dose of 150mg formulation of the polymer containing 10% paclitaxel the tumor volume didn't change during the first 12 days after treatment and than reached 3 cm3, twenty-one days after the treatment. When treated with 50mg of polymer containing 1% cis-platin up to 17th day the tumor volume was less then 1cm3 and after 21 day reached 3cm3. The toxicity of the polymers and formulations with paclitaxel was examined by subcutaneous delivery of the polymer to mice for different time periods. Histopathological examination of the tissue surrounding the implant showed minor inflammation one week after the injection and no inflammation three weeks following implantation. Implantation of the polymer without paclitaxel showed no adverse effects.

426.

PROGRESS TOWARDS TOTAL SYNTHESIS OF DOLLABELLANE MARINE DITERPENOID I. ***Pareshkumar K. Patel** and Branco S. Jursic, Department of Chemistry, University of New Orleans, 102 chemical science bldg, 2000 lakeshore dr, New Orleans, LA 70148, Fax: 5042806860, pkpatel@uno.edu*

Dolabellane diterpenoids, characterized by ordinary trans-bicyclo[9.3.0] tetradecane, are obtained primarily from marine sources and in many cases, exhibit antimicrobial, antitumor and antiviral activities. Dolabellane diterpenoid I was isolated from the Okinawan soft coral of the genus Clavularia. Its structure was determined based on spectroscopic analysis, chemical conversion and X-ray crystallographic analysis. This diterpenoid showed cytotoxic activity against tumor cells. Our current efforts toward synthesis of fragment 1 will be presented along with plans for completion of the synthesis of target molecule I.



427.

SAR STUDY OF NEW CORE-MODIFIED PORPHYRINS AS PHOTOSENSITIZERS FOR PHOTODYNAMIC CANCER THERAPY. ***Youngjae You**[1], Scott L Gibson[2], Russell Hilf[2], and Michael R. Detty[1]. (1) Department of Chemistry, The State University of New York, Buffalo, 630 Natural Sciences Complex, Buffalo, NY 14260, Fax: 716-645-6963, yjyou@nsm.buffalo.edu, (2) Department of Biochemistry and Biophysics, University of Rochester School of Medicine and Dentistry*

To observe the SAR relationships of 21,23-dithiaporphyrins with physicochemical and biological properties as photosensitizers, derivatives of 5,20-diphenyl-10,15-bis(4-carboxylatomethoxy)phenyl-21,23-dithiaporphyrin were prepared and their physicochemical and biological properties were evaluated. The structural change was focused on both the steric bulkness of 5- and 20-meso positions and the symmetry of the molecules. Minor impacts were observed in physicochemical properties such as the UV-VIS-near-IR absorption spectra, quantum yields for the generation of singlet oxygen and fluorescence. Some effects were shown in the values of the octanol/water partition coefficient. On the other hand, the modification at the meso positions resulted in significant differences in photosensitizing efficacy towards R3230AC cells: The smaller and unsymmetric porphyrins were better than the others. Of the synthesized compounds, 5-phenyl-20-(2-thienyl)-10,15-bis(4-carboxylatomethoxy-phenyl)-21,23-dithiaporphyrin showed the best phototoxicity: 68% cell kill at 100 nM and irradiation with 5 J/cm2 of 350-750-nm light.

428.

STRUCTURE AND RADIOPROTECTIVE FUNCTIONS OF LOW MOLECULAR WEIGHT SULFATED GALACTOSAN. ***Yi Li**, Navy 401 Hospital, 6 Minjiang Road, Qingdao 266071, China, wenjunm@ouc.edu.cn*

A wide attention has been taken because of the special structure and characterization of sulfated polysaccharides. In the paper, the low molecular weight sulfated galactosan from seaweed polysaccharides were obtained by the hydrolysis with acids, and were purified by ultra filter and chromatography. The low molecular weight sulfated galactosan contained alternate β-D-galactose and its derivation by α-1, 3 and β-1, 4 linkages. The animal experiments showed that the low molecular weight sulfated galactosan have radioprotective effect on

irradiated mice. It could significantly promote recovery of peripheral white blood cell, markedly increased thymus and spleen index, NK cytostatic activity and peritoneal macrophage phagocytes. Moreover, the low molecular weight sulfated galactosan significantly enhanced activities of the GSH-Px and SOD in blood, and decreased the activities of GPT and GOT in blood and the MDA level in liver. This research was funded by a grant (031070120) from Science and Technology Development Program of Shandong Province (China).

429.

STRUCTURE BASED DESIGN, SYNTHESIS AND EVALUATION OF BORONIC ACID BIOISOSTERES OF COMBRETASTATIN A-4. ***Yali Kong***[1]*, Jolanta Grembecka*[2]*, Michael C. Edler*[3]*, Ernest Hamel*[4]*, Susan L. Mooberry*[5]*, Michal Sabat*[1]*, and* ***Milton L. Brown***[1]*. (1) Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, VA 22904-4319, Fax: 434-924-0798, yk4n@virginia.edu, mlb2v@virginia.edu, (2) Molecular Physiology & Biological Physics, University of Virginia, (3) Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick Cancer Research and Development Center, (4) Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick Cancer Research and Development Center, (5) Department of Physiology and Medicine, Southwest Foundation for Biomedical Research*

Compounds that interact with β-tubulin represent important classes of clinically useful anticancer agents, and several new compounds are in late stage clinical trials. In this study, we designed aryl-boronic analogues of Combretastatin A-4. Preliminary docking studies of the x-ray structures of our aryl-boronic analogues **6** and **7** onto β-tubulin suggested that these compounds were inhibitors of the colchicine binding site. Evaluation of the compounds for inhibition of ^{3}H-colchicine revealed several analogues displaced the radioligand from the colchicine binding site, with compound **6** being the most potent. Compound **6** demonstrated 78 % inhibition at 5μμ in 1:1 ratio with ^{3}H-colchicine. Compound **6** also potently inhibited tubulin polymerization with an IC_{50} of 1.3μμ. Evaluation of compound **6** against human breast cancer cells (MCF7) revealed our boronic acid analogue to be extremely effective against cell proliferation with an IC_{50} of 11 nM. Altogether our results demonstrate that novel boronic acid analogues of Combretastatin A-4 are potent inhibitors of tubulin polymerization and human cancer cell proliferation and suggest that these compounds should be further studied for their potential use as new anticancer agents.

430.

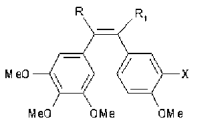
SYNTHESIS AND CYTOTOXICITY STUDIES OF EPOXIDE AND PYRAZOLE ANALOGS OF COMBRETASTATINS. *Toni Brown*[1]*, Regan LeBlanc*[1]*, John Dickson*[1]*, Herman Holt Jr.*[2]*, and* ***Moses Lee***[1]*. (1) Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, Fax: 864-294-3559, toni.brown@furman.edu, Moses.Lee@furman.edu, (2) Chemistry, University of North Carolina at Asheville*

A series of epoxide and corresponding pyrazole derivatives, of the structurally related chalcones and combretastatins, were synthesised and tested for in vitro cytotoxicity. Synthesis of these molecules was achieved by forming the epoxide of the relevant chalcone compound, prepared from crossed Aldol condensation of substituted acetophenones and benzaldehydes. The epoxide was then reacted with hydrazine to produce the desired pyrazole compounds in excellent yields. X-ray diffraction studies of the starting chalcone and epoxide, and subsequent pyrazole, were conducted for an example molecule and confirm strcutures of these compounds. In vitro cytotoxicity of each class of compounds was obtained using a 72 h continuous exposure MTT assay against two murine cancer cell lines; B16 and L1210. The effect of substitution in the A-ring is addressed: three methoxy groups versus two, generally increased cytotoxicity accross both cell lines. In the majority of cases, the pyrazoles are generally more active than the epoxides, with the most active, 5-(3′′-amino-4′′-methoxyphenyl)-3-(3′,4′,5′-trimethoxyphenyl)pyrazole, possessing an IC50 value of 2.4 and 5 microM (L1210 and B16, respectively). The development of these compounds provides useful information for drug design in the quest for novel, potent cytotoxic agents.

431.

SYNTHETIC MONO AND DIFLUORO COMBRETASTATIN AS POWERFUL ANTITUMORAL AGENTS. ***Mauro Marzi****, Giuseppe Giannini, marcella marcellini, Domenico Alloatti, teresa riccioni, massimo castorina, and claudio pisano, Department of Chemistry, Sigma-Tau s.p.a, ss pontina km 30,400, 00040 Pomezia (Roma), Italy, Fax: 0691393638, mauro.marzi@sigma-tau.it*

Combretastatins, originally derived from the Combretum caffrum tree, are known as powerful reversible inhibitors of tubulin polymerization. They find application as antitumor and antivascular agents. Currently, the prodrugs of Combretastatin A4 and its amino analogue AVE-8062, are in clinical trials. The substitution of one or both hydrogens present on the double bond of a combretastatin nucleus leads to a reduction of their biological activity (citotoxicity and/or tubulin binding). Recently, we synthesized a new class of combretastatins where one or both of these hydrogens were substituted with fluorine. Through total synthesis properly brominated fluorine-styrenes were prepared and subsequently condensed, via Suzuki reaction, with different boronic acids to obtain mono and difluoro combretastatin derivatives. These modifications did not determine a loss of activity, both in terms of potency and specificity, with regard to the molecular target. In fact, all synthesized compounds inhibited endothelial and tumoral cell proliferation as much as the reference compounds.



X= OH
R and R_1 = F (**ST2303**)
R = F, R_1 = H (**ST2966**)
R = H, R_1 = F (**ST3036**)

X= NO_2
R and R_1 = F (**ST2998**)
R = F, R_1 = H (**ST2969**)
R = H, R_1 = F (**ST3038**)

X= NH_2
R and R_1 = F (**ST2996**)
R = F, R_1 = H (**ST2971**)
R = H, R_1 = F (**ST3040**)

432.

SYNTHESIS, BIOLOGICAL EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF (–)-DICTYOSTATIN-1 AND ANALOGUES. ***Charitha Madiraju***[1]*, Brianne S. Raccor*[1]*, Youseung Shin*[2]*, Raghavan Balachandran*[1]*, Michael C. Edler*[3]*, Ernest Hamel*[3]*, Kenneth A. Giuliano*[4]*, Andreas Vogt*[4]*, Dennis P. Curran*[2]*, and Billy W. Day*[1]*. (1) Department of Pharmaceutical Sciences, University of Pittsburgh, 3501 Terrace Street, Pittsburgh, PA 15261, Fax: 412-624-1850, chmst41@pitt.edu, (2) Department of Chemistry, University of Pittsburgh, (3) Screening Technologies Branch, National Cancer Institute, (4) Department of Pharmacology, University of Pittsburgh*

(–)-Dictyostatin-1 is a 22-member macrolactone with microtubule (MT) stabilizing, mitotic block- and apoptosis-inducing activities whose full synthesis has been recently reported. A library of dictyostatins was synthesized and evaluated for MT and antiproliferative activities. Two analogues had remarkable antiproliferative activity against ovarian 1A9 and beta-tubulin mutated, paclitaxel-resistant cell lines with potencies comparable to that of the parent molecule. As seen with the parent compound, these analogues induced tubulin polymer formation at low temperatures. Radioligand displacement assays showed these two analogues to possess about 60% of the ability of the parent compound to inhibit [3H]paclitaxel from binding to tubulin polymer. The concentrations causing 50% tubulin polymerization were higher for the analogues as compared to that of the parent molecule. The SAR determined include: the macrolactone is important, but not a full requisite, for MT stabilization; the configuration of the hydroxyl at C19 has an important role; the configuration of the C6 and C14 methyls are important; anti positioning of the C6 and C7 methyls seems mandatory for MT stabilization; the natural E:Z geometry of the diene seems crucial; and, the C16 methyl is important for paclitaxel site binding but is not a factor in antiproliferative activity.

433.

STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF (–)-EPICATECHIN ANALOGUES AS DNA METHYLTRANSFERASE INHIBITORS. ***Yeng-Jeng Shaw****, Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, 500 West 12th Ave, Columbus, OH 43210, shaw.299@osu.edu, and Ching-Shih Chen, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University*

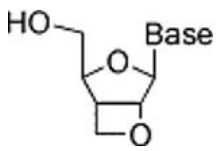
Hypermethylation of DNA CpG islands is an important epigenetic mechanism for aberrant gene silencing in cancer. Although nucleoside analogue inhibitors of

DNA methytransferases (DNMTs) can reverse abnormal DNA hypermethylation in cancer cells, their clinical utility is limited due to side effects. The development of nonnucleoside DNMTs that lack systemic toxicity is a promising approach to targeting epigenetic mechanisms for cancer therapy. (–)-Epicatechin analogues extracted from green tea inhibit DNMT. Among them, EGCG and ECG exhibit inhibitory activity with IC_{50} values of 20-30 μM. Our objective is to synthesize (–)-epicatechin derivatives that possess greater inhibitory potency and, thus, have value for development as novel anticancer agents. Based on the (–)-epicatechin scaffold, a series of derivatives have been synthesized and screened for activity in an in vitro recombinant DNMT assay system. Lead compounds with potent inhibitory activity will subsequently be evaluated in cancer cell-based models of viability, apoptosis and DNA methylation status.

434.

SYNTHESIS AND BIOLOGICAL EVALUATION OF 3,6-DIOXA-[3,2,0]BICYCLONUCLEOSIDES. ***Xingang Fang**, Department of Chemistry, Emory Univeristy, 1515 Dickey Drive, Atlanta, GA 30322, xfang2@emory.edu, Raymond F. Schinazi, Medical research 151H, Emory University School of Medicine/VA Medical center, and Dennis C. Liotta, Department of Chemistry, Emory University*

3,6-dioxa-[3,2,0]bicyclonucleosides and its tri(mono)phosphates are potential inhibitors of HCV polymerase, HIV and HBV reverse transcriptase and the mycobacterium tuberculosis thymidine monophosphate kinase. In this report, the thymine, 5-fluorocytosine, cytosine, 6-chloropurine and 5-fluorouracil derivatives of both enantiomers were synthesized and evaluated for their biological activities. Triphosphates of some selected nucleosides were synthesized and evaluated as well.



435.

SYNTHESIS AND BIOLOGICAL EVALUATION OF 5-(ALKYN-1-YL)-1-(P-TOLUENESULFONYL)URACIL DERIVATIVES. ***Zlatko Janeba**[1], Morris J. Robins[2], G. Andrei[3], R. Snoeck[3], J. Balzarini[3], and E. De Clercq[3]. (1) Department of Chemistry and Biochemistry, Northern Arizona University, PO Box 5698, Flagstaff, AZ 86011, Fax: 928-523-8111, zlatko.janeba@nau.edu, (2) Department of Chemistry and Biochemistry, Brigham Young University, (3) Rega Institute for Medical Research*

Bicyclic furanopyrimidine nucleosides and acyclic analogues are potential antiviral agents. During recent studies on cyclizations of 5-(alkyn-1-yl)uracil derivatives to form furo[2,3-d]pyrimidin-2(3H)-ones, we observed that the strongly electron withdrawing group at N1 of 5-ethynyl-1-tosyluracil completely inhibited the reaction, whereas N1 alkylated or glycosylated analogues were cyclized smoothly. 5-Ethynyl-1-tosyluracil exhibited some anti-HCMV activity, which suggested our investigation of the SAR of a series of 5-(alkyn-1-yl)-1-tosyluracils. However, 5-iodo-1-tosyluracil did not undergo Sonogashira coupling. Fortunately, coupling of terminal alkynes with 5-iodouracil followed by tosylation of these 5-(alkyn-1-yl)uracils at N1 afforded the desired compounds. We will present our syntheses and antiviral evaluations of the targeted 5-(alkyn-1-yl)-1-(p-toluenesulfonyl)uracils.

436.

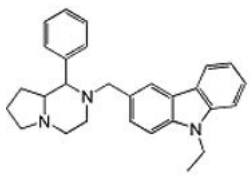
SYNTHESIS AND CELLULAR UPTAKE OF NOVEL NUCLEAR TARGETED CARBORANE PEPTIDES FOR BORON NEUTRON CAPTURE THERAPY. ***Paola Dozzo**[1], Stephen B. Kahl[1], E.A. Blakely[2], and Kathleen A. Bjornstad[2]. (1) Department of Pharmaceutical Chemistry, University of California at San Francisco, 513 Parnassus Ave., San Francisco, CA 94143, pdozzo@itsa.ucsf.edu, (2) Lawrence Berkeley National Laboratory*

Boron neutron capture therapy (BNCT) is a highly selective, binary cancer therapy in which administration of a tumor-seeking non-toxic boron containing compound is followed by its activation by a flux of thermal neutrons. The amount of B-10 required to achieve successful cell killing is highly dependent on the subcellular location of the boron atoms. If boron were to localize in the nucleus, a 10-100 fold increase in cell killing efficacy would be expected. We herein report the synthesis of a series of novel p-carborane containing peptides of variable lengths, which were covalently attached to the SV40 nuclear localization sequence and to a fluorescent probe. These conjugates were incubated with the human brain tumor cell line SF-767 and were shown to localize exclusively around the nucleus.

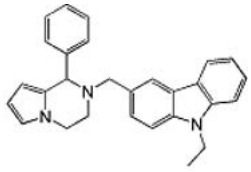
437.

SYNTHESIS AND CYTOTOXICITY OF 2-SUBSTITUTED-1-PHENYL-OCTAHYDROPYRROL[1,2-A]PYRAZINE. ***Yan Zhuang**, Staci N. Smith, and Charles D. Smith, P.O. Box 916, Apogee Biotechnology Corporation, Hershey, PA 17033*

N-Myristoyltransferase (NMT) attaches myristate to the N terminus of specific proteins, including some critical for tumor cell proliferation. We have shown that several compounds containing a cyclohexyl-octahydropyrrolo[1,2-a]pyrazine (COPP) moiety block tumor cell proliferation by inhibiting NMT . To further explore SARs, we prepared a series of 2-substituted analogs. In this procedure, a 1-phenyl-1,2,3,4-tetrahydro-pyrrolo[1,2-a]pyrazine (PTPP) nucleus was produced and then hydrogenated to generate the 1-phenyl-octahydropyrrolo[1,2-a]pyrazine (POPP) nucleus. Either scaffold could then be N-alkylated by a variety of alkyl bromides to provide the 2-substituted libraries. The POPP compounds were generally more cytotoxic toward human colon adenocarcinoma cells than were the corresponding PTPP congeners. The most potent compound of each series contained an N-ethyl carbazole group (shown below), which is consistent with our modeling studies that indicate that this component of the COPP series interacts with the peptide-binding site of NMT. Overall, these studies provide new synthetic routes to inhibitors of NMT.



POPP



PTPP

438.

SYNTHESIS OF 1-/ 2-SUBSTITUTED-[1,2,3]TRIAZOLO[4,5-G]PHTHALAZINE-4,9-DIONES AND EVALUATION OF THEIR CYTOTOXICITY. ***Hea-Young Park Choo**, Jinsung Kim, Hunjoo Park, and Sangkook Lee, School of Pharmacy, Ewha Womans University, Seoul 120-750, South Korea, Fax: 82-2-3277-2851, hypark@ewha.ac.kr*

The cytotoxicity of the heterocyclic quinones has been thoroughly studied and the antitumor activity of imidazoquinoxalinedione derivatives, imidazoquinolinedione derivatives, and imidazophthalazinedione derivatives was reported. Since Johnson and co-workers have reported that the number and position of nitrogen atoms are important for cytotoxicity, we anticipated higher activity on introduction of more nitrogens in the heterocyclic quinones. Here we report the synthesis and cytotoxicity of triazolophthalazinedione derivatives. Synthesis of 1-/ 2-substituted-[1,2,3]triazolo[4,5-g]phthalazine-4,9-diones has been achieved by the modified reaction of reported method using phthalazine-5,8-dione and 4-methoxybenzyl azide. The 1,3-dipolar addition of 4-methoxybenzyl azide to phthalazine-5,8-dione resulted in the formation of triazolophthalazine-4,9-dione derivative. Alkylation of triazole gave a mixture of two isomers and these isomers were separated by chromatography. The cytotoxicity was evaluated by a SRB (sulforhodamine B) assay against A549, SK-OV-3, SK-MEL-2, XF498 and HCT15. Most of the synthesized compounds showed very high cytotoxicity, considerably higher than that of the reference compound doxorubicin.

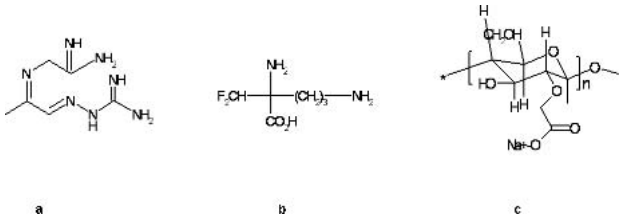
439.

SYNTHESIS OF CONTROLLED-RELEASE CHEMOTHERAPEUTIC AGENTS USING CARBOXYMETHYLCELLULOSE. ***Megan N. Nollenberger**, Department of Chemistry, Shippensburg University, 1871 Old Main Drive, Shippensburg, PA 17257, and Christine Martey-Ochola, Department of Chemistry, Shippensburg University*

The formation of peritoneal (intra-abdominal) adhesions is a common and dangerous side effect of general abdominal surgery, hernia repair, and radiation therapy, as well as many other invasive abdominal procedures. These adhesions may result in chronic pelvic pain, infertility and hemorrhaging. To reduce the

occurrence of these internal adhesions, the use of barrier polymers is becoming a more frequent and very successful solution. One such polymer is Sodium Carboxymethylcellulose (SCMC) (fig. 1), which is a very attractive candidate for nucleophilic drug substitutions due to the presence of the carboxymethyl residues. In this study, we monitor the release of chemotherapy agents, specifically Mitoguazone and Eflornithine (fig. 1), from the polymer-drug conjugate previously synthesized.

Figure 1. Structures of (a) Mitoguazone, (b) Eflornithine, and (c) SCMC



a b c

440.

SYNTHESIS OF NOVEL CYCLOBUTYL NUCLEOSIDE ANALOGS. ***Yongfeng Li*** *[1], Shuli Mao[1], Michael W Hager[1], Dennis C. Liotta[1], and Raymond F. Schinazi[2]. (1) Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, yli24@emory.edu, (2) Medical research 151H, Emory University School of Medicine/VA Medical center*

Several carbocyclic nucleoside analogs exhibit good biological activity as well as resistance to degradation by phosphorylases. In this poster, we present details about the synthesis using a variety of [2+2] approaches to produce cyclobutyl derivatives with the desired substitution patterns, and some of them exhibited potentially useful resistance profiles.

441.

SYNTHESIS, PURIFICATION, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF DIALKYLESTER GLUTATHIONE CONJUGATES WITH 3-METHYLENEOXINDOLE AS POTENTIAL THERAPEUTIC AGENTS. ***Karen DeBalsi*** *[1], Jeffery Bowen[2], and Edward J. Brush[1]. (1) Department of Chemical Sciences, Bridgewater State College, Bridgewater, MA 02325, kdebalsi@bridgew.edu, (2) Department of Biological Sciences, Bridgewater State College*

Mammalian glyoxalase I (GxI) converts the hemithioacetal formed from glutathione (GSH) and methylglyoxal, into the thioester, S-lactoylglutathione. Inhibitors of GxI are effective anti-cancer agents due to the subsequent increased concentration of cytotoxic methylglyoxal in tumor cells. The effectiveness of GxI inhibitors has been improved via the synthesis of GSH-dialkylester derivatives, which enhances their ability to cross the hydrophobic plasma membrane. The purpose of this research was the synthesis and characterization of the diethyl and dicyclopropyl GSH diester conjugates with 3-methyleneoxindole, GSMOI-(EE) and GSMOI-(CPE), respectively, and the fluorogenic evaluation of their plasma membrane permeability and GxI inhibition as evidenced by induction of apoptosis. Estimation of the lethal dose concentrations (LD50) on Clone 9 cells following 24hrs of treatment was 15micromolar with GSMOI-(EE), (42.9% ± 33.3, N = 4) and 100micromolar with GSMOI-(CPE), (62.2% ± 30.5, N = 5). This research was supported in part by the Research Corporation and Bridgewater Foundation.

442.

TUMOR-SPECIFIC DELIVERY OF NOVEL MAYTANSINOIDS: SYNTHESIS AND BIOLOGICAL EVALUATION. ***Sharon D. Wilhelm*** *[1], Wayne C. Widdison[1], Kathleen R. Whiteman[1], Elizabeth E. Roller[2], Emily E. Cavanaugh[1], Rita M. Steeves[1], Robert J. Lutz[1], Michele F. Mayo[1], Hongsheng Xie[1], and Ravi V. J. Chari[1]. (1) ImmunoGen, Inc, 128 Sidney Street, Cambridge, MA 02139, (2) Department of Chemistry, ImmunoGen, Inc*

Maytansinoids are highly cytotoxic agents. The anti-tumor activity of these potent agents can be greatly enhanced by targeted delivery. Modifications made to the C-3 ester moiety have enabled linkage of the agent, via disulfide bonds, to tumor-specific monoclonal antibodies. These conjugates show high specificity and cytotoxicity towards target cancer cells and efficacy in animal tumor models. Sterically hindered thiol and disulfide-containing maytansinoids have been synthesized. These novel maytansinoids have shown extraordinary potency, increased stability and provided an expanded therapeutic window when conjugated to cancer cell binding agents. The design, synthesis and biological evaluation of these maytansinoids will be presented.

443.

VIRGINIAMYCIN M1 CONFORMATION IN AQUEOUS SYSTEMS. ***Robert P. Metzger*** *[1], Jason Dang[2], Chai Ann Ng[2], Robert T. C. Brownlee[2], Mikael Bergdahl[1], and Frances Separovic[3]. (1) Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182-1030, Fax: 619 594 4634, rmetzger@sciences.sdsu.edu, (2) Department of Chemistry, LaTrobe University, (3) School of Chemistry, The University of Melbourne*

The antibiotic Virginiamycin consists of two molecules, Virginiamycin M1 (VM1) and S1 (VS1), which synergistically bind to bacterial 50S ribosomes and inhibit protein synthesis. We recently reported the 3-dimensional conformation of VM1 in chloroform, DMSO, and methanol (Dang et al, 2004a,b) differed markedly from the enzyme (Sugantino and Roderick ,2003)- and ribosomally (Hansen et al, 2003)-bound VM1 determined by x-ray crystallographic studies. We now report further NMR studies and subsequent molecular modeling of VM1 in deuterium oxide alone and with acetonitrile or methanol. Our results indicate that the 3 dimensional conformation of VM1 in deuterium oxide differs substantially from all of the other conformations reported to date. This indicates VM1 is a flexible compound and suggest that environmentally-determined conformations may contributes to VM1 effectiveness as an antibiotic. J. Dang, B.M. Bergdahl, F. Separovic, R.T.C. Brownlee and R.P. Metzger. Aus. J. Chem. 57 (2004), 415-418; J. Dang, F. Separovic, B. M. Bergdahl, R. T. C. Brownlee, and R. P. Metzger, Org. Biomol. Chem. 2 (2004), 2919-2924; M. Sugantino and S.L. Roderick. Biochemistry 41 (2002), 2209-2216; J.L. Hansen, P.B. Moore and T.A. Steitz. J. Mol. Biol. 330 (2003), 1061-1075

444.

ANTHRACYCLINES AFFECT CALCIUM ION BINDING TO CALSEQUESTRIN. ***Wendy K. Mercer****, Nico Cantone, Dawn J. Muhlestein, Henry A. Charlier Jr., and Susan E. Shadle, Department of Chemistry, Boise State University, 1910 University Drive, Boise, ID 83725, wendymercer@mail.boisestate.edu*

Anthracyclines, such as daunorubicin (Daun), are chemotherapeutic drugs used extensively in cancer treatment. Their use is, however, limited by a potentially lethal chronic cardiotoxicity related to the cumulative dose of drug administered. Previous studies indicate that Daun effectively inhibits calcium release from the sarcoplasmic reticulum. This inhibition has been hypothesized to be the result of Daun binding to the SR protein calsequestrin (CSQ), which is involved in the regulation of SR calcium. We have investigated the effects of anthracyclines on CSQ-calcium binding using equilibrium dialysis. Calcium concentrations in each dialysis half-cell were determined by atomic absorbance spectroscopy. Results were used to generate calcium binding curves for experiments using CSQ preincubated with anthracyclines. Data were fit to the Hill equation, from which the CSQ-calcium binding capacity, affinity, and cooperativity can be derived. Daun increases the calcium binding capacity of CSQ while decreasing the binding affinity and cooperativity. Supported by NIH/P20RR16454, NIH/R15HL68579-01

445.

