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MEDI 1

11β-Hydroxysteroid dehydrogenase type 1 as a therapeutic target in metabolic syndrome and beyond

Brian R Walker *and Jonathan R Seckl, Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, United Kingdom, Fax: +44-131-242-6779, B.Walker@ed.ac.uk*

11β-HSD1 catalyses regeneration of active cortisol from inert cortisone, thereby amplifying glucocorticoid receptor activation in adipose tissue, liver, CNS, and within the blood vessel wall. 11HSD1 is highly regulated and may adjust local cortisol concentrations independently of the plasma cortisol concentrations, eg in response to diet and local inflammation. Inhibition of 11HSD1 may reduce tissue cortisol concentrations without lowering circulating cortisol or the stress response, providing a new approach for treatment of metabolic syndrome, mild cognitive impairment, and in vascular remodelling following ischemia. Preclinical proof of principle data have been obtained in mice with transgenic overexpression of 11HSD1 in liver and adipocytes, targeted deletion of 11HSD1, and using novel selective 11HSD1 inhibitors. In humans, 11HSD1 is increased in adipose tissue in obesity, and non-selective inhibitors enhance insulin sensitivity and improve cognitive function. Results of clinical studies with novel potent selective 11HSD1 inhibitors are therefore eagerly awaited.

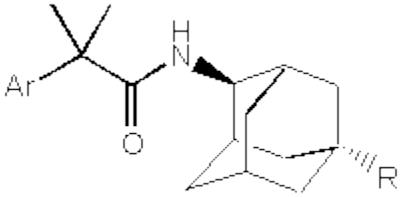
MEDI 2

Substituted adamantanamides as novel inhibitors of 11β-hydroxysteroid dehydrogenase type 1

Joannes TM Linders[1]*, Gustaaf HM Willemsens*[2]*, Danny Bellens*[2]*, Christophe Buyck*[3]*, Peter De Backker*[2]*, Kantharaj Ethirajulu*[4]*, Ron AHJ Gilissen*[4]*, Brian Hrupka*[2]*, Libuse Jaroskova*[1]*, Guy Van Lommen*[1]*, Ann Meulemans*[2]*, Karine Smans*[2]*, Pascal van Dorsselaer*[2]*, Louis Van der Veken*[1]*, and Monique Berwaer*[2]*. (1) Department of Medicinal Chemistry, Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica, Turnhoutseweg 30, B2340-Beerse, Belgium, Fax: +32-14605344, jlinders@prdbe.jnj.com, (2) Department of Internal Medicine, Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen*

Pharmaceutica, (3) Department of Molecular Informatics, Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica, (4) Department of ADME/Tox, Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica

11&beta-Hydroxysteroid dehydrogenase type 1 (11&beta-HSD1) catalyses the interconversion of inactive cortisone into bioactive cortisol principally in liver and adipose tissue. Increases in 11&beta-HSD1 activity and locally increased cortisol concentrations have been associated with visceral adiposity, which is a high-risk factor in developing the clinical features of the metabolic syndrome which includes insulin resistance, diabetes and hyperlipidemia. Transgenic mice overexpressing 11&beta-HSD1 specifically in adipose tissue, develop visceral adiposity, insulin resistance and diabetes, mimicking the clinical traits observed in human. 11&beta-HSD1-null mice exhibit improved lipid profile, hepatic insulin sensitivity and glucose tolerance, designating inhibition of 11&beta-HSD1 as a promising therapeutic approach for the development of new drugs to overcome the morbid conditions associated with the metabolic syndrome. We here report on the discovery of a series of trans-substituted adamantanamides (I), a novel class of 11&beta-HSD1 inhibitors that shows potent and selective inhibition of both mouse and human 11&beta-HSD1 in different cell preparations. The synthesis, structure activity relationships, as well as the optimization towards high metabolic stability, will be presented.



I

MEDI 3

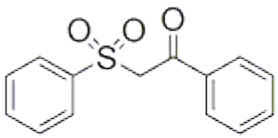
Beta-keto sulfones and beta-keto sulfonamides as selective inhibitors of the 11beta-HSD1

Jason S. Xiang[1], *Vipin Suri*[2], *Steve Tam*[3], *John C. McKew*[1], *Manus Ipek*[1], *Lihren Chen*[3], *Nelson Huang*[1], *Jianchang Li*[1], *Wei Li*[1], *Henry Lombart*[1], *Tarek Mansour*[1], *Jill Nunez*[3], *May Tam*[2], *Charlie Wu*[1], *Yuzhe Xin*[2], *Xin Xu*[4], *Yanling Zhang*[2], *and James Tobin*[2]. *(1) Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5682, jxiang@wyeth.com, (2) Cardiovascular & Metabolic Disease, Wyeth Research, (3) Department of Chemical and Screening Sciences, Wyeth Research, (4) Department of Drug Safety and 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.

We recently published our synthesis and biological evaluation of arylsulfonamidooxazoles as 11b-HSD1 inhibitors. In that paper, we also disclosed our serendipitous discovery of b-keto sulfone as potent 11b-HSD1 inhibitors. Presented herein is the synthesis and SAR study of that b-keto sulfone series. In addition, we will report a new b-keto sulfonamide series as 11b-HSD1 inhibitors. In our screening strategy, a cell-based assay was used as our primary assay to evaluate analogs. The mechanism by which b-keto sulfone and b-keto sulfonamides inhibit 11b-HSD1 activity was investigated. These studies are especially interesting because the keto functionality in these compounds may mimic the role of the ketone moiety in the endogenous 11b-HSD1 substrate cortisone.



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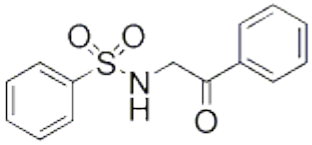


2

MEDI 4

Structural insights into 11B-HSD1 function and inhibition

David J Hosfield, *Structural Chemistry, Takeda San Diego, 10410 Science Center Dr, San Diego, CA 92121, Fax: 858-550-0526, david.hosfield@takedasd.com*

Human 11b-hydroxysteroid dehydrogenase type I (11b-HSD1) is an endoplasmic reticulum-localized membrane protein that catalyzes the interconversion of cortisone and cortisol. In adipose tissue, excessive cortisol production via 11b-HSD1 activity has been implicated in the pathogenesis of type II diabetes and obesity. Crystal Structures of the human type I isozyme, as well as those from murine and guinea pig species have recently been determined. Results from these studies, which have provided the molecular basis for understanding glucocorticoid interconversion and inhibitor binding, as well as novel insights into membrane localization and enzyme regulation in the cell, will be discussed.

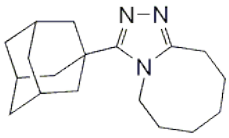
MEDI 5

Bicyclo[2.2.2]octyltriazole inhibitors of 11ß-HSD1

Sherman T. Waddell[1], *Xin Gu*[2], *Gina Santorelli*[2], *Donald Graham*[2], *and James Balkovec*[2]*. (1) Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., Inc, P.O. Box 2000, Rahway, NJ 07065-0900, tim_waddell@merck.com, (2) Department of Medicinal Chemistry, Merck Research Labs*

Abstract: Metabolic Syndrome, or Syndrome X, is now widely recognized by the medical community as being characterized by obesity, hyperlipidemia, hypertension and diminished sensitivity to insulin. Several compelling lines of evidence suggest that elevated levels of cortisol in key tissues, principally liver and adipose, underlie this condition. The enzyme 11ß-hydroxysteroid dehydrogenase-1 is highly expressed in these tissues and serves to convert inactive circulating cortisone into cortisol. Inhibition of this enzyme would therefore be expected to lower tissue cortisol levels and remedy the condition.

We have been engaged in a research effort to identify inhibitors of 11-ß HSD-1 which are suitable candidates for drug development. Specifically described will be the elaboration of screening lead 1 to the bicyclo[2.2.2]octyltriazole 2, a potent compound with poor PK and significant off-target activity, and on to the key milestone compound 3, which substantially addresses the deficiencies of 2.



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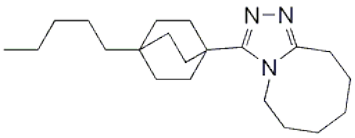

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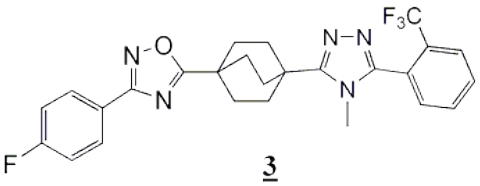


3

MEDI 6

11beta-Hydroxysteroid dehydrogenase type 1 (11beta-HSD1) inhibition prolonged memory retention in mice

Beatriz A. Rocha*, Regulatory Affairs International, Merck Research Laboratories, RY33-200, 126 E. Lincoln Ave, Rahway, NJ 07065, Fax: 732-594-5235, beatriz_rocha@merck.com*

Evidence from animal and human studies indicates that excess of glucocorticoids impairs cognitive function. 11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1), which is expressed in the hippocampus – a brain region that mediates memory retention – converts inactive corticosterone into active glucocorticoids, and recent results suggested that inactivation of this enzyme would enhance memory function in both men and mice. The 11beta-HSD inhibitor carbenoxolone was shown to improve cognitive function in healthy elderly men and type 2 diabetics (Sandeep et al. (2004) PNAS 101: 6734), and inactivation of the 11beta-HSD1 gene prevented aging-induced impairment in mice (Yau et al. (2001) PNAS 98: 4716). The present studies tested the hypothesis that selective inhibition of 11beta-HSD1 would improve memory retention in mice. Therefore, we tested the effect of the a selective 11beta-HSD1 inhibitor in the Novel Object Recognition and the Passive Avoidance tests in

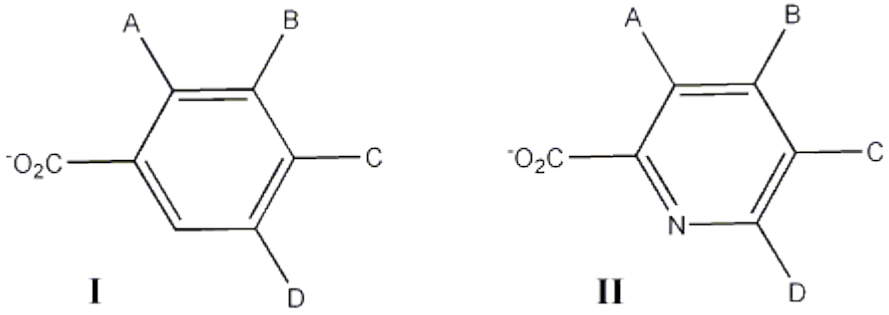
two inbred mouse strains, at young and old ages. Fourteen days of dosing (compound administered in feed at 10- or 3-mg/kg) significantly prolonged retention of passive avoidance in 4-month old 129/Sv and BALBc mice, and novel object recognition in 129/Sv mice. Similarly, 12 months of dosing restored acquisition and prolonged retention of novel object recognition in 14-month old BALBc mice. Taken together these results confirm the hypothesis that selective inhibition of 11beta-HSD1 enhanced cognitive function in young and old mice, and suggest that this mechanism might be relevant in treating aging-induced cognitive impairment.

MEDI 7

Novel inhibitors of Trypanosoma cruzi trans-sialidase

João Neres[1], *Pascal Bonnet*[1], *Richard A Bryce*[1], *Pravin L. Kotian*[2], *Alejandro Buschiazzo*[3], *Pedro Alzari*[3], *and Kenneth T. Douglas*[1]. *(1) School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom, Fax: 44-161-275-2481, joao.neres@manchester.ac.uk, (2) BioCryst Pharmaceuticals, Inc, (3) Unité de Biochimie Structurale, Institut Pasteur*

Chagas' disease, estimated to cause 50,000 deaths annually in Central and South America, is caused by the Trypanosoma cruzi parasite. The only drugs, nifurtimox and benznidazole, have limited efficacy. Surface sialylation plays a central role in evasion of T. cruzi trypomastigotes from host immune responses but T. cruzi is unable to synthesise sialic acids de novo, and uses trans-sialidase (TcTS) to catalyse the transfer of sialic acid molecules from host glycoconjugates to its own surface mucin-like glycoproteins. TcTS appears to be a key enzyme to its infective/invasive ability. The crystal structure of TcTS allows structure-based inhibitor design. Our initial approach uses benzoic acid derivatives, a framework which had been successful against Influenza neuraminidase. We also used virtual screening of databases using Dock 4.0. As a result, several benzoic acid and pyridine derivatives (I and II), were found to inhibit TcTS with IC50 values in the 100 μM range.



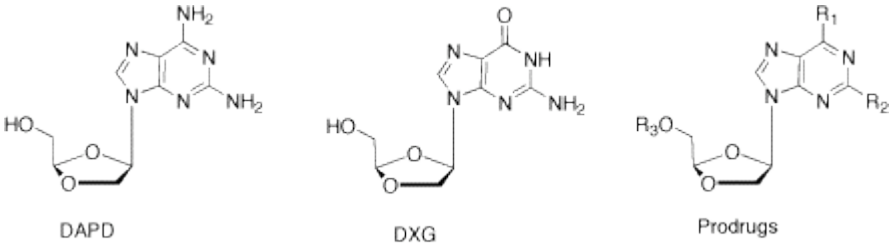
MEDI 8

Synthesis and anti-HIV activity of DAPD (Amdoxovir) prodrugs *in vitro* against M184 HIV mutant

Janarthanan Narayanasamy[1], *Ashoke Sharon*[1], *Manik R. Pullagurla*[1], *Raymond F. Schinazi*[2], *and Chung K. Chu*[1]. *(1) College of Pharmacy, The University of Georgia,*

Athens, GA 30602, Fax: 706-542-5381, jnarayan@mail.rx.uga.edu, (2) Emory University School of Medicine/Veterans Affairs, Atlanta, GA, 30033

DAPD (Amdoxovir), a produg of DXG, is active against 3TC- and AZT-resistant HIV mutants and is currently undergoing Phase II clinical trials for the treatment of HIV infection. The oral bioavailability of DAPD was estimated to be approximately 30% in monkey and rat. In order to further improve the anti-HIV activity and to improve the pharmacokinetic profiles of DAPD, we have synthesized a variety of lipophilic, acid stable, adenosine deaminase activated prodrugs of DAPD/DXG. We have also synthesized organic acid salts and 5'-O-valyl amino acid esters of DAPD to enhance the cell penetration. All the synthesized prodrugs were evaluated for their anti-HIV activity against LAI M184V mutant in PBM cells. Synthesis and anti-HIV activity of these prodrugs will be presented. (Supported by NIH AI 25899 and VA).

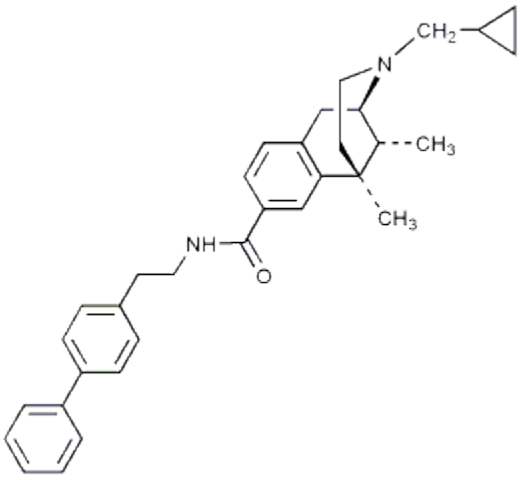


MEDI 9

Synthesis and evaluation of novel N-substituted derivatives of 8-carboxamidocyclazocine

Melissa A. VanAlstine[1], *Mark P. Wentland*[2], *Robert J. Kucejko*[2], *Rongliang Lou*[1], *Dana Cohen*[3], *Brian I. Knapp*[4], *and Jean M. Bidlack*[4]. *(1) Department of Chemistry, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, vanalm@rpi.edu, (2) Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, (3) Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester, (4) Department of Pharmacology and Physiology, School of Medicine and Dentistry, University of Rochester*

It was discovered that 8-carboxamidocyclazocine (8-CAC) has high affinity for mu and kappa opioid receptors. Preliminary structure-activity relationship studies for 8-CAC revealed that mono-substitution of the carboxamide nitrogen of 8-CAC with methyl or phenyl reduced the binding affinity considerably while dimethylation ablated the binding affinity. Results from other studies indicated that opioid receptors could accommodate aryl groups on the 8-position of the cyclazocine core structure. This led us to further probe the opioid receptor space for this hydrophobic pocket. The synthesis and opioid receptor binding of a series of aryl-containing N-mono-substituted analogs of the lead 8-CAC will be reported. High affinity binding to mu, kappa and delta opioid receptors was observed for the 8-[N-((4'-phenyl)-phenethyl)carboxamido] analog.



MEDI 10

Rational redesign of human butyrylcholinesterase for treatment of cocaine abuse

*Yongmei Pan, Daquan Gao, Wenchao Yang, Hoon Cho, Hsin-Hsiung Tai, and **Chang-Guo Zhan**, Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, KY 40536, Fax: 859-323-3575, zhan@uky.edu*

Molecular dynamics was employed to simulate the transition state for the first chemical reaction step (TS1) of cocaine hydrolysis catalyzed by human butyrylcholinesterase (BChE) and its mutants. The simulated results demonstrate that the overall hydrogen bonding between the carbonyl oxygen of (-)-cocaine benzoyl ester and the oxyanion hole of BChE in the TS1 structure for (-)-cocaine hydrolysis catalyzed by A199S/S287G/A328W/Y332G BChE should be significantly stronger than that in the TS1 structure for (-)-cocaine hydrolysis catalyzed by the wild-type BChE and other BChE mutants simulated. Thus, the transition-state simulations predict that A199S/S287G/A328W/Y332G mutant of BChE should have a significantly lower energy barrier for the reaction process and, therefore, a significantly higher catalytic efficiency for (-)-cocaine hydrolysis. The theoretical prediction has been confirmed by wet experimental tests showing a ~456-fold improved catalytic efficiency of A199S/S287G/A328W/Y332G BChE against (-)-cocaine. This is a unique study that an enzyme mutant is designed based on transition-state simulation. The encouraging outcome demonstrates that the novel design approach based on transition-state simulation is promising for rational enzyme redesign and drug discovery.

MEDI 11

Protein Arginine Deiminase 4: From mechanism to catalysis

***Paul R. Thompson**, Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, SC 29208, Fax: 803-777-9521, thompson@mail.chem.sc.edu*

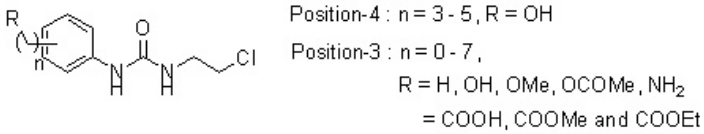
Protein Arginine Deiminase 4 (PAD4) is a calcium dependent transcriptional corepressor that catalyzes the deimination of arginine residues in a number of proteins, including Histones H2A, H3, and H4. We have initiated studies to characterize the molecular mechanism of PAD4 and develop inhibitors targeting this enzyme because its activity has been implicated in the onset and progression of Rheumatoid Arthritis; therefore PAD4 inhibitors are expected to represent lead compounds for the treatment of Rheumatoid Arthritis. In this paper, I will discuss our efforts to characterize the catalytic mechanism of PAD4 and our efforts to develop PAD4-selective inhibitors.

MEDI 12

Tubulin-binding antimitotic agents: Synthesis and structure activity-relationship study of N-phenyl-N'-(2-chloroethyl)urea series mimicking colchicinoids on microtubule assembly

***Sébastien Fortin**[1], Emmanuel Moreau[1], Michel Desjardins[2], Jean L. C. Rousseau[2], Jacques Lacroix[1], René C-Gaudreault[1], and Lakshmi P. Kotra[3]. (1) Unité de Biotechnologie et de Bioingénierie, Centre de recherche, C.H.U.Q., Hôpital Saint François d'Assise, Université Laval, Quebec, QC G1L 3L5, Canada, Fax: 418-525-4372, sebastien.fortin.1@ulaval.ca, (2) IMOTEP, (3) Departments of Pharmaceutical Sciences, Chemistry and Division of Cell & Molecular Biology, University of Toronto and Toronto General Research Institute*

Tubulin is a target for many anticancerous drug candidates. It includes compounds sharing a N-phenyl-N'-(2-chloroethyl)urea (CEU) scaffold. Unlike most anti-tubulin docking agents, these CEU compounds are protein monoalkylating agents that covalently bind on a tubulin amino acid in the colchicine-binding site through a nucleophilic substitution involving the N'-(2-chloroethyl)urea moiety. To improve CEU growth inhibition and &beta-tubulin alkylation, we designed several CEU series and evaluated three CEU susbtituents' properties, namely 1) position of the chain on the aromatic ring, 2) homologation of the substituting chain and 3) effect of a &omega-substituting oxygenated group. Twelve molecules (1e - 1h, 2e, 2f, 3e – 3g and 8e – 8g) were found to exhibit growth inhibition in the nanomolar range. Moreover, hydroxy compound 1f, methoxyl compound 2f and alkyl compound 8e with their 5 carbon atom chain substituent showed the highest affinity for tubulin, as demonstrated by an electrophoretic mobility shift assay of the alkylated &beta-tubulin.



MEDI 13

Development of chalcones as potential anticancer agents

Sylvie Ducki[1]*, Nicholas J. Lawrence*[2]*, James P. Snyder*[3]*, Benjamin Greedy*[2]*, Jérémie Fournier dit Chabert*[1]*, Grant Mackenzie*[1]*, James Nettles*[4]*, and Darren Cook*[1]*. (1) Biosciences Research Institute, University of Salford, Cockcroft Building, Salford M5 4WT, United Kingdom, Fax: 0161 295 5111, S.Ducki@Salford.ac.uk, (2) Drug Discovery Program, H Lee Moffitt Cancer Center & Research Institute, (3) Department of Chemistry, Emory University, (4) Lead Discovery Center, Novartis Institutes for Biomedical Research*

We have identified a phenylbutenone (IC_{50} K562 = 60 µM) from the Chinese mint *Scutellaria barbata*. Structure-activity relationship (SAR) studies led to the discovery of chalcone SD400 (IC_{50} K562 = 0.21 nM). *In vitro* biological studies allowed us to elucidate its mode of action: the drug interacts with tubulin, a protein that is essential for cell division and cell shape, at the colchicine-binding site and inhibits assembly into microtubules. In 2004, Ravelli published the structure of tubulin:colchicine, giving a much needed insight into the protein's structure and function. This structure helped us gain an understanding of how chalcone SD400 interacts with tubulin. This understanding has allowed us to design a new generation of chalcones which are powerful inhibitors of tubulin assembly. Pharmacokinetic studies have allowed us to optimise the drug-like propeties of these agents which are now ready to enter clinical trials.

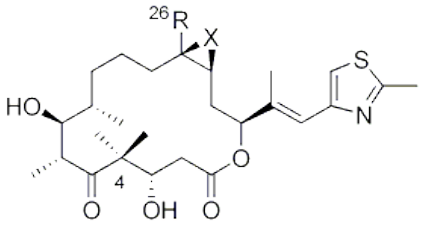
MEDI 14

Design, synthesis and biological investigation of conformationally constrained epothilone D analogs

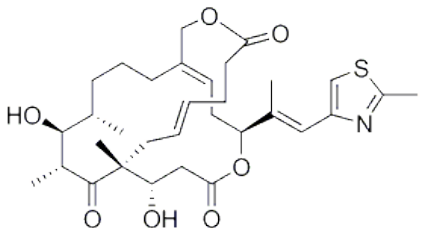
Thota Ganesh[1]*, Peggy Brodie*[1]*, Susan Bane*[2]*, Shubhada Sharma*[2]*, Rudravajhala Ravindra*[2]*, James P. Snyder*[3]*, and David G. I. Kingston*[1]*. (1) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, tganesh@vt.edu, (2) Department of Chemistry, State University of New York, (3) Department of Chemistry, Emory University*

Epothilones A and B are potential anti-cancer agents that exert a mechanism of action similar to that of the clinically proven drug paclitaxel (Taxol®). They bind to the same site as paclitaxel on microtubules, and enhance tubulin polymerization

more effectively than paclitaxel. The rational design of improved epothilone analogs would be greatly assisted by knowledge of the bioactive tubulin-binding conformation of the parent epothilones. Very recently one of us proposed a unique epothilone A conformation bound to tubulin based on ligand conformer deconvolution by NMR, SAR analysis and electron crystallographic density maps derived from Zn-stabilized tubulin sheets. Inspection of the model reveals the juxtaposition of the C4-Me and C26-Me groups. We have designed a conformationally constrained epoD derivative (5) with a bridge between the C4 and C26 methyl groups as one step in evaluating this proposed conformation. The synthesis of this bridged epothilone and its bioactivity will be presented.



1 R = H, X = O Epothilone A
2 R = Me, X = O Epothilone B
3 R = H, X = bond Epothilone C
4 R = Me, X = bond Epothilone D



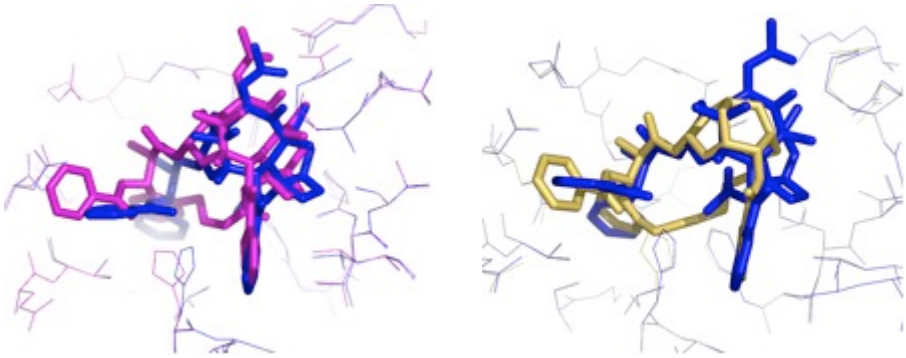
5

MEDI 15

Computational analysis of T-Taxol and highly modified taxane analogs

Ana A. Alcaraz[1]*, Thota Ganesh*[2]*, Chao Yang*[2]*, Shubhada Sharma*[3]*, Andrew Norris*[2]*, Anil K. Mehta*[1]*, Susan Bane*[3]*, David G. I. Kingston*[2]*, and James P. Snyder*[1]*. (1) Department of Chemistry, Emory University, 1515 Dickey Dr, Atlanta, GA 30322, Fax: 404-727-6586, aalcara@emory.edu, (2) Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, (3) Department of Chemistry, State University of New York*

Research around the anti-cancer drug paclitaxel (Taxol®) has shifted to the development of analogs with much greater potency, less toxicity and the ability to surmount resistance. Highly refined three-dimensional structures have proven to be decisive as guides to molecular design. The butterfly or T-form, as derived from ligand conformational fitting to the electron crystallographic density (*PNAS* **2001**, *98*, 5312-5316), has not only inspired a number of recent syntheses, but has uniquely led to bridged analogs with activity surpassing the parent drug in both tubulin polymerization and cytotoxicity assays (*PNAS* **2004**, *101*, 10006-10011). Subsequent chemical, biological and computational studies within our group have focused on two extremes: highly constrained bridged analogs and simplified taxanes, both derived from computational exploitation of the T-Taxol conformation. A number of computer-aided strategies and predictions employing conformational searching, protein docking and molecular dynamics will be presented along with synthetic and biological outcomes.



MEDI 16

Rediscovery of natural products using genomic screening

Akira Kawamura, *Angelika Brekman, Tal H Hasson, Anna Takaoka, and Stephanie Wolfe, Department of Chemistry, Hunter College – The City University of New York, 695 Park Avenue, 1305bN, New York, NY 10021, Fax: 212-772-5332, akawamur@hunter.cuny.edu*

Natural products, such as herbal components, are rich resources of potential therapeutic agents. Recently, our group developed a new screening methodology, called "genomic screening," to uncover previously overlooked biological properties of natural products. Genomic screening allows identification of compounds that can regulate cellular gene expression. Thus, compounds identified through genomic screening can serve as powerful molecular tools for functional genomics studies. In addition, such compounds could open up possibilities for transcription therapies based on cell-permeable small molecules. This presentation will first highlight the unique screening power of our methodology by comparing it to a traditional screening method, i.e., cytotoxicity-based screening. Then, latest results from our studies on several herbal medicine formulations will be presented to illustrate novel biological properties of compounds identified through genomic screening.

MEDI 17

Discovery and development of a small molecule antagonist of leukocyte function-associated antigen-1 (LFA-1)

Murali T. G. Dhar[1], *Suzanne J. Suchard*[1], *Dominique Potin*[2], *Michèle Launay*[2], *Francoise Monatlik*[2], *Patrice Malabre*[2], *Maud Fabreguettes*[2], *André Fouquet*[2], *Magali Maillet*[2], *Eric Nicolaï*[2], *Loïc Dorgeret*[2], *François Chevallier*[2], *Dominique Besse*[2], *Monique Dufort*[2], *François Caussade*[2], *Dawn K. Stetsko*[1], *Stacey Skala*[1], *Patricia M. Davis*[1], *Praveen Balimane*[1], *Karishma Patel*[1], *Zheng Yang*[1], *Punit Marathe*[1], *Pathanjali Kadiyala*[1], *Kim W. Mcintyre*[1], *Xiaoxia Yang*[1], *Jennifer Postelneck*[1], *Robert Townsend*[1], *Sam Chao*[1], *Leslie Leith*[1], *Zili Xiao*[1], *Arvind Mathur*[1], *Bang-Chi Chen*[1], *Daugh-Rurng Wu*[1], *Ravindra Tejwani*[1], *Denette Murphy*[1], *Russell S. Weiner*[1], *Murray McKinnon*[1], *Stephen Bai*[1], *Joel C. Barrish*[1], *Jeffrey A. Robl*[1], *and Edwin J. Iwanowicz*[1]. *(1) Bristol-Myers Squibb Pharmaceutical Research Institute, Route 206 and Provinceline Road,*

Princeton, NJ 08543-4000, Fax: 609-252-6804, murali.dhar@bms.com, (2) Cerep, 19 avenue du Quebec, 91951 Courtaboeuf cedex, France

LFA-1 (leukocyte function-associated antigen-1), is a member of the beta 2-integrin family and is expressed on all leukocytes. The LFA-1/ICAM interaction promotes tight adhesion between activated leukocytes and the endothelium, as well as between T cells and antigen-presenting cells. Evidence from both animal models and clinical trials provides support for LFA-1 as a target in several different inflammatory diseases. This presentation will describe the design, synthesis, in vitro and in vivo activity of a small molecule LFA-1 antagonist that is currently in phase II clinical trials for the treatment of inflammatory diseases.

MEDI 18

Design and synthesis of potent, selective, and orally efficacious DPP4 inhibitors accelerated by high-throughput structural biology

Stephen L. Gwaltney II*, Kathleen Aertgeerts, Jun Feng, Stephen W. Kaldor, Daniel B. Kassel, Melinda Manuel, Marc Navre, G. Sridhar Prasad, Lihong Shi, Robert J. Skene, Jeffrey A. Stafford, Mike Wallace, Rongda Xu, Sheng Ye, Zhiyuan Zhang, and David R. Webb, Department of Chemistry, Takeda San Diego, 10410 Science Center Drive, San Diego, CA 92121, Fax: 858-550-0526, stephen.gwaltney@takedasd.com*

DPP4 is a post-proline dipeptidyl aminopeptidase that belongs to the S9b peptidase family of proteolytic enzymes. DPP4 plays a significant role in maintaining glucose homeostasis by controlling the activity of the incretins glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Inhibition of DPP4 in wild-type or diabetic mice leads to increased levels of these peptides in the circulation, enhanced insulin secretion, and improved glucose tolerance. More importantly, it has been shown that a selective inhibitor of DPP4 improves plasma glucose levels in human type II diabetics. Takeda San Diego has solved the crystal structure of DPP4 and numerous complexes of inhibitors bound to DPP4. These data have guided the structure-based design and optimization of potent, selective, and orally efficacious inhibitors of DPP4. The discovery of the pyrimidinedione SYR-322, which is currently advancing in clinical trials, will be presented.

MEDI 19

Discovery of a potent CXCR2 receptor antagonist for the treatment of inflammatory disorders

Michael P. Dwyer[1]*, Younong Yu*[1]*, Jianping Chao*[1]*, Cynthia Aki*[1]*, Jianhua Chao*[1]*, Biju Purakkattle*[1]*, Diane Rindgen*[2]*, Richard Bond*[3]*, James Jakway*[3]*, R. William Hipkin*[3]*, James Fosetta*[3]*, Waldemar Gonsiorek*[3]*, Hong Bian*[3]*, Jay Fine*[3]*, J. Robert Merritt*[4]*, Laura L. Rokosz*[4]*, Bernd Kaiser*[4]*, Ge Li*[4]*, Wei Wang*[4]*, Tara Stauffer*[4]*, Lynne Ozgur*[4]*, and Arthur Taveras*[1]*. (1) Department of Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Rd, Kenilworth, NJ 07033-0539, Fax: 908-740-7152, (2) Department of Drug Metabolism, Schering-Plough Research Institute, (3) Department of Biological Research, Schering-Plough Research Institute, (4) Pharmacopeia Drug Discovery Inc*

Interleukin-8 (IL-8) and related CXC chemokines have been implicated in controlling the trafficking of neutrophils to the sites of inflammation. In addition, IL-8 levels are elevated in human body fluids in various inflammatory conditions such as chronic obstructive pulmonary disease (COPD), arthritis, and psoriasis where neutrophil infiltration is observed. To date, two G-coupled protein receptors have been identified that are activated by IL-8 (CXCR1 and CXCR1) making them attractive targets for therapeutic intervention for various inflammatory disorders. Herein, we report the discovery and SAR development of a series of potent, orally bioavailable CXCR2 receptor antagonists, which culminated in the identification of our current clinical candidate. This presentation will highlight the medicinal chemistry efforts toward the optimization of potency, selectivity, and pharmacokinetic profiles of this class of CXCR2 receptor antagonists toward our clinical candidate. In addition, predictive biological and preclinical data to support the selection of the lead candidate for clinical evaluation will be presented.

MEDI 20

Discovery of AMG 131: A selective modulator of PPAR-γ

Lawrence R. McGee[1], *Steven M. Rubenstein*[1], *Jonathan B. Houze*[1], *Guosen Ye*[1], *Yang Li*[2], *R. Marc Learned*[2], *Jin-Long Chen*[2], *Zhulun Wang*[3], *Alykan Motani*[2], *Pieter B. Timmermans*[2], *Hisashi Shinkai*[4], *Atushi Hagiwara*[4], *Noburo Furukawa*[5], *Jun Nishiu*[5], *and Motanao Nakamura*[6]. *(1) Medicinal Chemistry, Amgen San Francisco, LLC, 1120 Veterans Blvd., South San Francisco, CA 94080, Fax: 650-244-2015, mcgee@amgen.com, (2) Biology, Amgen San Francisco, LLC, (3) Structural Biology, Amgen San Francisco, LLC, (4) Chemical Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc, (5) Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc, (6) Pharmaceutical Frontier Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc*

PPAR-γ is a well-validated target for treatment of type 2 diabetes. However, the current thiazolidinedione-based therapies display a variety of undesirable side effects. By selecting for PPAR-γ ligands which act as partial agonists or antagonists, we discovered a new class of anti-diabetic agents. We plan to discuss the SAR which led to the identification of AMG 131, currently in a Phase 2 trial.

AMG 131 is a selective ligand for PPAR-γ, structurally distinct from the glitazone class, with unique properties in functional assays for receptor activation. X-ray crystallography reveals that AMG131 interacts with PPAR-γ through a distinct binding mode without the direct interactions to key residues that are characteristic of full agonists. The unique biochemical properties of AMG131 may provide a mechanistic basis for its distinct pharmacological profile.

AMG 131 shows potent anti-diabetic efficacy in animal studies with reduced adverse cardiovascular side effects relative to full agonists.

MEDI 21

Discovery of SCA-136, a novel 5-HT2C agonist, for the treatment of Schizophrenia

P. Siva Ramamoorthy[1], *Chad Beyer*[2], *Julie Brennan*[2], *John Dunlop*[2], *Stacey Gove*[1], *Steve Grauer*[2], *Boyd L. Harrison*[1], *Qian Lin*[2], *Jessica Malberg*[3], *Karen Marquis*[2], *Hossein Mazandarani*[2], *Mike Piesla*[2], *Claudine Pulicicchio*[2], *Sharon Rosenzwieg-Lipson*[2], *Ann-Marie Sabb*[1], *Lee Schechter*[2], *Gary Stack*[1], *and Jean Zhang*[2]. *(1) Chemical & Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, ramamos@wyeth.com, (2) Discovery Neuroscience, Wyeth Research, (3) Neuroscience, Wyeth Research*

At present, the most widespread treatments for schizophrenia are the 'atypical' antipsychotics, which in some cases, combine dopamine (D_2) receptor antagonism with serotonin ($5\text{-}HT_{2A}$) receptor antagonism. Despite the reported advances in efficacy and side-effect liability of atypical antipsychotics over typical antipsychotics, these compounds may not adequately treat all of the symptoms of schizophrenia and can be accompanied by problematic side effects such as weight gain. The $5\text{-}HT_{2C}$ receptor subtype has been implicated in a wide variety of conditions including schizophrenia and depression and as a consequence has received considerable attention as a target for drug discovery. Furthermore, $5\text{-}HT_{2C}$ agonists will be less likely to produce the body weight increases associated with some current atypical antipsychotics. This presentation will summarize medicinal chemistry efforts at Wyeth on a novel series of tetracyclic heterocycles that culminated in the discovery and development of SCA-136, a compound currently advancing in human clinical trials. SAR and pre-clinical properties of this unique $5\text{-}HT_{2C}$ agonist will be discussed.

MEDI 22

NVP-AEB071, an orally active inhibitor of early T cell activation for the prevention of graft rejection

Jürgen Wagner[1], *Rainer Albert*[1], *Nigel G. Cooke*[1], *Sylvain Cottens*[2], *Richard Sedrani*[2], *Maurice van Eis*[1], *Peter von Matt*[1], *Claus Ehrhardt*[2], *Jean-Pierre Evenou*[1], *Gerhard Zenke*[1], *Volker Brinkmann*[1], *Christian Beerli*[1], *Armin Brülisauer*[3], *Gisbert Weckbecker*[1], *Marc Bigaud*[1], *Charles Pally*[1], *Grazyna Wieczorek*[1], *Christoph Burkhart*[1], *Jianping Li*[1], *Barbara Nüsslein-Hildesheim*[1], *Christoph Heusser*[1], *Christian Bruns*[1], *Randall Morris*[1], *Gabriele Rummel*[4], *Wilhelm Stark*[4], *and Gottfried Baier*[5]. *(1) Department of Autoimmunity and Transplantation, Novartis Institutes of Biomedical Research, Basel CH-4002, Switzerland, juergen.wagner@novartis.com, (2) Protease Platform, Novartis Institutes of Biomedical Research, (3) DMPK, Novartis Pharma, (4) Protein Structure Unit, Novartis Institutes of Biomedical Research, (5) Institute of Medical Biology and Human Genetics, University of Innsbruck*

The presentation will include the disclosure of the structure of NVP-AEB071, an orally active inhibitor of early T cell activation for the prevention of graft rejection. The biological activity profile and related structure activity data will also be discussed.

MEDI 23

On the DNA interactions of thiophene compounds

Binh Nguyen[1], *Farial A. Tanious*[1], *Sirish Mallena*[1], *Arvind Kumar*[1], *David W. Boykin*[2], *and W. David Wilson*[1]. *(1) Department of Chemistry, Georgia State University, Atlanta, GA 30303, chebkn@langate.gsu.edu, (2) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University*

Previous results indicate that some thiophene-based diamidine compounds can enhance DNA binding better than furan analogs. To further elaborate on the DNA interactions of this class of compounds and to examine the influence of substituents, a new set of thiophene-based amidine compounds was synthesized. The DNA interactions were investigated by spectroscopic, calorimetric and biosensor techniques. Very different recognition patterns and energetic profiles were observed for different derivatives. Thermal melting analyses suggest that the thiophene compounds bind preferentially to A/T sequences. Biosensor binding affinity studies indicate that removing of one of the two amidine groups can significantly reduce the binding free energies. Calorimetric titrations indicate a small enthalpy contribution and slightly larger contribution of entropy to the binding energetics at 25 ºC. Enthalpy-entropy compensation leads to relatively unchanged binding free energies from 5 ºC to 45 ºC.

MEDI 24

Novel furandione with activity against cancer cell cultures and *Plasmodium falciparum*

Angela Hoffman*, Department of Chemistry and Physics, University of Portland, 5000 N Willamette Blvd, Portland, OR 97203, Fax: 503-943-7784, hoffman@up.edu, and Leah L. Frye, Schrödinger*

A novel furandione with a structure similar to usnic acid was purified from the culture of a *Phomopsis* species isolated from a New Guinea plant. This compound inhibited the growth of human carcinoma cell lines BroTo and A549 using the MTT cell proliferation assay with an IC_{50} of 10 µg/mL in 24 hours. A fluorescence-based assay against *P. falciparum* gave an IC_{50} of 3.4 µg/mL (chloroquine sensitive strain) and 4.6 µg/mL (chloroquine resistant strain) in 48 hours. Investigations are underway to determine the mechanism by which this compound inhibits growth of the cultured cell lines and the malaria organisms.

MEDI 25

Cucurbitacin analogs protect HepG2 and HSC-T6 liver cell lines against cytotoxicity and proliferation

Judit Bartalis*, Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD 57007, Fax: 804-751-0338, judit.bartalis@pmusa.com, and Fathi T. Halaweish, Chemistry & Biochemistry, South Dakota State University*

The cucurbitacin are highly oxygenated triterpenes found in various plants. Herbs containing them have been used successfully in folk medicine to heal acute or chronic liver disorders. Only a few in vivo studies have been conducted earlier on

cucurbitacin E, B, and iso-B, providing evidence of a wide range of activities. To gain further evidence at cellular level, we assessed these and other analogs bioactivity on cell lines representative of non- and parenchymal liver cells. The protective activity was measured on human hepatocyte-derived HepG2 cells against CCl4–induced toxicity, and the anti-proliferative activity on the serum-activated rat stellate cells, HSC-T6. Cytotoxicity (IC50) assays were conducted in parallel. For this purpose, 17 analogs had been isolated from two Cucurbitaceae plants and further modified structurally by acetylation (carbon 16) and alkylation (carbon 2). Ten analogs demonstrated significant protection on HepG2 cells (EC50 = 2.4 - 45.3 uM), with the rest indicating some degree of cytotoxicity. The anti-proliferative activity was highly significant for all analogs (EC50 = 0.02 - 4.12 uM). The level of hepatoprotection was similar to or higher than that of silybin on both cell lines and did not involve radical scavenging, anti-lipid peroxidation, or anti-hyaluronidase activities.

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MEDI 26

Supercritical fluid technology for enhanced drug delivery

***Pankaj Pathak**[1], Mohammed J. Meziani[2], Gaddamanugu L Prasad[3], and Ya-Ping Sun[2]. (1) Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson Univeristy, Clemson, SC 29634-0973, ppathak@clemson.edu, (2) Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, (3) Department of General Surgery, Wake Forest University*

The poor aqueous solubility of drug candidates presents a significant problem in drug development and related requirements such as bioavailability and a normal absorption pattern. Among various strategies to address the solubility issue, reducing the drug particle size (specifically nanosizing) has been identified as potentially effective and broadly applicable approach. We have developed a supercritical fluid processing technique, Rapid Expansion of a Supercritical Solution into a Liquid SOLVent (RESOLV), for the nanosizing of various water-insoluble drugs. The RESOLV process produces exclusively nanoscale (<100 nm) drug particles suspended in stable aqueous solutions. Drug particle size and morphology can also be controlled via altering and controlling the RESOLV experimental parameters. The cytoxicity profile of the nanoparticulate drug suspension monitored by human breast cancer cell line showed significantly higher tumoricidal activity compared to other formulations. Results from characterization of the drug nanoparticles by using DSC, XRD, SEM and biological testing are presented and discussed.

MEDI 27

Osmotic and mechanical properties of cartilage

Ferenc Horkay[1], *Emilios K. Dimitriadis*[2], *Iren Horkayne-Szakaly*[1], *David C. Lin*[1], *and Peter J. Basser*[1]*. (1) Laboratory of Integrative and Medical Biophysics, National Institutes of Health, NICHD, 13 South Drive, Bethesda, MD 20892, horkay@helix.nih.gov, (2) Instrumentation and Research Development Resource, Division of Bioengineering and Physical Science, ORS, National Institutes of Health*

Research done over the past decade has revealed osteoarthritis to be a complex disease process. Although recent research has elucidated much about the genetic and biochemical alterations associated with cartilage degeneration, little is known about the physical properties that govern the interactions among the macromolecules that constitute the cartilage matrix. A new experimental approach is proposed to determine the osmotic and elastic properties of biological tissue samples. The osmotic swelling of small tissue-engineered cartilage specimens was investigated by a new tissue osmometer. Atomic force microscope was used to image the surface and measure local elastic properties. The chemical composition of cartilage was determined by biochemical analysis. The osmotic results indicate that the swelling pressure decreases with increasing collagen content. This finding is consistent with the picture that the collagen network imposes an elastic pressure that counteracts the osmotic pressure of the glycosaminoglycans entrapped in the network.

MEDI 28

Synthesis and evaluation of α-Acetoxy Nitroso compounds as new nitroxyl donors

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

Nitric oxide (NO) has long been identified as a physiological regulator. Recently, nitroxyl (HNO/NO-, nitrosyl hydride/nitroxyl anion), the reduced form of nitric oxide has gained attention by demonstrating cardiovascular activity in a different mechanistic pathway from nitric oxide. Specifically, nitroxyl stimulates calcitonin gene-related peptide release while nitric oxide increases cyclic guanylate monophosphate (cGMP) and these results suggest the potential of nitroxyl donors for the treatment of heart failure. New nitroxyl donors will be of increasing importance as both biochemical and pharmacological tools and potential therapeutic agents. The goal of the research project is to synthesize new nitroxyl donors with unique mechanisms other than Angeli's salt, the most widely used nitroxyl donor. A variety of α-acetoxy nitroso compounds have been prepared and characterized. The ability of these compounds to release nitroxyl has been examined by GC headspace analysis, UV/vis and EPR spectroscopies. Kinetic decomposition profiles have been determined using UV/vis spectroscopy. In general, these compounds release nitroxyl more quickly in bases rather than neutral solutions. These compounds also cause vascular smooth muscle relaxation in a rat aortic ring assay.

MEDI 29

Design synthesis and evaluation of novel molecular transporters

*David E. Olson[1], **Joanne D. Kehlbeck**[1], and Barbara Danowski[2]. (1) Department of Chemistry, Union College, 807 Union Street, Schenectady, NY 12308, Fax: 518-388-6795, olsond@union.edu, kehlbeck@union.edu, (2) Department of Biological Sciences, Union College*

One important role of biological membranes is to exclude harmful substances from the interior of the cell. However, this defense mechanism also prevents the cellular uptake of many useful materials. The current design of therapeutics and tools for biotechnology limits their structural features, as they must be both soluble in polar extracellular fluids and capable of diffusing through the lipid bilayer of the cell. Fortunately, cationic molecular transporters solve these issues. This study sought to elucidate the mechanism by which molecular transporters are internalized by evaluating the effectiveness of a more highly conjugated analog of traditional guanidinium-rich molecular transporters. In addition, a technique exploiting the pH sensitivity of fluorescein was employed in an attempt to quench any fluorescence resulting from extracellular adhesion of these molecules.

MEDI 29

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MEDI 30

Functional characterization of a small molecule activation domain

Brian B. Brennan*, Sara J. Buhrlage, Aaron R. Minter, and Anna K. Mapp, Department of Chemistry, University of Michigan, 930 N University, Ann Arbor, MI 48109-1055, bbrenna@umich.edu*

Transcriptional activators play a critical role in the regulation of gene expression and small molecule replacements are both valuable mechanistic tools for the study of transcription and leads for a new therapeutic class. We recently reported the first small molecule that functions as a transcriptional activation domain. This molecule consists of an isoxadolidine scaffold that projects a mixture of hydrophobic and polar functional groups often observed in endogenous activation domains. Recent efforts have focused on elucidating the mode of action of these compounds. A positional mutagenesis experiment was carried out in which the polar and hydrophobic functional groups placed in varying orientations on the isoxazolidine scaffold then evaluated for function; this revealed that precise positioning of functional groups around the ring is not as important as a balance of polarity and hydrophobicity. Currently the function of the molecules in S. cerevisiae is under investigation to further probe the structural and mechanistic requirements for effective small molecule transcriptional activation domains.

MEDI 31

Development of protease sensors based on enhanced green fluorescent protein (EGFP)

Ning Chen*, Jin Zou, April Ellis, Wei Yang, and Jenny Yang, Department of Chemistry, Georgia State University, 50 Decatur Street, Atlanta, GA 30303, nchen1@student.gsu.edu*

Green fluorescent protein (GFP) has attracted tremendous attention due to the useful characteristics of its chromophore, which is opening a new era in the biology, medicine, and pharmaceutical fields. EGFP was derived from GFP with improvements of fluorescence intensity and thermosensitivity. In order to develop biosensors for tracking protease activities in vitro and in vivo, the EGFP-based protease sensors were designed for determining activities of trypsin, chymotrypsin, thrombin and caspase-3 by a grafting approach. Fluorescence of protease sensors has significant change upon protease cleavage both in vitro and in vivo. Due to the characteristics of GFP expression without cofactors in many organs, the protease sensors will

greatly contribute to the diagnosis of diseases rated to proteases activity and the tracking of kinetic process of protease activity during the diseases in vivo. Moreover, protease sensors targeted different signal peptides are useful for tracking protease activity in various cell apartments.

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MEDI 32

Design of synthetic immunostimulatory motifs as agonists of Toll-like receptor 9: Use of N^3-methyl-dC and N^1-methyl-dG

***P. Mallikarjuna Reddy**, FuGang Zhu, Yukui Li, YanPing Cong, Ekambar R. Kandimalla, and Sudhir Agrawal, Idera Pharmaceuticals, Inc, 345 Vassar Street, Cambridge, MA 02139, Fax: 617-679-5582, mputta@iderapharma.com*

Toll-like receptor 9 (TLR9) recognizes synthetic and bacterial DNAs containing unmethylated CpG motifs and triggers a Th1-type immune response through a cascade of cell signaling. Through structure-activity relationship studies we identified synthetic immunostimulatory motifs and novel DNA structures that induce potent immune responses. The combination of synthetic stimulatory motifs and novel DNA structures provided immune modulatory oligonucleotides (IMOs) with distinct immune responses compared with conventional CpG DNA. In continuation to understand CpG DNA-TLR9 recognition and develop potent synthetic immunostimulatory motifs, we synthesized IMOs containing N^3-methyl-dC or N^1-methyl-dG modifications in place of C or G in CpG dinucleotide. We have studied TLR9 activation and immunostimulatory properties of the IMOs containing *N*-methyl-dC and -dG. These in vitro and in vivo studies suggest that TLR9 recognizes IMOs

with N^1-methyl-dG in immunostimulatory motifs and induces potent immune responses.

MEDI 33

Antagonists of immunostimulatory CpG-DNA

Ekaterina M. Paliakov[1], *Martial Say*[1], *Maged M. Henary*[1], *Donald Macfarlane*[2], *Lori Manzel*[2], *Steven E. Patterson*[3], *Andrzej J. Bojarski*[4], *and Lucjan Strekowski*[1]. *(1) Department of Chemistry, Georgia State University, University Plaza, Atlanta, GA 30303, Fax: 4046511416, e_paliakov@yahoo.com, (2) Department of Internal Medicine, University of Iowa, (3) UMN Center for Drug Design, (4) Department of Medicinal Chemistry, Institute of Pharmacology*

Bacterial DNA, oligodeoxynucleotides, and phosphorothioate oligodeoxynucleotides with a CpG motif are immunostimulatory. The CpG driven response is strongly inhibited by 2-arylquinolin-4-amines. Evidence has been accumulating that practical drugs for treating rheumatoid arthritis and lupus erythematosus can be found within this class of compounds. Recently, a biological receptor for the quinoline antagonists has been identified as Toll-like receptor 9 (TLR9). Unfortunately, this information is of no help in the rational design of improved antagonists because the X-ray structure of TLR9 is not yet known. A large number of quinolines have been synthesized, assayed for their immunosuppressive activity, and analyzed by QSAR methodologies including Free-Wilson and CoMFA studies. The QSAR results have guided our efforts to find a highly active drug candidate. Several agents active at a concentration below 1 nM were designed and synthesized.

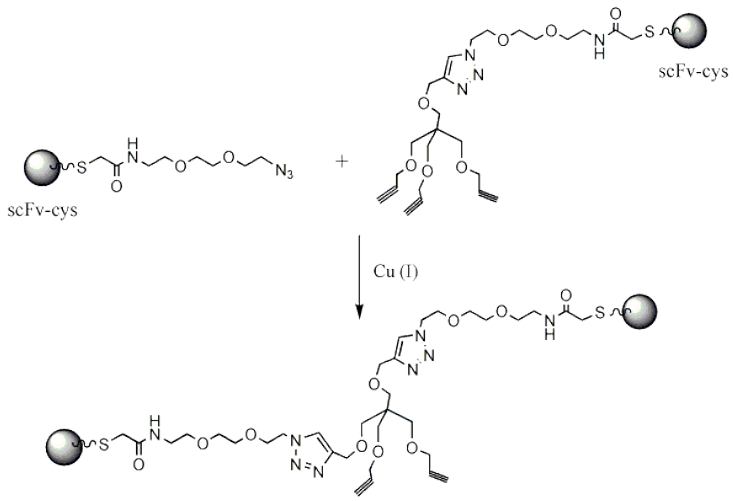
MEDI 34

Site-specific ligation of antibody scFv through Cu (I) catalyzed 1,3-dipolar cycloaddition

Wenjun Du[1], *Arutselvan Natarajan*[2], *Sally J. DeNardo*[3], *and Jacquelyn Gervay-Hague*[1]. *(1) Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, CA 95616, wjdu@ucdavis.edu, (2) Molecular Cancer Institute, University of California, Davis Medical Center, (3) Department of Internal Medicine, University of California, Davis Medical Center*

A new paradigm for radionuclide pretargeted systemic radiation therapy for cancer has shown early evidence of being achievable. However, developing a modular non-immunogenic molecule that can be efficiently produced and is applicable to many targeting drugs is challenging. Antibody fragments (scFv) provide effective modules for such multi-functional, multi-valent, anti-tumor; anti-radiochelate and high affinity have been developed. In order to pretarget tumor and catch the subsequently injected chelated radiometal, multi-valent PEG-scFv conjugates need to be efficiently produced. A series of Br-PEG-azide/alkyne linkers were designed and synthesized for site-specific conjugation and ligation with scFv-cys. Although scFv and Br-PEG-alkyne/azide conjugations gave an overall efficiency of 80%, the ligation of two scFvs by Cu (I) assisted "Click" reaction yielded <5%. Presumably, these small linkers suffer intrinsically from low effective concentration and substantial steric hindrance.

To overcome these "mathematical hurdles", a tri-alkyne containing linker was designed and synthesized. The Br-PEG-azide and Br-PEG-tri-alkyne linkers were first conjugated with two scFv-cys separately and "Clicked" together using copper (I) catalyzed 1,3-dipolar cycloaddition to give an excellent ligation efficiency of > 75% yield.



MEDI 35

Bivalent protein-binding agents based on the self-assembly of oligonucleotide-linked organic fragments

***Debarati M. Tagore**, Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06520, debarati.mazumder@yale.edu, and Katherine I Sprinz, Department of Molecular Biophysics and Biochemistry, Yale University*

A library of organic fragments on an oligonucleotide scaffold was generated for binding to streptavidin. Self-assembly by the annealing of two complementary functionalized oligonucleotides led to duplexes projecting two organic fragments on one end. In the presence of biotin as one of the library members, duplexes projecting one (monodentate) or two (bidentate) biotin molecules were detected through a novel polymerase chain reaction. Thermal denaturation experiments in the presence of streptavidin showed a 12ºC increase in the melting temperature of the bidentate biotin duplex as compared to the monodentate biotin duplex. Substitution of biotin with 2-iminobiotin led to the exclusion of all other duplexes by the bidentate 2-iminobiotin duplex in binding streptavidin.

MEDI 35

Bivalent protein-binding agents based on the self-assembly of oligonucleotide-linked organic fragments

***Debarati M. Tagore**, Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06520, debarati.mazumder@yale.edu, and Katherine I Sprinz, Department of Molecular Biophysics and Biochemistry, Yale University*

A library of organic fragments on an oligonucleotide scaffold was generated for binding to streptavidin. Self-assembly by the annealing of two complementary functionalized oligonucleotides led to duplexes projecting two organic fragments on one end. In the presence of biotin as one of the library members, duplexes projecting one (monodentate) or two (bidentate) biotin molecules were detected through a novel polymerase chain reaction. Thermal denaturation experiments in the presence of streptavidin showed a 12ºC increase in the melting temperature of the bidentate biotin duplex as compared to the monodentate biotin duplex. Substitution of biotin with 2-iminobiotin led to the exclusion of all other duplexes by the bidentate 2-iminobiotin duplex in binding streptavidin.

MEDI 36

Rational design of a calcium-dependent trigger

***Shun-yi Li**[1], Wei Yang[1], Anna L Wilkins[1], Julian A Johnson[2], Zhi-ren Liu[2], and Jenny J Yang[1]. (1) Chemistry, Georgia State University, 50 Decatur Street, Atlanta, GA 30303, sli@gsu.edu, (2) Biology, Georgia State University*

Calcium-dependent conformation change is a common mechanism for signal transduction. To understand the key factors related to conformation changes in calcium-binding protein, one de novo protein Ca.CD2.6D31 was designed based on the non-calcium-binding cell adhesion protein CD2. This protein was expressed in E. coli and purified. The protein has a pH-dependent conformational change and metal binding activity. The protein shows strong binding affinity to Tb3+, La3+ and Ca2+. Far-UV CD, tryptophan fluorescence, 1-anilinonaphthalene-8-sulfonic acid (ANS) fluorescence and NMR spectra of the protein underwent significant changes at different pH and in presence or absence of metal ions. These results suggest that the addition of negative charged residues destabilize the protein structure. Upon the neutralization of the negative charged ligand residues by lowering pH or metal binding, Ca.CD2.6D31 forms wild type CD2-like structure. Our study demonstrates that the design approach can be used to control protein structure and conformation by metal binding.

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MEDI 37

Cooperative response of synaptotagmin I C2A: Hypothesis for a calcium ion-driven molecular hammer

Jill A. Kertz[1]*, Paulo F. Almeida*[2]*, April A. Frazier*[3]*, Catherine M. Wieser*[1]*, David S. Cafiso*[4]*, and Anne Hinderliter*[1]*. (1) Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58105, jill.kertz.1@ndsu.edu, (2) Department of Chemistry and Biochemistry, University of North Carolina-Wilmington, (3) Department of Pharmacology, University of Iowa, (4) Department of Chemistry, University of Virginia*

In the current paradigm of neurological function, synaptotagmin I C2A domain is presumed to bind in a stepwise fashion: first, two calcium ions in solution, then acidic membrane, and finally a third calcium ion. In contrast, our analysis of C2A binding to membranes and cations suggests a fundamentally different role for synaptotagmin I in calcium ion-regulated exocytosis. We suggest two forms of cooperativity to modulate the responsiveness of synaptotagmin I C2A: the well-known cooperativity from a cation network that links the three cation binding sites and the less-characterized cooperativity from a membrane surface that reshuffles with each round of calcium ion–influx and subsequent fusion. The small cooperative interactions between lipids provide plasticity in how the membrane can become a more (or less) favorable protein-binding surface. Experimental fluorescence spectroscopy data was gathered over a variety of ligand conditions to test our model.

MEDI 38

Systematic exploration of dipyridamole analogs as potent inhibitors of equilibrative nucleoside transporters ENT1 and ENT2

Wenwei Lin *and John K. Buolamwini, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Suite 327, Memphis, TN 38163, Fax: 901-448-6828, wlin7512@yahoo.com*

Equilibrative nucleoside transporter (ENT) inhibitors have applications as coronary vasodilators, anticancer agents and antiparasitic agents. Dipyridamole is a potent ENT1 and ENT2 inhibitor, and it was introduced as an antianginal agent in 1959. Its activity is relatively low compared to the standard nitrobenzylmercaptopurine

riboside (NBMPR). However, dipyridamole is a good candidate for further exploration since it is used clinically. In this study, a series of dipyridamole analogues were systematically designed and synthesized, and their inhibitory activities against ENT1 and ENT2 were evaluated by flow cytometry and uptake assays. Compounds more potent than dipyridamole were identified, the best of which, 2,6-Diethanolamino-4,8-dihexamethyleneimino-pyrimido[5,4-d]pyrimidine, had a Ki value of 0.49 nM against ENT1 compared to 8.18 and 0.43 nM, for dipyridamole and NBMPR, respectively. With respect to ENT2, this compound exhibited an IC50 value of 90.8 nM campared to 308.0 and 1350 nM for dipyridamole and NBMPR, respectively. Supported by NIH grant number HL067479

MEDI 39

Design, synthesis, flow cytometric evaluation and 3-D QSAR studies with novel tetrahydroisoquinoline conformationally constrained analogs of nitrobenzylmercaptopurine riboside (NBMPR) as ENT1 nucleoside transporter inhibitors

***Zhengxiang Zhu**, Department of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, 847 Monroe Avenue Suite 327, Memphis, TN 38163, zzhu1@utmem.edu, and John K. Buolamwini, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center*

In the absence of a 3D structure of the ENT1 transporter we are using an indirect approach to probe the bioactive conformation of nitrobenzylmercaptopurine riboside (NBMPR), the prototype inhibitor. We have synthesized a series of conformationally constrained NBMPR analogues and found that the 7-NO2-tetrahydroisoquinoline analogue most captures the bioactive conformation. To further probe the bioactive conformation, we synthesized and evaluated another series of constrained analogues by forming a 5'-O, 8-linkage to lock the glycosidic bond. These novel compounds were also used to develop a pharmacophore model with PHASE, which was then used for CoMFA 3D QSAR modeling. The resulting model showed robustness and good predictive ability, with a predictive r2 of 0.606 for an external test set of 25 compounds. The results shed light on the bioactive conformation of NBMPR and provide a pharmacophore model for screening compound databases to discover novel inhibitors. Supported by NIH grant number HL067479.

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MEDI 40

Synthesis and flow cytometric analysis of novel 2-position analogs of Nitrobenzylmercaptopurine Riboside (NBMPR) as ENT1 nucleoside transporter ligands

Amol Gupte *and John K Buolamwini, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave, Suite 327, Memphis, TN 38163, Fax: 901-448-6828, agupte@utmem.edu*

Nucleoside transporters are membrane glycoproteins responsible for the cellular uptake and efflux of physiological nucleosides and synthetic analogs. Nucleoside transporter inhibitors have potential therapeutic applications in heart disease and stroke, cancer, viral infections and host tissue protection during chemotherapy. S6-(4-nitrobenzyl)mercaptopurine riboside (NBMPR) is a prototype inhibitor of the ENT1 nucleoside transporter. Although numerous studies exploring the S6-benzyl position have been undertaken, largely missing is a systematic structure-activity relationship study at the 2-position of NBMPR. A new series of 2-position substituted NBMPR analogs were synthesized and tested using flow cytometric method, for their ENT1 nucleoside transporter binding affinity, using SAENTA-fluorescein as the probe. The study provided us with valuable insights into the kind of substituents tolerated at the 2-position of NBMPR, which will be useful in subsequent design of ENT1 nucleoside transporter inhibitors. The synthesis and binding data will be presented and discussed. Supported by NIH grant number CA101856.

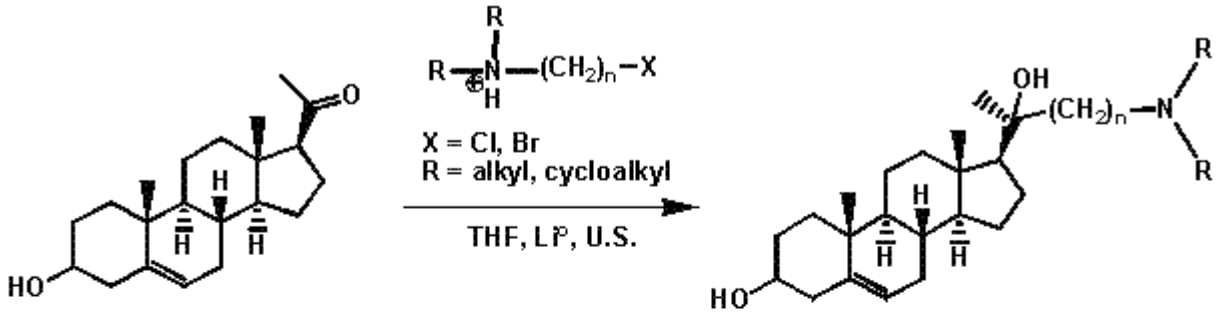
MEDI 41

Ultrasound-assisted organic synthesis of new azasteroids (One-pot synthesis)

Angela D. Bell*, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Bldg, Auburn, AL 36849, bellad1@auburn.edu, and EJ Parish, Department of Chemistry, Auburn university*

A number of steroidal structures containing nitrogen possess known biological and pharmaceutical values such as anti-microbial and anti-inflammatory properties.

Variation in the location of the nitrogen could prove to be critical to the compound's overall functionality. We have developed a one-step (one pot) synthesis of side-chain azasteroids which have provided a variety of new steroidal structures for biological evaluation. The key feature of this synthesis was the construction of modified steroidal side-chains using ultrasound techniques to streamline the synthetic procedures. This project has provided new side-chain azasteroids on a cholesterol ring system.

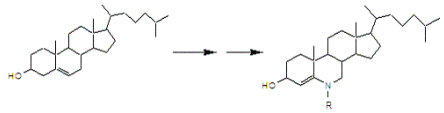


MEDI 42

A novel approach to the synthesis of 6-azasteroids

***Jyothi Gandikota**, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Building, Auburn University, Auburn, AL 36849, gandijs@auburn.edu, and Edward J. Parish, Department of Chemistry, Auburn University*

Azasteroids, which carry nitrogen in the C-6 position of the steroid nucleus, were found to possess antimicrobial and antifungal properties. We have designed a novel approach to the synthesis of new 6-azasteroids using a modified and streamlined procedure. The synthetic route we developed, did not require the use of ozone, which is generally used to cleave the B-ring of the steroids. The final products (R = alkyl,cycloalkyl) were available in good yields compared to the earlier synthetic procedures.

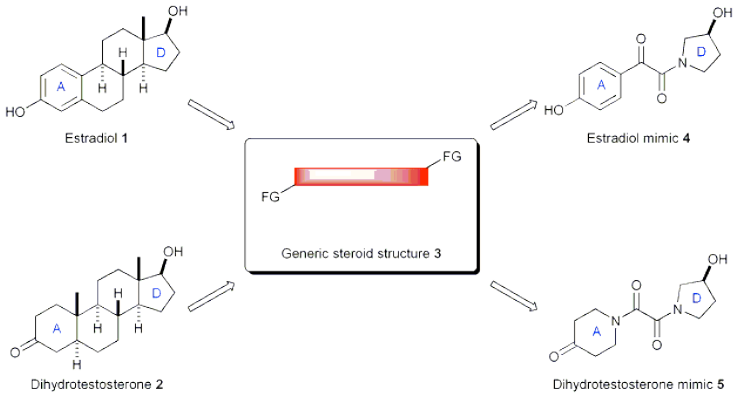


MEDI 43

Design, synthesis and evaluation of novel steroid mimics

Robert Strevens *and Nicholas C O Tomkinson, School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, United Kingdom, Fax: 0044-(0)2920874068, robstrevens@hotmail.com*

Steroids ellicit their diverse biological actions via different functionality located around the periphery of their rigid tetra-cyclic core. For example, estradiol (1) can be viewed as a phenolic and a secondary alcohol that are spatially fixed by a central lipophilic scaffold (3). Although steroids are chemically quite simple molecules, with relatively few functional groups, their stereochemical and architectural complexity renders them an exceedingly difficult target for chemical synthesis. We will report on a programme of work to prepare mimics for both androgenic (5) and estrogenic (4) activators that posess a central dicarbonyl moiety where the opposed dipole moments of the carbonyl groups spacially fix the A and D ring mimics in space. The molecules are simple to prepare and therefore amenable to the generation of a diverse range of compounds for biological evaluation. The synthesis and comprehensive SAR of both the A and D ring mimics of 4 and 5 will be presented.