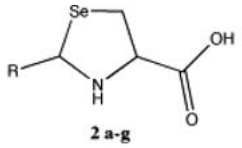
SUGAR DERIVATIVES OF POLYAMINE ANTHRAQUINONES; SYNTHESIS AND PRECLINICAL EVALUATION. ***D. Richard Ishmael Ishmael****, NatCel Dev, 3945 N. Walnut, Oklahoma city, OK 73105, ishdr@aol.com*

Various anticancer anthraquinones have been synthesized and tested over the past 30 years. One compound mitoxantrone is on the market and approved for treatment of leukemias, lymphomas and solid tumors such as breast and prostate. However mitoxantrone has adverse cardiac effects and is not as active as the anthracycline adriamycin. In an attempt to improve the efficacy of anthraquinone compounds, a program was undertaken to synthesize derivatives that might have impoved efficacy. It was found that 1,4-bis-aminoethylamino-5,8-dihydroxyanthraquinones had greater cell kill of a human breast cancer cell line. Further impovements in cytotoxicity were gained when sugar groups such as glucose fructose, galactose and glucoamine were added to the terminal amino groups. Structural confirmation of the compounds was undertaken.The cell line used in these studies was the BOT-2 human breast cancer cell line. Cytotoxicity was read as 100% cell kill. These compounds exibited increased cytotoxicity as compared to mitoxantrone.

446.

HEPATIC EFFECTS OF NOVEL SELENAZOLIDINE PRODRUGS OF SELENOCYSTEINE DEVELOPED AS POTENTIAL CANCER CHEMOPREVENTIVE AGENTS. ***Tarek Aboul-Fadl***[1], *Wael M. El-Sayed*[2], *Tenley Schofield*[2], *Jonathan Constance*[2], *John G. Lamb*[2], *Jeanette C. Roberts*[3], *and Michael R Franklin*[2]. *(1) Department of Medicinal Chemistry, University of Utah, 30 South, 2000 East, Room 201, Salt Lake City, UT 84112, Fax: 801 585 9119, Tarek.Aboul-Fadl@pharm.utah.edu, (2) Department of Pharmacology and Toxicology, University of Utah, (3) College of Pharmacy, University of Wisconsin*

Novel selenozolidines have been synthesized as potential cancer chemopreventive agents and their hepatic effects investigated. The prodrugs (2a-g) were synthesized by the reaction of selenocysteine with the appropriate carbonyl derivative. The lipophilicity of these compounds (expressed as Clog P values) ranged from -3.062 (2a) to -0.512 (2f) but hepatic effects appeared unrelated to this parameter. Male CF1 mice were treated daily for 7 days with equi-selenium (1.25 mg Se/kg) doses of each agent, by either the intraperitoneal (ip) or intragastric (ig) route and the effects compared with those of selenocystine. Hepatic parameters were determined 24 hours after the last dose. In general, few significant ($p < 0.05$) changes were seen with ig as compared to ip administration and the synopsis below documents the latter. Elevation of hepatic selenium concentration was only observed with 2d (1.6-fold), and liver toxicity (elevated sALT) was only observed with 2c. Elevated glutathione S-transferase activity was observed with 2b, 2c and 2g. For 2c, this was accompanied by elevations in mRNAs of Gst-alpha and Gst-mu, but for 2b, only changes in Gst-mu were observed. A decrease in glutathione peroxidase activity was observed for compounds 2e, 2f and 2g and an increase quinone oxidoreductase activity was observed for compounds 2c and 2d. For neither enzyme were changes in mRNA seen. Increases in Ugt mRNAs were observed for 2a, 2c, and 2d, but these did not result in changes in UDP-glucuronosyltransferase (UGT) activity. The mRNA increases were in Ugt1a1 and Ugt1a9 for 2a, and 2c, and Ugt1a6 and Ugt1a9 for 2d. Thioredoxin reductase activity was elevated by 2a, 2c, and 2d. Thioredoxin reductase activity was also the only parameter that was altered with selenocystine treatment.



2 a-g

R = a, H; b, CH_3; c, =O; d, C_4H_{11}; e, C_6H_5; f, Cyclo-C_6H_{11}; g, 2-Hydroxy-C_6H_4.

447.

HYPOXIA-SELECTIVE ANTICANCER AGENTS: PHOSPHATE DERIVATIVES OF KS119 (VNP40119). ***Xu Kevin Lin***, *Michael Belcourt, Li-Mou Zheng, Caroline Clairmont, Ala Nassar, Terrence W Doyle, and Ivan King, Vion Pharmaceuticals Inc, 4 Science Park, New Haven, CT 06511, Fax: 203-498-4211, xlin@vionpharm.com*

It has been increasingly of interest that hypoxia-selective drugs play positive roles in combining treatment with other clinical drug(s) or radiation for cancer therapy. More recently, we have developed a lead compound KS119 to address its hypoxia-selectivity from the class of the sulfonylhydrazine prodrugs (SHPs). In this unique sulfonylhydrazine class, CLORETAZINETM had been exhibited to be a novel alkylating agent for cancer therapy in Phase II human clinical trials; and it had been granted orphan drug designation from the FDA for treatment of acute myelogenous leukemia (AML). In this presentation, design and synthesis of two lead series of phosphate derivatives (KS119W and KS119S) of KS119 will be shown. Preclinical investigation has demonstrated that these newly synthesized anticancer agents are highly hypoxia-selective and have a promising activity of tumor inhibition in vivo with excellent pharmaceutical and pharmacokinetic properties. These phosphate derivatives of KS119 are optimized to give a clinical candidate.

448.

LIPOSOMAL LOADING OF WATER-SOLUBLE IRON(III) PORPHYRINS AS ANTICANCER DRUGS BASED ON SUPEROXIDE REACTIONS. ***Makoto Yuasa***[1], *Kenichi Oyaizu*[2], *Akihiko Ogata*[1], *Tomomi Hatsugai*[1], *Aritomo Yamaguchi*[1], *and Hiroyoshi Kawakami*[3]. *(1) Department of Pure & Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan, Fax: +81-4-7121-2432, yuasa@rs.noda.tus.ac.jp, (2) Institute of Colloid and Interface Science, Tokyo University of Science, (3) Department of Applied Chemistry, Tokyo Metropolitan University*

A novel design of anticancer drug delivery system, based on an electrostatic interaction of negatively charged liposomes and cationic metalloporphyrins will be reported. A lack of cytotoxicity of the iron(III) porphyrin-loaded liposomes and an efficient generation of a toxic hydroxyl radical from a superoxide anion radical through the iron(III)-catalyzed dismutation followed by the Fenton-like reaction allow for a targeted necrosis of tumor cells where the concentration of superoxide is locally increased as a result of the reduced activity of superoxide dismutase and catalase in tumor cells.

449.

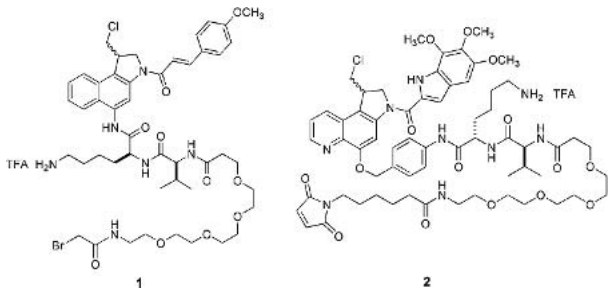
A NEW AND SELECTIVE G-QUARTET BINDING AGENT. ***Steven M. Firestine***[1], *Lisa Irish*[2], *David Bednarski*[3], *and Melanie J. Grubisha*[1]. *(1) Mylan School of Pharmacy, Duquesne University, 600 Forbes Ave, Pittsburgh, PA 15282, firestine@duq.edu, (2) Department of Chemistry, Florida Memorial College, (3) Graduate School of Pharmaceutical Sciences, Duquesne University*

We have recently synthesized a triazacyclopenta[b]phenanthrene compound (1) that has micromolar affinity for double stranded DNA. Equilibrium dialysis studies were conducted to determine if 1 was selective for other types of DNA structures. This study revealed that 1 bound selectively to G-quartet structures. To verify this result, studies on the stabilization of G-quadruplexes were conducted. Compound 1 readily promotes the formation and stabilization of G-quadruplexes in conditions that do not favor its formation. Further studies indicate that 1 competes with binding of the known G-quadruplex-binding porphyrin molecule TmPyP4. Using this information, we conducted a docking study of 1 to G-quartet DNA and this study revealed that 1 binds to the ends of G-quartet DNA and appears to interact with two of the four quanine residues. Future work will focus on the biological effects of 1 and synthetic efforts to increase its binding affinity and selectivity.

450.

DESIGN, SYNTHESIS AND IN VITRO EVALUATION OF DIPEPTIDE-BASED ANTIBODY MINOR GROOVE BINDER CONJUGATES. ***Scott C. Jeffrey***[1], *Michael Y. Torgov*[1], *Jamie B. Andreyka*[1], *Laura Boddington*[1], *Charles G. Cerveny*[1], *William A. Denny*[2], *Darin Gustin*[1], *Jennifer Haugen*[1], *Toni B. Kline*[1], *Minh T. Nguyen*[1], *and Peter D. Senter*[1]. *(1) Department of Chemistry, Seattle Genetics, 21823 30th Drive SE, Bothell, WA 98021, Fax: 425-527-4109, sjeffrey@seagen.com, (2) University of Auckland School of Medicine, Auckland Cancer Society Research Centre*

Antibody-drug conjugates (ADCs) were prepared from DNA minor groove binder drugs (MGBs) attached to monoclonal antibodies (mAbs) through peptide linkers designed to release drugs inside the lysosomes of target cells. Due to the hydrophobic nature of the MGBs, several measures were required to overcome antibody aggregation upon conjugation. These included the incorporation of a relatively hydrophilic valine-lysine dipeptide sequence and a tetraethylene glycol spacer between the mAb and dipeptide. This resulted in non-aggregated conjugates of 1 with up to 8 drugs/mAb. The approach was applied to the construction of the hydroxy aza-CBI drug-linker 2, which incorporated the para-amino benzyl ether self-immolative spacer. mAb ADCs comprised of 2 were monomeric with up to 8 drugs/mAb and released the hydroxy aza-CBI payload upon treatment with human cathepsin B. In vitro cytotoxicity assays established that both ADC formats were highly cytotoxic and effected immunologically specific cell kill at sub-saturating mAb concentrations.

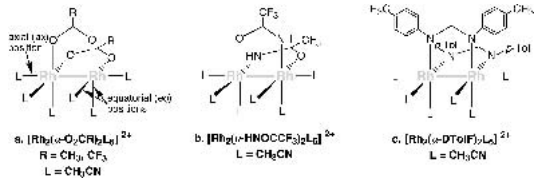


1 2

451.

EFFECT OF THE BRIDGING GROUPS OF DIRHODIUM(II,II) COMPLEXES ON THE EFFICIENCY OF TRANSCRIPTION INHIBITION IN VITRO. ***Helen Chifotides***[1]*, Kim R. Dunbar*[1]*, and Claudia Turro*[2]*. (1) Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77842-3012, Fax: 979-845-7177, chifotides@mail.chem.tamu.edu, (2) Department of Chemistry, Ohio State University*

Dirhodium compounds supported by various bridging groups have been shown to bind to DNA and inhibit T7-RNA polymerase in vitro. In the series of partially solvated complexes $Rh_2(\mu\text{-}O_2CCH_3)_2(NCCH_3)_6](BF_4)_2$, $Rh_2(\mu\text{-}O_2CCF_3)_2(NCCH_3)_6](BF_4)_2$, $Rh_2(\mu\text{-}HNCOCF_3)_2(NCCH_3)_6](BF_4)_2$ and $Rh_2(\mu\text{-}DTolF)_2(NCCH_3)_6](BF_4)_2$ inhibition of transcription appears to proceed predominantly via binding of the complexes to T7-RNA polymerase (T7-RNAP). The concentrations of the aforementioned complexes required to inhibit transcription by 50%, C_{inh50}, are similar to that measured for activated cisplatin and their values have been correlated to the lability of the bridging groups: the C_{inh50} value is highest for $Rh_2(\mu\text{-}DTolF)_2(NCCH_3)_6](BF_4)_2$ and lowest for $Rh_2(\mu\text{-}O_2CCF_3)_2(NCCH_3)_6](BF_4)_2$. The high value C_{inh50} for $Rh_2(\mu\text{-}DTolF)_2(NCCH_3)_6](BF_4)_2$ is attributed to steric factors as well.



452.

IMPROVED LIGAND BINDING TO G-G MISMATCH. ***Tao Peng****, Japan Science and Technology Agency, Sakyouku, Katsura, Kyoto University, Kyoto 615-8510, Japan, Fax: 81-75-3832759, taopengcn@msn.com, and Kazuhiko Nakatani, Department of Synthetic Chemistry and Biological Chemistry, 1Japan Science and Technology Agency, 2Kyoto University*

A new ligand, naphthyridine carbamate dimer (**NC**) possessing 2-amino-1,8-naphthyridines and a carbamate linker specially binds to guaninëCguanine (GCG) mismatch in duplex DNA, is much more thermally stable than naphthyridine dimer (**ND**) we reported previously. The half-life of **NC** is 2.5 times longer than that of **ND** at 80 ºC. **NC** is also much more stable than **ND** under alkaline conditions. In addition, **NC** has a larger increase of the melting temperature (T_m) by 5.4 ºC than **ND**. The K_a obtained between **NC** to the G-G mismatch was $>10^7$ M^{-1}, that is larger than the K_a for the **ND** binding to the G-G mismatch. While the binding of **ND** to G-G mismatch duplex proceeded with 1:1 and 2:1 fashion, **NC** showed exclusively 2:1 binding to the G-G mismatch. The improved stability and the binding of **NC** to the G-G mismatch would be suitable for the practical application.

453.

IMPROVED NUCLEIC ACID TRIGGERED PROBE ACTIVATION THROUGH THE USE OF A 5-THIOMETHYLURACIL PEPTIDE NUCLEIC ACID (PNA) BUILDING BLOCK. ***Jianfeng Cai****, Xiaoxu Li, and John Stephen Taylor, Department of Chemistry, Washington University in St. Louis, One brookings drive, St. Louis, MO 63130*

A number of years ago our group proposed a new concept for the design and synthesis of highly selective diagnostic and chemotherapeutic agents for cancers and viruses that we now call NATPA for nucleic acid triggered prodrug and probe activation (Proc. Natl. Acad. Sci. U.S.A., 2000, 97, 11159-11163). The idea is to use an mRNA or DNA sequence unique to the diseased cell to direct the association of a prodrug or probe component with a catalytic component which then catalyzes the release of a cytotoxic drug or probe. Most recently we developed a fluorogenic system based on peptide nucleic acid (PNA) that could be triggered by RNA (Bioconjug. Chem. 2003, 14, 679-683). Unfortunately, the rate of fluorescence activation was too slow to be useful for in vivo applications, which we attributed to poor aligmnent and constraint of the catalyst and substrate resulting from attachment to the terminal amino and carboxy groups. In an effort to improve the catalytic efficiency of this system, we synthesized a 5-thiomethyl U PNA building block which allows the catalytic and prodrug groups to be directly attached to the base and project into the major groove. Attachment of imidazole and a coumarin ester to thiomethyl U's on the ends of two PNAs resulted in a 6-fold enhancement in kcat compared to the previous system which we attribute to better alignment of the catalyst and substrate.

454.

INSIGHT INTO THE CISPLATIN MEDIATED APOPTOSIS IN CHO CELLS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY AND PHASE CONTRAST LIGHT MICROSCOPY. ***Leila Maurmann***[1]*, Maupali Dasgupta*[2]*, Lamis A. Joudah*[3]*, John Stalvey*[3]*, and Rathindra N. Bose*[1]*. (1) Department of Chemistry, Northern Illinois University, The Michael Faraday Laboratories - Northern Illinois University, DeKalb, IL 60115-2862, lmaurman@niu.edu, (2) Lerner Research Institute, (3) Kent State University*

Cisplatin, a widely used chemotherapeutic agent, induces apoptosis by causing arrest in the G2 phase of the cell cycle. Cells undergoing apoptosis exhibit characteristic morphological changes which are dependant on the concentration of cisplatin and the length of exposure. In an attempt to correlate morphological changes with biological markers, one and two dimensional 1H and 31P NMR experiments were conducted on cell lysate from Chinese Hamster Ovarian cells after treatment with cisplatin for 6, 12, 24 and 72 hours. Typical biological markers were monitored and compared with those from control experiments. The most prominent peaks on the 31P NMR spectra were assigned to phosphocholine, inorganic phosphate, phosphocreatine and NTP. An increase was observed in the level of phosphocholine, indicating disintegration of cell membranes due to apoptosis. These NMR data will be presented in correlation with the number of cells undergoing apoptosis and morphological changes observed by Phase Contrast light microscopy.

455.

MULTIPLE MOLECULAR DYNAMICS CRYSTAL SIMULATIONS OF DNA/POLYAMIDE COMPLEXES. ***Anne Loccisano****, Center for Computational Sciences, Duquesne University, Department of Chemistry and Biochemistry, 600 Forbes Avenue, Pittsburgh, PA 15282, Fax: (412)396-5683, loccisa780@duq.edu, Sarah A. Mueller-Stein, Department of Chemistry and Biochemistry, Center for Computational Sciences, Duquesne University, Steven M. Firestine, Mylan School of Pharmacy, Duquesne University, and Jeffrey D. Evanseck, Department of Chemistry & Biochemistry and Center for Computational Sciences, Duquesne University*

Minor groove binding polyamides that bind specific sequences of DNA offer one approach to artificial gene regulation. However, targeting some sequences is difficult, which is likely due to sequence-dependent structural variations of the minor groove. We are interested in carrying out multiple MD simulations in order to gain an understanding of how polyamides interact with DNA at the atomic level. In order to evaluate our methodology, six simulations of a DNA/polyamide complex have been performed for 10 ns each in the crystal environment. The helical parameters have been monitored and compared to the starting x-ray crystal structure in order to determine when these properties converge. The information gained from our simulations will provide a detailed understanding of how to equilibrate these systems, which in turn will enable a detailed understanding of how polyamides interact with DNA and the structural variations for specific sequences at the atomic level.

456.

READING THE LANGUAGE OF PYRROLE– AND IMIDAZOLE–CONTAINING POLYAMIDES THAT RECOGNIZE SPECIFIC DNA SEQUENCES. ***Karen Buchmueller***[1], *Peter Uthe*[1], *Cameron Howard*[1], *Minh Le*[1], *Kari Cox*[1], *Suzanna Bailey*[1], *David Matthews*[1], *Janna Register*[2], *Chrystal Bruce*[2], *Binh Nguyen*[3], *Caroline O'Hare*[4], *John Hartley*[4], *W. David Wilson*[3], *and Moses Lee*[1]. *(1) Chemistry, Furman University, 3300 Poinsett Highway, Greenville, SC 29613, karen.buchmueller@furman.edu, (2) Chemistry, Erskine College, (3) Department of Chemistry, Georgia State University, (4) Oncology, Royal Free and University College Medical School*

The ability of small molecules to bind specific DNA sequences is integral for their development into useful medicinal therapeutic agents and biosensors. Medicinal chemists have found that polyamides exhibit considerable promise in targeting specific sequences because of the versatility in which the molecules can be altered, but still retain DNA affinity. The recognition of specific cognate DNA base pairs (i.e. reading letters) by heterocyclic groups is now well established. However, it has become clear that a deeper understanding of the "polyamide language" is required because some polyamides show low binding affinity for their cognate DNA sequence, and others have low selectivity for their cognate DNA over non-cognate DNA. Towards elucidating the polyamide language, we have studied a class of pyrrole- and imidazole-containing triamides that contain an N-terminal formamido group by using several techniques that include surface plasmon resonance, circular dichroism, differential thermal melts and DNase I footprinting. The effects of the context in which the heterocyclic groups are arranged within the molecule on sequence specificity and affinity, as well as the sequence preference of the formamido group will be discussed. From these studies, we discovered a molecule (formamido-imidazole-pyrrole-imidazole) which binds DNA better than its parent compound, Distamycin A. The sequence selectivity, flanking sequence affect and general hydration profile of this molecule will also be presented.

457.

REGULATION OF TRANSCRIPTION BY SYNTHETIC DNA BENDING AGENTS. ***Steven M. Firestine****, Mylan School of Pharmacy, Duquesne University, 600 Forbes Ave, Pittsburgh, PA 15282, firestine@duq.edu, and David Bednarski, Graduate School of Pharmaceutical Sciences, Duquesne University*

Gene expression is regulated by a complex interplay between binding and the three-dimensional arrangement of transcription factors with RNA polymerase and DNA. Previous studies have supported a direct role of DNA bending and conformation on gene expression, suggesting that agents that induce bends in DNA may be able to control gene expression. To test this hypothesis, we examined the effect of triple helix forming oligonucleotide (TFO) bending agents on the transcription of luciferase in an in vivo transcription/translation system. We find that transcription is regulated only by a TFO that induces a 60° bend in the DNA. Related TFOs that do not induce bends in DNA have no effect on transcription. The effects of the TFO-mediated bend on gene expression is dependent upon the correct orientation (phase) of the bend relative to the start of the gene. When the bend is phased such that upstream DNA and RNA polymerase occur on the same face of the helix, an 80% increase in luciferase transcription is observed. When the bend occurs such that RNA polymerase and upstream DNA occur on opposite sides of the helix, a 50% decrease in transcription is observed. These results support the hypothesis that DNA bending agents may have the capability to regulate gene expression, opening up a previously undervalued avenue in research on the artificial control of gene expression.

458.

SYNTHESIS AND BIOLOGICAL STUDIES OF NCS-CHROM METABOLITE ISOSTERES: BINDING AGENTS FOR BULGED DNA MICROENVIRONMENTS. ***Yiqing Lin***[1], *Graham B. Jones*[1], *Geum-Sook Hwang*[2], *and Irving H. Goldberg*[2]. *(1) Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Ave., Boston, MA 02115, Fax: 617-373-8795, lin.yi@neu.edu, (2) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School*

Bulged structures (regions of unpaired bases) in nucleic acids have been the subject of intense interest, because they have been implicated as intermediates in a multitude of processes including RNA splicing, frame-shift mutagenesis, intercalator-induced mutagenesis, imperfect homologous recombination and as binding motifs for regulatory proteins in viral replications. Until now, few attempts have been made to prepare compounds with affinity for bulged sequences due to the lack of an available substrate that can effectively mimic the base pairing involved at a bulged site, which requires a unique wedge-shaped template. Using an enediyne natural product (NCS) metabolite NCSi-gb as lead compound, we developed a unique, convenient and versatile route for the synthesis of a series of bulged DNA binders and studied their binding modes by solution NMR. A full account of this research will be reported.

459.

THERMODYNAMIC AND STRUCTURAL BASES FOR THE BINDING OF THE BETTER-THAN-NATURE TRIHETEROCYCLIC POLYAMIDE F-IMPYIM TO ITS COGNATE SEQUENCE, ACGCGT. ***Karen Buchmueller***[1], *Peter Uthe*[1], *Suzanna Bailey*[1], *David Matthews*[1], *Kari Cox*[1], *Janna Register*[2], *Chrystal Bruce*[2], *Binh Nguyen*[3], *W. David Wilson*[3], *and Moses Lee*[1]. *(1) Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, karen.buchmueller@furman.edu, (2) Chemistry, Erskine College, (3) Department of Chemistry, Georgia State University*

Pyrrole and imidazole-containing polyamides are a class of small molecules that bind in the minor groove of DNA, many of which have shown both excellent sequence specificity and binding affinity for DNA. Recently, we have expanded the language of polyamide recognition of DNA beyond the recognition of single base pair "letters" to include two letter "words." In conjunction with this observation, we identified a molecule, formamido-imidazole-pyrrole-imidazole (f-ImPyIm), which has a stronger affinity for its cognate sequence, ACGCGT, than does its closely related natural product parent, Distamycin A, for its cognate the sequence, AAATTT (Keq = 2 × 10^8 and 2 × 10^7 M^-1, respectively). In addition, f-ImPyIm has great affinity by a factor of at least 10 fold, and often 3-4 orders of magnitude, for ACGCGT over all DNA sequences, including many that are closely related. The impressive binding affinity and sequence selectivity of f-ImPyIm are being investigated by studying the thermodynamic behavior and structural bases using isothermal microcalorimetry and molecular dynamic simulations with AMBER 7.0. In addition, the thermodynamic behavior of f-ImPyIm will be compared to that of its structural isomer, f-PyImIm (Keq = 8 × 10^5 M^-1 for its cognate ACCGGT) and its parent analog Distamycin A.

460.

ANTHRANILATE BASED ANALOGUES OF FARNESYL PYROPHOSPHATE. ***Mona A. Maalouf***[1], *Andrew J. Wiemer*[2], *Raymond J. Hohl*[3], *and David F. Wiemer*[1]. *(1) Department of Chemistry, University of Iowa, Iowa City, IA 52242-1294, Fax: 319-335-1270, mouna-a-maalouf@uiowa.edu, (2) Molecular Biology Program, University of Iowa, (3) Department of Internal Medicine, University of Iowa*

The Ras family of small G proteins plays a key role in an extensive network of cell signaling pathways, and mutated Ras has been linked to approximately 30% of all human cancers. To become biologically active Ras proteins must first undergo farnesylation through a reaction with farnesyl pyrophosphate catalyzed by farnesyl protein transferase (FTase, EC 2.5.1.58). As a result, the design and synthesis of FPP analogues as possible inhibitors or alternate substrates of FTase has attracted wide interest. To generate potential probes of Ras processing, we have synthesized a fluorescent analogue of farnesol and added monophosphate, vinyl phosphonate, α-hydroxy phosphonate, and bisphosphonate head groups to mimic the pyrophosphate moiety of FPP. The synthesis of these compounds, their interaction with FTase, and their impact on Ras expression, will be discussed.

461.

CHEMICAL TOOLS TO INDUCE ALTERED PROTEIN FARNESYL TRANSFERASE SPECIFICITY. ***Sarah A. Reigard****, Diwan S. Rawat, and Richard A. Gibbs, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, Fax: 765-494-1414, sreigard@pharmacy.purdue.edu*

Protein farnesyl transferase (FTase) is the enzyme responsible for the transfer of an isoprenoid from farnesyl pyrophosphate (FPP) to the cysteine of the CaaX box of proteins. FTase is responsible for farnesylating many GTPases including oncogenic mutant Ras, mediating membrane localization and biological activity. Thus FTase is an interesting molecule for cancer therapeutic development. We have developed tools having the ability to alter FTase specificity for peptide

substrate. FPP analogues, including a new class, 7-substituted analogues, have been synthesized via our vinyl triflate route. These compounds exhibit exciting biological activities, ranging from substrates to inhibitors, depending on peptide substrate. Peptide libraries of CaaX boxes of naturally occurring proteins were synthesized using standard Fmoc solid phase chemistry. Using FPP analogues and peptide libraries we can induce changes in FTase specificity. One compound 3-(methybutenyl)-FPP, is a substrate for FTase with certain peptides, but is an inhibitor of prenyl transfer to other peptides.

462.
PROTEIN FARNESYL TRANSFERASE PEPTIDE SELECTIVITY IS ALTERED OR ENHANCED BY ANILINOGERANYL ISOPRENOID LIPID ANALOGUES OF FARENSYL DIPHOSPHATE. ***Jerry M. Troutman**, Zhongwen Wang, Michael J. Roberts, Douglas A. Andres, and H. Peter Spielmann, Department of Biochemistry and Kentucky Center for Structural Biology, University of Kentucky, 800 rose st, UKMC - Combs 122, Lexington, KY 40536, jtrou0@uky.edu*

Protein farnesyl transferase (FTase) catalyzes the addition of a farnesyl group from farnesyl diphosphate (FPP) to a number of important cellular proteins including oncogenic Ras. The farnesyl moiety forms part of the binding pocket for a proteins C-terminal Ca1a2X motif in which C is the cysteine to be modified, a1 and a2 are typically aliaphatic residues and X is usually ser, met or gln. Fifty analogues of FPP were prepared and screened for the ability to transfer to four peptides representing the Ca1a2X motif of biologically important proteins. We found that specific structural features enhance the rate of peptide modification by FTase and that these structural features were not necessarily the same for all four of the peptides studied. This data suggests altered selectivity of analogues for protein substrates and therefore may present a useful strategy to target specific subsets of proteins based on a targets C-terminal Ca1a2X sequence motif.

463.
SOLID-PHASE SYNTHESIS OF LIPIDATED PEPTIDES ON CHLOROTRITYL RESIN: POTENTIAL INHIBITORS OF RAS-CONVERTING ENZYME 1 (RCE1). ***James L Donelson**[1], Sarah Hudon[2], Christine A. Hrycyna[2], and Richard A. Gibbs[1]. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47905, jimmyd@pnhs.purdue.edu, (2) Department of Chemistry, Purdue University*

Ras proteins undergo a series of post-translational modifications, including farnesylation by FTase, endoproteolytic cleavage of the terminal three residues by Rce1, and methylation of the resulting carboxyl group by Icmt. These modifications are essential for proper membrane targeting and functioning of Ras. This laboratory has developed a SPS method to synthesize biotinylated peptides representing the C-terminus of K-Ras4B. A diverse set of farnesyl analogs are attached to these peptides directly on the 2-chlorotrityl solid support. These peptides are then cleaved from the resin with hexafluoroisopropanol and assayed for Rce1 substrate or inhibitory activity. The farnesylated peptides are either assayed in the presence of both Rce1 and Icmt in a coupled radiometric assay, or an assay is used in which a dinitrophenyl lysine residue is incorporated into the peptide and the Rce1-mediated loss of the dinitrophenyl lysine is followed by HPLC.

464.
SONOGASHIRA COUPLING OF ISOPRENOID TRIFLATES WITH ALKYNES: SYNTHESIS AND BIOLOGICAL EVALUATION OF ISOPRENOID- BASED ICMT INHIBITORS. ***Surya K De**[1], Christine A. Hrycyna[2], and Richard A. Gibbs[1]. (1) Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47906, Fax: 765-494-1414, skd125@pharmacy.purdue.edu, (2) Department of Chemistry, Purdue University*

The Sonogashira coupling reaction has been used to introduce alkyne groups into an isoprenoid triflate. This key step has been utilized to produce novel N-acetylfarnesyl cysteine analogues as potential Icmt inhibitors. Since mutant Ras proteins are key causative agents in ~30% of human cancers, potent FTase inhibitors have been already developed as potential cancer chemotherapeutic agents. However, these inhibitors have a little effect on most Ras-transformed tumors, due to the alternative geranylgeranylation of N- and K- Ras. Therefore, there has been growing interest in the two post-prenylation enzymatic steps as alternative targets for the inhibition of Ras protein action. Carboxymethylation is a key step for the proper localization of Ras protiens. The synthesis and biological assay of this set of compounds will be presented.

465.
SUBSTRATE ANALOGUES MODULATE PROTEIN FARNESYL TRANSFERASE ACTIVITY. ***Amanda J. Krzysiak**, Sarah A. Reigard, Diwan S. Rawat, and Richard A. Gibbs, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 Stadium Mall Dr, Heine Pharmacy Building, West Lafayette, IN 47907, krzysiak@pharmacy.purdue.edu*

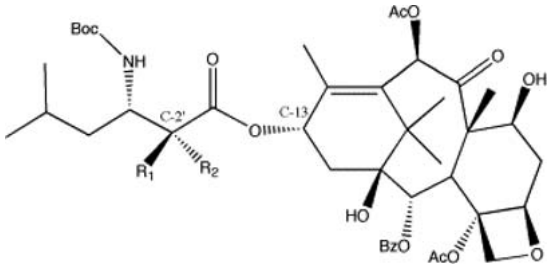
Protein prenylation is a post-translational modification required for proper localization and signaling of many proteins. Protein farnesyl transferase (FTase) is responsible for the transfer of a farnesyl moiety to a protein bearing a CaaX sequence at its C-terminus. The crystallographic characterization of the FTase reaction pathway has illustrated unique features of this enzyme. The FPP substrate forms part of the binding pocket for the CaaX peptide substrate, and before product release, another FPP substrate must enter this pocket. Potent inhibitors of FTase are advancing through clinical trials. Our laboratory has synthesized several substituted farnesyl analogues and has found both substrates and potent inhibitors. We have determined that varying the structure of FPP can alter the peptide substrate specificity of FTase. We have completed the screening of the several known CaaX boxes with our FPP analogues and found evidence that modulating the substituents on FPP alters the substrate/inhibitor profile for FTase.

466.
SYNTHESIS OF ALL-CARBON AFC AND ITS EVALUATION VERSUS ICMT: SULFUR IS IMPORTANT IN BINDING. ***Brian S. Henriksen**, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907, Fax: 765-494-1414, brian@pharmacy.purdue.edu*

Isoprenyl cysteine methyltransferase (Icmt) is a membrane bound enzyme that catalyzes the methyl esterfication of Ras. This methyl esterification is required for the proper localization of Ras to the plasma membrane. Mutant Ras is implicated in 30% human cancers, and 90% of pancreatic cancer. N-Acetyl farnesyl cysteine (AFC) is the minimal synthetic substrate for Icmt. SAR studies on AFC have been initiated with a goal of developing potent Icmt inhibitors as potential anti-cancer agents. These studies have necessitated the synthesis of two analogs, the desthio-AFC isostere (All-carbon AFC) and bis nor desthio AFC (BNDTC), where the sulfur was replaced by carbon , and the cysteine side chain was removed entirely, respectively. The first all-carbon AFCanalog was not a substrate and had a modestIC50 valueof 350 μM, and BNDTC was wholly unrecognized by Icmt. These results combined with previous work clearly demonstrates the importance of the sulfur in the pharmacophore model currently under development for the synthesis of Icmt inhibitors.

467.
BIOSTRUCTURAL ANALYSIS OF ALKYLATED C-2′ TAXOL ANALOGUES. ***Scott A. Johnson**[1], Shala Thomas[2], Paolo Dambruoso[3], Giovanni Appendino[3], Paraskevi Giannakakou[2], and James P. Snyder[1]. (1) Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, sajohn2@emory.edu, (2) Winship Cancer Institute, (3) Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, Italy*

The microtubule stabilizing agent Taxol and its C-2′ analogs show significant changes in cytotoxicity when modified at the C-2′ position on the C-13 sidechain. A computational analysis of the relative binding affinities of a series of C-2′ derivatives evaluated in beta-tubulin using the CScore family of scoring schemes (Sybyl 6.9) was performed. The work resulted in a complete lack of correlation between the assigned scores and actual activities. An alternative computational approach evaluated lipophilic interactions and quantitative steric contacts to develop a structure-activity relationship. It proved to be successful for predicting measured cytotoxicities within the C-2′ series and for assessing the activity of a bridged analog introduced by Sung and Ojima.



468.

DEVELOPMENT OF A NOVEL PROCESS FOR THE PRODUCTION OF PACLITAXEL AND ITS HOMOLOGUES FROM *TAXUS CANADENSIS* . *Zisheng Zhang, Yuheng Wang, and* ***Zdravko Duvnjak****, Department of Chemical Engineering, University of Ottawa, 161 Louis Pasteur, Ottawa, ON K1N 6N5, Canada, Fax: 613-562-5172, zzhang@uottawa.ca, duvnjak@genie.uottawa.ca*

Biologically active compounds from plants play an important role in human beings' relentless search for new drugs against diseases. Although some of those compounds can be synthesized by means of chemical synthesis, most of them, because of their complex structure, are produced through isolation and purification from plant materials. In this study, the production of paclitaxel by leaching from *Taxus canadensis* bark and needles is considered. The effects of various solvents and their amounts, the particle size of plant material and the extraction time in developing a new process for paclitaxel production were taken into account. A couple of interesting systems were developed and exploited in the production of this important anti-cancer compound and its homologues. Comparing to the published data, this newly developed process offers higher product yields, milder operating conditions, lower negative impact on the environment, and better economics.

469.

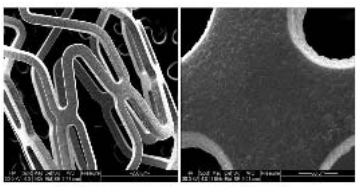
DEVELOPMENT OF TAXOIDS FOR USE AS IMMUNOCONJUGATES: AN SAR STUDY. ***Michael L. Miller***[1]*, Emily E. Cavanagh*[1]*, Tracy P. Marien*[1]*, Elizabeth E. Roller*[1]*, Erkan Baloglu*[1]*, Barbara A. Leece*[1]*, Yelena Kovtun*[1]*, Alain Commercon*[2]*, and Ravi V.J. Chari*[1]*. (1) Department of Chemistry, ImmunoGen, Inc, 128 Sidney Street, Cambridge, MA 02139, Fax: 617-995-2510, michael.miller@immunogen.com, (2) Chemistry Department, Sanofi-Aventis*

The targeted delivery of taxoids (Taxol® and Taxotere®) mediated by their attachment to monoclonal antibodies has emerged as a potentially powerful tool for the development of cancer specific chemotherapeutic agents. The preparation of such an immunoconjugate requires that the desired cytotoxic drug has high potency, acceptable solubility in the conjugation media, and a suitable functional group which may be linked to the antibody. In order to develop taxoids better suited for this purpose, we have performed a series of structure activity relationship (SAR) studies in which modifications at the C2 position were investigated. The design and synthesis of these taxoids and their biological data will be presented.

470.

ELECTROCOATING OF STAINLESS STEEL STENTS FOR THE EXTENDED RELEASE OF PACLITAXEL. ***Regina Okner***[1]*, Ishaiahy Danziger*[1]*, Raia Slivniak*[2]*, and Abraham J. Domb*[2]*. (1) Medicinal chemistry and Natural products, Hebrew University, School of Pharmacy, Faculty of medicine, Jerusalem 91120, Israel, Fax: 972-2-6757076, solnishko@md.huji.ac.il, (2) Medicinal Chemistry and Natural Products, Hebrew University*

Coating of conducting polymers onto metallic surfaces by electrochemical polymerization of designed pyrrole monomers was investigated. This process provided a very thin, uniform and adherent coating onto stainless steel surface. Coatings of up to 2 microns thick were prepared by electropolymerization of N-alkyl pyrrole monomers onto stainless steel 316L plates immersed in an acetonitrile solution at room temperature using a potentiometer. Stents were coated with N-alkyl pyrrole monomers to obtain a coating of about 0.6 microns. The coating was loaded with paclitaxel by immersion of the coated stent into paclitaxel ethanolic solution and solvent evaporation. Paclitaxel was released at a constant rate for over 20 days when placed in isotonic phosphate buffer solution at body temperature. Preliminary biocompatibility study didn't shows any inflammatory response without any noticeable morphology change. Studies now focus on improvement of the elasticity and stability of the coating. This process may have a potential clinical use in drug eluting stents.

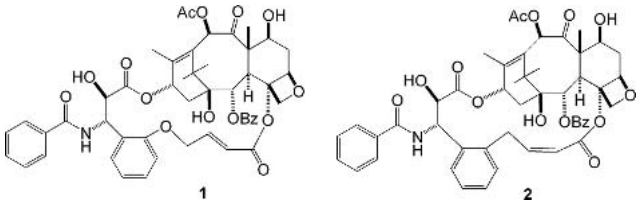


N-alkyl pyrrole based polymer on 316L stent

471.

EVALUATION OF THE BIOACTIVE TUBULIN-BINDING PACLITAXEL CONFORMATION: SYNTHESIS AND BIOLOGICAL EVALUATION OF C4 TO C3′-PHENYL BRIDGED PACLITAXEL ANALOGS. ***Thota Ganesh***[1]*, Susan Bane*[2]*, Rudravajhala Ravindra*[3]*, Natasha Shanker*[3]*, Ami S. Lakdawala*[4]*, James P. Snyder*[4]*, Andrew Norris*[1]*, and David G. I. Kingston*[1]*. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, tganesh@vt.edu, (2) Department of Chemistry, State University of New York, (3) Department of Chemistry, State University of New York at Binghamton, (4) Department of Chemistry, Emory University*

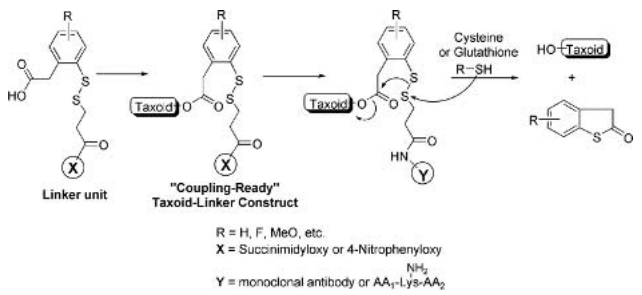
The important anticancer drug Taxol® (paclitaxel) binds to tubulin in a stoichiometric ratio and promotes its assembly into microtubules. The conformation of microtubule-bound drug has been the subject of intense study, and various suggestions have been made. In previous work (*Proc. Nat. Acad. Sci. USA* **2004**, *101*, 10006-10011) we presented experimental and theoretical evidence by the synthesis and analysis of bridged analogs **1** and **2** that Taxol adopts a T-shaped conformation when it is bound to tubulin. In this study we report additional experimental data and calculations that delineate the allowable parameters for effective taxol-tubulin interactions.



472.

NEW APPROACHES TO TUMOR-TARGETED CHEMOTHERAPY: DEVELOPMENT OF "COUPLING-READY" TAXOID-LINKER CONSTRUCTS. ***Xianrui Zhao***[1]*, Jin Chen*[1]*, Claude Commandeur*[1]*, and Iwao Ojima*[2]*. (1) Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, Fax: 631-632-7942, xizhao@ic.sunysb.edu, (2) Institute of Chemical Biology & Drug Discovery and Department of Chemistry, State University of New York at Stony Brook*

A new class of disulfide-containing linkers was designed and the protocol for the synthesis of "coupling-ready" taxoid-linker constructs was also established. The new disulfide-containing linker possesses a carboxylic acid terminus and an active ester terminus. The carboxylic acid terminus was coupled to a taxoid (an anticancer drug) to afford the corresponding "coupling-ready" taxoid-linker construct. Then, the construct was coupled with a lysine-containing tripeptide (a model of monoclonal antibody, tumor-targeting molecule) to form a complete conjugate. These linkers can be applied to any tumor targeting molecules and drugs in principle. Previous model studies have proved the designed efficient release mechanism, i.e., a taxoid was released from the conjugates *via* cleavage of the disulfide bond by cysteine or glutathione, forming the corresponding thiolactone. The key factors in this drug release process and the substituent effect on the kinetics in the model systems will be discussed.



473.

RATIONAL DESIGN, SYNTHESIS AND BIOLOGICAL TESTING OF A NOVEL FAMILY OF TUBULIN POLYMERIZATION INHIBITORS AS POTENTIAL ANTICANCER AGENTS. ***Xin I. Wang***[1], *Qiang Zhang*[2], *Youyi Peng*[1], *Susan M. Keenan*[2], *and William J. Welsh*[2]. *(1) Graduate School of Biomedical Sciences, Department of Pharmacology, University of Medicine and Dentistry of New Jersey, 675 Hoes Ln, Piscataway, NJ 08854, xin.wang@umdnj.edu, (2) Department of Pharmacology, University of Medicine and Dentistry of New Jersey*

The microtubule system of eukaryotic cells is widely regarded as a potent target for the development of anti-cancer agents. The α- and β-tubulin heterodimer is the building block of microtubules and the biochemical target for several chemotherapeutic agents. Many anti-tubulin drugs, such as colchicine and paclitaxel, frequently encounter limitations due to neural and systemic toxicity, poor water solubility and bioavailability, and complicated synthetic pathways and purification steps. We have rationally designed, synthesized and biologically evaluated a series of novel small-molecule compounds targeting the colchicine binding pocket in β-tubulin that show both strong in vitro anti-tubulin polymerization activity as well as cytotoxicity in the low nM range against human HeLa cervix, HCT-116 colon, ZR-75-1 breast, KB-31 cervix carcinoma cell lines. Moreover, these compounds also possess potent cytotoxicity against the multi-drug resistant (MDR+) cell line KB-V1. The present compounds are attractive from the standpoint of medicinal chemistry by virtue of their water solubility, ease of synthesis in high yield, and absence of stereochemistry. As such, these tubulin polymerization inhibitors represent promising candidates as anti-cancer therapeutic agents.

474.

RING C-SECOTAXOIDS AS ANTICANCER AGENTS: STRUCTURE-ACTIVITY RELATIONSHIPS AND TUBULIN BINDING MODE. ***Ana A. Alcaraz***[1], *James P. Snyder*[1], *Giovanni Appendino*[2], *Piergiorgio Bettoni*[2], *Alain Noncovich*[2], *Paraskevi Giannakakou*[3], *Shala Thomas*[3], *Gabriele Fontanta*[4], *and Luigi Gomez-Paloma*[5]. *(1) Department of Chemistry, Emory University, 1515 Dickey Dr, Atlanta, GA 30322, Fax: 404-727-6586, aalcara@emory.edu, (2) Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, (3) Winship Cancer Institute, (4) Indena S.p.A, (5) Dipartimento di Scienze Farmaceutische, Universita di Salerno*

The C-secotaxoid IDN 5390, currently in pre-clinical development, demonstrates ten-fold activity when compared to Taxol. It appears to be capable of promoting tubulin polymerization despite expected reduced binding affinity due to greater conformational flexibility. To investigate this new family of analogs, modifications of the primary and enolic hydroxyls and in the Southern portion of the molecule were pursued. The compounds' cytotoxicity was determined using a 1A9 human ovarian carcinoma cell line and the paclitaxel resistant cell line 1A9/PTX10. These studies demonstrate the similar structure-activity relationships of Taxol and IDN 5390, suggesting a common mechanism of action and a similar T-Taxol binding mode to tubulin. The 3D-QSAR minireceptor concept was applied to rationalize the biological data for the different analogs, and further docking of IDN 5390 and other selected analogs into the electron crystallographic structure of ?-tubulin was done for additional examination of the important interactions in the tubulin binding pocket. IDN 5390 is comparatively similar to Taxol when bound to the pocket of the protein, maintaining the T-taxoid binding conformation and important hydrophobic contacts. The compounds that do not show any activity do not maintain some the hydrophobic interactions that Taxol and IDN 5390 have with the binding pocket in the protein.

475.

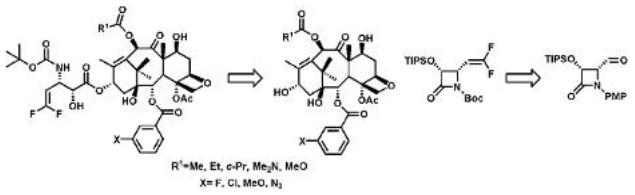
SYNTHESIS AND BIOLOGICAL EVALUATION OF HYDROLYTICALLY STABLE, BIFUNCTIONAL PACLITAXEL CONJUGATES. ***Carlo Ballatore***[1], *Simon E. Aspland*[2], *Rosario Castillo*[1], *Joel Desharnais*[1], *Trisha Eustaquio*[1], *Amos B. Smith III*[3], *and Angelo J. Castellino*[4]. *(1) Acidophil, LLC, 10931 North Torrey Pines Rd., Suite 104, La Jolla, CA 92037, Fax: 858-459-6672, cballatore@acidophil.com, (2) Biology, Acidophil, LLC, (3) Department of Chemistry, University of Pennsylvania, (4) Medicinal Chemistry, Acidophil, LLC*

Paclitaxel, a natural product originally isolated from Taxus brevifolia, is a widely used drug for the treatment of solid tumors. Its mechanism of action involves mitotic arrest through inhibition of microtubule depolymerization. Paclitaxel's major drawbacks are its lack of selectivity, the occurrence of drug resistance and poor water solubility. In an attempt to address these issues we have developed a methodology to attach a tumor-targeting moiety to paclitaxel at C-10 through a hydrolytically stable carbamate linkage while retaining the microtubule effect of the drug. The key step in the synthesis of the C10 carbamates is methyl iodide-mediated activation of a paclitaxel-carbonylimidazole intermediate. Representative compounds and biological data will be reported.

476.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL C3&PRIME;-DIFLUOROVINYL TAXOIDS. ***Larissa Kuznetsova***[1], *Paula Pera*[2], *Ralph J. Bernacki*[2], *and Iwao Ojima*[1]. *(1) Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, lkuznets@ic.sunysb.edu, (2) Grace Cancer Drug Center, Roswell Park Cancer Institute*

Unique properties of fluorine often critically increase the intrinsic activity, the chemical and metabolic stability, and the bioavailability of promising biologically active compounds. As our continuing studies on the structure-activity relationships (SAR) of fluorine-containing taxoids, we designed various C3-difluorovinyl taxoids. The synthesis and biological evaluations of these novel fluorine-containing taxoids will be discussed.



477.

TRI-METHOXY BENZYLIDENE SUBSTITUTED 1, 3-DIHYDRO-INDOL-2-ONE ANALOGS AS CYTOTOXIC AND ANTI-MICROTUBULE AGENTS. ***Bulbul Pandit***, *Ping Chen, Yanjun Sun, Pui-Kai Li, and April Hildebrand, Department of Medicinal Chemistry and Pharmacognosy, The Ohio State University, College of Pharmacy, 500 W 12th Avenue, Columbus, OH 43210, Fax: (614) 688-8556, pandit.6@osu.edu*

Compounds that contain 2-indolinone moieties have been reported to exhibit diverse pharmacological activities. We have recently reported an indoline 2-one containing compound [3-(3-Hydroxy-benzylidene)-6-methoxy-1, 3-dihydro-indol-2-one(OSU 111)]with anti-proliferative, anti-mitotic and apoptosis inducing activities. It displayed potent cytotoxicity with IC50 = 0.5-0.9 uM against human hormone independent prostate and breast carcinoma cell lines. The inhibition of proliferation correlated with in vitro polymerization inhibiting activity and cell cycle arrest in G2/M phase of prostate carcinoma cells. The compound was identified to be a colchicine site binder on tubulin. In our continuing research for potent 2-indolinone analogs, a series of di- and tri-methoxy benzylidene substituted 1, 3-dihydro-indol-2-one analogs were synthesized. Structure activity relationship study showed that 6-methoxy substitution on the dihydro-indol-2-one ring contributes to a major extent for maximal activity while trimethoxy-benzylidene group was optimal for activity. Structures, synthesis and biological activities of the tri-methoxy benzylidene substituted 1, 3-dihydro-indol-2-one analogs would be presented.

478.

CYTOTOXIC ACTIVITIES OF IMIDO-SUBSTITUTED 2-CHLORO-1,4-NAPHTHOQUINONE DERIVATIVES ON THREE HUMAN PROSTATE CANCER CELL LINES. ***Oladapo Bakare***, *Department of Chemistry, Howard University, 525 College Street, Howard University, Washington, DC 20059, Fax: 202-806-5442, obakare@howard.edu, Robert L. Copeland Jr., Department of Pharmacology, Howard University, Yasmine Kanaan, Department of Microbiology and Cancer Center, Howard University, and Leon H. Zalkow, School of Chemistry and Biochemistry, Georgia Institute of Technology*

Prostate cancer is the most common noncutaneous cancer in men. Although androgen ablation is highly effective palliative therapy, all patients eventually relapse due in part to the presence of androgen independent prostate cancer cells. It has been suggested that inhibition of Ras function in conjunction with standard hormone ablation therapy may prove beneficial in treating advanced

hormone-refractory prostate cancer. We have previously reported some imido-substituted 2-chloro-1,4-naphthoquinone analogs as MEK1 inhibitors of the Ras-MAPK pathway. In order to investigate their cytotoxicity, particularly in hormone-refractory prostate cancer cells, we have studied their effects on one androgen-dependent, LNCaP, and two androgen-independent, PC3 and DU145, human prostate cancer cell lines. The open chain analogs showed more potency in all three cell lines compared with the cyclic imido-substituted derivatives. For example, 2-chloro-3-diacetylamino-1,4-naphthoquinone and 2-chloro-3-dibutyrylamino-1,4-naphthoquinone showed the strongest inhibition in all cell lines with an IC50 of 6.0 μM and 7.5 μM, respectively, for PC3.

479.

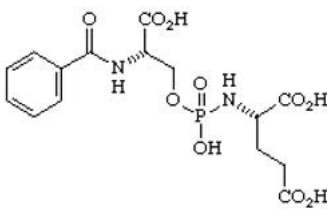
DESIGN AND SYNTHESIS OF CURCUMIN ANALOGUES AS ANTI-PROSTATE CANCER AGENTS. ***Li Lin***[1]*, Qian Shi*[2]*, Ching-yuan Su*[2]*, Charles C. -Y. Shih*[2]*, Kenneth F. Bastow*[1]*, and K. H. Lee*[1]*. (1) Division of Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill, School of Pharmacy, Chapel Hill, NC 27599-7360, llin@email.unc.edu, (2) Androscience Corporation*

Curcumin is the major constituent in the rhizome of Curcuma Longa (Zingiberaceae) commonly named as turmeric, which has been used as spice and indigenous medicine in Asia countries since over two thousand years ago. It possesses numerous biological activities. Curcumin was found to inhibit the growth of prostate cancer cells in vitro and in vivo. In our lab, we have been synthesizing curcumin analogues possessing anti-prostate cancer activity, and developed two potent anti-AR agents, dimethylated curcumin and 4-ethoxycarbonylethyl curcumin. In the continuing studies on the curcumin analogues, we aim to find more exciting analgoues as anti-prostate cancer agents and resolve some problems associated with the previously developed potent anti-AR curcumin analogues. In this study we have been focusing on the modifications on the phenyl rings as well as the linker of curcumin skeleton. Some conjugates were synthesized. The tautomerization problem of 4-ethoxycarbonylethyl curcumin have been resolved by designing enol-keto analogue and diketo analogue.

480.

MOLECULAR PRUNING STUDIES ON A PHOSPHORAMIDATE GAMMA-DIGLUTAMATE ANALOG INHIBITOR OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN. ***Jack Maung****, Department of Chemistry and Biochemistry, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132*

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, the role of its glutamate carboxypeptidase activity in prostate cancer has been relatively under explored. Known substrates for PSMA are limited to acidic dipeptides such as gamma-glutamyl derivatives of folic acid and the neuropeptide N-acetylaspartylglutamate (NAAG). Recently we identified a phosphoramidate analog of gamma-diglutamate as a potent inhibitor of PSMA (Ki = 5 nM). Molecular pruning of this inhibitor revealed the importance of the C-terminal glutamate, serine carboxylate, and the benzamide moiety. Synthesis and inhibition data for the diglutamate analogs of this pruning study will be presented.



481.

OPTIMIZING THE POTENCY OF PHENETHYLPHOSPHONAMIDATE INHIBITORS OF PROSTATE-SPECIFIC MEMBRANE ANTIGEN. ***David W. G. Wone***[1]*, Jenni A. Rowley*[1]*, Jack Maung*[1]*, Albert W. Garofalo*[2]*, and Clifford E. Berkman*[1]*. (1) Department of Chemistry and Biochemistry, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, Fax: 415-338-2384, david.wone@elan.com, (2) Chemistry, Elan Pharmaceuticals*

A notable discovery in prostate cancer research is the identification of an over-expressed membrane-bound, cell surface protein, namely, prostate-specific membrane antigen (PSMA). PSMA is strongly expressed on the surface of prostate cancer cells and is also expressed on the neovasculature of a variety of non-prostatic solid malignancies. Even though the enzymatic activities have been identified for PSMA, there are only a few published studies that have identified potent inhibitors of this enzyme. In this study, a series of eight substituted N-2-phenylethylphosphonyl derivatives of glutamic acid were prepared as putative inhibitors of PSMA. Specifically, the lead compound N-[hydroxy(2-phenyl)ethylphosphinyl]-L-glutamic acid was optimized based upon the Topliss approach. The most potent inhibitor of the set of eight compounds in the PSMA assay was the 3,4-dichlorophenyl analog. The synthesis and inhibition data of these N-2-phenylethylphosphonyl derivatives of glutamic acid will be presented.

482.

POLYMER SUPPORTED REAGENTS AND DIAGNOSTICS FOR BIOMEDICAL IMAGING: APPLICATIONS IN PROSTATE CANCER. ***Jiezhen Li****, Department of Chemistry, Northeastern Universty, 360 Huntington Ave., Boston, MA 02115, Fax: 617-373-8795, jiezhen.li@gmail.com, and Graham B. Jones, Department of Chemistry and Chemical Biology, Northeastern University*

Despite improvements in both primary surgical and radiation management of prostate cancer, it remains as the second leading cause of cancer mortality in men living in the United States. Improved response rates reported for combination chemotherapeutic regimens in metastatic disease, provides the basis for the use of combined systemic and local therapy initially in men with clinically localized prostate cancer who are at high risk for harboring occult micrometastatic disease. It is commonly recognized that improvements in our ability to deliver localized chemotherapies are needed and antibody mediated approaches have received considerable attention. Though interest in prostate stem cell antigen as a target for antibody conjugates has been investigated, there is considerable evidence to suggest prostate membrane specific antigen [PSMA] as a viable target. The focus of our investigation is to develop general protocols and platforms for the application of antibodies to this target. A key feature is the use of differentially functionalized polymers which promote internalization of conjugates and associated fluorophore tags to permit tracking.

483.

RADIOIMAGING AND RADIOTHERAPY OF PROSTATE AND BLADDER CANCER. ***A. Elizabeth Tedesco****, Zaihleen Keller, Pui Yuk Yan, and Ahamindra Jain, Chemistry, UC Berkeley, 325 Latimer Hall, Berkeley, CA 94720-1460, bethib002@hotmail.com*

TRP-M8 is a physiological receptor that detects cold temperaturechanges in the environment, which also happens to be found in elevated levelsin prostate and bladder cancer cells. TRP-M8 thus responds to drug ligands(e.g., menthol and icilin) that produce sensations of cold. The functional role, ifany, of TRP-M8 in prostate and bladder cancer cells is not known.However, the selective expression of TRP-M8 in the prostate makes this proteina target for diagnosis and treatment of prostate malignancies.The acid chloride derivative of menthol required forpreparation of the amides is readily-available from optically-pure menthol.4-Bromo-2-methylaniline is commercially available and following coupling to theacid chloride, halogen exchange assisted by tetrakis(triphenylphosphine)palladium(0) will afford the iodinated aryl amide. The iodinated compound will beconverted to the key trimethylstannane intermediate, again on treatment withpalladium (0) catalyst.