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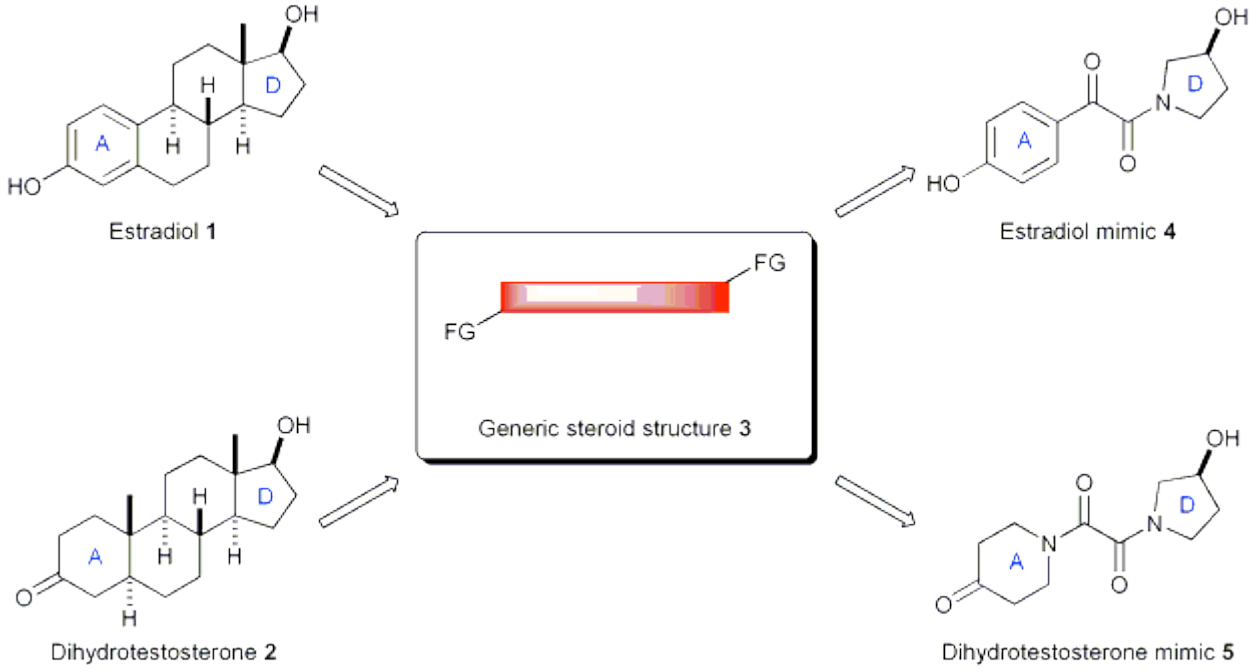


MEDI 44

Solution phase synthesis and solid phase approaches to laulimalide analogs

***Eric A. Tanifum**, Ian McAlexander, and Bradley S Davidson, Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322-0300, tanifum@cc.usu.edu*

Laulimalide presents a very promising lead structure for the development of new anticancer agents due to its potent growth inhibitory activity against several cancer cell lines, including multidrug-resistant cells. It shares the same microtubule-stabilizing mechanism with paclitaxel, the epothilones, discodermolide, and eleutherobin. Unlike the others, it has a distinct binding site on the microtubule. Recent studies show that laulimalide and two of its analogs act synergistically with paclitaxel and 2-methoyestradiol to inhibit proliferation. Several total syntheses of the natural product and a few structural analogs have been reported. The synthesis of novel structural analogs with variations on both the side chain and macrocyclic core of laulimalide will be presented. Progress towards the transfer of the solution phase chemistry to the solid phase will also be presented.

MEDI 45

An expedient and scalable preparation of activated arylnitroethanes: Key intermediates to biogenic amines

*Frederick A. Luzzio and **Marek T. Wlodarczyk**, Department of Chemistry, University of Louisville, 2320 South Brook Street, Louisville, KY 40292, Fax: 502-852-8149, faluzz01@gwise.louisville.edu, mtwlod01@gwise.louisville.edu*

The preparation of activated aryl-substituted phenethylamines can be effected by reduction of the corresponding nitro compounds, azide or nitrile intermediates as well as through reductive amination of carbonyl compounds. The requisite saturated nitro intermediates can be acquired through the Kornblum reaction or more commonly by reduction of nitrostyrenes which are obtained by dehydration of nitroaldol (Henry) intermediates. However, the reduction of nitrostyrenes, either by hydride reagents or by catalytic means, is an often fickle process and can result in mixtures of the desired nitro compounds along with amines and carbonyl compounds. We find that the best process for the reliable preparation of phenethylnitro compounds is the direct deoxygenation of the Henry (β-nitroalkanol) intermediates with triethylsilane/trifluoroacetic acid (TES/TFA). The β-nitroalkanol intermediates formed during the reaction of an activated (mono-, di-, trimethoxy, methylenedioxy) benzaldehyde with simple nitroalkanes conveniently possess a benzylic hydroxyl group which is amenable to the TES/TFA reduction. The overall process can be done on multigram scale and thereby enables direct conversion of a number of nitroalkanol intermediates to the corresponding phenethylnitro compounds.

MEDI 46

Microwave energy in accelerating reaction rate of solid-assisted solution phase synthesis

***Shahnaz Ghassemi**, Biotage, 1725 Discovery Drive, Charlottesville, VA 22911, Fax: 434 979-4743, SGhassemi@biotage.com*

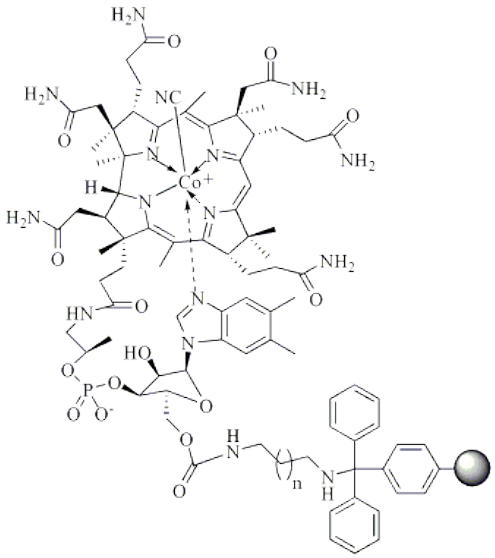
Bound reagents are functional polymers designed to perform synthetic transformations in same way as their solution counterparts. In this technique excess reagent and by-products remain attached to the solid, while product is in solution. Possible advantages of this technique are facile monitoring of reaction progress while reducing purification bottleneck. The disadvantage of this technique is the relative slow rate of reaction. Microwave irradiation has been used to overcome this problem and increase rate of reaction of solid-assisted solution phase synthesis. This presentation covers few examples of this technique in developing efficient and robust methods for the preparation of biologically interesting compounds.

MEDI 47

Solid phase synthesis and characterization of cyanocobalamin analogs

Narasimha R Ummaneni, *Marlito Gomes, and John S Williamson, Department of Medicinal Chemistry, The University of Mississippi, School of Pharmacy, 427 Faser Hall, University, MS 38677, Fax: 6629155638, ummaneni@olemiss.edu*

Higher eukaryotes require cobalamin (vitamin B12) as an essential cofactor for the methylation of uracil prior to DNA synthesis and cell replication. Cancer cells have an increased ability to transport and to sequester cobalamin in large excess over the amount required for the normal cellular metabolism and cell replication. This observation has been used to target the delivery of chemotherapeutic agents and radionulides to cancer cells by conjugation of a drug or radionuclide to cobalamin, there by enabling receptor-mediated endocytosis of the cobalamin-conjugate/transcobalamin complex (Smeltzer, C. C.; Cannon, M. J.; Pinson, P. R.; Munger Jr., J. D.; West, F. G.; and Grissom, C. B. Org. Lett., 2001, 3, 799 and the references cited there in). Here we report the first solid phase synthetic method to conjugate ribose-5'-hydroxyl group of cyanocobalamin (vitamin B12) with different types of bifunctional linkers. This method avoids the protection and deprotection steps for bifunctional linkers to be conjugated to cyanocobalamin and allows to isolating the pure products.

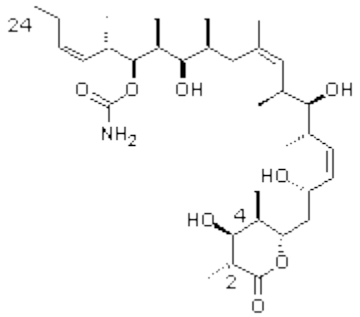


MEDI 48

Expanded investigations into the lactone region of (+)-discodermolide

Simon J. Shaw*, Kurt F. Sundermann, Mark A. Burlingame, Dan Zhang, Joseph Petryka, and David C. Myles, Department of Chemistry, Kosan Biosciences Inc, 3832 Bay Center Place, Hayward, CA 94545, shaw@kosan.com*

(+)-Discodermolide is a microtubule-stabilizing marine polyketide. It has undergone an early-stage human clinical trial using synthetic material, however, compounds that reduce the synthetic complexity of the molecule are desirable. Novel analogues of (+)-discodermolide were synthesized using the 23, 24-dihydrodiscodermolide scaffold in order to help define the minimum requirements for high potency. Cellular in vitro evaluation indicated that significant simplifications can be made, however, positioning of the lactone moiety is crucial for activity. (+)-23, 24-dihydrodiscodermolide

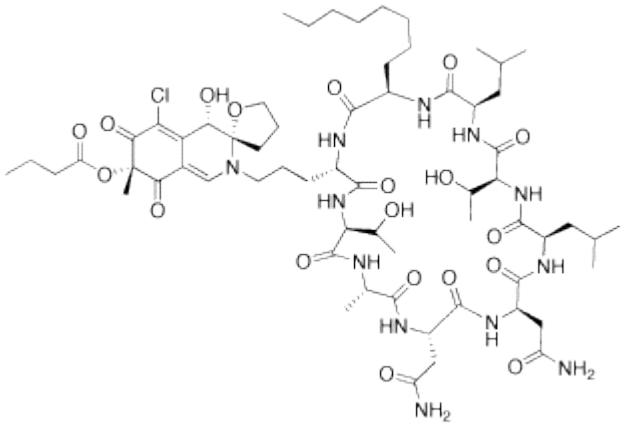


MEDI 49

Synthesis of chlorofusin chromophore

Michele N. Williams *and Sergey N. Savinov, Department of Chemistry, Purdue University, 560 Oval Dr, West Lafayette, IN 47907, Fax: 765-494-0239, mnwillia@purdue.edu*

Chlorofusin is a natural product that was found to inhibit the p53/MDM2 interaction (IC50=4.6μM). The structure of Chlorofusin includes a cyclic peptide linked to a highly functionalized tricyclic chromophore. The cyclic peptide alone was not found to be a potent inhibitor of the p53/MDM2 interaction, suggesting that the sidechain moiety is primarily responsible for the inhibitory activity. We are currently pursuing in parallel a synthetic approach and biological evaluation of the Chlorofusin sidechain to confirm its biological activity and to develop more potent analogs that may inhibit the p53/mdm2 protein-protein interaction, using a reverse two-hybrid system designed to report on the disruption of this interaction.



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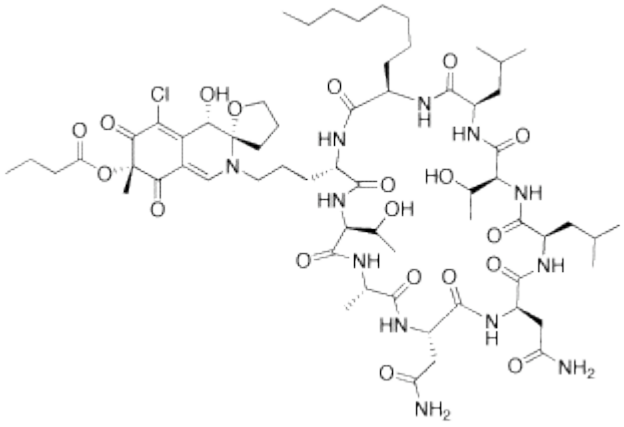
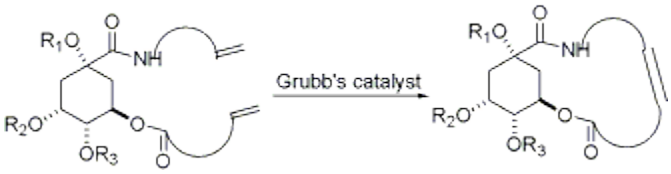


MEDI 50

Synthesis and biological evaluations of non-natural macrolides using ring closing olefin metathesis

Belhu B. Metaferia, *NIDDK/Laboratory of Bioorganic Chemistry, National Institutes of Health, 9000 Rockville Pike, Bldg 8-1A02, Bethesda, MD 20892, belhum@intra.niddk.nih.gov, and Carole A. Bewley, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, NIH*

Macrolides are among the most important classes of natural products in the development of new antibiotics and anti-cancer agents. In our ongoing effort to discover effective chemotherapeutic agents against Mycobacterium tuberculosis, we have developed a highly convergent synthesis of non-natural, quinic acid based macrolides of different ring sizes. The key strategy in the macrocyclization step utilizes ring closing olefin metathesis methodology. In this presentation, the synthesis and biological evaluations of these novel macrolide compounds will be discussed.

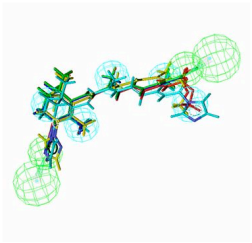


MEDI 51

Retinoic acid metabolism blocking agents: Chemical feature based pharmacophore

Purushottamachar P. Puranik[1], Jyoti B. Patel[1], Lalji K. Gediya[1], Omoshile O. Clement[2], and ***Vincent C. O. Njar***[3]*. (1) Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, School of Medicine, 685, West Baltimore Street, HSF-1, Room No. 563, Baltimore, MD 21201, Fax: 410-706-0032, porshottam@yahoo.com, (2) Informatics Division, Bio-Rad Laboratories, (3) Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, School of Medicine, 685, West Baltimore Street, HSF-1, Room No. 580 I, Baltimore, MD 21201, Fax: 4107060032, vnjar001@umaryland.edu*

Inhibitors of all-trans-retinoic acid (ATRA) metabolism enzymes (CYP26s), also called retinoic acid metabolism blocking agents (RAMBAs) are proving to be useful anticancer agents. In the search for new RAMBAs, we utilized IC50 values of known RAMBAs to generate a common feature-based pharmacophore model using the Catalyst HipHop approach. A total of ten Hypotheses were generated with high ranking scores (86.8 - 90.6). Hypothesis-2 and Hypothesis-3 were selected on the bases of GH analysis and knowledge of the ligand-enzyme interaction. These two hypos were used to retrieve potential new RAMBAs from the NCI and Maybridge databases. Two of the compounds retrieved using Hypo-3 (NCI0308597 and HST01914) were, tested for biological activity at 100 nM. These compounds showed 54.7% and 53.2 % inhibition of ATRA metabolism respectively. The activity results indicate that Hypo-3 with five hydrophobic and two hydrogen bond acceptor features is a good representative hypothesis for identifying new RAMBAs.



MEDI 52

Naturally occurring esterfication reactions with bryostatin

Giso Abadi[1], *Ken Jones*[2], *Thomas Manning*[2], *Dennis Phillips*[3], *Paul Groundwater*[1], *Lyn Noble*[1], *and Alan G. Marshall*[4]. *(1) Chemistry and Pharmacy, Sunderland University, Sunderland, United Kingdom, giso_17@yahoo.com, (2) Department of Chemistry, Valdosta State University, (3) Chemistry, University of Georgia, (4) Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University*

Twenty different structures of Bryostatin have been published. These structures share a commonality of having a central bryophan ring. For most structures the differences arise in two groups (R1 and R2) that are attached to the central bryophan ring via ester bonds. In this presentation we will discuss the impact that natural conditions such as UV light, I2, acidic and basic conditions have on the structure in the presence of some carboxylic acids (i.e. octanoic acid). Our argument is that simple esterfication reactions are responsible for the different forms of bryostatin, not different genetic strains of Bugula neritina (the host organism) or different strains of a symbiotic bacteria. We have also used MALDI-MS, and FT-ICR to study the composition of the sediment from the Bugula ecosystem and, from this data, postulate there are significant quantities of naturally occurring carboxylic acids to account for these reactions under natural conditions.

MEDI 53

Synthesis and characterization of naphthalimide modified chitosans

Whitney M. Wettlaufer *and Ronald E. Utecht, Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD 57007, Fax: 605-688-6364, Whitneywettlaufer@yahoo.com*

Chitosan is a naturally occurring biopolymer with bioadhesive properties. The implications for chitosan-based adhesives are vast, current studies revolve around its

applications as a soft tissue glue in vasculature, cornea and in muscle tissue of the urinary tract. This work focuses on the modification of chitosan with photo-activatable 4-amino-1,8-naphthalimides via the 4-amino functionality. We have studied compounds where the substitution in the imide position has been a simple alkyl amines or an amino acids. The nature of this substitution controls the photo reactivity and stability of the naphthalimide group. Substitution of simple alkyl amines such as butyl amine into the imide position results in a stable photo-activatable compound displaying tissue bond strengths significantly larger than current bioadhesives on the market. Substitution of amino acids such as glycine or isoleucine into the imide position results in a unstable compound with significantly lower tissue bonding efficacy.

MEDI 54

Reactivity of naphthalimide modified chitosan in biological applications

Courtney B. Wettlaufer *and Ronald E. Utecht, Department of Chemistry and Biochemistry, South Dakota State University, Brookings, SD 57007, Fax: 605-688-6364, c_wettlaufer@yahoo.com*

Chitosan is a naturally occurring biopolymer with bioadhesive characteristics. The implications for chitosan based adhesives are vast, and current studies revolve around its applications as a soft tissue glue in vasculature, cornea and in muscle tissue of the urinary tract. Our work has augmented the natural bioadhesive property of chitosan through modification of the chitosan with photoactivated naphthalimides. Our observed bond strengths between a processed pericardial collagen matrix and arterial tissue are 562 ± 146 g/cm2. These modified chitosans have been used in vivo to repair a damaged rabbit abdominal aorta without the use of sutures and without observed leakage. Toxicology studies have shown a promising biocompatibility profile for these compounds with both cytotoxicity assessed in a cell culture model and an intramuscular implant test. Our observed bond strengths are higher than strengths observed using commercial fibrin or cyanoacrylate based glues while providing greater ease of use.

MEDI 55

Synergistic enhancement of transdermal delivery using a pore-forming peptide and chemical enhancer

Yeuchun Kim*, Peter J. Ludovice, and Mark R. Prausnitz, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, 311 Ferst Drive, Atlanta, GA 30332, yckim@chbe.gatech.edu*

Drug delivery across skin is limited by the multilamellar extracellular lipid bilayers found in the skin's outer layer of stratum corneum. We hypothesize that magainin peptides can be used to disrupt these lipid bilayers and thereby increase skin permeability. Magainin peptides are known to spontaneously form transmembrane pores and ultimately disrupt bacterial cell membranes and, therefore, may be used to target stratum corneum lipids as well. In this study, we found that magainin peptides alone did not affect skin permeability, but the combination of magainin with

an anionic surfactant (N-lauroyl sarcosine) dissolved in ethanol-water mixtures increased skin permeability by more than an order of magnitude. These effects of magainin peptide on skin permeabilty showed a synergistic, as opposed to additive, effect. The molecular interactions of magainins with the surfactant, solvent, and skin lipids and proteins has been characterized using microscopy, calorimetry, infrared spectroscopy and X-ray diffraction to identify the mechanism.

MEDI 56

Metabolism of glyburide by microsomes from human liver and placenta: A study by HPLC-MS

Ravindran Selvan[1]*, Olga L. Zharikova*[1]*, Ronald A. Hill*[2]*, Tatiana N. Nanovskaya*[1]*, Gary D. V. Hankins*[1]*, and Mahmoud S. Ahmed*[1]*. (1) Department of Obstetrics & Gynecology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555, Fax: 409-747-1669, seravind@utmb.edu, (2) College of Pharmacy, University of Louisiana at Monroe*

Glyburide (Glibenclamide) is a second-generation sulfonylurea used for treatment of type-2 and gestational diabetes (GD). The use of glyburide in treatment of GD prompted investigation of its metabolism by the placenta. Therefore, the metabolism of glyburide by human placenta was compared to that by the liver utilizing microsomal preparations. The metabolites formed were identified by high-performance liquid chromatography-mass spectrometry (LC-MS). Total ion current (extracted at *m/z*510) revealed 6 major hydroxylated derivatives of glyburide in human liver and 5 in placenta. In liver, the predominant metabolites were the trans-4-hydroxycyclohexyl derivative and, to a lesser extent, two metabolites eluting next to each other, one being the cis-3-hydroxycyclohexyl product. In placenta, the trans-4-OH and cis-3-OH derivatives were also formed, but the predominant was the ethylhydroxy-glyburide as confirmed by its fragmentation pattern. The amounts of metabolites formed by placentas were lower than by the liver. Supported by the Obstetrical Pharmacology Research Network (OPRU/NICHD)

MEDI 57

Bioactive structure determination of drug-like molecules bound to proteins using an electron density restrained conformational search

Scott A. Johnson*, Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, sajohn2@emory.edu*

It has long been assumed that ligands bind to proteins in a reasonably low energy conformation. Recently published computational analyses suggest that the binding strain may well exceed 15 to 20 kcal/mol. Accordingly, significant effort has been made to study the nature and validity of these highly strained bioactive conformations through molecular mechanics. A new computer program which utilizes the experimentally derived electron density to restrain a force field mediated conformational search has been employed to study these high energy structures as well as several well defined low energy structures. This method has shown to minimize much of the force field parameterization bias and significantly lower

calculated binding energies of previously reported high energy ligands without significant structural change.

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MEDI 58

Investigation of the anti-oxidant potential of Microperoxidase-11

Dominic F. Qualley *and Stephen A. Woski, Department of Chemistry, The University of Alabama, Box 870336, Tuscaloosa, AL 35487, Fax: 205-348-9104, quall001@bama.ua.edu*

Microperoxidase-11 (MP-11), a proteolysis product of the protein cytochrome c, has been proposed as an anti-cataract agent. Its heme prosthetic group contains a redox-active iron that can potentially quench reactive oxygen species (ROS). However, this iron center can also act as a potential source of highly reactive radicals that can damage biomolecules such as DNA. This work investigates the generation of reactive oxygen species by MP-11 using the nicking of plasmid DNA as a probe. It was found that in the presence of biologically relevant reducing agents (ascorbate and thiols) and atmospheric oxygen, significant amounts of ROS are produced. The generation of these species is confirmed by the attenuation of DNA damage upon the addition of radical inhibitors. Experiments to examine the dependence of formation of DNA-damaging radicals upon MP-11 concentration will be described. These studies provide insight into the balance between radical formation by MP-11 and any potential protective effect.

MEDI 59

Improving the repeatability of proteolytic digestion with trypsin

Paul C. Kline[1], *David M. Bunk*[2], *Christian Arsene*[3], *Andre Henrion*[3], *William Burkitt*[4], *and Gavin O'Connor*[4]. *(1) Department of Chemistry, Middle Tennessee State University, MTSU Box X-102, Murfreesboro, TN 37132, Fax: 615-898-5182, pkline@mtsu.edu, (2) Analytical Chemistry Divsion, National Institute of Standards and Technology, (3) Physikalisch Technische Bundesanstalt, (4) LGC*

Digestion of proteins using trypsin and analysis of the resulting peptides by mass spectrometry plays a major role in the identification of clinically relevant proteins. Unfortunately many of the studies using this technique show highly variable results. This lack of repeatability is a major problem in the proteomics field of clinical biomarker discovery in which repeatable and validated measurements are necessary.

This study characterizes commercial preparations of the enzyme and the factors that affect the completeness of the tryptic digest. In addition a mass spectrometry-based assay has been developed to determine the extent to which non-specific proteolytic digestion occurs.

Our result indicate while the commercial preparations have comparable initial activities, there is a large degree of variability in the composition of the preparations.

MEDI 60

Discovery of potent purine type IL-12 production inhibitors

Lijun Sun*, Teresa Przewloka, Elena Kostik, Shijie Zhang, Weiwen Ying, David James, Yumiko Wada, Yaming Wu, Keizo Koya, and Mitsunori Ono, Synta Pharmaceuticals Corp, 45 Hartwell Avenue, Lexington, MA 02421, Fax: 781-274-8228, lsun@syntapharma.com*

Described herein is a class of novel purine compounds demonstrating potent inhibitory activities against interleukin-12 (IL-12), a p35/p40 heterodimeric cytokine that is believed to be a key mediator of autoimmune diseases such as rheumatoid arthritis, inflammation bowel disease, and psoriasis. We will discuss the design, synthetic challenges, and SAR studies. In vivo biological evaluations of lead compounds, including oral efficacy in animal models will be described.

MEDI 61

Role of point mutations causing antiandrogen withdrawal syndrome studied by molecular simulations and computational prediction approaches

William H. Bisson[1], *R. Abagyan*[1], *and Claudio N. Cavasotto*[2]. *(1) Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, wbisson@scripps.edu, (2) Molsoft LLC*

The primary treatment for prostate cancer still remains surgical castration followed by chemotherapy with antagonists to block the effects of androgens (antiandrogens). Unfortunately, the effect on patients is limited cause, over the time, tumors will develop clinical androgen independence that results in death (Antiandrogen

Withdrawal Syndrome, AWS). Androgen receptor (AR) point mutations are demonstrated to be present in androgen-independent prostate cancers. The homology models of the human wild type and W741L and T877A mutated forms of the AR-LBD in the agonist conformation were built and energetically minimized. The movement of the ligand-receptor complex was studied in the wild type and mutated forms of the AR-LBD by Molecular Dynamics. Flexible molecular docking was performed to follow the change in the binding orientation and energy of certain ligands related to particular mutations. The results obtained explain the role the specific point mutations in changing the biological response of bicalutamide and hydroxyflutamide in AWS.

MEDI 61

Role of point mutations causing antiandrogen withdrawal syndrome studied by molecular simulations and computational prediction approaches

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MEDI 62

Synthesis and evaluation of substrate analogs as mechanistic probes for isopentenyl diphosphate isomerase, type II

Joel R. Walker*, Steven C. Rothman, Jonathan Johnston, and C. Dale Poulter, Department of Chemisty, University of Utah, 315 South 1400 East, Salt Lake City, UT 84112, phd_med_chem@yahoo.com*

Isopentenyl diphosphate isomerase (IDI) catalyzes the interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The mechanism for the type I isomerase, which is found in eukaryotes and some bacteria, proceeds through a tertiary carbocation intermediate. Recently, a structurally unrelated enzyme, type II IDI, that catalyzes the same reaction was discovered in archaea and various bacteria. Unlike type I, the type II enzyme requires reduced flavin and divalent metal

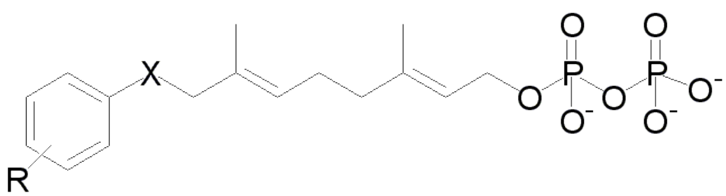
cofactors for activity and represents a novel drug target for antibacterial agents. Through the use of cyclopropyl, epoxide, and fluoro substrate analogs, the mechanism of type II IDI from *Thermus thermophilus* was investigated. The syntheses and pharmacological evaluations of the cyclopropyl, epoxide, and fluoro analogs are shown.

MEDI 63

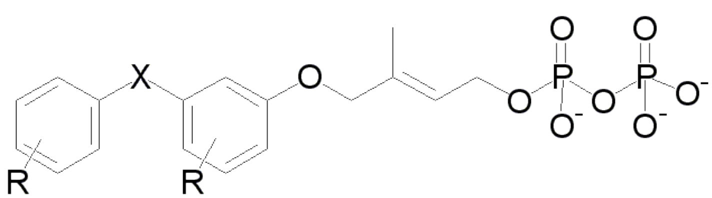
Synthesis of unnatural FPP analogs to study protein prenylation

H. Peter Spielmann, Department of Biochemistry and Kentucky Center for Structural Biology, University of Kentucky, Lexington, KY 40506, hps@pop.uky.edu, and ***Thangaiah Subramanian****, Department of Molecular and Cellular Biochemistry, University of Kentucky, 120 Combs Bldg, 800 Rose St., Lexington, KY 40536, Fax: 859-257-8940, sthan2@uky.edu*

Analogues of farnesyl diphosphate (FPP) are useful tools to study the substrate specificity of prenyltransferases and the downstream biological behavior of prenylated proteins. We report the synthesis and biochemical characterization of new unnatural FPP analogues where the terminal isoprene has been replaced by various substituted aromatic moieties. The nature of the specific substituents on the aromatic ring determines whether the compound acts as an inhibitor or alternative substrate for the enzymes protein farnesyltransferase or protein geranylgeranyltransferase type I. We also report the preparation and preliminary biochemical characterization of a new class of unnatural isoprenoid analogues where both the terminal and second isoprene of FPP have been replaced by aromatic moieties.



X = CH_2O, CH_2, S, O



X = O, OCH_2, CH_2

MEDI 63

Synthesis of unnatural FPP analogs to study protein prenylation

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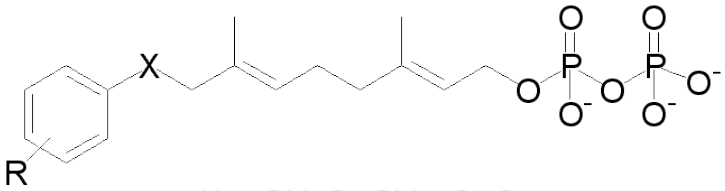
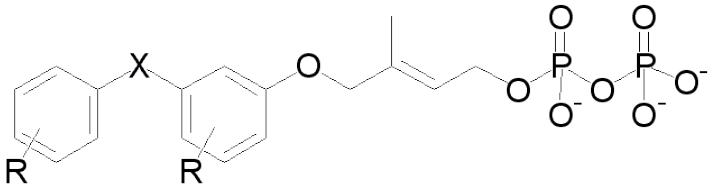


X = CH_2O, CH_2, S, O



X = O, OCH_2, CH_2

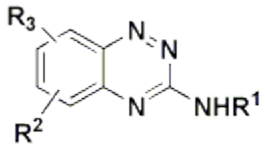
MEDI 64

Strategies involved in the construction of novel benzotriazine inhibitors of Src

Binqi Zeng[1]*, Kathy Barrett*[2]*, Jianguo Cao*[1]*, Colleen Gritzen*[1]*, Hong Gu*[3]*, John Hood*[2]*, Ge Li*[3]*, Chi Ching Mak*[1]*, Andrew McPherson*[1]*, Glenn Noronha*[1]*, Ved P. Pathak*[1]*, Joel Renick*[1]*, Chunbo Sha*[3]*, Feng Shi*[3]*, Yifeng Shi*[3]*, Richard Soll*[1]*, Ute Splittgerber*[1]*, and Suhan Tang*[3]*. (1) Medicinal Chemistry, TargeGen, Inc, 9393 Towne Centre Drive, suite 120, San Diego, CA 92121, Fax: 858-678-0160, zeng@targegen.com, (2) In-vitro Biology, TargeGen, Inc, (3) Wuxi PharmaTech Co. Ltd*

Src family inhibitors may be useful therapeutics for treatment of cancer, myocardial infarction, osteoporosis, stroke, neurodegeneration and other diseases. TargeGen has designed a novel class of highly potent benzotriazine Src inhibitors (>200 molecules with IC50 <30 nM). Driving initial hits from μM to low nM Src activity

required diverse convergent and divergent synthetic strategies for fine-tuning of R1, R2 and R3 moieties. Presented herein are the details of the synthetic methodology used to perform these elaborations, including use of the Buchwald-Hartwig (R1) and Suzuki-Miyaura (R3) cross coupling reactions, as well as the development of novel chemistries to modify the core (R2) and portions of R3, and R1 either prior to, or after attachment to the core. These synthetic methods enabled optimizations of sub-nM inhibitors of Src, and in at least two cases have lent themselves to the manufacture of several hundreds of grams of material needed for preclinical development.

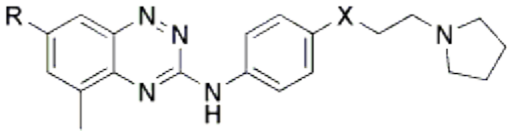


MEDI 65

Optimization of a novel Src inhibitor series to obtain sub-nM compounds

Chi Ching Mak[1]*, Kathy Barrett*[2]*, Jianguo Cao*[1]*, Colleen Gritzen*[1]*, John Hood*[2]*, Andrew McPherson*[1]*, Glenn Noronha*[1]*, Ved P. Pathak*[1]*, Joel Renick*[1]*, Richard Soll*[1]*, Ute Splittgerber*[1]*, and Binqi Zeng*[1]*. (1) Medicinal Chemistry, TargeGen, Inc, 9393 Towne Centre Drive, suite 120, San Diego, CA 92121, cmak@targegen.com, (2) In Vitro Biology, TargeGen, Inc*

Src family inhibitors may be useful therapeutics for the treatment of cancer, myocardial infarction, osteoporosis, stroke, neurodegeneration and other diseases. Dysregulated Src activity is associated with adhesion and cytoskeletal changes, altered gene expression, and increased cell proliferation. TargeGen has designed and optimized a novel series of benzotriazines that are used to target Src. The series was optimized to obtain sub–nM inhibitors of Src via elaboration of the R group, using a variety of substituted and unsubstituted alkyl, aryl and heteroaryl groups. Presented herein are the details for the design and synthesis of the inhibitors with the R group deep within the Src hydrophobic pocket, the resulting SAR, and the optimization that led to highly potent sub–nM Src inhibitors from this series.



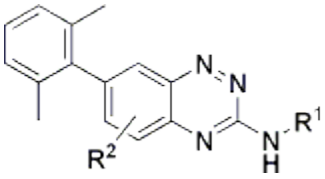
MEDI 66

Structure based design of novel, single-digit nM benzotriazine Src inhibitors

Andrew McPherson[1]*, Kathy Barrett*[2]*, Jianguo Cao*[1]*, Colleen Gritzen*[1]*, John Hood*[2]*, Chi Ching Mak*[1]*, Glenn Noronha*[1]*, Ved P. Pathak*[1]*, Joel Renick*[1]*, Richard Soll*[1]*, Ute Splittgerber*[1]*, and Binqi Zeng*[1]*. (1) Medicinal Chemistry, TargeGen, Inc, 9393 Towne*

Centre Drive, suite 120, San Diego, CA 92121, mcpherson@targegen.com, (2) In-vitro Biology, TargeGen, Inc

The tyrosine kinase Src plays a key role in vascular permeability, tumor progression and metastasis. Due to the pivotal role of Src in cell signaling pathways, Src inhibitors may be useful therapeutics for the treatment of cancers and other diseases including myocardial infarction, osteoporosis, stroke, and neurodegeneration. TargeGen has designed a series of novel benzotriazines that are used to target Src. Suzuki-Miyaura cross coupling chemistry was used to construct the 7-(2, 6-dimethylphenyl)-benzotriazine intermediates from the corresponding 2,6-disubstituted phenyl boronic acid and 7-bromo-benzotriazine cores. The series was optimized to obtain single-digit nM inhibitors of Src via elaboration of the R1 and R2 groups. The details of this campaign and the resulting SAR from these modifications will be presented.



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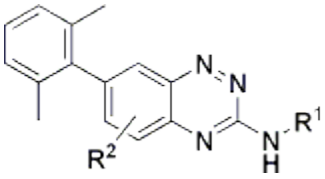


MEDI 67

Aza-peptide Michael acceptors: A novel class of potent and selective inhibitors targeting caspases

Özlem Dogan Ekici[1], *Zhao Zhao Li*[2], *Amy J. Campbell*[2], *Karen E. James*[2], *Juliana L. Asgian*[3], *Jowita Mikolajczyk*[4], *Guy S. Salvesen*[4], *Rajkumar Ganesan*[5], *Stjepan Jelakovic*[5], *Markus G Grutter*[5], *and James C. Powers*[2]. *(1) Department of Chemistry, Ohio State University, 100 W 18th Ave, Columbus, OH 43210, odekici@chemistry.ohio-state.edu, (2) School of Chemistry and Biochemistry, Georgia Institute of Technology, (3) Department of Chemistry, University of California, Irvine, (4) Program in Apoptosis and Cell Death Research, Burnham Institute, (5) Department of Biochemistry, University of Zurich*

Aza-peptide Michael acceptors are a novel class of irreversible inhibitors that are potent and specific for clan CD proteases. Aza-peptide Michael acceptors with an aza-Asp residue at P1 are excellent inhibitors of caspases-2, -3, -6, -7, -8, -9 and -10 with second order rate constants as high as 3,000,000 $M^{-1}s^{-1}$. Aza-peptide Michael acceptors designed with caspase specific sequences do not show any cross reactivity with clan CA cysteine proteases such as papain, cathepsin B, and calpains, demonstrating the selectivity of the inhibitors for clan CD proteases. There is also little to no reactivity toward other clan CD cysteine proteases including legumain, gingipain K, and clostripain. Aza-peptide Michael acceptors' potency and selectivity for caspases make them great candidates for potential use as probes in cellular function studies and as drugs.

MEDI 68

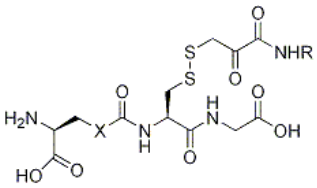
Design and synthesis of metabolically stable GSH-analogs as components of prodrugs and glyoxalase-I inhibitors

Swati S More *and Robert Vince, Department of Medicinal Chemistry, University of Minnesota, 8-125 WDH, 308 Harvard St. SE, Minneapolis, MN 55455, Fax: 612-624-0139, morex002@umn.edu*

The breakdown of glutathione by γ-glutamyl transpeptidase is a significant impediment towards the use of glutathione derivatives for a multitude of therapeutic applications. Efforts have been made to overcome this hurdle through the design of metabolically-stable glutathione analogs that retain crucial recognition elements. A practical methodology for the synthesis of these analogs was developed.

The first application of these analogs is their use as carriers in the delivery of antiparkinsonism drugs across the blood-brain barrier, through the GSH active-transport system. As with all prodrugs, an efficient release mechanism is paramount to its success. It should preferably be catalyzed by a selective enzymatic process, in addition to the byproducts of cleavage being non-toxic. Such constraints make mercaptopyruvate, the metabolite of cysteine, an especially attractive choice for the linker in prodrugs **I**.

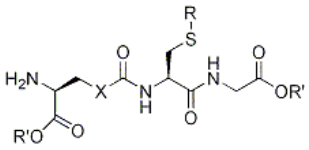
A second therapeutic area where our analogs find application is in the inhibition of glyoxalase I, an enzyme that is overexpressed by tumor cells and has been validated as a target for anticancer therapy. Following the successful recognition of our analogs **(II)** by this enzyme, efforts are being made to elaborate these analogs into zinc-chelating transition state mimics.



X= CH_2 (GSH), NH, O, S

R= dopamine, admantamine

I



X = NH, O, S

R = Transition state mimic

R' = Me, Et, n-Pr

II

MEDI 69

Protease inhibition by novel fluoropeptidomimetics: A mechanism-based design strategy

Lakshmi P. Kotra[1], *Subhash C. Annedi*[2], *Kanchana Majumder*[2], *Lianhu Wei*[2], *and Sheeba Samson*[2]. *(1) Departments of Pharmaceutical Sciences, Chemistry and Division of Cell & Molecular Biology, University of Toronto and Toronto General Research Institute, Molecular Design and Information Technology Centre, 19 Russell Street, Toronto, ON M5S 2S2, Canada, Fax: 416-978-8511, p.kotra@utoronto.ca, (2) Leslie Dan Faculty of Pharmacy, University of Toronto*

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 Ki 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. 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.

MEDI 69

Protease inhibition by novel fluoropeptidomimetics: A mechanism-based design strategy

Lakshmi P. Kotra[1], *Subhash C. Annedi*[2], *Kanchana Majumder*[2], *Lianhu Wei*[2], *and Sheeba Samson*[2]. *(1) Departments of Pharmaceutical Sciences, Chemistry and Division of Cell & Molecular Biology, University of Toronto and Toronto General Research Institute, Molecular Design and Information Technology Centre, 19 Russell Street, Toronto, ON M5S 2S2, Canada, Fax: 416-978-8511, p.kotra@utoronto.ca, (2) Leslie Dan Faculty of Pharmacy, University of Toronto*

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 Ki 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. 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.

MEDI 70

Metal mediated inhibition of methionine aminopeptidase by quinolinyl sulfonamides

Min Huang[1], *Sheng-Xue Xie*[1], *Ze-Qiang Ma*[1], *Robert P. Hanzlik*[2], *and Qi-Zhuang Ye*[1]. *(1) High Throughput Screening Laboratory, University of Kansas, 1501 Wakarusa Drive, Lawrence, KS 66044, Fax: 785-330-4332, mhuang@ku.edu, (2) Department of Medicinal Chemistry, University of Kansas*

Quinolinyl sulfonamides, such as N-(quinolin-8-yl)methanesulfonamide (1) and N-(5-chloroquinolin-8-yl)methanesulfonamide(2),were identified as potent methionine aminopeptidase (MetAP) inhibitors by high throughput screening of a diverse chemical library of small organic compounds. They showed different inhibitory potencies on Co(II)-, Ni(II)-, Fe(II)-, Mn(II)-, and Zn(II)-forms of Escherichia coli MetAP, and their inhibition is dependent on metal concentration. X-ray structures of E. coli MetAP complexed with 1 revealed that the inhibitor forms a metal complex with the residue H79 at the enzyme active site; the complex is further stabilized by an extended H-bond and metal interaction network. Analysis of the inhibition of MetAP by these inhibitors indicates that this is a typical mechanism of inhibition for many non-peptidic MetAP inhibitors and emphasizes the importance of defining in vitro conditions for identifying and evaluating MetAP inhibitors that will be capable of giving information relevant to the in vivo situation. Acknowledgments This research was supported by NIH Grants AI065898, RR015563, and RR016475.

MEDI 71

11-Beta hydroxysteroid dehydrogenase inhibitors

***Michael Siu**, Wendy Taylor, and Theodore O. Johnson, Medicinal Chemistry, Pfizer Global Research and Development, 10770 Science Center Drive, La Jolla, CA 92121*

Benzenesulfonylamino-pyridin-2-yl derivatives and related compounds as inhibitors of 11-beta-hydroxysteroid hydrogenase type 1 (11-beta-HSD-1) for the treatment of diabetes and obesity.

MEDI 72

Pyrazoloquinone substrates and inhibitors of carbonyl reductase

***Andrew Slupe**[1], Solomon Berhe[2], Oladapo Bakare[2], Choice Luster[1], and Henry A. Charlier Jr.[1]. (1) Department of Chemistry, Boise State University, 1910 University Drive, Boise, ID 83725-1520, AndrewSlupe@mail.boisestate.edu, (2) Department of Chemistry, Howard University*

Carbonyl reductase (CR) catalyzes the NADPH-dependent reduction of many carbonyl containing compounds, including anthracyclines. CR reduction of anthracyclines has been linked to both efficacy and cardiotoxicity associated with anthracycline anticancer therapy. Inhibition of CR during anthracycline therapy offers the potential to both increase the effectiveness of the drugs and to decrease the risk of cardiotoxicity. Understanding the substrate and inhibitor specificities of CR is paramount to developing inhibitors that could be used clinically to improve anthracycline therapy. Several pyrazoloquinone compounds were synthesized and screened for biological activity with CR. Several of the compounds tested were found to be substrates with a wide range of catalytic efficiencies. Four compounds were found to be inhibitors with IC50 values ranging from 3-5 micromolar. The pyrazoloquinones in this study represent a new class of substrates and inhibitors for CR and offer insights into the design of potential inhibitors. Supported by NIH/P20RR016454 and NIH/R15CA102119-01.

MEDI 73

Characterization of N-adenylated S-methyl-L-cysteine sulfoximine - a very potent inhibitor of human asparagine synthetase

***Jemy A. Gutierrez**[1], YuanXiang Pan[2], Michael Kilberg[2], and Nigel G. J. Richards[1]. (1) Department of Chemistry, University of Florida, Box 117200, Gainesville, FL 32611-7200, gutierr@chem.ufl.edu, (2) Department of Biochemistry & Molecular Biology, University of Florida College of Medicine*

Asparagine synthetase (AS) catalyzes the ATP-dependent conversion of L-aspartate to L-asparagine. AS inhibitors are targeted as drugs because of their potential in the treatment of drug-resistant acute lymphoblastic leukemia (ALL) and other solid tumors. Koizumi et. al. previously reported the synthesis of a sulfoximine that acts as competitive inhibitor to the ammonia-dependent AS in E. coli [Koizumi, M., et. al. (1999) J. Am. Chem. Soc. 121, 5799-5800.]. This sulfoximine is a transition state analog of the ammonia attack on B-aspartyl-AMP intermediate, and was tested with recombinant human AS. It exhibits slow binding inhibition with an overall Ki of 2.46

nM. Initial studies on drug-resistant MOLT-4 leukemia cells indicate that the sulfoximine acts as a cytostatic agent. Taken together, this transition state analog may be the most potent inhibitor of recombinant human AS to date, and present promising possibilities in the development of better treatments for drug-resistant ALLs.

MEDI 74

Modeling bioactivity for aspartic protease inhibitors using bioavailability and physicochemical molecular descriptors

Catharine J. Collar *and Levente Fabry-Asztalos, Department of Chemistry, Central Washington University, 400 East University Way, Ellensburg, WA 98926-7539, Fax: 509-963-1050, collarc@cwu.edu*

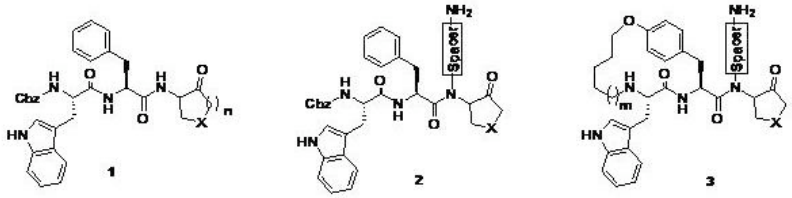
Aspartic proteases are therapeutic targets for several well-studied disease states, including HIV/AIDS, some cancers, Alzheimer's, and malaria. Ample experimental information on each allows for in-depth molecular modeling of bioactivities corresponding to known and potential inhibitory compounds. Our models predicted bioactivity, such as IC_{50}, EC_{50}, TC_{50}, and TI for test set inhibitors and potential inhibitory compounds. Active site binding specificities were also determined.

MEDI 75

Macrocyclic inhibitors of the serine protease plasmin: Development and biological activity

Fengtian Xue *and Christoher T. Seto, Department of Chemistry, Brown University, 324 Brook St., Providence, RI 02912*

The development and in vitro activity studies of cyclic ketone-based inhibitors of serine protease plasmin were described. Structure-activity studies of these inhibitors led to the discovery of three series of inhibitors 1-3. Inhibitor 1 contained a dipeptide Cbz-Trp-Phe that was designed to bind to the S3 and S2 subsites of the enzyme respectively, and a cyclic ketone moiety that can form a reversible covalent hemiketal bond with the active site serine residue of the enzyme. Our data showed that the involvement of a heteroatom O or a sulfone functionality in cyclopentanone ring can improve the potency of inhibitors by a factor of more than 1000 fold. Based on the structure of inhibitor 1, another family of inhibitor 2 was synthesized with an additional lysine mimicry to bind to the S1 subsite of plasmin. Different spacers were screened to improve the efficiency of the binding. Finally, the dipeptide moiety Cbz-Trp-Phe in 1 and 2 was replaced by a macrocyclic ring system to general inhibitor 3. The macrocyclic system can reduce peptide character of inhibitors, and can also help inhibitors form extended structure which favored binding. Inhibitors with various ring sizes were tested against plasmin, and the potency of inhibitors was further improved by introduction of the macrocyclic ring system.



MEDI 76

Molecular basis of the enhanced binding of "two-prong" inhibitors to Carbonic Anhydrase I

Joel A Kooren[1], *Abir Banjaree*[1], *Suad Nadi*[1], *Manas Haldar*[1], *Sanku Mallik*[2], *and D K Srivastava*[2]. *(1) Department of Chemistry, North Dakota State University, Fargo, ND 58102, Joel.A.Kooren@ndsu.edu, (2) Department of Chemistry and Molecular Biology, North Dakota State University*

Human Carbonic Anhydrase-I (hCA-I) is inhibited by a variety of "two-prong" inhibitors, which are constituted of benzenesulfonamide and iminodiacetate chelated copper ion (IDA-Cu^{2+}) attached with a tether residue. The binding affinities of these "two-prong" inhibitors are 1-2 orders of magnitude higher than the individual ligands. To ascertain the amino acid residue(s) involved in the binding of IDA-Cu^{2+}, we mutated His-67, His-200, and His-243 present on the surface of hCA-I via site-directed mutagenesis. Of these mutants, His-200 appears to be involved in the binding of IDA-Cu^{2+} moiety of the inhibitor which results in a significant increase in the binding affinity of the "two-prong" ligand as compared to the parent compound sulfonamide.

MEDI 76

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MEDI 77

Isozyme selective binding of a multi-pronged ligand with tumorigenic carbonic anhydrase XII vs. cytosolic carbonic anhydrase II

Daniel Roman Eiler[1], *Abir L. Banerjee*[1], *Bratati Ganguly*[1], *Manas Haldar*[2], *Melissa Buckle*[1], *Bidhan 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*

We provide evidence of designing an isozyme selective bi-functional ligand for the binding with tumorigenic carbonic anhydrase (CA) XII in preference to its cytosolic counterpart CA II. This ligand designing strategy is not based on optimizing accommodation of ligands within the active site pockets, but by attaching IDA-Cu2+ containing tether groups to the parent ligand, benzenesulfonamide, for interaction with surface exposed histidine residues of carbonic anhydrases. The structural features of CA XII and its cognate ligand reveal that the isozyme selectivity originated due to matching of the three IDA-Cu2+ moieties of the ligand with three complementary surface exposed histidine residues of the enzyme. effect of the isozyme selectivity for CA XII versus CA II was observed with a tri-dented IDA-Cu2+ ligand making it about 120 fold more selective for CA XII than for CA II. The isozyme selective ligand thus designed has the potential of disrupting the pH homeostasis in hypoxic tumors, leading to the promotion of apoptosis and suppression/ alleviation of invasion and metastasis.

MEDI 78

Pyrrole carboxamides as potential carbonic anhydrase inhibitors

Nishrin Marketwala[1], *David J. Malwitz*[1], *Daniel M. Ketcha*[1], *Albert Barrese III*[2], *and Brian C. Tripp*[3]. *(1) Department of Chemistry, Wright State University, 3640 Col. Glenn Hwy, Dayton, OH 45435, marketwala.2@wright.edu, (2) Biological Sciences Department, Wesrtern Michigan University, (3) Department of biological sciences, Western Michigan University*

Whereas most inhibitors of the carbonic anhydrase (CAI's) possess a sulfonamide functionality, the lack of isozyme specificity exhibited by members of this class prompt a search for non-sulfonamide CAIs. Recently, we initiated a screen of indoles and pyrroles possessing acyl-, carboxyl-, amido-, sulfonyl-, sulfamyl and oxime functionalities as potential inhibitors of CA II. The catalytic activity of human CA II was monitored by kinetic A348 measurements of its non-physiological 4-nitrophenyl acetate (4-NPA) esterase rate with a microplate spectrophotometer. The initial screen indicated that several compounds exhibited measurable inhibition of CA II with average inhibition values ranging from 58.6-14.3%. As slight inhibition was observed in the case of 1-(phenylsulfonl)pyrrole-2-carboxamide, and cognizant of the fact that pyrrole carboxamide derived sulfonamides had been demonstrated as CA inhibitors, we prepared N-protected analogues lacking the sulfonamide moiety. Thus, 1-(phenylsulfonyl)pyrrole-2-carbonyl chloride was treated with a variety of

benzyl amines wherein optimal activity was observed on the case of the meta-trifluoromethyl derivative.

MEDI 79

Dimethylamino-pyridinols and pyrimidinols as potent cyclooxygenase inhibitors: Mechanism of activity and toxicity

Tae-gyu Nam[1], *Hye-Young Kim*[1], *Olivier Boutaud*[2], *Derek A. Pratt*[3], *John A. Oates*[2], *and Ned A. Porter*[1]. *(1) Department of Chemistry, Vanderbilt University, Station B, 351822, Nashville, TN 37235, Fax: 615-343-5478, tae-gyu.nam@vanderbilt.edu, (2) Division of Clinical Pharmacology, Vanderbilt University School of Medicine, (3) Department of Chemistry, Queen's University*

Molecular targets of acetaminophen (ApAP), an analgesic and antipyretic, are prostaglandin H_2 synthases (PGHSs), or cyclooxygenases (COXs). We have recently proposed that the ability of ApAP analogs to donate an electron or a hydrogen atom, reflected in their ionization potential (IP) and O-H bond dissociation enthalpy (BDE), to the oxidized PGHS peroxidase correlates with thier PGHS inhibitory activity. We designed pyridinols and pyrimidinols analogs with lower IP an BDE making them the better electron and hydrogen atom donors than ApAP. These compounds showed about a 10-20 fold stronger PGHS inhibition than ApAP. Interestingly, the pyridinols inhibit PGHS peroxidase while one pyrimidinol activates it. ApAP causes severe hepatic necrosis when overdosed by the fomation of the toxic metabolite, N-acetyl-*p*-benzoquinone imine (NAPQI), which forms glutathione (GSH) adduct. We hypothesized that our analogs are less likely to form quinone imine species that are less likely to form GSH adduct. Horseradish peroxidase mediated oxidation experiment demonstrated our analogs form much less GHS adduct than ApAP.

MEDI 80

Nepafenac metabolite, Amfenac, inhibits VEGF-induced angiogenesis

John S. Penn[1], *Song Qilin*[1], *and* ***Charles A. Odonkor***[2]. *(1) Ophthalmology and Visual Sciences, Vanderbilt University School of Medicine, 800 Medical Center East, Nashville, TN 37232, john.penn@vanderbilt.edu, (2) Biology and Chemistry, Sewanee; The University of the South, 735 University Avenue, Sewanee, TN 37383, odonkca0@sewanee.edu*

In order to characterize the role of cyclooxygenease (COX)-1 and COX-2 in retinal angiogenesis and further develop therapeutic strategies for retinal angiogenesis, we have investigated the effects of different COX-1 and COX-2 inhibitors on rat Muller cells and human retinal microvascular endothelial cells (HRMEC). Amfenac is a potent non-selective COX-1 and COX-2 inhibitor. It is a metabolic product of nepafenac, which has the ability to penetrate the cornea and inhibit ischemia-induced retinal neovascularization. A COX-2- selective inhibitor, Celecoxib, and a COX-1 selective inhibitor, SC-560, were also employed in our studies. Our data indicate that the non-selective COX inhibitor, Amfenac, has the potential to inhibit retinal angiogenesis. Both Amfenac and Celecoxib inhibited rat Müller cell VEGF production stimulated by hypoxia, while SC-560, was ineffective. This suggests that COX-2 plays a critical role

in VEGF production in rat Müller cells. Amfenac also inhibited HRMEC behaviors induced by VEGF, including cell proliferation and capillary tube formation, both of which are critical to retinal angiogenesis. These results point to a critical role of COX-2 and its products both upstream and downstream of VEGF receptor activation. Specific targeting of COX enzymes may lead to potent inhibition of angiogenesis .

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MEDI 81

Inhibition of human group IIA Phospholipase A2 by Petrosaspongiolide M through a mechanism of protein-protein trans-inactivation

*Maria Chiara Monti[1], **Claudio N. Cavasotto**[2], Alessandra Tosco[1], Agostino Casapullo[1], Arturo Leone[1], Raffaele Riccio[1], Fabrizio Dal Piaz[1], Cosima Santomauro[1], R. Abagyan[3], and Luigi Gomez-Paloma[1]. (1) Dipartimento de Scienze Farmaceutiche, Università di Salerno, via Ponte don Melillo, Fisciano (Salerno) 84084, Italy, (2) Molsoft LLC, 3366 North Torrey Pines Court, S. 300, La Jolla, CA 92037, Fax: 858-625-2888, claudio@molsoft.com, (3) Department of Molecular Biology, The Scripps Research Institute*

The molecular mechanism for the inhibition of human group IIA secretory phospholipase A2 (sPLA2-IIA) by the natural anti-inflammatory sesterterpene Petrosaspongiolide M (PM) has been studied by mass spectrometry and computational biology techniques. We show that the amphiphilic PM is extracted

from phospholipid micelles by selectively reacting with Lys-67 of sPLA2-IIA forming an imine intermediate. This crucial covalent modification occurs at the enzyme-membrane interfacial binding surface (IBS). The nucleophilic attack of Lys-67 liberates in turn a free carboxyl group in PM, generating a PLA2-bound substrate analogue, capable of targeting the active site of another sPLA2-IIA molecule due to VDW/electrostatic complementarity and, importantly, ability to chelate the essential calcium ion. This unusual mode of action leads to a potent dual inhibition (disruption of the IBS in one PLA2 molecule and active site binding in another), which we have termed protein-protein trans-inactivation. This peculiar inhibition mechanism may lead to new therapeutic strategies utilizing PLA2 inhibition.

MEDI 82

Zinc metalloproteinase inhibitors with 1,2-dihydroxybenzene and 3-hydroxy-4-pyrone moieties as zinc binding groups

***Yonghao Jin**, Qiang Cao, Qing-Xiang A Sang, and Martin A Schwartz, Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, Fax: 850-644-8281, yjin@chem.fsu.edu*

Matrix metalloproteinases (MMPs) and tumor necrosis factor-? converting enzyme (TACE) are zinc endopeptidases that play very important roles in many physiological and pathological processes. It has been a challenging endeavor to develop inhibitors for these enzymes that have both favorable chemical and biological properties. The 1,2-dihydroxybenzene and 3-hydroxy-4-pyrone groups have been studied as zinc binding groups in inhibitor design. The design rationale, synthesis and inhibitory activites of a number of new inhibitors bearing these groups will be presented.

MEDI 83

Structure-based design of 5-Hydroxy-4-H-pyran-4-one derivatives as matrix metalloproteinase inhibitors

***Akash Khandelwal**, Yufen Zhang, and Stefan Balaz, Department of Pharmaceutical Sciences, North Dakota State University, Sudro Hall 8B2, Fargo, ND 58105, Fax: 7012318333, akash.khandelwal@ndsu.edu*

Matrix metalloproteinases (MMPs) are enzymes participating in remodeling of extracellular matrix and imbalance of their activities may lead to disease like cancer, arthritis etc. Inhibition of MMP-9 catalytic domain by 18 kojic acid (5-hydroxy-4-H-pyran-4-one) derivatives was tested using a fluorogenic peptide substrate in the 96-well format. The determined inhibition constants were used for binding affinity estimation studies. The coordinates of the MMP-9 active site were taken from x ray crystal structure (PDB file ID-1GKC). Kojic acid derivatives were docked into the active site of MMP-9 using FlexX. The complexes were then minimized using combined quantum mechanics/molecular mechanics (QM/MM) employing continuum solvent. The QM/MM energy and solvent-accessible surface area terms were then correlated linearly with binding affinity in a manner similar to linear interaction energy (LIE) approach. The developed model was able to explain ~90% of the

variation in experimental activity. The model will be used further for structure optimization.

MEDI 84

Pharmacophore modeling, QSAR, and CoMSIA studies of matrix metalloproteinase inhibitors

Haizhen Zhong *and J. Phillip Bowen, 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*

Matrix metalloproteinases (MMPs) are a family of zinc-containing, calcium-dependent enzymes involved in a series of disease processes, including cancer and arthritis. Many MMP inhibitors have been suspended during the clinical trials. These inhibitors include prinomastat, BMS-275291, and BAY 12-9566. Selectivity has been one of the most important factors that have been attributed to the failure of these MMP inhibitors. Inhibition of MMP-2 and MMP-9 has been considered a viable way to inhibit tumor growth and metastasis. MMP-2 and MMP-9 are considered to be proangiogenic. MMP-7 and MMP-12, however, are deemed as antiangiogenic factors. Inhibition of MMP-1 would cause the side effects of the MMP inhibitors that were pulled out from the clinical trials. Therefore, the selectivity toward MMP-2 and/or MMP-9 remains a challenge. We report herein the quantitative structure-activity relationship (QSAR) and comparative molecular similarity indices (CoMSIA) models for forty phenoxyphenyl sulfone N-formylhydroxylamines. The QSAR studies suggest: (1) Increase the logS and logP(o/w) to elevate the selectivity toward MMP-2/MMP-9; (2) Electrostatic interaction plays an important role for the selectivity. The pharmacophore models for the highly selective compounds are different from those models built from the lower selectivity inhibitors.

MEDI 84

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MEDI 85

Selectivity studies of diverse matrix metalloproteinase inhibitors using pharmacophore modeling and 3-D QSAR techniques

J. Phillip Bowen *and Haizhen Zhong, Center for Drug Design, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, 400 New Science Building, PO Box 26170, Greensboro, NC 27402-6170, Fax: 336-334-5402, jpbowen@uncg.edu*

The development of selective enzyme inhibitors has long been the goal of many medicinal chemists. After the suspension of clinical trials for prinomastat, BMS-275291, and BAY 12-9566, there is a new urgency to design highly selective inhibitors of matrix metalloproteinases (MMPs). MMP-2 and MMP-9 have been reported to be proangiogenic and therefore contribute to tumor growth and metastasis. Inhibition of MMP-2 and/or MMP-9, therefore, is an ideal pathway for anti-tumor drug design. Caution needs to be invoked for MMP-2 and MMP-9 inhibitor design, because inhibition of MMP-7, MMP-12, and MMP-1 would cause undesirable side effects. Pharmacophore modeling and three dimensional quantitative structure-activity relationship (3D-QSAR) studies were carried out with MOE and SYBYL software. These models were built based upon seventy-five inhibitors from a diverse set of scaffolds. Our model suggests that an increase in solubility would enhance the selectivity toward MMP-2 and MMP-9. Pharmacophore models and CoMSIA models will also be presented.

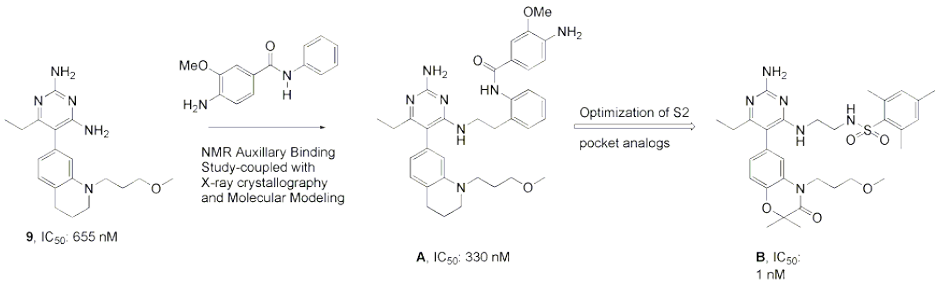
MEDI 86

NMR auxillary binding screen for lead optimization: Design of novel renin inhibitors that access both the S2 and S3 pockets of renin

Daniel D. Holsworth*, Michael Bury, Wayne L. Cody, Don Emerson, Chad Van Huis, Mehran Jalaie, Michael Kaufman, Patrick McConnell, Noel A. Powell, Parag Sahasrabudhe, Ronald W. Sarver, Vernkataraman Thanabal, and Erli Zhang, Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Fax: 734-622-3909, daniel.holsworth@pfizer.com*

Recently, a novel class of pyrimidine based renin inhibitors (9, IC50: 655 nM) have been discovered. Crystallographic analysis of 9 bound to the active site of renin demonstrated that 9 binds to the S3 pocket of renin, as well as, identified opportunities to access the S2 pocket of renin. An NMR auxillary binding screen was conducted to find small molecular “fragments” that bound in the S2 pocket, near compound 9/renin complex. Next, using the interligand NMR constraints, the design of a linker between the fragment and the 9/renin complex via molecular modeling

was accomplished. These efforts produced A, which possessed a modest increase in potency. Crystallographic analysis of A confirmed that both the S3 sub-pocket and the S2 pocket were occupied. Further optimization of S2/S3 pocket analogs via parallel synthesis produced compound B. The identification of fragments that bind to the S2 pocket via an NMR auxillary binding screen, the design of A by molecular modeling and crystallographic analysis, and the optimization of S2/S3 pocket analogs will be discussed.

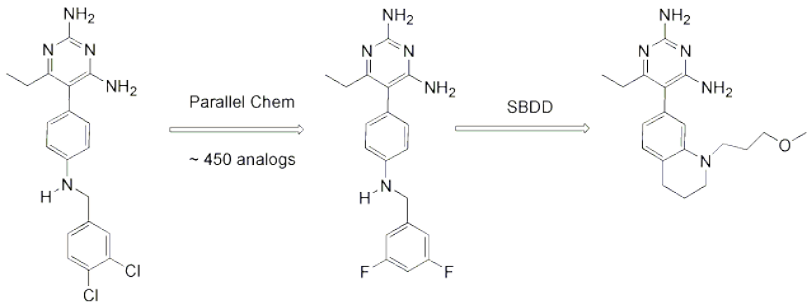


MEDI 87

Discovery of 6-ethyl-2,4-diaminopyrimidine-based small molecule renin inhibitors

Daniel D. Holsworth[1]*, Thomas Belliotti*[1]*, John Bryant*[1]*, Cuiman Cai*[2]*, Wayne L. Cody*[1]*, Dennis M. Downing*[1]*, Mehran Jalaie*[1]*, Aparna Kasani*[3]*, Tingsheng Li*[4]*, Samarendra Maiti*[3]*, Patrick McConnell*[1]*, Noel A. Powell*[1]*, Michael Ryan*[1]*, Rajandra Subedi*[3]*, Erli Zhang*[1]*, and Jeremy J Edmunds*[1]*. (1) Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Fax: 734-622-3909, daniel.holsworth@pfizer.com, (2) Michigan Laboratories, Pfizer Global Research & Development, (3) Naeja Pharmaceuticals, (4) Chemistry Department, NAEJA Pharmaceuticals*

Recently, we have discovered a novel class of small molecule renin inhibitors. Identification of a novel template (6, IC50: 27,000 mM) with low renin potency was obtained by high throughput screening. Optimization (~450 compounds) of 6 by parallel synthesis, in the absence of a crystal structure, produced 7 (IC50: 4000 nM). An X-ray crystal structure was obtained of 7 bound to the active site of renin. This structural information, coupled with crystal structure information from a previous chemical series was used to design a novel class of potent small molecule renin inhibitors (9, IC50: 655 nM). Crystallographic analysis, molecular modeling, synthesis and SAR of 9 will be presented.