484.

DATABASE SEARCHING AND PHARMACOPHORE STUDIES FOR INHIBITORS OF HMDM2: A PROMISING APPROACH FOR DRUG DISCOVERY?. ***Haizhen Zhong***[1], *J. Phillip Bowen*[1], *and Heather A. Carlson*[2]. *(1) Center for Drug Design, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, 401 New Science Building, PO Box 26170, Greensboro, NC 27402-6170, h_zhong@uncg.edu, (2) Department of Medicinal Chemistry, University of Michigan, Ann Arbor*

The interaction between human p53 and hMDM2 is an important event in controlling cell growth. Many studies have suggested that inhibiting the overexpressed hMDM2 in cancerous cells could release p53 from its complex with hMDM2 and initiate cell-cycle arrest or apoptosis. The MPS method was used to identify potential inhibitors for hMDM2. Two receptor-based pharmacophore models for the anticancer target, hMDM2, were developed based on 2000 snapshots from 2-ns MD simulations of hMDM2 alone and in complex with p53. Database searching against an NCI database of 32,557 structures with cancer test data gave 22 hits for the pharmacophore model developed from the p53-hMDM2 complex and 16 hits for the model developed from hMDM2. This suggests that inhibition of hMDM2 might be the mechanism of action for these molecules. Further comparison of these two lists shows that the uncomplexed hMDM2 pharmacophore matched more than half of the hits that were derived from complex-derived pharmacophore model. The biological activities of these hits against hMDM2 are currently under investigation. This methodology holds the promise of finding lead inhibitors even before crystal structures of bound protein-ligand complexes are solved. Using this promising new technique could shorten the length of time in drug discovery.

485.

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SMALL-MOLECULE INHIBITORS OF BCL-2 AND BCL-XL PROTEINS. ***Ke Ding***[1], *Zaneta Nikolovska-Coleska*[1], *Renxiao Wang*[1], *Manchao Zhang*[2], *Mei Lan Liu*[2], *Dajun Yang*[2], *York Tomita*[3], *and Shaomeng Wang*[1]. *(1) The Department of Internal Medicine and the Department of Medicinal Chemistry, The University of Michigan, 1500 E. Med.Center Dr, Ann Arbor, MI 48109-0934, keding@med.umich.edu, (2) The Department of Internal Medicine, University of Michigan Medical School, (3) Lombardi Cancer Center, Georgetown University*

The antiapoptotic Bcl-2 family proteins play a significant role in human malignancies and other proliferative diseases and they have become attractive targets to discover effective anticancer therapies. Gossypol was recently identified as a potent Bcl-2 and Bcl-xL inhibitor. Based on the three-dimensional structure of gossypol in complex with Bcl-xL protein and computational structure-based modeling, a series of novel small-molecule inhibitors were designed and synthesized. Some of these new inhibitors are much more potent than gossypol in their binding to Bcl-2 and in inhibition of cell growth in cancer cells with high levels of Bcl-2 or Bcl-xL protein. For example, Ke-iso-33 has a Ki value of 36 nM for its binding to Bcl-2 and has an IC50 value of 100 nM in inhibition of cell growth in both prostate cancer PC-3 cells and breast cancer MDA-MB-231 cells. These highly potent small-molecule inhibitors of Bcl-2/Bcl-xL may have great therapeutic potential as an entirely new class of anticancer agents, either alone or in combination with chemotherapeutic agents.

486.

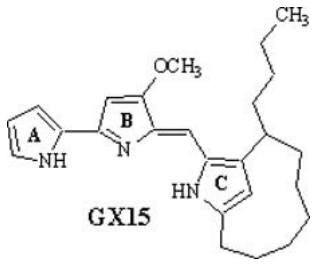
INDUCTION OF APOPTOSIS IN CANCER CELLS WITH SMALL MOLECULE INHIBITORS OF BCL-XL AND BCL-2. ***Chung-Wai Shiau***, *Chih-Cheng Yang, Kuen-Feng Chen, Jui-Wen Huang, Changshi Chen, and Ching-Shih Chen, Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Ave, Columbus, OH 43210, shiau.4@osu.edu*

Bcl-xL and Bcl-2 are antiapoptotic members of the bcl-2 family which are often overexpressed in cancers and which inhibit apoptosis by binding to proapoptotic proteins, such as Bak and Bax. Thus, Bcl-xL/Bcl-2 represent promising targets for anticancer drug development. We demonstrate that the peroxisome proliferator activated receptor-γ (PPARγ) agonist, troglitazone (TG), induces apoptosis in cancer cells by inhibiting Bcl-xL/Bcl-2, and that this activity is independent of PPARγ activation. A close structural analog of TG was synthesized, which was devoid of ability to activate PPARγ, but retained apoptosis-inducing activity. TG and its analogue induced apoptosis in PC-3 (PPARγ-expressing) and LNCaP (PPARγ-deficient) prostate cancer cells irrespective of PPARγ status. Fluorescence polarization assay showed that TG and its analogue disrupted interactions of Bcl-2 and Bcl-xL with a Bak BH3-domain peptide. These findings provide a proof of concept for the design of a new class of agents that induce apoptosis through targeting Bcl-xL and Bcl-2.

487.

PRELIMINARY SAR STUDY ON A NOVEL INHIBITOR OF THE APOPTOSIS SUPPRESSOR BCL PROTEINS. ***Laurent Bélec***[1], *Kenza Daïri*[1], *Lionel Dumas*[1], *Gerson G. Gonzalez*[1], *Jean-François Lavallée*[1], *Mathieu Lemay*[1], *Valérie Perron*[1], *Élise Rioux*[1], *Sasmita Tripathy*[1], *Angela Babineau*[2], *Sylvie Bailly*[2], *Helen Chan*[2], *Gang Chen*[2], *Gaétan Gagnon*[2], *Anne Jang*[2], *Abdelkrim Khadir*[2], *Murthy Madiraju*[2], *Richard Marcellus*[2], *Denis Paquette*[2], *Anne Roulston*[2], *Nancy Steenaart*[2], *Mark Watson*[2], *Zhiying Zhang*[2], *Giorgio Attardo*[1], *Pierre Beauparlant*[2], *and Gordon Shore*[1]. *(1) Department of Medicinal Chemistry, Gemin X Biotechnologies Inc, 3576 Avenue du Parc, suite 4310, Montréal, QC H2X 2H7, Canada, Fax: 514-281-1065, lbelec@geminx.com, (2) Department of Biology, Gemin X Biotechnologies Inc*

We have recently reported the discovery of **GX15**, a novel inhibitor of the Bcl-2 family of apoptosis suppressor proteins. This naturally occurring compound was shown to exhibit cytotoxicity in several cancer cell lines. The structure-activity-relationship (SAR) study has been initiated by preparing derivatives from the natural product and by total synthesis, targeting at first C ring modifications. In this poster, we would like to present our initial findings regarding the SAR obtained for cytotoxicity. The inhibitory profile towards Bcl antiapoptotic proteins will be presented for the best derivatives.



GX15

488.

STRUCTURE AND FUNCTION OF ING2 PLANT HOMEODOMAIN. *Foteini Davrazou*[1], *Or Gozani*[2], *Glenn D. Prestwich*[3], *and* ***Tatiana G. Kutateladze***[1]. *(1) Department of Pharmacology, University of Colorado HSC, Aurora, CO 80045, Fax: 303-724-3663, Foteini.Davrazou@uchsc.edu, Tatiana.Kutateladze@uchsc.edu, (2) Department of Cell Biology, Harvard Medical School, (3) Department of Medicinal Chemistry, University of Utah*

The putative tumor suppressor ING2 negatively regulates cell proliferation by enhancing acetylation of p53, a major tumor suppressor, which is mutated in about half of all human cancers. The PHD finger of ING2 recognizes phosphoinositides, and this interaction has been suggested to control the ability of ING2 to modulate the p53 function. Here, the solution structure of the ING2 PHD finger was elucidated by nuclear magnetic resonance spectroscopy. The PHD finger specificity for the phosphorylated inositol head groups was investigated. The binding site residues were identified by 1H/15N chemical shift changes in NMR spectra of the protein induced by inositol polyphosphates. Mutations of the C-terminal cluster of basic residues disrupted the inositol phosphate binding. Affinities of the wild-type and mutant PHD proteins for inositol phosphates were determined by fluorescence spectroscopy and NMR. The binding mode of PHD finger was compared to that of other phosphoinositide recognizing modules.

489.

THIAZOLYL HYDRAZONES AS NOVEL INHIBITORS OF EIF4E/EIF4G INTERACTION FOR CANCER THERAPY. ***Han Chen***[1], *Nathan Moerke*[2], *Fred Harbinski*[1], *Huseyin Aktas*[1], *Gerhard Wagner*[2], *Michael Chorev*[1], *and Jose A. Halperin*[1]. *(1) Laboratory for Translational Research, Harvard Medical School, One Kendall Square, Bldg. 600 3rd Floor, Cambridge, MA 02139, Fax: 617-621-6148, han_chen@hms.harvard.edu, (2) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School*

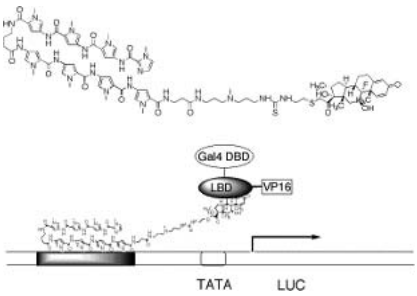
Eukaryotic initiation factor 4E (eIF4E) is a highly conserved 25 kDa cap-binding protein, which provides a docking site for binding of eIF4G (the scaffolding protein) within the eIF4E/eIF4G/eIF4A complex (eIF4F complex). Inhibitors of the

eIF4E/eIF4G interaction will prevent formation of eIF4F complex, and would predominantly inhibit translation of mRNAs involved in tumor growth and survival, as those mRNAs require elevated levels of eIF4F for efficient translation. For this reason inhibitors of eIF4E/eIF4G interaction are anticipated to possess therapeutic potential in the treatment of proliferative disorders, and are promising clinical candidates for anticancer drug development. A thiazolyl hydrazone derivative was identified in a cell free fluorescent polarization-based high throughput assay developed for screening chemical libraries for inhibitors of eIF4E/eIF4G interaction. This assay screens for compounds that competitively inhibit binding of fluorescent-labeled peptide derived from the consensus eIF4E binding motif in eIF4G to purified recombinant eIF4E. We will report on our structure-activity relationship studies to identify the active pharmacophore and to further improve the potency of the lead thiazolyl hydrazone.

490.

TRANSCRIPTION ACTIVATION BY A SYNTHETIC MOLECULE IN LIVING MAMMALIAN CELLS. ***Peng Yu***, *Bo Liu, and Thomas J. Kodadek, Department of Internal Medicine and Molecular Biology and the Center for Biomedical Inventions, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390, Peng.Yu@utsouthwestern.edu*

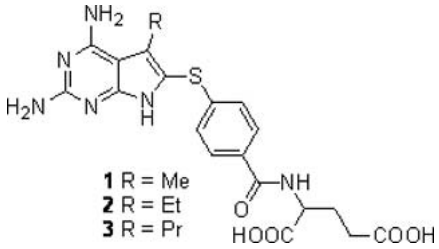
Synthetic molecules that promote the expression of specific genes would be powerful tools in biological research and could be potential drug candidates as well. The design of artificial transcriptional activators is based on the assumption that a synthetic molecule containing a DNA-binding motif and a transcriptional-complexes-targeting motif should mimic the biological function of a natural transcription activator. This principle has been proven in cell free systems. Here we report that a synthetic molecule is able to penetrate the mammalian cell membrane and up-regulates the expression of a reporter gene in living cells. A DNA-binding reagent imidazole-pyrrole hairpin polyamide is conjugated to a dexamethasone derivative. When this molecule penetrates the cell membrane, the dexamethasone moiety binds to a gene expression regulator, Gal4DBD-GRLBD-VP16, triggers the nuclear translocation of the complex, and activates the transcription of luciferase reporter with multiple hairpin polyamide binding sites in the promoter.



491.

DESIGN AND SYNTHESIS OF N-[4-(2,4-DIAMINO-5-PROPYL-7H-PYRROLO[2,3-*D*]PYRIMIDIN-6-YLSULFANYL)-BENZOYL]-L-GLUTAMIC ACID AS A POTENT INHIBITOR OF DIHYDROFOLATE REDUCTASE AND AS AN ANTITUMOR AGENT. *Aleem Gangjee[1], **Hiteshkumar Jain**[1], Sherry F. Queener[2], and Roy L. Kisliuk[3]. (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) Department of Pharmacology and Toxicology, Indiana University, (3) Department of Chemistry, School of Medicine, Tufts University*

N-[4-(2,4-diamino-5-methylpyrrolo[2,3-*d*]pyrimidin-6-yl)thio]benzoyl]-L-glutamic acid (**1**) was recently discovered by Gangjee *et al.* as a potent dual inhibitor of dihydrofolate reductase (DHFR) and thymidylate synthase (TS) with IC_{50} values in the 10^{-7} M range. Compound **1** also demonstrated potent antitumor activity against the growth of several NCI human tumor cell lines in culture with IC_{50} values of 10^{-8}-10^{-7} M or less. Similar results were obtained with the C5-ethyl homolog **2**. Molecular modeling suggests that homologation of the C5-methyl to a propyl could further enhance the binding to the target enzyme(s) and hence the antitumor activity. Thus compound **3**, the C5-propyl homolog of compounds **1** and **2** was designed and synthesized. The synthesis and biological activity of compound **3** will be presented and discussed.



492.

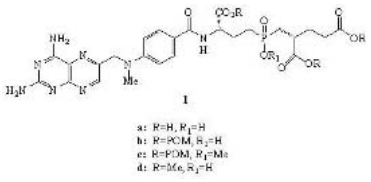
FOLATE TARGETED CHEMOTHERAPEUTICS AND THEIR SIZE-DEPENDENT PENETRATION INTO SOLID TUMOR MASS. ***Erina Vlashi**, **Walter A. Henne Jr.**, **Derek Doorneweerd**, **Andy R. Hilgenbrink**, and Philip S. Low, Department of Chemistry, Purdue University, 460 Oval Drive, West Lafayette, IN 47907, vlashi@purdue.edu, whennejr@purdue.edu, doornewe@purdue.edu, ahilgen1@purdue.edu*

Cell membrane associated folate receptor (FR) is overexpressed on a wide variety of cancers and thus serves as an attractive target to selectively deliver chemotherapeutic and imaging agents. To this end, we have constructed several folate tethered chemotherapeutic compounds that demonstrate IC50 values ranging from 4 -100 nM in FR+ cancer cell lines. However, the success of folate targeted delivery of toxic agents to FR+ tumors is dependent on their size and relative ease of penetration into solid tumor masses. To better understand the dynamics of folate-targeted macromolecules in solid tumor tissue, a series of rhodamine labeled polyethylene glycol (PEG)-folate conjugates of varying sizes were synthesized. These conjugates have been tested in vivo for their binding/penetrating ability into FR+ solid tumor tissue using two-photon microscopy. Accordingly, these studies will guide further refinements in dosing and optimal size of folate linked chemotherapeutic agents.

493.

PRODRUG STRATEGY FOR INTRACELLULAR INHIBITION OF FOLYLPOLYGLUTAMATE SYNTHETASE. ***Yan Feng*** *and James K. Coward, Departments of Medicinal Chemistry & Chemistry, University of Michigan, 930 N. University, Ann Arbor, MI 48109-1055, fengyan@umich.edu*

Folylpoly-γ-glutamate synthetase (FPGS, EC 6.3.2.17) catalyzes the ATP-dependent ligation of glutamic acid to reduced folates as well as to anticancer drugs such as Methotrexate, Lometrexol, and Alimta. FPGS deficiency leads to resistance in antifolate chemotherapy and also cells which are not viable unless grown in medium containing the products of one-carbon metabolism. Pseudopeptide **1a**, a mimic of the tetrahedral intermediate formed in the reaction, is a potent FPGS inhibitor. However, it is ineffective as an inhibitor of cell growth in culture, presumably due to its inability to cross the cell membrane. In order to overcome this transport barrier, **1a** has been converted to the lipophilic prodrugs, **1b-d**, as once inside the cell, hydrolysis of **1b-d** to **1a** should result in inhibition of FPGS. This research was supported by a grant from the National Cancer Institute (CA28097).

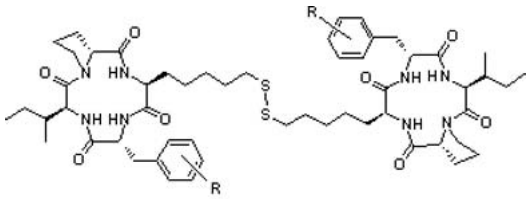


494.

DESIGN, SYNTHESIS AND HDAC INHIBITORY ACTIVITY OF CYCLIC TETRAPEPTIDES WITH MODIFICATIONS AT THE TYR RESIDUE. ***Binoy Jose**[1], Tamaki Kato[2], Norikazu Nishino[2], Yuko Sumida[1], and Minoru Yoshida[3]. (1) CREST Research Project, Japan Science and Technology Agency, Saitama 332-001, Japan, (2) Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, (3) Chem. Genet. Lab, RIKEN*

Histone deacetylases (HDACs) are integral nuclear isozymes that modulate the deacetylation of specific acetylated lysine residues. An increasing number of HDACs are being identified in different species, which is classified into three distinct classes. A number of HDAC inhibitors have been identified which include natural product trichostatin A (TSA), naturally occurring cyclic tetrapeptides and

synthetic compounds such as CHAPs. Of the reported HDAC inhibitors, TSA can inhibit HDAC1 and HDAC6 in almost equal intensity. Synthetic inhibitors with cyclic tetrapeptide cap and hydroxamic acid functional group (CHAP) and the natural compound TPX are less potent toward HDAC6 in comparison with HDAC1. Our recently reported inhibitor SCOP inhibited HDAC6 and HDAC4 more effectively than HDAC6 and HDAC8. Here we report the synthesis and inhibitory activity of more subtype HDAC inhibitors based on SCOP core structure with modifications at the Tyr residue. The Tyr residue was modified mainly with different substituents at o, m, and p positions of the phenyl group. The modifications were predominantly carried out using a trifluoromethyl group as the substituent.



R = 4-OMe, 3-OMe, 3,4-(OMe)$_2$, 2-CF$_3$, 3-CF$_3$, 4-CF$_3$

495.

DEVELOPMENT OF SULFONAMIDE COMPOUNDS AS POTENT METHIONINE AMINOPEPTIDASE TYPE II INHIBITORS WITH ANTIANGIOGENIC PROPERTIES. ***Megumi Kawai***[1], *Nwe Y. BaMaung*[1], *George S. Sheppard*[1], *Steve D. Fidanze*[1], *Scott A Erickson*[1], *William J. Sanders*[1], *Anil Vasudevan*[2], *Chang Park*[1], *Charles Hutchins*[1], *Kenneth M. Comess*[3], *Douglas M. Kalvin*[4], *Jieyi Wang*[1], *Qian Zhang*[1], *Pingping Lou*[1], *Lora Tucker-Garcia*[1], *Jennifer Bouska*[1], *Randy L. Bell*[1], *Richard Lesniewski*[1], *and Jack Henkin*[1]. *(1) Cancer Research, Global Pharmaceutical R & D, Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, IL, IL 60064, Fax: 847-937-8378, megumi.kawai@abbott.com, (2) Medicinal Chemistry Technology, Abbott Laboratories, (3) Department of Biological Screening, Abbott Laboratories, (4) Combinatorial Chemistry; Dept. 4CP, Abbott Laboratories*

Recently much attention has been focused on the biological role of methionine aminopeptidases (MetAPs). MetAPs fulfill a crucial role in the biosynthesis of proteins by removing the N-terminal methionine residue from nascent polypeptides. Eukaryotic cells contain two different types of MetAPs: type 1 and type 2. The importance of these MetAPs has been highlighted by findings that MetAP2 is a target molecule for anti-angiogenic/antitumor agents, such as fumagillin, ovacilin and TNP-470. We have screened molecules for inhibition of MetAP-2 as a novel approach toward antiangiogenesis and anticancer therapy using Affinity selection/Mass spectrometry (ASMS) employing MetAP2 loaded with Mn2+ as the active site metal. After a series of anthranilic acid sulfonamides with micromolar affinities was identified, chemistry efforts were initiated. The micromolar hits were quickly improved to potent nanomolar inhibitors by chemical modifications guided by insights from X-ray crystallography.

496.

SULFONAMIDES OF 5,6-DISUBSTITUTED ANTHRANILIC ACIDS AS POTENT INHIBITORS OF METHIONINE AMINOPEPTIDASE-2 (METAP2). ***Robert Mantei***[1], *Gary T. Wang*[1], *George S. Sheppard*[1], *Megumi Kawai*[2], *Jason S. Tedrow*[3], *David M. Barnes*[1], *Chang Park*[1], *Jieyi Wang*[1], *Qian Zhang*[1], *Pingping Lou*[1], *Lora A. Garcia*[1], *Melinda S. Yates*[1], *Jennifer J. Bouska*[1], *and Randy L. Bell*[1]. *(1) R47A, Cancer Research, Global Pharmaceutical R & D, Abbott Laboratories, 100 Abbott Park Road, Dept. R47A, AP10-307, Abbott Park, IL 60064, Fax: 847-935-5165, robert.a.mantei@abbott.com, (2) Cancer Research, Global Pharmaceutical R & D, Abbott Laboratories, (3) N/A*

Methionine aminopeptidase-2 (MetAP2) carries out post-translational processing of nascent proteins by cleaving the N-terminal methionine of substrate proteins. The aminopeptidase activity of MetAP2 appears to play an important role in cancer. MetAP2 is induced by growth factors, mitogens and oncogenes and is highly expressed in cancer cells. Small molecule MetAP2 inhibitors block cell proliferation and induce cell cycle arrest in tumor cell lines and activated endothelial cells, but have limited effects on normal cell types. MetAP2 inhibitors have also been shown to block tumor growth in animal models. We have developed a series of 5,6-disubstituted anthranilic acid sulfonamides that are potent inhibitors of MetAP2 activity. The design, synthesis, biological activities and structural analysis of these compounds will be discussed.

497.

MOLECULAR MODELING OF ASPARAGINE SYNTHETASE. ***Robert Humkey***, *Yun Ding, and Nigel G.J. Richards, Department of Chemistry, University of Florida, Box 117200, Gainesville, FL 32611-7200, humkey@qtp.ufl.edu*

The enzyme asparagine synthetase (AS) catalyzes the biochemical conversion of aspartate to asparagine. A direct correlation between the development of drug-resistance in leukemia and the over-expression of AS has recently been observed. This connection has led to the belief that an inhibitor of AS would be able to reverse the effects of drug resistance in leukemia cells. A molecular model of E. coli asparagine synthetase-B (AS-B) will be presented as the first complete structural insight into the critical active sites of this enzyme. The model is being used to examine the different effects of the binding of structurally analogous inhibitors and non-inhibitors on AS-B, as well as identifying the specific active site interactions that can be used to facilitate inhibitor binding.

498.

NOVEL INHIBITORS OF CARBONYL REDUCTASE. ***Berea Williams***, *Corianton L. Larson, Andrew Slupe, Kristofor Olson, Sanela Begic, Laura Lee, and Henry A. Charlier Jr., Department of Chemistry, Boise State University, 1910 University Drive, Boise, ID 83725-1520, willbere2000@yahoo.com*

Anthracyclines are effective antineoplasticagents, but are known to cause a potentially lethal chronic cardiomyopathy, which severely limits their use. Anthracycline cardiotoxicity has been linked to the formation of a metabolite catalyzed by carbonyl reductase (CR). Since the metabolite does not possess the antineoplastic properties of its parent anthracycline, the action of CR may also contribute to drug resistance. In an effort to prevent the CR derived formation of the cardiotoxic metabolite, CR inhibitor candidates were tested. Of the compounds that were tested, two were found to be noncompetitive inhibitors against both coenzyme and carbonyl substrates, with KI values in the low micromolar range. The inhibition patterns suggest that the inhibitors bind to multiple enzyme forms. Intrinsic protein fluorescence quenching studies demonstrated that the inhibitors bind to at least the free enzyme and to an enzyme/product binary complex with Kd values similar to the KI values. Supported by NIH/P20RR16454, NIH/R15CA102119-01.

499.

INVESTIGATION OF POLYAMINE ANALOGS ON THE GROWTH OF MCF-7 BREAST CANCER CELL LINES. *Michelle Piel,* ***Kristina Thornburg***, *Christopher Higgins, Francis Charles Mayville Jr., and Peter Leonard, Natural Science Department, DeSales University, 2755 Station Avenue, Center Valley, PA 18034, Fax: 610-282-0525, fcm0@desales.edu, fcm0@desales.edu*

In this study, we are synthesizing new polyamine analogs using 1,4-diaminobutane (putrescine) as the template. The new polyamine derivatives will contain two, three, or four carbon primers at the each amino end of the putrescine. 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 then 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.

500.

POLYAMINE CONJUGATES OF SERINE, 4-THIAZOLIDINONE AND THIAZOLIDINE-4-CARBOXYLIC ACID: SYNTHESIS AND GROWTH INHIBITORY EFFECTS ON HUMAN PROSTATE CANCER CELL LINES. ***Veeresa Gududuru***[1], *Eunju Hurh*[2], *James T. Dalton*[2], *and Duane D. Miller*[1]. *(1) Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 847 Monroe Ave, Memphis, TN 38163, (2) Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, OH 43210*

We showed that serine amide phosphates (SAPs), derivatives of lysophosphatidic acid (LPA) represent a class of cytotoxic phospholipids that are effective and potent in killing prostate cancer cells. Although many of these compounds showed significant cytotoxicity, they were non-selective. To improve the selectivity and antiproliferative activity of SAPs, we designed a new series of 4-thiazolidinone amides, in which the 4-thiazolidinone moiety was introduced as a phosphate mimic. However, these 4-thiazolidinone derivatives demonstrated less cytotoxicity in prostate cancer cells despite improved selectivity over nontumor cells. Further optimization of the thiazolidinone pharmacophore in terms of cytotoxicity and selectivity, led us to the discovery of third-generation 2-arylthiazolidine-4-carboxylic acid amides. These compounds were potent cytotoxic agents with IC50 values in the low micromolar concentration range and demonstrated enhanced selectivity in receptor-negative cells compared to SAPs and 4-thiazolidinone amides. During the course of structure-activity relationship studies of above class of compounds we were interested to investigate the effect of heteroatoms in the lipophilic alkyl side chain on potency and selectivity. It is also well known that polyamine containing compounds exhibit a number of biological activities and have been utilized as chemotherapeutic agents. Due to these reasons, we designed and prepared a series of compounds containing serine, 4-thiazolidinone and thiazolidine-4-carboxylic acid as head groups conjugated with naturally occurring polyamines like putrescine, spermidine and spermine. Short chain polyamines conjugated to serine, thiazoldinone, and thiazolidine carboxylic acid did not show activity up to 100 μM. As the length of polyamine moiety increased, cytotoxicity also increased in prostate cancer cells. One of the polyamine compounds tested demonstrated selective cytotoxicity against prostate cancer cells, but not in breast or ovarian cancer cells. Their synthesis and results of biological studies will be presented in this presentation.

501.