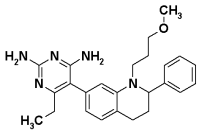
Compound No.:	6	7	9
Mw:	387	355	341
IC_{50} (nM):	27,000	4000	655

MEDI 88

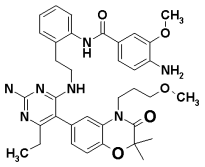
Binding thermodynamics of renin inhibitors: S2 vs. S3 sub-pocket analogs

Ronald W. Sarver[1]*, Fred L. Ciske*[1]*, Wayne L. Cody*[1]*, Jim Dyer*[1]*, Jeanne C Hagadorn*[1]*, Daniel D. Holsworth*[1]*, Mehran Jalaie*[1]*, Michelle Mastronardi*[2]*, Patrick McConnell*[1]*, Noel A. Powell*[1]*, John Quinn*[1]*, and Erli Zhang*[1]*. (1) Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105, ronald.w.sarver@pfizer.com, (2) Manpower*

Binding thermodynamics for several synthetic renin inhibitors were examined using isothermal titration calorimetry (ITC). Isothermal titration calorimetry measures the heat flow or enthalpy change that occurs when a molecule interacts with an enzyme. Using ITC to measure heat flow results in a binding isotherm from which the equilibrium binding constant (Kd), enthalpy change, entropy change, change in free energy, and stoichiometry are obtained, thus providing a complete thermodynamic picture for the binding interaction. Binding thermodynamics of several renin inhibitors that probe different pockets of the active site were measured. In particular, molecular additions to the pyrimidine ring template that extended into the S2 pocket dramatically changed the thermodynamic driving force for the binding interaction. Renin binding of the template, typified by 18, was enthalpically driven whereas binding of 19, with the S2 extension, was both enthalpically and entropically driven. The entropic gain for 19 was probably due to flexing of the protein to accommodate the extension into the S2 pocket and also removal of ordered water molecules from the pocket, but the interaction was also accompanied by a significant loss in enthalpy. Therefore, changes to orient polar functionalities for better electrostatic interactions were suggested for the S2 extension to regain the binding enthalpy of the core template. These and other modifications to the template suggested from analysis of the thermodynamics of several inhibitor interactions are presented along with the use of calorimetric data to help guide structure based drug design.



COMPOUND 18



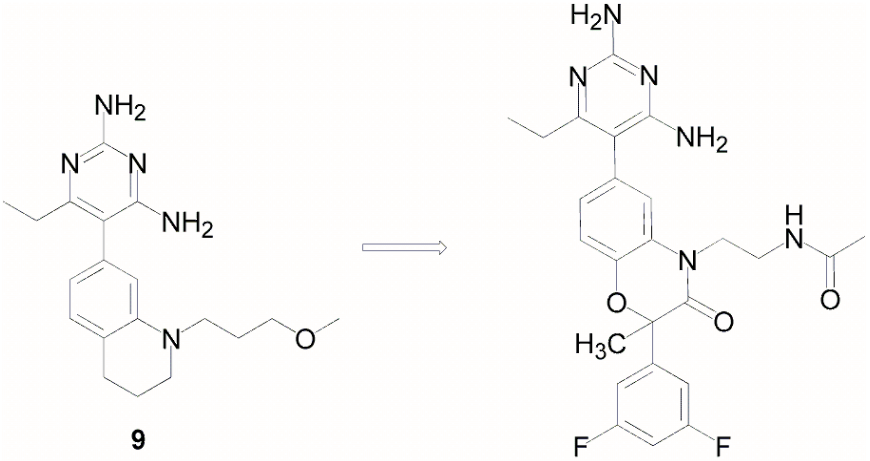
COMPOUND 19

MEDI 89

Rational design of 6-(2,4-diaminopyrimidinyl)-1,4-benzoxazinones as small molecule renin inhibitors

Noel A. Powell, *Fred L. Ciske, Cuiman Cai, Wayne L. Cody, Dennis M. Downing, Daniel D. Holsworth, Ken Mennen, Chad Van Huis, Mehran Jalaie, Michael Ryan, John Bryant, Wendy Collard, Suzie Ferreira, Patrick McConnell, Erli Zhang, and Jeremy J Edmunds, Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105*

We have recently discovered a novel class of small molecule renin inhibitors based on a 5-tetrahydroquinolinyl-6-ethyl-2,4-diaminopyrimidine scaffold (**9**, IC_{50} = 0.66 μM). A rational structure-based drug design approach was used to design a series of 6-(6-ethyl-2,4-diaminopyrimidinyl)-1,4-benzoxazinones that show improved renin potency (**12**, IC_{50} = 0.001 μM). The design, structure activity relationships, and X-ray crystallography analysis of this series will be discussed.



9

Renin IC_{50} = 0.66 μM

12

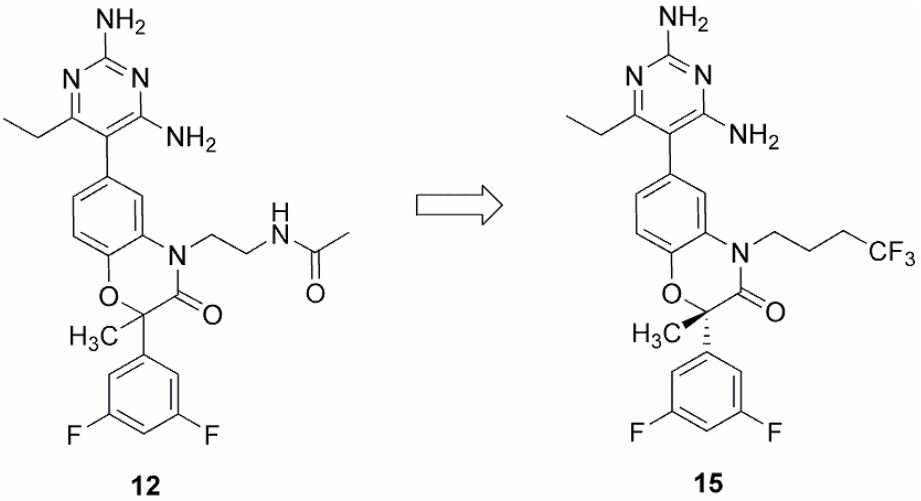
Renin IC_{50} = 0.001 μM

MEDI 90

Optimization of the PK properties of 6-(2,4-diaminopyrimidinyl)-1,4-benzoxazinone renin inhibitors

Noel A. Powell, *Fred L. Ciske, Cuiman Cai, Wayne L. Cody, Dennis M. Downing, Daniel D. Holsworth, Chad Van Huis, Mehran Jalaie, Michael Ryan, John Bryant, Wendy Collard, Suzie Ferreira, and Jeremy J Edmunds, Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105*

We have recently discovered a novel class of small molecule renin inhibitors based on a 5-(1,4-benzoxazinone)-6-ethyl-2,4-diaminopyrimidine scaffold exemplified by **12**. This series was characterized by poor PK parameters caused by rapid first pass metabolism and poor absorption. We will present our efforts to improve the PK parameters by blockade of the metabolic sites and improving absorption. These efforts resulted in **15**, which exhibits moderate renin potency and excellent oral bioavailability.

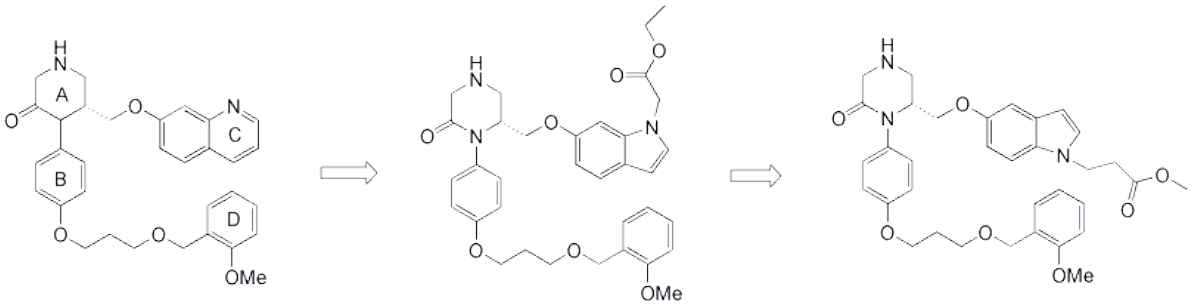


MEDI 91

Ketopiperazine-based renin inhibitors: Optimization of the "C" ring: Incorporation of indole rings

Cuiman Cai[1], *John Bryant*[2], *Xue Min Cheng*[2], *Wayne L. Cody*[2], *Wendy Collard*[2], *Dennis M. Downing*[2], *Noe Erasga*[2], *Suzie Ferreira*[2], *Eric Hall*[3], *Daniel D. Holsworth*[2], *Mehran Jalaie*[2], *Aparna Kasani*[3], *Chitase Lee*[2], *Tingsheng Li*[3], *Samarendra Maiti*[3], *Patrick McConnell*[2], *Noel A. Powell*[2], *Mohammad Rahim*[3], *Michael Ryan*[2], *Michael A. Stier*[2], *Rajandra Subedi*[3], *Erli Zhang*[2], *and Jeremy J Edmunds*[2]. *(1) Michigan Laboratories, Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105, cuiman.cai@Pfizer.com, (2) Michigan Laboratories, Pfizer Global Research & Development, (3) Naeja Pharmaceuticals*

Optimization of the "C" ring of 1 by installing substituted indole rings attached at the 5- or 6-position generated novel, potent ketopiperazine-based renin inhibitors. An X-ray crystal structure of 2 bound to the renin active site was utilized for further design of "C" ring analogs that addressed the S3 sub-pocket of renin. The SAR, synthesis, crystallographic studies, and CYP3A4 activity of this series of compounds will be presented.

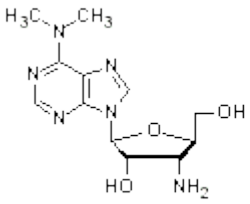


MEDI 92

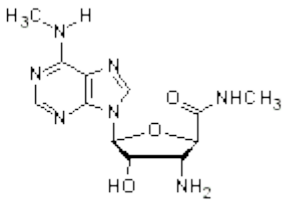
L-Adenosine analogs as cardioprotective agents

Giuseppe Gumina[1], *Harinath Kasiganesan*[1], *Renee P. Wong*[1], *Gary Wright*[2], *and Craig C. Beeson*[1]. *(1) Department of Pharmaceutical Sciences, Medical University of South Carolina, 280 Calhoun Street, PO Box 250140, Charleston, SC 29425, Fax: 843-792-1617, gumina@musc.edu, (2) Department of Pharmaceutical Science, Medical University of South Carolina*

Cardiovascular disease is America's leading killer with almost one million victims every year, mainly from heart attack and stroke. For this reason, new preventive therapeutic agents are needed to complement the management of risk factors. However, in order for a drug to be used in prophylactic therapy, it should be very safe, i.e. display virtually no toxicity. Adenosine shows protective effect against oxidative stress and ischemia. This effect is due to interaction with at least four receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. Activation of A_1 and A_3 receptors plays a major role during ischemic preconditioning. Activation of A_{2A} receptors is also beneficial because it reduces postischemic inflammation. Therefore, agonists to adenosine receptors have the potential to become cardioprotective drugs. All previously reported agonists are analogs of naturally configured D-adenosine. As such, they have the potential to interact with metabolic enzymes such as kinases and phosphorylases with consequent deactivation and potential toxicity. On the other hand, L-nucleosides have proven to possess favorable features, such as low toxicity and high metabolic stability. Two L-nucleosides, L-3'-amino-3'-deoxy-N^6-dimethyladenosine (L-3'-ADMdA) **1**, previously synthesized in our laboratory, and the novel L-3'-amino-3'-deoxy-N^6-methyladenosine-5'-*N*-methyluronamide (L-3'-AM-MECA) **2**, show cardioprotection on an ischemic reperfusion model using perfused murine hearts. Furthermore, both compounds were able to enhance protective metabolic responses in L6 myoblasts exposed to chemical ischemia. Taken together, these studies strongly suggest that **1** and **2** act as agonists to A_1 and/or A_3 adenosine receptors. Notably, L-3'-ADMdA and L-3'-AM-MECA are the first L-nucleosides that show biological activity besides antiviral or antitumor effect.



L-3'-ADMdA, 1



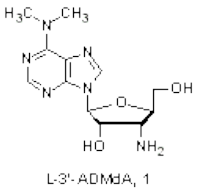
L-3'-AM-MECA, 2

MEDI 92

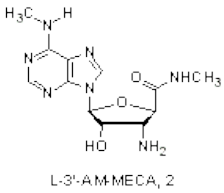
L-Adenosine analogs as cardioprotective agents

Giuseppe Gumina[1], *Harinath Kasiganesan*[1], *Renee P. Wong*[1], *Gary Wright*[2], *and Craig C. Beeson*[1]. *(1) Department of Pharmaceutical Sciences, Medical University of South Carolina, 280 Calhoun Street, PO Box 250140, Charleston, SC 29425, Fax: 843-792-1617, gumina@musc.edu, (2) Department of Pharmaceutical Science, Medical University of South Carolina*

Cardiovascular disease is America's leading killer with almost one million victims every year, mainly from heart attack and stroke. For this reason, new preventive therapeutic agents are needed to complement the management of risk factors. However, in order for a drug to be used in prophylactic therapy, it should be very safe, i.e. display virtually no toxicity. Adenosine shows protective effect against oxidative stress and ischemia. This effect is due to interaction with at least four receptor subtypes: A_1, A_{2A}, A_{2B} and A_3. Activation of A_1 and A_3 receptors plays a major role during ischemic preconditioning. Activation of A_{2A} receptors is also beneficial because it reduces postischemic inflammation. Therefore, agonists to adenosine receptors have the potential to become cardioprotective drugs. All previously reported agonists are analogs of naturally configured D-adenosine. As such, they have the potential to interact with metabolic enzymes such as kinases and phosphorylases with consequent deactivation and potential toxicity. On the other hand, L-nucleosides have proven to possess favorable features, such as low toxicity and high metabolic stability. Two L-nucleosides, L-3'-amino-3'-deoxy-*N*^{6}-dimethyladenosine (L-3'-ADMdA) **1**, previously synthesized in our laboratory, and the novel L-3'-amino-3'-deoxy-*N*^{6}-methyladenosine-5'-*N*-methyluronamide (L-3'-AM-MECA) **2**, show cardioprotection on an ischemic reperfusion model using perfused murine hearts. Furthermore, both compounds were able to enhance protective metabolic responses in L6 myoblasts exposed to chemical ischemia. Taken together, these studies strongly suggest that **1** and **2** act as agonists to A_1 and/or A_3 adenosine receptors. Notably, L-3'-ADMdA and L-3'-AM-MECA are the first L-nucleosides that show biological activity besides antiviral or antitumor effect.



L-3'-ADMdA, 1



L-3'-AM-MECA, 2

MEDI 93

Synthesis and evaluation of hypolipidemic properties of some novel derivatives of thieno oxazine and 4-hydroxyindole-2-carboxylic acid

*Sankar Mohan, Kalusani A. Reddy, Javed Iqbal, Debnath Bhuniya, and **Saibal K. Das**, Metabolic Disorders Group, Discovery Research, Dr. Reddy's Laboratories Ltd, Bollaram Road, Miyapur, Hyderabad 500049, India, Fax: +91-40-23045438, sankarm@sfu.ca, saibalkumardas@drreddys.com*

Hypercholesterolemia and hypertriglyceridemia are associated with an increased incidence of atherosclerosis, coronary artery and heart diseases, the well-recognized surrogate factor for cardiovascular deaths. Among the existing drugs for combating dyslipidemia, statins such as atorvastatin and fibrates such as fenofibrate, bezafibrate and clofibrate are most widely used. Statins act via inhibition of HMG CoA reductase enzyme arresting cholesterol biosynthesis. The predominant effect of statins is lowering of low-density lipoprotein (LDL) cholesterol and it has minimum effect on triglyceride (TG). However, fibrates act on dietary lipids and have demonstrated favorable reductions in coronary heart disease (CHD) and cardiac mortality by lowering circulating TG and LDL and raising high-density lipoprotein (HDL) cholesterol in human. Though fenofibrate is reasonably safe, in general, fibrates still have concern in increasing muscle toxicity especially when used in combination with statins. Therefore, there is an unmet medical need to discover safer fibrate analogues with higher potency. Such compounds may also be used in combination with Ezetimibe. Continuous organizational efforts along with aforesaid considerations have prompted us to initiate a research program based on phenoxy and phenylsulfanyl acetic acid derivatives and study their profiles as triglyceride lowering agents. Two novel series (fig. 1) were developed with carboxamidomethyl linker utilizing thieno oxazinone and indole carboxylic acid. One compound in each series has been identified with significant triglyceride lowering activity. The SAR will be discussed.

†DRL Publication No. 484-A

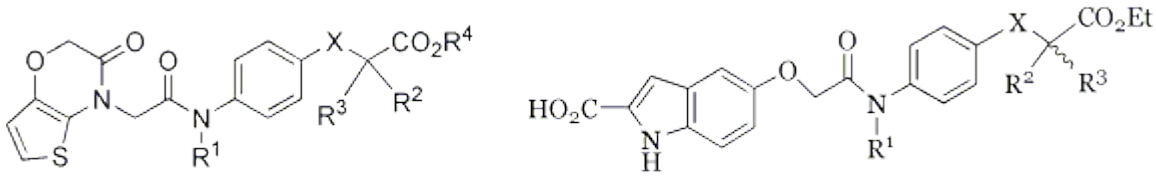


Fig. 1

MEDI 94

Novel chemoenzymatic oligomers of cinnamic acid that inhibit coagulation enzymes utilizing antithrombin dependent and independent mechanisms

Brian Lawrence Henry[1]*, Bernhard H. Monien*[1]*, Arjun Raghuraman*[2]*, Michael Hindle*[3]*, and Umesh R. Desai*[1]*. (1) Department of Medicinal Chemistry, Virginia Commonwealth University, 410 N. 12th St., Blackwell Smith Bldg. Room 551, Richmond, VA 23298-0540, henrybl@vcu.edu, (2) Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia*

Commonwealth University, (3) Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University

The occurrence of thrombosis in several pathophysiological conditions creates a huge need for anticoagulation therapy. This work describes the synthesis, characterization and biochemical analysis of chemoenzymatically synthesized oligomers of cinnamic acid derivatives as anticoagulants. Peroxidase-catalyzed oxidative coupling of 4-hydroxycinnamic acid derivatives followed by sulfation gave oligomers composed of 4–9 units with an average of 1–3 sulfate groups per chain. APTT and PT tests displayed a concentration-dependent prolongation of clotting time, which compares favorably with the profile determined for a low-molecular-weight heparin. More interestingly, the unsulfated oligomers were found to inhibit both factor Xa and thrombin in the absence of antithrombin. This anticoagulation improved nearly 2–3-fold in the presence of the serpin inhibitor suggesting a unique ability of these oligomers to inhibit the two procoagulant enzymes in a direct and indirect manner, a property not found in LMWH. These oligomers could serve as extremely interesting anticoagulant lead compounds.

MEDI 95

Design, synthesis and biological evaluation of small molecule heparin/heparan sulphate (H/HS) mimics as glycosaminoglycane (GAG) - protein interactions inhibitors

*Eun-Ang Raiber[1], James A. Wilkinson[1], **Sylvie Ducki**[1], Malcolm Lyon[2], and John Gallagher[2]. (1) Biosciences Research Institute, University of Salford, Cockcroft Building, Salford M5 4WT, United Kingdom, Fax: 0161 295 5111, E.A.Raiber@pgr.salford.ac.uk, S.Ducki@Salford.ac.uk, (2) Cancer Research UK Department of Medical Oncology, Christie Hospital NHS Trust*

Cancer growth involves the proliferation and invasion of cancer cells, the stimulation of angiogenesis, and the metastasis of tumour cells. Key factors in these cellular activities are growth factors such as hepatocyte growth factor / scatter factor (HGF/SF) and fibroblast growth factors (FGFs). These signalling events involve the binding of the GFs to their receptor, a transmembrane tyrosine kinase. This binding can only take place in the presence of H/HS. Given the potential significance of therapies based on control of GAG-protein interactions, the development of structurally simpler mimics is an attractive area for investigation.

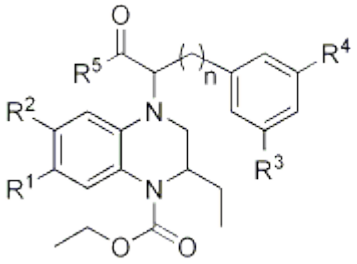
Based on previous studies of bioactive heparin fragments, we designed and synthesized small, non-sugar, aromatic molecules as mimics of H/HS, to bind to the GF without promoting receptor activation and downstream signalling. Here we present the design and synthesis of these compounds and the interesting discoveries we have made by evaluating their biological properties against GFs.

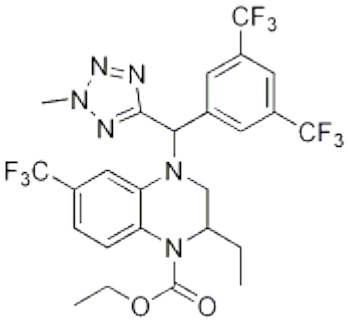
MEDI 96

Ester and tetrazole substituted tetrahydoquinoxalines as potent cholesterol ester transfer protein inhibitors

C. Todd Eary, *Zachary S. Jones, Robert D. Groneberg, Laurence E. Burgess, David A. Mareska, Mark D. Drew, James F. Blake Jr., Ellen R. Laird, and Devan Balachari, Array BioPharma, 3200 Walnut Street, Boulder, CO 80301, Fax: 303-381-6662, teary@arraybiopharma.com*

Cholesterol ester transfer protein (CETP) is a plasma glycoprotein which performs a critical function of transferring cholesterol ester between lipoprotein particles. Blocking this protein in vitro and in vivo results in an increase in plasma high density lipoprotein cholesterol (HDL-C), "the good cholesterol". We will describe a series of tetrahydroquinoxalines as potent inhibitors of CETP. This presentation will include an analysis of the SAR at several points on the molecule (R1 – R4) and the impact of stereochemistry on potency. We will also present the SAR at the ester group (R5 = OR) and bioisosteres for the ester which include alkyl tetrazoles.



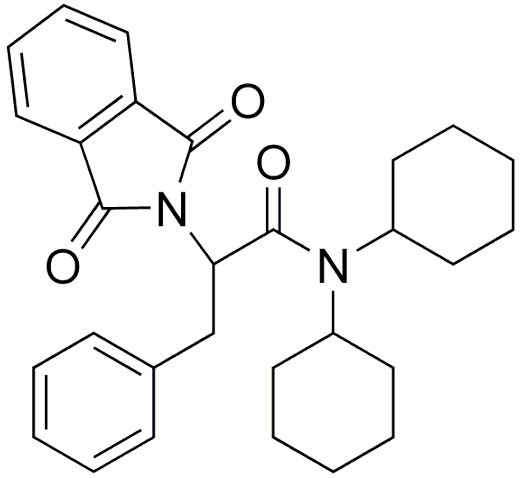


MEDI 97

Synthesis and biological evaluation of novel, orally bioavailable antagonists of FXR

Jonathan B. Houze, *Joshua Gergely, Yi Xia, Sharon McKendry, Bei Shan, Hua Tu, Margrit Schwarz, Matthew Wright, Qiuping Ye, and Hoa Le, Amgen Inc, 1120 Veterans Blvd., South San Francisco, CA 94080, Fax: 650-244-2015, jhouze@amgen.com*

The farnesoid X receptor (FXR) is a ligand activated transcription factor in the nuclear receptor gene superfamily that plays a key role in cholesterol metabolism, lipid homeostasis, and absorption of dietary fat. Activation of FXR by bile acids leads to the upregulation of several genes, including the ileal bile acid binding protein (I-BABP) and SHP-1. In turn, SHP-1 downregulates expression of several genes including that of CYP7A which catalyzes the rate-determining step for the formation of bile acids from cholesterol. High-throughput screening identified an alpha-substituted tertiary amide (I) as a potent FXR antagonist. Efforts to improve the potency and pharmacokinetic profile of compound I led to the identification of analogs that decreased levels of I-BABP and increased levels of CYP7A when dosed orally in mice. Suitable analogs of compound I could serve as biological tools to further elucidate the utility of FXR modulators for the treatment of metabolic disorders and cardiovascular disease.



I

MEDI 97

Synthesis and biological evaluation of novel, orally bioavailable antagonists of FXR

Jonathan B. Houze*, Joshua Gergely, Yi Xia, Sharon McKendry, Bei Shan, Hua Tu, Margrit Schwarz, Matthew Wright, Qiuping Ye, and Hoa Le, Amgen Inc, 1120 Veterans Blvd., South San Francisco, CA 94080, Fax: 650-244-2015, jhouze@amgen.com*

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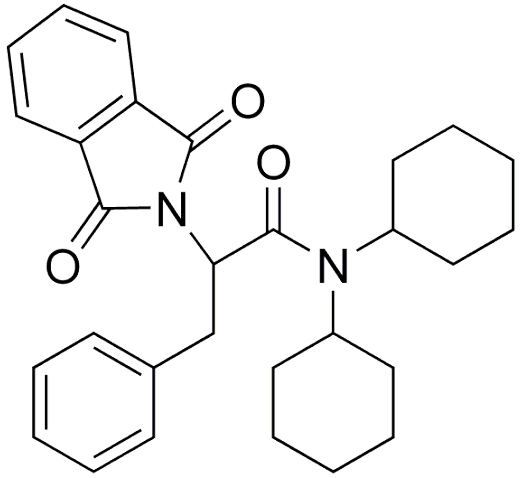


I

MEDI 98

Design and synthesis of a series of novel 3-benzyl-quinolines as LXR modulators

Ronald C. Bernotas[1], *Robert Singhaus*[1], *Ponnal Nambi*[2], *Elaine Quinet*[2], *John W. Ullrich*[1], *Horace Fletcher III*[1], *Liang Chen*[2], *Anita Halpern*[2], *Qiang-Yuan Liu*[2], *Dawn Savio*[2], *Ray J Unwalla*[1], *Anna Wilhelmsson*[3], *Annika Goos-Nilsson*[3], *Crina Ursu*[3], *Erik Arnelof*[3], *Johnny Sandberg*[3], *Christopher Enroth*[3], *Tomas Hansson*[3], *and Jay Wrobel*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Fax: 484-865-9399, bernotr@wyeth.com, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth Research, (3) Karo Bio AB*

Liver X receptors (LXRs) are nuclear receptors that helps control cholesterol-lipid metabolism by regulating the gene expression of proteins involved in cholesterol efflux from cells. The endogenous ligands are oxidized sterols including oxycholesterols. LXR agonists may have the potential to lower cholesterol levels without increasing triglycerides. As part of a program focused on LXR agonists, we have identified a series of 4-aryl-3-benzylquinolines with excellent LXR affinity and functional activity. The synthesis, biological activity, and SAR of these and related quinolines will be described.

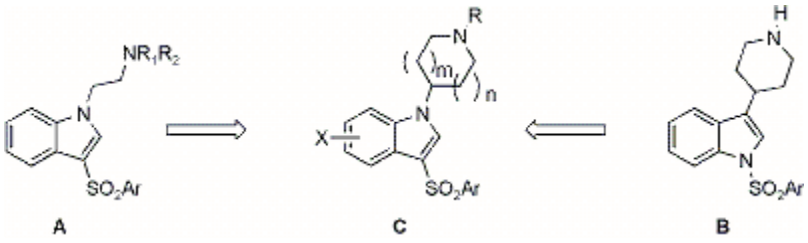
MEDI 99

3-Arylsulfonyl-1-(azacyclyl)-1H-indoles as 5-HT$_6$ ligands

Ronald C. Bernotas[1], *Rajesh A. Shenoy*[2], *Van-Duc Le*[2], *Ping Chen*[2], *Albert J. Robichaud*[3], *Guo Ming Zhang*[4], *Deborah L. Smith*[4], *and Lee E. Schechter*[5]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Fax: 484-865-9399, bernotr@wyeth.com, (2) Albany Molecular Research Inc, (3) Department of Medicinal Chemistry, Chemical and Screening Sciences, Wyeth*

Research, (4) Department of Neuroscience, Wyeth Research, Princeton, (5) Neuroscience, Wyeth Research

1-Aminoethyl-3-arylsulfonyl-1H-indoles **A** are 5-HT_6 receptor ligands with modest activity in a 5-HT_6 cyclase assay. Constrained 3-(4-piperidinyl)-1-arylsulfonyl-1H-indoles **B** have good 5-HT_6 affinity. We sought to constrain the basic side chain as part of a ring to make 1-(azacyclyl)-3-arylsulfonyl-1H-indoles **C**, incorporating a piperidinyl or pyrrolidinyl ring system. We will describe the synthesis of these compounds as well as their 5-HT_6 binding and in vitro functional activity. Depending on the arylsulfonyl, ring type, and other substitution, high affinity 5-HT_6 ligands were identified.



MEDI 100

Discovery and SAR of phenyl-acetic acid based substituted quinolines as liver X receptor (LXR) agonists

Michael Collini[1], *Baihua Hu*[1], *James Jetter*[1], *Christopher P Miller*[1], *Ray J Unwalla*[1], *James C. Keith Jr.*[2], *Valerie Clerin*[2], *Elaine Quinet*[2], *Dawn Savio*[2], *Anita Halpern*[2], *Michael Basso*[2], *Liang Chen*[2], *Qiang-Yuan Liu*[2], *Irene B. Feingold*[3], *Mathias Farnegardh*[4], *Annika Goos-Nilsson*[4], *Anna Wilhelmsson*[4], *Tomas Hansson*[4], *Ponnal Nambi*[2], *and Jay Wrobel*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Rd, Collegeville, PA 19426, Fax: 484-865-9399, CollinM2@wyeth.com, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth Research, (3) Department of Drug Safety and Metabolism, Wyeth Research, (4) Karo Bio AB*

The liver X receptors (LXRα and LXRβ) are nuclear receptors that transcriptionally regulate lipid metabolism in various tissues such as liver and macrophages. Lipid regulation in macrophages is central to the formation of atherosclerotic plaques that lead to cardiovascular disease. It has been shown that LXRs can stimulate reverse cholesterol transport from macrophages by upregulating ABCA1. The ABCA1 protein is critical in the efflux pathway of cholesterol and this has made LXRs an interesting target for discovery. The design, synthesis, biological activity, and SAR of (phenoxymethyl-phenyl)-acetic acid substituted quinolines will be reported.

MEDI 101

Futher modification on phenyl acetic acid based quinolines as liver x receptor (LXR) agonists

James Jetter[1], *Baihua Hu*[1], *Michael Collini*[1], *Christopher P Miller*[1], *Ray J Unwalla*[1], *James C. Keith Jr.*[2], *Valerie Clerin*[2], *Elaine Quinet*[2], *Dawn Savio*[2], *Anita Halpern*[2], *Michael Basso*[2], *Liang Chen*[2], *Qiang-Yuan Liu*[2], *Irene B. Feingold*[3], *Mathias Farnegardh*[4], *Annika Goos-Nilsson*[4], *Anna Wilhelmsson*[4], *Tomas Hansson*[4], *Ponnal Nambi*[2], *and Jay Wrobel*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Rd., Collegeville, PA 19444, Fax: 484-865-9399, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth Research, (3) Department of Drug Safety and Metabolism, Wyeth Research, (4) Karo Bio AB*

Liver X receptors (LXRα and LXRβ), originally identified from liver as orphan receptors, are members of the nuclear hormone receptor super family and are involved in the regulation of cholesterol and lipid metabolism. They are ligand-activated transcription factors and bind to DNA as obligate heterodimers with retinoid X receptors. Activation of LXRs in macrophages results in the expression of several genes involved in lipid metabolism and reverse cholesterol transport including ABCA1, ABCG1 and ApoE. Thus LXR modulators (agonists) can potentially mediate a two-pronged effect (removal of cholesterol from the macrophages and inhibition of vascular inflammation) resulting in the inhibition of atherosclerotic lesion and therefore represents a novel target for the treatment of cardiovascular disease. The synthesis, biological activity, and SAR of phenyl acetic acid based quinolines will be described.

MEDI 102

Design and synthesis of 4-aryl-3-methylquinolines as LXR modulators

Robert Singhaus[1], *Ronald C. Bernotas*[1], *John W. Ullrich*[1], *Horace Fletcher III*[1], *Mike Basso*[2], *Ponnal Nambi*[2], *Liang Chen*[2], *Anita Halpern*[2], *Qiang-Yuan Liu*[2], *Elaine Quinet*[2], *Dawn Savio*[2], *Jim Keith*[2], *Valerie Clerin*[2], *Ray J Unwalla*[1], *Irene Feingold*[3], *Anna Wilhelmsson*[4], *Annika Goos-Nilsson*[4], *Crina Ursu*[4], *Erik Arnelof*[4], *Johnny Sandberg*[4], *Tomas Hansson*[4], *Yingru Zhang*[1], *Oliver McConnell*[1], *and Jay Wrobel*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Singhar@wyeth.com, (2) Department of Cardiovascular and Metabolic Diseases, Wyeth Research, (3) Department of Drug Safety and Metabolism, Wyeth Research, (4) Karo Bio AB*

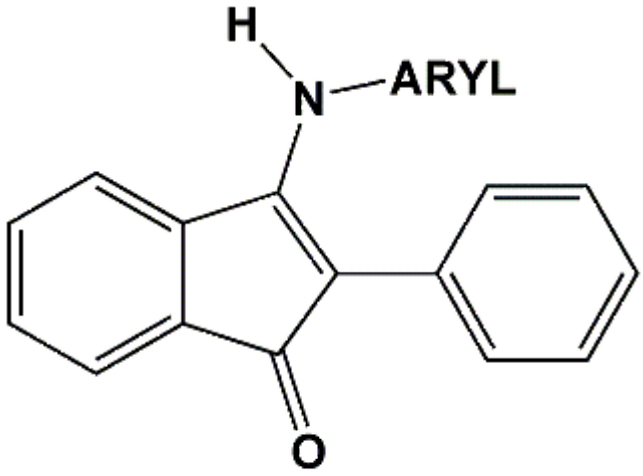
The modulation of gene expression by nuclear receptors has considerable potential in cardiovascular disease therapy. For example, cholesterol-lipid metabolism is partly regulated by the production of several proteins involved in cholesterol efflux from cells. Control of gene expression and hence protein production is exerted by liver X receptors (LXRs) which have various oxidized sterols as their endogenous ligands. In order to modulate gene expression, we have targeted novel LXR agonists leading to the identification of a series of high affinity LXR agonists incorporating a 4-aryl-3-methylquinoline core. We will present the syntheses, biological activity and SAR of these ligands.

MEDI 103

Discovery of 2-Phenyl-3-arylamino-inden-1-ones as Novel LXR Agonists

*Jinqian Liu[1], **Rajiv Sharma**[1], Stephen Shuttleworth[1], Peter Coward[2], Patrick Escaron[2], Jean Danao[2], Anne Chai[2], Andrew Shiau[2], Karen Siegler[2], Derek Piper[3], Martin Harrison[3], Paul Faulder[3], Nigel Walker[3], Zhulun Wang[3], Haoda Xu[3], and Lynn Miao[2]. (1) Medicinal Chemistry, Amgen Inc, 1120 Veterans Blvd, S. San Francisco, CA 94080, Fax: 650-244-2015, rajivs@amgen.com, (2) Biology, Amgen Inc, (3) Structural Biology, Amgen Inc*

Non-steroidal LXR agonists have been shown to increase expression of ABCA1 and raise the HDL levels in mice. These findings have provided sufficient biological rational to pursue LXR as potential target for cardiovascular diseases. In this presentation, we discuss the discovery of a series of 2-phenyl-3-arylamino-inden-1-one derivatives (1) as potent LXR agonists. Details of the synthesis and optimization of analogs of 1 will be discussed. X-ray crystallography studies were used to understand the binding orientation of analogs of 1, relative to that of known LXR agonists.



(1)

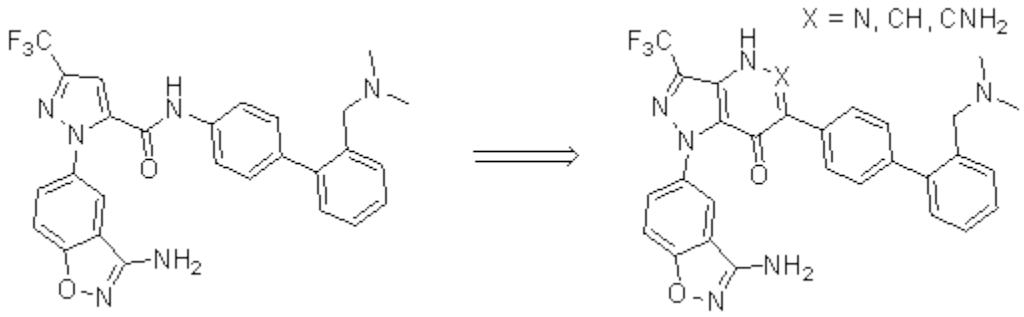
MEDI 104

Preparation of bicyclic pyrazoloheterocycles as highly potent, selective inhibitors of coagulation Factor Xa

***Yun-Long Li**, John M. Fevig, Qi Han, Joseph M. Luettgen, Robert M. Knabb, Ruth R. Wexler, and Patrick Y. S. Lam, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 5400, Princeton, NJ 08543-5400, yunli@incyte.com*

Factor Xa, a trypsin-like serine protease, holds the central position that links the intrinsic and extrinsic mechanism in the blood coagulation cascade. Direct inhibition of factor Xa has emerged as an attractive strategy for the discovery of novel antithrombotic agents. Recently, we reported the discovery of a series of potent pyrazole factor Xa inhibitors. Here, we describe the syntheses and biological activities of factor Xa inhibitors with bicyclic pyrazoloheterocyclic cores, designed to mask the potential hydrolysable amide functionality of pyrazole factor Xa inhibitors.

Several compounds with the new bicyclic cores are selective and sub-nanomolar factor Xa inhibitors.



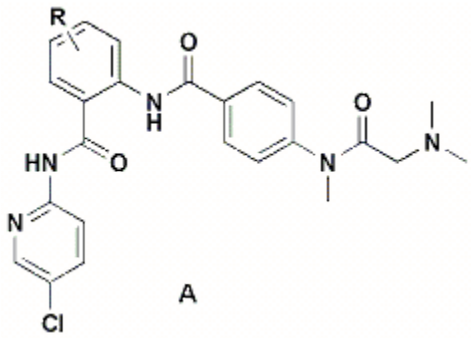
[1]Current address: Incyte Corporation, Experimental Station, Route 141 and Henry Clay Road, Wilmington, DE 19880.

MEDI 105

Novel anthranilamides as potent coagulation factor Xa inhibitors

Wei Han*, Zilun Hu, XiangJun Jiang, Donald J. Pinto, Alan R. Rendina, Joseph M. Luettgen, Kan He, Pancras C. Wong, Robert M. Knabb, Ruth R. Wexler, and Patrick Y. Lam, Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ 08543, wei.han1@bms.com*

Factor Xa, a serine protease, plays an important role in blood coagulation due to its central role in the coagulation pathways. Inhibition of factor Xa interrupts thrombin formation without impairing platelet haemostatic function. Thus factor Xa inhibitors are useful in the treatment of thrombosis without the increased risk of bleeding associated with currently available anticoagulants. In this poster, the design, synthesis, in vitro and in vivo activities of anthranilamide-based factor Xa inhibitors (A) will be disclosed.



MEDI 106

Discovery, synthesis, and SAR of highly potent and selective inhibitors of coagulation factor Xa containing 1,1-disubstituted phenylcyclopropyl P4 moieties

Jennifer X. Qiao[1], *Sarah R. King*[1], *Donald J. Pinto*[2], *Michael J. Orwat*[1], *Richard S. Alexander*[1], *Angela M. Smallwood*[1], *Kan He*[2], *Alan R. Rendina*[2], *Joseph M. Luettgen*[1], *Robert M. Knabb*[1], *Ruth R. Wexler*[1], *and Patrick Y. Lam*[1]. *(1) Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 5400, Princeton, NJ 08543-5400, Fax: 609-818-6810, jennifer.qiao@bms.com, (2) Bristol-Myers Squibb*

Thromboembolic diseases remain the leading cause of death in developed countries. Conventional antithrombotic therapies using either heparin or warfarin have several limitations. One approach to discover safer and efficacious oral anticoagulants is to inhibit thrombin generation by targeting the inhibition of coagulation factor Xa (fXa). Recently, we disclosed a series of pyrazole-fused bicyclics containing *ortho*-substituted *bi*-aryl P4 moieties as highly potent and selective fXa inhibitors. Aiming for structural diversity, we later on discovered that 1,1-disubstituted phenylcycloalkyl analogs can serve as surrogates of *ortho*-substituted *bi*-aryls. In this presentation, we will describe the synthesis and SAR of bicyclic pyrazole fXa inhibitors containing a variety of 1,1-disubstituted phenylcycloalkyl moieties which occupy the aryl binding S4 pocket of fXa.

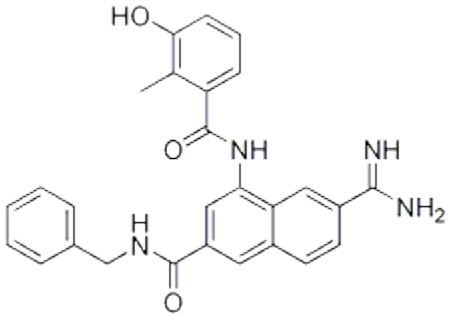
MEDI 107

Structure-based design, synthesis, and optimization of small molecule inhibitors for factor XIa

Zihong Guo, *Thomas Bannister, Rebecca Noll, Scott Magee, Pamela Nagafuji, Cassandra Celatka, Pramod Pandey, Lei Jin, Michael Rynkiewicz, Frank Bibbins, Joan Gorga, James Strickler, Harold V. Meyers, Robert Babine, and Sherin S. Abdel-Meguid, Department of Medicinal Chemistry, Daiichi Asubio Medical Research Laboratories LLC, 1 Kendall Square, Building 700, Cambridge, MA 02139, zihong.guo@gmail.com*

Thrombotic diseases are the leading cause of mortality and morbidity in the United States and other Western countries. Current therapies for thrombotic diseases require parenteral administration or careful monitoring to achieve desired efficacy and minimize excessive bleeding. Therefore, there is a medical need for well-tolerated anticoagulants with a wider therapeutic window to combat these diseases. Coagulation factor 11a plays an important role in the coagulation cascade. Recent reports suggested that factor 11a reduces thrombus growth significantly in a baboon thrombosis model without bleeding problems, and individuals with factor 11a genetic deficiency do not show severe bleeding problems. Thus, inhibition of factor 11a could be effective in blocking or limiting clot formation in deep vein and arterial thrombosis

without affecting the hemostatic response to more severe injury. Previously we achieved the development of a peptide mimetic candidate for preclinical studies. In-vivo pharmacological studies revealed this inhibitor effectively reduced clot size without altering bleeding times. Herein we report the design and synthesis of a series of potent, selective naphthamidine inhibitors of factor 11a for potential use as antithrombotic therapeutics.



IC_{50} = 76 nM

MEDI 108

Structure-based design and isothermal titration calorimetry studies of tetrapeptides [D-Phe-Pro-D-Arg-P1'-CONH2] inhibitors of thrombin

Cristina Clement[1]*, Richard S Magliozzo*[2]*, and Manfred Philipp*[1]*. (1) Chemistry Department, Lehman College, City University of New York, 250 Bedford Park BLVD West, Bronx, New York City, NY 10468, cclement_us@yahoo.com, (2) Department of Chemistry, Brooklyn College and the Graduate Center of the City University of New York*

A structure-activity relationship (SAR) for reversible inhibitory activity toward thrombin of tetrapetides from series D-Phe-Pro-D-Arg-P1'-CONH2 is reported. The significant differences between the inhibitory constants (Kis) of different tetrapeptides (varying from 2 to 500 fold) suggest that the interaction between the amino acid at the P1' position and the S1' pocket in thrombin is very specific and requires selective hydrophobic (Ala, Ile, Val, Met) and polar amino-acids (such as Ser, Thr, Gln, Cys). These differences in the binding affinities were confirmed both kinetically and through isothermal titration calorimetry (ITC). Isothermal titration calorimetry (ITC) experiments for lead compounds were performed using an ITC instrument from Microcal with the built-in Microcal-Origin software. Titration of peptide inhibitors into thrombin was conducted for all lead compounds and was characterized by specific heat of binding with exothermic and endothermic components. The ITC results confirmed the kinetic data, which showed that peptides are specific inhibitors for thrombin. In all cases the dissociation constant (Kd) determined from ITC experiments had the same order of magnitude as the Kd determined from kinetics studies (low micromolar-hundreds of nanomolar range). The ITC binding data were best fitted to a "sequential binding sites" model suggesting that the peptides might bind to thrombin with a positive cooperativity. The ITC results are consistent with the kinetics of thrombin inhibition which showed that the peptides are not binding only to the active site (competitive inhibition) but there is at least one more independent or "linked" binding site (the kinetics showed mixed inhibition for most of the peptides investigated through ITC experiments). For

most peptides the enthalpy of binding was the driving force for the observed free energy of interaction between inhibitors and thrombin.

MEDI 109

Exploring the potential of comparative molecular field analysis (CoMFA) in predicting anti-estrogen activity

Patrick Joseph *and John S. Cooperwood, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32301, patrick1.joseph@famu.edu*

Tamoxifen is a non-steroidal anti-estrogen drug that is widely used in the treatment of breast cancer. It contributed significantly to the reduction in breast cancer mortality rate, but its partly estrogenic activity and adverse side effects have limited its use. Consequently, there is an intense interest in finding ways to develop anti-estrogen agents that are better than tamoxifen. We synthesized seven different 17-oximino-3-(O-alkylated)-estrone derivatives. **PURPOSE:** To determine whether Comparative Molecular Field Analysis (CoMFA) was able to sufficiently predict the order of potency of the estrone derivatives relative to tamoxifen. **METHODS:** Sybyl 7.0/CoMFA was used to predict the order of potency of the estrone derivatives relative to tamoxifen. CoMFA achieved this by relating the concentration at which 50% inhibition (IC_{50}) of MCF-7 breast cancer cells to the structural features of certain agents. This model was then used to predict the order of potency of the estrone derivatives relative to tamoxifen. The experimental IC_{50}s of the estrone derivatives was determined by their ability to inhibit MCF-7 breast cancer cells. **RESULTS:** The theoretical data generated from CoMFA was compared to the experimental data. **CONCLUSION:** If CoMFA is able to accurately predict the order of potency of the estrone derivates, then it could be used as a guide in the further development of anti-estrogen agents that are better than tamoxifen.

MEDI 109

Exploring the potential of comparative molecular field analysis (CoMFA) in predicting anti-estrogen activity

Patrick Joseph *and John S. Cooperwood, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32301, patrick1.joseph@famu.edu*

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derivatives relative to tamoxifen. The experimental IC_{50}s of the estrone derivatives was determined by their ability to inhibit MCF-7 breast cancer cells. **RESULTS:** The theoretical data generated from CoMFA was compared to the experimental data. **CONCLUSION:** If CoMFA is able to accurately predict the order of potency of the estrone derivates, then it could be used as a guide in the further development of anti-estrogen agents that are better than tamoxifen.

MEDI 110

17-Arylcarbamidomethyl-4-azasteroids as selective androgen receptor modulators

William P. Dankulich*, Helen J. Mitchell, George D. Hartman, and Robert S. Meissner, Department of Medicinal Chemistry, Merck Research Labs, 770 Sumneytown Pike, West Point, PA 19486, Fax: NA*

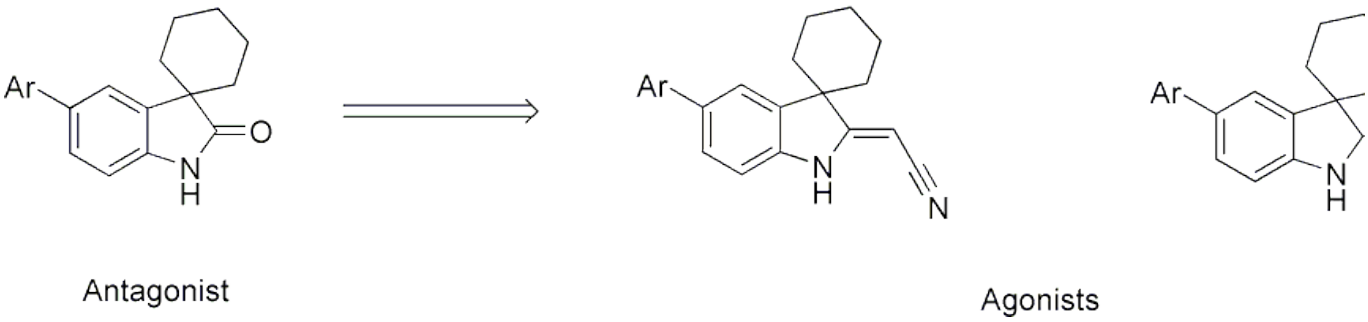
The androgen receptor is a member of the nuclear receptor superfamily and is responsible for mediating the physiological action of endogenous androgen ligands such as the agonist dihydrotestosterone (DHT). A selective androgen receptor modulator, or SARM, that would be useful for the treatment of osteoporosis should demonstrate robust osteoanabolic action while having limited effects on skin and reproductive tissues. Our SARM program was initiated around the premise that such selectivity would be possible with compounds that elicit partial agonism in context-dependent transcription assays. To that end, a novel series of 17-arylcarbamidomethyl-4-azasteroids were developed from a 17-hydroxy-4-azasteroid lead. By evaluating SAR in our transactivation assays, we were able to produce compounds that demonstrated osteoanabolism with low virilization potential in our OVX rat model.

MEDI 111

New cyano-eneamine derived progesterone receptor agonists

Casey C. McComas[1]*, Jeff Cohen*[2]*, Andrew Fensome*[1]*, Edward Melenski*[1]*, Ray J Unwalla*[1]*, Richard C. Winneker*[2]*, Jay Wrobel*[1]*, Zhiming Zhang*[2]*, and Yuan Zhu*[2]*. (1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Fax: 484-865-9398, mccomac@wyeth.com, (2) Women's Health and Musculoskeletal Biology, Wyeth Research*

During the course of our studies on progesterone receptor (PR) modulators, based upon the 3,3-cyclohexyl-5-aryl-oxindole template we were interested in making changes to the amide functionality. We discovered that changing this region of the molecule to a cyanoenamine moiety resulted in compounds displaying potent PR agonist activity in functional cell based assays. In this poster, the synthesis and structure activity relationships (SAR) of this class of 5-arylcyanoenamines will be discussed.



Antagonist

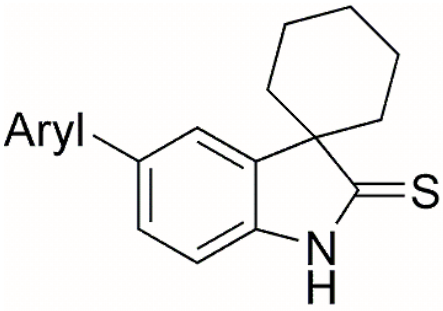
Agonists

MEDI 112

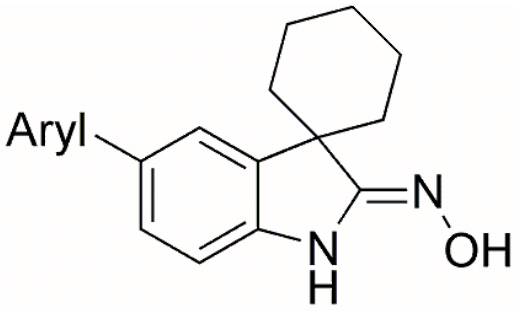
N-Hydroxyamidines as novel progesterone receptor agonists

Michael Marella[1], *Jeff Cohen*[2], *Andrew Fensome*[1], *Edward Melenski*[1], *Raymond Unwalla*[1], *Richard C. Winneker*[2], *Z Zhang*[2], *Yuan Zhu*[2], *and Jay Wrobel*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, marellm@wyeth.com, (2) Women's Health and Musculoskeletal Biology, Wyeth Research*

During the course of our studies with the progesterone receptor (PR) agonists 1 we were interested in modifying the thio-amide moiety. While initially prepared as an intermediate, the N-hydroxyamidines 2 were found serendipitously to have functional PR agonist activity in a cell based assay. This presentation will describe the synthesis and in vitro as well as in vivo activity of this novel series of compounds 2 as PR agonists.



1



2

MEDI 113

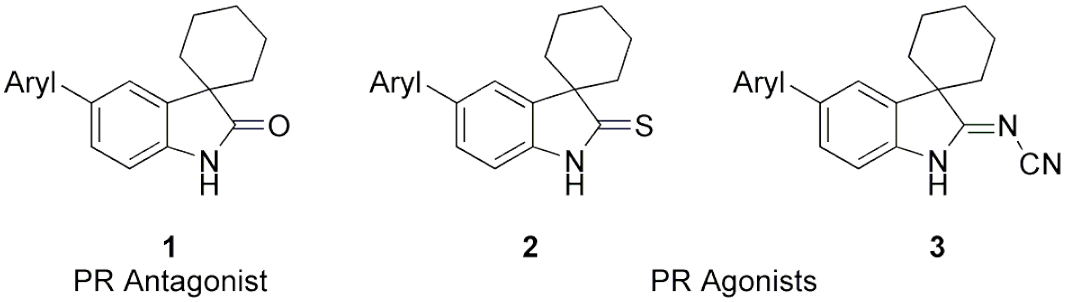
New cyano-amidine based progesterone receptor agonists

Andrew Fensome[1], *Reinhold Bender*[1], *Jeff Cohen*[2], *Horace Fletcher III*[1], *Susan Lockhead*[3], *Michael Marella*[1], *Edward Melenski*[1], *Andrea Olland*[1], *Ray J Unwalla*[1], *James, M. Wilhelm*[1], *Richard C. Winneker*[2], *Yuan Zhu*[2], *Zhiming Zhang*[2], *and Jay*

Wrobel[1]. (1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Fax: 484-865-9398, fensoma@wyeth.com, (2) Women's Health and Musculoskeletal Biology, Wyeth Research, (3) Drug Safety and Metabolism, Wyeth Research

Non-steroidal progesterone receptor (PR) modulators have received attention in the last few years, with many new structural classes being disclosed. Compounds in this class may have utility in female contraception and hormone therapy as well as in the treatment of malignant and non-malignant disease.

We have previously published in this area with PR modulators derived from a common oxindole platform. In particular we have found that this template is very sensitive to small functional group changes with respect to the amide group itself, oxo-amides 1 were functional antagonists of the PR, whereas thio-amides 2 were found to be agonists. We have sought to expand upon this theme and found that the cyanoamidine moiety also provides PR agonists in cell-based assays. In this poster we will discuss the synthesis, SAR, pharmacology, binding mode and pharmacokinetics of PR agonists utilizing the cyanoamidine functionality 3.



1 **2** **3**

PR Antagonist PR Agonists

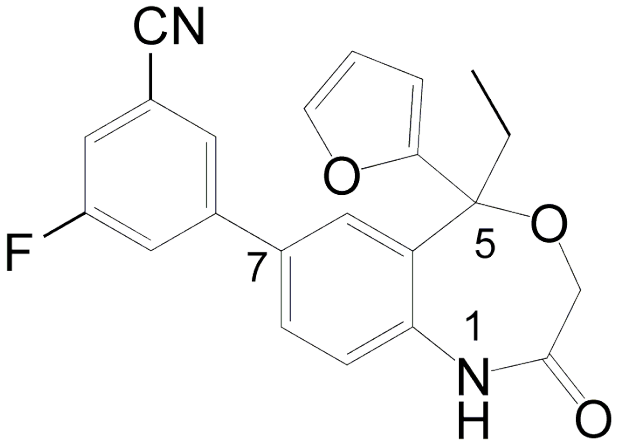
MEDI 114

7-Phenyl-1,5-dihydro-benzo[e][1,4]oxazepin-2-ones as nonsteroidal progesterone receptor antagonists

Jeffrey C. Kern[1]*, Eugene A. Terefenko[1], Andrew Fensome[1], Ray J Unwalla[2], Jay Wrobel[1], Zhiming Zhang[3], Yuan Zhu[3], Jeff Cohen[3], Richard C. Winneker[3], and Puwen Zhang[1]. (1) Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, Fax: 484-865-9398, KernJ@wyeth.com, (2) Department of Medicinal Chemistry, Wyeth Research, (3) Women's Health and Musculoskeletal Biology, Wyeth Research*

We have recently reported several series of aryl substituted benzene-fused 5- and 6-membered heterocycles as progesterone receptor (PR) modulators. Among these series, the 6-membered benzoxazin-2-one and benzoxazin-2-thione templates were the more interesting scaffolds and led to discovery of a number of potent PR agonists and antagonists that were advanced to development. In a continuation of this work we decided to examine the SAR of benzoxazin-2-one ring-expansion analogs, 7-aryl benzo[1,4]oxazepin-2-ones as PR modulators. A number of 7-aryl benzo[1,4]oxazepinones with various substitutions at the 5-position as well as different 7-aryl groups were prepared and evaluated in the T47D cell alkaline phosphatase assay and in the ovariectomized mature female rat decidualization

model. In this poster, the synthesis, in vitro SAR of 7-phenyl benzo[1,4]oxazepinones, and in vivo activity of several potent analogs, such as 1, will be discussed.



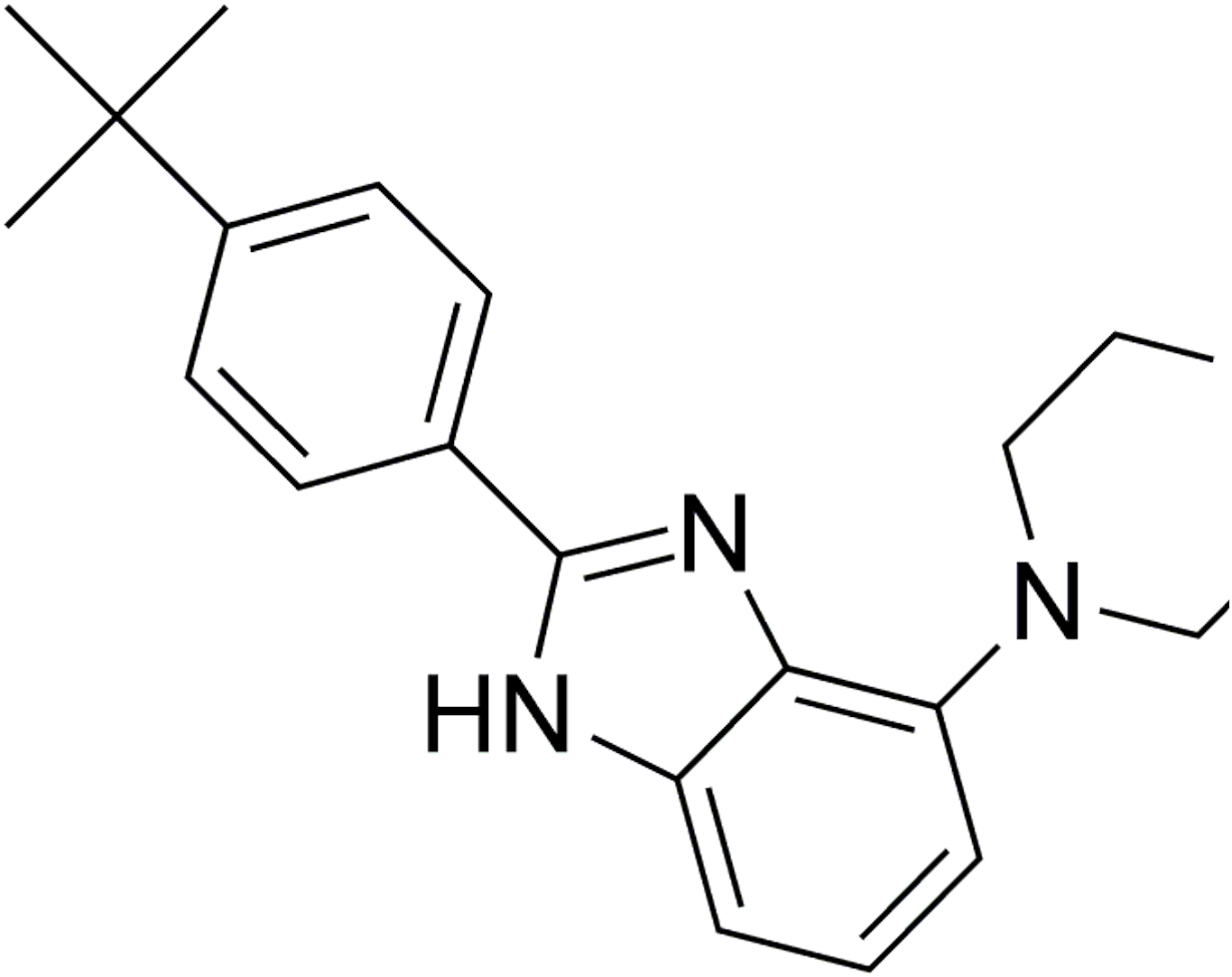
1, Alk. Phos. IC_{50} 12.0 nM

MEDI 115

Synthesis and SAR of imidazopyridines and related analogs as Gonadotropin-Releasing Hormone (GnRH) antagonists

John F. Mehlmann[1], *Charles W. Mann*[2], *Joseph T. Lundquist IV*[1], *Diane B. Hauze*[2], *Jeffrey C. Pelletier*[2], *Joshua E. Cottom*[3], *Linda Shanno*[3], *Murty V. Chengalvala*[3], *Irene B. Feingold*[4], *and Jay Wrobel*[5]. *(1) Department of Chemical and Screening Sciences, Wyeth Research, 500 Arcola Rd, Collegeville, PA 19426, (2) Department of Chemical & Screening Sciences, Wyeth Research, (3) Department of Women's Health and Musculoskeletal Biology, Wyeth Research, (4) Department of Drug Safety and Metabolism, Wyeth Research, (5) Chemical and Screening Sciences, Wyeth Research*

Gonadotropin-Releasing Hormone (GnRH) stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland, which in turn stimulates the release of sex hormone steroids from the gonads of both genders. The regulation of GnRH is seen as a means of treating sex hormone related disorders such as endometriosis, breast cancer, prostate cancer, and precocious puberty in children. Currently, only peptide-based GnRH antagonists and superagonists administered through intramuscular and subcutaneous routes have been approved. Our goal is to develop small molecule GnRH antagonists for oral administration. To this end, imidazopyridine, imidazopyrimidine, and pyridine derivatives of 2-(1-*t*-butylphen-4-yl)-4-(piperazin-1-yl)benzimidazole have been prepared and tested for their activity in both human and rat binding and functional assays. The structure-activity-relationships (SAR) of these compounds will be presented.



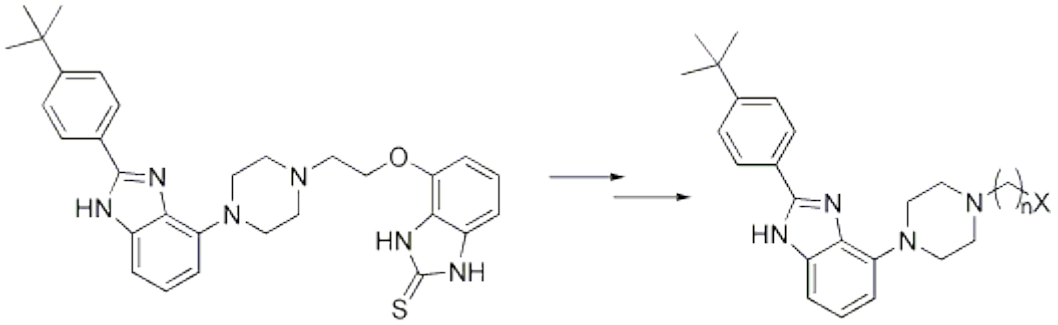
MEDI 116

Structure activity relationships of the linker group in 2-phenyl-4-piperazinyl-benzimidazole antagonists of the Gonadotropin-Releasing Hormone Receptor (GnRH)

Diane B. Hauze[1], *Jeffrey C. Pelletier*[1], *Charles W. Mann*[1], *Daniel Green*[1], *John Rogers*[1], *Murty V. Chengalvala*[2], *Joshua E. Cottom*[2], *Linda Shanno*[2], *and Irene B. Feingold*[3]. *(1) Department of Chemical & Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, hauzed@wyeth.com, (2) Department of Women's Health and Musculoskeletal Biology, Wyeth Research, (3) Department of Drug Safety and Metabolism, Wyeth Research*

Our program aimed at developing an orally efficacious small molecule antagonist of the GnRH receptor began with a partially optimized lead molecule containing a 2-(4-t-butylphen-1-yl)-4-(4-piperazin-1-yl)imidazole. Structure-activity-relationship (SAR)

studies centering around the replacement of the ethoxy linker unit and optimization of the distance between the key pharmacophores to improve human and rat GnRH antagonist activity will be presented.

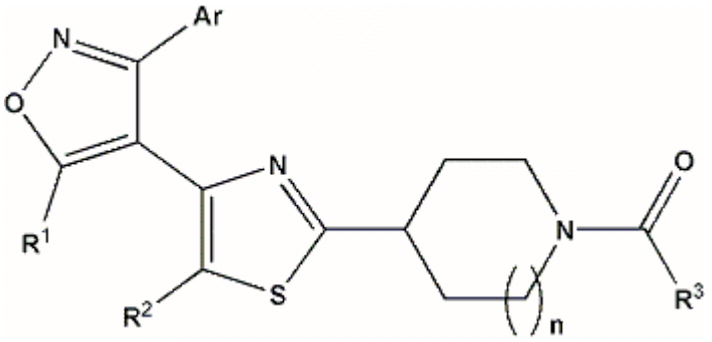


MEDI 117

Small molecule ligands of the Follicle Stimulating Hormone receptor

Jonathan A. Covel*, Eric Babych, Rena Hayashi, Brian Hofilena, Jason Ibarra, Michelle Pulley, Vincent J. Santora, Paul Sheehan, Robert R. Webb, Carolyn Wellman, Karin Lehmann-Bruinsma, Steve Chang, Lara Collins, Hongmei He, Thuy Le, James Leonard, Chen Liaw, Kong Namvong, Lawrance Tam, and Tim Walton, R&D, Arena Pharmaceuticals Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-453-7210, jcovel@arenapharm.com*

Infertility is a worldwide problem afflicting millions. It can arise from low levels of follicle stimulating hormone (FSH), a pituitary gonadotropin which acts through a Gs-coupled GPCR. FSH receptors are found in the membrane of ovarian granulosa cells and are essential for folliculogenesis. FSH itself can be obtained from urine or produced from recombinant DNA technology. However, treatment with FSH is costly and inconvenient. To overcome these issues, we initiated a program to identify small molecule FSH modulators for the treatment of infertility. SAR development began around a lead structure containing a thiazole-piperidine core which was discovered from a proprietary, Constitutively Activated Receptor Technology (CART™) assay. The synthesis and biological activity of several analogs from this effort will be described.



MEDI 118

Design, synthesis, and biological evaluation of small molecule peptide mimetics targeting the melanocortin receptors

James P. Cain, *Alexander V. Mayorov, Minying Cai, Dev Trivedi, Kevin B. Chandler, Bahar Tan, Yeon Sun Lee, and Victor J. Hruby, Department of Chemistry, University of Arizona, 1306 E. University Blvd, Tucson, AZ 85721, jcain@email.arizona.edu*

The functions of melanocortin receptors (MCRs) are critical to myriad biological activities, including pigmentation, steroidogenesis, energy homeostasis, erectile activity, and inflammation. These G Protein-Coupled Receptors (GPCRs) are targets for drug discovery in a number of areas, including cancer, pain, and obesity therapeutics. The melanocortin receptors are unique in that endogenous agonists (α-, β-, and γ-MSH, ACTH) and antagonists (agouti and agouti-related protein) are known. Nonetheless, relatively few potent and selective small molecule ligands for the MCRs are available. This problem is addressed with a ligand-based rational design strategy. Following this approach, a focused library of piperazine-based peptide mimetics has been synthesized. Analogs were chosen to examine the effect of variations in the stereochemistry, the distance between key pharmacophore elements, substituents on a crucial phenyl group, and lipophilic appendages to the core structure. Data from binding and functional assays will be presented.