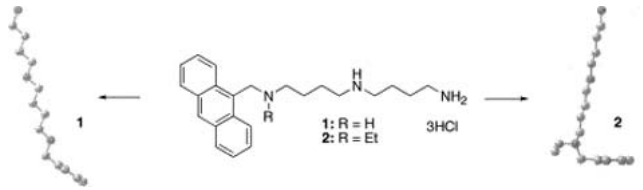
POLYAMINO-HETEROCYCLE CHELATING AGENTS WITH CYTOTOXIC ACTIVITY IN TUMOR CELLS: STRUCTURE-ACTIVITY RELATIONSHIP OF IMIDAZOLE, THIAZOLE AND PYRIDYL DONOR GROUPS. ***Roy P. Planalp***[1], *Matt Childers*[1], *Daniel P Kennedy*[1], *Allison Lindell*[1], *Grant Broker*[2], *Robin D. Rogers*[2], *Martin W. Brechbiel*[3], *Rong Ma*[4], *Frank M. Torti*[5], *and Suzy V. Torti*[4]. *(1) Department of Chemistry, University of New Hampshire, Durham, NH 03824, Fax: 603-862-4278, roy.planalp@unh.edu, (2) Department of Chemistry and Center for Green Manufacturing, University of Alabama, (3) Chemistry Section, Radiation Oncology Branch, (4) Department of Biochemistry, Wake Forest Univ Health Sciences, (5) Department of Cancer Biology, Wake Forest University Health Sciences*

The structure-activity relationships of novel tripodal hexanitrogen-donor chelators are reported. The chelators are based on the two triamine frameworks, *cis, cis*-1,3,5-triaminocyclohexane (tach) and tris(2-aminoethyl)amine (tren) and the four pendant groups 2-aminoethyl, imidazolemethyl, thiazolemethyl and pyridylmethyl (where the pyridyl group may be alkylated). One member of this family is tachpyridine (N,N′,N′′-tris(2-pyridylmethyl)-*cis,cis*-1,3,5-triaminocyclohexane; tachpyr), a potent hexadentate iron chelator under preclinical investigation as a potential anti-cancer agent. Tachpyr induces apoptosis in cultured cancer cells by triggering a mitochondrial pathway of cell death that is p53-independent. The strength of Fe(II) chelation by a number of these chelators has been determined as tachpyr trenpyr > tachthiazole > tachimidazole. Cytotoxicity of the chelators has been studied by a mitochondrial function assay of the response of cultured breast cancer cells to chelator treatment. The order of toxicity is tachpyr > trenpyr > tachthiazole > tachimidazole as indicated by IC_{50} values (2 μM, 25μM, 56 μM and 80 μM respectively). Studies of metal-binding selectivity in this family show that tachpyr chelates Fe(II) preferentially to Zn(II) (stability constant log $K_f([Zn(tachpyr)]^{2+})$ 15 and log $K_f([Fe(tachpyr)]^{2+})$ >= 20. The kinetic and thermodynamic factors influencing metal-binding selectivity and the role of metal chelation in cytotoxicity will be discussed.

502.

SYNTHESIS AND BIOLOGICAL EVALUATION OF DIHYDROMOTUPORAMINE DERIVATIVES IN CELLS CONTAINING ACTIVE POLYAMINE TRANSPORTERS. ***Navneet Kaur***, *Fred Breitbeil III, and Otto Phanstiel IV, Department of Chemistry, University of Central Florida, P.O. Box 162366, Orlando, FL 32816-2366, Fax: 407-823-2252, nkaur@mail.ucf.edu*

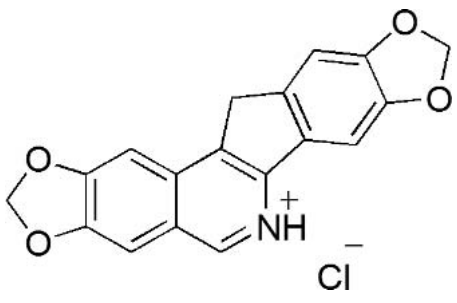
Dihydromotuporamine C (a known anti-invasive cytotoxic agent) and its 4,4-triamine analogue were synthesized using ring-closing methathesis (RCM) methods in good yield. Comparison of their biological activities (K_i determinations in L1210 cells and IC_{50} determinations in L1210, CHO, and CHO-MG cells) revealed that the motuporamine derivatives do not use the polyamine transporter (PAT) for cellular entry. Molecular modeling studies suggested that their inability to use the PAT was due to the presence of a N^1-tertiary amine center, which imparted a particular molecular shape. Bioevaluation of a *N*[1]-(anthracen-9-ylmethyl)-*N*[1]-(ethyl)-homospermidine (**2**, a computed shape mimic) confirmed this suspected trend. A new computational screen was developed, which refined an earlier PAT model and identified the molecular shapes necessary for PAT use (e.g., shovel, **1**) and dihydromotuporamine C mimicry (e.g., golf putter, **2**).



503.

NEW INDENOISOQUINOLINIUM SALTS, *N*-6-DESALKYLINDENOISOQUINOLINES, AND 5,11-DIKETOINDENOISOQUINOLINES: DESIGN AND SYNTHESIS OF TOPOISOMERASE I INHIBITORS AS ANTICANCER AGENTS. ***Alexandra S. Ioanoviciu***[1], *Smitha Antony*[2], *G. Kohlhagen*[3], *Y. Pommier*[4], *Bart Staker*[5], *Lance Stewart*[5], *and Mark Cushman*[6]. *(1) Department of Medicinal Chemistry and Molecular Pharmacology and Purdue Cancer Center, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, aioanovi@purdue.edu, (2) Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, (3) Laboratory of Molecular Pharmacology, National Cancer Institute, (4) Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, (5) BioStructures Group, deCODE genetics, Inc, (6) Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University*

The focus of our research is the design and synthesis of new indenoisoquinolines pertaining to three structural subgroups: *N*-6-desalkylindenoisoquinolines, indenoisoquinolinium salts and 6,11-diketoindenoisoquinolines bearing different substituents on the nitrogen atom. These compounds are thought to act *via* the same mechanism of action as camptothecin. The recently solved crystal structure of topoisomerase I-DNA in complex with an inhibitor belonging to the *N*-6-desalkylindenoisoquinoline subclass has provided insight into the interactions that stabilize the cleavage complex. Several *N*-6-desalkylindenoisoquinolines have displayed high cytotoxicities and inhibited topoisomerase I. Two structural moieties that have been associated with enhanced antitumor effect are the isoquinoline aromatic system, which intercalates with DNA, and hydrophilic side chains attached to the heterocyclic nitrogen atom. 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.



504.

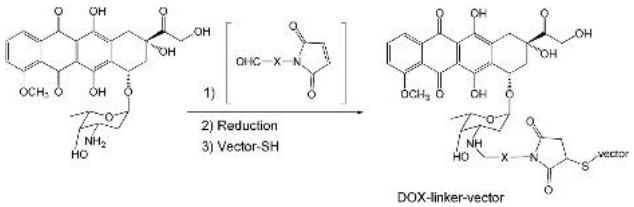
ON THE BINDING OF INDENO[1,2-C]ISOQUINOLINES IN THE DNA-TOPOISOMERASE I CLEAVAGE COMPLEX. ***Xiangshu Xiao***[1], *Smitha Antony*[2], *Yves Pommier*[2], *and Mark Cushman*[1]. *(1) Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University, West lafayette, IN 47907, xsxiao@pharmacy.purdue.edu, (2) Laboratory of Molecular Pharmacology, National Cancer Institute, NIH*

Indenoisoquinolines are a novel class of polycyclic non-camptothecin topoisomerase I inhibitors with potent cytotoxicity in various cancer cell cultures. A number of different indenoisoquinolines have been developed in our laboratory with comparable biological activities to camptothecin. However, they are more chemically stable and also produce more stable ternary cleavage complexes than camptothecin. To further understand the structure-activity relationships of indenoisoquinolines, a binding model for indenoisoquinolines in the DNA "cleavage complex" was generated and validated by quantum mechanics calculation and biological evaluation of a pair of discriminating stereoisomers. The quantum mechanics calculation method was also employed to analyze the structure-activity relationships of different indenoisoquinolines with differently modulated electron density in terms of electrostatic interactions and electron transfer. The results presented here can fairly well explain the known structure-activity relationships of indenoisoquinolines.

505.

UNIVERSAL DOXORUBICIN-LINKERS FOR THE PREPARATION OF CONJUGATES THAT RETAIN TOPOISOMERASE II ACTIVITY. ***Chengzao Sun****, Medicinal Chemistry, Acidophil LLC, 10931 North Torrey Pines Rd., Suite 104, La Jolla, CA 92037, Fax: 858-459-6672, csun@acidophil.com, Simon E. Aspland, Biology, Acidophil, LLC, and Angelo J. Castellino, Medicinal Chemistry, Acidophil, LLC*

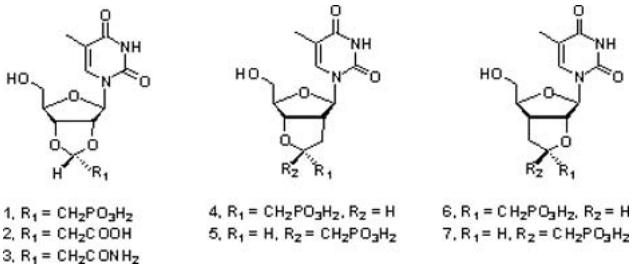
The basic nitrogen on the 3′-N position of Doxorubicin (DOX) is known to be a prerequisite for its Topoisomerase II activity. Preparation of 3′-N-alkyl conjugates of DOX generally involves reductive alkylation or nucleophillic substitution reactions that are laborious and low yielding. We report the use of N-alkyl-Maleimides as universal linkers for the preparation of doxorubicin conjugates through subsequent Michael addition of thiol containing vectors. Representative conjugates and biological results will be reported.



506.

SYNTHESIS AND EVALUATION OF MULTISUBSTRATE BICYCLIC PYRIMIDINE NUCLEOSIDE INHIBITORS OF HUMAN THYMIDINE PHOSPHORYLASE. ***Amy L. Allan***[1], *Patricia L. Gladstone*[1], *Melissa L. P. Price*[1], *Stephanie A. Hopkins*[1], *Jose C. Juarez*[1], *Fernando Doñate*[1], *Robert J. Ternansky*[1], *Bruce Ganem*[2], *Yingbo Li*[2], *Weiru Wang*[2], *and Steven E. Ealick*[2]. *(1) Attenuon, LLC, 11535 Sorrento Valley Road Suite 401, San Diego, CA 92121, Fax: 858-720-1086, allan@attenuon.com, (2) Department of Chemistry and Chemical Biology, Cornell University*

Tumor growth and metastasis are angiogenesis-dependent processes. Thymidine phosphorylase (TP) has been implicated in angiogenesis and chemotaxis in human tumors and inhibitors of TP have been shown to reduce tumor growth and metastasis in animal models. Ganem *et al.* recently reported compound **1** as an efficient inhibitor of TP activity (IC_{50} = 236 nM). This compound can be categorized as a multisubstrate analogue, binding both to the nucleoside and phosphate binding sites. A series of analogues based on compound **1** were synthesized and evaluated in a human TP enzymatic assay. Concern over the lability of the acetal functionality in **1** encouraged us to assess the activity and stability of the 2′- and 3′-carbon analogs **4** and **6** and their corresponding diastereomers **5** and **7**. Computational docking studies provided some insight into the binding orientation of these compounds, as well as the observed experimental trends in binding affinity.



507.

THREE-DIMENSIONAL QSAR STUDIES ON HUMAN THYMIDINE KINASE-1 SUBSTRATES. ***Achintya K. Bandyopadhyaya***[1], *Jayaseharan Johnsamuel*[1], *Youngjoo Byun*[1], *Ashraf S. Al-Madhoun*[2], *Staffan Eriksson*[2], *and Werner Tjarks*[1]. *(1) Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, 500 W 12th Avenue, 416 Parks Hall, Columbus, OH 43214, bandyopadhyaya.1@osu.edu, (2) Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences*

Thymidine kinase 1 (TK1) is an important target enzyme for antiviral and anticancer chemotherapy. In the recent years, a large number of nucleoside derivatives have been developed as the substrates for TK1. Many of these nucleosides were 3-carboranyl thymidine analogs (3CTAs), which showed significant accumulation and retention in the various types of tumor cells both in vitro and in vivo. Therefore, 3CTAs were considered as potential agents for boron neutron capture therapy (BNCT), a binary chemo-therapeutic method for the treatment of cancer. Biological data obtained from studies with non-boronated and boronated thymidine- and 2′-deoxyuridine derivatives were now used to develop the 3D-QSAR models of their TK1 substrate characteristics. The derived models showed predictive capabilities and a high level of internal consistency. Contour maps obtained from CoMFA and CoMSIA models correlated with the experimentally developed SAR. The obtained models may be utilized for the development of improved 3CTAs for BNCT.

508.

COMBINED PROTEIN HOMOLOGY MODELING – PHOTOAFFINITY LABELING APPROACH FOR IDENTIFICATION OF THE PROPAFENONE BINDING DOMAINS OF THE ABC-TRANSPORTERS LMRA AND P-GLYCOPROTEIN. ***Karin Pleban***[1], *Stephan Kopp*[2], *Michael Peer*[2], *Gerhard F. Ecker*[1], *and Peter Chiba*[2]. *(1) Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, Vienna A-1090, Austria, karin.pleban@univie.ac.at, (2) Institute of Medical Chemistry, Medical University of Vienna*

P-glycoprotein (P-gp, ABCB1) and its functional and structural bacterial homologue LmrA confer multidrug resistance to therapeutically targeted cells. Propafenone-type substrates, which are inherently photoactivatable in a pharmacophoric substructure, were used in affinity labeling studies. Component peptide fragments containing the covalently bound photoligand were identified by MALDI-TOF mass spectrometry. Frequency distribution analysis of labeled fragments allowed identification of transmembrane helices which are involved in substrate binding. Protein homology models indicated that both transporters bind their substrates at the transmembrane-domain : transmembrane-domain interfaces. The dynamics of the transport process were explored by quantifying changes in the labeling pattern at different steps of the transport cycle. Inverse changes in labeling of TM-segments 5 and 6 upon ATP-binding and hydrolysis suggest a concerted repositioning of these helices during transport. These data seem to conform to a first intermediate resolution structure of P-gp, in which the á-helical structures of the membrane spanning domains are visible.

509.

APPLICATION OF P-GP PHARMACOPHORE MODELS IN DATABASE SCREENING. ***Cheng Chang****, Biophysics Program, Ohio State University, 1614 Sparks Rd, Sparks, MD 21152, chang.440@osu.edu, Sean Ekins, GeneGo Inc, and Peter Swaan, Department of Pharmaceutics, University of Maryland at Baltimore*

Currently a large number of P-gp pharmacophore models derived from different subsets of substrates and inhibitors exist. Despite the availability of a large number of computational models for P-gp, little progress has been made in applying the models to assist in the drug discovery process. In an effort to

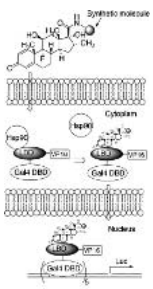
compare different P-gp pharmacophore models and apply the models as database filters, two P-gp digoxin inhibition models and one P-gp substrate model were generated and used to search a database of 576 known drugs. For the regenerated digoxin model search, 40 drugs were predicted to inhibit digoxin transport by P-gp. 25 drugs (5 from the training set) were identified to be substrates of P-gp through literature searching. 15 untested drugs fulfilling the pharmacophore requirements were also retrieved for future experimental verification. For the updated digoxin inhibition pharmacophore model 32 drugs (4 from the training set) of 68 hits were known P-gp substrates. For the substrate model (a Catalyst HipHop model derived from digoxin, verapamil and vinblastine), 4 of the 6 returned hits were known P-gp substrates including digoxin. The successfully validated P-gp pharmacophore models could be widely applied as consensus database filters to identify possible P-gp substrates and inhibitors during the screening process for lead discovery.

510.
GENERATION OF PREDICTIVE QSAR-MODELS FOR INHIBITORS OF P-GLYCOPROTEIN USING SIMILARITY BASED DESCRIPTORS. ***Barbara Zdrazil***[1]*, Dominik Kaiser*[1]*, Stephan Kopp*[2]*, Peter Chiba*[2]*, and Gerhard F. Ecker*[1]*. (1) Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, Vienna 1090, Austria, Fax: 431-4277-9551, barbara.zdrazil@univie.ac.at, (2) Institute of Medical Chemistry, Medical University of Vienna*

The recently introduced SIBAR-descriptors are based on calculation of similarity values for each compound of the training set to each compound of a reference set. These similarity values are then used for QSAR-studies. To further explore the applicability of this approach, we performed QSAR-studies on a 391 compounds data set of P-glycoprotein inhibitors using in total 31 'classical' 2D- and 3D- molecular descriptors as implemented in MOE and compared the results to those obtained with SIBAR. Six different reference data sets, consisting of twenty compounds each, were used. We obtained models with good predictive capacity both in leave one out cross validation runs and for the external test set with Q^2 values between 0.54 and 0.70. The SIBAR-descriptors showed at least equal performance to classical multiple linear regression analysis, whereby the results highly depend on the nature of the reference data set used (i.e. P-gp ligands, chemicals, ...).

511.
HIGH-THROUGHPUT ASSAY FOR ASSESSING THE CELL PERMEABILITY OF COMBINATORIAL LIBRARIES AND COMPOUND COLLECTIONS. ***Bo Liu****, Department of Internal Medicine and Molecular Biology and the Center for Biomedical Invertions, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390, Fax: 214-648-1450, bo.liu@utsouthwestern.edu, Peng Yu, Department of Internal Medicine and Molecular Biology and the Center for Biomedical Inventions, University of Texas Southwestern Medical Center, and Thomas J. Kodadek, Department of Internal Medicine and Molecular Biology and the Center for Biomedical Inventions, University of Texas Southwestern Medical Center at Dallas*

We report a high-throughput assay to measure the cell permeability of large numbers of compounds. The compounds were conjugated to a dexamethasone derivative through amide bond formation and the entry of these chimeras into living mammalian cells was evaluated. The cells contain a Gal4 DNA binding domain-glucocorticoid receptor ligand binding domain (GR LBD)-VP16 activation domain fusion protein that is trapped in the cytoplasm by heat shock protein 90 (hsp90). If a dexamethasone conjugate enters cells, it binds to GR LBD, allows the fusion protein to translocate into the nucleus and activate a Gal4-responsive luciferase reporter. The degree of the cell permeability of the test compounds is thus quantitatively described by the luciferase expression level.



512.
PREDICTING IN-VITRO PERMEABILITY OF ANTIMYCOTICS USING QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) MODELS. ***Denise Mills*** *and Subhash C. Basak, Center for Water and the Environment, Natural Resources Research Institute, University of Minnesota, 5013 Miller Trunk Hwy, Duluth, MN 55811, Fax: 218-720-4328, dmills@nrri.umn.edu*

Quantitative structure-activity relationship (QSAR) models were developed for the prediction of bovine hoof membrane permeability, in an effort to gain insight into the rate of penetration of antimycotics through the nail plate. Numerical descriptors based on chemical structure were calculated for a set of 14 drugs, mainly antimycotics. The descriptors were then placed into one of three classes based on level of complexity and demand for computational resources. Models using the various classes of structural descriptors were developed using ridge regression, principal component regression, and partial least squares. Results indicate that dermal permeability of antimycotics can be modelled based on structural descriptors alone, without the need for experimental data. As such, predictions can be made about the permeability of hypothetical compounds of similar structure not yet synthesized. However, additional data is required to allow for reliable modelling of human dermal permeability.

513.
SCREENING FOR DRUG-MEMBRANE INTERACTIONS USING ARRAYS OF SUPPORTED LIPID BILAYERS WITH VARYING LIPID COMPOSITIONS. ***Michael Mayer****, Department of Biomedical Engineering and Chemical Engineering, University of Michigan, Gerstacker Bldg., Room 1107, 2200 Bonisteel Blvd, Ann Arbor, MI 48109-2099, Fax: 734-763-4371, mimayer@umich.edu, and Sheereen Majd, Department of Biomedical Engineering, University of Michigan*

Screening arrays of lipid bilayers for binding of drugs makes it possible to explore pharmacologically-relevant drug-membrane interactions. Ideally many copies of an array of different bilayers could be exposed to different drug candidates. Techniques for rapid preparation of multiple copies of arrays of supported lipid bilayers with different lipid composition are, however, limited. Here, we present a simple stamping technique for parallel and repetitive formation of arrays of different lipid bilayers using patterned agarose stamps. We inked individual posts (diameter 200-1000 μm) on hydrogel stamps manually with minute volumes (~1-3 μL) of different suspensions containing liposomes with varying lipid composition. During stamping, islands of lipid bilayers formed by diffusion of small unilamellar liposomes (diameter 20-80 nm) through the agarose stamp and subsequent spreading of liposomes onto the regions of contact with a glass slide. We demonstrated that agarose stamps inked with liposome suspensions can be used for rapid (contact time 7 s), repetitive stamping of more than 100 copies of the same pattern without the need for re-inking. We stamped supported lipid bilayers with gradients in phosphatidylcholine from chicken egg (EPC) and phosphatidylserine (PS) on clean glass slides. Fluorescence recovery after photobleaching (FRAP) experiments revealed that the lipids in the supported bilayers were fluid at room temperature. Patterned spots of bilayers were stable in PBS buffer for several days. The technique presented here uses biocompatible agarose stamps and does not require drying of the molecules to be stamped; it might therefore be amenable to stamping of proteoliposomes. We demonstrate that arrays of supported lipid bilayers with varying compositions of lipids bind drugs differently and may therefore be useful for screening of drug-membrane interactions.

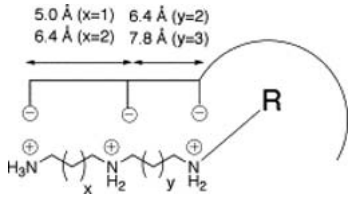
514.
TOWARDS PREDICTIVE ADME PROFILING OF DRUG CANDIDATES: LIPOPHILICITY AND SOLUBILITY. ***Gennadiy Poda****, Structural & Computational Chemistry, Pfizer Global R & D, 700 Chesterfield Parkway West, Mail Zone BB4G, Chesterfield, MO 63017, Gennadiy.I.Poda@pfizer.com, and Igor Tetko, GSF - Forschungszentrum fuer Umwelt und Gesundheit, GmbH, Institute for Bioinformatics*

To reach the target receptor or enzyme in the human body, drugs have to pass numerous membrane barriers by passive diffusion or carrier-mediated uptake. To achieve that drugs have to be soluble both in water and lipids. This makes lipophilicity and solubility the two major properties responsible for absorption and bioavailability of drugs. Reliable prediction of these parameters would significantly facilitate selection of drug candidates from virtual libraries. Within the ALOGPS approach, a statistical ensemble of associative neural networks trained on the dataset of publicly available data globally maps input parameters

to the target property. The final tuning of the model is done using a self-learning feature of the ALOGPS based on a user-defined set of the data and was shown to remarkably improve the accuracy in logD and solubility predictions for proprietary compounds. Thus, the ALOGPS combines the best properties of both global and local models.

515.
CELL-SELECTIVE DRUG DELIVERY USING THE POLYAMINE TRANSPORTER. ***Otto Phanstiel IV***[1], *Navneet Kaur*[1], *and Jean-Guy Delcros*[2]. *(1) Department of Chemistry, University of Central Florida, P.O. Box 162366, Orlando, FL 32816-2366, Fax: 407-823-2252, ophansti@mail.ucf.edu, (2) Groupe Cycle Cellulaire, Université Rennes 1*

Several N^1-arylalkylpolyamines containing various aromatic ring systems were synthesized. These ranged in size from N^1-benzyl, N^1-naphthalen-1-ylmethyl, N^1-2-(naphthalen-1-yl)ethyl, N^1-3-(naphthalen-1-yl)propyl N^1-anthracen-9-ylmethyl, N^1-2-(anthracen-9-yl)ethyl, N^1-3-(anthracen-9-yl)propyl, and pyren-1-ylmethyl. The polyamine architecture was also altered and ranged from diamine, triamine and tetraamine systems. IC_{50} cytotoxicity studies were conducted in L1210 (murine leukemia), Chinese Hamster Ovary (CHO) and its polyamine transport-deficient mutant (CHO-MG) cell lines. K_i values for spermidine uptake were also determined in L1210 cells. The size of the N^1-arylalkyl substituent as well as the polyamine sequence used had direct bearing on the observed cytotoxicity profiles. In summary, there are clear limits to the size of N^1-substituents, which can be accommodated by the polyamine transporter. A direct correlation was observed between polyamine-conjugate uptake and cytotoxicity. In this regard, a cytotoxicity model was proposed, which describes a hydrophobic pocket of finite dimensions adjacent to the putative PAT triamine-binding site (a presumed tri-anion of set dimensions).



516.
GC/MS ANALYSIS OF HYDROXYUREA METABOLISM TO HYDROXYLAMINE BY RAT LIVER. ***Mamudu Yakubu***[1], *Jinming Huang*[2], *Daniel B. Kim-Shapiro*[3], *and S. Bruce King*[2]. *(1) Department of Chemistry, Elizabeth City State University, 1704 Weeksville Rd, Elizabeth City, NC 27909, Fax: 252-335-3508, myakubu@mail.ecsu.edu, (2) Department of Chemistry, Wake Forest University, (3) Department of Physics, Wake Forest University*

Hydroxyurea is an approved treatment for sickle cell disease however, its mechanism of action remains to be completely described. Recent experiments have provided *in vivo* evidence for the formation of nitric oxide (NO), a known vasodilator, from hydroxyurea. GC/MS analysis of the metabolic product of hydroxyurea treated with rat liver followed by derivatization with acetone shows the formation of acetone oxime. Incubation of ^{15}N-hydroxyurea and rat liver gave ^{15}N-acetone oxime indicating the origin of the oxime is the hydroxyurea molecule. EPR spectroscopy of the incubation of rat liver and hydroxylamine gave a triplet heme(Fe^{2+})-NO complex within 30 minutes. The spectrum is identical to that of hydroxyurea reacting with rat liver and shows that hydroxylamine, derived from hydroxyurea, serves as the source of the NO. These results clearly show the ability of rat liver to metabolize hydroxyurea to hydroxylamine and demonstrate a pathway for NO production from hydroxyurea.

517.
HERG MUTANT PANEL FOR LEAD OPTIMIZATION OF COMPOUNDS WITH HERG LIABILITY. *Mark W. Nowak,* ***Niki M. Zacharias****, Ashutosh A. Kulkarni, John B. Nicholas, Sue Dee Sahba, Baljit S. Lally, Heinte P. Lesso, Steven J. Reyes, Elisha D. Mackey, Nima W. Shiva, and Paul B. Bennett, Neurion Pharmaceuticals, 180 North Vinedo Ave., Pasadena, CA 91107, Fax: 626-685-5983, markn@neurionpharma.com, nikiz@neurionpharma.com*

Avoiding cardiac liability associated with blockade of hERG (human ether a-go-go) is a costly and time consuming challenge for drug development. To abolish hERG block, we have developed unique tools to elucidate the structural determinants governing compound interactions with hERG. We believe with an understanding of how and where a compound is binding to hERG, a medicinal chemist will then be able to design out hERG blockade for that particular compound. We have evidence that various combinations of binding interactions (hydrogen-binding, cation-π, ion pairing, and hydrophobic) at numerous distinct protein side chains are involved in hERG blockade. Furthermore, our data indicate that the channel binding interactions can vary greatly even for structurally related hERG blockers. We present studies utilizing our proprietary mutagenesis methodology that reveal distinct binding modes for compounds that block hERG. Such knowledge can be applied early in drug discovery to remove hERG interactions associated with lead candidates.

518.
NOVEL DRUG DELIVERY VEHICLES: SYNTHESIS AND BIOLOGICAL EVALUATION OF DENDRIMERS BASED ON MELAMINE. ***Hui-Ting Chen***[1], *Michael F. Neerman*[1], *Alan R. Parrish*[2], *and Eric E. Simanek*[1]. *(1) Department of Chemistry, Texas A&M University, College Station, TX TX 77843-325, htchen@mail.chem.tamu.edu, (2) Department of Toxicology and Medical Pharmacology, Texas A&M University Health Science Center*

Dendrimer **1** based on melamine was designed to possess AB_4 surface groups and conveniently synthesized in 5g scale in 5 linear steps at 56% yield. In the course of searching better drug carriers, **1** was modified to obtain dendrimer **2-7**, and their cytotoxicity and hemolytic activity was evaluated. While exposing to cells, the anionic dendrimers displayed concentration dependant activity. In comparison, cationic and carboxylated dendrimers were much less toxic. To obtain a biocompatible dendrimer, the surface of **2** was functionalized with PEG to give **7**, which shows no toxicity via iv administration. In addition, **7** is able to encapsulate hydrophobic drugs such as methotrexate, or taxol. It doesn't only release drugs slowly but also enhance efficacy of drugs. Furthermore, the accumulation of **7** in cancer cells was observed in cell uptake experiments. Briefly, melamine based dendrimers provide persuasive results for drug delivery and dendrimer **7** is the most potential candidate.

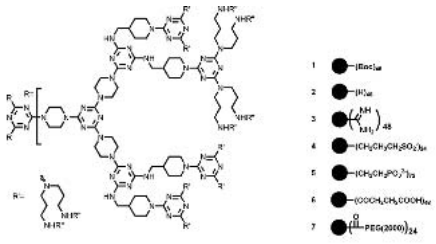


Chart 1. Dendrimer 1 (R=Boc) in Atomic Detail and 1-7 Shown Schematically with R Identified

519.
PREVALENCE OF SCAFFOLDS IN P450 INHIBITORS. ***Richard Kho***[1], *Keith Koch*[1], *Mark R. Hansen*[2], *Jason Hodges*[2], *and Charles Sanglimsuwan*[2]. *(1) Triad Therapeutics, PO Box 910302, San Diego, CA 92191, rkho@interking.net, (2) Altoris, Inc*

The prediction of ADME properties remains a challenge using any kind of computational technique due to the paucity of experimental data. Emphasis has been on the prediction of ADME properties for individual compounds using either global molecular descriptors, three-dimensional pharmacophore models, and docking algorithms. In the current study, P450 inhibition was analyzed in terms of scaffolds present in the data. Inhibition data for 1280 pharmacologically relevant compounds was generated and analyzed. The prevalence of scaffolds was studied and substructures were correlated with biological activity. We were able to readily identify some scaffolds that appear to be relevant for inhibition of a panel of P450 enzymes. The results could lead to improved evaluation of lead compounds for adverse metabolic effects.

520.
DEVELOPMENT OF ALPHA-HELIX MIMETICS DESIGNED TO DISRUPT PROTEIN-PROTEIN INTERACTIONS. ***Jessica M. Davis*** *and Andrew D. Hamilton, Department of Chemistry, Yale University, 225 Prospect St, P.O. Box 208107, New Haven, CT 06520, jessica.davis@yale.edu*

Alpha-helices on protein surfaces function as recognition devices for protein-protein interactions. Mimicking α-helices with small molecules has proven an

effective means of disrupting these interactions. Our work has previously shown that a terphenyl scaffold can mimic the *i*, *i+3* or *i+4*, and *i+7* residues of an α-helix. Proof of concept came from successfully disrupting the Bcl-x_L/Bak complex by mimicking the critical α-helical region of Bak. The low solubility of the terphenyl scaffold in polar solvents lead to the development of the oligoamide foldamer and terephthalamide scaffolds. Based on the success of previous scaffold designs, novel α-helix mimetics employing terpyridine and piperazinyl-pyrimidine scaffolds have been designed.

521.

GENERATING β-TURNS USING 1,1′-FERROCENEDICARBOXYLIC ACID AS A TEMPLATE. *Timothy P. Curran and* ***Mark V. Silva****, Department of Chemistry, Trinity College, 300 Summit Street, Hartford, CT 06106, Fax: 860-297-5129, timothy.curran@trincoll.edu, mark.silva@trincoll.edu*

β-turns are thought to nucleate the start of β-sheets. As such, conformationally constrained peptides that nucleate β-turns can be used to generate derivatives of bioactive peptides that possess β-sheet structures. Inclusion of a transition metal in the conformational constraint would provide a spectroscopic marker that could be used to locate the peptide derivative in vivo. In this work the use of 1,1′-ferrocenedicarboxylic acid to nucleate a β-turn structure has been probed. The 1,1′-ferrocenedicarboxylic acid was converted to the diacid chloride, which was then reacted with Boc-Lys-Lys-OMe to generate a metallacyclicpeptide in which the two lysine amines form amides with the acylferrocene. The metallacyclicpeptide can then be incoroporated into larger peptides using traditional peptide synthesis methods. The synthesis of the metallacyclicpeptide, and its incorporation into larger peptides will be presented, along with spectroscopic data that shows that the metallacyclicpeptide nucleates a β-turn.

522.

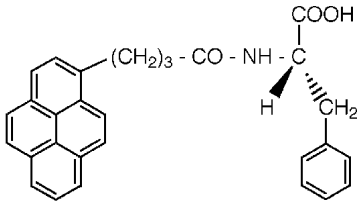
METALLACYCLICPEPTIDES - CYCLIC PEPTIDES THAT INCORPORATE METAL ATOMS. *Timothy P. Curran and* ***Richard S. H. Yoon****, Department of Chemistry, Trinity College, 300 Summit Street, Hartford, CT 06106, Fax: 860-297-5129, timothy.curran@trincoll.edu, richard.yoon@trincoll.edu*

Cyclicpeptides are often used to probe structure-activity relationships for peptides and their receptors. Work in our lab is focused on developing novel ways to form cyclicpeptides that incorporate metal atoms (metallacyclicpeptides). Using the metal atom in the ring offers two potential advantages. First, the chemistry for making the cyclic species could be direct and easy to accomplish. Second, the metal atom provides a convenient spectroscopic marker for locating the cyclicpeptide. We have found that alkynylpeptides will react with $W(CO)_3(dmtc)_2$ (dmtc=dimethyldithiocarbamate) to yield bis(alkynylpeptide) complexes. As an extension of this work, we have prepared peptides bearing two alkyne groups (dialkynylpeptides). The two alkyne groups were positioned at the N- and C-termini of the peptide. These dialkynylpeptides were reacted with $W(CO)_3(dmtc)_2$ under high dilution conditions in order to prepare metallacyclicpeptides - a cyclicpeptide that incorporates the tungsten in the ring. This poster will detail the synthesis of the dialkynylpeptides, and the synthesis and conformational behavior of the metallacyclicpeptides.

523.

ARTIFICIAL PHOTOPROTEASES: SITE-SPECIFIC PHOTOCLEAVAGE OF PROTEINS BY CO(III) COMPLEXES. *Challa. V. Kumar and* ***Jyotsna Thota****, Department of Chemistry, University of connecticut, Unit 3060, North Eagileville Road, Storrs, CT 06268, c.v.kumar@uconn.edu*

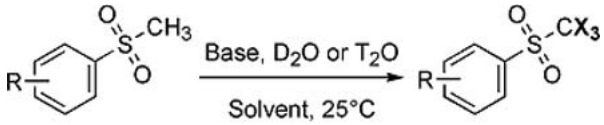
Artificial peptidases that cleave protein backbone with high selectivity will prove useful in protein structural studies and in studying, protein-metal, protein-DNA, protein-solid and protein-protein interactions. In the recent past, our group has shown that N-(1-phenylalanine)-4-(1-pyrine)butyramide (Py-Phe) cleaves proteins at specific sites. As a next step, we decided to design strategies to target metal binding sites on proteins, and develop methods to locate metal binding sites on proteins. Here, it is reported that hexaamminecobalt(III) chloride (CoHA), pentaamminechlorocobalt(III) chloride (CoPACl), pentaamminebromocobalt(III)bromide (CoPABr) and tetraammine carbonatocobalt(III) nitrate (CoTA) cleave lysozyme, when irradiated at 310 nm, into two fragments of approximate molecular masses of 11 kDa and 3 kDa. The yields of photoproducts increased with time of irradiation, as well as the concentration of the metal complex. Irradiation at wavelengths below 300 nm resulted in self-cleavage of the protein. Irradiations at wavelengths higher than 310 nm resulted in cleavage of protein by CoPACl, CoPABr and CoTA but not CoHA indicating that excitation into the charge transfer bands of the metal complexes are responsible for the cleavage. To test the possibility that ligand generated free radicals are involved in these reactions, CoTA/lysozyme irradiation was carried out in the presence of selected quenchers. 2-Propanol, for example, quenched the product formation and the observed quenching constant is consistent with protein photocleavage via the generation of the carbonate radical. N- terminal sequencing of the 11 kDa fragment indicated N-terminus of lysozyme, and from the N-terminal sequencing of 3 kDa fragment we conclude that lysozyme is cleaved betweent Trp 108 and Val 109. Thus, these data provide the very first examples of photocleavage of proteins by transition metal complexes.



524.

BASE-CATALYZED DEUTERIUM AND TRITIUM LABELING OF ARYL METHYL SULFONES AND ARYL METHYL KETONES. APPLICATIONS TO DRUG DEVELOPMENT. ***John Scheigetz****, Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire - Dorval, QC H9R 4P8, Canada, Fax: 514-428-4900, john_scheigetz@merck.com, Carl Berthelette, Department of Medicinal Chemistry, Merck Frosst Canada, Chun Li, Medicinal Chemistry Department, Merck Frosst Canada & Co, and Robert Zamboni, Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research*

A method is presented for conveniently tritiating the aryl methyl sulfones of compounds identified as potent and selective inhibitors of human Cox-2 and as DP receptor antagonists. A base-catalyzed exchange reaction was conducted with deuterated water and the total deuterium incorporation ranging from 46 to 99% was calculated using mass spectrometry. Results from these exchanges were used as guidelines for tritium labeling giving specific radioactivities in the range of 28 to 120 mCi/mmol (1.03 GBq/mmol to 4.43 GBq/mmol). The scope of the exchanges was extended to methyl aryl ketones.



X= **D** or **T**

525.

CHARACTERIZATION AND ANTIOXIDATIVE ACTIVITIES OF AGAR-DERIVED OLIGOSACCHARIDES. ***Wenjun Mao*** *and Ligen Wu, Marine Drugs and Foods Institute, Ocean University of China, 5 Yushan Road, Qingdao 266003, China, wenjunmqd@hotmail.com*

The agar-derived oligosaccharides were prepared by hydrolysis with acids, and were separated by Superdex 30 column chromatography. The molecular weight of these oligosaccharides was determined by ESI-MS. The NMR spectra characteristics of oligosaccharides with different degree of polymerization were compared. The scavenging effects on superoxide anion radicals and hydroxyl radicals of agar-derived oligosaccharides by the chemiluminescence's method were studied. In addition, agar-derived oligosaccharides are fed to mice following whole-body X-ray irradiation. The activities of GSH-Px, SOD in blood, and MDA level in the liver were determined. The results showed that the agar-derived oligosaccharides had strong scavenging effect on superoxide anion radicals, and much weaker effect on hydroxyl radicals. Moreover, the LMWSG

significantly enhanced activities of the SOD in blood, and decreased the activities of the MDA level in liver. This research was funded by a grant (031070120) from Science and Technology Development Program of Shandong Province (China).

526.

EFFECT OF 2′,5′-LINKED NUCLEIC ACID ON SIRNA ACTIVITY. ***Thazha P. Prakash**, Bryan Kraynack, Brenda Baker, Muthiah Manoharan, Eric E. Swayze, Richard H. Griffey, and Balkrishen Bhat, Department of Medicinal Chemistry, Isis Pharmaceuticals, 2292 Faraday Ave, Carlsbad, CA 92008, Fax: 760-929-0036, tprakash@isisph.com*

Synthetic small interfering RNAs (siRNA) when transfected into cell culture are known to incorporate into RNA-induced silencing complex (RISC) and trigger silencing of specific gene and this mechanism is emerging as a powerful tool for gene regulation. However their utility is limited as therapeutic applications primarily due to their poor metabolic stability. Backbone modifications are known to stabilize oligonucleotides against metabolic degradation and alter duplex stability. The 2′,5′-linked oligonucleotides are known to be more stable to nucleolytic degradation than 3′,5′-linked oligonucleotides. It has been reported that 2′,5′-DNA and RNA form stable duplexes with complementary RNA. Here we have evaluated oligonucleotides with 2′-structural isomer of natural nucleic acids for siRNA mediated target reduction. We have evaluated siRNA constructs with 2′,5′- inter nucleotide linked RNA and DNA as sense or antisense strand. The results from these studies will be presented.

527.

EFFECT OF SULFUR OXIDATION STATE AND HYDROPHOBICITY ON THE BINDING KINETICS OF TRIFLUOROMETHYL KETONE-CONTAINING CARBOXYLESTERASE INHIBITORS. ***Craig E. Wheelock**[1], Zecheng Ying[1], Paul D. Jones[1], Michael E. Colvin[2], Marilyn M. Olmstead[3], and Bruce D. Hammock[1]. (1) Department of Entomology and Cancer Reseach Center, University of California Davis, Briggs Hall, One Shields Ave, Davis, CA 95616, and Kyoto University, Ugi, Kyoto 611-0011, Japan, Fax: 530-752-1537, craig@kuicr.kyoto-u.ac.jp, (2) School of Natural Science and Engineering, University of California, (3) Department of Chemistry, University of California*

Carboxylesterases hydrolyze numerous pharmaceuticals and xenobiotics. These enzymes are potently inhibited by trifluoromethylketone-containing (TFK) inhibitors. Structure-activity relationship studies showed that sulfur inclusion beta to the carbonyl increases inhibitor potency. However, the sulfur oxidation state affects inhibitor binding kinetics. The bimolecular rate constants (k_i) of 12 different TFK-containing inhibitors containing different sulfur oxidation products (thioether, sulfoxide, and sulfone) coupled to varying aliphatic chains were measured. Results showed that within a given set of sulfur oxidation products attached to the same alkyl group, the sulfoxide and sulfone analogs had lower k_i values than the thioether. However differences in k_i decreased in inhibitors containing longer alkyl groups, showing that hydrophobic effects upon k_i negate influences of the sulfur oxidation state. Crystallographic and ab initio calculation studies were used to further explore this phenomenon. This study provides insight into carboxylesterase inhibition and explains the binding kinetics of the most potent inhibitors observed to date.

528.

MIF IMBALANCE IN THE SEMINAL FLUID IS PREDICTIVE OF MALE INFERTILITY: DEVELOPMENT OF HOME DIAGNOSTIC SCREENING TEST FOR MALE INFERTILITY. ***Yousef Al-Abed**, Laboratory of Medicinal Chemistry, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, Fax: 1-516-365-5090, yalabed@nshs.edu*

Macrophage migration inhibitory factor (MIF) is a protein that was discovered in the early 1960s as a product of activated lymphocytes that inhibited the random migration of macrophages. Recent studies have shown that MIF is released from the Leydig cells of the testis and others indicated that MIF may play a role in reproduction. In our ongoing study, we measured MIF levels in the seminal ejaculate of more than 150 subjects and concluded that MIF level is indicative of the fertility status. MIF was isolated and characterized by mass spectrometry and Western analysis. In our presentation, we will focus on the correlation between MIF levels and infertility, and also on how to utilize a unique enzymatic activity of MIF to develop a diagnostic tool to measure the fertility status.

529.

MODIFIED POLYELECTROLYTE CAPSULES AS SMART ANTIOXIDANT SYSTEMS FOR DRUG AND ENZYME ENCAPSULATION. *Tatsiana Shutava[1], Dmitry Shchukin[2], **Zhiguo Zheng**[1], and Yuri Lvov[1]. (1) Institute for Micromanufacturing, Louisiana Tech University, 911 Hergot Ave, Ruston, LA 71272, Fax: 318-257-5104, tshutava@latech.edu, zzh002@latech.edu, (2) Dept. of Interfaces, Max Planck Institute of Colloids and Interfaces*

A new type of protective semipermeable microcontainer, capable of preventing oxidation of encapsulated material by low molecular weight and radical oxidizing agents, was demonstrated. Layer-by-Layer (LbL) technology is based on sequential deposition of oppositely charged polyelectrolytes, nanoparticles and enzymes, allowing the formation of multilayer films with nanometer precision over inorganic templates, enzyme complexes or drug microcrystals. Active layers with target properties were introduced into LbL films and capsules and their inhibitory influence on H2O2-induced oxidation of bovine serum albumin encapsulated in polyelectrolyte microcapsules and discoloration of hemoglobin/poly(styrene sulfonate) films was shown. New types of capsules on the basis of tannic acid, a well known radical scavenger, and polycations, were obtained and their activity in prevention of free radical induced damage of encapsulated substances was investigated. Protective polyelectrolyte films and capsules can find applications as delivery and depot systems in medicine, drug industry, and biotechnology.

530.

MTMOIV, A KEY BAEYER-VILLIGER TYPE OXYGENASE OF THE MITHRAMYCIN BIOSYNTHETIC PATHWAY. ***Miranda P. Gibson**, Md. Nur-e-alam, Chenchen Wang, and Jürgen Rohr, College of Pharmacy, University of Kentucky, 725 Rose St., Lexington, KY 40536, Fax: 859-257-7585, mgibs3@email.uky.edu*

The biosynthetic pathway of the aureolic acid antitumor drug Mithramycin by *Streptomyces argillaceus* has previously been characterized to show that an oxidative cleavage of Premithramycin B by the monooxygenase MtmOIV leads to the final step of the biosynthesis through a Baeyer Villiger oxidation. Several Mithramycin derivatives have also been isolated as a result of the MtmOIV oxidation, Mithramycin SK, SDK, and SA. MTM-SK in particular has been shown to exhibit a higher therapeutic index than MTM. The kinetics of the MtmOIV oxidation has been studied monitoring the depletion of the cofactor NADPH under physiological conditions to find the Km and Vmax, and several intermediates of the MtmOIV reaction have been isolated and characterized. Crystals of the MtmOIV protein were generated, which will serve to solve the crystal structure and future design of this important key enzyme of mithramycin biosynthesis.

531.

PERIPHERAL SEQUENCE ELEMENTS KEY TO SELF-CLEAVAGE ACTIVITY OF NATURAL HAMMERHEAD RIBOZYMES BY FORMING TERTIARY CONTACTS. ***Manami Roychowdhury Saha**, Sugata Roychowdhury, and Donald H Burke, Department of Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405, Fax: 812-855-6590, mroychow@indiana.edu*

Hammerhead ribozymes (hRz) are small, naturally occurring RNA motifs found mostly in viroids, where they site-specifically cleave RNA during replication. Recent work by Khvorova (1), by De la Peña (2) and by our lab (3) showed that tertiary contacts outside the catalytic core stabilize natural hRz at physiological Mg2+ concentrations. The natural hammerheads cleave well in intracellular assays compared to the minimalist hRzs. The magnesium ion-induced folding occurs in a single step for the natural hRzs, a contrast to the two-step folding seen upon disruption of the loop I and II interaction4. We are exploring the contributions of peripheral sequence elements to catalysis by natural and engineered hRz. Artificial hammerheads containing tertiary stabilizing motifs (TSM) cleave in absence of magnesium with the addition of spermidine or cobalt hexammine and they are more stable at high temperatures. Searches for TSM-containing hammerhead and hammerhead-like sequences in animal database gave several potential hits in Schistosomes, crickets and even rodents and humans. Kinetic studies of the cricket hammerhead show the influence of TSM in the catalytic activity of the molecule under physiological magnesium concentrations (0.5 mM). Mutational studies suggest potential structural models of tertiary contacts in cricket hammerhead. 1)Khvorova A, Lescoute A, Westhof E, Jayasena SD. (2003) Sequence elements outside the hammerhead ribozyme catalytic core enable intracellular activity. 10(9), 708-12. 2)De la Peña M, gago

S, Flores R. (2003) Peripheral regions of natural hammerhead ribozymes greatly increase their self-cleavage activity. 22(20) 5561-70. 3)Saksmerprome V, Roychowdhury-Saha M, Jayasena S, Khvorova A, Burke DH. (2004) Artificial tertiary motifs stabilize trans-cleaving hammerhead ribozymes under conditions of sub-millimolar divalent ions and high temperatures.

532.

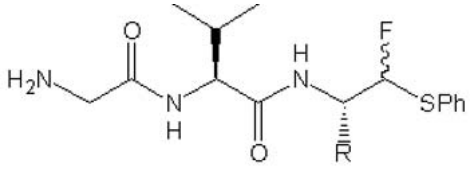
PROGRESS TOWARD PHOTORELEASABLE CAGED BIOCIDES. ***Robert G. Brinson*** *and Paul B. Jones, Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, brinrg1@wfu.edu*

The development of photoreleasable bioactive aldehydes from organic and water soluble 1-allyloxy-9,10-anthraquinones is presented. These caged biocides can be photochemically generated upon irradiation with visible light in the presence of water or other nucleophilic solvents under both aerobic and anaerobic conditions. Bioactive aldehydes generated include trans-4-hydroxy-2-nonenal (4-HNE) and acrolein. 4-HNE was produced in up to 100% yield and isolated in 91 % yield. These aldehydes were produced independent of oxygen and may have potential applications to photodynamic therapy. The synthesis, photochemistry, and biological activity of these systems will be presented.

533.

PROTEASE INHIBITION BY NOVEL FLUOROPEPTIDOMIMETICS: A MECHANISM-BASED DESIGN STRATEGY. ***Lakshmi P. Kotra****, Subhash C. Annedi, Kanchana Majumder, and Sheeba Samson, Leslie Dan Faculty of Pharmacy, University of Toronto, Molecular Design and Information Technology Centre, 19 Russell Street, Toronto, ON M5S 2S2, Canada, Fax: 416-978-8511, p.kotra@utoronto.ca*

Designer fluoropeptidomimetics as protease inhibitors against chymotrypsin are revealed. The key peptidomimetic region in the inhibitors contains a "-CHF-S-" moiety and is designed to mimic the tetrahedral oxyanion species during the hydrolysis of a peptide bond. Fuoropeptidomimetics containing bulky substitutions at P1 (1 and 2) exhibited time-dependent loss of activities against chymotrypsin, up to 67 and 79% with a K_i of 63 and 120 uM, respectively. Designed fluoropeptidomimetics in aqueous methanol underwent defluorination to form corresponding methyl ether and/or oxazole derivatives after cyclization in several hours to days. In the case of dipeptide mimetics, decomposition pattern is complex compared to that in the corresponding tripeptides. Bulkier alkyl substitutions at C-2 position exhibited enhanced aqueous stability. In case of *N*-phthaloyl protected monomer of the fluoropeptidomimetic, fluorine elimination was not observed even after 4 days. Nature of "-CHF-S-" moiety and the stabilities of various fluoropeptidomimetics in aqueous solutions are disclosed in detail, which serve for a rational design of new generation of fluoropeptidomimetics.



1-5

R = CH_2Ph, CH_2(cyclohexyl), $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $CH(CH_3)C_2H_5$

534.

RAPID DIVERSITY-ORIENTED SYNTHESIS OF FIVE-MENBERED IMINOCYCLITOLS IN MICROTITER PLATES FOR IN SITU SCREENING OF GLUCOSIDASE INHIBITORS. ***Pi-Hui Liang***[1]*, Chung-Yi Wu*[1]*, and Chi-Huey Wong*[2]*. (1) Genomics Research Center, Academia Sinica, No. 128, Academia Road Section 2, Nan-Kang, 115, Taipei, Taiwan, Fax: -2-27898670, (2) Department of Chemistry, Scripps Research Institute*

The iminoalditol 2,5-dideoxy-2,5-imino-D-manitol (DMAP), a natural product, is known as a potent reversible inhibitor of D-glucosidase and invertase. In order to study this interesting structure information of DMAP, the DMAP derivative 1 was generated as a core structure and which a beta-aminomethyl group was attached to the C1 position for the subsequent amide bond formation and screening inhibition activities in situ for different sources of alpha/beta-glucosidases and beta-N-acetyl glucosaminidase. These results provided important structure information of C1 position for glucosidase. In particular, acetamido group at C1 position was essential for anti-N-acetyl glucosaminidase activity. Furthermore, N-aminioalkyl derivatives 2 were synthesized and subjected to amide bond formation to afford series II compounds. Screening in situ without further purification, potent and selective inhibitors for N-acetyl glucosaminidase and N-acetyl hexosaminidase were found. Herein, we reported the structure-activity relationship for five-menbered iminocyclitols in various enzyme glycosilic hydrolysis.

535.

RAPID DISCOVERY OF POTENT INHIBITORS USING DIVERSITY-ORIENTED SYNTHESIS FOLLOWED BY IN SITU SCREENING. ***Ashraf Brik*** *and Chi-Huey Wong, Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC-338, La Jolla, CA 92037, Fax: 858-784-2409, ashraf@scripps.edu*

In this seminar I will present a useful approach to rapidly identify potent inhibitors against a target enzyme using diversity-oriented synthesis in microtiter plates. The method allows for the in situ screening of a focused library, generated from a common core, without purification or isolation of any of the synthesized compounds. The method is a general means and has been used to target several enzymes including HIV protease (wild type and its mutants), SARS Coronavirus protease, Sulfotransferase and Alpha-Fucosidase. In all of these examples, potent inhibitors were identified and the selected compounds were isolated and their Ki values were determined.

536.

SILVER/DENDRIMER NANOCOMPOSITES AS BIOMARKERS: FABRICATION, CHARACTERIZATION, IN VITRO TOXICITY AND INTRACELLULAR DETECTION. *Wojciech Lesniak*[1]*, Xiangyang Shi*[2]*, Anna Bielinska*[2]*, Katarzyna Janczak*[2]*, Kai Sun*[3]*, James R. Baker Jr.*[4]*, and* ***Lajos P Balogh***[1]*. (1) Center for Biologic Nanotechnology, University of Michigan, 200 Zina Pitcher Pl, Kresge-II, Rm 4010, Ann Arbor, MI 48109-0533, Fax: 734-615-0621, wlesniak@med.umich.edu, (2) Center for Biologic Nanotechnology, University of Michigan, Ann Arbor, (3) Electron Microbeam Analysis Laboratory(North Campus), University of Michigan, (4) Center for Biologic Nanotechnology, Department of Internal Medicine, University of Michigan*

We have developed water-soluble, biocompatible, fluorescent, and photostable silver/dendrimer nanocomposites that have a potential to be used for *n vitro*i cell labeling. A PAMAM_E5.NH_2 dendrimer was used as a template to prepare first a silver-dendrimer complex in aqueous solution at biologic pH=7.4. Conversion into nanocomposites was achieved by irradiating the solution of the $[(Ag+)_{25}$-PAMAM] complexes by UV light to reduce the bound Ag+ to zero-valent Ag(0) atoms trapped in the dendrimer network. Other templates with positive, neutral and negative surface charges were also used. Results indicate that the {$(Ag(0))_{25}$-PAMAM} silver/dendrimer nanocomposites dominantly form single particles of 4-5 nm with the same surface charge as the host. The dendrimer nanocomposites proved to be fluorescent. Toxicity testing of the nanocomposites revealed behaviors similar to the template dendrimers. Intracellular internalization of the silver nanocomposites and cell labeling capabilities was confirmed by confocal microscopy.

537.

TRIGGERED RELEASE OF LIPOSOME CONTENTS BY MMP-9. ***Aaron Krueger****, Nihar Sarkar, Abir L. Banerjee, Keith Benton, Sanku Mallik, and D K Srivastava, Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105, Aaron.Krueger@ndsu.nodak.edu*

Matrix Metalloproteinases (MMPs) are a group of 26 Zn^{2+} dependent endopeptidases responsible for upkeep of the extracellular matrix. Although MMPs assist with many necessary functions, over expression may result in disease processes often associated with cancer cell metastasis and angiogenesis. Due to this dichotomy, targeted inhibition, rather than complete inhibition is more feasible. To target MMP-9, liposomes incorporating a lipid conjugate of a synthetic collagen mimetic peptide (a substrate for MMP-9), have been prepared. These liposomes release the encapsulated dye (5-carboxyfluorescein) in the presence of MMP-9. In the absence of the enzyme, no leakage was observed.

538.
CONSTRUCTION OF CLASSIFICATION MODELS FOR THE VIRTUAL SCREENING. ***Natalia Grinevich**, Analytical Chemistry, Ukrainian National Pharmaceutical University, 3 Aviatsionnaya St, Kharkov 31024, Ukraine, zalot@rambler.ru*

The role of statistical classification modeling in basic virtual screening methods in the modern pharmaceutical chemistry is very important. Some theoretical and practical aspects of classification model construction, including new techniques for training databases creation, calculation and selection of molecular descriptors, the construction and analysis of the model will be presented.

539.
EFFECT OF VAPOR PHASE PRE-MINIMIZATION VERSUS MD SIMULATIONS ON DOCKING CALCULATION PERFORMANCE. ***Ian G. Welsford** and Michael J. McManus, BioSciences Group, Fujitsu America Inc, 200 Lowder Brook Rd, Suite 2100, Westwood, MA 02090, Fax: 781-326-7179, iwelsford@us.fujitsu.com*

We report studies designed to compare vapor phase-based QM methods (LocalSCF, MOZYME) versus MD approaches (GROMACS) for optimizing the 3D structure of proteins prior to docking. Five varying proteins were used (1VAG,1C3P, 1PMN, 1GSZ, 1M52). LocalSCF and MOZYME were run using BioMedCAChe using FastDock, a PMF scoring function. GROMACS was run on a desktop PC running LINUX. Trials were run in quintuplicate and RMS errors were analyzed using an ANOVA followed by post hoc comparisons. We found that LocalSCF and MOZYME produced similar results for docking scores which deviated significantly from those obtained by GROMACS. Significantly lower RMS errors were obtained using MOZYME when analyzing metal containing proteins (e.g. 1VAG and 1C3P), while LocalSCF produced lower RMS errors in multimeric proteins (e.g. 1PMN). These preliminary data support the hypothesis that optimization methods should be matched to the particular protein family in order to obtain optimal docking results.

540.
NEW COMPUTATIONAL TOOL FOR MEDICINAL CHEMISTS THAT USES DESIGN "RULES-OF-THUMB". ***Kent D. Stewart**, Global Pharmaceutical Research & Development, R-46Y, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, Fax: 847-937-2625, kent.d.stewart@abbott.com, and Craig A. James, Moonview Consultants, Ltd*

This poster presentation will give details of a new computational tool in corporate-wide use at Abbott Laboratories. The name of the web-based application is Drug Guru™ (Drug Generation Using Rules) and indicates that the new analogs designed in this program derived from applying medicinal chemistry "rules-of-thumb" to an input structure. These rules have been selected from the history of medicinal chemistry and converted into SMIRKS computer format which interconverts SMILES representations of the structures. An example of a "rule-of-thumb" is to replace every amide in a structure with a retro-amide. The output of the program is automatically ranked according to ClogP, PSA, Reactivity, and Solubility parameters. An example of the utility of Drug Guru™ in a drug discovery program will be given.