MEDI 119

Synthesis, pharmacology and molecular modeling for the development of selective low molecular weight modulators for glycoprotein hormone receptors

Susanna Moore[1], *Holger Jaeschke*[1], *Susanne Neumann*[1], *Gunnar Kleinau*[1], *Stefano Costanzi*[2], *Jian-Kang Jiang*[3], *John Childress*[1], *Bruce M. Raaka*[1], *Anny Colson*[2], *Ralf Pashke*[4], *Gerd Krause*[5], *Craig J. Thomas*[6], *and Marvin C. Gershengorn*[1]. *(1) NIDDK Clinical Endocrinology Branch, National Institutes of Health, Bldg 50 Room 4133, 9000 Rockville Pike, Bethesda, MD 20892, Fax: 301-480-4214, mooresu@niddk.nih.gov, (2) Computational Chemistry Core Laboratory, NIDDK, National Institutes of Health, (3) Chemical Biology Core Facility, NIDDK, National Institutes of Health, National Institutes of Health, (4) Universität Leipzig, Medizinische Klinik und Poliklinik III, (5) Structural Bioinformatics and Molecular Design, Institute for Molecular Pharmacology, (6) NIDDK Chemical Biology Core, National Institutes of Health*

Thyroid stimulating hormone receptor (TSHR) and luteinizing hormone receptor (LHR) belong to the glycoprotein hormone receptor family of seven transmembrane-spanning receptors and exhibit high homology within their transmembrane cores. TSHR regulates growth and function of thyroid follicular cells. Low molecular weight modulators may have therapeutic potential for both thyroid cancer and hyperthyroidism. Recently, a thienopyrimidine was identified as a partial agonist for LHR and TSHR, and we provided evidence for its binding pocket within the

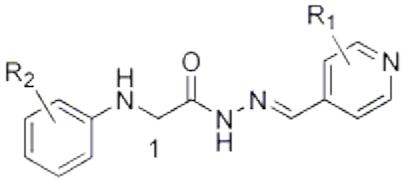
transmembrane core of these receptors. Here we present structure activity relationships for several new substituted pyrimidines that provide insight into the chemical properties required for agonist and antagonist activity as well as selectivity at TSHR and LHR. Docking studies using homology models of TSHR and LHR suggest possible interactions between amino acids within the receptor binding domains and functional groups of these ligands. These docking results provide clues to understanding selectivity and may aid in the design of improved ligands.

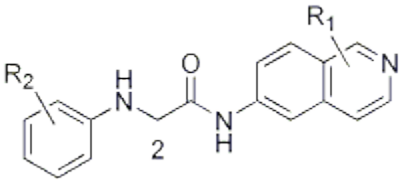
MEDI 120

Small molecule inhibitors of G-Protein Coupled Receptor Kinase-2 (GRK-2)

*Marcos L. Sznaidman[1], Allen Eckhardt[1], Jeff Yingling[1], Robert Oakley[1], Christine Hudson[1], Shuntai Wang[1], Michael Peel[2], **Thomas E. Richardson**[2], Clare L. Murray[2], B. N. Narasinga Rao[2], Brian H. Heasley[2], and Paresma R. Patel[2]. (1) Xsira Pharmaceuticals, P.O.Box 14769, Research Triangle Park, NC 27709-4769, msznaidman@Xsira.com, (2) SCYNEXIS, Inc, P.O. Box 12878, Research Triangle Park, NC 27709-2878, tom.richardson@scynexis.com*

GRK-2 is a member of the small family of serine/threonine protein kinases generally known as G-protein coupled receptor kinases (GRKs). These kinases bind and phosphorylate agonist-activated GPCRs to initiate mechanisms leading to impaired receptor signaling, or desensitization. Desensitization is believed to underlie the development of tolerance that limits the effectiveness of many drugs such as morphine in pain treatment and bronchodilators in asthma treatment. Most of the known GRK-2 inhibitors are small peptides, polyanions and small molecules with modest activity. Here we discuss the design and synthesis of a class of hydrazides (1) and isoquinolines (2) with low nanomolar activity against GRK-2. In this study we present the SAR as well as modeling studies that show the key interactions between these molecules and the GRK-2 enzyme required for activity. These molecules serve the dual purpose of validation tools for investigating the role of GRK2 activity in the development of drug tolerance and as starting points for further drug development.



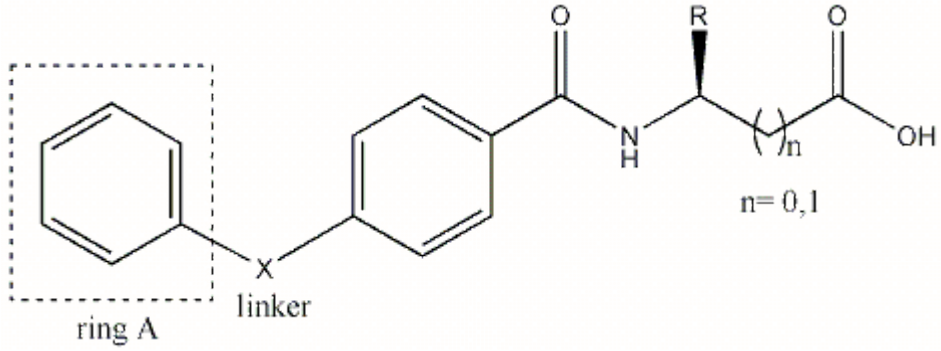


MEDI 121

4-Benzoylamino-propionic acid derivatives as novel antagonists of VLA-1

Kyla L. Bjornson[1], *Michael Pete VanBrunt[2], Ramesh A. Kasar[1], Kerry W. Fowler[1], Gabrielle R. Kolakowski[1], Irina C. Jacobson[2], Edith A. S. Harris[3], Li-ming Sui[3], Mark L. Lupher Jr.[3], Donald S. Staunton[3], Francine Farouz[2], and Eugene D. Thorsett[2]. (1) ICOS Corporation, 22021 20th Ave SE, Bothell, WA 98021, (2) Medicinal Chemistry, ICOS Corp, (3) Biology, ICOS Corp*

In an on-going effort to generate novel VLA1 antagonists with improved PK properties we identified a 4-benzoylamino-propionic acid scaffold as a novel starting point. A systematic evaluation of substitution around the A-ring system and linker (X) identified key structural elements of the pharmacophore. Subsequent modifications were successful in improving potency, ultimately resulting in sub-micromolar VLA-1 inhibitors. A comprehensive structure-activity relationship study of this work will be presented.

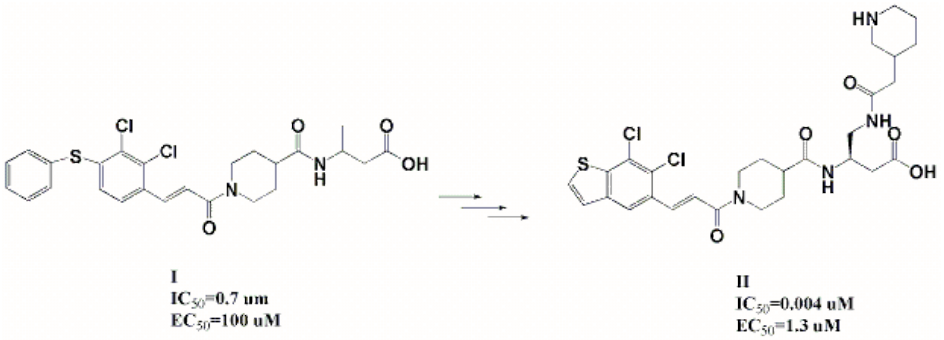


MEDI 122

Identification of the potent small molecule inhibitors of VLA-1 integrin

Gabrielle R. Kolakowski[1]*, Kyla L. Bjornson*[1]*, Angela Judkins*[1]*, Ramesh Kasar*[1]*, Michael Pete VanBrunt*[1]*, Irina C. Jacobson*[1]*, Edith A. S. Harris*[2]*, Li-ming Sui*[2]*, Mark L. Lupher Jr.*[2]*, Donald S. Staunton*[2]*, Kelly Hensley*[2]*, Francine Farouz*[1]*, and Eugene D. Thorsett*[1]*. (1) Medicinal Chemistry, ICOS Corp, 22021 20th Ave SE, Bothell, WA 98021, ijacobson@icos.com, (2) Biology, ICOS Corp*

VLA-1 (very late antigen-1) is an integrin heterodimer composed of an alpha chain (CD49a, á1) and a beta chain (CD29, â1). Alpha1 contains an I domain which is structurally similar to the I domains of LFA-1 and Mac-1. VLA-1 has been shown to bind to the extracellular matrix proteins collagen (preferentially to type IV collagen) and laminin. In the course of our work we have discovered potent, selective and efficacious small molecule inhibitors of VLA-1. In this paper we will detail the structure activity relationship work from the initial lead I, identified in a high throughput screening effort, to the discovery of a potent series of antagonists of VLA-1. Compound II selected from that series was found to be efficacious in the DTH animal model. Synthesis, binding data, structure activity relationships, PK and DTH results will be presented.

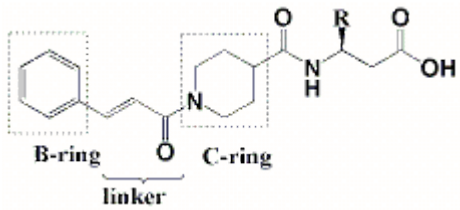


MEDI 123

Synthesis and structure-activity relationship of the potent antagonists of VLA-1 integrin

Michael Pete VanBrunt[1], *Ramesh Kasar*[1], *Gabrielle R. Kolakowski*[1], *Irina C. Jacobson*[1], *Edith A. S. Harris*[2], *Li-ming Sui*[2], *Mark L. Lupher Jr.*[2], *Donald S. Staunton*[2], *Francine Farouz*[1], *and Eugene D. Thorsett*[1]. *(1) Medicinal Chemistry, ICOS Corp, 22021 20th Ave SE, Bothell, WA 98021, ijacobson@icos.com, (2) Biology, ICOS Corp*

VLA-1 (very late antigen-1) is an integrin heterodimer composed of an alpha chain (CD49a, á1) and a beta chain (CD29, â1). Alpha1 contains an I domain which is structurally similar to the I domains of LFA-1 and Mac-1. VLA-1 has been shown to bind to the extracellular matrix proteins collagen (preferentially to type IV collagen) and laminin. A series of analogs was developed based on the lead structure I and evaluated for VLA-1 activity. These new analogues have provided information regarding the structural components that are necessary for the optimization of biological properties. The synthesis and structure activity relationship around rings B and C, linker area and side chain (R) will be presented.



MEDI 124

Tetrahydroquinolines as CRTH2 antagonists

Jiwen Liu[1], *Yingcai Wang*[1], *Ying Sun*[1], *Lucy Tang*[2], *Derek Marshall*[3], *George Tonn*[4], *and Julio C. Medina*[5]. *(1) Chemistry, Amgen Inc, 1120 Veterans Blvd., South San Francisco, CA 94080, jiwenl@amgen.com, (2) Biology, Amgen Inc, (3) (4) PKDM, Amgen Inc, (5) Department of Chemistry, Amgen Inc*

CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) is a G protein coupled receptor expressed on eosinophils, basophils, and T helper 2 (Th2) lymphocytes. CRTH2 activation by its ligand, prostaglandin D2 (PGD2), is known to induce eosinophil degranulation and recruitment of lymphocytes to inflammatory sites. In addition, PGD2 is released by mast cells in large amounts during asthmatic responses. Therefore, it has been postulated that blocking CRTH2 could be therapeutically valuable in the treatment of asthma, allergic rhinitis and other allergic diseases. In this presentation, we will disclose a series of tetrahydroquinoline derivatives as high affinity CRTH2 antagonists and we will discuss the optimization of their potency and their pharmacokinetic properties.

MEDI 125

Structure activity relationship of uridine 5'-diphosphate analogs at the human P2Y6 receptor

Pedro Besada[1], *Dae Hong Shin*[1], *Stefano Costanzi*[2], *Hyojin Ko*[1], *Christophe Mathe*[3], *Julien Gagneron*[3], *Gilles Gosselin*[3], *Savitri Maddileti*[4], *T. Kendall Harden*[4], *and Kenneth A. Jacobson*[1]. *(1) Molecular Recognition Section, NIDDK, NIH, 9000 Rockville Pike, Bethesda, MD 20892, pedrop@intra.niddk.nih.gov, (2) Computational Chemistry Core Laboratory, NIDDK, National Institutes of Health, (3) Laboratoire de Chimie Organique Biomoleculaire de Synthese, Université Montpellier II, (4) School of Medicine, Univ. of North Carolina*

A structure activity and molecular modeling study of the uracil nucleotide-activated P2Y6 receptor has been developed. A series of UDP analogues bearing substitutions at the level of the ribose moiety, the uracil ring, and the diphosphate has been synthesized and assayed for activation of the human P2Y6 receptor. The ribose ring has been modified by replacing the 2'-hydroxyl with different functional groups. Alternatively, the ribose has been substituted with a 2-oxabicyclohexane ring, which fixed the sugar moiety in a rigid South conformation. The uracil ring has been modified at the 4-position, with the synthesis of 4-substituted-thiouridine 5'-diphosphate analogues, as well as at positions 3 and 5. The effect of modifications at the level of the phosphate chain has been studied by preparing a cyclic 3',5'-diphosphate analogue and several dinucleotides. This work provides a better understanding of the P2Y6 receptor essential to design new selective and potent compounds.

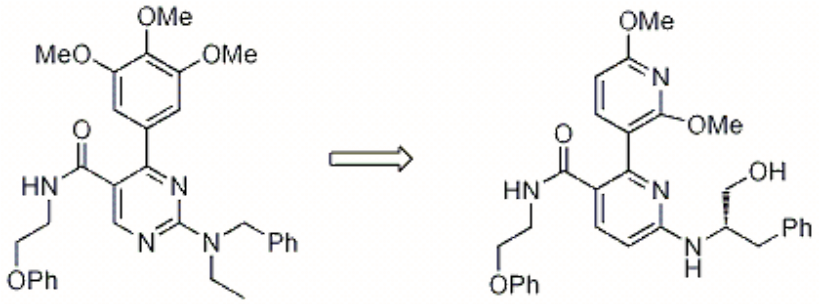
MEDI 126

Discovery and structure-activity relationships of tri-substituted pyrimidines/pyridines as novel calcium-sensing receptor antagonists

wu Yang, *Zheming Ruan, Katy Van Kirk, Yufeng Wang, Christopher Cooper, Dharmpal Dodd, Zhengping Ma, Brian Arey, Ramakrishna Seethala, Jean Feyen, and John Dickson, Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 5400, Princeton, NJ 08643, wu.yang@bms.com*

The calcium-sensing receptor (CaR) is a G-protein coupled receptor (GPCR) expressed on the surface of parathyroid cells. This receptor is a key regulator in bone

metabolism through its control of endogenous parathyroid hormone (PTH) release. Intermittent PTH administration has been shown to promote bone growth; therefore, a small molecule CaR antagonist and/or a negative allosteric modulator should act as an oral anabolic agent for the treatment of osteoporosis. A novel series of tri-substituted pyrimidines/pyridines that function as calcium-sensing receptor antagonists are reported. Compound 1, which exhibits sub-micromolar potency against CaR, was identified from high-throughput screening. Investigation of the structure-activity relationships around two pendant regions and the heterocyclic core resulted in the identification of compound 40, having improved potency and solubility over the original lead. In an acute in vivo model in rats, compound 40 demonstrated efficacy at promoting PTH release.



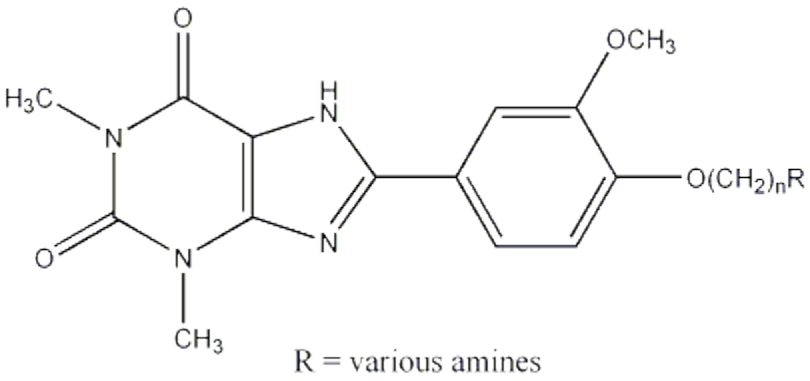
1 (HTS Lead) **40**

MEDI 127

Synthesis of 8-[4-(aminoalkoxy)-3-methoxyphenyltheophylline derivatives as potent adenosine receptor antagonists

Ranju Bansal[1]*, Gulshan Kumar*[1]*, Alan L. Harvey*[2]*, and Louise Young*[2]*. (1) University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh- 160014, India, Fax: 91-172-2541142, ranju29in@yahoo.co.in, (2) Strathclyde Institute for Drug Research, University of Strathclyde*

Adenosine plays a significant role in pathophysiology of human asthma and selective adenosine receptor antagonists are of interest for its management. The ability of theophylline to block the adenosine receptors has attracted much attention. Many studies have been carried out with the aim of designing selective theophylline derivatives with better therapeutic index. This has resulted in design and synthesis of many 8-phenyltheophyllines as selective adenosine receptor antagonists. Following a similar approach, we synthesized a number of 8-(aminoethoxyphenyl)theophylline derivatives by treating 5,6-diamino-1,3-dimethyluracil with various aminoalkylated vanillin derivatives to afford corresponding benzylidines, which on subsequent cyclization using thionyl chloride gave target compounds. The compounds have been tested in binding assays using cloned human adenosine A1 and A2a receptors using [^{3}H] DPCPX and [^{3}H]ZM241385, respectively. The synthesized xanthine derivatives exhibited significant adenosine receptor affinity with approximately 1000 times more selectivity for A2a vs A1 receptors.



MEDI 128

Synthesis, lead optimization, and structure-activity relationship studies of thiazolidinone CFTR inhibitors

Nitin D. Sonawane*, Jie Hu, Shannon Sullivan, and Alan S. Verkman, Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, 1246 Health Sciences East Tower, San Francisco, CA 94143-0521, Fax: 415-665-3847, ndsonawane@yahoo.com*

Cholera toxin-induced intestinal fluid secretion involves active chloride secretion by Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) chloride channels present on enterocytes. We previously identified by high throughput screening a thiazolidinone class of CFTR inhibitors. The compound 3-[(3-trifluoromethyl)phenyl]-5-[(4-carboxyphenyl)methylene]-2-thioxo-4-thiazolidinone, ($CFTR_{inh}$-172) blocked CFTR by a voltage-independent mechanism, and reduced enterotoxin-mediated intestinal fluid secretion. One limitation of $CFTR_{inh}$-172 was its relatively low water solubility (~20 μM). Here we report the synthesis, lead optimization, and SAR studies of thiazolidinone CFTR inhibitors with improved water solubility and favorable physicochemical characteristics. To improve water solubility, various analogues were synthesized using strategies such as introduction of water solubilizing groups, adding groups to reduce lattice energy, modification of thiazolidinone core, and exploring chemical space systematically. Analogue syntheses and SAR studies resulted several water soluble compounds which blocked CFTR chloride channel and reduced cholera toxin-mediated intestinal fluid secretion. Synthesis and SAR and *in vivo* antidiarrheal studies of these compounds will be presented.

MEDI 129

Discovery of a novel piperidinyl-sulfonyl benzoic ester, active as CB1 agonist

N. Lambeng[1], F. Lebon[2], B. Christophe[1], M. Grossmann[1], M. Burton[2], M. De Ryck[1], and ***L. Quere****[2]. (1) CNS Research, UCB, (2) Chemical Research, UCB, rue du Foriest, braine l'alleud 1420, Belize, luc.quere@ucb-group.com*

The endocannabinoid system seems to be involved in a rising number of pathological conditions. CNS responses to cannabinoids are mainly mediated by the G protein-

coupled CB1 receptor, which is known to couple preferentially to Gi/Go G proteins. Due to its presynaptic distribution, and its coupling to various systems, CB1 receptor represents an ideal natural tool for modulating the neurotransmitter release. Therapeutic interest for searching CB1 agonists mainly lies in developing drugs for treating pain (chronic & acute), multiple sclerosis, tremor, anxiety/mood disorders, sleep disorders, seizures and for neuroprotection. Two products are already available on this growing (yet still controversial) market, namely Marinol and Nabilone as well as Sativex which is supposed to become available soon. In an effort to discover new CB1 agonists, we developed a high-throughput screening assay for identification of CB1 modulators using CHO-K1 cells stably expressing mitochondrially-targeted Aequorin, G(alpha)16 and the human CB1 receptor (Euroscreen). Validation of the HTS was performed with competition studies against [3H]CP 55,940 and GTPgammaS binding experiments. One compound with an IC50 in the low nanomolar range was identified as a full agonist, and was further evaluated in secondary assays for selectivity and biological activity. A preliminary SAR has been obtained around this potent agonist which can be used to further characterize this family of sulfonyl benzoic esters and further optimize in vivo pharmacological profile and ADME properties.

MEDI 130

Identification of an allosteric binding site at the cannabinoid CB1 receptor

Teresa S. Barber*, Dow P. Hurst, and Patricia H. Reggio, Chemistry and Biochemistry, University of North Carolina Greensboro, 1000 Spring Garden St., Greensboro, NC 27403, Fax: (336)334-5402, tsmcmill@uncg.edu*

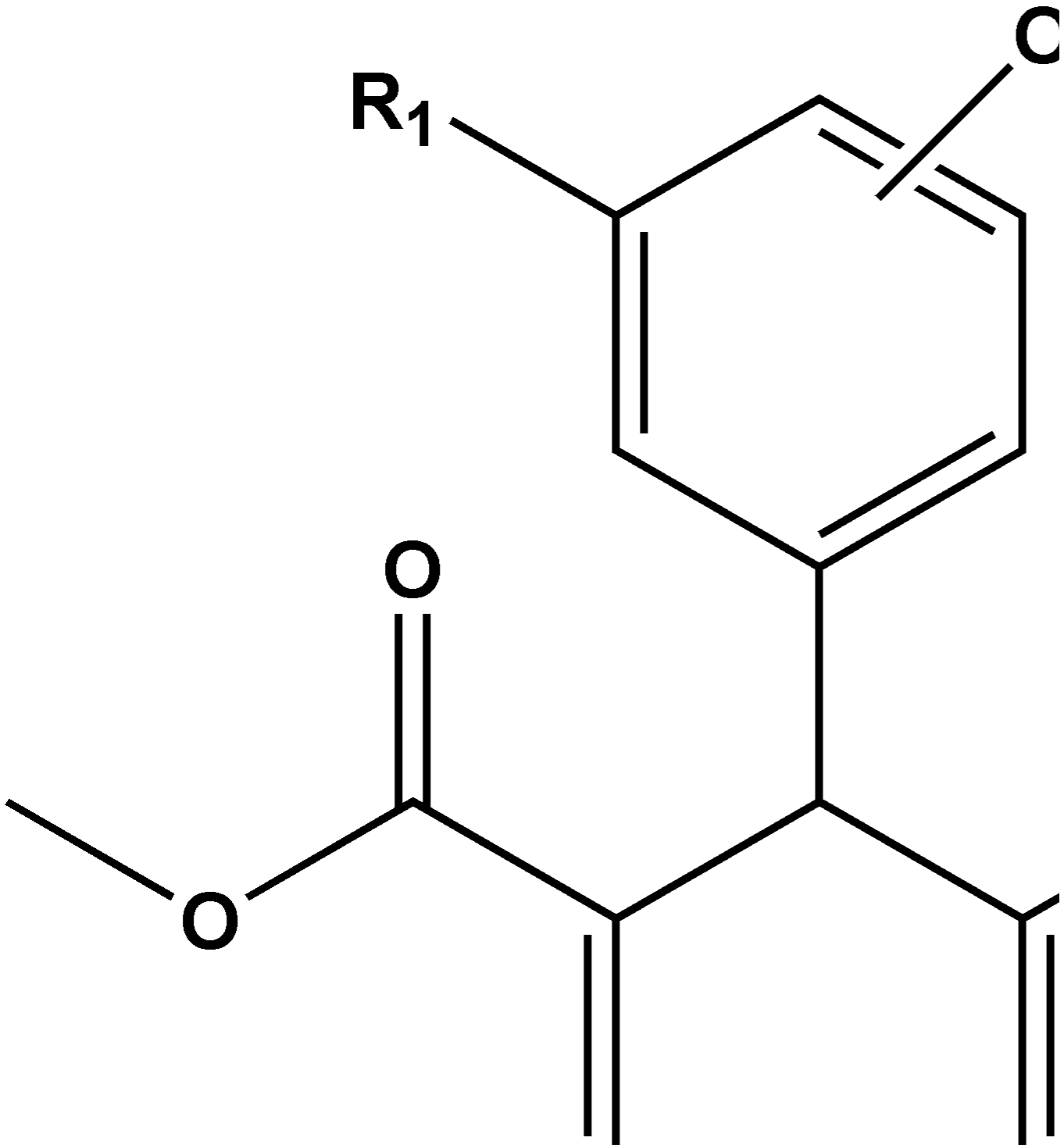
The cannabinoid CB1 receptor contains an allosteric binding site recognized by Org27759 (3-ethyl-5-fluoro-1H-indole-2-carboxylic acid [2-94-dimethylamino-phenyl)-ethyl]-amide), Org27569 (5-chloro-3-ethyl-1H-indole-2-carboxylic acid [2-(4-piperidin-1-yl-phenyl)-ethyl]-amide), and Org29647 (5-chloro-3-ethyl-1H-indole-2-carboxylic acid (1-benzyl-pyrrolidin-3-yl)-amide, 2-enedioic acid salt). In equilibrium binding assays, the Org compounds significantly increase the binding of CB1 agonist [3H]CP 55,940, indicative of a positively cooperative allosteric effect. The same compounds cause a significant, but incomplete, decrease in the specific binding of CB1 inverse agonist [3H]SR141716, indicative of a limited negative binding cooperativity (Price et al. Mol Pharmacol. 68:1484(2005)). Spartan Monte Carlo conformational searches revealed that the global min for each Org compound was a folded conformation, with extended conformations for each within 0.70 kcal/mol of the global min. A binding site for each was sought in a CB1 activated state (R*) model. Preliminary docking experiments indicate that these compounds can interact at the TMH5-6 interface, stabilizing W6.48 in a trans chi 1 conformation. [Support: DA03934 and DA00489]

MEDI 131

Synthesis and pharmacological evaluation of some amide derivatives of 4-phenyl-1,4-dihydropyridine carboxylic congeners

Gaurav Narang[1]*, Priyanka Jain*[1]*, Alan. L. Harvey*[2]*, Rosalia Carron*[3]*, and Ranju Bansal*[1]*. (1) University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh- 160014, India, Fax: 91-172-2541142, narang77@yahoo.com, (2) Strathclyde Institute for Drug Research, University of Strathclyde, (3) Departament of physiology and pharmacology, University of Salamanca*

4-Aryl-1,4-dihydropyridines (DHP) exemplified by nifedipine have been used in general medical practice worldwide for the treatment of hypertension and vasospastic angina for over two decades. Recently, hybrid structures like vanidipinedilol and lercanidipine containing active principals of two or more drugs in a single molecule are of much interest for the development of potent antihypertensives free from side effects. As a part of our ongoing efforts to develop new antihypertensive agents with improved therapeutic profile, we have synthesized some newer dihydropyridines possessing both calcium channel blocking and vasodilatory properties. These structures are also lacking a nitro functionality, which usually imparts chemical instability and mutagenicity to the molecules. *In-vitro* calcium channel blocking activity has been evaluated by measuring the inhibitory response at L-type calcium channels activated by veratridine. Many compounds exhibited moderate to significant calcium channel blockade. When assessed on isolated rat thoracic aortic rings precontracted by phenylephrine / KCl (30 mM), most of the compounds produced a concentration-dependent inhibition of the contractile response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MEDI 132

SAR of the N-terminal histidine of GLP-1 in interactions with GLP-1R

Rebecca Roush[1], *Martin Beinborn*[2], *and David R. Haines*[1]. *(1) Department of Chemistry, Wellesley College, 21 Wellesley College Rd, Wellesley, MA 02481, Fax: 781-283-3642, rroush@wellesley.edu, (2) Department of Medicine, New England Medical Center*

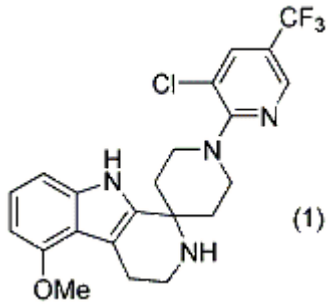
The N-terminal histidine of GLP-1 is known to be important in the binding of GLP-1 to GLP-1R, and essential to the ability of GLP-1 to activate GLP-1R. We have designed a series of heterocyclic histidine analogs, based on modifications of hydrogen bonding potential and pKa, which we have incorporated into GLP-1 in place of the N-terminal histidine. It is evident from early results that the binding potential/biological activity of N-terminally modified GLP-1s varies greatly with the heteroatom position and pKa. These findings, together with ongoing analyses, provide further insight into the structural requirements of receptor-agonist interaction.

MEDI 133

Discovery and optimization of a series of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole derivatives as CXCR3 antagonists

Liusheng Zhu, *Feng Xu, Tassie L. Collins, and Julio C. Medina, Chemistry Department, Amgen SF, 1120 Veterans Boulevard, South San Francisco, CA 94080, liusheng@amgen.com*

The CXCR3 receptor and its ligands MIG (CXCL9), IP-10 (CXCL10) and ITAC (CXCL11) have been implicated in a variety of inflammatory and autoimmune diseases. Cells expressing CXCR3 have been identified in diseased tissue from transplant rejection, psoriasis, rheumatoid arthritis and multiple sclerosis patients. Moreover, the ligands for CXCR3 (MIG, IP-10, ITAC) are upregulated within many of these tissues. Screening of our chemical library led to the discovery of a novel series of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole derivatives as CXCR3 antagonists. Here we describe the optimization of this series that led to the discovery of potent antagonists exemplified by (1).



(1)

MEDI 134

Estrogen's role in retina neuroprotection and optic nerve regeneration in the adult zebrafish (Danio rerio)

Jessica L. Fulton[1]*, Daniel F. McCain*[2]*, and James E. Turner*[2]*. (1) Department of Biology, Virginia Military Institute, New Science Building, Lexington, VA 24450, Fax: 1-540-464-7761, fultonjl@vmi.edu, (2) Department of Chemistry, Virginia Military Institute*

Estrogen has been shown to act as a neuroprotectant, slowing the progress of such conditions as Alzheimer's disease. Telost fish, such as the zebrafish, possess the capacity to regenerate damaged parts of their central nervous system, such as the retina and optic nerve resulting in the return of vision which is not the case with mammals. It has been hypothesized that this regenerative capacity is due to the fact that these fish have 100-1000 fold more brain aromatase activity than do mammals, including humans. This study begins to test estrogen's role in neural regeneration by inducing damage to the retina of the zebra fish via an optic nerve crush and then analyzing the concentrations of estrogen and aromatase under pre- and post- crush conditions using ELISA and enzyme assays. Results revealed an eighteen-fold increase in estrogen levels at fourteen days after optic nerve crush, a time when the regenerative response is metabolically maximized. These results have significant implications as to estrogen's role in successful repair and regenerative responses in the brain and may lead to clinical and pharmacological applications resulting in successful treatments for many neurological diseases.

MEDI 135

Allosteric modulation of group I metabotropic glutamate receptors: From structure to function

Tanja Weil*, Department Medicinal Chemistry & Drug Design, Merz Pharmaceuticals, Eckenheimer Landstrasse 100, Frankfurt 60318, Germany, Fax: 0049-695962150, Tanja.Weil@merz.de, Christopher G. Parsons, Department In vitro Pharmacology, Merz Pharmaceuticals, and Valerjans Kauss, Latvian Institute of Organic Synthesis*

We will present our recent advances towards the understanding of allosteric modulators interacting with the transmembrane domaine of group I metabotropic glutamate receptors (mGluR1 and mGluR5). For this purpose, functional and binding assays were established and validated. Then, based on structural information of already existing mGluRI modulators, discriminating pharmacophore models of both mGluR1 and mGluR5 were developed. Successful virtual screenings of compound libraries lead to the discovery of a new chemical scaffold revealing moderate activity for mGluR1. Optimization of this scaffold followed by careful SAR analysis yielded highly active antagonists for mGluR1 as well as negative and positive modulators of mGluR5. Focused compound libraries were synthesized to increase selectivity as well as to provide a deeper understanding of crucial interaction points within the scaffold. The mode of action of positive and negative modulation as well as the binding site of these novel compounds was further analyzed via a mutation analysis within the

transmembrane domain. Our results on mGluRI will be also compared with the mechanism of action of positive and negative modulation of other GPCRs.

MEDI 136

1-(2-Aminoethyl)-3-(arylsulfonyl)-1-H-7-azaindoles as Novel 5-HT6 receptor ligands

Simon N. Haydar[1], *Schuyler Antane*[1], *chen Ping*[2], *Jonathan L. Gross*[1], *Bob McDevitt*[1], *Van-Duc Le*[2], *Jessica Malberg*[3], *Albert J. Robichaud*[1], *Rajesh A. Shenoy*[2], *Deborah L. Smith*[3], *Guo Ming Zhang*[3], *and Lee E. Schechter*[3]. *(1) Department of Medicinal Chemistry, Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, haydars@wyeth.com, (2) Albany Molecular Research Inc, (3) Neuroscience, Wyeth Research*

The 5-HT6 receptor is one of the latest G-protein coupled receptors (GPCR) to have been identified in the serotonin family. The central nervous system (CNS) localization of the 5-HT6 receptors and their affinity for CNS drugs have created intense interest in identifying selective 5-HT6 receptors modulators as tools for studying the receptor and its' potential therapeutic applications. Novel 1-(aminoethyl)-3-(arylsulfonyl)1-H-7-azaindoles were prepared and several analogs within this class have been identified as high-affinity 5-HT6 receptor ligands functioning as full agonists. The synthesis and structure activity relationship of this potent class will be discussed.

MEDI 137

Substituted 3-arylsulfonyl-7-azaindoles as novel 5-HT$_6$ agonists

Robert E. McDevitt[1], *Schuyler A. Antane*[1], *Chad E. Beyer*[2], *Ping Chen*[3], *Jonathan L. Gross*[1], *Simon N. Haydar*[1], *Zoe. A. Hughes*[2], *Van-Duc Le*[3], *Omar Mabrouk*[2], *Jessica E. Malberg*[2], *Albert J. Robichaud*[1], *Rajesh A. Shenoy*[3], *Deborah L. Smith*[4], *Xintao G. Wang*[5], *Guo Ming Zhang*[4], *and Lee. E. Schechter*[2]. *(1) Department of Medicinal Chemistry, Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, mcdevib@wyeth.com, (2) Neuroscience, Wyeth Research, (3) Albany Molecular Research Inc, (4) Department of Neuroscience, Wyeth Research, Princeton, (5) Albany Molecular Research Inc®*

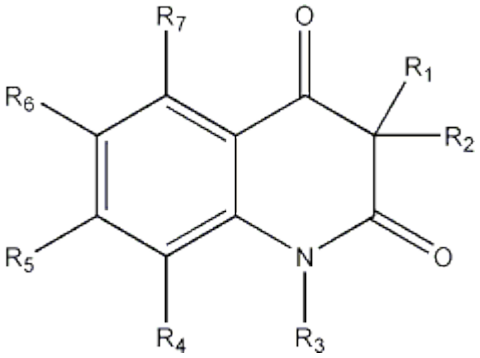
In recent years much investigation has been focused on higher-order serotonin receptors in the hopes of elucidating their functional roles. 5-HT$_6$ receptors are exclusively localized within the CNS and positively coupled to an adenylate cyclase second messenger system. The ability of 5-HT$_6$ receptors to regulate neurotransmitter release has further demonstrated the potential for this receptor in the treatment of a number of disease targets such as cognition, anxiety and depression. Our earlier work identified novel 3-Arylsulfonyl-7-Azaindoles as potent ligands of 5-HT$_6$ receptors. Herein we describe the synthesis and effects of substitutions on the 7-Azaindole core. Select examples within this series were revealed to be full 5-HT$_6$ agonists based on cAMP accumulation studies and were shown to be active in a rat behavioral model of anxiety.

MEDI 138

N-Substituted-1*H*-quinoline-2,4-diones as novel 5-HT_6 receptor antagonists

Chul Min Park[1], *Sunhee Kang*[1], *Sukjin Song*[1], *Jinil Choi*[1], *Wookyu Park*[2], *Nosang Park*[1], *and Churlmin Seong*[1]. *(1) CNS Research Team, Bioorganic Division, Korea Research Institute of Chemical Technology (KRICT), Daejeon 305-606, South Korea, Fax: 82-42-861-1291, cmsung@krict.re.kr, (2) Pharmaceutical Screening Research Team, Korea Research Institute of Chemical Technology (KRICT)*

The serotonin (5-HT) superfamily of receptors currently consists of seven classes (5-HT_{1-7}) that include approximately fourteen human subclasses. The 5-HT_6 receptor is one of the most recently identified serotonin receptors and is positively coupled to the adenylate cyclase secondary messenger system. The CNS localization of the 5-HT_6 receptors and their affinity for CNS drugs have sparked considerable effort to understand the role of this receptor in treatment of CNS disorder, including schizophrenia, depression, and impaired learning and memory. As a part of ongoing work aimed at the discovery of new 5-HT_6 receptor antagonists, novel classes of *N*-substituted-1*H*-quinoline-2,4-dione were designed, synthesized and identified to exhibit high binding affinity for 5-HT_6 receptors. This presentation will discuss the discovery of KR-055014 and KR-055015 which show high binding affinity towards the 5-HT_6 receptors and excellent selectivity over other serotonin subtype receptors and neurotransmitter receptors.



MEDI 139

SUVN-501: Novel 5-HT6 receptor antagonist enhances extracellular levels of acetylcholine – an in vivo brain microdialysis study

Ramakrishna V. S. Nirogi, *Vishwottam N. Kandikere, Koteshwara Mudigonda, Gopinadh Bhyrapuneni, Rajesh S. Omtri, Nageswararao Muddana, and Jagadeeshchandra Reddy, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 914023541152, nvsrk@suven.com*

5-HT6 receptors are expressed in brain regions associated with learning and memory; blockade of their function increases central cholinergic and glutamatergic neurotransmission and enhances cognitive process. Animal studies have suggested

that acetylcholine plays an important role in learning and memory. SUVN-501 is a novel 5-HT6 receptor antagonist. Effective lead-optimization of critical physico-chemical properties has led to the high brain penetration index. The present study investigated the neurochemical effects of SUVN-501 in the rat hippocampus. The effect of SUVN-501 on extracellular levels of acetylcholine in the hippocampus was examined using in vivo brain microdialysis in freely moving rats. SUVN-501 (3 mg/kg, i.p.) produced a significant increase in extracellular levels of acetylcholine. These results further support the rationale for the use of 5-HT6 receptor antagonists in the treatment of cognitive dysfunction associated with psychiatric diseases.

MEDI 140

3-(Amino)alkoxy indoles: Potent and selective 5-HT6 receptor antagonists

Ramakrishna V. S. Nirogi*, Anand V Daulatabad, Sarika A Daulatabad, Sandeep B. Bhosale, Narendra Varma Gaddiraju, Vishwottam N. Kandikere, Rama S. Kambhampati, and Vikas S. 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 5-HT6 antagonists in the treatment of Alzheimer's disease, Parkinson's disease and other neurodegenerative disorders has been well demonstrated in the animal models of cognition. The lack of desirable pharmacokinetic properties required for these CNS agents is one of the main reason for full characterization of functional and physiological usefulness of these molecules in human therapy. Our continuing efforts towards design and discovery of selective 5-HT6 antagonists have led 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 5-HT6 receptor ligands with Ki in the range of 1 - 5 nM, when tested by the in-vitro radio-ligand binding techniques. Synthesis, physicochemical properties and the in-vitro binding data along with the SAR will be discussed.

MEDI 141

Substituted aminomethyl indoles: Selective 5-HT6 receptor ligands

Ramakrishna V. S. Nirogi*, Amol D. Deshpande, Sarika A Daulatabad, Anand V Daulatabad, Rama S. Kambhampati, and Vikas S. Shirsath, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, nvsrk@suven.com*

Various research groups have demonstrated the usefulness of 5-HT6 antagonists in the treatment of 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 our ongoing programme for selective 5-HT6 ligands, we have designed a series of 5-HT6 ligands on a chemically

novel skeleton. Attempts have been made to impart the drug like properties to these molecules by optimizing their physicochemical properties. Our primary hit in this series was found to have the Ki in the micromolar range. Various structural modifications and optimization led to the compounds with higher binding affinity and selectivity. Synthesis, physicochemical properties, in-vitro binding data along with the preliminary SAR will be discussed.

MEDI 142

Novel substituted piperazines: New chemical class of selective 5-HT6 receptor antagonists

Vikas S. Shirsath, *Amol D. Deshpande, Adi R. Dwarampudi, Venugopala Rao Bhatta, Sandeep B. Bhosale, Srinivasulu Kota, Vishwottam N. Kandikere, Rama S. Kambhampati, and Ramakrishna V. S. Nirogi, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, svikas@suven.com*

5-HT6 receptor have been implicated in syndromes that affect cognition, such as Schizophrenia, Dementia, Alzheimer's, Parkinson's disease and other neurodegenerative disorders. Various structurally diverse small molecules have been known to bind selectively at 5-HT6 receptors. Some attempts are in progress for the clinical proof of concept for 5-HT6 antagonists as a new therapeutic class. Hitherto we report a new chemical class of 5-HT6 antagonists which have been designed, keeping in mind the physicochemical properties required for achieving the desired pharmacokinetic and CNS penetration properties of the molecules. Our effective lead generation and optimization methods have resulted in a series of potent 5-HT6 receptor antagonists with Ki in the range of 1 - 5 nM, when tested by the in-vitro radio-ligand binding assays. Synthesis, physicochemical properties, in-vitro binding data along with the SAR will be discussed.

MEDI 143

Novel chemical class of selective 5-HT6 receptor ligands: Conformationally restricted tryptamines

Rama S. Kambhampati, *Prabhakar Kothmirkar, Jagadish B. Konda, Trinath R. Bandyala, Srinivasulu Kota, Vikas S. Shirsath, and Ramakrishna V. S. Nirogi, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 91-40-23541152, krsastri@suven.com*

There has been increasing interest in the role of 5-HT6 receptors in higher cognitive processes such as memory. Manipulation of 5-HT6 receptor activities alters the transmission of several neurotransmitters important in memory. In-spite of availability of numerous potent and selective in-vitro 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 our research programme to identify and develop selective 5-HT6 ligands, we have designed 5-HT6 ligands on a chemically novel skeleton. Attempts have been made to impart the drug like properties to these molecules by optimizing their

physicochemical properties. Our primary hit was found to have the Ki in the micromolar range. Our effective lead optimization strategies have resulted in the molecules with Ki in the range of 1 - 5 nM at the 5-HT6 receptor. Synthesis, physicochemical properties, in-vitro binding data together with SAR will be discussed.

MEDI 144

Structure-activity relationships and modeling studies in the group of 2-(4-methylpiperazino)pyridines as serotonin receptor agents

Aldona Raszkiewicz[1], *Beata Duszynska*[2], *Andrzej J. Bojarski*[2], *Maria H. Paluchowska*[2], *Mateusz Nowak*[2], *Marcin Kolaczkowski*[3], *Markus W. Germann*[1], *and Lucjan Strekowski*[1]. *(1) Department of Chemistry, Georgia State University, Peachtree Center Ave, Atlanta, GA 30302, cheaer@langate.gsu.edu, (2) Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Science, (3) Department of Pharmaceutical Chemistry, Collegium Medicum, Jagiellonian University*

On the basis of the results obtained for 4,6-disubstituted-2-(N-methylpiperazino)pyrimidines, investigated previously as serotonin 5-HT_{2A} and 5-HT_7 receptor ligands, we have designed and synthesized a new group of analogous piperazinopyridine derivatives. Structure-activity relationship studies clearly showed the important role of heteroaryl substituents, especially 3-thienyl and 3-furyl, for a high 5-HT_7 affinity. Automated docking to homology model of 5-HT_7 receptor placed ligands between helices 2, 3 and 7 and revealed a strong interaction with the indole of Trp7.40.

MEDI 145

5-HT_{2A} Receptor inverse-agonists: Design and structure-activity relationship of novel pyrazole derivatives

Sonja Strah-Pleynet*, Bradley R. Teegarden, Susan D. Selaya, William Thomsen, Hazel Reyes, Jonathan Foster, Frederique Menzaghi, and Kevin Whelan, Arena Pharmaceuticals, Inc, 6166 Nancy Ridge Drive, San Diego, CA 92121, sstrah@arenapharm.com*

Serotonin (5-HT) mediates a variety of pharmacological responses in the central and peripheral nervous system. Modulation of 5-HT receptors has been actively pursued for a treatment of numerous disease states. Specifically, 5-HT_{2A} inverse-agonists are known to alleviate negative symptoms of schizophrenia as well as influence sleep patterns. Previously, we reported several potent and selective 5-HT_{2A} inverse-agonists, containing diphenylurea or diarylamine moieties. In continuation of our research in this area, a series of novel pyrazole derivatives has been designed and evaluated for its activity at the 5-HT_{2A} receptor. In this paper, the design, synthesis and structure-activity relationship of the new series will be presented.

MEDI 146

Serotonin 5-HT2 receptor agonists with topical ocular hypotensive activity

***Abdelmoula Namil**, Jennifer Ward, Marsha Mc Laughlin, Najam Sharif, John Liao, Jesse May, and Mark Hellberg, Chemistry Research, Alcon Research Ltd, 6201 South Freeway, Fort Worth, TX 76134, Fax: 817-615-3391, abdelmoula.namil@alconlabs.com*

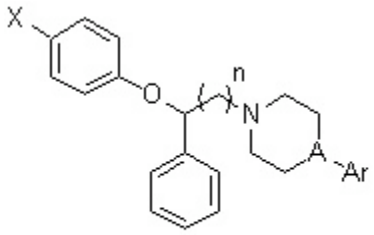
New analogues of (R)-2-(4-Iodo-2,5-dimethoxy-phenyl)-1-methyl-ethylamine (R-DOI), in which the iodo atom was replaced by different methoxy alkyl chains were prepared. The length of the chain connecting the methoxy group to the 4-position of the phenyl ring was varied from one to three carbons. Functional studies showed that R-methoxypropyl analogue was at least as potent as R-DOI in the 5-HT2A-mediated calcium mobilization assay. These acyclic ethers demonstrated high binding affinity at 5-HT2A and behaved as partial agonists. Connecting the methoxy alkyl chain to the 3-position of the phenyl ring led to a new series of cyclic ethers, isochroman analogues, that retain high binding affinity and high agonist potency. Single R-isomers were either prepared by asymmetric synthesis, or separated by chiral column HPLC. One example of each class, acyclic and cyclic ethers, was tested for its ability to lower intraocular pressure (IOP) in laser induced ocular hypertensive cynomolgus monkeys. Both representatives were found to be potent ocular hypotensive agents. Synthetic strategy as well as binding and activity data will be presented.

MEDI 147

Synthesis and biological evaluation of novel bi-functional selective serotonin reuptake inhibitors

***M. Graciela Miranda**, Department of Chemistry and Biochemistry, Baylor University, One Bear Place # 97348, Waco, TX 76798-7348, Fax: 254-710-4272, Maria_Miranda@baylor.edu, and Kevin G. Pinney, Department of Chemistry and Biochemistry, Center for Drug Discovery, Baylor University*

The research presented herein focuses on the design, synthesis and biological evaluation of novel bi-functional molecules that, by exhibiting an enhanced activity towards the 5-HT2A receptors while keeping a highly selective inhibition of serotonin reuptake, provide synergism in their potential efficacy over a wider variety of both depressive and anxiety disorders. We have prepared two distinct families of fluoxetine homologues which combine portions of that molecule with functionalized piperazines and piperidines. Biological evaluation shows that, in fact, some of the synthesized molecules exhibit the desired dual activity.



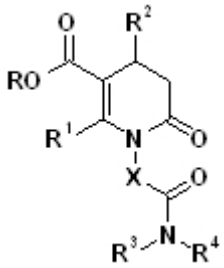
Novel Bi-Functional SSRIs

MEDI 148

Discovery and synthesis of novel class of dopamine transporter inhibitors using an in silico lead multiplying design approach

Laszlo Urge[1], *György Dormán*[2], *Ákos Papp*[1], *Agota Bucsai*[1], *Ferenc Darvas*[1], *Maria Sasvari-Szekely*[3], *Istvan Sziraki*[4], *and Toshio Fujita*[5]. *(1) ComGenex Inc, 7 Zahony u, Budapest, H-1031, Hungary, Fax: +361-214-2310, laszlo.urge@comgenex.hu, (2) 7 Zahony u, ComGenex, Inc, (3) Institute of Med. Chem. Mol. Biol. Pathobiochem, Semmelweis University, (4) IVAX Drug Research Institute, (5) EMIL Project*

The major goal in the hit-to-lead process is to discover novel chemotypes that provides suitable starting point for further optimization. We present here our 'Lead Multiplier' approach that generates novel lead classes against particular targets. It utilizes a unique medicinal chemistry knowledge base with bioanalogous library enumerator (EMIL- Example Mediated Innovation for Lead evolution), similarity search tools, in silico ADME filtering, chemical feasibility and diversity analysis. Selective inhibition of neurotransmitter transporters are very important since dopamine transporter inhibitors are useful against attention deficit/hyperactivity disorders while the others treat mainly depression. Starting from known inhibitors and using various cycles and filters of our lead multiplying approach a library containing 13 chemotypes was tested for DAT inhibition at 10 ìM using striatal synaptosomes from rat brain. Selecting the best 4 chemotypes finally dihydropyridone analogs (prepared by modified Hantzsch synthesis) showed highly selective inhibition (at 100 nM) with 2.8 % overall hit rate.



MEDI 149

Ucb 46331, a new nutative antidepressant agent: Pharmacological properties in vitro and in vivo

C Genicot[1], *B. Fuks*[2], *B. Christophe*[2], *R. Grimée*[2], *O. Berton*[2], *D. Dassesse*[2], *A. Matagne*[2], *M. Guyaux*[2], *T. Waegemans*[2], *B. Kenda*[1], *and Y. Lamberty*[2]. *(1) Chemical Research, UCB, rue du Foriest, braine l'alleud 1420, Belgium, christophe.genicot@ucb-group.com, (2) CNS Research, UCB*

ucb 46331, a N-aryl-N-benzyl-4-aminopiperidine, is the outcome of drug discovery efforts aimed at identifying molecules with a 'triple' mechanism of action as putative antidepressant drugs. In vitro, ucb 46331 binds to serotonin reuptake sites with an affinity similar to fluoxetine. ucb 46331 also inhibits [3H]pyrilamine binding to histaminergic H1 receptors and [3H]ketanserine binding to 5-HT2 receptors and interacts to a lesser extent with alpha1 adrenoreceptors and dopamine D2 receptors. In synaptoneurosomes, ucb 46331 potently inhibits 5-HT uptake (71% at 10 µM). In vivo, ucb 46331 is able to significantly increase cortical serotonin levels in the rat (from doses of 3.8 and 12 mg/kg after intraperitoneal and oral administration respectively) as revealed by microdialysis studies, thus confirming its blocking effect on brain serotonin reuptake sites. ucb 46331 also inhibits DOI-induced behaviour in mice (ED50 = 2mg/kg) after intraperitoneal administration, which confirm its 5-HT2 antagonistic activity. The moderate affinity and functional activity of ucb 46331 at dopaminergic D2 and adrenergic alpha1 receptors was not reflected in vivo. Indeed, ucb 46331 neither antagonised apomorphine-induced climbing in the mouse nor altered heart rate and blood pressure in the rat. Finally, after oral administration, ucb 46331 was found to be effective in the forced swimming test in mice (from a dose of 12 mg/kg), a behavioural screening model sensitive to antidepressant agents. Together, these results indicate that ucb 46331 is an orally active potential antidepressant drug which is able to inhibit brain serotonin reuptake sites and to behave as an antagonist at H1 and 5-HT2 receptors.

MEDI 150

AEG33783 Target Identification: 2-(2-aminoethylsulfanyl)-5-phenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfone and sulfoxide molecular probes

Patrick Bureau[1], *Stephen J. Morris*[2], *Guillaume Levesque*[2], *Genvieve Doucet*[2], *James B. Jaquith*[1], *Jon Durkin*[1], *and John W. Gillard*[1]. *(1) Department of Chemistry, Aegera Therapeutics Inc, 810 chemin du Golf, Verdun (Montreal), QC H3E 1A8, Canada, patrick.bureau@aegera.com, (2) Department of Molecular Biology, Aegera Therapeutics Inc*

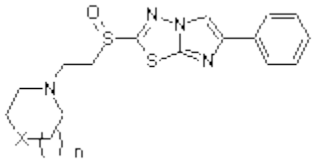
The treatment of peripheral neuropathies and polyneuropathies induced by diabetes and chemotherapy represent a growing unmet need in neurology and cancer treatment. The AEG33783 family of neuroprotective agents have demonstrated in vivo efficacy in several animal models of peripheral neuropathy and represent a novel approach to the treatment of Diabetic Neuropathy (DN) and chemotherapy induced neuropathy (CTIN). A putative target for AEG33783 has been identified using various photoaffinity, biotinylated and bead conjugate derivatives. A mechanistic correlation between HSP70 upregulation and indirect JNK inhibition is presented.

MEDI 151

6-Phenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfones and sulfoxides as novel neuroprotective agents

Scott Jarvis[1], *James B. Jaquith*[1], *Patrick Bureau*[1], *Stephen J. Morris*[2], *Guillaume Levesque*[2], *Genvieve Doucet*[2], *Jon Durkin*[1], *and John Gillard*[1]. *(1) Department of Chemistry, Aegera Therapeutics Inc, 810 chemin du Golf, Verdun (Montreal), QC H3E 1A8, Canada, (2) Department of Molecular Biology, Aegera Therapeutics Inc*

The treatment of peripheral neuropathies and polyneuropathies induced by diabetes and chemotherapy represent a growing unmet need in neurology and cancer treatment. Many chemotherapeutic agents such as the taxanes, epithilone derivatives, the vinca alkaloids, proteosome inhibitors and the platins, induce dose limiting peripheral neuropathies. In conjunction with SAR studies of the AEG33783 series of neuroprotective agents, a family of 6-phenylimidazo[2,1-b]-1,3,4-thiadiazole-2-sulfones and sulfoxides were prepared and evaluated in in vitro models of peripheral neuropathy. This novel family of agents protected primary SCG neurons against neurotoxic insults including NGF withdrawal and treatment with Taxol or cisplatin.



MEDI 152

Poly(ADP-ribose) glycohydrolase (PARG) inhibitors as novel agents for the treatment of Parkinson's disease

Xianjun Liu, *Karson S. Putt, and Paul J. Hergenrother, Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Ave, Urbana, IL IL61801, liu5@uiuc.edu*

Parkinson's disease is a debilitating neurological disorder that results from the loss of dopaminergic neurons in the substantia nigra. Currently, there is no cure for Parkinson's disease and the few treatment options, such as dopamine replacement with levodopa, lose their efficacy over time. There is a dire need to discover new protein targets and small molecule therapeutics for this disease. Poly(ADP-Ribose) Glycohydrolase (PARG) inhibitors have great potential as neuroprotective agents in Parkinson's disease treatment. A new class of small molecules that inhibit PARG has been found. A novel PARG inhibitor was identified from a high-throughput screen of a 22,000-membered compound collection. The best hit from this screen, PIP-1, inhibits PARG with an IC50 of 0.49 μM. This compound also shows excellent protection of differentiated PC-12 and SK-N-SH cells from MPP+ cell culture. Derivatives of the identified inhibitor were synthesized by solid-phase to determine structure-activity relationships and to identify more potent inhibitors. Through these experiments, we have identified new small molecule inhibitors and have identified a new therapeutic target for Parkinson's disease.

MEDI 153

Fluoro and trifluorosemicarbazones as novel anti-convulsant drug

***Manjusha Verma**, Pharmacy, University of Georgia, 325, PBS, UGA, Athens-30602, GA, vermamanjusha@yahoo.com, and **Krishna Nand Singh**, Applied Chemistry, Institue of Technology, Banaras Hindu University, Applied Chemistry, I. T., B. H. U, Varanasi 221005, India, knsinghbhu@yahoo.co.in*

Since anticonvulsant activity has been displayed by various urea derivatives, hydrazones and amides, a logical strategy was the incorporation of the urea, hydrazo and amidic groups into single functional entity namely semicarbazido group. During the last five years, semicarbazones have emerged as novel anticonvulsant entities. A pharmacophore model has been suggested for anticonvulsant semicarbazones. The structural requirements in the semicarbazone series are a lipophilic aryl ring, a distal aryl ring and a hydrogen-bonding domain.The lipophilic aryl ring with chloro, bromo or nitro group has been found to be essential for anticonvulsant activity. The distal aryl ring is also implicated at the binding site. Thus, in the present study, special emphasis has been given to the synthesis of fluoro and trifluoromethyl substituted semicarbazones of a variety of aromatic aldehydes, ketones and isatins with the aim of protecting metabolic hydroxylation and inactivation. In view of the discussion, a series of substituted isatin semicarbazones and related bioisosteric semicarbazones have been synthesised to meet the structural requirement essential for anticonvulsant properties. The evaluation of anticonvulsant activities of the synthesized semicarbazones for maximum electroshock (MES) test, subcutaneous metrazole seizure pattern test (ScMet) and neurotoxicity along with isatin, phenytoin, carbamazepine and valporic acid as reference has been studied.

MEDI 154

WITHDRAWN

MEDI 155

Hypothesis-driven, structure-based drug design using isoxazole chemistry to target the N-methyl-D-asparate receptor

***Elizabeth F. Scott**[1], Monika Szabon[2], Jared K. Nelson[2], and Nicholas R. Natale[2]. (1) Department of Chemistry, Central Washington University, 400 East University Way, Ellensburg, WA 98926, scottel@cwu.edu, (2) Department of Chemistry, University of Idaho*

N-methyl-D-asparate (NMDA), a sub-type of glutamate receptor (GluR), is involved with learning and memory. Previous studies suggest that detrimental changes to receptors in the hippocampus during the aging process impair memory function. We have proposed to extend our catalytic asymmetric synthesis, previously applied to AMPA receptor ligands, to prepare molecules that will bind to this receptor sub-type. This project specifically explores the application of isoxazole chemistry developed in

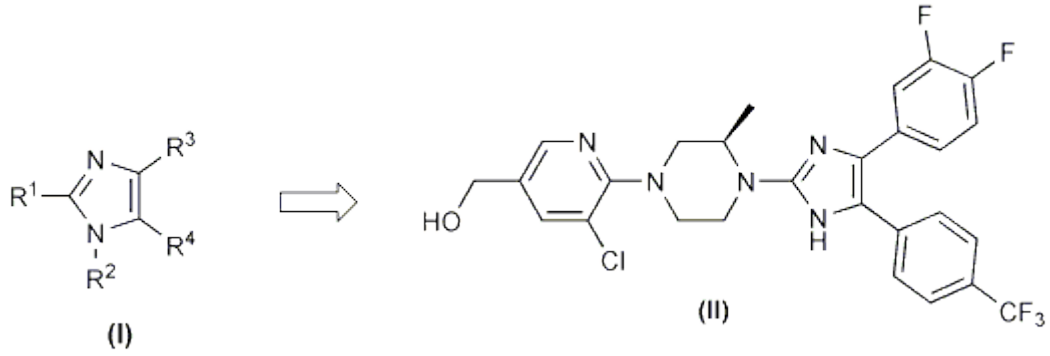
our laboratories towards ligands that will bind to and help distinguish between the receptors in the neurological system. As a proof-of-concept study for the critical bond forming reaction in our quest for NMDA selective ligands, the racemic control of the Strecker synthesis was accomplished. The characterization of the products will be described.

MEDI 156

Structure-activity relationship (SAR) investigations of substituted imidazole analogs as TRPV1 antagonists

Vu V. Ma[1], *Vijay K. Gore*[1], *Ning Xi*[1], *Vassil I. Ognyanov*[1], *Rami Tamir*[2], *James J. S. Treanor*[2], *Narender R. Gavva*[2], *and Mark H. Norman*[1]. *(1) Department of Chemistry Research & Discovery, Amgen Inc, Thousand Oaks, CA 91320, vma@amgen.com, (2) Department of Neuroscience, Amgen Inc*

Vanilloid receptor-1 (VR1, TRPV1) is a member of the transient receptor potential (TRP) family of ion channels. TRPV1 functions as a ligand-gated nonselective cation channel that is activated by capsaicin, low pH, heat, and endogenous ligands such as anandamide, 12-HPETE, and OLDA. These receptors are expressed in peripheral sensory neurons that are involved in nociception and neurogenic inflammation. Antagonists of the TRPV1 receptor have been reported to be effective in animal models of inflammatory and neuropathic pain. A series of substituted imidazoles (I) were synthesized to investigate the SAR of this novel class of TRPV1 antagonists. These studies led to the identification of a highly potent and orally bioavailable TRPV1 antagonist (II).

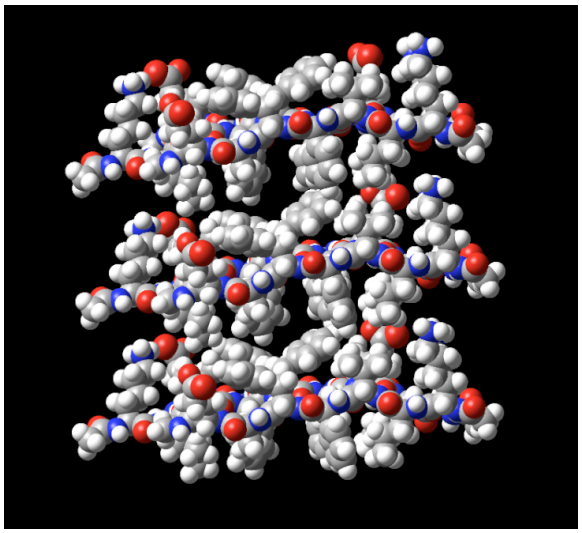


MEDI 157

Mutation studies and molecular models of amyloid-ß(16-22): A provocative role for water cavities

Yan Liang[1], *Ashutosh S. Jogalekar*[2], *James P. Snyder*[2], *and David G. Lynn*[1]. *(1) Departments of Chemistry and Biology, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, yliang2@emory.edu, (2) Department of Chemistry, Emory University*

Amyloid-ß (Aß) peptide deposition as fibrils in brain tissue is associated with Alzheimer's disease. It is important to understand fibril formation pathways during amyloid peptide self-assembly, a process proposed to be dominated by hydrophobic interactions. To focus on the endpoint of assembly, the hydrophobic core of amphiphilic Aß(16-22) (i.e. LVFFA) has been chosen as a model system. Several unnatural hydrophobic mutants installed at Phe19 and Phe20 undergo self-assembly. CD spectroscopy indicates predominately ß-sheet structure. Microscopy shows the resulting fibers to correspond to different morphologies, implying hydrophobic interactions coupled to hydrogen bond formation are critical factors for controlling self-assembly. Attempts to characterize possible structural relationships between the natural and mutant forms have been made by developing several fiber models with particular attention to hydrophobic side-chain interactions. An unusual feature of the mutant models is the relative degree of cavity-formation and the variable potential for water migration within the fibers.



MEDI 158

Novel gamma-secretase transition state analogs as Aß inhibitors

Pierre-Emmanuel F-X. Broutin[1]*, Vincent Laporte*[1]*, Christopher M. Lincoln*[1]*, Jason A. Carr*[1]*, Michael Mullan*[2]*, and Pansham Bakshi*[1]*. (1) Chemistry, Roskamp Institute, 2040 Whitfield Avenue, Sarasota, FL 34243, Fax: 941-752-2948, pbroutin@rfdn.org, (2) Director, Roskamp Institute*

Alzheimer's disease (AD) is the most common form of dementia in elderly people. Cerebral deposition of amyloid ß (Aß) is an early and invariant step in AD pathogenesis. The 38-43 residues Aß peptide is formed through proteolytic processing of the integral membrane Aß precursor protein (APP) by the sequential action of two aspartyl proteases: ß- and gamma-secretases which consequently have emerged as important therapeutic targets. Herein, we will present the synthesis and SAR studies of novel transition state peptidomimetics designed on the basis of existing knowledge of gamma-secretase active site. These transition state analogues were assayed for both Aß inhibition as well as for APP intracellular domain (AICD) inhibition in cell-based assay. We found 1:1 correlation with respect to their IC50's in

both assays suggesting same enzyme is responsible for both gamma- and epsilon-cleavages.

MEDI 159

Synthesis and pharmacological evaluation of 9- and 10-substituted cytisine derivatives: Novel ligands for acetylcholine receptors

Sheela K. Chellappan[1]*, Yingxian Xiao*[2]*, Kenneth J. Kellar*[2]*, and Alan P. Kozikowski*[1]*. (1) Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, sheelakc@uic.edu, (2) Department of Pharmacology, Georgetown University*

Neuronal nicotinic receptors (nAChRs) constitute therapeutically relevant targets for the treatment of neurodegenerative disorders and other central nervous system disorders including schizophrenia and pain. nAChRs are also important targets for the discovery of medications for use in smoking cessation. (-)-Cytisine, a natural chiral quinolizidine alkaloid, is reported to behave as a potent partial agonist at the alpha4beta2 nAChR and to possess low nanomolar binding affinity for this subtype. We have focused our attention on chemically modifying cytisine to create nicotinic ligands of enhanced subtype selectivity. The synthesis, and pharmacological evaluation of novel 9-and 10-substituted cytisine derivatives that show derivatives with high alpha4beta2 subtype selectivity will be presented.

MEDI 160

Thienopyridyl ureas: A new series of TRPV1 antagonists active in models of inflammatory and neuropathic pain

Sean C. Turner[1]*, Tammie K. Jinkerson*[2]*, Arthur Gomtsyan*[2]*, Heath McDonald*[2]*, Prisca Honore*[2]*, Carol T. Wismer*[2]*, Kennan C. Marsh*[3]*, Jill Wetter*[3]*, Michael F. Jarvis*[2]*, Connie R. Faltynek*[2]*, and Chih-Hung Lee*[2]*. (1) Neuroscience Research, Global Pharmaceutical Research and Development, Abbott GmbH & Co. KG, Ludwigshafen 67061, Germany, Fax: 49-621-5892443, sean.turner@abbott.com, (2) Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, (3) Global Pharmaceutical Research & Development, Abbott Laboratories*

The capsaicin sensitive TRPV1 receptor is a member of the mammalian transient receptor potential (TRP) channel family and is highly expressed on small diameter (C-fiber) nociceptive sensory neurons. It is also expressed at lower levels in other non-neuronal tissues such as skin and bladder. This receptor has been called a polymodal detector of noxious stimuli since it can be activated in several ways. Low pH, heat and naturally occurring ligands such as capsaicin and resinoferotoxin activate TRPV1 causing burning pain sensation. TRPV1 antagonists continue to be an attractive target for the discovery of novel analgesic agents. Interest in these compounds is supported by the finding that TRPV1 receptor knock-out mice do not develop thermal hyperalgesia following acute inflammation. Herein we report a new series of thienopyridyl ureas that demonstrated in vitro activity in blocking capsaicin

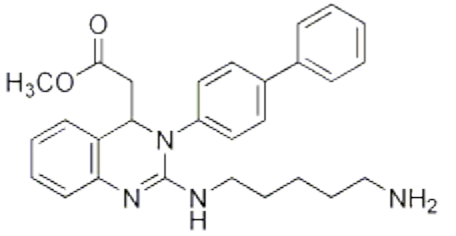
activation of TRPV1. The most potent derivatives from this series were also found to be active in both inflammatory and neuropathic in vivo pain models.

MEDI 161

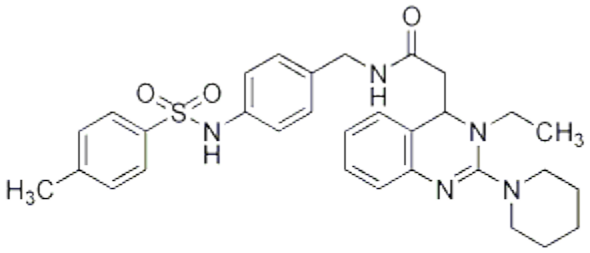
3,4-Dihydroquinazolines as novel and selective T-type calcium channel blockers

Jae Y. Lee[1]*, Seong J. Park*[2]*, Sung J. Park*[1]*, Min J. Lee*[1]*, and Ji Y. Jeon*[1]*. (1) Department of Chemistry, Kyung Hee University, 1 Hoegi-Dong, Dongdaemoon-Gu, 130-701 Seoul, South Korea, Fax: +82-2-966-3701, ljy@khu.ac.kr, (2) Life Sciences Division & Department of Chemistry, Korea Institute of Science and Technology & Korea University*

Calcium channels (CCs) play an important role in the transduction of action potential to the cytosol. An influx of calcium ion is essential for muscle contraction, neurotransmitter, and hormonal release. Among voltage-gated calcium channels, T-type channels are strongly associated with the generation of rhythmical firing patterns in the mammalian CNS and implicated in pathogenesis of epilepsy and neuropathic pain. However, only limited progress has been made to date in the quest to identify both potent and selective compounds except kurtoxin and mibefradil for T-type channel blockade. Therefore, we sought to discover novel small molecule compound with high potency and selectivity for T-type channels. To serve this purpose, a library of 3,4-dihydroquinazoline derivatives was synthesized and a brief structure-activity relationship will be described. From these studies, we have identified three compounds: KYS05044, KYS05064, and KYS05071 which exerted potent blocking actions on T-type, but no effects on N-type calcium channels in the low micromolar range compared to mibefradil.



KYS05044
(IC_{50} = 0.56 ± 0.10 μM)
(Selectivity = > 100 for T/N-type channel)



KYS05064
(IC_{50} = 0.96 ± 0.22 μM)
(Selectivity = > 100 for T/N-type channel)

MEDI 162

RNAi in mammalian cells: Transcripts, targets and technology

Natasha Caplen*, Gene Silencing Section, Center for Cancer Research, National Cancer Institute, NIH, 37 Convent Drive, Bethesda, MD 20892, Fax: 301-480-1717, ncaplen@mail.nih.gov*

Gene silencing in mammalian cells through RNA interference (RNAi) has become an invaluable tool for the study of biological processes. RNAi is mediated through the action of small ~21 nucleotide duplex RNAs, known as small interfering RNAs (siRNAs), which form part of an endogenous enzyme complex, termed the RNA-induced silencing complex (RISC). More specifically, a single strand of the siRNA duplex is loaded into RISC where it provides stringent guidance for the catalytic cleavage of complementary mRNA transcripts. We are employing RNAi for the exploration of cancer-associated pathways including the validation of anti-cancer targets, the potential identification of new targets, the development of novel model systems, and the elucidation/validation of integral components of cancer related phenotypes. This presentation will discuss the development of RNAi-based technologies and will describe specific examples of how RNAi can be used to study gene regulation, identify potential molecular targets, and help understand gene-drug interactions.

MEDI 163

Understanding and improving the properties of siRNA for drug discovery

***William S. Marshall**, Dharmacon Inc, 2650 Crescent Dr, Suite 100, Lafayette, CO 80026, Fax: 303-604-3287, marshall.b@dharmacon.com*

Gene silencing by small interfering RNA (siRNA) has emerged as a useful technology to specifically eliminate targeted gene expression and allow for assessment of gene function. Because the RNAi machinery plays a fundamental role in controlling cellular fate and homeostasis, it has become apparent that treatment of cells with siRNA can lead to unintended "off-target" events that can complicate gene functional analysis and development of siRNA therapeutics. We have conducted detailed mechanistic studies of siRNA action in cells in order to more fully understand the basis for both potency and "off-target" activity. The results of these studies have lead to bioinformatic and chemical modification strategies that significantly enhance the properties of siRNA. Coupling this knowledge with pharmacokinetic and biodistribution advancements is key to further imparting drug like properties on siRNA. Application of these strategies should broaden the application of siRNA across the drug discovery process and positively impact their direct application as therapeutic agents.

MEDI 164

Structure-activity relationship studies of siRNAs

***Eric E. Swayze**, Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc, 1896 Rutherford Rd., Carlsbad, CA 92008, Fax: 760-603-4654, eswayze@isisph.com*

RNA interference (RNAi) has emerged as a powerful antisense mechanism for inhibition of gene expression. As an antisense based therapeutic, siRNAs should be held to the standards set by the current generation of antisense therapeutics, which have shown pharmacology in both animal models and human clinical trials. While unmodified siRNAs are sufficient in cell culture when transfected with cationic lipids, it appears that chemical modifications or formulations will be required to achieve

therapeutic effects in animals. We have focused on optimizing the stability of siRNAs, while maintaining or improving the intrinsic potency of the RNAi mechanism. To accomplish this goal, we studied the positional effects of chemical modifications known to impart beneficial properties to oligonucleotides, and have developed an understanding of the chemical SAR of various siRNAs. The resulting information was used to design stable, active siRNAs. Details of these studies, as well as preliminary pharmacokinetic studies will be presented.

MEDI 165

Therapeutic applications of chemically modified siRNAs

Chandra Vargeese, *Chemistry Group, Sirna Therapeutics, Inc, 2950 Wilderness Place, Boulder, CO 80301, Fax: 303-449-8829, vargeesec@sirna.com*

Sirna Therapeutics is focused on in vivo delivery of chemically modified siRNA to reduce expression of genes with important clinical and commercial potential. We have examined the efficacy of chemically modified siRNA incorporated in lipid nanoparticles targeted to hepatitis B virus (HBV) in an in vivo mouse model of HBV replication. Chemically modified siRNA targeted to a highly conserved site in the HBV RNA was incorporated in lipid nanoparticles and administered by intravenous injection into mice carrying replicating HBV. We will present data demonstrating knockdown of hepatitis B virus and endogenous disease-related genes in vivo with formulated chemically modified siRNAs. In addition, we address interactions of formulated siRNA with components of the innate immune system in vivo.

MEDI 166

Translating RNA interference into drugs

Muthiah Manoharan, *Alnylam Pharmaceuticals, 300 Third Street, Cambridge, MA 02142, Fax: 617-551-8102, mmanoharan@alnylam.com*

A critical requirement for achieving safe and efficacious RNAi therapeutics is introduction of "drug-like" properties, such as stability, cellular delivery, and tissue bioavailability, into synthetic siRNAs to improve in vivo pharmacological properties. Recently we demonstrated that conjugation of siRNAs with cholesterol has tremendous potential to treat diseases by silencing the expression of otherwise non-druggable proteins. Extending these findings, we modified potential endo- and exonuclase cleavage sites by appropriate sugar and backbone modifications and improved in vivo serum and tissue half-live of siRNA duplexes and enhanced in vivo efficacy which led to siRNA compounds that silence target mRNA expression in tissues of interest by >80%. These results represent a significant advance in the development of siRNA therapeutics via appropriate chemical modifications. We will summarize our chemical conjugation strategies for delivery and our efforts for scale-up and manufacture of siRNAs.

MEDI 167

Lead generation and optimization strategies for the development of orally efficacious fXa inhibitors

Michael R. Wiley, *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285*

The search for novel antithrombotics has emerged as one of the most active areas in drug discovery due to the large number of patients afflicted each year with thrombotic diseases, coupled with the therapeutic limitations of warfarin. This effort has largely been focused on the identification of direct, orally-available inhibitors of coagulation enzymes; trypsin-like serine proteases whose disease linkage has been validated in animal models with selective, protein-based inhibitors. Given the attractiveness of many of these targets, we pursued a lead generation strategy focused on the identification of inhibitors with the greatest potential for yielding an oral drug, and prioritized the corresponding biological targets on that basis. This strategy was used to explore chemical leads from a variety of sources and ultimately led to the selection of fXa as our primary target for drug discovery. This presentation will highlight lessons learned from SAR studies on a series of D-amino acid based fXa inhibitors we explored in collaboration with colleagues at Protherics, Ltd, as well as the methods used to prioritize the inhibitor characteristics deemed most important for the selection of LY517717 as a candidate for clinical investigation.

MEDI 168

Development of factor VIIa-TF complex inhibitors for the treatment of cardiovascular disease

Wendy B. Young, *Celera, 180 Kimball Way, South San Francisco, CA 94080*

Several structural classes of highly selective and potent factor VIIa/Tissue Factor complex inhibitors were generated through structure-based design. The rationale for gaining selectivity for the fVIIa/TF complex versus related trypsin-like serine proteases will be outlined. Additionally, the pharmacokinetic and pharmacodynamic properties of lead analogs in several preclinical species as well as the efficacy (reduction of thrombus formation) in an arterial baboon thrombosis model will be described. Work toward orally bioavailable inhibitors will also be presented.

MEDI 169

Design and synthesis of novel himbacine based thrombin receptor (Par-1) antagonists

Mariappan V. Chelliah[1], *Samuel Chackalamannil*[1], *Yan Xia*[1], *Keith Eagen*[1], *Hsingan Tsai*[1], *Martin Clasby*[1], *Xiaobang Gao*[1], *William J. Greenlee*[1], *Ho-Sam Ahn*[2], *George Boykow*[2], *Carolyn Foster*[2], *Yunsheng Hsieh*[2], *Jacqueline Agans-Fantuzzi*[2], *Matthew Bryant*[2], *and Madhu Chintala*[2]. *(1) Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Rd, Kenilworth, NJ 07033, mariappan.chelliah@spcorp.com, (2) Biological Research, Schering-Plough Research Institute*

Thrombin, a serine protease, plays a major role in the activation various cell types such as platelets and smooth muscle cells in addition to its central role in hemostasis. The cellular pathway involves proteolytic cleavage of specific cell-surface receptors known as protease activated receptors (PARs). Four subtypes of PARs are known (PAR-1, PAR-2, PAR-3, PAR-4), among which PAR-1 plays a key pathophysiological role in thrombotic disorders. PAR-1, also known as the thrombin receptor, is widely distributed in the human platelets, endothelial cells and smooth muscle cells. Activation of PAR-1 by thrombin causes platelets to bind to fibrinogen, which results in the formation of a thrombus at the injury site. A thrombin receptor antagonist is expected to selectively interrupt the cellular activation of thrombin without affecting its enzymatic generation of fibrin. This is expected to result in the identification of a safe antithrombotic drug with less bleeding side effect. We will present the SAR studies on a novel series of PAR-1 antagonist based on a himbacine lead which resulted in very potent analogs with good efficacy after oral dosage.

MEDI 170

Design and synthesis of a novel orally efficacious PAI-1 inhibitor for the treatment of fibrinolytic impairment

Scott C. Mayer[1]*, Thomas Antrilli*[2]*, Ann Aulabaugh*[3]*, John A. Butera*[1]*, David L. Crandall*[2]*, Hassan Elokdah*[4]*, Stephen Gardell*[2]*, Eric Gundersen*[4]*, James Hennan*[2]*, Girija Krishnamurthy*[3]*, Geraldine R. McFarlane*[1]*, Gwen Morgan*[2]*, and Robert Swillo*[2]*. (1) Chemical and Screening Sciences, Wyeth Research, CN 8000, Princeton, NJ 08543, Fax: 732-274-4505, mayers@wyeth.com, (2) Cardiovascular and Metabolic Diseases Research, Wyeth Research, (3) Chemical and Screening Sciences, Wyeth Research, (4) Chemical & Screening Sciences, Wyeth Research*

Plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor, regulates fibrinolysis through its modulation of plasmin. Under normal physiological conditions, PAI-1 acts as an inhibitor of both urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA) leading to limited plasmin generation. Elevated levels of PAI-1 have been associated with diseases of fibrinolytic impairment. In acute thrombosis, PAI-1 plays a role in clot stabilization, while in diseases such as atherosclerosis and cancer PAI-1 regulates tissue remodeling. Therefore, inhibition of PAI-1 could restore the central role of plasmin. Our work led to the discovery of a novel series of substituted naphthyl indoles as PAI-1 inhibitors. Of these, PAI-749 binds PAI-1 with high affinity and exhibits oral efficacy in preclinical models of arterial and venous thrombosis. Due to its in vitro potency, in vivo oral efficacy and its safety in preclinical animal models, PAI-749 has advanced to clinical trials. This presentation will highlight the synthetic strategies and structure-activity relationship studies leading to the discovery of the naphthyl indole series as well as the preclinical data of PAI-749 leading to its advancement to clinical trials for the treatment of fibrinolytic disorders.

MEDI 171

TAFIa Inhibitors for treatment of thrombosis

***Julian Blagg**, Discovery Chemistry, Pfizer Global Research and Development, Sandwich Laboratories, Sandwich, Kent, TN CT13 9NJ, United Kingdom, Fax: 0044 1304 651987, julian.blagg@pfizer.com*

Thrombin Activatable Fibrinolysis Inhibitor (TAFI) has emerged as a key molecular link between coagulation and fibrinolysis. Activation of the TAFI zymogen by thrombin/thrombomodulin generates an unstable basic carboxypeptidase (TAFIa) that inhibits fibrinolysis through removal of C-terminal lysine residues from the surface of a fibrin clot. Inhibition of TAFIa has the potential to enhance endogenous fibrinolysis and represents a novel anti-thrombotic approach that does not rely on direct inhibition of coagulation. The presentation will describe the medicinal chemistry programme that led to the discovery of a novel series of imidazole-propionic acids that are potent inhibitors of TAFIa and exhibit excellent selectivity over related enzymes, such as plasma carboxypeptidase N. Clinical data will be disclosed which confirms the potential of UK-396,082 as an oral TAFIa inhibitor in humans.

MEDI 172

Progress on the etiology, modeling and treatment of Parkinson's disease

***Michael A. Schwarzschild**, Neurology Service, Massachusetts General Hospital, MassGeneral Institute for Neurodegenerative Disease, 114 16th Street, Boston, MA 02129, Fax: 617-724-1480, michaels@helix.mgh.harvard.edu*

Although the cause(s) of Parkinson's disease (PD) remain largely unknown, it is clear that its characteristic gradual slowing of normal movements results from the progressive loss of dopaminergic neurons projecting from the substantia nigra to the striatum. Current therapies can substantially imrpove the motor symptoms of PD by various dopamine replacement strategies. However they do not necessarily address the underlying degeneration of dopaminergic and other neurons. Moreover, standard pharmacotherapy is limited by development of abnormal involuntary movements (dyskinesias) that can eventually develop as the disease advances, as well as psychosis and other adverse events. Using increasingly refined laboratory models of disease progression as well as PD symptoms, multiple novel therapeutic targets have emerged in pursuit of improved antipakrinsonian treatment.

MEDI 173

Adenosine A2A antagonists: New therapeutic approach against Parkinson's disease

***Junichi Shimada**, Medicinal Chemistry Research Laboratories, Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan, Fax: +81-559-86-7430, junichi.shimada@kyowa.co.jp*

Adenosine modulates a great variety of biological functions both in nervous system and peripheral tissues. Most of these effects appear to be mediated via specific cell

surface receptors (A1, A2A, A2B and A3). In contrast to the wide distribution of the A1 and A2B receptors in brain, A2A receptors appear to be confined to the striatum, nucleus accumbens, and olfactory tubercle. The discrete distribution of the A2A receptor suggests a specific functional role of the A2A receptor in neuronal communication in basal ganglia.

We have reported that 1,3,7-trialkylxanthine derivatives substituted with (E)-8-styryl groups act as selective A2A-antagonists both in vitro and in vivo. In particular, (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1H- purine-2,6-dione (KW-6002, Istradefylline) was found to be a potent A2A antagonist. SAR of xanthine derivatives and pharmacological data of KW-6002 including recent clinical results will be presented.

MEDI 174

Novel triazolopyrazine, triazolopyrimidine, and triazolotriazine derivatives as potent and selective adenosine A2a receptor antagonists

Gnanasambandam Kumaravel[1], *Thomas Engber*[2], *Chi B Vu*[3], *Hairuo Peng*[3], *James E Dowling*[1], *Gang Yao*[3], *Jeffery T Vessels*[1], *Daniel Scott*[1], *and Russell C Petter*[3]. *(1) Department of Medicinal Chemistry, Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142, Fax: 617-679-2616, Gnanasambandam.kumaravel@biogenidec.com, (2) Department of Pharmacology, Biogen Idec, (3) N/A*

Adenosine exerts diverse pharmacological effects and endogenous adenosine is presumed to be an important physiological regulator in mammalian systems. Many of the effects of adenosine appear to result from interaction with four cell surface adenosine receptors termed A1, A2a, A2b and A3. The adenosine A2a receptors are highly expressed in the striatum and play an important role in regulating motor function. Selective adenosine A2a antagonists have been shown to improve motor disabilities in preclinical animal models of Parkinson's disease, and one antagonist has been shown to be effective in human clinical trials for Parkinson's disease. Our main objective was to identify novel, potent, selective and in vivo active A2a antagonists based on various triazolo heterocycles. This talk will focus on the discovery of these compounds, the synthesis, structure-activity relationships observed and their efficacy in various animal models of Parkinson's disease.

MEDI 175

Dual-mechanism MAO-B inhibitors and A2A antagonists in Parkinson's disease therapy

Cornelus J. Van der Schyf*, Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center, 1300 Coulter Drive, Amarillo, TX 79106, Fax: 806-356-4034, neels.vanderschyf@ttuhsc.edu, Jacobus P. Petzer, Department of Chemistry, Virginia Tech, Kay P. Castagnoli, Department of Chemistry, Virginia Polytechic Institute and State University, Jiang-Fan Chen, Department of Neurology, Boston University, Michael A. Schwarzchild, Department of Neurology, Harvard Medical School, and N. Castagnoli Jr., 107 Davidson Hall, Virginia Polytechnic Inst. & State University*

Most drugs used in managing Parkinson's disease (PD) enhance nigrostriatal dopaminergic action through many mechanisms. Compounds that simultaneously act on several of these mechanisms suggest that a single structure may offer the combined benefits of addressing multiple pharmacologic targets, each with significant benefits in PD. Caffeine and other antagonists of the adenosine A2a receptor provide symptomatic relief in PD and have been found to be neuroprotective in PD models. A second class of compounds that is neuroprotective in PD models includes inhibitors of monoamine oxidase B (MAO-B), an enzyme that regulates levels of brain neurotransmitters, including dopamine. We examined several styrylxanthinyl derivatives for overlapping A2a antagonist and MAO-B inhibitory properties and established that many of the compounds tested exhibit such a unique dual mechanism. The results of these studies offer an opportunity to design multimodal drugs that may have enhanced therapeutic and neuroprotective potential in the treatment of PD.

MEDI 176

α-Synuclein misfolding and aggregation: drug discovery target for Parkinson's disease

Hiroshi Kase *and Hiromi Nonaka, Fuence Co., Ltd, RIKEN W207, Hirosawa, Wakoshi, Saitama 351-0198, Japan, Fax: +81-48-462-1169, kase@fuence.co.jp*

Neurodegenerative diseases have common cellular and molecular mechanisms including protein aggregation and inclusion body formation. The earlier steps in the cascade of aggregation may be directly tied to the pathogenesis. We have developed a novel ‘mechanochemcal method', which can monitor, in real time, the earlier aggregation steps, and we have used it as an efficient tool for the screening of therapeutics and diagnostics aimed at protein conformational diseases. In Parkinson's disease (PD), α-synuclein, a major constituent of Lewy bodies, plays a central role in PD pathogenesis. We have developed a screening system for agents to restore α-synuclein aggregation using our mechanochemcal method, and found FNC-1101. The compound interacted with the β-sheet structure in α-synuclein and dose-dependently inhibited the earlier steps of α-synuclein aggregation. The compound could be a promising lead for drugs to retard the progression of PD, but it could also be a potential diagnostic marker for PD.

MEDI 177

Second generation MLK inhibitors

Robert L. Hudkins*, Medicinal Chemistry, Cephalon, Inc, 145 Brandywine Parkway, West Chester, PA 19380, Fax: 610-738-6558, rhudkins@cephalon.com*

Our research has focused on developing potent, selective inhibitors of mixed lineage kinases (MLKs) for the treatment of neurodegenerative diseases. The MLKs are a critical upstream activating component of the stress-activated protein kinase-signaling cascade regulating JNK activation and subsequent cJun phosphorylation leading to neuronal cell death. Several lines of evidence indicate that neuronal apoptosis may be an important mechanism contributing to the progression of

disability in Parkinson's and Alzheimer's diseases. Although the few available therapies afford some degree of symptomatic relief, none prevents the progression of the disease or delays the pathological neuronal cell death associated with the disease. CEP-1347 was the first compound from this program that advanced to late clinical evaluation for Parkinson's disease. Although it displayed an impressive preclinical profile in several in vitro and in vivo models, the semi-synthetic natural product does not display ideal pharmacokinetic properties. Presented will be SAR and structural studies of family selective, synthetic second generation MLK inhibitors with improved properties.

MEDI 178

Coronary artery disease risk factors: Successes, failures and future directions

***Kevin G. Peters**, Cardiovascular Research, Procter & Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, OH 45040, peters.kg@pg.com*

Despite advancements in prevention and treatment, coronary artery disease remains the major cause of death in the US and is on the rise world wide. Targeting risk factors such as hypertension and LDL cholesterol has led to significant decreases in the incidence of initial and recurrent coronary events. However, even with aggressive LDL lowering the incidence of recurrent cardiovascular events remains about 20% in the two years following acute myocardial infarction. This persistent, residual cardiovascular risk has led to an intense search for additional cardiovascular risk factors. Recently, focus has centered on HDL and insulin resistance as two key risk factors. Data supporting these risk factors as important contributors to the development of coronary artery disease will be discussed. Hopefully, by defining and developing approaches to address these and other emerging risk factors, progress will continue toward the goal of minimizing the impact of coronary artery disease.

MEDI 179

Discovery of orally bioavailable, non-steroidal mineralocorticoid receptor antagonists: A tale of three platforms

***P. K. Jadhav**[1], Donald P. Matthews[1], Jonathan Green[1], Kevin Fales[1], Kostas Gavardinas[1], Douglas Gernert[1], Michael G. Bell[1], David A Neel[1], Peter S. Lander[1], Timothy A. Grese[1], Anthony G Borel[2], Chen Zhang[2], Sally Kelley[2], Karen Zimmermann[2], and Mitchell I. Steinberg[2]. (1) Discovery Chemistry Research & Technologies, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, pkjadhav@lilly.com, (2) Lilly Research Laboratories, Eli Lilly and Company*

Mineralocorticoid Receptor is a member of the steroid subfamily of Nuclear Hormone Receptors (NHR). It is so named for its role in regulation of mineral ions such as sodium, potassium, and magnesium. Aldosterone is an endogenous ligand of MR. Elevated levels of Aldosterone have been implicated in various cardiovascular disorders including high blood pressure, cardiac and perivascular fibrosis, and potentiation of catecholamines. MR blockade is emerging as an important mechanism of action for identification of therapeutic agents for the treatment of cardiovascular

diseases. Spironolactone and eplerenone are the only two steroid based MR antagonists available for the treatment of mineralocorticoid excess. We have identified and optimized potent, selective and orally active MR antagonists based on three structurally distinct platforms viz. oxindole, indole and dibenzosuberane. The evolution of SAR leading to orally bioavailable MR antagonists which are efficacious in the aldosterone infusion animal model of hypertension will be discussed.

MEDI 180

Synthesis and structure activity relationship of potent, selective piperidine-based PPARα agonists

Cheryl M Hayward[1], *Scott W. Bagley*[1], *William A. Hada*[1], *Ernest S. Hamanaka*[1], *Charles Aldinger*[2], *Bruce Auerbach*[2], *Omar L. Francone*[2], *Mehrdad Haghpassand*[2], *Christopher D. Kane*[2], *H. James Harwood Jr.*[2], *Lori Royer*[2], *Kimberly Stevens*[2], *and Janice D. Wagner*[3]. *(1) Cardiovascular and Metabolic Diseases, Medicinal Chemistry, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, Fax: 860-715-4483, cheryl.m.hayward@pfizer.com, (2) Cardiovascular and Metabolic Diseases, Discovery Biology, Pfizer Global Research and Development, (3) Department of Pathology, Wake Forest University*

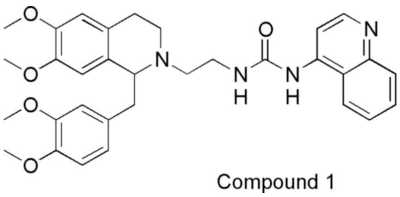
The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the superfamily of nuclear receptors. Three subtypes (α, β, γ) have been identified and display distinct physiological functions based on their tissue distribution and gene expressions. Each subtype is the focus of intense pharmaceutical pursuit. PPARα is implicated in the regulation of lipid and lipoprotein metabolism and is responsible, at least in part, for the hypolipidemic activity of the fibrates, which have been used clinically to treat dyslipidemia for several decades. However, the fibrates are weak and non-selective PPARα agonists. Potent, selective agents would be expected to provide more robust effects and to further delineate the specific physiological effects of the subtype. A series of potent PPARα-selective piperidine-based agonists is described including their synthesis, structure activity relationship and pharmacological effects.

MEDI 181

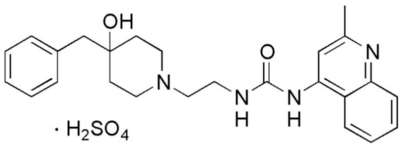
Structure activity relationship of quinolyl-ureas as Urotensin-II receptor antagonists: The discovery of palosuran

Jörg Velker, *Hamed Aissaoui, Christoph Binkert, Magdalena Birker-Robaczewska, Céline Boukhadra, Daniel Bur, Martine Clozel, Walter Fischli, Patrick Hess, Boris Mathys, Keith Morrison, Claus Müller, Oliver Nayler, Alexander Treiber, Michael W. Scherz, and Thomas Weller, Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, Allschwil 4123, Switzerland, jorg.velker@actelion.com*

Palosuran is the first orally active urotensin-II receptor antagonist to be described. We will present the discovery of palosuran from the first screening hit and discuss the structure-activity relationship of related quinolyl-urea derivatives.



Compound 1



Palosuran

Urotensin-II (U-II) is a cyclic peptide that has been described as the most potent vasoconstrictor known. It is highly conserved across species but was first isolated from fish urophysis and extensively studied for its role in osmoregulation. It signals through the G-protein coupled receptor GPR14, now named UT receptor. The UT receptor is found in skeletal muscle, vascular smooth muscle, cerebral cortex, kidney cortex and heart. U-II is a potent vasoconstrictor, regulates pancreatic insulin release, and may also have pathophysiological roles in cardiac and skeletal muscle. We sought an orally active low-molecular weight UT receptor antagonist, an essential tool for further characterizing the physiological and pathophysiological roles of the U-II system.

A random screening campaign identified compound 1 as a potent UT receptor antagonist. Compound 1 had been initially synthesized in the search for orexin receptor antagonists. Variations in the quinolyl and the urea moieties revealed a sensitive structure-activity relationship at the UT receptor. Modifications of the tetrahydroisoquinoline moiety were largely tolerated and used to optimize pharmacological parameters. Palosuran proved to have an optimal combination of properties for selection as a clinical development candidate.

MEDI 182

Pathway selective modulators in the elucidation of anti-atherosclerotic effects of LXR

William J. Zuercher, *Discovery Research, GlaxoSmithKline, Five Moore Drive, Research Triangle Park, NC 27709, william.j.zuercher@gsk.com*

The Liver X Receptors, LXRα and LXRβ, are ligand-activated transcription factors from the nuclear receptor superfamily. In vitro and in vivo studies have demonstrated the potential of LXR ligands in the treatment of cardiovascular disease. The LXRs are known to promote reverse cholesterol transport (RCT) and inhibit inflammatory response pathways, each of which may contribute to an anti-atherosclerotic effect. Herein we describe the identification of pathway selective LXR modulators that will be valuable tools for determining the relative contribution of the RCT and inflammation components in the anti-atherosclerotic activity of the LXRs.

MEDI 183

The battle against HIV: A worthy fight

Joel R. Huff, *Merck Research Laboratories (Retired), 1123 River Rock, New Braunfels, TX 78130, joel.huff2@gmail.com*

Human immunodeficiency virus infects more than forty million people worldwide, with almost a million of those living in the United States. The pharmaceutical industry has worked for two decades developing drugs to combat this pandemic. Their efforts have resulted, for many, in converting an almost certain death sentence into a manageable, chronic disease. Work within Merck Research Laboratories to develop inhibitors for the three virally encoded enzymes, protease, reverse transcriptase, and integrase, will be described along with lessons learned and applied to other drug discovery problems.

MEDI 184

Structure-based design of a series of potent inhibitors of human ƒ"-secretase

***Joseph P. Vacca**, Department of Medicinal Chemistry, Merck Research Laboratories, WP14-3, West Point, PA 19486, joe_vacca@merck.com*

ƒ"-secretase (ƒ"-site APP cleaving enzyme or BACE-1), a novel type I transmembrane aspartyl protease whose identity remained elusive until 1999, is generally accepted as the key enzyme that commits APP catabolism to the amyloidogenic pathway. As such ƒ"-secretase inhibition is considered an attractive therapeutic target for the treatment and prevention of Alzheimer's Disease. A high throughput screening effort resulted in the identification of a low micromolar non-peptide BACE-1 inhibitor and its mode of binding was subsequently defined through crystallographic determination of the enzyme-inhibitor complex. This resorcinol-derived inhibitor was shown to interact with the BACE-1 catalytic dyad in an unprecedented manner among aspartyl protease inhibitors. Additionally, this complex revealed a heretofore unknown S3 sub-pocket within ƒ"-secretase that was created by the inhibitor. The unique binding mode of this compound coupled with the structural information gleaned therein has guided our drug design effort toward more potent and selective BACE-1 inhibitors. Herein we describe the development of a series of low nanomolar, cell-permeable BACE-1 inhibitors that demonstrably block the production of a secreted ƒ"-secretase amino terminal fragment (sAPP_NF) in cell culture. In addition to impressive cellular activity this series of inhibitor also display enhanced selectivity towards other aspartyl proteases.

MEDI 185

Discovery of sertraline (Zoloft®)

***B. Kenneth Koe**, Charles A. Harbert, Reinhard Sarges, Albert Weissman, and Willard M. Welch, Pfizer Inc (retired), Groton, CT 06340, kenkoe@pol.net*

Sertraline was discovered in an era of drug research, before intensive high throughput screening, when chemists and biologists formulated rational hypotheses concerning the activity of psychotherapeutic agents and synthesized and evaluated new chemicals based on the perceived molecular requirements for a common biological activity. Our ultimate success also depended significantly on delving empirically into ancillary neuropharmacology guided by unexpected findings. Thus, lometraline (N,N-dimethyl-8-chloro-5-methoxy-1-aminotetralin), a structural

modification of the potential tricyclic neuroleptic pinoxepin, emerged not as an antipsychotic but a potential anxiolytic. Attaching a 4-phenyl moiety to N-methyl-1-aminotetralin led to a new property—inhibition of norepinephrine, dopamine, and serotonin transporters, found only in the *trans*-1R,4S isomer, tametraline, a potential antidepressant. Introducing substituents, particularly electronegative atoms like 3,4-dichloro into the 4-phenyl ring enhanced the potency of tametraline, but, remarkably, made the *cis*-1S,4S isomer (sertraline) a selective serotonin reuptake inhibitor (SSRI). Sertraline became Zoloft®, a leading psychotherapeutic drug for treating depression and anxiety.

MEDI 186

The human nature of molecules

***Johann Gasteiger**, Department of Organic Chemistry, Computer-Chemie-Centrum, University of Erlangen-Nuremberg, Naegelsbachstrasse 25, 91052 Erlangen, Germany, Fax: +49-9131.8526566, gasteiger@chemie.uni-erlangen.de*

The international language of chemistry is a two-dimensional structure diagram. However, molecules are three-dimensional species much like human beings. I have always appreciated the beauty of molecules and, from the very beginning of my work in chemoinformatics, have resisted any attempt to dissect molecules into fragments. Rather, I have tried to respect the integrity of molecules and have treasured that they have a three-dimensional structure, have a skin, change shape, and have left- and right hands.

Computational approaches to the generation of 3D molecular models, to the calculation of molecular surface properties, to the generation of multiple conformations, and to the quantification of molecular chirality will be presented. It will be shown how these structure representations can be used to investigate the relationships between molecular structure and physical, chemical, and biological properties of compounds with particular emphasis on drug design.

MEDI 187

Reflections on predicting preclinical PK from physical properties

***Yvonne Martin**, D-47E, AP10/2, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60065-6100, Fax: 847-937-2625, yvonne.c.martin@abbott.com*

Although many have presented rules-of-thumb to predict animal and human pharmacokinetics from molecular structure, do these rules stand up to detailed scrutiny? What are the lessons learned?

MEDI 188

Protein medicinal chemistry, optimization of endocrine hormones

***Richard D. DiMarchi**, Chemistry, Indiana University, 800 E. Kirkwood Avenue, Bloomington, IN 47405, Fax: 812-856-3358, RDiMarch@indiana.edu*

The scientific work from the DiMarchi group was central to the discovery and the commercial development of a number of peptide and protein based medicines, such as Forteo®, rGlucagon®, Humalog® Humatrope®, Humulin® , and Xigris®. Humalog represents the first biosynthetic hormone optimized by rDNA technology approved as a human medicine. It established the precedent that endogenous hormones were not optimized for use as drugs, and that through insightful structural modification a more efficacious and safer protein could be developed. The recent emergence of new technologies in protein biosynthesis is dramatically enlarging the structural space that can be utilized by protein medicinal chemists. Our current work is focused on the exploration of non-natural amino acids as a means to further enhance the pharmacological properties of biosynthetic proteins. Glucagon and insulin represent two hormones where we have successfully employed this strategy.

MEDI 189

Therapeutic applications of costimulation blockade in transplantation

***Kenneth A. Newell**, Department of Surgery, Emory Transplant Center, Emory University, Atlanta, GA 30322, Fax: (404) 727-3660, Kenneth.Newell@emoryhealthcare.org*

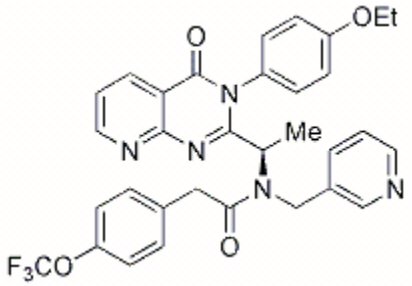
While immunosuppressants introduced over the past two decades have improved the outcome of transplantation, their non-specific effects produce a global state of immunosuppression that increases the risks of infection and malignancy while drug-specific toxicities contribute to hypertension, diabetes, hyperlipidemia, and renal insufficiency. To avoid these complications, agents that target lymphocytes are being developed. Among these new agents are those that modify T cell costimulatory signals. Complete T cell activation requires at least two signals; one transduced when the TCR recognizes peptides presented by MHC molecules and a second, non-antigen specific signal delivered by cell surface costimulatory molecules. A classic example of a costimulatory pathway is CD28 expressed by T cells and its ligands CD80 and CD86 (B7.1 and B7.2) expressed on APCs. Experimental work in the early 1990s demonstrated that blockade of the CD28 pathway (with or without blockade of a second costimulatory molecule CD154) prolonged graft survival in several experimental models. However, subsequent work demonstrated that CD8+ T cell and memory T cell function was not dependent on these molecules. Recently additional costimulatory molecules have been identified most of which belong to either the TNF receptor superfamily (i.e., 4-1BB, OX40, and CD27) or to the B7 superfamily (i.e., ICOS). In addition to molecules that provide positive costimulatory signals, a number of "inhibitory" costimulatory molecules have been identified (i.e., CTLA4 and PD1). This presentation will provide an overview of the biology of these pathways and of immunosuppressive strategies, both experimental and clinical, using costimulation blockade to inhibit allo- and autoimmunity.

MEDI 190

Optimization and biological profile of 2,3-substituted quinazolin-4-ones as potent CXCR3 antagonists

Julio C. Medina[1], *Tassie L. Collins*[2], *Michael Johnson*[2], *An-Rong Li*[2], *Zice Fu*[2], *Jiwen Liu*[2], *Alan Huang*[2], *George Tonn*[2], *Daniel Dairaghi*[3], *Christopher Lawrence*[2], *Georges Hollander*[4], *Luca Piali*[4], *Thomas Schall*[3], *Tim Sullivan*[2], *and Qiuping Ye*[2]. *(1) Amgen SF, 1120 Veterans Boulevard, South San Francisco, CA 94080, Fax: (650)244-2015, (2) Amgen SF LLC, (3) ChemoCentryx, (4) Basel University*

CXCR3 is a chemokine receptor associated with the recruitment of leukocytes from the peripheral blood into inflamed tissue. The ligands for CXCR3 are Mig (CXCL9), IP10 (CXCL10) and ITAC (CXCL11). CXCR3 and its ligands are found in increased levels in samples of diseased tissue taken from patients suffering from organ transplant rejection, inflammatory bowel disease, multiple sclerosis, psoriasis and rheumatoid arthritis. Therefore, it has been postulated that blockade of CXCR3 may play a beneficial role in the treatment of these diseases. In this presentation we will describe the optimization of the potency and pharmacokinetic properties of a series of 2,3-substituted quinazolin-4-ones with potent CXCR3 antagonism that led to the discovery of the clinical candidate AMG 487. In addition, we will also discuss the efficacy of these compounds in several in vivo models.



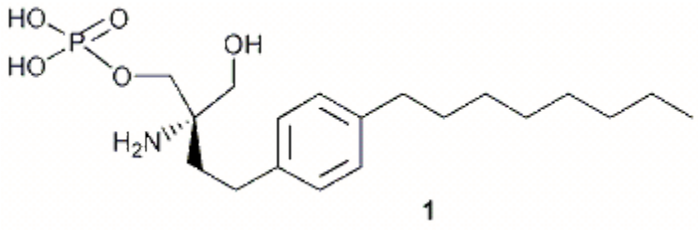
MEDI 191

Role of sphingosine-1-phosphate receptor modulators in the prevention of transplant rejection

Rainer Albert[1], *Christian Beerli*[1], *Volker Brinkmann*[1], *Peter Bühlmayer*[1], *Christian Bruns*[1], ***Nigel Cooke***[1], *Peter Ettymayer*[2], *Eric Francotte*[1], *Nathanael Gray*[3], *Danilo Guerini*[1], *Klemens Hoegenauer*[2], *Klaus Hinterding*[1], *Peter Nussbaumer*[2], *Barbara Nüsslein-Hildesheim*[1], *Charles Pally*[1], *Shifeng Pan*[3], *Carsten Spanka*[1], *Markus Streiff*[1], *Sven Weiler*[1], *Trixie Wagner*[1], *Frédéric Zécri*[1], *and Marcus Zollinger*[1]. *(1) Novartis Institute for Biomedical Research, Basel, Switzerland, nigel_graham.cooke@novartis.com, (2) Novartis Institute for Biomedical Research, Vienna, Austria, (3) Genomics Institute of the Novartis Foundation, La Jolla, CA 92121*

FTY720 is a novel immunomodulator which is highly effective in animal models of transplantation and autoimmunity. In vivo phosphorylation of FTY720 in rats and humans results exclusively in the (S)-configured FTY720 mono-phosphate 1. FTY720 mono-phosphate 1 signals as an agonist through four of the five sphingosine-1-phosphate (S1P) receptors. This presentation describes the SAR, in-vitro and in-vivo

characterization of sub-type selective S1P receptor modulators and the use of these compounds to investigate the role of S1P receptors in the prevention of acute rejection of transplanted organs in rodents. The contribution of S1P-1 and S1P-3 receptor agonism to the transient reduction in heart rate observed with FTY720 will also be discussed.



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MEDI 192

Identification and characterization of acridone based Inosine 5'-Monophosphate Dehydrogenase (IMPDH) inhibitors for the prevention of solid organ transplant rejection

TG. Murali Dhar[1], *Scott H. Watterson*[1], *Ping Chen*[1], *Yufen Zhao*[1], *Zhongqi Shen*[1], *Henry H. Gu*[1], *Zili Xiao*[1], *Catherine A. Fleener*[2], *Katherine Rouleau*[2], *Mary Obermeier*[3], *Connie Kliwinski*[3], *Jennifer Postelnek*[1], *Joel C. Barrish*[1], *Jeff A. Robl*[1], *Robert Townsend*[2], *and Edwin J. Iwanowicz*[1]. *(1) Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, Fax: (609) 252-7410, murali.dhar@bms.com, (2) Immunology, Inflammation and Pulmonary Discovery, Bristol Myers Squibb Pharmaceutical Research Institute, (3) Metabolism and Pharmacokinetics Department, Bristol-Myers Squibb Pharmaceutical Research Institute*

Inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo synthesis of guanosine nucleotides, catalyzes the irreversible NAD-dependent oxidation of inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP). CellCept™(MMF), a prodrug of mycophenolic acid (MPA) has clinical utility due to its inhibition of IMPDH, for the treatment of transplant rejection. The overall clinical benefit of MMF is limited by what is generally believed to be compound-related dose limiting gastrointestinal (GI) toxicity. Thus, development of an IMPDH inhibitor with a novel structure and different pharmacokinetics may reduce the GI toxicity and allow for increased efficacy. This presentation will detail the discovery and SAR of acridone based IMPDH inhibitors which potently and specifically inhibits IMPDH type II and type I enzymes in vitro. One of the compounds in this series, BMS-566419, effectively prolonged graft survival in the rat heterotopic heart transplant model as a single agent or synergistically in combination with other agents.

MEDI 193

CCR5 Blockade modulates alloimmunity in primates

***Julie A. DeMartino**, Merck Research Laboratories, RY80-A22, P.O. Box 2000, Rahway, NJ 07065, Fax: 732-594-7177, Julie_DeMartino@merck.com*

Potent and selective small molecule CCR5 antagonists like Merck's CMPD 167 ((N-[(1R,3S,4S)-3-((4-(3-benzyl-1-ethylpyrazol-5-yl)piperidin-1-yl)methyl)-4-(3-fluorophenyl)cyclopentan-1-yl]-N-methyl-D-valine) have best been characterized in primates as HIV-1 antiviral therapies, but to date the anti-inflammatory properties of these agents, which target a chemotactic cytokine receptor expressed on macrophages and activated T-cells, have been less well investigated. We explored the immunomodulatory effects of CMPD 167 in an established cynomolgus monkey cardiac allograft model since recruitment of CCR5-bearing cells from the peripheral blood to the allograft is a hallmark of the rejection process and since renal transplant patients on standard immunosuppressive therapy and homozygous for a 32 base pair deletion mutation encoding a non-functional CCR5 receptor purportedly demonstrate enhanced long term graft survival. We found marginal effect of CCR5 monotherapy in blunting acute rejection despite evidence that peri-operative stress responses and recruitment of CCR5-bearing leukocytes into the graft were attenuated. In contrast, we found that CMPD 167-Cyclosporin A combination therapy further prolonged graft survival, delayed alloantibody production, and suppressed cardiac allograft vasculopathy relative to CsA monotherapy. CCR5 therefore represents an attractive therapeutic target to attenuate post-surgical stress responses and favorably modulate pathogenic alloimmunity in primates, including man.

MEDI 194

Design, synthesis and SAR of novel, heterocyclic Factor VIIa inhibitors

***Roopa Rai**, Aleksandr Kolesnikov, Paul A. Sprengeler, Steven Torkelson, Tony Ton, John Hendrix, Robin Stephens, William D. Shrader, Bradley A. Katz, Christine Yu, Ronnel Cabuslay, Ellen Sanford, Jim Janc, Erik Gjerstad, and Wendy B. Young, Celera, 180 Kimball Way, South San Francisco, CA 94080, roopa.rai@celera.com*

Direct inhibition of the Factor VIIa/Tissue-factor complex is a validated strategy for the development of anticoagulant drugs. The design, synthesis and structure activity relationships of novel heterocyclic Factor VIIa inhibitors containing 5-aminopyrrolo[3,2-b]pyridine as the P1 element will be described.

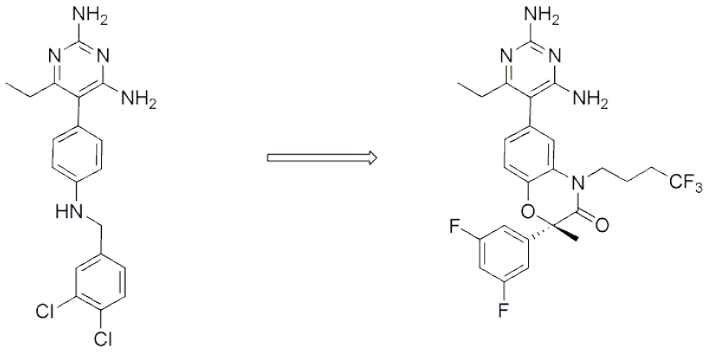
MEDI 195

Discovery of novel orally bioavailable renin inhibitors by structure based drug design

***Jeremy J Edmunds**[1], Thomas Belliotti[1], John Bryant[1], Cuiman Cai[1], Xue Min Cheng[1], Fred L. Ciske[1], Wayne L. Cody[1], Wendy Collard[1], Dennis M. Downing[1], Noe Erasga[1], Suzie Ferreira[1], Eric Hall[2], Daniel D. Holsworth[1], Mehran Jalaie[1], Aparna Kasani[2], Michael Kaufman[1], Chitase Lee[1], Tingsheng Li[2], Samarendra Maiti[2], Patrick McConnell[1], Ken Mennen[1], Robert Ostroski[1], Noel A. Powell[1], John Quin III[1], Mohammad Rahim[2], Michael Ryan[1], Michael A. Stier[1], Rajandra Subedi[2], Chad Van Huis[1], and Erli Zhang[1]. (1) Michigan Laboratories, Pfizer Global Research &*

Development, 2800 Plymouth Road, Ann Arbor, MI 48105, Jeremy.Edmunds@pfizer.com, (2) Naeja Pharmaceuticals

This oral presentation will highlight the progress that has been made at Pfizer relative to the design and synthesis of orally available renin inhibitors. As such we will disclose the synthesis, structure, renin inhibitory activity, CYP and dofetilide activity, in vivo blood pressure lowering, in vivo pharmacokinetic behavior in rats/dogs, and x-ray crystal structures of principally a heterocyclic series of renin inhibitors. These pyrimidine compounds were discovered by high throughput screening and a structure based drug design optimization. The series is characterized by compounds with MW of approx. 500 amu, good bioavailability in rats and dogs, half life predictive of once a day dosing in humans, and IC50s in the nanomolar level for renin. This will be the first disclosure of this series of compounds, and will likely generate a great deal of enthusiasm for renin as an anti-hypertensive target.



6 (HTS hit)
IC_{50} = 26 uM

15
IC_{50} = 0.048 uM
T1/2 = 2.9 h
Bioavail. = 74%

MEDI 196

Bicyclo[2.2.2]octyltriazole inhibitors of 11ß-hydoxysteroid dehydrogenase type 1: Pharmacological agents for the treatment of metabolic syndrome

Milana Maletic[1]*, Aaron Leeman*[1]*, Steven S. Mundt*[2]*, Hratch J. Zokian*[2]*, Kashmira Shah*[2]*, Jasminka Dragovic*[1]*, Kathryn A. Lyons*[1]*, Sam L. Koprak*[2]*, Gloria C. Koo*[2]*, Kang Cheng*[2]*, Eugene Y. Tan*[3]*, Rolf Thieringer*[2]*, Anne Hermanowski Vosatka*[2]*, Martin S. Springer*[2]*, James M Balkovec*[1]*, and Sherman T. Waddell*[1]*. (1) Department of Medicinal Chemistry, Merck Research Laboratories, Merck & Co., Inc, P.O. Box 2000, Rahway, NJ 07065, Fax: 732-594-9473, milana_maletic@merck.com, (2) Department of Atherosclerosis and Endocrinology, Merck Research Laboratories, Merck & Co., Inc, (3) Department of Drug Metabolism, Merck Research Laboratories, Merck & Co., Inc*

Metabolic syndrome is comprised of a cluster of health problems of metabolic origin that include abdominal obesity, hypertension, elevated fasting glucose, dyslipidemia, and atherosclerosis. In recent years compelling evidence has emerged suggesting that elevated levels of cortisol in adipose and liver tissues may contribute to the development of metabolic syndrome. Intracellular glucocorticoid concentrations in these tissues are regulated by an NADPH-dependent reductase 11ß-hydroxysteroid

dehydrogenase type 1 (HSD1), which, *in vivo*, converts cortisone to cortisol. A potent and selective inhibitor of HSD1 may, therefore, be a novel agent for treatment of the health problems associated with metabolic syndrome. We have developed a series of highly potent and selective bicyclo[2.2.2] octane triazole inhibitors of human and murine HSD1. Several of the compounds show excellent oral activity in a murine pharmacodynamic model and show efficacy in murine models of diabetes and atherosclerosis. The development of this series will be presented and the *in vivo*profiling of one compound will be highlighted.

MEDI 197

SAR study on a novel series of pyridocarbazole-based Melanin Concentrating Hormone Receptor-1 antagonists: Discovery of T0910792, a potent, selective and orally bioavailable agent that is efficacious in obesity models

Leping Li[1], *Pingchen Fan*[1], *Xiaoqi Chen*[1], *Jeff Mihalic*[1], *Ying Fu*[1], *Kang Dai*[2], *Lingming Liang*[2], *Michael Reed*[2], *Mathhew Wright*[3], *Pieter Timmermans*[3], *Jin-Long Chen*[2], *and Juan Jaen*[1]. *(1) Department of Chemistry, Amgen Inc, 1120 Veterans Blvd., South San Francisco, CA 94080, Fax: (650) 244-2015, lepingl@amgen.com, (2) Department of Biology, Amgen Inc, (3) Department of Pharmacokinetics, Amgen Inc*

Melanin concentrating hormone (MCH), a cyclic nonadecapeptide found in the CNS of all vertebrates, has been shown to play an important role in controlling eating behavior in mammals. Intraventricular injection of MCH increases food intake and body weight gain in rodents. Mice lacking expression of MCH are lean, hypophagic and hypermetabolic. Rodents have only one (MCHR1) of the two MCH receptors (MCHR1 and MCHR2). Targeted disruption of the MCHR1 gene in mice results in resistance to diet-induced obesity, , hyperactivity and altered metabolism. A unique alkaloid lead (compound 1, 2,11-dimethyl-2,3,4,4a,5,6,11,11a-octahydro-1H-pyrido[4,3-b]carbazole) was discovered in our laboratories. We have developed an asymmetric synthesis and demonstrated that the (4aR, 11R, 11aS)-configuration possesses the highest level of activity. We have also found that maintaining the basicity of the nitrogen atom on the piperidine ring and the hydrogen-bond donating capability of the tetrahydrocarbazole NH are essential for high affinity to MCHR1. The judicious introduction of water- and lipid-solublizing groups such as a tetrahydropyran with optimal spacers not only enhanced MCHR1 binding affinity but also significantly improved pharmacokinetic properties. Several potent antagonists of MCHR1 (IC50s < 1 nM) exhibit high selectivity over a broad panel of GPCRs and are orally bioavailable with excellent CNS permeability, an attribute that is essential for a CNS-targeting agent. T0910792, a leading candidate compound, has been shown to be efficacious in various in vivo anti-obesity models. Herein, we report the SAR development toward improving the potency, selectivity and overall pharmacokinetic properties and the in vitro and in vivo profiling of T0910792.

MEDI 198

Synthesis and biological activity of PTK 0796: A novel semisynthetic tetracycline in Phase I clinical trials

Mark L. Nelson[1], *Mohamed Ismail*[1], *Todd Bowser*[1], *Laura Honeyman*[1], *Ann Macone*[1], *Beena Bhatia*[1], *Atul Verma*[1], *Mark Grier*[2], *Joel Berniac*[1], *Rachid Mechiche*[1], *Kwasi Ohemeng*[1], *Pat Cannon*[1], *Janice Donatelli*[1], *David McKenney*[2], *and Stuart B. Levy*[1]. *(1) Paratek Pharmaceuticals, Inc, 75 Kneeland St, Boston, MA 02111, Fax: 617-275-0039, (2) Paratek Pharmaceuticals*

Antibiotic resistant bacteria have decreased the effectiveness of numerous antibiotics, including the tetracycline family of therapuetics. Our synthetic modifications of second generation tetracyclines has resulted in the production of numerous new classes of tetracyclines, novel 3rd generation compounds with activity against both Gram negative and Gram positive bacteria, both in vitro and in vivo. Our studies show that out of these classes, the position C9 aminomethylcyclines (AMCs) and derivative subsets, show potent activity against a broad spectrum of bacteria, are non-cytotoxic to mammalian cells, and possess activity in several animal infection models of disease, including S. pneumoniae. One compound, designated PTK 0796, has been chosen for development and is currently in Phase I human clinical trials.

MEDI 199

The development of AEG33783, a novel JNK pathway inhibitor, which prevents neuropathy induced by a wide variety of chemotherapeutic agents in animal models

James B. Jaquith[1], *Kimberly E. Hewitt*[2], *Stephen J. Morris*[3], *Alain Boudreault*[4], *Jon Durkin*[1], *and John Gillard*[1]. *(1) Department of Chemistry, Aegera Therapeutics Inc, 810 chemin du Golf, Verdun (Montreal), QC H3E 1A8, Canada, Fax: 514-288-9280, james.jaquith@aegera.com, (2) Department of Pharmacology, Aegera Therapeutics Inc, (3) Department of Molecular Biology, Aegera Therapeutics Inc, (4) Department of Biochemistry, Aegera Therapeutics Inc*

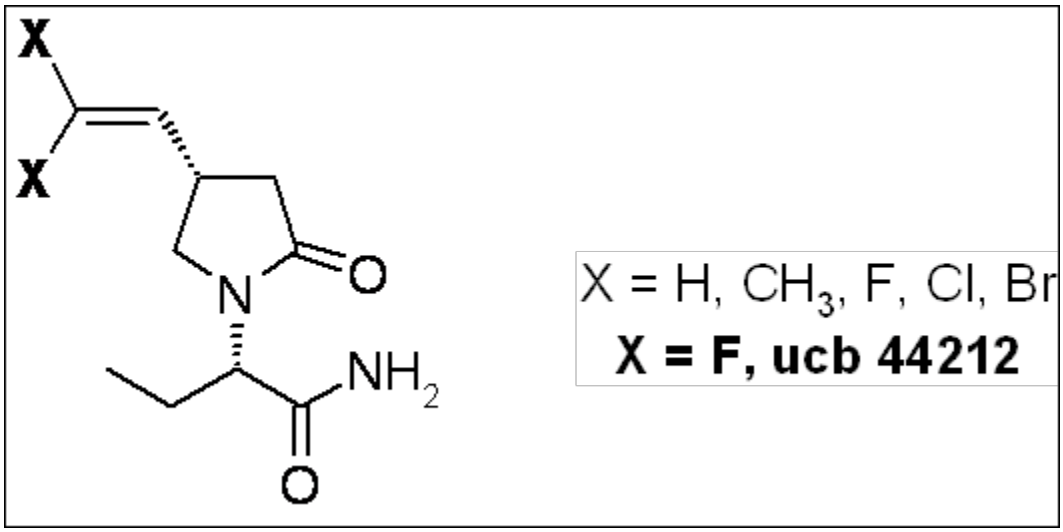
Peripheral neuropathies are a frequent and serious consequence of chemotherapy. AEG33783 is a neuroprotective Clinical Development candidate that protects primary SCG neurons from apoptosis induced by NGF withdrawal and from treatment with anti-cancer agents. Animal models utilizing paclitaxel, cisplatin or oxaliplatin demonstrated that AEG33783 attenuates the damaging effects of CT agents on nerve conduction velocity, without interfering with cancer chemotherapy, or affecting the pharmacokinetic profile of a co-administered chemotherapy. AEG33783 displays dose linearity pharmacokinetics with rapid tissue distribution. AEG33783 blocks apoptotic responses of neurons to chemotherapeutic stress by indirectly inhibiting the JNK pathway through HSP70 induction. In vivo analyses confirm these effects, showing that AEG33783 induces HSP70 expression in sciatic nerve, and attenuates cyclin D1 expression in dorsal root ganglia (DRG) neurons in response to cisplatin. AEG33783 represents a novel therapeutic strategy for the treatment of chemotherapy induced neuropathy.

MEDI 200

Discovery of Seletracetam: A new pyrrolidone derivative with potent antiepileptic properties and high tolerability in rodent models of Epilepsy

B. Kenda[1], *A. Matagne*[2], *P. Talaga*[1], *and P. Michel*[1]. *(1) Chemical Research, UCB, rue du Foriest, braine l'alleud 1420, Belgium, Fax: +3223862704, benoit.kenda@ucb-group.com, (2) CNS Research, UCB*

Levetiracetam (KeppraTM, UCB S.A.), a structural analogue of piracetam, is currently the fastest growing of the new add-on treatments of refractory partial onset seizures in adults. This drug combines significant efficacy and high tolerability with a unique mechanism of action. A CNS-specific binding site for Levetiracetam (LBS for Levetiracetam Binding Site) has now been identified as the Synaptic Vesicle Protein of type 2A (SV2A). It probably exerts a major role in the antiepileptic properties of KeppraTM. Indeed, there is a strong correlation between the affinity of a compound for SV2A and its ability to protect against seizures in an audiogenic mouse model of epilepsy. In advance of this discovery, we used this novel molecular target in a drug discovery program aiming at the discovery of ligands with significant affinity to SV2A. SAR around KeppraTM has been investigated in detail through more than 1000 molecules. Among many other substituents investigated at the 4 position of the pyrrolidinone, the dibromovinyl moiety was prepared as a precursor for the acetylenic derivative. It proved surprisingly potent in vitro as ligand for SV2A and in vivo in mice models of epilepsy (audiogenic seizure-prone mice and corneally kindled mice). Despite the rather unusual type of substituent and some misgivings about its possible toxicity or mutagenicity, these results led us to synthesise several 4-vinyl derivatives. All dihalovinyl compounds showed the same exceptional pharmacological behaviour in mice as well as various rat models of epilepsy. Selection of the 4-difluorovinyl derivative ucb 44212 (Seletracetam) was based on both pharmacological and in vivo and in vitro DMPK data. No evidence of mutagenic potential was observed and conventional safety and toxicity studies permitted human administration. A clinical Phase I program is currently well underway and acute administration in epileptic patients is scheduled for the early 2005.



MEDI 201

PRX-03140: The discovery and development of a novel 5HT4 partial agonist for the treatment of Alzheimer's disease

Ashis K. Saha[1], *Oren, M Becker*[2], *Silvia Noiman*[2], *Pradyumna Mohanty*[1], *Dongli Chen*[1], *Mercedes Lobera*[1], *Laurence Wu*[1], *Yael Marantz*[2], *Boaz Inbal*[2], *Alex Heifetz*[2], *Shay Bar-Haim*[2], *Dale S. Dhanoa*[1], *and Sharon Shacham*[1]. *(1) Chemistry, Predix Pharmaceuticals, 4 Maguire Road, Lexington, MA 02421, Fax: 781-372-3267, asaha@predixpharm.com, (2) Predix Pharmaceuticals Ltd*

Alzheimer's disease (AD) leads to severe cognitive decline and has devastating consequences on the world population. One in 10 Americans over age 65 suffer from AD and prevalence increases to five in 10 over age 85. It is estimated that 15 million people world wide suffer from Alzheimer's disease. Current treatments for Alzheimer's disease that target cerebral cholinergic systems have limited effectiveness in alleviating cognitive deficits or providing neuroprotection, while producing undesirable side effects. The main classes of drugs currently in use to treat AD are acetylcholinesterase inhibitors, such as donepezil (Aricept) and galantamine (Reminyl). However, response rate to these drugs is insufficient with, for example, only 1/3rd of patients responding to donepezil. It is hence generally believed that there is a significant unmet medical need for a well-tolerated, effective treatment for Alzheimer's disease that can be given once daily. Although dysfunction and death of cholinergic neurons comprise the central neuropathology observed in AD, there is increasing evidence that serotonin neurotransmission is altered in AD, including alteration of serotonin 5-HT4 receptor function. Stimulation of 5-HT4 receptors is associated with both effects on APP processing and on the release of acetylcholine. Development of a treatment that stimulates cerebral 5-HT4 receptor activity therefore has the potential for greater clinical benefits than acetylcholinesterase inhibition alone in alleviating AD. Here we present the discovery and development of PRX-03140, a new 5-HT4 partial agonist with a dual cholinergic/disease-modifying mechanism for the treatment of Alzheimer's disease. PRX-03140 has been well tolerated in over 100 patients and healthy volunteers, and has showed the desired alterations in brain wave activity in patients with mild-to-moderate Alzheimer's disease. Phase II trial in Alzheimer's disease patients is scheduled to start in 2006. Details of SAR and pre-clinical data will be disclosed during the presentation.

MEDI 202

Inhibition of signal transducer and activator of transcription 3 in intact cells by peptidomimetic prodrugs targeting the SH2 domain

John S. McMurray[1], *Pijus K. Mandal*[2], *David R. Coleman*[1], *Warren Liao*[3], *Zhiyong Ren*[3], *and Xiaomin Chen*[3]. *(1) Department of Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, 1515, Holcombe Blvd., Houston, TX TX 77030, Fax: 713-834-6230, jsmcmurr@audumla.mdacc.tmc.edu, (2) Neuro-Oncology, The University of Texas M. D. Anderson Cancer Center, (3) Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center*

Signal transducer and activator of transcription 3 (Stat3) is a latent transcription factor that relates signals from cell surface receptors directly to the nucleus. Stat3 is constitutively active in several cancers and is considered a target for anticancer drug design. We are developing inhibitors targeted to the SH2 domain of Stat3. From our lead compound, Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH2, we developed two high affinity peptidomimetic inhibitors, GAD10 and SM58, with IC50 values of 168 and 68 nM,

respectively, in a fluorescence polarization assay. To enhance activity in vivo, the phosphate was replaced with the phosphatase-stable phosphonodifluoromethyl group, and the phosphonate oxygens were capped with carboxyesterase-labile pivaloyloxymethyl (POM) groups for cell penetration. We present here SAR leading to our inhibitors. We also show that the peptidomimetic prodrugs inhibit Stat3 phosphorylation and expression of a Stat3 reporter gene in intact cells, and growth of breast tumor cells with IC50 values of 1 – 25 microM.

MEDI 203

A-60444: The discovery and development of a novel inhibitor of RSV

G Stuart Cockerill[1], *Malcolm C Carter*[2], *Elisa Henderson*[2], *Richard Kelsey*[2], *Verity Dowdell*[2], *Lara Wilson*[3], *Dagmar Alber*[3], *Rachel Harland*[4], *Julie Dent*[4], *Ray Pickles*[5], *Sue Grieve*[4], *Jeremy Stables*[1], *and Ken Powell*[1]. *(1) Research Director, Arrow Therapeutics, 7 Trinity Street, London SE1 1DB, United Kingdom, scockerill@arrowt.co.uk, (2) Medicinal Chemistry, Arrow Therapeutics, (3) Virology, Arrow Therapeutics, (4) Developement, Arrow Therapeutics, (5) Pulmonary and Critical Care Medicine, University of North Carolina at Chapel Hill*

Human respiratory syncytial virus (RSV) is the most common viral pathogen causing respiratory disease in infants, immuno-compromised and elderly patients worldwide. A-60444, 3-(S)-(1-(2-Fluoro-phenyl)-3-(2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-urea is a novel Benzodiazepine antiviral possessing potent activity against RSV. This compound has submicromolar activity against RSV laboratory strains (A and B) and clinical isolates. This talk describes the discovery of A-60444 and subsequent mode of action elucidation. Analysis of A-60444 resistant mutants generated *in vitro* suggested that A-60444 targets the RSV nucleocapsid (N) protein that is essential for viral replication. Phase I clinical studies showing that oral A-60444 administered for up to 7 days was well-tolerated and achieved sustained plasma concentrations above the antiviral *in vitro* IC_{90} values will also be described. In conclusion, we describe the discovery of a potent anti-RSV agent which achieves plasma concentrations, we believe, required to inhibit infection safely in human studies after daily oral dosing.

MEDI 204

Development of a selective, orally active adenosine A2a receptor antagonist for the treatment of Parkinson's disease: Sch 420814

Bernard Neustadt[1], *J Hao*[1], *N Lindo*[1], *William J. Greenlee*[2], *Andrew W. Stamford*[3], *Ennio Ongini*[4], *J Hunter*[5], *Angela Monopoli*[4], *R Bertorelli*[4], *S Fredduzzi*[4], *F Impagnatiello*[4], *Carolyn Foster*[6], *Leyla Arik*[6], *Jean Lachowicz*[7], *Kwokei Ng*[8], *R White*[7], *K. I. Feng*[9], *and D Hesk*[3]. *(1) CNS/CV Chemical Research, Schering-Plough Research Institute, K-15-2B-2800, 2015 Galloping Hill Road, Kenilworth, NJ 07033-1300, bernard.neustadt@spcorp.com, (2) Chemical Research, Schering-Plough Research Institute, (3) CV/CNS Chemical Research, Schering-Plough Research Institute, (4) CNS Department of Biological Research, Schering-Plough Research Institute, (5) CV/CNS Biological Research, Schering-Plough Research Institute, (6) CNS Department of Biological Research, Schering Plough Research Institute, (7) CV &CNS*

Biology, Schering-Plough Research Institute, (8) Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute, (9) Pharmaceutical Research, Schering Plough Research Institute

Abstract text not available.

MEDI 205

Is heat shock protein 90 the cancer chaperone?

***Len Neckers**, Urologic Oncology Branch, National Cancer Institute, Bldg. 10, Room 1-5940, Bethesda, MD 20892-1107, Fax: 301-402-0922, neckers@nih.gov*

Heat shock protein 90 (Hsp90) is a molecular chaperone whose association is required for stability and function of a growing number of signaling proteins that have been implicated in cancer cell survival, including several mutated proteins that are cancer-specific. A growing body of evidence suggests that cancer cells are particularly dependent on Hsp90 for their growth and survival, and thus are more sensitive to the effects of its inhibition than are non-transformed cells. Identification of small molecule Hsp90 inhibitors has been key to unraveling the complex web of interactions made between Hsp90 and its many client proteins. Some of these drugs have also entered the clinic and their efficacy as anti-cancer agents is being examined in a large number of Phase I and Phase II clinical trials. In this presentation I will review the function of Hsp90 in modulating multiple cancer cell signaling nodes. I will also describe our recent studies examining the physical properties regulating interaction of Hsp90 with a client tyrosine kinase, as well as the role of post-translational modification of Hsp90 in regulating its function. I will discuss the C-terminus of the chaperone as a second pharmacologically accessible inhibitory site. Finally, I will highlight several possible clinical applications of Hsp90 inhibitors.

MEDI 206

Design and development of purine-scaffold inhibitors of the heat shock protein 90

***Gabriela Chiosis**, Program in Molecular Pharmacology&Chemistry and Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York ave RRL301b, New York, NY 10021, Fax: 212-794-6236, chiosisg@mskcc.org*

Hsp90 is a chaperone protein that allows cancer cells to tolerate the many components of dysregulated pathways. We have pioneered the development of synthetic Hsp90 inhibitors. Making use of the specific fold adopted by ATP upon binding to Hsp90, we were able to design the purine-scaffold class derivatives with Hsp90 inhibitory activities. The first synthesized derivative of this class, **PU3**, bound Hsp90 with moderate affinity and exhibited biological activities in the 50 μM concentration range (ChemBio 2001). Further efforts focused at improving the potency of this agent, led to the synthesis of several compounds, such as **PU24FCl**, with improved activity and tumor selectivity (BioorgMedChem2002, ChemBio2004).

Recently we have disclosed the synthesis of nanomolar potency water soluble derivatives of the PU-class, **PU-H71** and **PU-DZ8** (EC_{50}SKBr3 Hsp90 binding = 30 nM; IC_{50}SKBr3 growth inhibition = 36 nM and EC_{50} SKBr3 Hsp90 binding = 50 nM; IC_{50}SKBr3 growth inhibition = 57 nM, respectively)(JMedChem2005). These agents equipotently affect multiple tumor-specific aspects of oncogenesis regulated by the chaperone. When administered to mice bearing human cancer xenografted tumors, they result in pharmacologically relevant concentrations and accordingly, in modulation of Hsp90-client proteins in tumors. In concordance with their higher affinity for tumor cells, PUs are retained in tumors while cleared rapidly from normal tissue. Long-term administration of PUs to human tumor xenografted mice leads to anti-tumor activity without toxicity to the host.