541.
SELECTION OF A DIVERSE SET OF DRUGS BASED ON MULTIVARIATE DESIGN. ***Christian Sköld**[1], Susanne Winiwarter[2], Johan Wernevik[2], Fredrik Bergström[2], Hans Lennernäs[3], Torbjörn Lundstedt[1], Anders Hallberg[1], and Anders Karlén[1]. (1) Department of Medicinal Chemistry, Division of Organic Pharmaceutical Chemistry, Uppsala University, BMC, Box 574, SE-751 23 Uppsala, Sweden, Fax: +46-18-4714474, Christian.Skold@orgfarm.u.se, (2) Department of Drug Metabolism and Pharmacokinetics & Bioanalytical Chemistry, AstraZeneca R&D Mölndal, (3) Department of Pharmacy, Uppsala University*

A multivariate analysis of drugs on the Swedish market has been made using 691 compounds derived from FASS (Swedish drug index). Based on this analysis we have selected a small, diverse set of 24 compounds in the physicochemical property space. The variables used for the analysis are general physicochemical descriptors such as size, lipophilicity, polarity, hydrogen bond capacity, etc. The selection was made by multivariate design and factors such as commercial availability, price and ease of analytical analysis were considered in the selection. We believe that this commercially available dataset can be used for many different purposes. It can for example be used as a benchmark dataset when validating different experimental techniques or as a small dataset that describes drugs in general.

542.
USEFUL CYCLOALKYLAMINE PHARMACOPHOR BUILDING BLOCKS. ***Jonathan Havel**, James Solomon, Christophe Guillon, and Ned Heindel, Department of Chemistry, Lehigh University, 6 East Packer Avenue, Bethlehem, PA 18015, jjh8@Lehigh.EDU*

The cycloalkylamine pharmacophor is found in tumoricidal amino acids mimics and in anticonvulsants (e. g., phenylcyclohexylamines such as PCP). Synthetic approaches that place an amino function on a tertiary carbocyclic ring, and which could thus generate intermediates to such pharmaceuticals, are decidedly limited. While successful in many other cases, the Ritter approach (HCN substitution onto protonated tertiary alcohols), led invariably in our hands to elimination not substitution; cycloalkenes not cycloalkylamines. The Curtius rearrangement, however, from tertiary cycloalkyl carboxylates has been telescoped to a one-pot, three-step synthesis which generates the pharmaceutical building blocks consistently in > 60% yields.

543.
COMPARISON OF SCAFFOLDS FOUND IN SCREENING LIBRARIES. ***Mark R. Hansen**, Robin Friedman, Charles Sanglimsuwan, and Kazuhiro Komatsu, Altoris, Inc, 5820 Miramar Rd #207, San Diego, CA 92121*

SARvision, is a new tool for scaffold perception that identifies the scaffolds contained in a chemical dataset automatically and organizes them hierarchically, according to frequency of occurrence and biological activity. We used this software to analyze the prevalence of different scaffolds in commercial libraries. The results are compared to privileged structures found in drug databases, and toxicological databases. The analysis allows the identification of scaffolds that are more suited for biological screening and that have a higher likelihood of being developed into drugs.

544.
CIRCULAR DICHROISM SPECTROSCOPY STUDY: SECONDARY STRUCTURE OF ORGANOPHOSPHORUS ACID ANHYDROLASE (OPAA) IN SOLUTION AND IN LANGMUIR-BLODGETT FILM. ***Liang Zhao**[1], Xiaojun Ji[1], Jianmin Xu[1], Tu chen Cheng[2], and Roger M Leblanc[1]. (1) Department of Chemistry, University of Miami, 1301 Memorial Drive, Room 315, Coral Gables, FL 33124, Fax: 3052841881, leonliangzhao@hotmail.com, (2) U.S. Army Edgewood Chemical Biological Center*

We studied the secondary structure of organophosphorus acid anhydrolase (OPAA) by circular dichroism (CD) in the far-UV region. The secondary structure was well defined when the pH value was at the isoelectric point (6.8). The secondary structure of OPAA solution was obtained from pH value 4.6 to 10. We obtained a quantitative estimation of the secondary structure from the CD spectra of OPAA solution by CDPro software. The fraction of secondary structure showed that the helix structure is partially lost when the pH values are increased or decreased from isoelectric point. We studied the thermal stability of the secondary structure of OPAA solution from 10 °C to 56 °C. The α-helix was almost destroyed when the temperature reached 56 °C. We also prepared and characterized the Langmuir and Langmuir-Blodgett (LB) film of the enzyme OPAA at the air-water interface by Circular Dichroism. The Langmuir- Blodgett film and dry film showed that the molecular arrangement played a dominant role in the thermal stability of OPAA.

545.
DETECTION OF CARBON-FLUORINE BONDS BY RAMAN SPECTROSCOPY IN SELECTED FLUORINE-CONTAINING BLOOD SUBSTITUTES. ***Dale F. Shellhamer**[1], Olga Sharts[2], Michael O'Hagan[2], Leo P. Avakyants[3], Samvel Sarkisyan[2], Victor Contreras[4], and Robert P. Metzger[5]. (1) Department of Chemistry, Point Loma Nazarene University, 3900 Lomaland Dr, San Diego, CA 92106, Fax: 619-849-2598, dshellhamer@ptloma.edu, (2) Fluorotronics, Inc, (3) Department of Physics, Moscow State University, (4) Department of Chemistry & Biochemistry, San Diego State University, (5) Department of Chemistry and Biochemistry, San Diego State University*

A newly developed Pulsed Copper Vapor Laser Raman Spectrometer has been used to detect the C-F bond in fluorine-containing blood substitutes with minimal sample preparation. Pulsed excitation and time delay technology eliminate interference with the detection of the C-F signal. Using this technique, the blood substitute perfluorobron was found to give one strong absorbance peak at 720 cm-1 and small peaks at 1195, 1301 and 1367 cm-1. Typical

spectra of a number of perfluorocarbon blood substituents in aqueous solutions and in solutions simulating serum, plasma and urine are presented. The results show the great utility of the Pulsed Copper Vapor Laser Raman Spectrometer in pharmacokinetic studies of blood substitutes.

546.

FLUORO-RAMAN FOR DRUG DISCOVERY AND PHARMACEUTICAL ANALYSIS. ***Olga Sharts**, Fluorotronics, Inc, 10871 Fuerte Dr, San Diego County, La Mesa, CA 91941, olgasharts@cox.net, and Leo P. Avakyants, Department of Physics, Moscow State University*

Pulsed Laser Isochronic Raman & Fluorescence Method & Apparatus (PLIRFA) is a broad platform technology and a general method of detection and characterization of compounds, products, and biological molecules in liquid and solid states, containing -C-F,= CF2, - CF3 bond (s) as novel molecular Raman labels. Characteristic Raman signal of C-F bonds was observed in the 550-850 nm at (=510.6 & 578.2 nm using pulsed copper vapor laser . Samples were measured under backscattering or fiber optic set-up via glass vials and quartz cuvette, or via flow cell: fluorous compounds, 3-fluoro-aniline, Perfluorobron (blood substitute), pertrifluorobutylamine. Method provides ultrasensitive detection, does not require sample preparation. It is ideally suited for fluorous separations and drug discovery. It is intended for the pharmaceutical, biomedical industries, molecular and medical diagnostics, screening of the drug candidates, in the medical and clinical studies of the effects of fluorinated drugs.

547.

SITE-SELECTIVE FLUORESCENT PROBES FOR CELLS AND ORGANELLES. ***Kristy A. McNitt**[1], **Grant J. Sormunen**[2], Lori L. Scardino[2], Elizabeth M. Ott[2], Scott C. Hartsel[2], and David E. Lewis[3]. (1) Department of Chemistry, University of Wisconsin-Eau Claire, Eau Claire, WI 54702, mcnittka@uwec.edu, (2) Department of Chemistry, University of Wisconsin - Eau Claire, Eau Claire, WI 54702, sormungj@uwec.edu, (3) Department of Chemistry, U. of Wisconsin - Eau Claire*

The use of fluorescence has revolutionized the study of a wide range of biological processes, including cellular metabolism. The key to using fluorescence for screening of drug candidates, for example, at the cell culture level is to ensure that the fluorescent probe is highly specific for the target, and this is often accomplished by conjugation to a target-specific adjuvant. We have developed several simple naphthalimide dyes that have shown excellent localization characteristics in organelles (e.g. lysosomes) and membrane domains (e.g. high-cholesterol microdomains), and which show superior bleach resistance. The synthesis of these dyes, and their localization will be discussed, as will progress towards the development of new dyes for localization in other subcellular organelles (e.g. mitochondria).

548.

SMALL MOLECULE VS GENETICALLY ENCODED FRET SENSORS: PROS AND CONS. ***Carsten Schultz**, Oliver Wichmann, Andreas Schleifenbaum, and Justin Brumbaugh, Gene Expression Programme, European Molecular Biology Laboratory, Meyerhofstr. 1, 69117 Heidelberg, Germany, Fax: 001-49-6221-387-206, schultz@embl.de*

Biochemical experiments are shifting increasingly from the test tube to living cells. This development is mainly made possible by fluorescent probes that replace e.g. radioactivity-based assays. Accordingly, the demand for fluorescent probes has vastly increased over the past decade. While the rapid development of fluorescent proteins from jelly fish and corals fosters the construction of genetically encoded probes, small molecule probes are mostly provided by chemistry laboratories. Which type of probe will serve the needs better? The pros and cons will be discussed with the help of two current examples from our lab. One is a genetically encoded FRET probe based on pleckstrin that was recently shown to reliably monitor changes in PKC-induced phosphorylation levels in living cells with high specificity (Schleifenbaum et al., JACS 2004, 126, 11786). The other is a yet unpublished small molecule FRET probe that monitors the activity of phospholipase A2 (PLA2) in living cells.

549.

NEW METHOD FOR MACRODIOLIDE FORMATION USING SELENOL ESTER. *Liulan Shen, Han-Seo Mun, and **Jin-Hyun Jeong**, College of Pharmacy, Kyung Hee University, 1# Heogi-dong, Dongdaemoon-ku, Seoul, South Korea, Fax: 82-2-961-0357, liulanx@hanmail.net, jeongjh@khu.ac.kr*

A novel macrocyclization for macrodiolide with a selenium and aluminum complex has been developed. ω-Hydroxy methyl selenol ester was produced from ω-hydroxy methyl selenol ester via dimethylaluminum methylselenolate ($Me_2AlSeMe$, 1) in medium-diluted condition. Corey-Nicoulao condition using pyridyl sulfide gave a mixture of 68% macrolide and 25% macrodiolide. Nevertheless, our condition provided only macrodiolide in a 70% yield. In the mean time, macrolactonization in solid phase for monomer macrolide has been investigated. This can offer only macrolide without the presence of macrodiolide.

550.

EFFICIENT STRATEGY FOR THE SYNTHESIS OF SPARSE MATRIX LIBRARIES USING INTEGRATED ROBOTICS. ***Moustafa El-Araby**, Anne Vergnon, Elena Arvanitis, Richard Pottorf, and Dennis J. Hlasta, Combinatorial Chemistry, Johnson & Johnson Pharmaceutical Research & Development, 8-Clarke Drive, Cranbury, NJ 08512, Fax: 609-655 6930, melaraby@prdus.jnj.com*

Combinatorial libraries synthesized from sets of monomers (X*Y*Z) in a full matrix always contain a percentage of compounds with undesired properties (LogP, MW, etc). These undesirable compounds are typically generated from monomer combinations rather than poor selections of monomers. Therefore, elimination of some monomers is not useful particularly when they are known to possess useful pharmaceutical properties. We describe herein the use of common technologies to perform high-throughput synthesis in a sparse matrix, which excludes undesirable compounds. The initial selection of monomers for the virtual library is performed *in-silico* using DiscoverWorks® and 3DX® programs, which filters out compounds with undesired properties. We have developed a protocol on the Gilson 215 that takes the list of desired monomers in the sparse matrix and delivers the proper reagents and monomers into Bohdan Miniblocks® to perform the synthesis. This strategy is exemplified in a small virtual library containing 240 compounds (10 diarylmethyl mesylates and 24 amines), which led to the preparation of a sparse matrix of 189 compounds with lead-like properties. We also applied this strategy to a known library to demonstrate the efficiency of our methods to exclude unwanted compounds during library synthesis.

551.

C-LITHIATION / ALKYLATION OF TRIMETHYLAMINE CYANOBORANE. ***Khuloud J Takrouri**[1], Jehoshua katzhendler[1], and Morris Srebnik[2]. (1) Departement of Medicinal Chemistry and Natural Products, Hebrew University in Jerusalem, Ein Kerem Campus, Jerusalem 91120, Israel, (2) Department of Medicinal Chemistry and Natural Products, Hebrew University in Jerusalem*

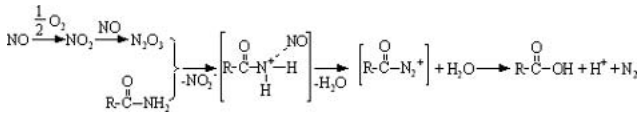
C-lithiation of trimethylamine cyanoborane readily occurs with 1.5 equiv of s-BuLi. The C-lithiated complex, reacts with various electrophiles such as alkyl iodides, aldehydes, ketones, allyl bromide and bromotrimethylsilane, to give the corresponding products, in excellent conversion and in good yields. This is the first example of C-lithiation / alkylation of an amine cyanoborane and allows the preparation of amine cyanoboranes not readily available before. Molecular structure for one of the prepared compounds was determined by X-ray crystallography.

552.

DEAMIDATION OF PEPTIDES IN AEROBIC NITRIC OXIDE SOLUTION. ***Li Kong**[1], Brett M. Showalter[1], Joseph E. Saavedra[2], Gregory S. Buzard[3], and Larry K. Keefer[1]. (1) Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at Frederick, Frederick, MD 21702, Fax: 301-846-5946, kongl@ncifcrf.gov, (2) Basic Research Program, SAIC-Frederick, Inc, (3) BRP, SAIC-Frederick, Inc., National Cancer Institute at Frederick*

Hydrolytic deamidation of protein Asn and Gln residues to Asp and Glu can have significant biological consequences. We hypothesize that a wholly different mechanism of deamidation might occur in the presence of aerobic nitric oxide (NO) (see equation). Accordingly, we examined the deamidating ability of aerobic nitric oxide toward two model peptides, Ser-Glu-Asn-Tyr-Pro-Ile-Val and Lys-Trp-Asp-Asn-Gln, incubating them with the NO-generating compound, $Et_2N[N(O)NO]Na$ (DEA/NO, 5~40 mM), in aerobic pH 7.4 buffer at 37 °C.

Ser-Glu-Asp-Tyr-Pro-Ile-Val, Lys-Trp-Asp-Asp-Gln and Lys-Trp-Asp-Asn-Glu were detected within 5 min, accumulating in yields of up to ~10% within 5 h. The extents of deamidation showed marked dependence on both pH and DEA/NO loading. DEA/NO solutions preincubated to exhaust the NO before the peptides were added did not induce detectable deamidation. The data are consistent with the hypothesis that NO exposures can lead to nitrosative deamidation of peptides, a pathway which differs from the established hydrolytic deamidation mechanism. (NO1-CO-12400)



553.

DESIGN AND SYNTHESIS OF NEW BICYCLIC DIKETOPIPERAZINES AS SCAFFOLDS FOR RECEPTOR PROBES OF STRUCTURALLY DIVERSE FUNCTIONALITY. ***Pedro Besada**, Liaman Mamedova, Craig J. Thomas, Stefano Costanzi, and Kenneth A. Jacobson, NIDDK, NIH, 9000 Rockville Pike, Bethesda, MD 20892, pedrop@intra.niddk.nih.gov*

Diketopiperazines (DKPs) are a common motif in various biologically active natural products, and hence they may be useful scaffolds for the rational design of receptor probes and therapeutic agents. We constructed a new bicyclic scaffold that combines a DKP bridged with a 10-membered ring. In this way we obtained a three-dimensional molecular skeleton, with several amendable sites that provide a starting point to design a new combinatorial library having diverse substituent groups. We developed a general scheme that will allow us to test rings of varying sizes, linkages, and stereochemical parameters. The DKP derivatives were tested for activity in astrocytoma cells expressing receptors coupled to phospholipase C. The new class of DKP derivatives shows utility as pharmacological tools, and further mechanistic studies are needed to explore the spectrum of action of these compounds.

554.

EFFICIENT METHOD FOR THE SOLID-PHASE SYNTHESIS OF TRISUBSTITUED GUANIDINES. ***Jan Urban** and Vincent J. Huber, 8841 Helen James Ave., San Diego, CA 92126, jurban@kemia.com*

Aryl and alkyl substituted guanidines are of considerable interest in the pharmaceutical industry. As such, a number of different methods have been reported for their synthesis. Herein, we describe a new solid phase synthesis of trisubstituted guanidines starting from a resin bound amine. In the course of this synthesis, a functionalized amine resin was reacted with either an isothiocyanate, or thiocarbonyldiimidazole followed by a primary amine, thereby forming a disubstituted thiourea. This initial product was then oxidized under mild conditions to give a dialkylformamidinosulphonic acid. Subsequent treatment with another amine directly led to the core guanidine moiety. Cleavage from the resin gave the desired guanidine, generally in excellent yield and purity. Synthetic details and relevant examples will be disclosed in our report. Present affiliation: J.U., Kemia Inc., 5871 Oberlin Dr., Suite 100, San Diego, CA, 92121; V.J.H, Asahi Kasei Pharma Co., 632-1 Mifuku Ohito-cho Tagata-gun, Shizuoka-ken, 410-2321, Japan.

555.

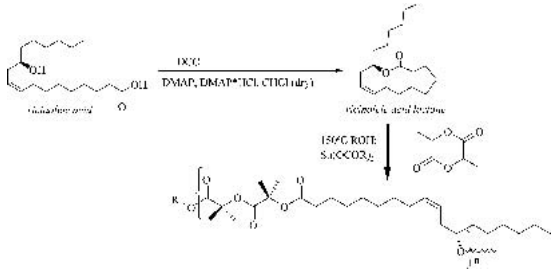
EPOXIDATION AND NUCLEOPHILIC ADDITION TO 4, 4A, 5, 6-TERTRAHYDRONAPHTHALEN-2-ONE: A CONVENIENT STEREOSELECTIVE SYNTHESIS STRATEGY. ***Ze Li**, Department of medicinal chemistry, University of Mississippi, P.O.Box 6284, University Avenue, University, MS 38677, plize@olemiss.edu*

The synthesis of 4, 4a, 5, 6-tertrahydronaphathalen-2-one is described. Epoxidation of this bicyclic compound shows stereo-preference presumably due to constraint of cyclic ring geometry. Nucleophilic addition to the epoxide mediated by Lewis acid exhibits remarkable regioselectivity and diastereoselectivity. It makes possible that regioselectively adding bulky substituents to cyclic compounds in energetically unfavorable axial configures.

556.

MACROLACTONES AND POLYESTERS FROM RICINOLEIC ACID. ***Raia Slivniak** and Abraham J. Domb, Medicinal Chemistry and Natural Products, Hebrew University, School of Pharmacy, Faculty of Medicine, Jerusalem 91120, Israel, Fax: 972-2-6757076, chipmonk@pob.huji.ac.il*

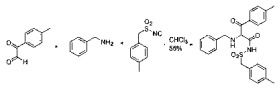
Ricinoleic acid lactones were synthesized from pure ricinoleic acid at a 75% yield. Polymerization of the ricinoleic acid lactones with catalysts commonly used for ring opening polymerization of lactones, under different reaction conditions, resulted in oligomers. Copolymerization with lactide (LA) by ring opening polymerization, using Sn(Oct) as catalyst, yielded block-copolyesters with molecular weights in the range of 5,000 and 16,000. In vitro degradation of P(LA-RA)s with up to 20% w/w of RA, slowly degraded and released only ~ 7% of its lactic acid content in 60 days, while pure PLA under similar conditions released more then 20% of its lactic acid content. On the other hand, copolyesters containing more then 20% w/w of RA degraded and released lactic acid faster then pure PLA due to low crystallinity of the copolymers.



557.

NEW SUBSTRATES FOR UGI THREE-COMPONENT CONDENSATION. ***Qiang John Yu**, Fengping Wei, Jinlin Wang, Liangfu Huang, Zhiqiang Fang, and Wuping Ma, Synchem, Inc, 1700 Mount Prospect Rd, Des Plaines, IL 60018, qyu@synchem.com*

In-house developed arylglyoxals, instead of traditional aldehydes, can act as substrates of Ugi multi-component reactions (MCR) with isocyanide and amine to construct a new series of multi functionalized drug-like molecules. One typical example is illustrated below. The success of this MCR one pot synthesis provides an efficient approach to build chemical library for drug discovery. Further derivations on these scaffolds are possible to create “super libraries”.



558.

REDOX REACTIONS OF BIOACTIVE PHENAZINES: VOLTAMMETRIC AND SPECTROPHOTOMETRIC STUDY. ***Dragic Vukomanovic**, Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, N. Darmouth, MA 02747, Fax: 508-999-9167, dvukomanovic@umassd.edu*

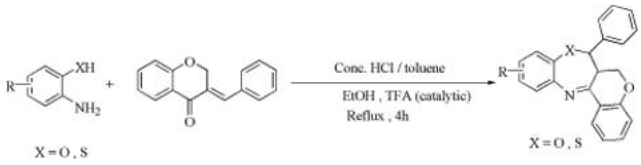
Extracellular electron transfer may be a general mechanism whereby microorganisms generate energy for cell growth and/or maintenance. Perhaps P. *Aeruginosa* generates a small redox-active molecule Pyocyanin (Pyo) to shuttle electrons between reduced and oxidized compounds and biofilms and this electron transfer is likely related to some virulence factors. It is known that the leading cause of morbidity and mortality in cystic fibrosis, for example, continues to be lung infections with P. *aeruginosa* biofilms with Pyo as the active compound. Reduction and protonation of the natural antibiotics Pyo and 1-hydroxyphenazine (1-HP) may play a crucial role in their biological activities. Our computational, electrospray mass spectrometric, and adsorptive stripping voltammetric studies indicated that divalent metals can induce de-methylation of Pyo and that bubbling of nitric oxide into an unbuffered aqueous Pyo solution

causes Pyo's protonation, likely its reduction and ultimately its de-methylation and formation of 1-HP. Since solution redox reactions are biologically more relevant than those observed in gas phase (mass spectrometry) and at the surface of electrodes (adsorptive voltammetry), charge transfer reactions of Pyo and 1-HP were also studied by direct voltammetry (diffusion controlled process), bulk electrolysis and spectrophotometry, at the micro-molar level.

559.

SYNTHESIS OF 1,5-BENZOTHIAZEPINES AND 1,5-BENZOOXAZEPINES VIA CYCLIZATION OF TRANS-3-BENZYLIDENECHROMAN-4-ONES AND 2-AMINOTHIOPHENOLS. ***Edward R. Biehl**, Department of Chemistry, SMU, Dallas, TX 75275, Fax: 2147684089, ebiehl@mail.smu.edu, Hongming Zhang, Department of Chemistry, Southern Methodist University, and Ramadas Sathunuru, Chemistry, SMU*

Synthesis of 1,5-Benzothiazepines and 1,5-Benzooxazepines via Cyclization of *trans*-3-Benzylidenechroman-4-ones and 2-Aminothiophenols Certain 1,5-benzothiazepine and benzooxazepines are important cardiovascular drugs that act as calcium channel blockers for e.g. Diltiazem. There are also indications from the chemical literature that certain substituents on an aromatic ring fused with the 1,5-benzothiazepine nucleus may serve as potential pharmacophores. We report the synthesis of a variety of substituted tetracyclic 1,5-benzothiazepines, benzooxazepines by the reaction of *trans*-3-Benzylidenechroman-4-ones and 2-aminophenols.



560.

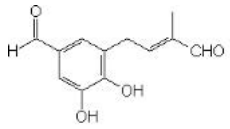
SYNTHESIS OF CYCLIC ANILINES BY REDUCTIVE REARRANGEMENT OF O-SILYLATED KETOXIMES USING BORANE / BORON TRIFLUORIDE. ***Margarita Ortiz-Marciales**[1], Sandraliz Espinosa[2], Luis D. Rivera[3], Melvin De Jesús[4], Orlando E. Casanova[5], Josue A. Benjamin[1], Sandra E. Rodriguez[1], and Wilbert Correa[1]. (1) Chemistry, University of Puerto Rico-Humacao, CUH Station, 100 Rd 908, Humacao, PR 000791-4300, Fax: (787) 850-9422, mr_ortiz@webmail.uprh.edu, (2) Chemistry, University of Puerto Rico, Humacao, (3) Department of Chemistry, University of Puerto Rico- Humacao, (4) Department of Chemistry, University of Puerto Rico, Humacao, (5) Deparment of Chemistry, University of Puerto Rico- Humacao Campus*

Cyclic anilines are very important organic compounds used as intermediates for the synthesis of a variety of pharmaceutical products. The reduction of aromatic oxime ethers by borane have been known to afford the hydroxyl amine or the amine depending on the structure and reaction conditions. However, in previously work, we demonstrated the formation of N-alkyl anilines in the reduction of aromatic O-silylated oximes with borane in THF under reflux conditions. Presently, we are investigating the reduction of O-tert- butyldimethylsilyl-, and O-triisopropylsilyl ketoximes of indanone, tetralone and chromanone, with borane catalyzed by BF3-ethearate. The bulkiness of the substituents on the silicon atom, the size of the aliphatic ring and the presence of alkoxy substituents on the aromatic ring were found to play an important role in the aniline formation. This study will contribute to the development of a new synthesis for tetrahydroquinolines, benzazepines and benzoxazines compounds.

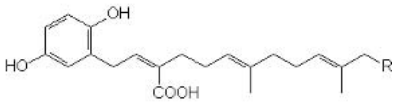
561.

SYNTHESIS OF PRENYLATED AROMATIC COMPOUNDS. ***Sina I. Odejinmi** and David F. Wiemer, Department of Chemistry, University of Iowa, Iowa City, IA 52242-1294, sina-odejinmi@uiowa.edu*

The natural products montadial A (**1**) and the ganomycins (**2** and **3**) are prenylated aromatic compounds with a variety of biological activities. For example, montadial A has strong cytotoxic activity against lymphocytic leukemia of mice and promyelocytic human leukemia, and ganomycins A and B have antimicrobial activity against several Gram-positive and Gram-negative bacteria. We will present efforts directed at the total synthesis of these compounds, where the key steps include coupling the prenyl units to the aromatic cores through reactions such as halogen metal exchange, Claisen rearrangement, and olefin metathesis.



1 Montadial A

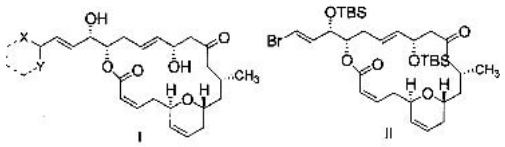


2 R= OH Ganomycin A
3 R = H Ganomycin B

562.

SYNTHESIS OF SIDE-CHAIN MODIFIED DERIVATIVES OF (–)-LAULIMALIDE. ***Junfa Fan**, Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260, juf3@pitt.edu, and Scott G. Nelson, Deparment of Chemistry, University of Pittsburgh*

To establish a library of side-chain modified derivatives of (–)-laulimalide, which are pictured as structure (I), a common precursor vinyl bromide(II) was enantioselectively synthesized with asymmetric Aldol reaction and Yamachuchi lactonization as key steps. Mild Stille coupling reaction conditions developed by Fu was found to be the best way to incorporate diverse side-chain on the structure(I).



563.

STUDY ON FLASH CHROMATOGRAPHY PERFORMANCE USING DIFFERENT SAMPLE LOADING METHODS. ***Jack Liu**, Discovery Chemistry Group US, Biotage, 1725 Discovery Drive, Charlottesville, VA 22911, Fax: 434-979-4743, Jliu@biotage.com*

The purification efficiency is a function of loading mass and sample volume in flash chromatography. Excessive sample mass and dissolving solvents have a direct impact on the quality of the purification. Sample resolution degrades as column becomes overloaded. When a column is overloaded with dissolving solvent, sample peak broadens significantly causing the loss of resolution. This paper demonstrates a new sample loading technology that allows dry-loading to minimize the dilution effect of the dissolving solvent while the sample mass is maximized. The study showed that in the 'dry' loading method, dissolving solvent was first removed under vacuum and then separation was performed that significantly improves peak resolution while the 'wet' loading separation resulted in collapsed resolution with sample carry-over and peak fronting. Removal of dissolving solvent also minimizes the disruption of elution process when a stronger solvent is required to dissolve the sample. It is hoped that results illustrated in this paper will be useful to chemists for better practice of flash chromatography.

564.

USE OF ISCO'S REDISEP SPECIALTY MEDIA COLUMNS FOR THE SEPARATION OF LOW-SOLUBILITY COMPOUNDS. ***Veronica D. Thomason**, Chromatography Department, Teledyne - Isco, 4700 Superior Street, Lincoln, NE 68504, Fax: 402-465-3089, vthomason@teledyne.com*

In addition to C-18 reversed phase, specialty media represent convenient alternative stationary phases for synthetic organic chemists considering flash chromatography purification of insoluble compounds. Efficient and convenient tactics exploiting Teledyne Isco's RediSep C-18 reversed phase and various specialty columns for challenging flash chromatography purification of medium to high polarity compounds will be described.