MEDI 207

Rationally designed high-affinity 2-amino-6-halopurine Hsp90 inhibitors which exhibit potent antitumor activity

Srinivas R. Kasibhatla[1]*, Kevin Hong*[1]*, Marco A. Biamonte*[1]*, David Bush*[2]*, Patricia L. Karjian*[2]*, John L Sensintaffar*[2]*, Adeela Kamal*[2]*, Rachel E. Lough*[2]*, John Brekken*[2]*, Karen Lundgren*[2]*, Roy Grecko*[3]*, Gregg A. Timony*[4]*, Yingqing Ran*[3]*, Robert Mansfield*[4]*, Lawrence C. Fritz*[5]*, Ed Ulm*[3]*, Francis J. Burrows*[2]*, and Marcus F. Boehm*[1]*. (1) Department of Medicinal Chemistry, Conforma Therapeutics Corp, 9393 Towne Centre Dr, Suite 240, San Diego, CA 92121, skasibhatla@conformacorp.com, (2) Department of Biology and Pharmacology, Conforma Therapeutics Corp, (3) Department of Pre-Clinical Development, Conforma Therapeutics, (4) Department of Pre-Clinical Development, Conforma Therapeutics Corp, (5) Conforma Therapeutics Corp, Conforma Therapeutics Corp*

Heat shock protein 90 (Hsp90) is a molecular chaperone protein implicated in stabilizing the conformation and maintaining the function of numerous cell signaling proteins. Since many oncogenic proteins are more dependent on Hsp90 in maintaining their conformation, stability and maturation than their normal counterparts, inhibition of Hsp90 function is emerging as an exciting new strategy for the treatment of cancer. Furthermore, recent data showing that Hsp90 exists in an activated form in malignant cells, and in a latent inactive form in normal tissues, suggests that inhibitors selective for the activated form could provide a high therapeutic index. Herein we present the discovery of highly potent (IC50 = 9 nM in a HER-2 degradation assay) and highly specific Hsp90 inhibitors. These inhibitors display excellent antiproliferative activity against various tumor cell lines (IC50 = 30 nM in MCF7 cells). When administered orally, compounds from this series cause degradation of client proteins, induction of apoptosis and inhibition of tumor growth in several human tumor xenograft models. The design, synthesis, structure activity relationship studies and in vivo oral efficacy of this series that culminated in identification of CNF2024 as the clinical candidate, will be discussed.

MEDI 208

Managing the Hsp90-mediated protein folding process with small molecules

***Brian S. J. Blagg**, Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045-7562, Fax: 785-864-5326, bblagg@ku.edu*

Hsp90 is a molecular chaperone required for the refolding of denatured proteins and the conformational maturation of nascent polypeptides. Numerous oncogenic proteins are dependent upon the Hsp90 protein folding machinery to achieve their biologically native structures. Consequently, multiple oncogenic pathways can be simultaneously derailed by Hsp90 inhibition. The Hsp90-mediated protein folding process is dependent upon the hydrolysis of ATP to furnish the energy required for the folding of protein substrates. There are two ATP binding sites; one of which binds the natural products geldanamycin and radicicol, while the other binds novobiocin. Using a combination of rational drug design and high-throughput screening, new inhibitors of the N-terminal binding site have been developed. Since the C-terminus of Hsp90 has not been determined by x-ray crystallography, a structure–activity guided approach has been used to provide new inhibitors of the C-terminal nucleotide binding pocket. The development of both N- and C-terminal inhibitors will be presented along with biological results that suggest Hsp90 inhibitors may have potential use beyond the treatment of cancer.

MEDI 209

Semisynthetic analogs of geldanamycin: Chemistry and biology

***David C. Myles**, Kosan Biosciences, Inc, 3832 Bay Center Place, Hayward, CA 94545, myles@kosan.com*

Hsp90 is an essential protein that chaparones multiple growth-regulatory signaling proteins. Because many of the client proteins of Hsp90 are important in signal transduction and transcription, inhibitors of this enzyme have potential utility for use in cancer chemotherapy. Two such compounds, KOS-953 and KOS-1022, are currently undergoing extensive clinical evaluation. This talk will discuss the medicinal chemistry and biology of these and related semisynthetic analogs of the potent Hsp90 inhibitor geldanamycin

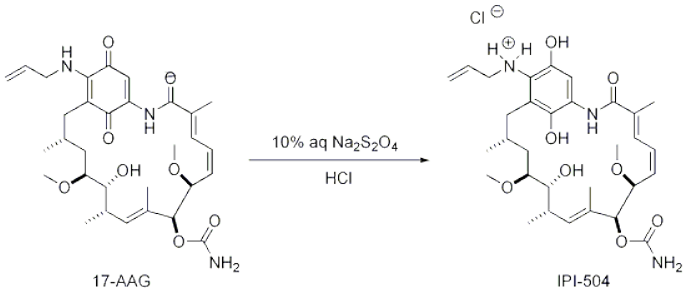
MEDI 210

Synthesis and biological evaluation of IPI-504, an aqueous soluble analog of 17-AAG and potent inhibitor of Hsp90

***James R. Porter**, Jie Ge, Emmanuel Normant, Janid Ali, Marlene S. Dembski, Yun Gao, Asimina T. Georges, Louis Grenier, Roger Pak, Jon Patterson, Jens R. Sydor, Jim Wright, Julian Adams, and Jeffrey K. Tong, Infinity Pharmaceuticals, Inc, 780 Memorial Drive, Cambridge, MA 02139, Fax: 617-453-1001, jporter@ipi.com*

IPI-504 is the hydroquinone hydrochloride salt of 17-allylamino-17-demethoxy-geldanamycin (17-AAG), an Hsp90 inhibitor that is currently in clinical trials for the treatment of cancer. IPI-504 demonstrates high aqueous solubility (>200 mg/mL). Interestingly, in vitro and in vivo IPI-504 interconverts with 17-AAG and exists in a

pH and enzyme-mediated redox equilibrium. This occurs due to oxidation of the hydroquinone (IPI-504) to the quinone (17-AAG) at physiological pH and the reduction of 17-AAG by quinone reductases such as NQO1 to IPI-504. Here we report the design and synthesis of the stabilized hydroquinone IPI-504 and its inhibitory effect against Hsp90 and Grp94. Although IPI-504 was originally designed to be a soluble prodrug of 17-AAG, the hydroquinone is more potent than the quinone in the biochemical Hsp90 binding assay. Various hydroquinone analogs have been prepared to investigate the structure activity relationship of hydroquinone binding to Hsp90. Hydroquinone and quinone forms of 17-AAG metabolites show comparable binding affinities for Hsp90 and in cancer cell lines, hydroquinone analogs elicit specific responses consistent with Hsp90 inhibition. The desirable pharmacological properties as well as in vitro and in vivo activity of our lead compound, IPI-504, has led to the initiation of Phase I clinical trials in multiple myeloma.



MEDI 211

Immobilized artificial membrane chromatography: A useful tool for predicting membrane permeability

Adeboye Adejare *and Ahmed El-Gendy, Department of Pharmaceutical Sciences, Philadelphia College of Pharmacy, University of the Sciences in Philadelphia, 600 South 43rd Street, Philadelphia, PA 19104-4495, Fax: 215-895-1161, a.adejar@usip.edu*

The partitioning of structurally diverse and functionally unrelated set of 21 compounds was studied on immobilized artificial membrane (IAM) column using high performance liquid chromatography (HPLC). The aqueous capacity factor (log KIAMW) values of the compounds were correlated with the corresponding logarithm of octanol/water partition coefficient (log P) and blood/brain partition coefficient (log BB) values to determine whether molecular binding on IAM columns can predict permeability of drugs through biological membranes such as intestinal membrane and blood brain barrier. Good correlation was obtained between log KIAMW and log P of the compounds ($r = 0.952$). Log KIAMW values of the compounds also showed an acceptable correlation with log BB ($r = 0.767$). Though many in vitro physicochemical based techniques are available to predict membrane permeability; IAM.HPLC seems to be a promising tool for easy and reproducible prediction.

MEDI 212

Increasing hERG margin of G-protein coupled receptor inhibitors by optimization of non-desolvation component of potency

Igor Shamovsky[1], *Stephen Connolly*[2], *Laurent David*[3], *Svetlana Ivanova*[3], *Bo Norden*[3], *Brian Springthorpe*[2], *and Klaus Urbahns*[3]. *(1) Department of Medicinal Chemistry, AstraZeneca R&D, S-22187 Lund, Sweden, Fax: 4646-337119, Igor.Shamovsky@AstraZeneca.com, (2) Department of Medicinal Chemistry, AstraZeneca R&D Charnwood, (3) Department of Medicinal Chemistry, AstraZeneca R&D Lund*

The inhibition of hERG potassium ion channel by commonly used medications, particularly basic lipophilic amines, is a major hurdle in the development of new drugs. The development of hERG-free drugs is a challenging problem because of poor understanding of the amazing structural diversity of hERG blockers. G-protein coupled receptors (GPCRs) are implicated in many pathological conditions, including inflammation, autoimmune disorders, atherosclerosis, osteoporosis, COPD and asthma, which makes them important targets for therapeutic intervention. Binding sites of GPCRs represent lipophilic surfaces with few polar residues, accordingly lipophilicity of inhibitors is necessary to maintain high potency. On the other hand, compound lipophilicity plays a fundamental role in binding to hERG channel. Thus, to develop hERG-free GPCR antagonists, it is crucial to achieve selectivity by utilizing direct ligand-protein interactions, which are not related to desolvation. We suggest a general strategy to develop hERG-free GPCR antagonists based on hERG baseline lipophilicity relationships and fragment-based QSAR analysis, which allow us to subtract the average contribution of lipophilicity to hERG potency and focus on outliers, thereby revealing both intrinsic hERG binding and intrinsic hERG non-binding molecules and their fragments. Intrinsic hERG binders (alternatively non-binders) represent molecular fragments that make significantly higher (alternatively lower) contribution to hERG potency (pIC50) than to compound lipophilicity (logD). The strategy is to gain selectivity against hERG by using polar interactions with the target protein, while avoiding intrinsic hERG binders. Binding modes of a number of identified intrinsic hERG binders, which interact with the hERG ion channel unusually efficiently, are obtained by molecular modeling using a hERG homology model. Three types of GPCRs and several chemical classes of antagonists will be used to illustrate advantages of this strategy.

MEDI 213

Filling out grey areas in passive absorption predictions

Alanas Petrauskas, Pranas Japertas, Remigijus Didziapetris, and ***Dimitri Bondarev****, Pharma Algorithms Inc, 591 Indian Rd., Toronto, ON M6P 2C4, Canada*

This study presents a mechanistic QSAR analysis of Human Intestinal Absorption (HIA) that takes into account its dependence on multiple kinetic and thermodynamic parameters for ionizable drugs. While parameters such as molecular weight, LogP and pKa are known to affect absorption on a qualitative level, our goal was to derive exact quantitative relationships. The HIA database from Pharma Algorithms Inc. was used to select 400 compounds with %HIA values that were reasonably free of various unwanted effects (dose-dependencies, limited solubility, poor chemical stability, active transport, first-pass effect). Multiple steps of non-linear fitting were

performed. The first step involved an analysis of HIA of non-electrolyte drugs to determine non-electrolyte factors. Further steps involved analyses of various types of electrolytes aimed at the determination of corresponding charge factors. The resulting predictive model explained over 95% of all analyzed HIA values. It also was consistent with the liposome/water partitioning of various electrolytes.

MEDI 214

Novel approach to lead optimization based on physicochemical properties

*Karim Kassam[1], **Daria Jouravleva**[2], and Eduard Kolovanov[1]. (1) Advanced Chemistry Development, Inc, 110 Yonge Street, 14th Floor, karim.kassam@acdlabs.com, (2) Advanced Chemistry Development, Inc, 110 Yonge Street, 14th Floor, daria@acdlabs.com*

Biological activity of a drug is contingent on its ability to cross one or more barriers in order to get into the intended sites of action. The ability to deliver the drug to the target site is greatly influenced by the compound's physicochemical properties (logP, pKa, aqueous solubility, etc.). By combining physicochemical property predictors and a critically evaluated database of biologically-acceptable substituents (with Hammett parameters), medicinal chemists can quickly account for all possible physicochemical effects of the proposed structural modification and, as a result, reduce the number of analogues that need to be synthesized to achieve optimal exposure at the site of action. Examples of lead optimization for improving aqueous solubility and eliminating BBB penetration will be outlined in the presentation.

MEDI 215

Scaffold hopping: A drug discovery integrative approach

***Ismael Zamora**[1], Manuel Pastor[2], Jorge A. Naranjo[2], and Gabriele Cruciani[3]. (1) Lead Molecular Design,s.l, Francesc Cabanes i Alibau, 1-2 2º-1ª, Sant Cugat del Valles, 08190 Barcelona, Spain, ismael.zamora@telefonica.net, (2) Research Unit on Biomedical Informatics (GRIB), IMIM, Universitat Pompeu Fabra, (3) Molecular Discovery Ltd*

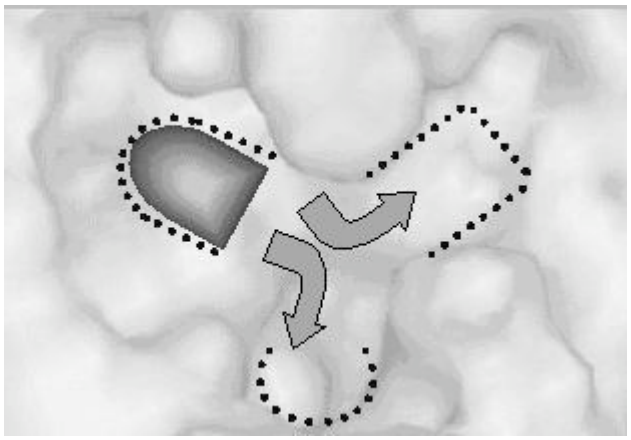
The computational procedure proposed enables the ranking of a collection of scaffolds from a database by comparison to the query introduced by the user. The methodology is based on an extension of the biosesterism concept able to perform Scaffold HOPping. The method aims to induce the collaboration and integration of Medicinal Chemistry, Computational Chemistry and ADME areas in the drug discovery process. Medicinal Chemistry: The scaffolds introduced in the databases are considered by their chemical reactivity assuring them their synthetability. Computational Chemistry: These scaffolds are basically described by fingerprints and GRID derived descriptors. ADME: Each scaffold can be characterized by the VolSurf derived ADME predicted properties like Caco2 passive permeability, Blood Brain Barrier, solubility in water or in 2% DMSO solution, unspecific binding to plasmatic proteins, volume of distribution and metabolic stability towards CYP3A4. Six different cases studies will be shown to demonstrate the use of this technique in the case of cdk2 kinase, HIV protease, steroid and adenosine derivatives synthesis.

MEDI 216

Fragment-to-candidate discovery using high throughput X-ray crystallography

Gordon Saxty, *Department of Medicinal Chemistry, Astex Therapeutics, 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, United Kingdom, Fax: +44(0)1223226201, g.saxty@astex-therapeutics.com*

Fragment-based approaches to lead discovery are gaining interest in many labs as a complementary approach to high through-put screening. [1], [2], [3] Astex's approach to drug discovery involves screening of low molecular weight fragments that have typical molecular weights between 120-250 Da and efficient binding interactions (typically mM - 30 uM). These fragments can then be rapidly manipulated into nM leads via fragment growing or fragment linking strategies using high throughput X-ray technology to provide structural information about the protein-ligand interactions and the orientations of ligands in the protein structure. The advantages of a fragment-based technique include the requirement to screen and synthesise only small numbers of compounds and high success rates in generating chemical series with lead-like properties. The talk will include examples of fragment growing strategy. For example, from our cyclin dependant kinase programme, where a mM fragment was 'grown' into a pM lead with in vivo efficacy in a tumour model. This compound is now in Phase 1 clinical trials. This illustrates Astex's Fragment-to-Candidate approach to drug discovery.



MEDI 217

Design and synthesis of kinase inhibitors of anaplastic lymphoma kinase (ALK) using IGF1R protein-inhibitor co-crystal X-ray structure based homology models

Thomas R. Webb[1], *Rongshi Li*[2], *Tong Zhu*[2], *Stephan W. Morris*[3], *Liquan Xue*[3], *Qin Jiang*[3], *Jian Wang*[2], *Xiaoli Cui*[3], *Danny McGee*[2], *Vidyasagar Gantla*[2], *Zheng Yan*[2], *Jason C. Pickens*[2], *Sergey Zozulya*[2], *and Douglas McGrath*[2]*. (1) Research and Development, ChemBridge Corporation and ChemBridge Research Labs, 16981 Via*

Tazon, Suite G, San Diego, CA 92127, Fax: 858-451-7401, (2) ChemBridge Research Laboratories (CRL), (3) Departments of Pathology and Hematology-Oncology, St. Jude Children's Research Hospital

Anaplastic Lymphoma Kinase (ALK) is a promising new target for the therapy of certain cancers such as anaplastic large-cell lymphoma (ALCL). We have identified a series of novel pyridones as kinase inhibitors of ALK by application of a stepwise process involving in vitro screening of a novel targeted library followed by iterative template modification with computational ranking of virtual libraries. We have discovered ALK selective inhibitors with improved potency and selectivity. The details of the design process and synthesis of these novel pyridones, along with their enzymatic and cell based activities will be discussed. Additionally we have obtained an X-ray-inhibitor co-crystal structure of a construct of the highly homologous kinase domain of the insulin-like growth factor receptor (IGF1R). We will discuss the interpretation of these results and their implications for ALK inhibitor design based on a homology model of ALK with inhibitors bound, which we have derived from this structure.

MEDI 218

Medicinal chemist directed pharmacophore framework-based ligand design

Thomas R. Webb*, Jian Wang, Douglas E. McGrath, and Ruben Venegas, Research and Development, ChemBridge Corporation and ChemBridge Research Labs, 16981 Via Tazon, Suite G, San Diego, CA 92127, Fax: 858-451-7401*

Abstract: Current commonly applied methods for *de novo* design involves the *in silico* screening of virtual or real compound libraries by pharmacophore queries, docking, or structure based ligand assembly methods. One of the major limitations of these methods is that the molecules that are newly designed may not be reasonable synthetic targets. We propose a novel method that is applicable to highly targeted ligand or scaffold design; this method separates the ligand design process into several steps. First a library of possible wildcard molecular frameworks is enumerated. Then, a geometry search of the featureless pharmacophore of interest against the framework library is carried out. The 'hit' frameworks are annotated with the features of the pharmacophore and then ranked based on their complexity (e.g. number of rings). Each 'framework and feature annotation' represents a very large number of possible compounds that can be converted to synthetically attainable structures with inspection/modification by a medicinal chemist. These designed molecules can then be checked and ranked based on the quality of their fit with the pharmacophore. The high ranking hits then become synthetic or acquisition targets for the project of interest. This method allows the medicinal chemist to guide the process of design and selection of synthetic targets so that optimal use of medicinal/synthetic expertise can be applied. We call this method "retro-pharmacophoric annotated framework design" (RAFD). This presentation will show some examples of the application of this method to the design of ligands for kinases and GPCRs.

MEDI 219

Docking simulations of pyrazole class of aurora-2 kinase inhibitors

***Tanaji T Talele**, Department of Pharmaceutical Sciences, College of Pharmacy & Allied Health Professions, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, Fax: 718-990-1877, talelet@stjohns.edu, and Mark L. McLaughlin, Department of Chemistry, University of South Florida*

The binding mode of a novel series of Aurora-2 kinase inhibitors was investigated employing a molecular docking approach utilizing Glide. Crystallographic bound inhibitor 8 was accurately predicted by our docking protocol. Prediction of the binding mode of compounds 1-8 was not altered by the presence or absence of explicit water molecules, while compounds 9-25 showed altered binding modes. Our docking results suggested that inhibitors 1-25 assume an inverted V shape conformation within the active site of Aurora-2 kinase. We propose that chemical modification of these inhibitors to obtain an inverted Y shape would lead to highly potent and selective Aurora-2 kinase inhibitors for the treatment of cancer. The detailed docking simulations will be discussed along with the design of novel Aurora-2 kinase inhibitors. Comparison of Aurora-2 kinase active site to that of other closely related kinases will be discussed in an effort to specifically exploit Aurora-2 kinase for anticancer drug discovery.

MEDI 220

Structure-based design of long-acting PDE5 inhibitors

***Andrew S. Bell**, Michael J Palmer, Kevin N. Dack, Ian R Marsh, Andrew I Morrell, and David G Brown, Sandwich Chemistry, Pfizer Global Research & Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom, Fax: 860 686 1704, andy.bell@pfizer.com*

Inhibition of phosphodiesterase type 5 (PDE5) inhibits the breakdown of cGMP allowing the levels of cGMP, and hence smooth muscle relaxation, to be maintained. Our first generation PDE5 inhibitor, sildenafil, (Viagra™, Revatio™) has proved to be a highly effective treatment for male erectile dysfunction and pulmonary hypertension. In designing a second-generation PDE5 agent, we identified improved selectivity and a long duration of action as key attributes to aim for. We will present the application of co-crystal structures of PDE5 to design novel, selective PDE5 inhibitors with the potential for once–daily dosing in man. We will also describe the use of parallel synthesis to deliver leads meeting our design criteria.

MEDI 221

Molecular pharmaceutics strategies for targeting transporters and enzymes in the GI tract

Gordon L. Amidon, *College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, MI 48109, glamidon@umich.edu*

Recent advances in understanding the molecular mechanisms associated with intestinal membrane transporters and enzymes have enabled the rational design and optimization of bioavailable drugs and delivery systems. Amino acid prodrugs that are substrates to the oligopeptide transporter, hPEPT1, have been successfully applied to improve the delivery of a number of drugs with otherwise limiting physicochemical properties, selectivity or undesireable side effects. More recently, a novel human serine hydrolase, BPHL, has been exploited to catalyze the hydrolytic activation of the antiviral prodrugs, valacyclovir and valganciclovir. These and other molecular pharmaceutics approaches will be presented, each of which offer considerable potential to the medicinal chemist in designing "deliverability" into new molecular entities to improve overall therapeutic index and downstream success rates.

MEDI 222

XP 13512, a transported prodrug of gabapentin, overcomes the saturable oral absorption of Neurontin® in humans

Stephen P. Raillard, *Chemical Development, Xenoport, Inc, 3410 Central Expressway, Santa Clara, CA 95051, stephen.raillard@xenoport.com*

Clinical data will be presented for XP13512, a prodrug of gabapentin currently undergoing Phase 2 studies for the treatment of restless legs syndrome and neuropathic pain disorders. XP13512 was designed to be actively absorbed along the length of the GI tract by high-capacity transport pathways. This has permitted development of an oral sustained-release formulation of XP13512 that provides higher levels of gabapentin in the blood for a longer period of time compared with administration of Neurontin®.

MEDI 223

Human PEPT1 pharmacophore distinguishes between binding and transport

Teresa N. Faria[1], *Balvinder S. Vig*[1], *Terry R. Stouch*[2], *Julita K. Timoszyk*[1], *Yong Quan*[1], *Doris A. Wall*[1], *and Ronald L. Smith*[1]. *(1) Biopharmaceutics R&D, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903, teresa.faria@bms.com, (2) Computer-Assisted Drug Design, Lexicon Pharmaceuticals*

The human intestinal oligopeptide transporter (SLC 15A1, PEPT1) facilitates the absorption of di- and tripeptides and many peptidomimetic drugs such as beta-lactam and cephalosporin antibiotics, ACE inhibitors, renin inhibitors and amino acid esters of nucleosides. In this study, a large number of peptides were selected to investigate the structural features required for PEPT1 transport. Binding affinity was determined in a Gly-Sar uptake inhibition assay whereas functional transport was ranked in a membrane depolarization assay. Although most of the peptides tested could bind to PEPT1, not all were substrates. As expected, single amino acids and

tetrapeptides could not bind to or be transported by PEPT1. The extent of dipeptide transport was variable and, unexpectedly, some dipeptides were not transported by PEPT1. Dipeptide transport was influenced by charge, hydrophobicity, size, and sidechain flexibility. Charge or extreme bulk in either dipeptide position 1 or 2 tended to decrease or eliminate transport. These results identify key features required for PEPT1 transport, in contrast to most previously described pharmacophores, which are based on inhibition of a known substrate.

MEDI 224

Folate receptor targeted design of anticancer drugs with improved tumor selective delivery

***Christopher P. Leamon**, Endocyte, Inc, 300 Kent Avenue, West Lafayette, IN 47906, chrisleamon@endocyte.com*

Many human cancers express high levels of the folate receptor (FR), a membrane protein that delivers folates and folate-drug conjugates inside cells via a non-destructive endocytosis mechanism. This uptake pathway has successfully been exploited to deliver a wide variety of pharmacologically active molecules to FR-positive cells and tumors, including radiodiagnostic imaging agents, chemotherapeutics, and nanoparticulates. In early 2006, the first folate-targeted chemotherapeutic, EC145, will enter phase 1 clinical trials for the treatment of refractory cancer. Importantly, two additional agents are expected to begin clinical testing before the end of that same year. Owing to these recent advances, the technology behind folate-targeted chemotherapy will be presented.

MEDI 225

Engineered polymers for targeted delivery of siRNA

***Jeremy D. Heidel**, Colando Pharmaceuticals, 1710 Flower Avenue, Suite 100, Duarte, CA 91010, jheidel@calandopharma.com, and Mark E. Davis, Department of Chemical Engineering, California Institute of Technology*

RNA interference (RNAi) is becoming the method of choice for target validation studies that involve gene inhibition. While localized delivery of siRNA is now used in early clinical trials (direct injection into the eye), many diseases will require systemically delivered therapies, e.g., metastatic cancer. Numerous issues must be addressed when considering the systemic delivery of siRNA as a generalized gene inhibition strategy against human disease. In order to have repeatable, systemic dosing of siRNA that provides a cost effective therapy, we believe that non-viral delivery systems must be employed. We will show that a cyclodextrin-based polymeric delivery system can provide systemic delivery of non-chemically functionalized siRNA at doses and via routes of administration that are applicable to human therapy. Effective doses in animals are at least an order of magnitude below those used with chemically modified siRNAs lacking delivery systems, and this feature provides for a more cost-acceptable therapeutic. The delivery system contains a targeting ligand to enhance delivery to the desired tissue and an optimized siRNA sequence to provide potent and long-lasting gene inhibition. The

delivery system protects unmodified siRNA from degradation in serum and does not produce an immune response. Results illustrating all of these essential features of the therapeutic will be presented.

MEDI 226

Liquid chromatographic tandem mass spectrometry method for the quantification of Miglitol in human plasma

Vishwottam N. Kandikere*, Manoj Shukla, Koteshwara Mudigonda, Santosh Maurya, Ravikumar Boosi, and Ramakrishna V. S. Nirogi, Discovery Research, Suven Life Sciences Ltd, Serene Chambers, Road No 7, Banjara Hills, Hyderabad 500034, India, Fax: 914023541152, knvishu@suven.com*

Miglitol (Glyset) is the first oral alpha-glucosidase inhibitor for use in the management of non-insulin-dependent diabetes mellitus. In this study, we developed and validated a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of miglitol in human plasma. Following protein precipitation, miglitol was separated using an isocratic mobile phase on a reversed phase phenyl column and analyzed by MS in the multiple reaction monitoring mode using [M+H] ion m/z 208/146. Recovery from plasma was 40.5% and the limit of quantification was 100 ng/mL. The method is accurate and reproducible and has been successfully used to analyze human plasma samples for application in pharmacokinetic, bioavailability or bioequivalence studies. The observed maximum plasma concentration of miglitol (100 mg oral dose) is 1740 ng/mL, time to observed maximum plasma concentration is 3.5 h and elimination half-life is 2.5 h.

MEDI 227

Mapping the p53-hdm2 protein-protein interaction by structure-guided peptidomimetics

Patrick Chene[1], Pascal Furet[1], and ***Carlos Garcia-Echeverria****[2]. (1) Oncology Research, Novartis Institutes for BioMedical Research, Basel, Switzerland, (2) Oncology Research, Novartis Institutes for BioMedical Research, WKL-136.13.16, Basel 4002, Switzerland, Fax: 41-61-696-6929, carlos.garcia-echeverria@novartis.com*

A long-standing goal of medicinal chemists is to develop approaches for the *de novo* design of inhibitors based on knowledge of a protein's 3D-structure. In the case of the p53-hdm2 protein-protein interaction, an X-ray crystal structure of the complex between the amino-terminal domain of hdm2 and the 15-residue transactivation domain peptide of p53 showed that hdm2 possesses a deep hydrophobic cleft into which the p53 peptide binds as an amphiphatic α-helix. The 3D-structure also revealed that this protein-peptide interaction is dominated by the van-der-Waals contacts mediated by only three residues: Phe, Trp and Leu. Although these three amino acids insert deep into the hdm2 cleft, they bury no more than about 500 Å of surface, suggesting the possibility to identify low-molecular mass compounds able to target this protein surface. The lack of success in our high through-put screening

campaign for the p53-hdm2 protein-protein interaction prompted us to determine the amino acid specificities of hdm2's binding pockets in order to establish a pharmacophore model, and select or design scaffolding molecules. A variety of approaches, including both phage-display libraries and structure-guided peptidomimetics, were used to identify amino acid side-chains that formed key contacts with the hdm2 protein as well as residues that may be structurally important but more appropriate for chemical modification. Although the final compound, with a peptidic backbone and a molecular weight of 1210, did not qualify as a lead, it provided proof-of-concept for this therapeutic approach in cellular settings. Thus, the untagged peptide was capable of inducing p53 activation and apoptosis only in cells expressing wild-type p53 and high endogenous levels of hdm2 protein.

MEDI 228

Small-molecule antagonists of the p53-mdm2 interaction

Binh T. Vu[1], *Bradford Graves*[1], *Lyubomir T. Vassilev*[2], *Daisy Carvajal*[2], *Zoran Filipovic*[2], *Christine Lukacs*[1], *Christian Klein*[3], *Ursula Kammlott*[1], *Frank Podlaski*[4], *Weiguo Qing*[2], *Kathryn Packman*[2], *Norman Kong*[1], *Emily Liu*[1], *Kathleen Dillon*[1], *Anthony Specian Jr.*[1], *Gerald Kaplan*[1], *Sung-Sau So*[1], *Don Emerson*[1], *David Fry*[1], *Kyungjin Kim*[1], *Steven G. Mischke*[1], *Bingbing Wang*[1], *John Roberts*[1], *and Nader Fotouhi*[1]*. (1) Discovery Chemistry, Hoffmann-La Roche, Inc, 340 Kingsland Street, Nutley, NJ 07110, binh_t.vu@roche.com, (2) Discovery Oncology, Hoffmann-La Roche, Inc, (3) Molecular Biology, Roche Penzberg, (4) Roche Discovery Technologies, Hoffmann-La Roche, Inc*

The p53 tumor suppressor is a potent transcription factor with powerful growth suppressive and pro-apoptotic activity. Under non-stressed conditions, cellular p53 is tightly controlled by MDM2 which inhibits its transcriptional activity and promotes its degradation. Overexpression of MDM2 can impair the tumor suppressor function of p53 and has been found in many human cancers. Inhibition of MDM2-p53 interaction can stabilize p53 and may offer a novel strategy for cancer therapy. We have developed the first potent and selective small-molecule antagonists of MDM2 that activate the p53 pathway in cancer cells both in vitro and in vivo. Crystal structures of their complexes with human MDM2 confirmed that these compounds bind at the p53 binding pocket of MDM2 and effectively mimic the interaction of critical amino acid residues from the p53 protein. Incubation of cancer cells with low micromolar concentrations of the MDM2 antagonists stabilized p53 and activated p53-regulated genes (e.g. p21, MDM2) only in cells with wild-type p53. This led to arrest of cell cycle progression in G1 and G2 phases, followed by induction of apoptosis. MDM2 antagonists administered orally to nude mice bearing established human cancer xenografts suppressed tumor growth by 90% compared to vehicle controls. These experiments suggested that small-molecule inhibitors of MDM2-p53 interaction may have therapeutic utility in the treatment of human tumors expressing wild-type p53.

MEDI 229

Computational Studies and peptidomimetic design for the p53-HDM2 complex

Heather A. Carlson[1]*, Haizhen Zhong*[2]*, and Anna L. Bowman*[1]*. (1) Department of Medicinal Chemistry, University of Michigan, Ann Arbor, 428 Church Street, Ann Arbor, MI 48109, carlsonh@umich.edu, (2) Center for Drug Design, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro*

The interaction between p53 and HDM2 is a key event in controlling cell growth. Many studies have suggested that a p53 mimic would be sufficient to inhibit HDM2 to reduce cell growth in cancerous tissue. In order to design a potent p53 mimic, molecular dynamics (MD) simulations and the MM/GBSA method were used to examine binding in the p53-HDM2 complex. Our estimates of the free energy of binding for a model p53-HDM2 complex were -7.4 kcal/mol, which is in very good agreement with the experimentally determined values. We have used the information from our studies of p53-HDM2 to design a β-peptide mimic of p53. MD simulations of the mimic bound to HDM2 estimate a free energy of binding of -8.8 kcal/mol. The mimic was compared to other inhibitors that block the formation of the p53-HDM2 complex. Interesting similarities and the differences point to ways that inhibitors of the system may be improved. Lastly, an additional hydrophobic pocket is noted in the interior of HDM2. It may be possible to design new inhibitors to take advantage of that pocket.

MEDI 230

Discovery and structure-based design of benzodiazepinedione inhibitors of the HDM2:p53 complex

Bruce L. Grasberger*, Tianbao Lu, Juan Jose Marugan, Daniel J. Parks, Carsten Schubert, Holly K. Koblish, Maxwell D. Cummings, Kristi A. Leonard, Pierre Raboisson, Karen L. Milkiewicz, Raul R. Calvo, Louis V. LaFrance, Robert R. Donatelli, Diane Maguire, Theodore E. Carver, Jennifer Lattanze, Carol F. Franks, Shuyuan Zhao, Kannan Ramachandren, Ingrid C. Deckman, and Anna C. Maroney, Johnson & Johnson Pharmaceutical Research & Development LLC, 665 Stockton Drive, Exton, PA 19341, Fax: 6104588249, bgrasber@prdus.jnj.com*

HDM2 binds to an α-helical transactivation domain of p53, inhibiting its tumor suppressive functions. We have used a miniaturized thermal denaturation assay to screen chemical libraries and discover a novel series of benzodiazepinediones that bind to HDM2 and inhibit its association with p53. The X-ray crystal structure of benzodiazepinedione inhibitors bound to HDM2 reveals their α-helix mimetic properties. This structural information was used in the design and synthesis of compounds with improved cellular activity that, in combination with doxorubicin, decrease tumor growth in a mouse xenograft.

MEDI 231

Beta-hairpin protein epitope mimetics that inhibit the p53-HDM2 interaction

John Anthony Robinson*, Department of Chemistry, University of Zurich, Winterthurerstrasse 190, Zurich 8057, Switzerland, robinson@oci.unizh.ch*

This work will show how ß-hairpin peptidomimetics of the alpha-helical epitope on p53 can be designed that bind tightly to the p53-binding site on HDM2. The ß-hairpin is used as a scaffold to display energetically hot residues in an optimal array for interaction with HDM2. The initial lead ß-hairpin mimetic (IC50 = 125 μM) was optimized to afford cyclo-(L-Pro-Phe-Glu-6ClTrp-Leu-Asp-Trp-Glu-Phe-D-Pro) (where 6ClTrp = L-6-chlorotryptophan), which has an almost 1000-fold higher affinity (IC50 = 140 nM). Insights into the origins of this affinity maturation came from structure-activity studies and an X-ray crystal structure of the inhibitor/HDM2 complex at 1.4 Å resolution. The crystal structure confirms the ß-hairpin conformation of the bound ligand, and reveals that a significant component of the affinity increase arises through new aromatic/aromatic stacking interactions that side chains around the hairpin make with groups on the surface of HDM2.

MEDI 232

WITHDRAWN

MEDI 233

Development of a chemical process for an antiviral drug candidate

Richard P. Polniaszek[1], *Xi Chen*[1], *Ken Crawford*[1], *Azar Dastgah*[2], *Eric Dowdy*[1], *Arnold Gutierrez*[1], *Mari Iwamoto*[1], *Michael Mitchell*[2], *Christina Schmidt*[1], *Jianying Wang*[2], *Richard Yu*[1], *and LinHua Zhang*[1]. *(1) Process Research, Gilead Sciences, 333 Lakeside Drive, Foster City, CA 94404, Fax: 650-522-5326, rpolniaszek@gilead.com, (2) Medicinal Chemistry, Gilead Sciences*

Abstract text not available.

MEDI 234

Evolution of a synthetic process from discovery through early clinical development

Gregory S. Wayne*, Wenke Li, Timothy B. Towne, Steven J. Wittenberger, Brian Kotecki, Steven M. Hannick, and Bryan S. Macri, GPRD Process Chemistry, Abbott Laboratories, R450, R8-115, 1401 Sheridan Rd, North Chicago, IL 60064, Fax: 847-938-2258, greg.wayne@abbott.com*

An enabling synthetic route capable of producing kilogram quantities of Active Pharmaceutical Ingredient (API) for Phase 1 and toxicology studies was initially developed, based on the original discovery route. This chemistry was successfully demonstrated on 10 kilogram scale. However, to meet the needs of future clinical development a new route was developed which overcame the limitations of the enabling route; chiral resolution, protection/deprotection steps, intellectual property issues, and Pd removal. This 2nd generation route is shorter and higher yielding and has been demonstrated on 25 kilogram scale.

MEDI 235

Integration of science and business in early-phase chemical process development for active pharmaceutical ingredients

*Tony Zhang, Eli Lilly and Company, zhang@lilly.com, and **Keith DeVries**, Eli Lilly & Company, keith_devries@lilly.com*

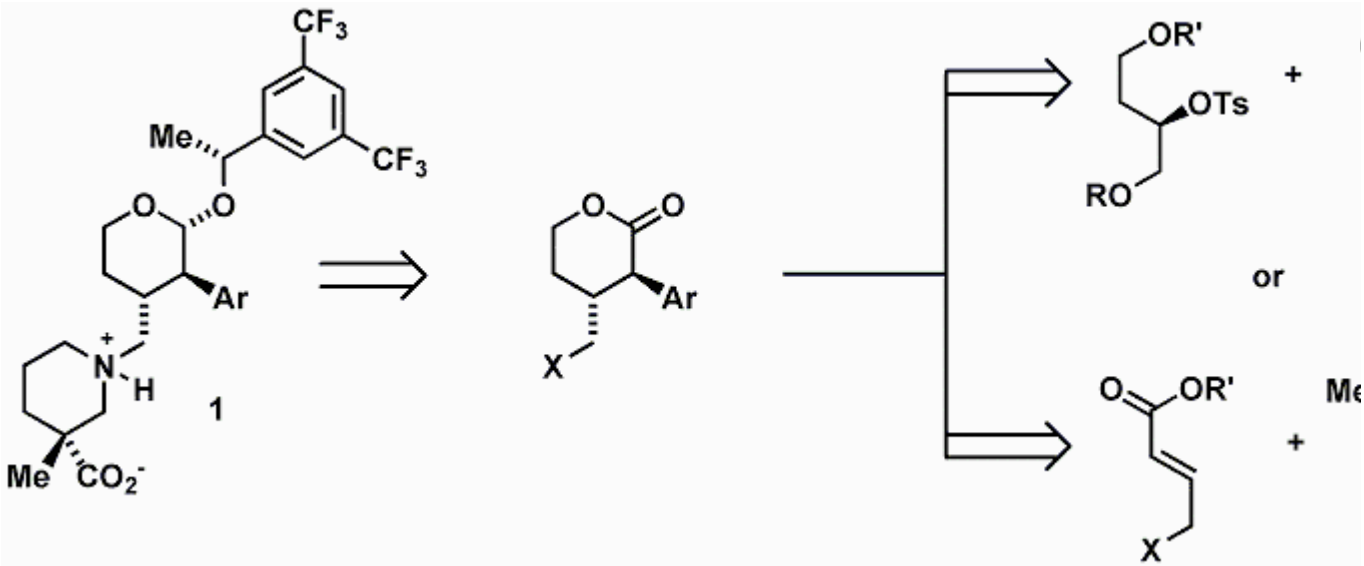
The changes in the socioeconomical environment for research based pharmaceutical companies are translating into multiple challenges for the chemical process R&D divisions. The balance between material delivery to enable toxicological, formulation, and clinical studies, and the exploration for the ultimate manufacturing process requires deliberate considerations. Several examples of chemical process research efforts will be used to illustrate the important roles of innovation, risk taking, and third party network for early phase API development.

MEDI 236

Synthesis of tetrahydropyran NK_1 receptor antagonists

***Mark A. Huffman**, Department of Process Research, Merck & Co., Inc, P.O. Box 2000, Rahway, NJ 07065, mark_huffman@merck.com*

Three practical asymmetric routes were developed to synthesize tetrahydropyran-containing NK_1 receptor antagonists 1. The first route sets the core stereochemistry through stereospecific enolate alkylation with a chiral 2° alkyl sulfonate. The second and third generation syntheses employ asymmetric conjugate addition to α,β-unsaturated esters using amides of the chiral auxiliary pseudoephedrine.

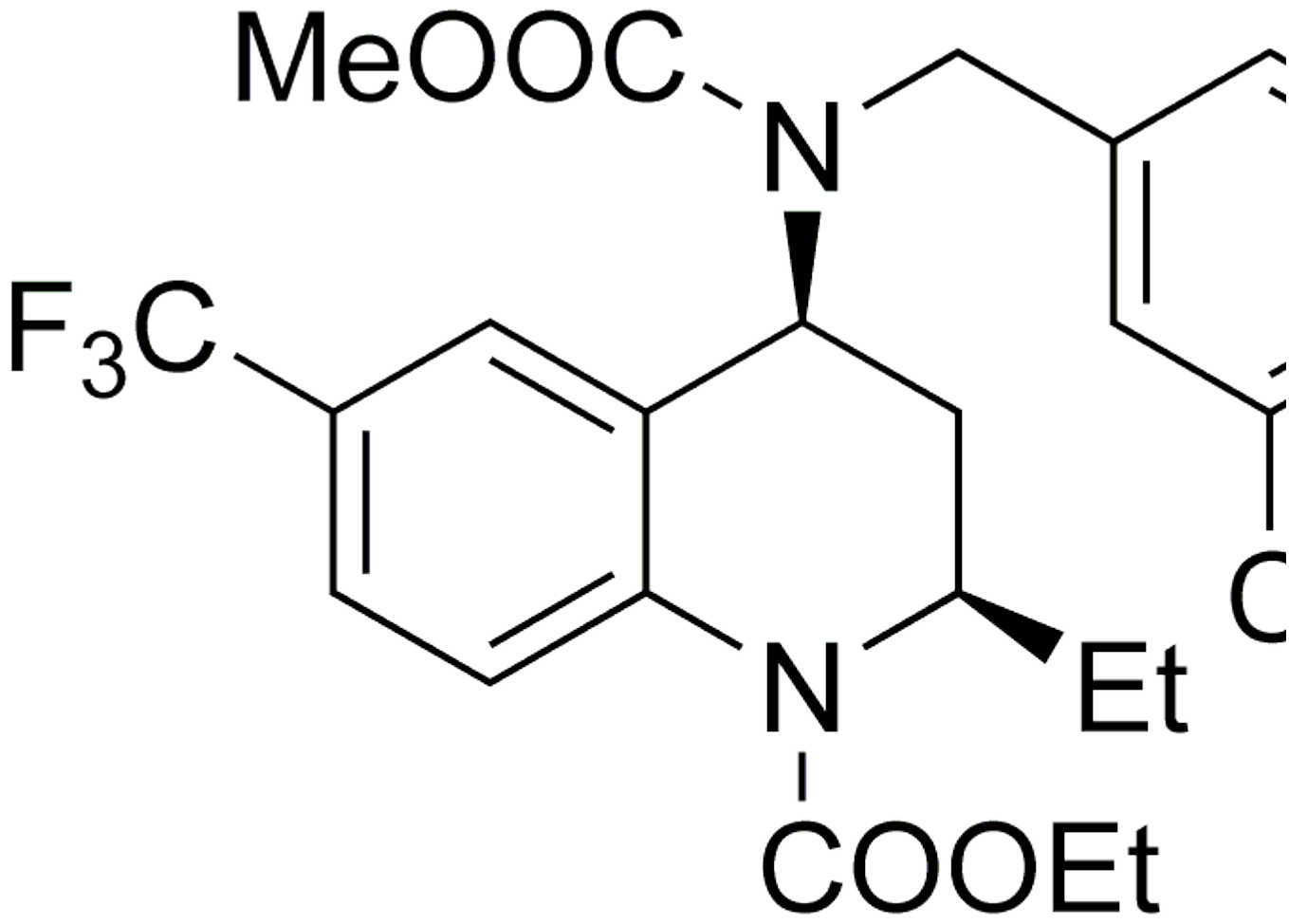


MEDI 237

Synthesis of the CETP inhibitor torcetrapib

Robert W. Dugger, *Chemical Research and Development, Pfizer Inc, Eastern Point Road, Groton, CT 06340, Fax: 860-441-5540, robert.w.dugger@pfizer.com*

Torcetrapib (CP-529,414) is a clinical candidate for the prevention of atherosclerosis via CETP inhibition. The early process improvements directed towards a scalable synthesis of this compound will be described. The events leading to the discovery of an improved, chiral synthesis will also be presented.



MEDI 238

Anticoagulant activity of the sulfated polysaccharides from marine algae

Wenjun Mao *and Huijuan Zhang, Marine Drugs and Foods Institute, Ocean University of China, 5 Yushan Road, Qingdao 266003, China, wenjunmqd@hotmail.com*

The leading causes of death are now diseases that involve heart and blood vessels and as a consequence thrombosis. The current efforts are to develop specific and

potent antithrombotic agents. Heparin has been used for anticoagulant more than 50 years, but heparin has several side effects, such as development of thrombocytopenia, hemorrhagic effect and so on. One abundant source of new anticoagulant is the sulfated polysaccharides from marine algae. The sulfated polysaccharides from seaweeds contain a variety of sulfated galactans and sulfated fucans, which are among the most abundant non-mammalian sulfated polysaccharides found in nature. These sulfated polysaccharides are potent thrombin and factor Xa inhibitors mediated by anthithrombin or heparin cofactor II. The observations show that the sulfated polysaccharides from marine algae might be used as anticoagulants and therapeutic reagents for thrombosis. The relationship between the sulfated polysaccharides structure and its anticoagulant activity is interesting and useful for the design of new anticoagulant. Therefore further studies have been conducted on algae anticoagulant polysaccharides.

MEDI 239

Investigation of N-alkyl-N-alkyloxycarbonylaminomethyl promoiety as novel bioreversible prodrugs for phenol and imide containing drugs

Susruta Majumdar *and Kenneth B. Sloan, Department of Medicinal Chemistry, University of Florida, P O Box 100485, Gainesville, FL 32610, Fax: 352-392-9455, susrutam@ufl.edu*

Several N-alkyl-N-alkyloxycarbonylaminomethyl derivatives of a model phenolic drug, acetaminophen and an imide theophylline, were synthesized. Diffusion cell experiments using hairless mouse skins were carried out and flux (amount of drug permeating per unit area per unit time) was measured. All derivatives were found to be enzymatically labile and thus can function as prodrugs.The N-methyl-N-methyloxycarbonylaminomethyl derivative of acetaminophen and the N-methyl-N-ethyloxycarbonylaminomethyl derivative of theophylline prodrugs showed a 2.1 and 1.6 fold increases in flux compared to acetaminophen and theophylline alone. N-alkyl-N-alkyloxycarbonylaminomethyl promoiety thus acts as novel prodrugs of phenol and imide containing drug molecules and increases their permeation through biological membranes like the skin.

MEDI 239

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ethyloxycarbonylaminomethyl derivative of theophylline prodrugs showed a 2.1 and 1.6 fold increases in flux compared to acetaminophen and theophylline alone. N-alkyl-N-alkyloxycarbonylaminomethyl promoiety thus acts as novel prodrugs of phenol and imide containing drug molecules and increases their permeation through biological membranes like the skin.

MEDI 240

Mild, efficient and regioselective synthesis of fatty ester derivatives of 1-β-D-arabinofuranosylcytosine via Novozym 435–catalyzed acylation in ionic liquid-containing systems

*Xiao-feng Li, **Min-hua Zong**, and Hong Wu, College of Biological Sciences and Biotechnology, South China University of Technology, 1 Wushan Street, Guangzhou 510640, China, Fax: +86-20-2223-6669, lxfbio317@126.com, btmhzong@scut.edu.cn*

A facile synthesis of 5′-*O*-monoesters of 1-β-D-arabinofuranosylcytosine, more powerful antitumor drugs, was successfully performed for the first time using enol esters ($C_nH_{2n+1}COOCH=CH_2$, n=2-18) as acyl donors and Novozym 435 as the biocatalyst in ionic liquid (IL)-containing systems. Novozym 435 exhibited a high regioselectivity towards the 5′-hydroxyl of 1-β-D-arabinofuranosylcytosine, giving 5′-*O*-monoesters exclusively. Both the cation and the anion of ILs have a significant effect on the reaction, and 10% (v/v) C4MIm•PF_6-THF was the most suitable medium for this purpose, giving higher initial rate and substrate conversion than other IL-containing systems examined while keeping the regioselectivity above 99.5%. Besides, elongating the alkyl chain of the enol ester resulted in lower reaction rate, but had little effect on the substrate conversion and regioselectivity (96.0% and 99.5%, respectively in all cases assayed). IL-containing system seems to be an efficient alternative to conventional organic solvents for the preparation of 5′-*O*-monoesters of 1-β -D-arabinofuranosylcytosine.

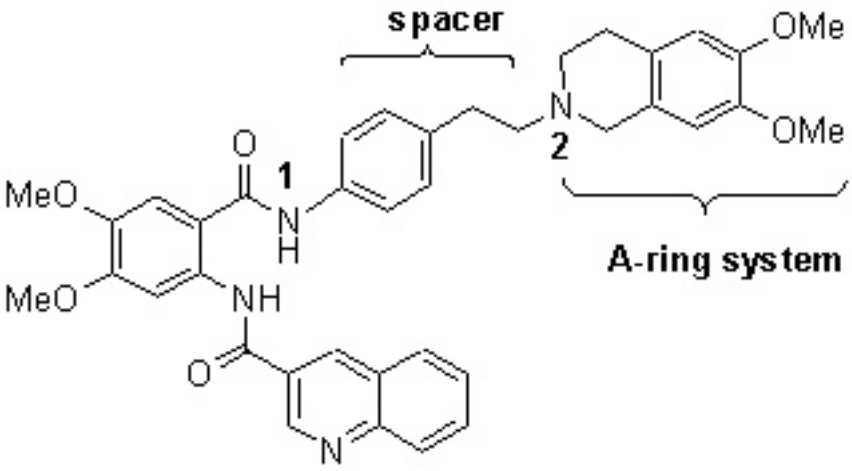
MEDI 241

In vitro activity of new MDR anthranilamide modulators and their effect on CYP450

Philippe Labrie[1]*, Shawn. P. Maddaford*[2]*, Suman Rakhit*[2]*, and Rene C. Gaudreault*[3]*. (1) Faculty of Pharmacy and Instituts des Biomateriaux et des technologies, Hopital St-François D'Assise, Laval University, Laval university, Quebec, QC G1K 7P4, Canada, plabrie16@hotmail.com, (2) Chemistry and Computer Sciences Building, York University, MCR Research Inc, (3) Instituts des Biomateriaux et des technologies, Hopital St-François D'Assise, Laval University*

Synthesis and in vitro activity of the novel MDR anthranilamide modulators have been performed to assess the importance on their potency of inhibition P-glycoprotein (P-gp) of the aromatic spacer group between both nitrogen atoms 1 and 2. In that context, we have evaluated the effect of the flexibility, of the chain length, of the chirality and the presence of an arylpiperazinyl group. Fifteen molecules were

found to inhibit the resistance due to overexpression of P-glycoprotein in CEMVLB500. The EC50(VLB) of the resistance assessed by the P-gp inhibition were between 59 and 1345nM. Biotransformation studies of these inhibitors were also conducted using several cytochrome P-450 isoforms and indicate that these compounds inhibit the activity of a CYP450 subset different than the one affected by XR9576. Compound P24 is more potent (EC50(VLB)=59±35nM) than verapamil (EC50(VLB)=1155±629nM) and comparable to XR9576, but affect more the CYP450 subset increasing cytosolic accumulation of chemotherapeutic drugs.



MEDI 242

Design and synthesis of triazole-containing macrocylclic tetrapeptide as novel Grb2 SH2 domain ligands

Zhen-Dan Shi[1], *Karen M. Worthy*[2], *Robert J. Fisher*[2], *and Terrence R. Burke Jr.*[1]. *(1) Laboratory of Medicinal Chemistry, CCR, NCI, NIH, Bidg.376, Boyles Street, Frederick, MD 21702, Fax: 301-846-6033, shiz@ncifcrf.gov, (2) Protein Chemistry Laboratory, SAIC-Frederick*

Growth factor receptor-bound protein 2 (Grb2) is an adaptor protein that provides critical connectivity between receptor protein-tyrosine kinases and Ras signal transduction. It has been shown to be involved with several cancers including breast cancer and kidney cancer. Therefore, disruption of Grb2 function by high affinity synthetic ligands may potentially afford new therapeutics. Using double bonds as the linkage of cyclic peptide, we recently prepared a large number of macrocyclic peptide mimetics that exhibited low nanomolar affinity in cellular assays. In order to develop other approaches to the formation of macrocyclic peptide, we prepared novel Grb2 inhibitors by utilizing [3+2] cycloaddition reactions of azide with terminal alkyne called click chemistry. This novel Grb2 platform represents newly application of click chemistry in peptidomimetic design. Detailed herein is the design, synthesis and biological evaluation of these unique agents.

MEDI 243

Development of potent and selective inhibitors of FLT–3 kinase with good pharmacokinetic profiles

Robert M. Grotzfeld*, Shamal A. Mehta, Zdravko V. Milanov, Andiliy G. Lai, Kelly G. Sprankle, Maiko Ezawa, Qi Chao, Brian Campbell, Joyce K. James, Michael F. Gardner, Merryl D. Cramer, Miles A. Fabian, Todd A. Carter, Anne Marie Velasco, Julia M. Ford, Mark Floyd, Pietro Ciceri, Darren E. Insko, Sanna Herrgard, Corey E. Atteridge, Lisa M. Wodicka, Daniel K. Treiber, David J. Lockhart, Patrick P. Zarrinkar, Shripad Bhagwat, and Hitesh K. Patel, Ambit Biosciences Corp, 4215 Sorrento Valley Boulevard, San Diego, CA 92121, Fax: 858-334-2199, rgrotzfeld@ambitbio.com*

FMS-like tyrosine kinase-3 (FLT-3) plays an important role in the development of blood cells and is also highly expressed in several hematological malignancies, including acute myeloid leukemia (AML). The single most commonly mutated gene in AML is FLT-3 kinase, and resulting activating mutations are associated with poor prognosis in leukemia patients. Selectively targeting FLT-3 kinase therefore offers a significant therapeutic approach for the treatment of AML as well as other hematopoietic malignancies associated with FLT-3 kinase mutations. Ambit Biosciences has previously reported the discovery of highly specific picomolar FLT-3 kinase inhibitors that target FLT-3, PDGFRb, and c-KIT. Here, we present recent development efforts on the improvement of the pharmacological profile of these compounds, as well as progress towards the development of a clinical candidate.

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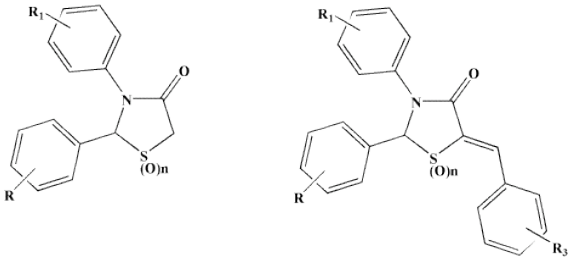
MEDI 244

Inhibition cyclin dependent kinase 4 (CDK4) activity by thiazolidinones

Venkat R Pallela, *Srinivas R Natala, Stephen C Cosenza, Muralidhar R Mallireddigari, Nabissa Pappathi, Balaiah Akula, Vinaykumar Billa, E. Premkumar Reddy, and MV. Ramana Reddy, Fels Institute for Cancer Research, Temple University School of Medicine, 3307, North Broad Street, Philadelphia, PA 19140-5101, pallela@temple.edu*

Cyclins and cyclin dependent kinases (CDKs) play an important role in the regulation of cell cycle events. Abnormal phosphorylation of CDKs is a hallmark of cancer and for this reason there is a growing interest in the use of CDK inhibitors as drugs. An increasing body of evidence has shown a link between multiple deregulations of CDKs and tumor development. This evidence has led to an intense search for small molecule inhibitors of the CDK family as an approach to cancer chemotherapy. Several CDK inhibitors such as flavopiridol, 7-hydroxystaurosporine (UCN-01), roscovitine (CYC202), 2-aminothiazole derivative BMS-387032 and PD0332991 have been reported as anti-cancer agents. Among these, some nonspecific CDK inhibitors such as UCN-01 and flavopiridol have entered in to clinical trials for treatment of various cancers. As a next generation of CDK inhibitors, some of the molecules showed a promising selectivity towards a specific CDK and caused cell cycle arrest without undesirable toxicity. One of the kinase that is activated in G1 phase of cell cycle is CDK4 and specific inhibition of CDK4 by a small molecule can cause cell cycle arrest in the G1 phase in tumor cells without affecting normal cells. Our interest in finding the inhibitors of CDK4/cyclin D led us to investigate a series of substituted thiazolidinones. We present the synthesis, structure-activity relationships, and in vitro biological evaluation of a series of 2,3-diphenyl-1,3-thiazolidin-4-one and 5-benzylidene-2,3-diphenyl-1,3-thiazolidin-4-one.



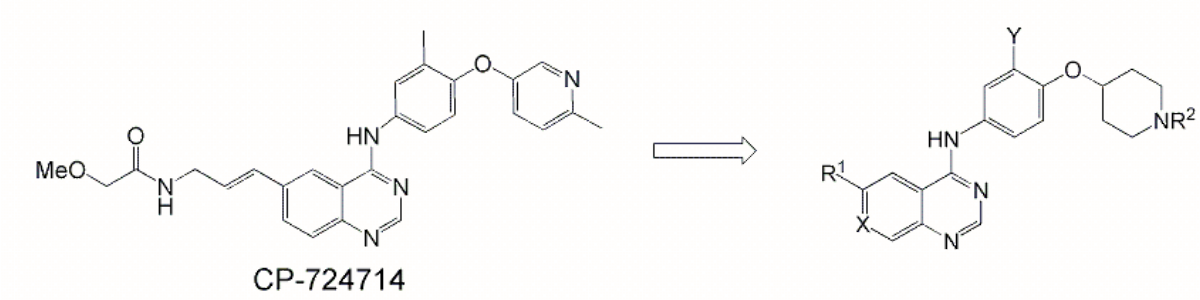
MEDI 245

Synthesis and biological evaluation of selective ErbB2 (Her2) inhibitors for the treatment of cancer

Goss S. Kauffman, *Blaise Lippa, Joel Arcari, Tricia A. Kwan, Samit K. Bhattacharya, Leslie R Pustilnik, Chunyan Su, James D. Moyer, Ling Ma, Mary Campbell, and Stefan Steyn, Department of Cancer Research, Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, Fax: 860-686-0605, goss.s.kauffman@pfizer.com*

ErbB2 is a tyrosine kinase that is an important target for the treatment of breast cancer. The erbB2 inhibitor CP-724714 was synthesized at Pfizer and is currently in clinical trials. Due to the interest in this important cancer target, efforts at Pfizer

have been invested in the discovery of additional erbB2 inhibitors. This poster will describe the synthesis and biological evaluation of a new series of novel erbB2 antagonists. Several of these new inhibitors have shown improved erbB2 selectivity, decreased clearance and enhanced solubility, while retaining excellent kinase and cell potency.



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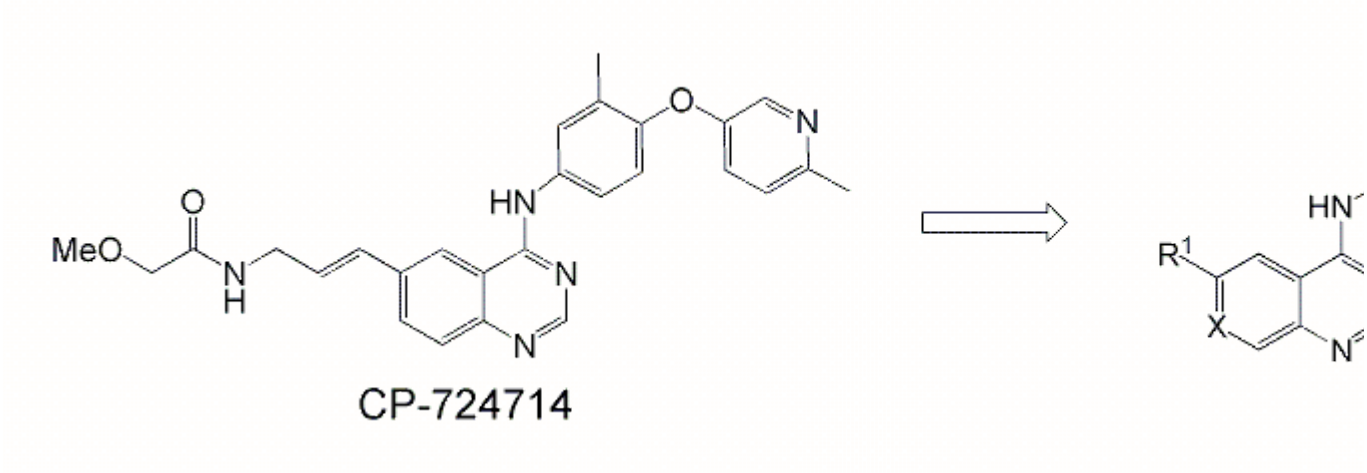


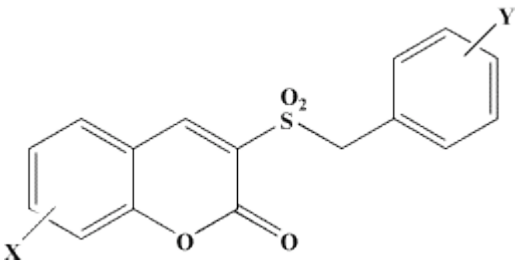
MEDI 246

Benzylsulfonyl coumarins: Novel ErbB2 kinase inhibitors

Muralidhar R Mallireddigari[1], *Stephen C Cosenza*[1], *Venkat R Pallela*[1], *Balaiah Akula*[1], *Stanley C Bell*[2], *E. Premkumar Reddy*[1], *and MV. Ramana Reddy*[1]. *(1) Fels Institute for Cancer Research, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140-5101, mmreddy67@yahoo.com, (2) Department of Medicinal Chemistry, Onconova Therapeutics Inc*

Epidermal growth factor receptor kinase family includes EGFR, ErbB2, ErbB3 and ErbB4 and over expression and constitutive activation of EGFR and ErbB2 have been implicated in wide variety of human cancers. Inappropriate activation and over expression of EGFR and ErbB2 in many tumors has become an attractive target in cancer therapy and triggered extensive efforts to identify new molecules that specifically inhibit or abolish the kinase activity of these receptors. Specific antibodies and low molecular weight tyrosine kinase inhibitors of EGFR and ErbB2 are approved for the treatment of breast and lung cancers.

In our attempts to identify specific tyrosine kinase inhibitors of ErbB2, we designed and synthesized a series of novel benzyl sulfonyl coumarins and studied their activity in vitro and in vivo. The cytotoxicity analysis in ErbB2 +ve BT 474, SKBr3 and –ve DU145, BT 20 cell lines, in vitro inhibition of ErbB2 tyrosine phosphorylation and kinase activity studies showed that these compounds are selective inhibitors of erbB2 kinase.



MEDI 247

In silico identification of novel chemical scaffolds as inhibitors of EGFR tyrosine kinase activity

Claudio N. Cavasotto[1], *Maria A. Ortiz*[2], *R. Abagyan*[3], *and F. Javier Piedrafita*[2]. *(1) Molsoft LLC, 3366 North Torrey Pines Court, S. 300, La Jolla, CA 92037, Fax: 858-625-2888, claudio@molsoft.com, (2) Sidney Kimmel Cancer Center, (3) Department of Molecular Biology, The Scripps Research Institute*

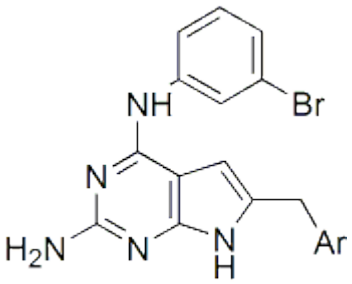
To the best of our knowledge, the crystal structure of EGFR has been used for the first time to identify novel inhibitor chemotypes by in silico screening of a large virtual chemical library followed up by experimental validation. We identified several compounds that inhibited EGFR tyrosine kinase activity. Amongst them, a C(4)-N(1) substituted pyrazolo[3,4-d]pyrimidine (MSK-039) was discovered as an ATP-competitive low-micromolar inhibitor of EGFR tyrosine kinase activity. Importantly, MSK-039 as well as other EGFR inhibitors were found to inhibit proliferation of A431 epidermoid carcinoma cells. The predicted binding mode of MSK-039 opens a new avenue towards the optimization of novel chemical entities to develop potent and selective inhibitors of EGFR signaling.

MEDI 248

Synthesis of 2-amino-4-substituted-6-arylmethyl pyrrolo[2,3-*d*]pyrimidines as inhibitors of receptor tyrosine kinases

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, ***Sudhir Raghavan****, Division of Medicinal Chemistry, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, raghavans@duq.edu, and Michael Ihnat, Department of Cell Biology, The University of Oklahoma Health Science Center*

Angiogenesis plays a pivotal role in tumor growth and metastasis. Several growth factor receptor tyrosine kinases (RTKs) are directly or indirectly involved in angiogenesis. Certain tumors overexpress RTKs which include PDGFR, FGFR, VEGFR, IGFR and EGFR. Recently erlotinib, an EGFR inhibitor, has been approved for non-small cell lung cancer and several other RTK inhibitors are currently in clinical trials. Gangjee *et al.* designed and synthesized 2-amino-4-(3'-bromoanilino)-6-benzylsubstituted pyrrolo[2,3-*d*]pyrimidines of general structure **1** as inhibitors of RTKs. These analogs demonstrated that variation of substituents in the phenyl moiety controlled both the potency and specificity of inhibitory activity against various RTKs. The 6-(2',4'-dichloro)benzyl analog was found to be a selective EGFR kinase inhibitor while the 6-(2'-methyl)benzyl analog was found to be a selective VEGFR-2 kinase inhibitor with submicromolar activity in whole cell assays. Using these analogs as lead compounds, we have explored substitutions on the 4-position to evaluate their effect(s) on the specificity and potency of RTK inhibition. The design and synthesis of these compounds will be presented and discussed.



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MEDI 249

Exploring Akt kinase using enzymatic and biophysical methods

Girija Krishnamurthy[1], *Keith Pitts*[1], *Meichu Lo*[1], *Ker Yu*[2], *Lisa Doliveira*[3], *and Ed Salaski*[1]. *(1) Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown, Pearl River, NY 10965, krishng@wyeth.com, (2) Oncology Research, Wyeth Research, (3) Chemical and Screening Sciences, Wyeth Research*

Akt is a serine/threonine kinase that plays a crucial role in cell survival signaling and its activation is linked to tumorigenesis. We report here the results of the enzymatic characterization studies and the initial results exploring a fluorescence binding assay to determine the binding affinity of the inhibitors of Akt. Using a coupled enzyme assay, we obtained kinetic constants for Akt2 using a 10-mer substrate, GSK3a, and demonstrated that staurosporine competes with ATP, as expected. The binding experiments using the intrinsic tryptophan fluorescence of Akt2 showed that staurosporine interacts specifically with high affinity to both the active and unactivated enzymes. Further, we developed a direct binding assay by taking advantage of the strong intrinsic fluorescence of staurosporine to use it as a fluorescence polarization (FP) probe. The affinity of staurosporine was 100 nM to unactivated Akt2, and about 5 nM to the active form. The difference in staurosporine affinity towards the active and unactivated enzymes suggests that the binding affinity is enhanced at the ATP site due to conformational changes that result from the phosphorylation of Thr308.