565.

C18 FLASH COLUMNS IN RAPID ISOLATION OF ORGANIC COMPOUNDS. ***Shahnaz Ghassemi**, Biotage, Inc, 1725 Discovery Drive, Charlottesville, VA 22911, Fax: 434-979-4743, SGhassemi@biotage.com*

Reversed-phase C18 Flash purification has been used for rapid isolation of drug like compounds. The column life time, compounds retention, peaks shape and

loading capacity were compared using a direct injection versus pre-absorption of reaction mixture onto C18

566.

DETERMINATION OF MODAFINIL IN PLASMA AND URINE BY REVERSED PHASE LIQUID CHROMATOGRAPHY. *Harvey A. Schwertner, Clinical Research, Wilford Hall Medical Center, 2200 Bergquist Dr, Lackland Air Force Base, TX 78236, harvey.schwertner@lackland.af.mil, and **Suk Bin Kong**, Department of Chemistry, University of the Incarnate Word, 4301 Broadway, San Antonio, TX 78209, Fax: 210 829 3153, kong@universe.uiwtx.edu*

Modafinil (Provigil) is a new wake-promoting drug that is being used for the management of excessive sleepiness in patients with narcolepsy. In this study, we developed a high-performance liquid-chromatographic procedure (HPLC) for the quantitative analysis of modafinil in plasma and urine. Modafinil was extracted from urine and plasma with ethyl acetate and ethyl acetate-acetic acid (100:1, v/v), respectively, and analyzed on a C18 reverse phase column with methanol-water-acetic acid (500:500:1, v/v) as the mobile phase. Recoveries from urine and plasma were 80.0 and 98.9 %, respectively and the limit of quantitation was 0.1 mg/mL at 233 nm. Forty-eight two-hour post-dose urine samples from sham controls and from individuals taking 200 or 400 mg of modafinil were analyzed without knowledge of drug administration. The analytical procedure is accurate and reproducible and can be used for therapeutic drug monitoring, pharmacokinetic studies, and drug abuse screening.

567.

NEW METHOD EXTRACTING SALICYLIC ACID FROM PLASMA AND FOR ITS ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. *Harvey A. Schwertner[1], **Suk Bin Kong**[2], and Elizabeth L. Richter[2]. (1) Clinical Research, Wilford Hall Medical Center, 2200 Bergquist Dr, Lackland Air Force Base, TX 78236, harvey.schwertner@lackland.af.mil, (2) Department of Chemistry, University of the Incarnate Word, 4301 Broadway, San Antonio, TX 78209, Fax: 210 829 3153, kong@universe.uiwtx.edu*

A number of methods have been developed for the analysis of plasma salicylic acid concentrations. Most of these methods rely on protein precipitation or extraction with halogenated hydrocarbons or ethyl ether. In this study, a salt-solvent combination of sodium chloride and ethyl acetate was developed for the extraction of salicylic acid from plasma. This extraction solvent was found to result in greater recoveries and less co-extractable interferences than the previously used solvents such as chloroform-isopropanol or methylene chloride. Analysis of plasma salicylate was performed using reverse-phase high-performance liquid-chromatography (HPLC) with photodiode array detection at 236 and 300 nm. Recoveries were 80 ± 4% and the lower limit of quantitation was 0.1 µg/mL. With-day and between-day coefficients of variation were 2.0 and 4.9%, respectively, at a concentration of 1.0 µg/mL. The method was used to determine the pharmacokinetics of several forms of acetylsalicylic acid and was found to result in accurate and reproducible results.

568.

SUPER CRITICAL FLUID (SFC) AND HPLC FOR THE ANALYSIS OF CHIRAL PHARMACEUTICALS. ***Amanda L Jenkins** and **Michael A. Burns**, HPLC and SFC Applications Development, Jasco Inc, 8649 Commerce Drive, Easton, MD 21601, Fax: 410-822-7526, ajenkins@jascoinc.com, mburns@jascoinc.com*

Chiral analysis has become critical in drug development because in many cases one enantiomer of a drug can have profoundly different biological effects from the other. This has prompted regulatory changes and the demand for single isomers. HPLC has now become one of the most important techniques for chiral separations; however Supercritical Fluid Chromatography (SFC) is beginning to gain recognition because of its speed and efficiency. SFC is a normal phase technique that uses liquid CO2 as the main component in the mobile phase. This permits high flow rates and shorter analysis times (at least 2-3 times faster than HPLC) without sacrificing resolution. Since the CO2 is not present after separation, waste production is dramatically reduced and the need for post run sample purification almost eliminated. This application examines the speed, efficiency and sensitivity of SFC and HPLC for the analysis of chiral pharmaceuticals and controlled substances.

569.

SUPERCRITICAL FLUID CHROMATOGRAPHY OF IONIC ANALYTES. ***Jun Zheng**[1], Larry Taylor[1], and J. David Pinkston[2]. (1) Department of Chemistry, Virginia Tech, 107 Davidson Hall, Blacksburg, VA 24061, juzheng1@vt.edu, (2) Procter & Gamble Pharmaceuticals*

Addition of a small amount of polar solvent (e.g. modifier) which contains an ionic component (e.g. additive) to a CO_2 mobile phase has shown major improvement in the elution of ionic analytes via packed column SFC. Our study initially focused on the elution of sodium dodecylbenzene sulfonate. The additives studied were alkylammonium acetates. Conventional and Deltabond cyanopropyl and bare silica were the stationary phases. The effect of additive type and concentration on retention were investigated. Sodium 4-octylbenzenesulfonate and sodium p-toluenesulfonate were also studied. The study then turned to the use of sodium alkylsulfonates as mobile phase additives to elute ammonium salts. Propranolol hydrochloride and benzyltrimethylammonium- and cetylpyridinium-chloride were successfully eluted from the Deltabond phase after 5 minutes with a sulfonate additive. To gain insight into the elution mechanism(s), solid state NMR of the silica stationary phase has been performed. Modification of the stationary phase and ion pairing with the analyte are two possible elution mechanisms being considered.

570.

REMOVAL OF TFA FROM ORGANIC SOLUTIONS USING POLYMERIC SPE DEVICES. ***Paul A Boguszewski**[1], Andrew F Coffey[1], John W Davies[1], Alasdair A MacDonald[2], **Aubrey J Mendonca**[2], and Frank P Warner[1]. (1) Polymer Laboratories Ltd, Essex Road, Church Stretton, Shropshire SY6 6AX, United Kingdom, Fax: (+44) 01694 722171, SPS@polymerlabs.com, (2) Polymer Laboratories Inc, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, Fax: 413 253 2476, SPS@polymerlabs.com*

Library compounds when stored as trifluoroacetate salts either as solutions or in solid forms have often been shown to be less stable in long-term storage than neutralised analogues. Whether the trifluoroacetate salt is present as a result of cleavage from a solid support or from post-preparative HPLC, the long-term storage of such compounds can be problematic. Removal of the trifluoroacetic acid (TFA) either using solution phase neutralization followed by liquid–liquid extraction or using basic silica materials can be an expensive and time-consuming process. Herein we report the use of a novel polymeric SPE material which can be used to remove TFA from organic and HPLC solvent systems and also can be used to convert already isolated trifluoroacetate salts into their respective free bases.

571.

INVESTIGATION OF THE EFFECT THAT DIFFERENT DRYING METHODS HAVE ON THE MECHANISM OF THEOPHYLLINE RELEASE FROM MICROCRYSTALLINE CELLULOSE BEADS. ***Patricia Cruz**[1], **Kristin Kurek**[1], Francis Charles Mayville Jr.[1], and Rodney J. Wigent[2]. (1) Natural Science Department, DeSales University, 2755 Station Avenue, Center Valley, PA 18034, Fax: 610-282-0525, fcm0@desales.edu, fcm0@desales.edu, (2) Chemistry and Biochemistry Department, University of the Sciences in Philadelphia*

Samples of microcrystalline cellulose, MCC, and 10% theophylline were granulated, extruded and marumerized into wet sustained release beads. These wet beads were then exposed to several different drying methods including: freeze-drying, convection oven drying, and exposure to four different humidity conditions. The rate of theophylline release from the MCC bead systems was measured by dissolution methods using distilled water and 0.1 molar hydrochloric acid as the solvent systems. The control for this experiment was the convection oven dried beads. The results observed, based on these dissolution studies, suggest that the rate of theophylline release from each MCC dried bead system, either increases, decreases or follows the same release rate as the control sample. Which further suggests that the rate of release of theophylline from each MCC sample depends on the drying method used to dry the wet bead systems.

572.
METHODS FOR PRODUCING POLYMERIC DRUG LOADED ULTRASOUND CONTRAST AGENTS. ***Odelia Mualem Burstein**, School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut St., Philadelphia, PA 19104, Fax: 215-895-4983, om37@drexel.edu, and Margaret A Wheatley, Department of Chemical Engineering, Drexel University*

The objectives of this research are to develop methods to produce polymeric ultrasound (US) contrast agents (CA) with therapeutic capabilities serving as drug carriers, and study the preparation factors affecting their echogenicity in the medical imaging range. Although about 30 million US diagnostic scans are performed yearly, discrimination between diseased and normal tissue is impossible without CA. Combining imaging and drug delivery functions would enable targeting a drug into a specific site, triggering its release at the right time, enabling high local drug doses delivery, greatly reducing undesired effects as in the case of systemic administration. This work describes methods to produce and load hollow Poly-lactic acid microcapsules with drugs of different chemical characteristics, by absorption or incorporation. In vitro and In vivo dose and time response echogenicity of the microcapsules are presented, showing US enhancement of 12-23dB, a half life of 12-15min and US triggered drug released for 6min.

573.
PRACTICAL ASYMMETRIC SYNTHESIS OF A POTENT PDE4 INHIBITOR VIA STEREOSELECTIVE ENOLATE ALKYLATION OF A CHIRAL ARYL-HETEROARYL SECONDARY TOSYLATE. ***Cheng-yi Chen**[1], Paul D. O'Shea[2], Weirong Chen[1], Philippe Dagneau[2], Lisa F. Frey[1], Edward J. J. Grabowski[1], Karen M Marcantonio[1], Robert A. Reamer[1], Lushi Tan[1], Richard D. Tillyer[1], Amelie Roy[2], Xin Wang[2], and Dalian Zhao[1]. (1) Department of Process Research, Merck Research Laboratories, P. O. Box 2000, Rahway, NJ 07065, Fax: 732-594-5170, cheng_chen@merck.com, (2) Merck Frosst Centre for Therapeutic Research*

A practical, chromatography-free catalytic asymmetric synthesis of a potent and selective PDE4 inhibitor is described. Catalytic asymmetric hydrogenation of thiazole ketone afforded the corresponding alcohol in excellent enantioselectivity. Activation of the alcohol via formation of the corresponding p-toluenesulfonate followed by an unprecedented displacement with the lithium enolate of ethyl-3-pyridyl acetate N-oxide generated the required chiral trisubstituted methane. The displacement reaction proceeded with inversion of configuration and without loss of optical purity. Conversion of esters to the PDE4 inhibitor was accomplished via a one-pot deprotection, saponification and decarboxylation sequence in excellent overall yield.

574.
APPLICATION OF NEW TECHNOLOGIES IN PHARMACEUTICAL PROCESS RESEARCH AND DEVELOPMENT. ***Jennifer L. Rutherford**, Groton/New London Laboratories, Pfizer Inc, Groton, CT 06340, Fax: 860-686-5168, jennifer.l.rutherford@pfizer.com*

Case studies will illustrate the utilization of automated technologies in the development of a scalable drug synthesis. Process chemistry challenges are tackled with automated parallel reactors, in situ monitoring techniques, and non-traditional reaction technologies, such as flow reactors.

575.
ENZYMATIC SYNTHESIS OF PHARMACEUTICAL INTERMEDIATES. ***Brian Morgan**, Grace DeSantis, Nelson Barton, David P. Weiner, William A. Greenberg, and Mark J. Burk, Diversa Corporation, 4955 Directors Place, San Diego, CA 92121, bmorgan@diversa.com*

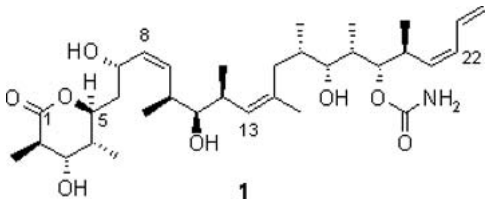
Biocatalysis has become increasingly important for the preparation of chiral fine chemical and pharmaceutical intermediates. However, to fully exploit the potential of biocatalysis, access to novel enzymes with high activity, enhanced thermal and pH stability, and high chemo- regio- and enantioselectivity is necessary. Using high-throughput screening of genomic DNA libraries prepared from samples from diverse environments, we have assembled a collection of enzymes that catalyze a range of useful transformations. The challenges involved in progressing these enzymes from the initial discovery phase to the final process conditions will be discussed using aldolases, amidases, esterases and nitrilases as examples.

576.
EVOLUTION OF A MANUFACTURING ROUTE FOR A HIGHLY POTENT DRUG CANDIDATE. ***Ambarish K. Singh**, Process Research and Development, Bristol Myers Squibb Pharmaceutical Research Institute, One Squibb Drive, P.O. Box 191, New Brunswick, NJ 08903-0191, ambarish.singh@bms.com*

The evolution of a strategy towards a practical synthesis of an advanced drug candidate will be discussed.

577.
FROM DEEP-SEA SPONGE TO PILOT PLANT: THE LARGE SCALE TOTAL SYNTHESIS OF THE MARINE NATURAL PRODUCT (+)-DISCODERMOLIDE. ***Stuart J. Mickel**, Novartis Pharma AG, Lichtstrasse, 4002 Basel, Switzerland, Fax: 41 61 696 29 57, stuart_john.mickel@pharma.novartis.com*

A small, but structurally diverse collection of naturally occurring non-taxane microtubule stabilizing agents (MTS) has been discovered over the last decade. These include the epothilones (EPO), eleutherobin, laulimalide, and discodermolide. (+)-Discodermolide (**1**) is a novel polyketide natural product first isolated from extracts of the marine sponge *Discodermia dissoluta* by researchers at Harbor Branch Oceanographic Institution (HBOI). Discodermolide stabilizes microtubules faster and more potently than any of the other known MTS agents, is a potent inhibitor of tumor cell growth *in vitro* including paclitaxel- (PTX) and EPO-resistant cells. Discodermolide also demonstrates significant human tumor growth inhibition in hollow fiber and xenograft mouse models (including paclitaxel-resistant tumors). Discodermolide is currently undergoing Phase 1 clinical trials.



1

This presentation will discuss in some detail the strategy and tactics that lead to the production of 60 g of (+)-discodermolide for phase 1 clinical trials. Several of the key steps in the synthesis will also be presented with respect to scalability and problems encountered. Some workable solutions to the difficulties will be presented.

578.
DESIGN, SYNTHESIS AND SAR OF NOVEL PHOSPHONATES AS POTENT AND SELECTIVE FBPASE INHIBITORS WITH ORAL EFFICACY IN RODENT MODELS OF TYPE 2 DIABETES. ***Qun Dang**, Mark D. Erion, K. Raja Reddy, Srinivas Rao Kasibhatla, M. Rami Reddy, and Paul D. van Poelje, Metabasis Therapeutics, Inc, 9390 Towne Centre Drive, San Diego, CA 92121, dang@mbasis.com*

Hepatic glucose output is often upregulated in type 2 diabetes (T2DM) and is a significant contributor to both postprandial and fasting hyperglycemia. Increased gluconeogenesis (GNG) accounts for this increased hepatic glucose output, suggesting that inhibitors of the GNG pathway might be potential drug candidates for T2DM. Fructose-1,6-bisphosphatase (FBPase) is a key rate-limiting enzyme of GNG and is a well known target for T2DM. Previous efforts targeting FBPase, however, were unable to find potent, specific and cell-permeating inhibitors. Herein we present the discovery of a series of low molecular weight heterocyclic phosphonates that mimic AMP and are potent inhibitors of FBPase. The initial series of compounds was identified using structure-based drug design. Key pharmacophores were identified by SAR analysis of several series of heterocyclic FBPase inhibitors (FBPaseIs) and were used to discover a series of inhibitors with low nanomolar inhibitory potency, high FBPase specificity and potent oral glucose lowering activity in rodent models of type 2 diabetes. Recently, a compound discovered from this program successfully completed a second Phase IIA clinical trial. The design, synthesis, SAR and in vivo efficacy of FBPaseIs will be presented.

579.

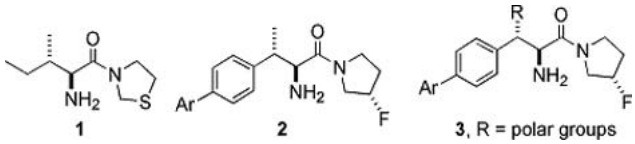
DISCOVERY OF NOVEL PTP1B INHIBITORS FROM A NON-HTS HIT. ***Steven Kirincich***[1], *Bruce Follows*[1], *Alessandro Moretto*[1], *Zhao-Kui Wan*[1], *Douglas P. Wilson*[1], *Michael J Smith*[1], *Dave Erbe*[2], *James Tobin*[2], *Yan-Ling Zhang*[2], *May Tam*[2], *Wei-Xin Xu*[1], *Diane Joseph-McCarthy*[1], *Kenneth Foreman*[1], *Steve Tam*[1], *and Jinbo Lee*[1]. *(1) Department of Chemical and Screening Sciences, Wyeth, 200 Cambridge Park Drive, Cambridge, MA 02140, skirincich@wyeth.com, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth*

Type 2 diabetes currently affects over 200 million people worldwide and can ultimately lead to a multitude of complications resulting from elevated plasma glucose levels. Recent work on PTP1b (protein tyrosine phosphatase) knockout mice have shown that this enzyme plays a role in the insulin receptor signal transduction pathway, and therefore suggests that a PTP1b antagonist could prove beneficial in the treatment of this disease. Our HTS efforts to identify a novel lead proved unsuccessful, but some fortuitous hand-picking from the corporate database provided a verified PTP1b inhibitor. The SAR of a series of compounds based on this lead structure will be discussed.

580.

DISCOVERY OF POTENT AND SELECTIVE ORALLY BIOAVAILABLE β-SUBSTITUTED PHENYLALANINE DERIVED DIPEPTIDYL PEPTIDASE IV INHIBITORS. ***Scott D. Edmondson***[1], *Anthony Mastracchio*[1], *Joseph L. Duffy*[1], *George J. Eiermann*[2], *Huaibing He*[1], *Barbara Leiting*[3], *Joseph F. Leone*[1], *Kathryn A. Lyons*[1], *Amanda M. Makarewicz*[1], *Reshma A. Patel*[3], *Aleksandr Petrov*[2], *Joseph K. Wu*[3], *Nancy A. Thornberry*[3], *and Ann E. Weber*[1]. *(1) Department of Medicinal Chemistry, Merck & Co. Inc, PO Box 2000, RY 123-236, Rahway, NJ 07065, scott_edmondson@merck.com, (2) Department of Pharmacology, Merck & Co. Inc, (3) Department of Metabolic Disorders, Merck & Co. Inc*

Inhibition of dipeptidyl peptidase IV (DPP-IV) has emerged as a promising new approach for the treatment of type 2 diabetes mellitus. DPP-IV inhibitors offer several potential advantages over existing therapies including decreased risk of hypoglycemia, potential for weight loss, and the potential for regeneration and differentiation of pancreatic β-cells. The conversion of the ethyl group of **1** to a biaryl group (**2**) increased potency against DPP-IV as well as selectivity over DPP8 and DPP9. Incorporation of polar aryl groups into **2** further increased DPP-IV potency and provided additional selectivity over off-target enzymes such as quiescent peptidyl peptidase (QPP) and hERG. Unfortunately, the latter benefits were achieved at the expense of diminished rat pharmacokinetic profiles. This presentation will show that the introduction of polar substituents at the β-position (**3**) improves the potency, selectivity, and pharmacokinetic profiles of this promising new series of DPP-IV inhibitors.



581.

MIF KNOCKOUT MICE ARE RESISTANT TO THE DEVELOPMENT OF TYPE 1 DIABETES. ***Yousef Al-Abed***[1], *Ivana Cvetkovic*[2], *Djordje Miljkovic*[2], *Christine Metz*[1], *Ferdinando Nicoletti*[3], *and Stanislava Stosic-Grujicic*[2]. *(1) Laboratory of Medicinal Chemistry, North Shore-LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030, Fax: 1-516-365-5090, yalabed@nshs.edu, (2) Institute for Biological Research "Sinisa Stankovic', (3) University of Catania*

We have recently shown that MIF protein is 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 reduces histopathological changes in the islets of pancreas and suppresses the development of hyperglycaemia. 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 secretion by islets of pancreas and by peritoneal macrophages. Our successful approach to prevent or treat the development of type 1 diabetes using anti-MIF treatments, prompted us to examine the role of MIF deletion in the disease process. In contrast to wild-type mice, we found that mice lacking MIF gene are resistant to the development of type 1 diabetes using the streptozotocin approach.

582.

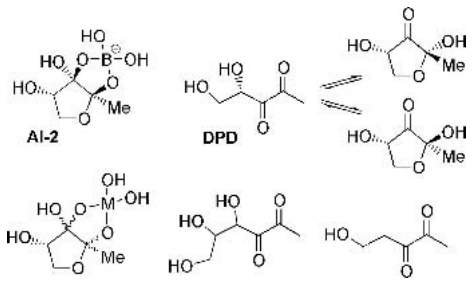
CLUSTERING OF BETA-LACTAM ANTIBIOTICS TO PREDICT CROSS-REACTIVITIES IN ALLERGIC PATIENTS. ***R Terreux***[1], *O preaud*[1], *A roziere*[2], *M Domard*[1], *and J F Nicolas*[2]. *(1) LCMP2, ISPB, University Claude Bernard Lyon1, 8 av. Rockefeller, lyon 69373, France, raphael.terreux@univ-lyon1.fr, (2) U503, INSERM*

Penicillin allergy is a major healthcare problem and can be life-threatening. 46 beta-lactam (penicillins and cephalosporins) molecules were modelled and cluster analysis calculations were performed and computed using the COmFA method. The cluster analysis allowed to select 3 families of b-lactams comprising few molecules to make a set. Each molecule set was tested by prick-test in a cohort of 50 patients allergic to b-lactams and allergic responses were coded into cross reactivity percentage for each molecule. A QSAR study was made to predict potent allergy for drug. Using the same QSAR result, the plot of higher weight probe of the structure activity relationship gives information about allergenic molecule domains.

583.

EVALUATION OF DPD AND SYNTHETIC ANALOGS IN AI-2 BASED QUORUM SENSING. ***Michael M. Meijler***, *Kathleen M. McKenzie, Colin A. Lowery, Longwu Qi, and Kim D. Janda, Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, Fax: 858-784-2590, meijler@scripps.edu*

Quorum sensing is one of the chemical methods that bacteria use to communicate. Cell-to-cell communication is used by single-cell organisms to coordinate their behavior and function in such way that they can adapt to changing environments and allows them to compete with multicellular organisms. A universal signaling molecule, called autoinducer-2 (AI-2), is utilized by both Gram-negative and positive bacteria. The structure of AI-2, as a complex with the V. harveyi sensor protein LuxP, has recently been determined by x-ray crystallography as the ring-closed form of 4,5-dihydroxy-2,3-pentanedione (DPD), chelating boric acid to form a furanosyl borate diester. Previously we have described the first chemical synthesis of DPD in enantiopure form and confirm that this molecule induces bioluminescence in V. harveyi with an activity identical to that reported for AI-2. This synthesis of DPD adds an important new means of investigation to the field of quorum sensing. DPD is stable at low concentrations in buffer solution and can be used to study bacterial coordination of gene expression, biofilm formation and other quorum sensing regulated processes. We also have synthesized and evaluated synthetic analogs of DPD and several metal complexes with this molecule. Our findings may provide the foundation for a structure and mechanism based approach to develop innovative antimicrobial therapy.



584.

STEREOCHEMICAL PREFERENCES OF AUTOINDUCER ANALOGS OF PSEUDOMONAS AERUGINOSA QUORUM SENSING REGULATORS LASR AND RHLR. ***Geetanjali J Jog***, *Chemistry, University at Buffalo, State University of New York, Buffalo, NY 14260, gjjog@buffalo.edu, and Hiro-aki Suga, Department of Chemistry, University at Buffalo, The State University of New York*

Pseudomonas aeruginosa, an opportunistic pathogen, is a common cause of infections in immunocompromised individuals and individuals with cystic fibrosis. The production of a variety of virulence factors as well as biofilm formation is governed by a mechanism known as Quorum Sensing (QS) i.e.

cell-density regulated expression of genes. Treatment of Pseudomonal infections is limited due to the development of antibiotic resistance and the presence of these bacteria in a protected biofilm environment. Autoinducer analogs having the ability to interfere with the binding of the natural autoinducer with its regulatory protein would shut down further activation of QS cascade and production of virulence factors. In order to expand our understanding with respect to the stereochemical requirements of an agonist and antagonist, we carried out the present study. This involved synthesis of pure enantiomers of cis and trans amino alcohols, and further coupling with respective side-chains to give the requisite 3OC12 or C4 derivatives. Herein we discuss the stereochemical preferences of these autoinducer analogs.

585.

STRUCTURE-BASED DESIGN OF NONPEPTIDIC INHIBITORS FOR THE MALARIAL PROTEASE PLASMEPSIN II. ***Fraser Hof***[1], *Andri Schütz*[1], *Daniel Bur*[2], *and François Diederich*[1]. *(1) Laboratorium fuer Organische Chemie, ETH Zurich, ETH Hoenggerberg, CH-8093 Zurich, Switzerland, fraser.hof@org.chem.ethz.ch, (2) Molecular Modeling, Actelion Pharmaceuticals Ltd*

Malaria infects up to 500 million people annually. In the search for new treatments for increasingly prevalent resistant infections, attention has turned to the parasites' enzymatic consumption of hemoglobin as a source of amino acids for growth and development. Plasmepsin II is one such enzyme, and is a promising target for the development of new antimalarials. Using structure-based design, we have developed a new class of plasmepsin II inhibitors that take advantage of the deep hydrophobic *flap* pocket that is revealed only when the enzyme undergoes a large binding-induced conformational shift. The best such inhibitor to date displays an IC50 of 185 nM, in comparison to a value of 14800 nM for a control compound lacking the *flap* binding element. This family of inhibitors engages the catalytic aspartate dyad with a rigid bicyclic diamine - a novel motif for the inhibition of aspartic proteases.

586.

DRUG GURU: A NEW TOOL FOR MEDICINAL CHEMISTS. ***Kent D. Stewart***, *Global Pharmaceutical Research & Development, R-46Y, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, Fax: 847-937-2625, kent.d.stewart@abbott.com, and Craig A. James, Moonview Consultants, Ltd*

Drug Guru™ (Drug Generation Using Rules) is a computer program that applies medicinal chemistry "rules-of-thumb" to an input structure to design new analogs. After entering the structure of interest, the chemist is presented with a list of analogs along with historical precedent of conception. As examples, every benzene ring is converted to a thiophene ring and every amide is made into a retro-amide. Several hundred of these rules have been captured from medicinal chemistry programs over the last 50 years and programmed into a web-based application that is distributed corporate-wide. Some rules, such as the carboxylate-to-tetrazole rule, correspond to well known isostere replacements. Other rules, such as ring modification, metabolism blocking, or solubility increasing rules are more complex. Calculated physical properties, incorporated directly into the software, are useful for prioritizing output. The Drug Guru™ software package and its use in drug discovery programs will be described in this presentation.

587.

DISCOVERY OF NOVEL CARBOXYLATED, HETEROARYL-SUBSTITUTED CHALCONES AS INHIBITORS OF VCAM-1 EXPRESSION FOR USE IN CHRONIC INFLAMMATORY DISEASES. ***Charles Q Meng***, *Liming Ni, Kimberly J Worsencroft, Zhihong Ye, M. David Weingarten, Jacob E Simpson, Jason W. Skudlarek, Elaine M Marino, Ki-Ling Suen, Charles Kunsch, Randy B Howard, Cynthia L Sundell, Martin A Wasserman, and James A Sikorski, AtheroGenics, Inc, 8995 Westside Pkwy, Alpharetta, GA 30004, Fax: 678-336-2501, cmeng@atherogenics.com*

Vascular cell adhesion molecule-1 (VCAM-1) is a key regulator of leukocyte trafficking to sites of inflammation and has been implicated in numerous inflammatory diseases such as asthma. Reported in this presentation are lead evolution and SAR studies that resulted in the discovery of a series of carboxylated, heteroaryl-substituted chalcone compounds as novel, drug-like inhibitors of cytokine-induced VCAM-1 expression. Selected compounds in this series reduced airway inflammation and improved lung function in animal models of asthma, and therefore have the potential to treat human asthma.