MEDI 249

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MEDI 250

Synthesis and biological evaluation of dimeric imatinib analogs as potent tyrosine kinase inhibitors

Mikell A. Paige[1], *Miki Newman*[2], *Thomas Kindler*[3], *D. Gary Gilliland*[3], *John H. Bushweller*[2], *and Milton L. Brown*[1]. *(1) Department of Chemistry, University of Virginia, McCormick Road, Charlottesville, VA 22904, map9d@virginia.edu, (2) Department of Molecular Physiology and Biological Physics, University of Virginia, (3) Harvard Institutes of Medicine*

Mutations in protein tyrosine kinases (PTK) have been implicated in the transforming process of cancer cells. Imatinib mesylate (gleevec) is a selective tyrosine kinase inhibitor that is currently used in the clinic for the treatment of chronic myeloid leukemia (CML). Several oncogenic kinases are activated by homo-oligomerization of the protein. To directly target this oligomerization property, we have developed a series of divalent imatinib analogs with varying chain lengths between two imatinib core molecules. Rapid access to these dimeric imatinib analogs utilizing click chemistry is presented. These analogs were found to be potent ABL-kinase inhibitors. Furthermore, these analogs showed selectivity and chain-dependent potency to cells that over-expressed FIP1L1-PDGFRalpha.

MEDI 251

Design, syntheses and SAR of inhibitors targeting the T315I-ABL mutation

Jianguo Cao[1], *Kathy Barrett*[2], *Richard M. Fine*[3], *Colleen Gritzen*[1], *John Hood*[2], *Jason Kang*[3], *Dan Lohse*[2], *Chi Ching Mak*[1], *Andrew McPherson*[1], *Glenn Noronha*[1], *Ved P. Pathak*[1], *Joel Renick*[1], *Richard Soll*[1], *Ute Splittgerber*[1], *Binqi Zeng*[1], *and Hong Zhu*[2]. *(1) Medicinal Chemistry, TargeGen, Inc, 9393 Towne Centre Drive, Suite 120, San Diego, CA 92121, Fax: 858-678-0762, jcao@targegen.com, (2) In-vitro Biology, TargeGen, Inc, (3) Biopredict, Inc*

Although imatinib has been a remarkable success for treatment of CML, a significant proportion of patients develop resistance due to mutations. The T315I (gatekeeper) mutation stands out among the >40 clinically identified mutations because it shows highest prevalence (>20%), and maintains resistance to all recently developed BCR/ABL inhibitors (AMN107, BMS354825, AP23464, PD180970) that potently target most other mutations. Using modeling and structure-based design, TargeGen

leveraged a dual SRC and ABL inhibitor series to potently target the T315I-ABL mutant. A key feature that characterizes this targeted design involves manipulation of groups deep within the ABL hydrophobic pocket, circumvention of the gatekeeper T315I-residue, and distal optimization to fine-tune for both SRC and ABL. We will discuss the SAR and modeling that led to nM inhibitors of mutant T315I-ABL. Representative compounds inhibit wt-ABL or mutant-BCR/ABL activity in cells as confirmed by reduction of phosphorylation of endogenous BCR/ABL-targeted proteins (BCR/ABL, ABL, STAT5 and CrkL).

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MEDI 252

Transcriptional regulation of VPACR-1 by the tumor suppressor Ikaros

Aaron Krueger*, Rebecca Hermann, and Glenn Dorsam, Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105, Aaron.Krueger@ndsu.edu*

Greater understanding of tumor suppressor function should significantly improve cancer therapies. Recombinant Ikaros, a known tumor suppressor, downregulates the antiproliferative receptor VPACR-1 in fibroblast NIH-3T3 cells. Ikaros represses transcription by a DNA-binding-dependent or non-dependent mechanism. Due to several Ikaros binding motifs in the VPACR-1 promoter, we hypothesize that Ikaros downregulates VPACR-1 by a DNA-binding-dependent mechanism. Therefore, DNA-binding necessity will be examined by mutating Ikaros DNA binding domains by PCR

mutagenesis and comparing VPACR-1 levels by qPCR in a transient NIH-3T3 system. Presently, four different Ikaros isoforms have been cloned into pCMV-Tag2B expression vector, analyzed by endonuclease digestion analysis, visualized by agarose gel electrophoresis, and confirmed by DNA sequencing. From this starting point, we expect a new level of insight into the interaction between tumor suppressors and their gene targets. This research was funded by NIH 5K01DK64828-2 and the Center for Protease Research.

MEDI 253

Condensation and uptake of therapeutic oligonucletides to breast cancer cells with dendrimers and dendrimer modified gold nanoparticles

Alex Chen, Department of Chemistry, Rutgers University, 73 Warren Street, Newark, NJ 07102, Fax: 973-353-1264, TJ Thomas, Medicine, University of Medicine and Dentistry of New Jersey, and ***Huixin He****, Chemistry, Rutgers University, 73 Warren street, Newark, NJ 07102*

The efficacy of 5 generations (G1 to G5) of polypropylenimine(PPI) dendrimers to condense therapeutic oligonucleotides (ONs) into nanoparticles was studied. The structure-activity relationships for the ONs condensation were revealed. A "zipping" mechanism, for the first time, was proposed for the ONs condensation. Confocal microscopic analysis showed that the nanoparticles formed with G4 and G5 dendrimers could undergo facile cellular uptake in a breast cancer cell line, MDA-MB-231, whereas particles formed with G1 – G3 dendrimers lacked this property. Zeta potential measurements showed that the particles were positively charged with also increased with higher generation dendrimers, which may be the reason for the different uptake properties of the nanoparticles. To enhance uptake ability of lower generation dendrimers, a novel gold nanopartcles approach was developed to catalyze the condensation of ONs while maintaining their non-toxic property.

MEDI 254

Cancer cell diagnostics and therapy using gold nanoparticles

Xiaohua Huang*, School of Chemistry and Biochemistry, Georgia Institute of Technology, 770 Sate Street, Atlanta, GA 30332, Fax: 4048947452, gtg202j@mail.gatech.edu, Ivan H El-Sayed, Otolaryngology-Head and Neck Surgery, Comprehensive Cancer Center, University of California at San Francisco, and Mostafa A. El-Sayed, Laser Dynamics Lab, School of Chemistry and Biochemistry, Georgia Institute of Technology*

Gold nanoparticles have great potential for biological applications due to their simple preparation, easy bioconjugation, potential noncytotoxicity and their size and shape controlled optical properties. Gold nanoparticles strongly absorb and scatter visible and near infrared light because of the strongly enhanced electric fields at the surface. This provides the potential of designing novel optically active reagents for simultaneous molecular imaging and photothermal cancer therapy. In our work, gold nanoparticles are conjugated with anti-epidermal growth factor receptor (anti-EGFR) antibodies that specifically target EGFR on the cell surface. Using micro-absorption

spectroscopy and light scattering imaging, cancerous (HOC 313 and HSC 3) and noncancerous cells (HaCat) can be differentiated due to the overexpression of EGFR on the surface of cancer cells (1). By irradiating the cells with a CW laser, much lower laser energies are needed to cause cancer cell destruction than the healthy cells due to the specific binding of the antibody conjugated gold nanoparticles to the cancer cells (2).

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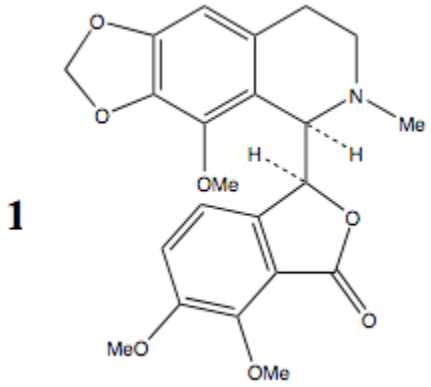
MEDI 255

Solution multi-conformational analysis of the antitussive and potential anti-cancer agent noscapine

***Ashutosh S. Jogalekar**, Pahk Thepchatri, Bing Wang, Aiming Sun, and James P. Snyder, Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, ajogale@emory.edu*

Conformational analysis of the antitussive and potential anti-cancer agent Noscapine (1) in solution has been performed by a combination of molecular mechanics and 1- and 2-D NMR spectroscopy. Molecular structures were generated by exhaustive low-mode conformational searches with two force fields. Experimental interatomic distance averages were obtained by the careful integration of NMR-NOESY cross peaks derived from mixing time build-up curves and an internal distance standard. A key torsion angle average was derived by 3JHCCH measurement. The NAMFIS methodology (NMR Analysis of Molecular Flexibility In Solution), integrating both conformational datasets and averaged NMR-based geometries, was subsequently used to deconvolute the averaged NMR spectra in two solvents into two sets of solution conformations with specific predicted mole fractions (i.e. populations, %). The resulting conformer populations will be described and compared with several solid state X-ray crystal structure determinations of noscapine and analogs. Solvent effects on noscapine's conformational profile will be discussed.



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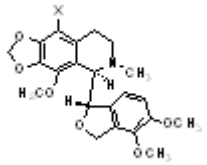
MEDI 256

Synthesis and biological evaluation of cyclic-ether analogs of noscapine that improved anti-tumor efficacy in vitro

Suryanarayana Vangapandu[1], *Ritu Aneja*[1], *Manu Lopus*[2], *Dulal Panda*[2], *and Harish C. Joshi*[1]. *(1) Laboratory for Drug Discovery and Research, Department of Cell Biology, Emory University School of Medicine, 615 Michael st, Atlanta, GA 30322, svangap@emory.edu, (2) School of Biosciences and Bioengineering, Indian Institute of Technology, Mumbai, India*

Many microtubule-interfering anticancer drugs such as taxanes and vincas in clinic today face challenges including toxicities and tumor recurrences due to drug-resistance. Noscapine, on the other hand, is well-tolerated in humans, did not show any detectable toxicity in tissues with frequently dividing cells such as hematopoetic, gut, and spleen and no signs of neurotoxicity in post-mitotic neurons. It is orally available and regress breast and lymphoid tumor xenografts in nude mice models. Here we present novel tubulin-binding semi-synthetic noscapine analogs that showed selective and potent anti-cancer acitivity and displayed 15-20 fold lower IC50 against many human tumor cell lines. Furthermore, they are also effective against a variety of drug-resistant cancer cells that are resistant to vincas and taxanes. Novelty also lies in the ability of these compounds to selectively arrest cancer cells that succumb

to apoptosis without affecting the normal cells. Thus, noscapinoids have a great promise in the clinic.

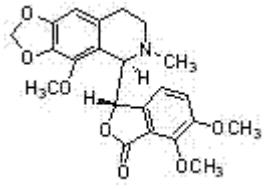


MEDI 257

Synthesis of potent microtubule-interfering halogenated noscapine analogs that inhibit cellular proliferation and perturb mitosis

Ritu Aneja[1]*, Suryanarayana Vangapandu*[1]*, Manu Lopus*[2]*, Dulal Panda*[2]*, and Harish C. Joshi*[1]*. (1) Laboratory for Drug Discovery and Research, Department of Cell Biology, Emory University School of Medicine, 615 Michael Street, Atlanta, GA 30322, raneja@emory.edu, (2) School of Biosciences and Bioengineering, Indian Institute of Technology, Mumbai, India*

We have previously identified the naturally-occurring non-toxic antitussive phthalideisoquinoline alkaloid, noscapine as a microtubule-interacting agent that binds tubulin, arrests mitosis and induces apoptosis. Here we present high yield efficient synthetic methods and an evaluation of anti-tumor potential for a series of halogenated noscapine analogs that are predicted by in silico design to have a lower free-energy configuration in the bound state of its target molecule, tubulin. Our results show that all analogs have higher tubulin-binding activity and possess greater cytotoxicity than noscapine. They act by selective G2M arrest followed by apoptotic cell death with much higher efficiency than noscapine. More importantly, the bromo analog demonstrated ability to significantly inhibit melanoma progression in vivo and conferred a significant survival advantage as compared to noscapine. Based upon the promise of the lead compound noscapine, this work might have wide-ranging implications for preclinical and clinical studies of many cancer types.



MEDI 258

Synthesis and biophysical evaluation of minor-groove binding C-terminus modified polyamide derivatives of distamycin

Toni Brown[1]*, Zarmeen Taherbhai*[2]*, Jim Sexton*[2]*, Arden Sutterfield*[2]*, Mark Turlington*[2]*, Justin Jones*[2]*, Lindsay Stollings*[2]*, Michelle Stewart*[2]*, Karen Buchmueller*[2]*, Hilary*

*Mackay[1], Caroline O'Hare[3], John Hartley[3], Binh Nguyen[4], David Wilson[4], and **Moses Lee**[1]. (1) Department of Chemistry, Hope College, Natural Sciences Division, 35E. 12th. Street, Holland, MI 49422, Fax: 616-395-7923, lee@hope.edu, (2) Chemistry, Furman University, (3) Oncology, Royal Free & University College Medical School, (4) Department of Chemistry, Georgia State University*

Polyamides bind to the minor groove of DNA. These molecules are being heavily researched in an attempt to understand a 'language of DNA recognition'. Distamycin, formamido-tripyrroleamide-amidine (f-PyPyPy-am), has been shown to bind to regions of A/T rich sequences in duplex DNA with high binding affinity. A wealth of research has been performed to expand this recognition to include G/C base pairs. As a result the stacked heterocycles pairing rules were discovered. A stacked Py/Py pairing was found to favor binding to an A•T or T•A base pair. However, a stacked Im/Py pairing binds preferentially to a G•C base pair; Py/Im for C•G, and the Im/Im pairing favors a T•G mismatched base pair. In subsequent studies, a formamido polyamide containing imidazole moieties and a dimethylamino C-terminus (f-ImPyIm) has been shown to bind to its cognate ACGCGT sequence with superior affinity over the f-PyPyPy molecule for its AAATTT sequence. In order to gain further insight into the molecular recognition of polyamides with DNA, it was apparent that a systematic study on the DNA binding properties of C-terminus modified polyamides needs to be conducted. Accordingly, in this presentation, we will describe the synthesis and biophysical evaluation of f-PPP and f-IPI triamides with different C-terminus moieties, including methylpiperazine and a spermidine analog. The DNA interacting properties of these compounds were ascertained by a series of biochemical (DNase I footprinting) and biophysical studies (DNA melts, CD, and isothermal titration calorimetry).

MEDI 259

Synthesis and biological evaluation of cyclohexenone derivatives of combretastatin-A4 as potential tubulin inhibitors

*John Dickson[1], Khyati Baxi[1], Toni Brown[2], Regan LeBlanc[1], Lori Forrest[1], Patrick Nolan[2], Herman Holt Jr.[3], William Pennington[4], and **Moses Lee**[2]. (1) Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, (2) Department of Chemistry, Hope College, Natural Sciences Division, 35E. 12th. Street, Holland, MI 49422, Fax: 616-395-7923, lee@hope.edu, (3) Chemistry, University of North Carolina at Asheville, (4) Chemistry Department, Clemson University*

Vascular targeting agents, such as combretastatin A-4 (CA-4) and the phosphate prodrug (CA-4P), inhibit tumor growth by specifically targeting the tumor microvasculature and occluding blood flow to it. The antitumor activity of CA-4 is derived from its ability to interact at the colchicine binding site of tubulin thus preventing the polymerization of tubulin to form microtubules, a component of the cytoskeleton. The aryl rings of these compounds have been shown to exist in a twisted conformation. In contrast pyrazole derivatives possess a planar structure and the cytotoxicity of these compounds is reduced. In order to test this hypothesis, 3,5-(diaryl)-6-(ethyoxycarbonyl)-2-cyclohexenones and 3,5-(diaryl)-2-cyclohexenones were designed as potential anti-cancer agents. The synthesis, molecular mechanics calculations, x-ray crystallography studies, and biological activity of a range of derivatives were performed. Cytotoxicity towards murine and human cancer cell lines

was established, and it was found to be correlated to the twisted conformation observed in the structural analyses. These results demonstrate that shape is an important factor in the design of new, potent anti-cancer agents based on the combretastatins.

MEDI 259

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MEDI 260

Synthesis and evaluation of LVF-I-141: An intercalator-polyamide hairpin designed to target the inverted CCAAT box 2 in the topoisomerase II alpha promoter

*Lloyd Flores[1], Andrew Staples[1], Hilary Mackay[2], Cameron Howard[1], Peter Uthe[1], Jim Sexton[1], Karen Buchmueller[1], Caroline O'Hare[3], Daniel Hochhauser[3], John Hartley[3], David Wilson[4], and **Moses Lee**[2]. (1) Department of Chemistry, Furman University, 3300 Poinsett Hwy, Greenville, SC 29613, (2) Department of Chemistry, Hope College, Natural Sciences Division, 35E. 12th. Street, Holland, MI 49422, Fax: 616-395-7923, lee@hope.edu, (3) Oncology, Royal Free & University College Medical School, (4) Department of Chemistry, Georgia State University*

Topoisomerase IIα (TopoIIα) is an important cellular target for chemotherapeutic agents such as etoposide and doxorubicin. Low levels of TopoIIα gene expression in

confluent cells correlate with relative resistance to these agents. The inverted CCAAT box 2 (ICB2) on the TopoIIα promoter is essential for the down regulation of promoter activity that is recognized by the transcription factor NF-Y. It has been suggested thereby that confluence-arrested cancer cells could be resensitized to TopoIIα drugs by inhibiting NF-Y binding to ICB2. The synthesis and DNA binding properties of an intercalator-polyamide hairpin (LVF-I-141) designed to target the ICB2 sequence promoter are described. The polyamide backbone PyImIm-γ-PyPyPy was designed to bind to the sequence 5'-ATTGG following the established polyamide pairing rules. A napthalimide moiety attached to the C-terminus of the polyamide was incorporated to provide additional binding affinity via DNA intercalation. In addition, this moiety will aid molecular recognition through preferential binding to GC base pairs. Biophysical studies were performed to establish DNA binding affinity and preferential binding to ICB2. DNase I footprinting analysis demonstrated that LVF-I-141 bound to the ICB2 and ICB3 sites with greater affinity than ICB1. Thermal denaturation studies confirmed these results revealing the highest degree of stabilization with ICB2 and 3 (4.1 and 4.6ºC vs. 0.6ºC for ICB1). Circular dichroism studies confirmed minor groove binding and indicated a 1:1 binding stoichiometry. Surface plasmon resonance studies demonstrated strong binding to ICB2 (2.5e7 M-1) with no binding to ICB1. Fluorescence emission titration confirmed intercalative binding. It is concluded that LVF-I-141 selectively binds to the ICB2 site via intercalation and minor groove binding and warrants further investigation as a model compound for the regulation of gene expression.

MEDI 261

Chalcones as powerful inhibitors of tubulin assembly

Sylvie Ducki[1], *Grant Mackenzie*[1], *Benjamin Greedy*[2], *Nicholas J. Lawrence*[2], *Jérémie Fournier dit Chabert*[1], *James Nettles*[3], *and James P. Snyder*[4]. *(1) Biosciences Research Institute, University of Salford, Cockcroft Building, Salford M5 4WT, United Kingdom, Fax: 0161 295 5111, S.Ducki@Salford.ac.uk, (2) Drug Discovery Program, H Lee Moffitt Cancer Center & Research Institute, (3) Lead Discovery Center, Novartis Institutes for Biomedical Research, (4) Department of Chemistry, Emory University*

We have identified a phenylbutenone (IC_{50} K562 = 60 µM) from the Chinese mint *Scutellaria barbata*. Structure-activity relationship (SAR) studies led to the discovery of chalcone SD400 (IC_{50} K562 = 0.21 nM). *In vitro* biological studies allowed us to elucidate its mode of action: the drug interacts with tubulin, a protein that is essential for cell division and cell shape, at the colchicine-binding site and inhibits assembly into microtubules.

In 2004, Ravelli published the structure of tubulin:colchicine, giving a much needed insight into the protein's structure and function. This structure helped us gain an understanding of how chalcone SD400 interacts with tubulin. This understanding has allowed us to design a new generation of chalcones which are powerful inhibitors of tubulin assembly. Pharmacokinetic studies have allowed us to optimise the drug-like propeties of these agents which are now ready to enter clinical trials.

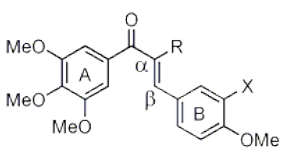
MEDI 262

Chalcone derived indenones and indanones as potent inhibitors of tubulin polymerization

Nicholas J. Lawrence[1], *Simon Armitage*[1], *Sylvie Ducki*[2], *Benjamin Greedy*[1], *and Darren Cook*[2]*. (1) Drug Discovery Program, H Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, MRC 4West, Tampa, FL 33612-9416, Fax: 813-979-6700, Lawrennj@moffitt.usf.edu, (2) Biosciences Research Institute, University of Salford*

We will present studies of novel tumor targeting compounds related to combretastatin A-4 and α,β-unsaturated analogs. We have shown that some chalcones are able to inhibit tubulin assembly by binding to the colchicine-binding site of tubulin. The impact of research in this area is high since Combretastatin A-4P elicits irreversible vascular shutdown within solid tumors, leaving normal vasculature intact. In this way tumors are starved of oxygen and nutrients and their constituent cells die. It is becoming clear that agents that act in this manner will have a significant impact on the clinical management of cancer.

Preliminary modelling and crystallographic studies led us to postulate that molecules adopting the *s-trans* rather than the *s-cis* conformation will bind more strongly to tubulin. Chalcones **1** possessing an α-H preferentially adopt an *s-cis* conformation while those bearing a group other than H at the α-position adopt an *s-trans* arrangement. We therefore sought to design systems that act as surrogates of *s-trans* enones. The design and synthesis of conformationally constrained surrogates will be presented. The first generation analogs are based on directly linking the β-carbon atom to the A-ring. In general the cytotoxic activity is retained in both indanones **3** and indenones **2**. A structure activity relationship for both series will be presented. A preliminary molecular model for the mode of binding of the agents to tubulin will be presented.



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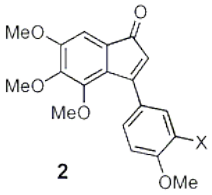

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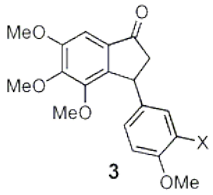


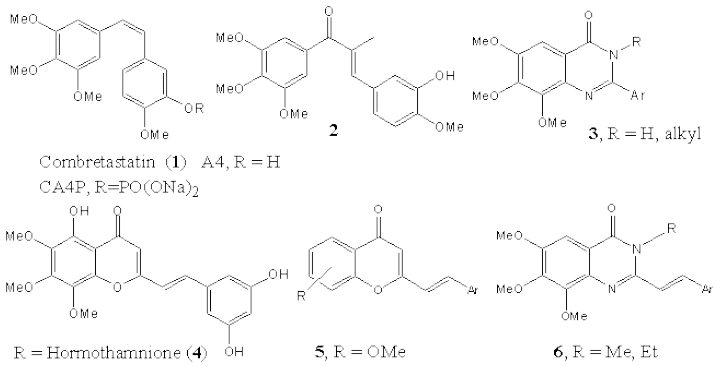
3

MEDI 263

New inhibitors of tubulin polymerization bearing chromone or quinazoline scaffolds based on combretastatin A4 and hormothamnione

Nicholas J. Lawrence[1], ***Roberta Pireddu***[1], *Sylvie Ducki*[2], *Darren Cook*[2], *and Abdul Hannan*[3]*. (1) Drug Discovery Program, H Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, MRC 4West, Tampa, FL 33612, Fax: 813 979 9748, Lawrennj@moffitt.usf.edu, pireddr@moffitt.usf.edu, (2) Biosciences Research Institute, University of Salford, (3) Department of Chemistry, Cardiff University*

Novel anticancer agents that target tumour vasculature as a consequence of their anti-tubulin properties have been investigated. The importance of tubulin as a target has been underlined by the discovery that the clinical candidate combretastatin A4P (**1**) displays potent and selective toxicity towards tumor vasculature. Our interest in tumor targeting compounds focuses on compounds related to **1**. A detailed structure-activity relationship (SAR) study conducted in our group led to the development of CA4-like chalcones as inhibitors of tubulin polymerization. We have prepared a new series of quinazoline **3** related to the α-methyl chalcone **2** as putative tubulin binding ligands. The chemical aspect of the second part of the project is based on the styrylchromone natural product Hormothamnione (**4**). Hormothamnione is an exceptionally potent cytotoxin and possesses remarkable structural similarity to the chalcones. We have prepared series of styrylchromones **5** and quinazolinones **6** related to Hormothamnione. The compounds have been tested for cytotoxicity, tubulin binding and vascular targeting properties in order to establish a structure activity relationship and mode of action.



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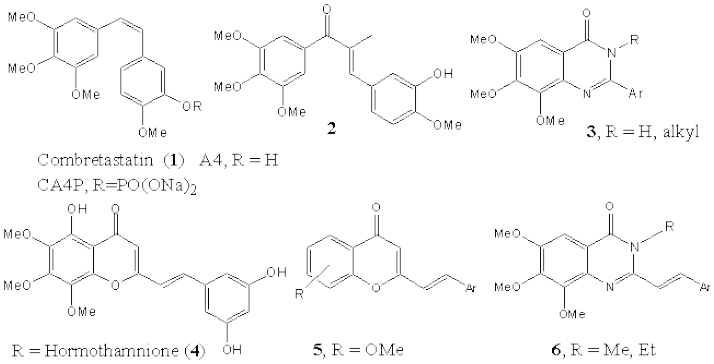


MEDI 264

Azinomycin B: Mode of action and biosynthesis

Gilbert Thomson Kelly *and Coran M. H. Watanabe, Department of Chemistry, Texas A&M University, M.S. 3255, College Station, TX 77843, gkelly@mail.chem.tamu.edu*

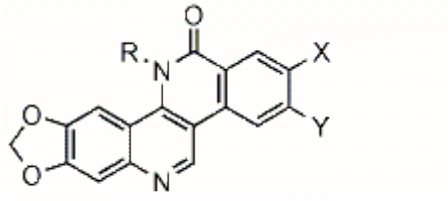
Since the isolation of Azinomycin B in 1954 from soil bacteria and structure confirmation in 1986, this natural product has been a synthetic target primarily for its potent anti-tumor activity. The bicyclic aziridine and epoxide in the molecule provide clues to the likely mode of action, DNA alkylation. The suspected reactivity was confirmed in a series of in vitro experiments by Coleman et al. We are investigating the global impact of Azinomycin B treatment in a yeast model with special emphasis on DNA damage response, the resulting cell cycle effects, and cellular localization of the compound. In addition, we are investigating the biosynthesis of Azinomycin B. Towards this end we are pursing labeled metabolite feeding experiments and are elucidating the Azinomycin B biosynthetic cluster with DNA sequencing and genetic manipulation.

MEDI 265

Synthesis and evaluation of biological activities of 8- and 9- amino derivatives of 5*H*-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthridine-6-ones as TOP I-targeting agents

Lisa S Sharma[1]*, Sudhir K. Singh*[2]*, Yuan-chin Tsai*[3]*, Angela L. Liu*[3]*, Leroy F. Liu*[3]*, and Edmond J. LaVoie*[1]*. (1) Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854-8020, Fax: (732) 445-6312, lssharma@eden.rutgers.edu, (2) Jubiliant Organosys, (3) The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Department of Pharmacology*

5*H*-8,9-Dimethoxy-5-[2-(N,N-dimethylamino)ethyl]-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one (ARC-111, Topovale®) is highly active in vivo as an antitumor agent by either parenteral or oral administration. The presence of the 2-(N,N-dimethylamino)ethyl substituent at the 5-position allows for the formation of a citrate salt that greatly enhances its aqueous solubility. The earlier studies on 8- and 9-amino-2,3-methylenedioxy-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones were extended to include varied water-solubilizing derivatives at these positions to assess their influence on relative biological activity. Reduction of 8- and 9-nitro-2,3-methylenedioxy-5*H*-dibenzo [*c*,*h*][1,6]naphthyridin-6-ones possessing either a 5-(n-butyl) or 5-[2-(N,N-dimethylamino)ethyl] alkyl side chain provided the respective amine derivatives. These arylamines were converted to their N-[2-(N,N-dimethylamino)ethyl] or N-(N,N-dimethylglycinyl) derivatives. In the present study, the relative TOP1-targeting activity and cytotoxicity of these dibenzo[*c*,*h*][1,6]naphthyridin-6-ones are compared to that observed for ARC-111.



R= $CH_2CH_2N(CH_3)_2$ or $CH_2CH_2CH_2CH_3$

X= OCH_3; Y= OCH_3

When Y= H:
X= NO_2; NH_2; $NHCH_2CH_2N(CH_3)_2$ or $NHCOCH_2N(CH_3)_2$

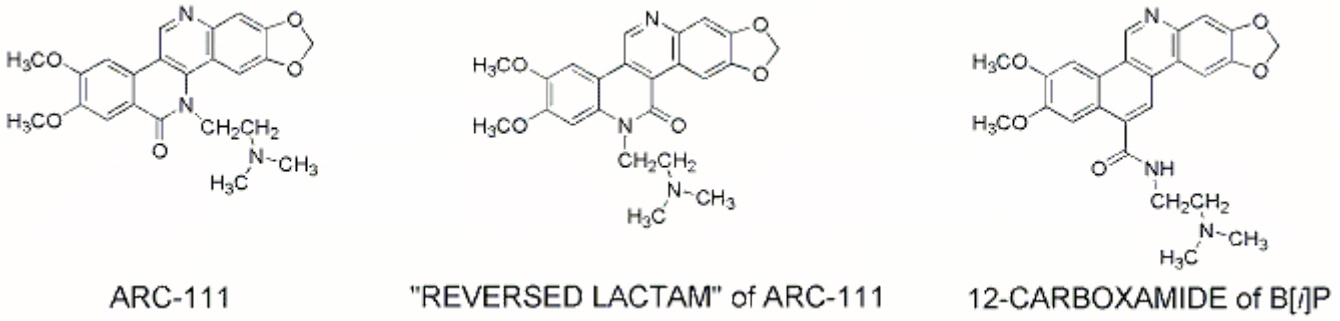
When X= H:
Y= NO_2; NH_2; $NHCH_2CH_2N(CH_3)_2$ or $NHCOCH_2N(CH_3)_2$

MEDI 266

Synthesis and evaluation of N-substituted 5-[2-(N-alkylamino)ethyl]dibenzo[*c*,*h*] [1,6]-naphthyridines as novel topoisomerase I-targeting antitumor agents

Wei Feng[1], *Satyanarayana Mavurapu*[1], *Yuan-chin Tsai*[2], *Angela Liu*[2], *Leroy F. Liu*[2], *and Edmond J. LaVoie*[1]. *(1) Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854-8020, Fax: 732-445-6312, wfeng@eden.rutgers.edu, (2) Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School*

Several dibenzo[*c*,*h*][1,6]naphthyridine derivatives related to ARC-111 (Topovale®) have been identified as potent topoisomerase I (TOP1)–targeting agents. Recently, "reversed lactam" analogues of ARC-111 and structurally-related 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxamide derivatives were also identified as potent TOP1-targeting agents. These novel non-camptothecin TOP1-targeting agents exhibited potent cytotoxicity in several human tumor cell lines with IC_{50} values frequently ranging from 0.15 to 3 nM. 5-[(2-N-alkylamino)ethyl]-dibenzo[*c*,*h*][1,6]naphthyridines, where the N-alkyl substituent of the secondary amine was CH_3, CH_2CH_3, or $CH(CH_3)_2$, were alkylated to form tertiary amines, N-alkyl(R), wherein R = CH_2CN, $CH_2C{\equiv}CH$, CH_2CF_3, and CH_2CONH_2. This study provides insight into the relative TOP1-targeting activity, cytotoxicity, and in vivo efficacy of these novel non-camptothecin TOP1-targeting agents.

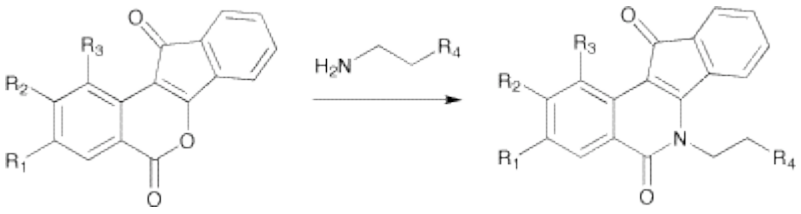


MEDI 267

Synthesis of Benz[d]indeno[1,2-b]pyran-5,11-diones: Versatile intermediates for the design and synthesis of topoisomerase I inhibitors

Andrew Morrell[1], *Smitha Antony*[2], *Glenda Kohlhagen*[2], *Yves Pommier*[2], *and Mark Cushman*[1]. *(1) Department of Medicinal Chemistry and Molecular Pharmacology and the Purdue Cancer Center, Purdue University, Heine Pharmacy Building, 575 Stadium Mall Drive, West Lafayette, IN 47907, morrell@pharmacy.purdue.edu, (2) Laboratory of Molecular Pharmacology, National Cancer Institute, NIH*

A method has been developed that relies upon a two step, one pot condensation between phthalide and 2-carboxybenzaldehyde to provide benz[d]indeno[1,2-b]pyran-5,11-dione in a multi-gram fashion. Furthermore, the method has been generalized to incorporate substituted 2-carboxybenzaldehydes (phthaldehydic acids) to furnish the corresponding substituted benz[d]indeno[1,2-b]pyran-5,11-diones. Treatment of these compounds with a primary amine allows rapid access to various N-substituted indenoisoquinolines, whose in vitro anticancer activity and topoisomerase I inhibition have been evaluated.



MEDI 268

Studies on the inhibition of kinesin motors by the marine natural product adociasulfate-2

Khalilah G. Reddie[1], *Donald R. Roberts*[2], *and Timothy M. Dore*[1]. *(1) Department of Chemistry, University of Georgia, Athens, GA 30602-2556, khalilah@chem.uga.edu, (2) Center for Advanced Ultrastructural Research, University of Georgia*

The inhibition of kinesin-1 motor protein's microtubule stimulated ATPase activity by a sulfated hydroquinone, adociasulfate 2 (AS-2), was investigated using molecular docking, fluorescence anisotropy, dynamic light scattering, enzyme kinetics, and transmission electron microscopy. The results obtained suggest that the inhibitory form of the highly chiral natural product is an aggregate that mimics the structures of microtubules and leads to non-specific recognition by kinesin, resulting in inhibition of the enzyme. The aggregate based inhibition, described as promiscuous, is novel for natural products.

MEDI 269

Novel carbohydrate-tethered carboplatin analogs

*Mo Hunsen, **Kelly P. Burke**, and Christopher R. D'Ardenne, Department of Chemistry, Kenyon College, Tomsich Hall, Gambier, OH 43022, Fax: 740-427-5731, hunsenm@kenyon.edu*

Worldwide, malignant disease is a leading cause of mortality, accounting for nearly 550,000 deaths per year in the United States alone. Although the mechanisms underlying malignancy are better understood, the death rate due to cancer has only decreased by 0.26 percent since 1950. Once cancer becomes widespread, the most effective treatment is chemotherapy, with drugs such as cisplatin and carboplatin. Though platinum drugs are effective in mediating the induction of apoptosis; they are limited by toxicity, resistance (inherent or acquired), low solubility, and relatively limited activity profile. However, efforts to develop new platinum anticancer drug candidates have yet to effectively circumvent the aforementioned limitations of platinum treatment. In this study, we report novel carboplatin analogs incorporating carbohydrate carrier ligands in an attempt to reduce resistance and toxicity, while increasing solubility and tumor specific delivery.

MEDI 270

Novel antineoplastic diterpene-benzoates from the Fijian red alga Callophycus serratus

***Amy L. Lane**[1], Anne C. Prusak[2], Mark E. Hay[2], Terry W. Snell[2], Rachel A. Giese[1], Kenneth I. Hardcastle[3], Craig R. Fairchild[4], William Aalbersberg[5], Carmen Raventos-Suarez[4], and Julia M. Kubanek[6]. (1) School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, AmyLane@gatech.edu, (2) School of Biology, Georgia Institute of Technology, (3) Department of Chemistry, Emory University, (4) Pharmaceutical Research Institute, Bristol-Myers Squibb Co, (5) Institute of Applied Sciences, University of the South Pacific, (6) School of Biology and School of Chemistry and Biochemistry, Georgia Institute of Technology*

Red macroalgae are well-known for the production of brominated metabolites, including terpenoids and phenols, yet some taxa within the Rhodophyta remain relatively unstudied. Herein, we report the discovery of unusual diterpene-benzoate natural products representing three novel carbon skeletons, from the red alga Callophycus serratus collected in Fiji. Included among the new compounds were 15- and 16-membered macrolides as well as non-macrocyclic diterpene-benzoic acid

structural motifs, all apparently sharing the same 27-carbon biosynthetic precursor. Biological activities of these compounds included moderate antibacterial, antiviral, and antineoplastic effects via specific apoptotic cell death.

MEDI 270

Novel antineoplastic diterpene-benzoates from the Fijian red alga Callophycus serratus

Amy L. Lane[1], *Anne C. Prusak*[2], *Mark E. Hay*[2], *Terry W. Snell*[2], *Rachel A. Giese*[1], *Kenneth I. Hardcastle*[3], *Craig R. Fairchild*[4], *William Aalbersberg*[5], *Carmen Raventos-Suarez*[4], *and Julia M. Kubanek*[6]. *(1) School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, AmyLane@gatech.edu, (2) School of Biology, Georgia Institute of Technology, (3) Department of Chemistry, Emory University, (4) Pharmaceutical Research Institute, Bristol-Myers Squibb Co, (5) Institute of Applied Sciences, University of the South Pacific, (6) School of Biology and School of Chemistry and Biochemistry, Georgia Institute of Technology*

Red macroalgae are well-known for the production of brominated metabolites, including terpenoids and phenols, yet some taxa within the Rhodophyta remain relatively unstudied. Herein, we report the discovery of unusual diterpene-benzoate natural products representing three novel carbon skeletons, from the red alga Callophycus serratus collected in Fiji. Included among the new compounds were 15- and 16-membered macrolides as well as non-macrocyclic diterpene-benzoic acid structural motifs, all apparently sharing the same 27-carbon biosynthetic precursor. Biological activities of these compounds included moderate antibacterial, antiviral, and antineoplastic effects via specific apoptotic cell death.

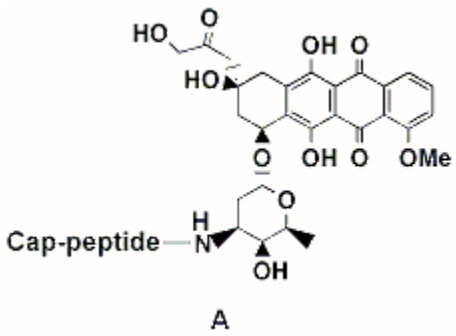
MEDI 271

Discovery of MMP activated peptide-doxorubicin prodrugs as anti-tumor agents: Part I

Wei Han[1], ***XiangJun Jiang***[1], *Zilun Hu*[1], *Nilsa Graciani*[2], *Charles F. Albright*[1], *Eddy Yue*[3], *Mingzhu Zhang*[4], *Randine Dowling*[1], *Pearl Huang*[5], *Allen Oliff*[5], *Robert A. Copeland*[5], *George L. Trainor*[1], *Andrew P. Combs*[3], *and Steven P. Seitz*[1]. *(1) Pharmaceutical Research Institute, Bristol-Myers Squibb, Princeton, NJ 08543, wei.han1@bms.com, xiang-jun.jiang@bms.com, (2) Wyeth Pharmaceutical, (3) Incyte Pharmaceuticals, (4) Neurocrine Biosciences, (5) GlaxoSmithKline*

Doxorubicin is an anthracycline natural product that is frequently used to treat tumors such as breast cancer, liver cancer, soft-tissue sarcomas, and non-Hodgkin's lymphoma. Like other cytotoxic drugs, the therapeutic efficacy of doxorubicin is limited by unwanted toxicity to nontumor tissues. A promising approach to overcome these limitations, and thus increase therapeutic efficacy, is to use tumor associated enzymes to deliver the cytotoxic drug specifically to the tumor. Based on this concept, a cytotoxic drug is masked as nontoxic prodrug. Upon administration, the prodrug is selectively activated to regenerate the toxic parent drug at the tumor site. In this poster, we will disclose the design, synthesis and biological evaluation of

matrix metalloproteinase (MMP) activated peptide-doxorubicin prodrugs (A) as anti-tumor agents.



A

MEDI 272

Discovery of MMP activated peptide-doxorubicin prodrugs as anti-tumor agents: Part II

*Wei Han[1], **Zilun Hu**[1], XiangJun Jiang[1], Charles F. Albright[1], Shu-Yun Zhang[2], Nilsa Graciani[3], Mingzhu Zhang[4], Swamy Yeleswaram[5], Pearl Huang[2], Alan Oliff[2], George L. Trainor[1], Andrew P. Combs[5], and Steven P. Seitz[1]. (1) Bristol-Myers Squibb, Princeton, NJ 08543, wei.han1@bms.com, zilun.hu@bms.com, (2) GlaxoSmithKline, (3) Wyeth Pharmaceutical, (4) Neurocrine Biosciences, (5) Incyte Pharmaceuticals*

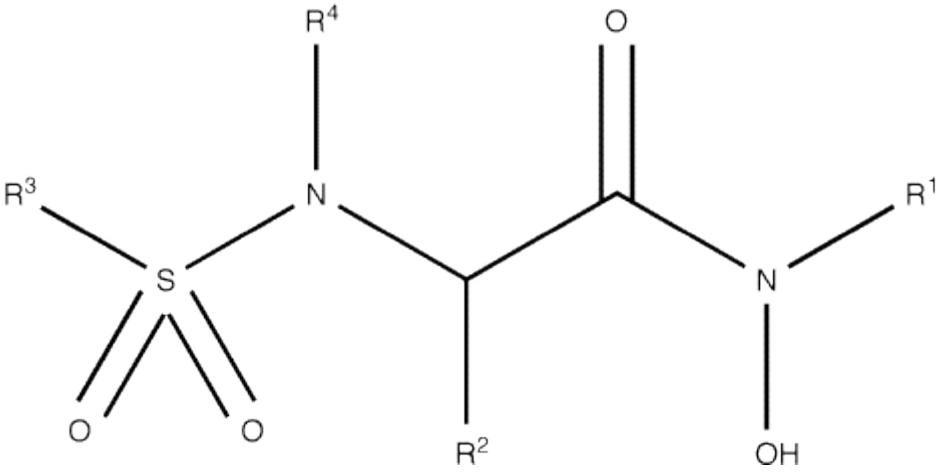
Except for heart and coronary artery disease, cancer is the principle cause of death in the Western world. Most of current anticancer drugs are restricted in their dosage due to toxicity to normal cells. Selective delivery of drug to the tumor through use of prodrugs is one way to overcome this limitation. Cleaved by tumor specific enzymes, prodrugs can be administered at higher doses to maintain drug levels in tumor while reducing exposure in nontumor tissue. Thus the therapeutic index of such drugs may be increased. In the first part of our presentation, we discussed the design, synthesis and in vitro evaluation of MMP activated peptide-doxorubicin prodrugs as potential anticancer agents. In this poster, the in vivo efficacy in animal models and the pharmacokinetic profile of select compounds will be disclosed.

MEDI 273

Polymer-supported N-derivatized-O-linked hydroxylamine for concurrent solid-phase synthesis of diverse N-alkyl and N-H hydroxamates, inhibitors of cell proliferation and migration of highly invasive breast cancer cells MDA-MB-231

***Keith J. Stanger**, Walther Cancer Institute, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, IN 46556-5670, Fax: 574-631-6652, kstanger@nd.edu, Viktor Krchnak, Department of Chemistry & Biochemistry, University of Notre Dame, and Daniel Sliva, Cancer Research Laboratory, Methodist Research Institute*

A general method for the solid-phase concurrent synthesis of diverse N-alkyl and N-H hydroxamates was developed, based on a N-derivatized O-linked hydroxylamine. For the synthesis of NH hydroxamates, protection of the nitrogen by 2,4-dimethoxybenzyl group eliminates side-reactions caused by the presence of the hydroxamate NH group. This protecting group is acid labile and removed simultaneously during acid-mediated cleavage of the product from the resin. The methodology was used to synthesize a set of more than 50 compounds based on structure 1. Purified compounds were tested for inhibition of cell proliferation and migration of the highly invasive breast cancer cell line MDA-MB-231. Structural changes at the four positions, R1 to R4, were made to evaluate the compounds ability to inhibit cell proliferation. N-alkylated versions were shown to be the most active inhibitors of proliferation of invasive breast cancer cells.



MEDI 274

Synthesis and testing of both reversible and irreversible selective androgen receptor modulators (SARMs) for prostate cancer

Dong Jin Hwang[1]*, Jun Yang*[2]*, Michael L. Mohler*[1]*, James T. Dalton*[2]*, and Duane D. Miller*[1]*. (1) Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, 847 Monroe Ave Suite # 327, Memphis, TN 38163, djhwang@utmem.edu, (2) Division of Pharmaceutics, Ohio State University*

Prostate cancer (CaP) is series disease for men in the United States and growing up to developed countries. Androgens, majorly testosterone (T) and dihydrotestosterone (DHT), play important roles not only in the growth of CaP, but also in the development and maintenance of normal prostate tissue. The replacements of T or DHT for prostate cancer therapy are powerful and efficient methods. To the target, we have developed and reported several non-steroidal types' selective androgen receptor modulators (SARMs) until now. In this study by extension of our previous isothiocyanato ligands, we prepared several new non-steroidal ligands and tested on prostate cancer lines including androgen dependent/independent cells. We investigated the B-ring part of R-bicalutamide skeleton with ortho-substitution, one-more chain of 4-substitution, and alkyl chained B-ring for CaP. These novel reversible/irreversible SARMs represent a new class of androgen receptor targeting agents (ARTA). These ARTA compounds demonstrated growth inhibitory activity

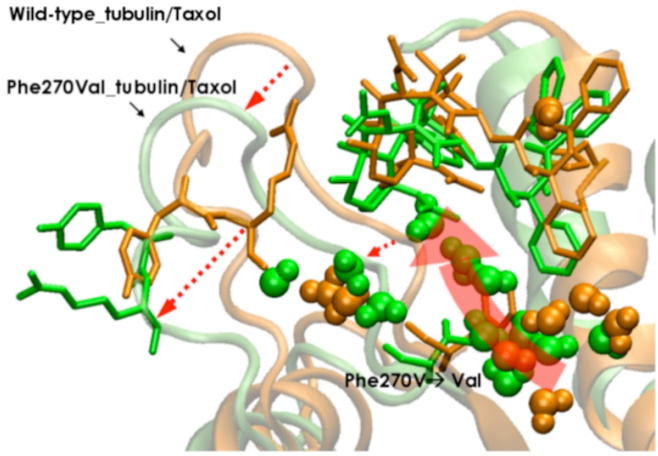
against PC cell lines (LNCaP, DU145, PC-3, PPC-1 and TSU) and tested on a normal cell line (CV-1) in vitro.

MEDI 275

An explanation for the resistance of the tubulin/microtubule Phe272Val mutant to taxol: Molecular dynamics simulation in water

suwipa Saen-Oon *and James P. Snyder, Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, suwipa@euch4e.chem.emory.edu*

Among the anti-tubulin drugs, Taxol is a uniquely effective anti-cancer agent. The compound stabilizes microtubules and subsequently leads to mitotic arrest and cell death. Despite Taxol's cytotoxicity, side-effects and drug–resistance limit its efficacy. In the context of cell-based resistance, Giannakakou et al. demonstrated that the b-Phe270Val mutation near the M-loop causes a 24-fold resistance against Taxol along with a lack of tubulin polymerization in the resistant cells. In the present work, molecular dynamics simulations with explicit water molecules was used to study the mobility of the tubulin/Taxol complex. The Phe270Val mutation induces greater structural flexibility in the protein particularly around the “M-loop”, suggesting a basis for the reduced binding affinity of Taxol. The observed loop flexibility can be traced to a buried water cluster absent in the native protein. Recognition of the role of water in resistance may contribute to overcoming it in tumorogenic cells fortified by acquired mutation.



MEDI 275

An explanation for the resistance of the tubulin/microtubule Phe272Val mutant to taxol: Molecular dynamics simulation in water

suwipa Saen-Oon *and James P. Snyder, Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, suwipa@euch4e.chem.emory.edu*

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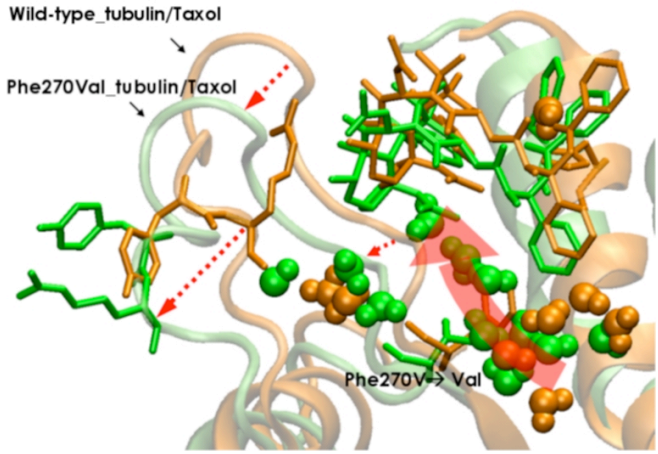


MEDI 276

Cytotoxic activities of peptide conjugates targeting ribonucleotide reductase

Prasant Deb[1]*, X Liu*[2]*, Y Yen*[2]*, and B. S. Cooperman*[1]*. (1) Department of Chemistry, University of Pennsylvania, 231 South 34th Street, Philadelphia, PA 19104, pdeb@sas.upenn.edu, (2) Department of Medical Oncology and Therapeutic Research, City of Hope National Medical Center*

Mammalian ribonucleotide reductase (mRR) catalyzes the reduction of nucleoside diphosphates (NDPs) to deoxynucleoside diphosphates (dNDPs), the rate-limiting step in the de novo synthesis of dNTPs, and hence of DNA, and as such is a clear target for therapeutic agents. Active mRR depends on the association of two different subunits, mR1 and mR2. The original lead molecule for a peptide inhibitor of mRR was the heptapeptide N-AcFTLDADF, denoted P7, corresponding to the C-terminus of mR2, which competes with mR2 for binding to mR1. Optimization of linear peptides targeting mR1, based on both modeling and screening, has resulted in the identification of shorter tri- and tetrapeptides having similar potency vs. P7, and of hexapeptides with substantially increased potency vs. P7, leading to a new lead linear peptide Fmoc(N-Me)PhgLDChaDF, denoted Fmoc-P6 and cyclic peptide AcFc[ELDK]DF. However, these peptides show little or no in-vivo activity (up to 100 μM compound) AcFc[ELDK]DF and Fmoc-(N- CH3)FLDChaDF (P6Cha), raising the question of whether they were taken up into the target cell. Here we report on the

synthesis of fluorescent polyarginine and folic acid conjugates of these peptides, their uptake into mammalian tumor cells, and their cytotoxic activities.

MEDI 277

Inhibitors of the polo-like protein kinase Plk-1

Thomas Nixey[1], *John Boylan*[2], *C. Hulme*[3], *David Powers*[4], *Adrian Smith*[1], *and Andrew Wong*[2]*. (1) Chemistry Research and Development, Amgen, Inc, One Amgen Center Drive, MS 29-1-B, Thousand Oaks, CA 91320, Fax: 805-480-1346, tnixey@amgen.com, (2) Cancer Biology, Amgen, Inc, (3) High-Throughput Medicinal Chemistry and Analytical Technologies, Discovery Chemistry and Research Technologies, Eli Lilly and Company, (4) Department of HTS and Molecular Pharmacology, Amgen Inc*

Plk-1 is a highly conserved serine/threonine nuclear kinase with expression and activity tightly regulated during mitosis. Over expression of Plk-1 is associated with increased proliferation rate and poor prognosis in NSCLC, melanoma, head and neck tumors, and ovarian cancer. Plk-1 is an attractive target for the treatment of certain solid tumors because biological down-regulation predicts that a Plk-1 inhibitor will result in mitotic catastrophe and apoptotic cell death in tumor cells but reversible mitotic arrest in non-transformed cells. Selective inhibitors of Plk-1 would therefore offer the potential for a therapeutic anticancer agent devoid of the toxic side effects associated with current agents. We herein disclose a novel series of inhibitors of the Plk-1 enzyme.

MEDI 278

Synthesis halogenated or methylated in position ortho- or meta- of phenyl ring, derivatives of 2-(5-bromo-2-methyl-4-nitro-1H-imidazol-1-yl)-1-phenylethanones as new anticancer agents

Marcin Wierzchowski*, Department of Chemical Technology of Drugs, Faculty of Pharmacy, University of Medical Sciences Poznan Poland, ul. Grunwaldzka 6, Poznan 60-780, Poland, Fax: 0048618546609, mwierzch@amp.edu.pl, and S. Sobiak, Department of Chemical Technology of Drugs/Faculty of Pharmacy, University of Medical Sciences*

High anticancer activity of phenacyl derivatives of nitroimidazole prompted us to explain which structural components are the most responsible for the promising activity (Figure 1). Our recent observation and SAR analysis indicate, that nitro group and bromine atom in position 4 or 5 imidazole and carbonyl group together with halogenated in position para phenyl ring seems to be most important moieties for growth of activity of title compounds.

Our main goal in this work was synthesis of new compounds with migrated halogen or alkyl group from para position to *ortho* or *meta* in phenyl ring. The following new

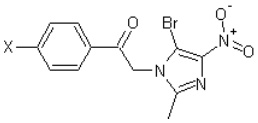
compounds have been obtained: 2-(5-bromo-2-methyl-4-nitro-1*H*-imidazol-1-yl)-1-(3-halogenophenyl)ethanone (Figure 2A), 2-(4-bromo-2-methyl-5-nitro-1*H*-imidazol-1-yl)-1-(3-halogenphenyl)ethanone (Figure 2B), 2-(5-bromo-2-methyl-4-nitro-1*H*-imidazol-1-yl)-1-(2-halogenophenyl)ethanone (Figure 3A),

2-(4-bromo-2-methyl-5-nitro-1*H*-imidazol-1-yl)-1-(2-halogenophenyl)ethanone (Figure 3B) and corresponding methyl compounds.

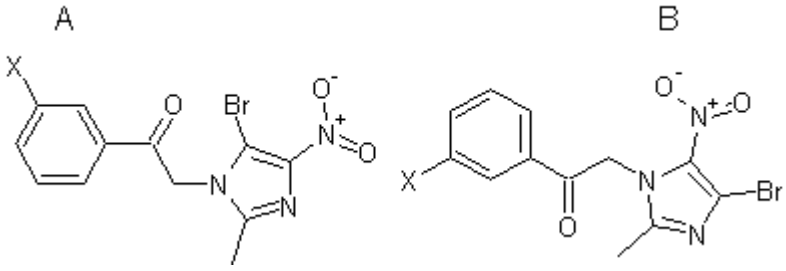
The procedure of synthetic work consisted of the following reactions:

1. Bromination of ketones with Br_2 in $CHCl_3$ at room temperature.
2. Reaction of suitable amount 5(4)-bromo-2-methyl-4(5)-nitroimidazole with halogenated ketones in presence of $NaHCO_3$ and anhydrous $MgSO_4$ in DMF as solvent

Figure 1.Basic active compounds

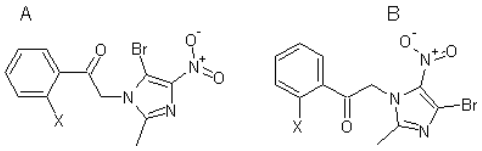


R = H, CH_3, F, Cl, Br, NO_2
Figure 2. New synthesized compounds with substituent in position *meta* in phenyl ring



X= CH_3, F, Cl, Br

Figure 3.New synthesize compounds with substituent in position *ortho* in phenyl ring



X= CH_3, F, Cl, Br

MEDI 279
Molecular modeling and synthesis of mono- and bis-(5-bromo-2-methyl-4-nitro-1H-imidazol-1-yl) derivatives of aromatic diketones
Marcin Wierzchowski, *Department of Chemical Technology of Drugs, Faculty of Pharmacy, University of Medical Sciences Poznan Poland, ul. Grunwaldzka 6, Poznan 60-780, Poland, Fax: 0048618546609, mwierzch@amp.edu.pl, and S. Sobiak, Department of Chemical Technology of Drugs/Faculty of Pharmacy, University of Medical Sciences*

In our department, high anticancer activity of phenacyl derivatives of nitroimidazole has been observed. This information prompted us to explain which structural components are the most responsible for the promising activity (Figure 1).
Our main goal in this work was to synthesize new compounds (Figure 2) with additional phenyl ring and carbonyl group and predict its molecular properties.

Figure 1.Basic active compounds

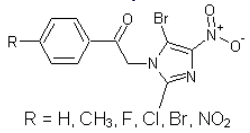
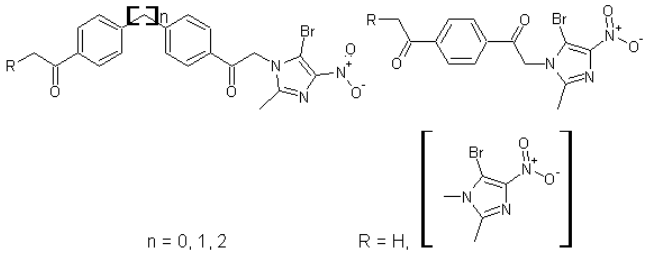


Figure 2. New synthesized compounds



Crystallographic data and structures build de novo in HyperChem® 7.0 were used for conformational analysis. Energy potential surface were investigated by NDDO methods. Recognized conformers were checked by IR. Further research was made by applying ab initio methods in gas state: H-F, DFT and Second Order Perturbation Theory - Møller-Plesset by PCGamess 6.4 program. We used basis sets MINI. STO-3G, 3-21G(d), 6-31G(d), 6-31G(d,p), 6-311G(d) and 6-311G(d,p. Molecular parameters like electron density, charges on atoms, dipole moment, HOMO, LUMO etc. and compared with corresponding properties of 2-(5-bromo-2-methyl-4-nitro-1H-imidazol-1-yl)-1-phenylethanones (Figure 1)

MEDI 280
HTS for Hsp90 inhibitors: New scaffolds and elucidation of structure-activity relationships
M. Kyle Hadden*, Christopher Avila, and Brian S. J. Blagg, Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045, Fax: 785-864-5362, haddenmk@ku.edu*
The 90 kDa family of heat-shock proteins (Hsp90) has become a major target for anticancer drugs because of its role as a molecular chaperone in multiple oncogenic signaling pathways. We recently developed and optimized a high-throughput assay that monitors the inherent N-terminal ATPase activity of Hsp90 and used it to screen a structurally diverse library of ~25,000 compounds for inhibitory activity. Several compounds containing the same molecular scaffold were shown to be potent, selective inhibitors of Hsp90. Rational modification of these scaffolds has produced several compounds with improved inhibitory activity in a variety of cell-based assays. Continued optimization of these molecules is currently underway and the results from these studies will be described.

MEDI 280
HTS for Hsp90 inhibitors: New scaffolds and elucidation of structure-activity relationships

***M. Kyle Hadden**, Christopher Avila, and Brian S. J. Blagg, Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045, Fax: 785-864-5362, haddenmk@ku.edu*
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MEDI 281
Development of Hsp90-based chemotherapeutic agents
***Kwon Ho Hong**, **Timothy R. Welch**, and Brian S. J. Blagg, Department of Medicinal Chemistry, University of Kansas, 1251 Wescoe Hall Drive, Lawrence, KS 66045-7583, khhong77@ku.edu, twelch@ku.edu*
New inhibitory scaffolds of the 90 kDa Heat Shock Proteins (Hsp90) have been developed. Since Hsp90 is required for the conformational maturation of more than forty oncogenic proteins, Hsp90 inhibition represents a novel approach toward the development of anti-cancer agents. Through the use of rational drug design and high-throughput screening, new scaffolds have been prepared and tested for their ability to cause the degradation of Hsp90-dependent client proteins. The synthesis, structure-activity relationships, and biological evaluation of these new compounds will be presented.

MEDI 282
Hsp90 Inhibitors from rationally designed chimeric compounds
***Gang Shen** and Brian S. J. Blagg, Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4077, Lawrence, KS 66045-7582, Fax: 785-864-5326, gangshen@ku.edu*
Geldanamycin and radicicol are natural products that are potent Hsp90 N-terminal inhibitors. Radester, a chimeric compound comprised of the geldanamycin quinone ring and resorcinol moiety of radicicol, was prepared along with more than 20 analogs to determine structure-activity relationships. These novel compounds were evaluated in several human cancer cell lines and their protein degradation and anti-proliferation activities were determined. Utilizing data obtained from the first generation of compounds, new Hsp90 inhibitors have been prepared and their syntheses and biological activities will be reported.

MEDI 283
Novobiocin analogs: Small molecule inhibitors of the Hsp90 protein folding machinery
*Brian S. J. Blagg[1], **Joseph A. Burlison**[2], and Donna J. Lubbers[1]. (1) Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045-7562, Fax: 785-864-5326, bblagg@ku.edu, (2) Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045-7652, Fax: 785-864-5326, burlison@ku.edu*
Hsp90 is an emerging target for the development of cancer chemotherapeutics for which geldanamycin derivatives have entered clinical trials. In contrast to geldanamycin, which binds to the N-terminal ATP binding pocket of Hsp90, novobiocin binds to the C-terminus. However, novobiocin exhibits a weak inhibititory

activity against Hsp90 and improved analogues have only recently been reported. In an effort to elucidate structure-activity relationships for novobiocin and Hsp90, new novobiocin analogues have been produced and screened against several cell lines. A presentation of the biological properties exhibited by these molecules and their syntheses will be described.

MEDI 283

Novobiocin analogs: Small molecule inhibitors of the Hsp90 protein folding machinery

*Brian S. J. Blagg[1], **Joseph A. Burlison**[2], and Donna J. Lubbers[1]. (1) Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045-7562, Fax: 785-864-5326, bblagg@ku.edu, (2) Department of Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045-7652, Fax: 785-864-5326, burlison@ku.edu*

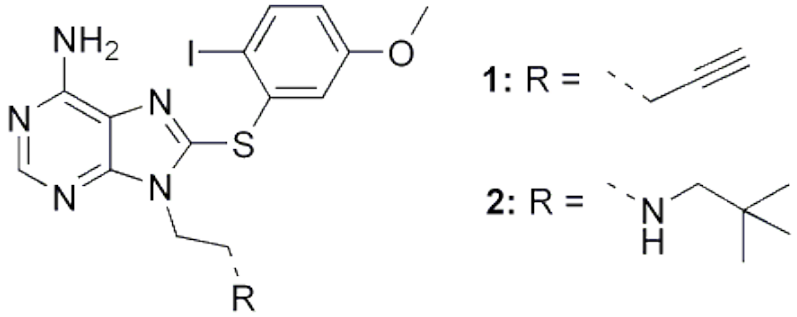
Hsp90 is an emerging target for the development of cancer chemotherapeutics for which geldanamycin derivatives have entered clinical trials. In contrast to geldanamycin, which binds to the N-terminal ATP binding pocket of Hsp90, novobiocin binds to the C-terminus. However, novobiocin exhibits a weak inhibititory activity against Hsp90 and improved analogues have only recently been reported. In an effort to elucidate structure-activity relationships for novobiocin and Hsp90, new novobiocin analogues have been produced and screened against several cell lines. A presentation of the biological properties exhibited by these molecules and their syntheses will be described.

MEDI 284

Inhibitors of the heat shock protein 90 (Hsp90): Orally active 8-sulfanyladenines

Marco A. Biamonte[1]*, Jiandong Shi[1], Kevin Hong[1], David C. Hurst[1], Lin Zhang[1], Junhua Fan[1], David J. Busch[2], Patricia L. Karjian[2], Angelica A. Maldonado[2], John L Sensintaffar[2], Yong-Chin Yang[2], Adeela Kamal[2], Rachel E. Lough[2], Karen Lundgren[2], Francis J. Burrows[2], Gregg A. Timony[3], Marcus F. Boehm[1], and Srinivas R. Kasibhatla[1]. (1) Department of Medicinal Chemistry, Conforma Therapeutics Corp, 9393 Towne Centre Dr, Suite 240, San Diego, CA 92121, Fax: 858-657-0343, mbiamonte@conformacorp.com, (2) Department of Biology and Pharmacology, Conforma Therapeutics Corp, (3) Department of Pre-Clinical Development, Conforma Therapeutics Corp*

The molecular chaperone Heat Shock Protein 90 (Hsp90) folds and maintains the proper conformation of "client" proteins. It is emerging as a valuable target for the treatment of cancer because Hsp90 inhibition simultaneously interferes with a number of critical oncogenes, namely HER-2, ER, AR, Bcr-Abl, Raf-1, etc. In addition, since Hsp90 exists in an activated form in tumor cells, inhibitors selective for this form may exhibit a greater therapeutic index than current chemotherapeutic agents. Adenine derivative **1**, our first-generation Hsp90 inhibitor, is relatively potent (IC50 = 300 nM) but is not water-soluble and lacks oral bioavailability. We now report that insertion of an amino functionality in the N-9 side chain provides aqueous solubility. SAR studies culminated with the neopentylamine **2**, which was the first Hsp90 inhibitor to be both potent in a HER-2 degradation assay (IC50 = 90 nM) and orally bioavailable (F= 50%). In mice, the amine **2** and close analogs induced the expected pharmacodynamic response (degradation of client proteins, up-regulation of Hsp70) and inhibited tumor growth in xenograft models upon oral administration.



MEDI 285

Design, synthesis, and structure–activity relationships of 7'-substituted benzothiazolothio- and pyridinothiazolothio -purines as potent heat shock protein 90 inhibitors

Lin Zhang[1], *Junhua Fan*[1], *Khang Vu*[1], *Kevin Hong*[1], *Jean-Yves Le Brazidec*[1], *Jiandong Shi*[1], *Marco Biamonte*[1], *David J. Busch*[2], *Rachel E. Lough*[2], *Roy Grecko*[3], *Yingqing Ran*[3], *John L. Sensintaffar*[2], *Adeela Kamal*[2], *Karen Lundgren*[2], *Francis J. Burrows*[2], *Robert Manfsield*[3], *Gregg A. Timony*[3], *Ed Ulm*[3], *Srinivas R. Kasibhatla*[1], *and Marcus F. Boehm*[1]. *(1) Medicinal Chemistry, Conforma Therapeutics, 9393 Towne Centre Drive, suite 240, San Diego, CA CA92129, Fax: 858-657-0343, lzhang@conformacorp.com, (2) Department of Biology and Pharmacology, Conforma Therapeutics, (3) Department of Pre-Clinical Development, Conforma Therapeutics*

A novel class of benzo- and pyridino- thiazolothiopurines was found to have potent heat shock protein 90 (Hsp90) inhibitory activity. The benzothiazole moiety is exceptionally sensitive to substitutions on the aromatic ring with a 7'-substituent essential for activity. Many of these compounds exhibit low nanomolar inhibition activity in a Her-2 degradation assay (28-150 nM), good aqueous solubility and oral bioavailability in mice. In-vivo efficacy experiments demonstrate that compounds of this class inhibit tumor growth in xenograft models of human cancer via oral administration.

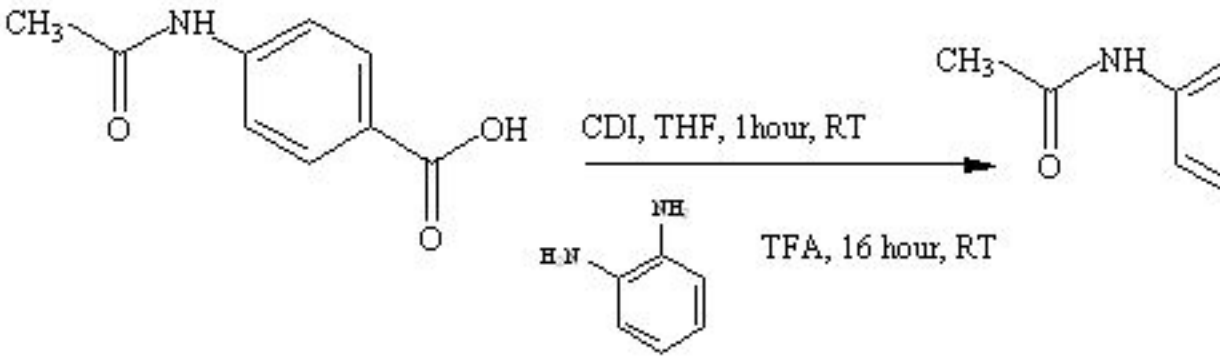
MEDI 286

A new facile and expeditious one pot synthesis of CI-994 and its inhibitory effect alone or in combination with ATRA/RAMBAs on the growth of MDA-MB-231 human breast cancer cells

Lalji K. Gediya[1], *Jyoti B. Patel*[1], *Purushottamachar P. Puranik*[1], *Jhalak Mehta*[1], *and* ***Vincent CO. Njar***[2]. *(1) Pharmacology and Experimental Therapeutic, University of Maryland, School of Medicine, 685, West Baltimore street, HSF-I, Room No. 563, Fax: 410-706-0032, lgedi001@umaryland.edu, (2) Pharmacology and Experimental Therapeutic, University of Maryland, School of Medicine, 685, West Baltimore street, HSF-I, Room No. 580 I, Fax: 410-706-0032, vnjar001@umaryland.edu*

CI-994 or N-acetyldinaline [4-(acetylamino)-N-(2-amino-phenyl)bezamide], a histone deacetylase inhibitor (HDACi) is an antitumor cytostatic agent currently undergoing clinical trials for pancreatic cancer and myeloma. We have developed a one pot synthesis of CI-994 from commercially available acetamidobenzoic acid in 80% yield. Acetamidobenzoic acid was first converted into its imidazolide by reaction with CDI which was then condensed with 1,2 –phenylenediamine in presence of TFA to give CI-994. Effect of CI-994 alone and in combination with ATRA on MDA-MB-231 hormone-independent breast cancer cell line was studied and the IC50 values of CI-994 and ATRA were 7.0 and 20.0 μM, respectively. The combination both agents was synergistic with respect to MDA-MB-231 cell growth inhibition. CI-994 was also used

to synthesize mutual prodrugs with ATRA and RAMBAs. These data will also be presented.



MEDI 287
Synthesis and structure-activity relationship of carbazole sulfonamides as a novel class of potent antimitotic agents against solid tumors

Lai-xing Hu[1]*, Zuo-rong Li*[2]*, Jian-dong Jiang*[2]*, and David W. Boykin*[3]*. (1) Department of Chemistry, Georgia State University, Atlanta, GA 30302, Fax: 404-651-1416, chelxh@langate.gsu.edu, (2) Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China, (3) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University*

Tubulin-binding agents can disrupt the cell cycle at the M-phase and lead to cell death and tumor regression. Combretastatin A-4 (CA-4) is a natural product which strongly inhibits polymerization of tubulin by binding to the colchicine binding site. Its water-soluble phosphate prodrug CA-4P, has unique antivascular activity, is now in phase II clinical trials. We synthesized two series of carbazole sulfonamides – CA-4 analoges and evaluated them for antiproliferative and tubulin activity. Many compounds have strong cytotoxicity with IC50 values of 0.02-0.03 ug/ml against CEM leukemia cells in vitro. Preliminary studies of mode of action revealed that these compounds can arrest tumor cell cycle at M-phase and induces apoptotic cell death by increasing expression of p53 and promoting bcl-2 phosphorylation. The lead compound 9-Ethyl-N-(3,4,5-trimethoxyphenyl)-carbazole-3-sulfonamide (3a) showed significant antitumor activity in two human xenograft MCF-7 and Bel-7402 models. SAR information for these two series compounds will be presented.

MEDI 288

Honokiol and Magnolol: New potent antitumor compounds

Franck Amblard*, Laboratory of Biochemical Pharmacology, Emory University, VA Medical Center, 1670 Clairmont road, Decatur, GA 30033, Fax: 404-728-7726, famblar@emory.edu, David Delinsky, Laboratory of Biomedical Pharmacology, NA, Jack L. Arbiser, Department of Dermatology, Emory University School of Medicine, and Raymond F. Schinazi, Veterans Affairs Medical Center, Emory University*

Simple biphenyl neolignans, honokiol and magnolol, which can be extracted from magnolia trees, have been used in traditional Chinese medicine for thousand years for treatment of anxiety and stroke but their potential as anti-tumor agents has not been evaluated. Moreover, the difficulty in separating these closely structurally related compounds from the tree extract makes pure honokiol and magnolol very expensive. This encouraged us to develop a simple separation of honokiol and magnolol based on the selective chemical protection of magnolol followed by flash chromatography. These pure compounds were then subjected to a bioassay to determine there anti-angiogenesis and anti-tumor property. Honokiol showed potent anti-angiogenic and anti-tumor properties in vitro and was active against angiosarcoma in a mouse model without major toxicity making it a promising candidate for further preclinical testing; The need for a significant quantity of honokiol for future clinical studies makes our economical purification method very attractive.

MEDI 289

Synthesis and biological evaluation of selective aromatase expression regulators in breast cancer cells

Bin Su[1]*, Serena Landini*[2]*, and Robert W. Brueggemeier*[2]*. (1) Medicinal chemistry, College of pharmacy, The Ohio State University, 500 West12th Ave., Columbus, OH 43210, Su.112@osu.edu, (2) Medicinal Chemistry, College of pharmacy, The Ohio State University*

Aromatase, which converts androgens to estrogens, is a particularly attractive target in the treatment of estrogen receptor positive breast cancer. The enzyme is encoded by the CYP19 gene whose expression is in a tissue-specific manner. Prostaglandin E2 (PGE2), the major product of cyclooxygenase-2 (COX-2), stimulates aromatase gene

expression via protein kinase A and C signaling pathways. COX-2 selective inhibitor nimesulide can decrease aromatase activity from the transcriptional level in breast cancer cells. The synthesis and biological evaluation of a series of nimesulide analogs as potential selective aromatase expression regulators are described. Several novel sulfonanilide compounds can selectively decrease aromatase activity and enzyme gene expression at low micromole concentrations in breast cancer cells.

MEDI 289

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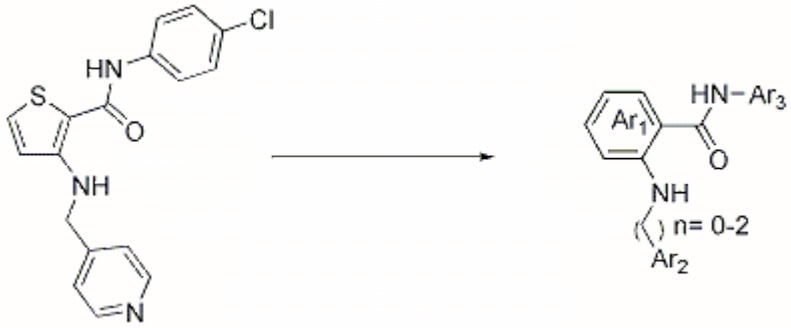
MEDI 290

KDR inhibitors: From in vitro potency to in vivo efficacy

Celia Dominguez[1]*, Leon Smith II*[2]*, Qi Huang*[1]*, Chester Yuan*[1]*, Tae-Seong Kim*[1]*, Lynn Cai*[1]*, Andrew Tasker*[1]*, Rashid Syed*[1]*, Michael Zhang*[3]*, Timothy Harvey*[1]*, Sesha Neervannan*[4]*, Angela Coxon*[5]*, Anthony Polverino*[3]*, and Richard Kendall*[3]*. (1) Chemistry Research & Discovery, Amgen Inc, One Amgen Center Drive, MS 29-1B, Thousand Oaks, CA 91320, Fax: 805-480-1337, celiad@amgen.com, (2) Cardiovascular Disease Chemistry, BMS Research Institute, (3) Cancer Biology, Amgen Inc, (4) PKDM, Amgen Inc, (5) Cancer Pharmacology, Amgen Inc*

Abstract: Inhibition of tumor-induced angiogenesis is a promising strategy in anticancer drug research. Angiogenesis, the recruitment of new blood vessels, is a crucial mechanism required for both tumor growth and metastasis. Enhanced understanding of the molecular mechanisms underlying the angiogenesis process has led to the discovery of a variety of pharmaceutical agents with anti-angiogenic activity. The potential application of these angiogenesis inhibitors is currently under intense clinical investigation. Decades of investigation suggests that vascular endothelial growth factor (VEGF) and its receptors, in particular VEGFR2 or kinase insert-domain-containing receptor (KDR), play a critical role in tumor-associated angiogenesis. Therefore, KDR represents a good target for therapeutic intervention. Herein we report the progression of KDR inhibitors from screening hits to in vivo efficacy.



MEDI 291

Structural insights into the interaction of $p14^{Arf}$ and Hdm2

Sivashankar G. Sivakolundu[1], *Amanda Nourse*[2], *and Richard W. Kriwacki*[1]. *(1) Department of Structural Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38103, sivashankar.sivakolundu@stjude.org, (2) Hartwell Center for Bioinformatics and Biotechnology, St. Jude Childrens Research Hospital*

$p14^{Arf}$ (Arf) and Hdm2 are oncoproteins involved in regulation of the transcriptional activity of the tumor suppressor protein, p53. Hdm2 negatively regulates the transcriptional activity of p53 by (i) binding to its transactivation domain and (ii) promoting its proteasome-mediated degradation. Mitogenic signals activates Arf, which binds to Hdm2 and inhibits the Hdm2-dependent degradation of p53. Disruption of the Arf/Hdm2/p53 pathway results in cancer in mice and humans. Both Arf and the Arf-interacting domain of Hdm2 (residues 210 – 304) are unstructured in solution. However, upon binding to each other they form a supramolecular complex that has a β-sheet secondary structure. In the present study, we have used a small, N-terminal peptide fragment of Arf (9 residues) that interacts with Hdm2 but does not trigger the formation of supramolecular complexes. We mapped Arf 9mer/Hdm2 interactions by monitoring Arf-9mer binding-induced chemical shift perturbations of the ^{15}N-HSQC spectrum of Hdm2. We have further analyzed this interaction by introducing an MTSL spin-label at various positions of Hdm2 through site-directed mutagenesis and by monitoring the effects of spin-label enhanced relaxation on the ^{15}N-HSQC spectrum of Hdm2 in the presence and absence of the Arf-9mer peptide. To complement these studies, we applied additional analytical methods to gain a more comprehensive understanding of the physical properties of the Arf peptide-Hdm2 complex, including diffusion NMR, CD, and analytical ultracentrifugation techniques. The results indicate that Arf interacts with regions of Hdm2 that are consistent with those identified previously. However, several segments of Hdm2 previously not known to interact with Arf were also perturbed significantly. This study provides new insights into the mechanism of Arf-Hdm2 interactions.

MEDI 292

Synthesis of new water soluble Parthenolide analogs as potential anti-tumor agents

Daneli Lopez[1], *Peter A. Crooks*[2], *and Sundar Neelakantan*[2]. *(1) Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, P.O. Box 23346, San Juan, PR 00931-3346, PR, ilenad4@hotmail.com, (2) College of Pharmacy, University of Kentucky*

Recent studies have shown that Parthenolide (PTL) induces robust apoptosis in primary human acute myelogeneous leukemia cells (AML), while sparing normal cells. PTL is more specific to leukemia cells, but non-toxic to bone marrow cells and it preferentially targets AML progenitors and stem cell populations. However, PTL have some disadvantages since it is poorly soluble in water and does not have good

bioavailability. A Michael Addition Reaction was used to attach water-soluble amino acids and sugars to PTL to improve its pharmacological characteristics. The PTL amine is regenerated in an *in vivo* Retro-Michael reaction, and the sugars and amino acids generated are endogenous compounds, which are non-toxic to humans. Several water-soluble PTL analogs: L-Lysine, N-Methylglucamine and Glucosamine, were synthesized and purified successfully by column chromatography. The proton NMR spectrum and GC-MS confirmed the structure and purity of each compound respectively. These compounds will be evaluated for anti-tumor activity.

MEDI 293

Phosphoramidate derivatives of hydroxy steroids as inhibitors of prostate-specific membrane antigen

Lisa Yong Wu[1], *Yoko Toriyabe*[2], *Marc O. Anderson*[3], *and Clifford E. Berkman*[3]. *(1) Chemistry & Biochemistry, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, Fax: 415-338-6802, lisawu44@yahoo.com, (2) Department of Chemistry and Biochemistry, San Francisco State Univeristy, (3) Department of Chemistry and Biochemistry, San Francisco State University*

Prostate cancer cells over-express the membrane-bound, cell surface protein prostate-specific membrane antigen (PSMA). Its enzymatic activities have been identified but the role of this enzyme remains conjectural. Using simple hydrophobic phosphoramidate derivatives of glutamic acid as inhibitors of PSMA, we have previously found evidence for the existence of a hydrophobic binding site remote from the enzyme's catalytic center. In order to explore the possibility for specificity of this hydrophobic binding site, we have prepared a series of glutamate-containing phosphoramidate derivatives of various hydroxyl steroids. The inhibition profiles of these inhibitors compared to simple hydrophobic analogs, as well as results from molecular docking studies into the PSMA active site will be presented.

MEDI 294

4-Aryl-4*H*-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high throughput screening assay, structure-activity relationships of fused rings at the 7, 8-positions

William Kemnitzer[1], *Shailaja Kasibhatla*[2], *Songchun Jiang*[2], *Hong Zhang*[1], *Jianghong Zhao*[2], *Real Denis*[3], *Henriette Gourdeau*[3], *Ben Tseng*[2], *John Drewe*[2], *and Sui Xiong Cai*[2]. *(1) Maxim Pharmaceuticals Inc, 6650 Nancy Ridge Drive, San Diego, CA 92121, bkemnitzer@maxim.com, (2) Maxim Pharmaceuticals, Inc, (3) Shire Biochem Inc*

We have recently reported the discovery of 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4H-chromene (1a) as potent apoptosis inducers using a novel cell- and caspase-based HTS assay and the SAR of the 4-position with a dimethylamino group in the 7-position. These chromenes were found to be active in the HUVEC tube formation assay, suggesting that this series of compounds may have antivascular activity, and several compounds were also found to be highly active in anti-cancer in vivo tumor models. More recently, we have described the SAR of the 7-position and the 5, 6, 8-positions of 4-aryl-4H-chromenes as inducers of apoptosis. The SAR revealed that disubstitution at the 7,8-positions was preferred over the 5,6 or 6,7-positions. Herein we will report in detail the chemistry, in vitro and in vivo characterization of compounds with a fused ring at the 7, 8-positions.

MEDI 295

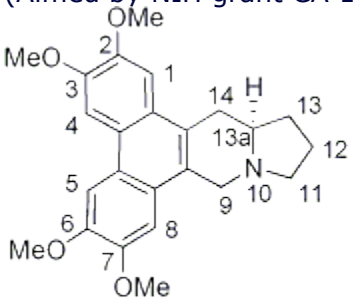
Elucidation of the structural elements required for the anti-cancer activity of an amaryllidaceae constituent pancratistatin

***Marcia A. Ogasawara**, Madhuri Manpadi, and Alexander Kornienko, Department of Chemistry, New Mexico Institute of Mining and Technology, 801 Leroy Place, Socorro, NM 87801, mogasawara@gmail.com*
Pancratistatin, one of the many constituents of the Amaryllidaceae plants, has been extensively evaluated and is recognized as a potential antineoplastic agent. Recent studies have shown it selectively targeting cancerous cells without being cytotoxic to healthy ones, which further stimulates our interest in this natural product. The mechanism of action of this compound is currently unknown and its pharmacophore is yet to be established. We synthesized various structurally simplified analogues of pancratistatin and performed their biological evaluation. This paper will present data on growth inhibitory and apoptosis inducing properties of these compounds in a number of human cancer cell lines. These results will be examined with the data obtained from the testing of the natural product itself and conclusions will be drawn on the structural elements required for the anti-cancer activity of this promising medicinal agent.

MEDI 296

New phenanthrene-based tylophorine derivatives (PBTs) as a novel class of antitumor agents

***Linyi Wei**, Kenneth F. Bastow, Arnold Brossi, and Kuo-Hsiung Lee, Division of Medicinal Chemistry and Natural Products, Division of Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7360, Fax: 919-966-3893, weily@email.unc.edu*
Tylophorine (1) and related phenanthroindolizidine alkaloids, also known as tylophora alkaloids, have been isolated principally from plants of the family Asclepiadaceae, including members of the genus Tylophora. These compounds have been targets of synthetic modification because of their profound cytotoxic antitumor activity. However, to date, this compound class has not been successfully developed for clinical use in cancer due to the CNS toxicity reported in 1966. In this study, novel water-soluble phenanthrene-based tylophorine derivatives (PBTs) were designed, synthesized and evaluated as potential antitumor agents. These compounds contain a core phenanthrene structure and can be synthesized efficiently in excellent yield. The newly synthesized PBTs were evaluated for cytotoxic activity against the A549 human cancer cell line. Several PBTs showed superior activity profiles with EC50 values in the sub-micromolar range, which are comparable to those of currently used antitumor drugs. A structure-activity relationship (SAR) study was also explored to facilitate the further development of this new compound class.
(Aimed by NIH grant CA 17625 awarded to Dr. K. H. Lee)



(-)-R-Tylophorine (**1**)

MEDI 297

Design, synthesis and biological studies of an intercalator-alkylator hybrid compound that interacts with the c-MYC G-quadruplex

Chandana Sharma Joshi[1], *Daekyu Sun*[2], *Jadrian Rusche*[2], *and Laurence H. Hurley*[2]. *(1) Department of Chemistry, The University of Arizona, 1306 E University Blvd., Tucson, AZ 85721, Fax: 520-626-5623, csharma@u.arizona.edu, (2) College of Pharmacy, The University of Arizona*

An intercalator-alkylator hybrid compound (HybP4; Figure1) was designed, synthesized and evaluated for interaction with the c-MYC G-quadruplex. c-MYC oncogene is overexpressed in more than 60% of cancers. The silencer element of this gene, the nuclease hypersensitivity element III1 (NHE III1) upstream of the P1 promoter, uniquely forms a parallel-type G-quadruplex structure at the guanine rich site and a chair-type i-motif structure at the cytosine rich site. A G-quadruplex consists of guanine tetrads formed by Hoogstein base pairing between four guanines, and an i-motif has similar base pairing between two cytosine residues. It has been shown that cationic porphyrins like 5,10,15,20-tetra-(N-methyl-4-pyridyl)porphyrin chloride (TMPyP4) stabilize the G-quadruplex structure and repress the gene transcription. These ligands intercalate below a guanine-tetrad and stabilize the quadruplex. A hybrid compound (HybP4) with a cationic porphyrin moiety as an intercalator and a nitrogen mustard (chlorambucil) as an alkylator was designed and synthesized. Circular Dichroism studies show that HybP4 interacts with the c-MYC G-quadruplex at a lower mole equivalent than TMPyP4. Further, in vitro studies were done to evaluate both intercalating and alkylating properties of HybP4. Interesting cleavage pattern that sheds light on the intercalation and alkylation of the c-MYC gene will be presented.

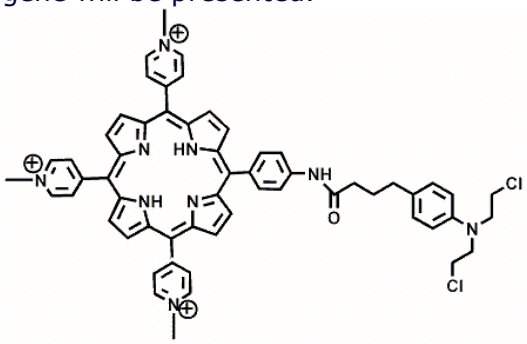


Figure1: HybP4

MEDI 298

DNA photocleavage by acridine and phenazine-based chromophores

Earl J. Fields Jr.[1], *María-José Fernández*[2], *Antonio Lorente*[2], *and Kathryn B. Grant*[1]. *(1) Department of Chemistry, Georgia State University, P.O. Box 4098, Atlanta, GA 30302-4098, Fax: 404-651-1416, efields2@student.gsu.edu, (2) Departamento de Química Orgánica, Universidad de Alcalá de Henares*

Photodynamic therapy (PTD) is a new and promising treatment approach in which a light source is utilized to activate a photosensitizing agent that selectively destroys diseased tissues. Our research is focused on the development of new, non-porphyrin photonucleases for use in PDT. Towards this end, the present study evaluates the photo-induced DNA cleaving abilities of a series of acridine and phenazine-based compounds. Their extended, aromatic ring systems are expected to intercalate between adjoining base pairs in the DNA double-helix. Once irradiated by light of the appropriate wavelength, strand breakage, or nicking of plasmid DNA, is achieved at micromolar concentrations (pH 7.0 and 22 ≡C). Two of the photonucleases chelate metal, and thereby exhibit modulated levels of DNA photocleavage in the presence of

copper(II). This is significant in light of the broad distribution of copper in biological systems.

MEDI 299

Selective binding of a heterocyclic diamidine compound with human telomeric G-quadruplex DNA

Elizabeth W. White[1]*, Rupesh K Nanjunda*[1]*, Arvind Kumar*[1]*, Mohamed A. Ismail*[2]*, David W. Boykin*[2]*, and W. David Wilson*[1]*. (1) Department of Chemistry, Georgia State University, 50 Decatur Street, Atlanta, GA 30303, ewhite7@student.gsu.edu, (2) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University*

Stabilization of the quadruplex conformation of telomeres by small molecules has been shown to inhibit telomerase, resulting in cancer cell death. Since essentially all known quadruplex binders are derived from duplex intercalators, many exhibit limited selectivity for quadruplex over duplex structures. Lack of selectivity over duplex DNA can result in cytotoxicity and compound loss. Several physicochemical techniques were used to investigate the interaction of heterocyclic diamidines with the human telomere and other quadruplex- and duplex-forming sequences. Our results show that a furan-based compound binds as a stacked species to the human telomere and sequences with similar structures, but not to duplex DNA or other quadruplexes. This compound also binds the human telomere with higher affinity than with duplex DNA. This compound may provide a starting point for the design of unique compounds with high affinity and selectivity for human telomeric DNA, leading to enhanced telomerase inhibition with decreased side effects.

MEDI 300

Prediction and design of small molecule inhibitors of human killer-cell immunoglobulin-like receptor (KIR) 2DL4

Sivanesan Dakshanamurthy*, Melaku Gedil, Carolyn Hurley, and Nagarajan Pattabiraman, Department of Oncology, Lombardi Cancer Center, Georgetown University, Researvoir Road, E401, NRB, washington DC, DC 20057, sd233@georgetown.edu*

Human killer-cell immunoglobulin-like receptors (KIR) are expressed on the surface of natural killer (NK) cells and modulate NK cell mediated cytotoxicity of tumor cells. These receptors deliver activating or inhibitory signals that depend, in part, on binding to HLA ligands. KIR2DL4 is one of 15 different polymorphic receptors and exhibits several unique features. We predicted the 3D structure of the KIR2DL4 protein complexed with its putative ligand HLA-G. KIR provides two acidic residues (E187, D135) and HLA contributes two basic residues (K146, R145) to the interface to form a salt bridge in addition to the hydrogen bond interactions. The predicted KIR2DL4/HLA-G structure was subsequently utilized to discover small-molecule inhibitors. By pharmacophore based virtual screening of small molecule database, we identified several inhibitors and by visual inspection, we selected top 15 compounds. We strongly believe that these inhibitors will interrupt the KIR2DL4 receptor signaling, and act as a potential antitumor agent.

MEDI 301

Novel BIR binding ligands as XIAP antagonists: High potency anti-cancer agents

Alain Laurent[1]*, James B. Jaquith*[1]*, Patrick Bureau*[1]*, Scott Jarvis*[1]*, Danielle Boulais*[2]*, Alain Boudreault*[3]*, Jonathan Cole*[4]*, Kimberly E. Hewitt*[2]*, Lori Jerome*[2]*, Danielle Labit*[2]*, Sandra Larouche*[2]*, Guillaume Levesque*[4]*, Sharon Lin*[3]*, Joanie Lussier*[2]*, Andrea Romeo*[2]*, Andrew Manning*[3]*, Stephen J. Morris*[4]*, Farid Arab Said*[4]*, Jon Durkin*[1]*, and*

John Gillard[1]. (1) Department of Chemistry, Aegera Therapeutics Inc, 801 chemin du Golf, Verdun (Montreal), QC H3E 1A8, Canada, Fax: 514-288-9280, alain.laurent@aegera.com, (2) Department of Pharmacology, Aegera Therapeutics Inc, (3) Department of Biochemistry, Aegera Therapeutics Inc, (4) Department of Molecular Biology, Aegera Therapeutics Inc
The Inhibitor of Apoptosis Protein (IAP) family of proteins plays a crucial role in regulating apoptosis, primarily by regulating caspase activity via the highly structured BIR (Baculovirus Inhibitory Repeat) motifs and by causing ubiquitination of binding partners through the E3 ligase motif. In the IAP family, XIAP is an important cancer target; XIAP antisense AEG35156, shows potent antitumor activity in various xenograft models and in multiple clinical trials. For a small molecule inhibitor approach, blocking interactions of XIAP with caspases through its BIR domains represent exceptional targets for modulation of anti-apoptotic properties. However, because of contrasting BIR activities within and between various IAPs, the impact of BIR selectivity for small molecule binders must be established. A series of novel BIR ligands with high binding affinity to IAP BIRs have been synthesized. In vitro and pharmacokinetic characterization of a representative set of pre-clinical XIAP antagonist is reported.

MEDI 301

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The Inhibitor of Apoptosis Protein (IAP) family of proteins plays a crucial role in regulating apoptosis, primarily by regulating caspase activity via the highly structured BIR (Baculovirus Inhibitory Repeat) motifs and by causing ubiquitination of binding partners through the E3 ligase motif. In the IAP family, XIAP is an important cancer target; XIAP antisense AEG35156, shows potent antitumor activity in various xenograft models and in multiple clinical trials. For a small molecule inhibitor approach, blocking interactions of XIAP with caspases through its BIR domains represent exceptional targets for modulation of anti-apoptotic properties. However, because of contrasting BIR activities within and between various IAPs, the impact of BIR selectivity for small molecule binders must be established. A series of novel BIR ligands with high binding affinity to IAP BIRs have been synthesized. In vitro and pharmacokinetic characterization of a representative set of pre-clinical XIAP antagonist is reported.

MEDI 302

Synthesis of 2-fluoroethoxyestradiol, an analog of 2-methoxyestradiol, evaluation and labeling with fluorine-18 as a PET tracer

Jiyoung Mun[1]*, Yuefang Wang[2], Paraskevi Giannakakou[2], and Mark M. Goodman[1]. (1) Department of Radiology, Division of Radiological Sciences, Emory University, 1364 Clifton Road NE, Atlanta, GA 30322, Fax: 404-778-5550, jmun@emory.edu, (2) Winship Cancer Institute, Emory University*

2-Methoxyestradiol is an endogenous human metabolite, and currently in clinical I/II trials for breast, metastatic breast and prostate cancer. Two analogs of 2-methoxyestradiol (2ME2) were synthesized as candidates of cancer drugs that can also serve as PET tracers. 2-Fluoroethoxyestradiol (2FEE2) and 3-fluoropropanoxyestradiol (2FPE2) were synthesized from β-estradiol in five steps with the overall yield of 28.7% and 15.6%. 2FEE2 showed twice more potent cytotoxicity than 2ME2 in human glioma cell line, LN229, and human ovarian carcinoma cell line, 1A9. Microtubule depolymerization was observed with 2FEE2 and 2FPE2 in human glioma cell lines, LN229 and U87. 2FEE2 showed more efficient HIF-1α inhibition than 2ME2, whereas 2FPE2 showed less HIF-1α inhibition in human glioma cell line, LN229, in a dose dependent manner. 2-[^{18}F]fluoroethoxyestradiol was synthesized by combining the precursor 2-hydroxy-3,17β-*O*-bis(methoxymethyl)estradiol and [^{18}F]fluoroethylbrosylate and followed by deprotection. The decay-corrected radiochemical yield was 8.3% from the end of bombardment with over 98% radiochemical purity.

MEDI 303

Iodine-124 produced by the 124Te(p,n)124I reaction: Specific Activity and Te(IV) determination

Atilio I. Anzellotti*, Alexander Yordanov, Sami Rizvi, Robert Ylimaki, and Maxim Kiselev, Eastern Isotopes, Inc, Eastern Isotopes, Inc./IBA S.A., Virginia Commonwealth University, Richmond, P.O. Box 980131, Richmond, VA 23298-0131, aanzellotti@vcu.edu*

Iodine-124 (124I) is an attractive isotope of iodine due to its complex radioactive decay scheme and convenient half-life (4.18 d). With the increasing use of positron emission tomography (PET) in nuclear medicine, medical oncology, pharmacokinetics and drug metabolism, 124I-labeled radiopharmaceuticals could be most useful for PET imaging.1 Furthermore, the 4.18 d half-life would permit their use in PET facilities far away from the radionuclide production centers. Limited availability of this radionuclide so far has been a hindrance to its wider development and clinical use. Sodium [124I]iodide is already reportedly used for diagnosis and treatment planning in thyroid disease. Also [124I]m-iodobenzylguanidine ([124I]MIBG) is a promising PET tracer for cardiovascular imaging and for diagnosis/treatment planning in malignant diseases such as neuroblastoma, paraganglioma, pheochromocytoma, and carcinoid. The achievement of a radiopharmaceutical preparation possessing the optimal specific activity and radiochemical purity always requires a radiohalogen starting material with no-carrier-added (NCA) specific activity as close to the theoretical carrier-free (CA) specific activity as possible. Another important characteristic of 124I batches would be a minimal Te(IV) content, since it is widely believed that Te(IV) can potentially interfere with radioiodination reactions. We have tested batches of 124I produced by the 124Te(p,n)124I reaction and have estimated their specific activity and Te(IV) content.

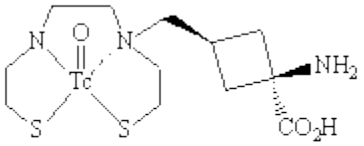
MEDI 304

Synthesis, radiolabeling, and *in vitro* characterization of *syn* and *anti* ^{99m}Tc MACBC•BAT as possible tumor imaging agents

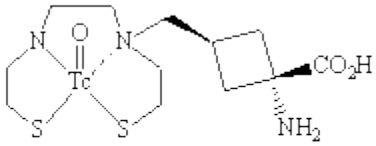
Anthony M. Giamis*[1], Weiping Yu[1], Vernon M. Camp[1], Zhaobin Zheng[2], Ronald J. Voll[1], Andrew O. DePompei[1], and Mark M. Goodman[1]. (1) Department of Radiological Sciences, Emory University Hospital, 1364 Clifton Road NE, Atlanta, GA 30322, Fax: 404-727-3488, agiamis@emory.edu, (2) Department of Neurosurgery, Emory University Hospital*

The non-natural non-metabolized amino acids *syn / anti* 1-[N-[(1,1-dimethylethoxy)carbonyl]amino]-3-[N,N'-bis[2-[[(4-

methoxyphenyl)methyl]thio]ethyl]-1,2-ethanediamine]methyl-1-cyclobutanecarboxylic acid 1,1-dimethylethyl ester (**MACBC•BAT**) were synthesized, radiolabeled with ^{99m}Tc, deprotected and evaluated *in vitro* as potential **SPECT** tumor imaging agents against human tumor cell lines (A549, MDA MB468, DU145, SKOV3, U87). Both *syn*- and *anti*- ^{99m}Tc **MACBC•BAT** were obtained with 3-20% radiolabeling yields and radiochemical purity over 99% measured by radiometric TLC. The cell uptake in the human tumor cell lines ranged from 2.8 to 14.4 % CPM / 5e5 cells. These findings suggested that these *syn*- and *anti*- ^{99m}Tc **MACBC•BAT** amino acids enter these tumor cells *in vitro* primarily via ACS amino acid transport. These results support the further study of *syn*- and *anti*- ^{99m}Tc **MACBC•BAT** as **SPECT** imaging agents. Research supported by Nihon Medi-Physics Co., Ltd..



^{99m}Tc *syn*-MACBC-BAT



^{99m}Tc *anti*-MACBC-BAT

MEDI 305

Synthesis of carbon-11 labeled triphenylmethylamides as novel potential PET tracers for melanoma cancer detection

Mingzhang Gao *and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, migao@iupui.edu*

A class of small molecules triphenylmethylamides (TPMAs) has been developed as potential antimelanoma agents by Dothager *et al*. (JACS 2005, 127, 8686-8696), in which some TPMAs were found to potently induce apoptosis in melanoma cells through G1 cell cycle arrest and dramatically reduce the level of active nuclear factor κ-B (NFκB) in the cell. We are interested in the development of cancer imaging agents. TPMAs labeled with a positron emitting radionuclide carbon-11 may enable non-invasive monitoring of cancer proliferation and apoptosis in melanoma cells and NFκB, and melanoma cancer response to chemotherapy using positron emission tomography (PET) imaging technique. In our effort to further develop potential chemotherapeutic agents as diagnostic agents, we have synthesized two carbon-11 labeled TPMA compounds with excellent potency, *N*-(4-[^{11}C]methoxyphenyl)-2,2,2-triphenyl-acetamide and 3-phenyl-(*R*)-2-(2,2,2-triphenyl-acetylamino)-propionic acid [^{11}C]methyl ester. The reference standards *N*-(4-methoxyphenyl)-2,2,2-triphenyl-acetamide and 3-phenyl-(*R*)-2-(2,2,2-triphenyl-acetylamino)-propionic acid methyl ester were prepared from triphenylacetyl acid via 2 steps. The desmethylation of the reference standards provided the precursors *N*-(4-hydroxyphenyl)-2,2,2-triphenyl-acetamide and 3-phenyl-(*R*)-2-(2,2,2-triphenyl-acetylamino)-propionic acid, which were labeled by *O*-[^{11}C]methylation using [^{11}C]methyl triflate under basic conditions to give target radiotracers.

MEDI 306

Synthesis of [^{11}C]hemicholinium-15 and [^{18}F]hemicholinium-15 as new PET ligands for the high-affinity choline transporter in heart

Mingzhang Gao*, Timothy R. DeGrado, Bruce H. Mock, and Qi-Huang Zheng, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, migao@iupui.edu*

Like hemicholinium-3, hemicholinium-15 (HC-15) is an inhibitor of high-affinity choline transport (HACT). HC-15 labeled with a positron emitting radionuclide either

carbon-11 or fluorine-18 may enable non-invasive monitoring of HACT transporter density in the heart to study cardiac parasympathetic innervation using positron emission tomography (PET) imaging technique. In our effort to develop PET heart imaging agents, we have synthesized two new radiotracers, [^{11}C]hemicholinium-15 ([^{11}C]HC-15) and [^{18}F]hemicholinium-15 ([^{18}F]HC-15). The precursor compound for radiolabeling (4-methyl-2-phenyl-morpholine-2-ol) was prepared from the reaction of 2-bromoacetophenone with 2-(methylamino)ethanol. Reference standard HC-15 was prepared either from the methylation of the precursor with methyl iodide or from the reaction of 2-bromoacetophenone with *N*,*N*-dimethylethanolamine. The carbon-11 labeled target tracer [^{11}C]HC-15 was prepared by *N*-[^{11}C]methylation of the precursor using [^{11}C]methyl triflate and isolated by a simplified silica solid phase extraction (SPE) purification procedure with 50-65% radiochemical yields. The fluorine-18 labeled target tracer [^{18}F]HC-15 was prepared by *N*-[^{18}F]fluoromethylation of the precursor using [^{18}F]fluoromethyl triflate followed by a fast silica SPE purification with 20-30% radiochemical yields.

MEDI 307

Total synthesis of an anticancer drug Iressa

*Ji-Quan Wang, Mingzhang Gao, and **Qi-Huang Zheng**, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, jiqwang@iupui.edu, qzheng@iupui.edu*

Iressa (Gefitinib) is a trademark of the AstraZeneca group of companies, which is an orally active inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-tk) for cancer chemotherapy. Iressa labeled with a positron emitting radionuclide either carbon-11 or fluorine-18 may enable non-invasive evaluation of cancer EGFR-tk levels and cancer response to Iressa therapy by using positron emission tomography (PET) imaging technique. We are interested in the synthesis of cancer imaging agent carbon-11 or fluorine-18 labeled Iressa. In our effort to develop novel PET tracers, we have initialed a synthetic endeavor of reference standard Iressa. There is very little synthetic information appeared in the literature. Wishing to study this compound in this laboratory, we have developed an improved synthetic approach for the synthesis of Iressa using a modification of the patent procedure [Gilday, John Peter; Moody, David. PCT Int. Appl. (2004), 32 pp. WO 2004024703 A1]. Consequently, Iressa was synthesized starting from morpholine and 3-hydroxy-4-methoxybenzaldehyde via 9 steps in 11% overall chemical yield.

MEDI 308

Synthesis of [^{123}I]I-Xeloda as a novel potential SPECT imaging agent for cancer detection

*Ji-Quan Wang, Xiangshu Fei, Bruce H. Mock, Mingzhang Gao, and **Qi-Huang Zheng**, Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L3-202, Indianapolis, IN 46202, Fax: 317-278-9711, jiqwang@iupui.edu, qzheng@iupui.edu*

Xeloda (5'-deoxy-5-fluoro-*N*^{4}-(pentyloxycarbonyl)cytidine, capecitabine) (Hoffman LaRoche) is the first and only fluoropyrimidine to be approved for use as second-line therapy in metastatic breast cancer, colorectal cancer, and other malignancies. Xeloda labeled with radionuclides fluorine-18 and iodine-123 may enable non-invasive monitoring of cancer thymidine phosphorylase (TP) levels and cancer response to Xeloda therapy by using positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging techniques. In our previous work, we have synthesized PET tracer [^{18}F]Xeloda. In this ongoing study, we synthesized I-Xeloda (5'-deoxy-5-iodo-*N*^{4}-(pentyloxycarbonyl)cytidine) and labeled it with iodine-123 as a new SPECT tracer [^{123}I]I-Xeloda. The key intermediate 5-deoxy-

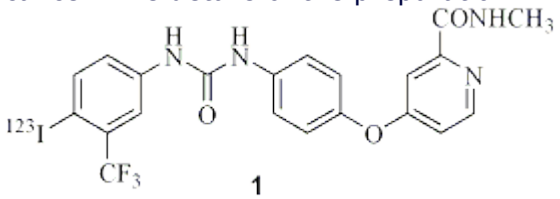
1,2,3-tri-*O*-acetyl-β-*D*-ribofuranoside was prepared from *D*-ribose via 5 steps. The reference standard I-Xeloda was prepared from the intermediate and 5-iodocytosine via 3 steps, and it was radiolabeled with Na[^{123}I]I/HOAc through iodine isotope exchange reaction to afford the target molecule [^{123}I]I-Xeloda.

MEDI 309

Synthesis of iodine-123 labeled raf kinase inhibitor: A potential SPECT agent

George W. Kabalka, Departments of Chemistry and Radiology, University of Tennessee, Buehler Hall, Knoxville, TN 37996-1600, Fax: 865-974-2997, kabalka@utk.edu, ***Arjun R. Mereddy****, Departments of Chemistry and Radiology, The University of Tennessee, Buehler Hall, Knoxville, TN 37996-1600, Fax: 865-974-3260, amereddy@mc.utmck.edu, and Hildegard Schuller, Department of Pathology, Veterinary Teaching Hospital, University of Tennessee College of Veterinary Medicine*

Lung cancer a leading cause of death in the United States. Previous reports in literature have shown Raf kinase inhibitors are promising agents for treatment of hyperproliferative disorders such as cancer. We have developed a no-carrier-added, radioiodinated analogue of BAY 43-9006, **1**, for use as a single photon emission tomography (SPECT) imaging agent for potential use in the early detection of lung cancer. The details of the preparation will be discussed.



1

MEDI 310

Synthesis and biological evaluation of carbon-11 labeled 2β-carbomethoxy-3α-(3'-fluoro-4'-methylphenyl)-nortropane: Candidate PET ligand for the norepinephrine transporter

Fanxing Zeng[1]*, Nachwa Jarkas*[1]*, Jeffrey S. Stehouwer*[1]*, Ronald J. Voll*[1]*, Larry Williams*[1]*, John R. Votaw*[1]*, and Mark M. Goodman*[2]*. (1) Department of Radiology, Emory University, 1364 Clifton Road, NE, Atlanta, GA 30322, Fax: 404-727-3488, fzeng@emory.edu, (2) Department of Radiology, Division of Radiological Sciences, Emory University*

The norepinephrine transporter (NET), a specific marker of noradrenergic neurons, plays a critical role in regulating neurotransmitter concentration at noradrenergic synapses as well as terminating noradrenergic neurotransmission by reclaiming norepinephrine (NE) from the extracellular space. The NET has been recognized in the involvement of several neurological and psychiatric disorders and is an established molecular target for the treatment of depression, anxiety disorder, and attention-deficit/hyperactivity disorder (ADHD). Imaging agents suitable for visualization and quantification of the NET by emission tomography techniques would present unique opportunities to define the function and pharmacology of the NET in the living human brain and would therefore provide a useful tool to diagnose and monitor response to therapy in depressive illness and other psychiatric disorders. A series of candidate NET ligands of 3a-4'-substituted phenyl nortropane analogues were synthesized and characterized in terms of their affinity for human monoamine transporters. The most promising compound, 2b-carbomethoxy-3a-(3'-fluoro-4'-methylphenyl)-nortropane was radiolabeled with carbon-11 and evaluated with PET imaging study in a rhesus monkey.

MEDI 311

MicroPET imaging of the brain serotonin transporter with [^{11}C]*m*ZBrENT

Jeffrey S. Stehouwer[1]*, Nachwa Jarkas*[1]*, Fanxing Zeng*[1]*, Ronald J. Voll*[2]*, Larry Williams*[1]*, John R. Votaw*[1]*, and Mark M. Goodman*[2]*. (1) Department of Radiology, Emory University, 1364 Clifton Road NE, Atlanta, GA 30322, jstehou@emory.edu, (2) Department of Radiological Sciences, Emory University Hospital*

As part of ongoing research in our laboratories to develop nortropane-based serotonin transporter (SERT) positron emission tomography (PET) imaging agents we have been investigating the monoamine transporter binding of 2β-carbomethoxy-3β-(3′-((Z)-2-bromoethenyl)phenyl)nortropane (*m*ZBrENT) and the microPET imaging properties of [^{11}C]*m*ZBrENT. *m*ZBrENT was synthesized and it's binding to the human serotonin, dopamine, and norepinephrine transporters was determined to be (K_i, nM): SERT 0.19, DAT 32.6, NET 38.0, respectively. MicroPET imaging with [^{11}C]*m*ZBrENT in an anesthetized cynomolgus monkey showed high uptake in the SERT-rich regions of the brain with the following ratios to cerebellum uptake at 65 and 105 min post-injection, respectively: Caudate = 1.26, 1.48; putamen = 1.39, 1.67; thalamus = 1.35, 1.69; midbrain = 1.66, 2.02; pons = 1.33, 1.54; medulla = 1.49, 1.85; occipital lobe = 1.05, 1.27; and frontal lobe = 0.77, 0.89. *m*ZBrENT has shown promise as a candidate SERT PET imaging agent and further studies are being performed.

MEDI 311

MicroPET imaging of the brain serotonin transporter with [^{11}C]*m*ZBrENT

Jeffrey S. Stehouwer[1]*, Nachwa Jarkas*[1]*, Fanxing Zeng*[1]*, Ronald J. Voll*[2]*, Larry Williams*[1]*, John R. Votaw*[1]*, and Mark M. Goodman*[2]*. (1) Department of Radiology, Emory University, 1364 Clifton Road NE, Atlanta, GA 30322, jstehou@emory.edu, (2) Department of Radiological Sciences, Emory University Hospital*

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MEDI 312

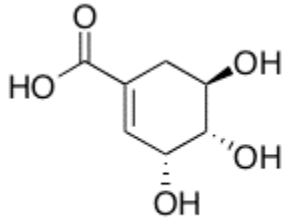
Three dimensional QSAR studies and pharmacophore modeling of aromatic enones as Chalcone analogs

Hari S. K. Paila*, Haizhen Zhong, and J. Phillip Bowen, Center for Drug Design, Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, 401 New Science Building, Greensboro, NC 27402, phskalyan@yahoo.com*

Curcumin was isolated from the commonly used spice turmeric, and it has been shown to inhibit the bFGF-induced endothelial cell proliferation in vitro and also to inhibit angiogenesis in vivo. Structural modification through the truncation of the

central region of curcumin gave rise to more compact analogs. Our laboratory has reported the synthesis and biological evaluation of sixty-three enones – chalcone analogs. Some of these enones yielded promising activities equaling or exceeding curcumin's ability to inhibit endothelial cell growth in vitro. Here we present our latest studies on pharmacophore modeling, and quantitative structure-activity relationships for these sixty-three compounds. The QSAR models suggest that increasing logP(o/w) and logS would augment the activities.

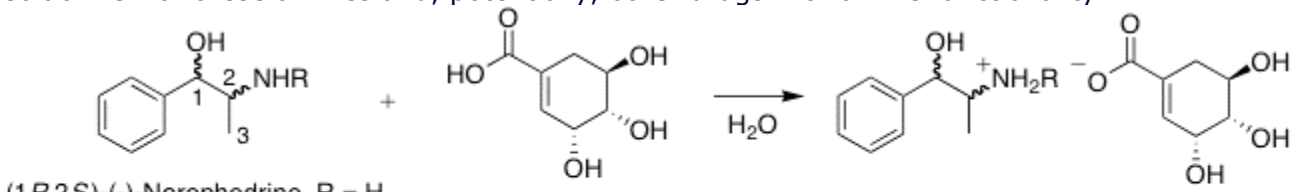
MEDI 313
Isolation of shikimic acid from *Liquidambar styraciflua*
***Margaret Scheuermann**, W.M. Keck Science Center, Scripps College, and **Thomas Poon**, Joint Science Department, Claremont McKenna, Pitzer, and Scripps Colleges, W.M. Keck Science Center, 925 N. Mills Ave., Fax: 909-621-8588, tpoon@jsd.claremont.edu*
Shikimic acid, the starting material in the commercial synthesis of the antiviral agent Oseltamivir and an important intermediate in the biosynthesis of aromatic amino acids in plants, was successfully isolated from the seeds of *Liquidambar styraciflua*, commonly known as the sweetgum tree. Using simple organic laboratory techniques (solid-liquid extraction, hot gravity filtration, decolorization, and distillation *in vacuo*), 400-700 mg of shikimic acid could be obtained from 28-32 g of seed, representing a 1.3-1.8% recovery. The isolation procedure is also amenable to scale-up (0.5 kg seeds yielding approx. 4 g of shikimic acid). *Liquidambar* is native to North America and is found in 39 states of the continental U.S., making it an abundant and renewable source of shikimic acid with the potential to increase the availability of this important natural product for future applications.



Shikimic Acid

MEDI 314
Solubilization of aminophenylpropanols using shikimic acid
***Shandi Ramirez**[1], Margaret Scheuermann[1], Michael Fujinaka[2], and **Thomas Poon**[3]. (1) W.M. Keck Science Center, Scripps College, Claremont, CA 91711, (2) W.M. Keck Science Center, Claremont McKenna College, (3) Joint Science Department, Claremont McKenna, Pitzer, and Scripps Colleges, W.M. Keck Science Center, 925 N. Mills Ave., Claremont, CA 91711, Fax: 909-621-8588, tpoon@jsd.claremont.edu*
Four new ionic analogs (**1-4**) of (±)-norephedrine and (±)-pseudoephedrine were synthesized by reacting each drug with the plant metabolite, shikimic acid. The resulting diastereomeric salts were characterized using differential scanning calorimetry, electrospray high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy. Solubility determinations with **1-4** in various buffered and unbuffered aqueous media, reveal a high degree of solubility. For example, in unbuffered water, the solubilities of **1-4** range from 0.48 – 0.72 g/mL, which are comparable to current formulations of norephedrine and pseudoephedrine with HCl or H_2SO_4. We have also explored the synthesis of covalent analogs of (±)-norephedrine and shikimic acid, which offer tempered solubility in

water, but enhanced lipophilicity. Our results show that shikimic acid is a versatile solubilizer for these amines and, potentially, other drugs with amine functionality.

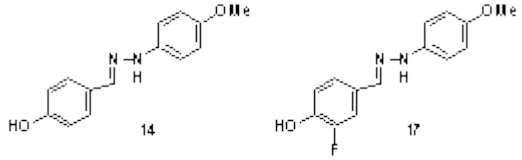


(1*R*,2*S*)-(-)-Norephedrine, R = H
(1*S*,2*R*)-(+)-Norephedrine, R = H
(1*R*,2*R*)-(-)-Pseudoephedrine, R = CH_3
(1*S*,2*S*)-(+)-Pseudoephedrine, R = CH_3

Shikimic Acid

1-4

MEDI 315
Phenolic hydrazones are potent inhibitors of MIF proinflammatory activity and increase survival in severe sepsis
Darrin Dabideen, *Kai Fan Cheng, Bayan Aljabari, and Yousef Al-Abed, Laboratory of Medicinal Chemistry, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Fax: 516-365-5090, ddabidee@nshs.edu*
MIF is a proinflammatory cytokine and has been implicated in sepsis and type 1 diabetes. A series of phenolic hydrazones were developed, synthesized and initially evaluated for their inhibition of MIF tautomerization of D,L-dopachrome methyl esters. Among the phenolic hydrazones tested compound 14 and its fluoro derivative 17 showed superior inhibition of MIF tautomerase activity. The ability of 17 to inhibit the proinflammatory activity of MIF was demonstrated in vivo by the survival of animals in severe sepsis.



MEDI 315
Phenolic hydrazones are potent inhibitors of MIF proinflammatory activity and increase survival in severe sepsis
Darrin Dabideen, *Kai Fan Cheng, Bayan Aljabari, and Yousef Al-Abed, Laboratory of Medicinal Chemistry, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Fax: 516-365-5090, ddabidee@nshs.edu*
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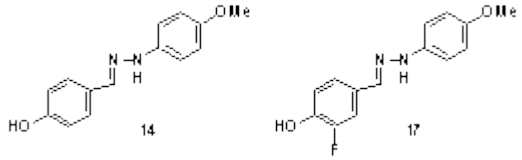
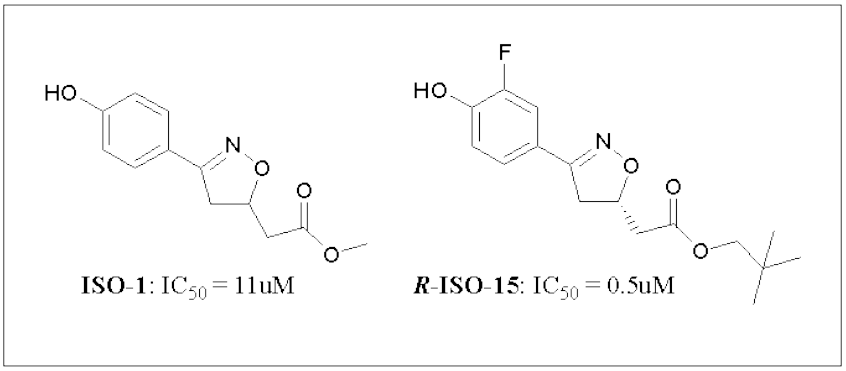


MEDI 316

Critical modifications onto ISO-1 scaffold improve its potent inhibition of MIF activity

Kai Fan Cheng, *Darrin Dabideen, Bayan Aljabari, and Yousef Al-Abed, Laboratory of Medicinal Chemistry, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Fax: 516-365-5090, kcheng2@nshs.edu*

Sepsis is an inflammatory disorder that is a major cause of morbidity and mortality and the leading cause of death in non-cardic intensive care units. Our recent studies have clearly defined macrophage migration inhibitory factor (MIF) as a critical factor in the pathophysiology of sepsis. We designed a molecule to fit into the catalytic site of MIF and showed the (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) is a potent inhibitor of MIF tautomerase activity. We also showed that abolition of MIF activity during sepsis by ISO-1 or antibodies improves cardio-circulatory efficiency and prevents the lethality associated with sepsis. Based on the scaffold of ISO-1, two critical modifications and a chiral resolution of isomers were examined. R-ISO-15 is 20-folds more potent than the parent compound ISO-1. R-ISO-15 inhibits MIF tautomerase and biological activities with an IC50 of 500 nM.

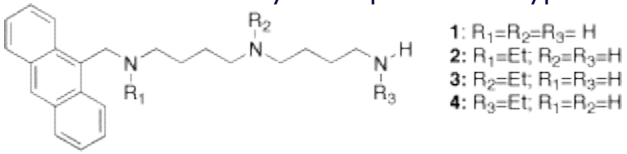


MEDI 317

Influence of N-substituents on the transport behavior of polyamine conjugates

Navneet Kaur[1], *Nathan Weagraff*[1], *Jean-Guy Delcros*[2], *Dan Yiqiang Wang*[1], *and Otto Phanstiel IV*[1]*. (1) Department of Chemistry, University of Central Florida, P.O. Box 162366, Orlando, FL 32816-2366, Fax: 407-823-2252, nkaur@mail.ucf.edu, (2) Groupe Cycle Cellulaire, Université Rennes 1*

Several *N*[1]-anthracenylmethyl-*N*-ethyl-substituted triamines were synthesized and screened for their ability to deliver a toxic anthracene moiety via the polyamine transporter (PAT) to murine leukemia (L1210) cells, Chinese hamster ovary (CHO) cells, and a mutant PAT-deficient CHO cell line (CHO-MG). Respective mono *N*-ethylation at each of the three available nitrogen centers of *N*[1]-anthracenylmethyl-homospermidine (**1**) had a dramatic effect both on the molecular shape preferences (molecular modeling) and PAT-targeting ability of these derivatives (**2-4**). A direct correlation was found between cytotoxicity and the ability of the polyamine conjugate to use the PAT for cellular entry. A survey of these conjugates led to the identification of a general PAT-selective motif, which was effective in targeting the PAT for cellular entry into specific cell types.

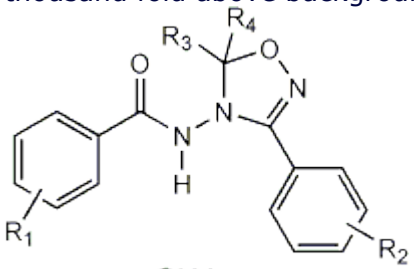


MEDI 318

Oxadiazolines as gene switch ligands

*Robert E. Hormann, Orestes Chortyk, Christine S. Thompson, Jennifer L. Friz, Dean E. Cress, and **Bing Li**, RheoGene Inc, Norristown, PA 19403*

Ligand inducible gene expression systems (gene switches) have potential applications in human gene therapy and the production of therapeutic proteins. The oxadiazolines are a new class of gene switch ligands for the ecdysone receptor (EcR). A library of 5,5-disubstituted-oxadiazolines has been prepared by solution phase synthesis and assayed in the EcR-based gene expression systems. Representatives of this class induced marker reporter genes at levels several hundred to thirty thousand fold above background with EC_{50} values in the range of 300 nM-5 μM.



OXA

MEDI 318

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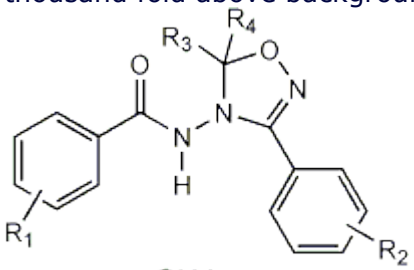


OXA

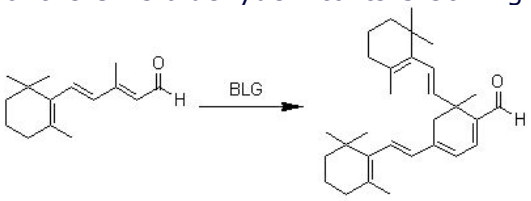
MEDI 319

Investigation of the biological roles of 1,4-disubstituted and 1,2,4-trisubstituted cyclohexadienes

***Bennie J. Bench**, Chaomin Liu, and Coran M. H. Watanabe, Department of Chemistry, Texas A&M University, College Station, TX 77843, bbench@mail.chem.tamu.edu*

Over the past 60 years, beta-lactoglobulin, the principle whey protein found in milk, has been extensively investigated by both biophysical and biochemical methods. To this day, there is no specific biological function ascribed to this protein. In the early 1990's, it was discovered that if ƒ"-lactoglobulin was incubated with C-15 aldehyde, the protein catalyzed self-condensation of this aldehyde into a C-30 ring fused dimer (Figure). However, no follow-up studies were conducted to access biological function.

We are mimicking this reaction (Figure) by using proline as a catalyst to develop a library of 1,4-disubstituted and 1,2,4-trisubstituted cyclohexadienes. Other aspects of this project include the mechanistic investigation of the proline-mediated reaction as well as the biological investigation of the beta-lactoglobulin enzymatic conversion of the C-15 aldehyde into its C-30 ring-fused dimer adduct.



MEDI 320

Evaluation of alkyloxycarbonyloxymethyl and alkylcarbonyloxymethyl prodrugs as permeation-enhancing derivatives of phenol-containing drugs

Joshua D. Thomas *and Kenneth B. Sloan, Department of Medicinal Chemistry, University of Florida, P O Box 100485, Gainesville, FL 32610, Fax: 352-392-9455, joshthom@ufl.edu*

Given the success of alkyloxycarbonyloxymethyl (AOCOM) and alkylcarbonyloxymethyl (ACOM) prodrugs at improving the bioavailability of imide, thioamide, carboxylic acid, and amide-containing drugs, there are surprisingly few examples of this approach being applied to phenolic drugs. In the present study, a series of AOCOM and ACOM prodrugs of acetaminophen, a model phenol, have been synthesized and their ability to improve the permeation of acetaminophen across hairless mouse skin in vitro has been evaluated. The results of these experiments suggest that ACOM and AOCOM prodrugs are capable of improving the flux (amount of drug permeating per unit area per unit time) of poorly permeable phenol-containing drugs.

MEDI 321

Synthesis of PUGNAC analogs and the development of fluorogenic substrates toward the selective study of O-GlcNAcase

Melissa Perreira[1]*, Eun Ju Kim*[2]*, John A. Hanover*[2]*, and Craig J. Thomas*[3]*. (1) Chemical Biology Core Facility, National Institute of Digestive and Diabetes and Kidney Disorders, National Institutes of Health, Bethesda, MD 20982, perreiramelissa@hotmail.com, (2) Laboratory of Cell Biochemistry and Biology, National Institute of Diabetes and Digestive and Kidney Disorders, National Institutes of Health, (3) Chemical Biology Core Facility, NIDDK, National Institutes of Health, National Institutes of Health*

The ubiquitous post-translational modification that entails the addition or elimination of O-linked N-acetylgucosamine (O-GlcNAc) on nuclear and cytoplasmic proteins has been well documented. While the catalytic mechanisms of action of O-GlcNAcase and O-GlcNAc transferase enzymes are not well understood, a number of lines of

evidence suggest that disruption of cellular O-GlcNAc levels might be linked to type II diabetes, cancer, and Alzheimer's. Examination of the function, regulation and mechanism of each enzyme requires the design and synthesis of small molecules that selectively blocks its activity. There are a few known inhibitors of O-GlcNAcase, such as O-(2-acetamido-2-deoxy-D- glucopyranosylidene)amino-N-phenylcarbamate (PUGNAC) and straptozotocin. To better understand the inhibition of O-GlcNAcase, we synthesized PUGNAC and several derivatives generating two oxime stereoisomers (E and Z) for each substrate. All studies were performed with a highly sensitive fluorogenic substrate, fluorescein di-?-D-N-acetylglucosamine we have developed. The information obtained was used in our development of new fluorogenic substrates that recognize O-GlcNAcase but not lysosomal hexosaminidase A and B, allowing for the study of O-GlcNAcase independently.

MEDI 322

Facile removal of metal species using polymeric SPE materials functionalized with uronium ligands

Aubrey J Mendonca[1], *Paul A Boguszewski*[2], *Andrew F Coffey*[2], *John W Davies*[2], *Alasdair A MacDonald*[1], *and Frank P Warner*[2]. *(1) Polymer Laboratories Inc, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, Fax: 413 253 2476, SPE@polymerlabs.com, (2) Polymer Laboratories Ltd*

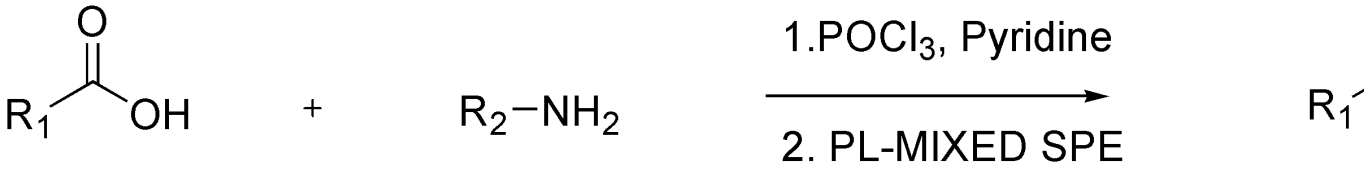
The diversity and reliability of organometallic reactions has increased greatly over the last decade and it is commonplace to see such reactions in a high throughput and process chemistry environment. In particular the removal of active catalyst species from final products is critical to allow reliable high throughput screening. There have been several examples of materials developed for the removal of palladium and ruthenium, but few for the effective removal of species such as mercury, nickel, rhodium and copper. It is known that ureas, thioureas and guanidines are good ligands for these metals and we have developed a range of monodispersed polymeric SPE materials functionalized with the appropriate chemical motif. With just one pass through an SPE device, metal catalytic species can be removed to single figure ppm levels, where they will not interfere with high throughput screening results.

MEDI 323

Effective clean up of amidation reactions using a tri-functional SPE device

Aubrey J Mendonca[1], *Paul A Boguszewski*[2], *Andrew F Coffey*[2], *John W Davies*[2], *Oliver Guth*[3], *Alasdair A MacDonald*[1], *and Frank P Warner*[2]. *(1) Polymer Laboratories Inc, Amherst Fields Research Park, 160 Old Farm Road, Amherst, MA 01002, Fax: 413 253 2476, SPE@polymerlabs.com, (2) Polymer Laboratories Ltd, (3) Research Chemistry Fungicides Monheim, Bayer CropScience Monheim*

A variety of methods are available to the synthetic chemist for the formation of an amide bond. The predominant strategy is the use of a coupling reagent either in soluble form or bound to an inert solid phase. Examples of such coupling agents include carbodiimides, active ester reagents and highly activated isouronium salts. Often an excess of the reagent and one of the components is required to drive the reaction to completion. In certain cases, however, this tactic fails as these commonly used reagents are insufficiently active to form the required amide bond. For such difficult cases, we will describe an innovative, general method for amide bond formation. This utilizes equimolar quantities of acid and amine in the presence of phosphorous oxychloride and pyridine. After completion of the reaction, the mixture is passed through a specially engineered SPE device that removes all un-reacted components and by-products to afford a solution of the purified amide product in an anhydrous form.



MEDI 324
Optimized flash chromatography purification: From TLC to large scale in three steps
Veronica D. Thomason, *Chromatography Department, Teledyne - Isco, 4700 Superior Street, Lincoln, NE 68504, Fax: 402-465-3089, vthomason@teledyne.com*
Method development for optimal flash chromatography is a time consuming step for synthetic organic chemist. A quick approach for developing optimal chromatographic purification utilizing the Rf to gradient calculator and Scale-up feature, developed by Teledyne Isco for use with their Combiflash automated purification systems, will be discussed.

MEDI 325
Shape similarity values as tool for QSAR-studies on inhibitors of P-glycoprotein
Gerhard F Ecker[1]*, Elisabet Gregori*[2]*, Barbara Zdrazil*[1]*, Stephan Kopp*[3]*, Peter Chiba*[3]*, Jordi Mestres*[4]*, and Ferran Sanz*[5]*. (1) Department of Medicinal Chemistry, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria, Fax: 431-4277-9551, gerhard.f.ecker@univie.ac.at, (2) Chemogenomics Laboratory, Research Group on Biomedical Informatics (GRIB), Municipal Institute of Medical Research (IMIM), (3) Institute of Medical Chemistry, Medical University of Vienna, (4) Chemogenomics Laboratory, Research Unit on Biomedical Informatics (GRIB), Municipal Institute of Medical Research (IMIM), (5) Research Unit on Biomedical Informatics (GRIB), IMIM, Universitat Pompeu Fabra*
Polyspecific transport pumps, such as P-glycoprotein (P-gp), are versatile model systems for development of new methods for in silico screening of promiscuous targets and antitargets. Recently we published the successful application of similarity-based descriptors (SIBAR) on a set of propafenone-type inhibitors of P-gp. Our studies were based on calculation of euclidian distances between a set of 20 reference compounds and the training set compounds in a 31 dimensional descriptor space. These similarity values (= SIBAR descriptors) were used as input vector in QSAR analyses. Herein we extended the approach to the use of shape similarity values as implemented in the software package MIMIC. Using a set of structurally highly diverse compounds from the SPECS-library as reference sets, a q2-value of 0.48 was obtained for our in house data set comprising 293 P-gp inhibitors. However, predictivity varied strongly between structurally related subsets of compounds, with benzopyranes showing highest (q2 = 0.70), propafenones medium (q2 = 0.48) and pyrazolones lowest (q2 = 0.02) predictivity. Supported by grants from the Austrian Science Fund (grant 17014) and from the HPC-Europa Transnational Access program.

MEDI 326
Predictive models for P-glycoprotein substrates based on VSA-descriptors
Gerhard F Ecker[1]*, Silke U. Schindler*[1]*, Barbara Zdrazil*[1]*, and Peter Chiba*[2]*. (1) Department of Medicinal Chemistry, University of Vienna, Althanstrasse 14, A-1090*

Vienna, Austria, Fax: 431-4277-9551, gerhard.f.ecker@univie.ac.at, (2) Institute of Medical Chemistry, Medical University of Vienna
With the increasing knowledge on the physiological role of P-glycoprotein for bioavailability and brain uptake the focus of interest changed from design of inhibitors to prediction of substrate properties. This involves mainly the areas of anticancer agents and cns-active compounds. Due to the broad substrate specificity and fuzzy SAR-pattern, only a few models for P-gp substrate prediction have been published so far. They rely on support vector machines, decision tree analysis or simple filter rules. We used the VSA-descriptors developed by Labute for a set of 139 structurally and functionally diverse P-gp substrates and non-substrates. Binary QSAR as implemented in MOE gave a model with a cross-validated accuracy of 0.78 for the training set and 0.74 and 0.81 for two external test sets, which is in the range of previously published models. Due to the high speed of VSA-descriptor calculation, this is a versatile model for high throughput in silico filtering of large compound libraries.

MEDI 327
Structure-activity analysis of P-glycoprotein transport and inhibition, and development of predictive algorithms
*Alanas Petrauskas, **Pranas Japertas**, Remigijus Didziapetris, and Dimitri Bondarev, Pharma Algorithms Inc, 591 Indian Rd., Toronto, ON M6P 2C4, Canada*
Prediction of P-glycoprotein substrate specificity and inhibition can be viewed as a constituent part of a compound's pharmaceutical profiling. P-gp transport is class-specific towards various types of natural compounds. For example, transport of peptides, alkaloids and some steroids must be considered separately from other compounds. P-gp inhibition is less sensitive to such effects. In this work, a novel probabilistic cheminformatics approach was taken to analyzing experimental data from a number of in vitro and in vivo assays. It is based on the following steps: (i) dynamic generation of fragmental descriptors, (ii) logistic PLS representing a combination of logistic regression and partial least squares analysis, and (iii) multiple bootstrapping procedures. In addition, rule-based classification with physicochemical descriptors as well as molecular substructures was performed to build a predictive model independent of the fragmental model. The two methods are complementary and can be used to generate consensus predictions.

MEDI 328
Modeling plasma protein binding and volume of distribution
*Alanas Petrauskas, Pranas Japertas, Remigijus Didziapetris, Kiril Lanevskij, and **Dimitri Bondarev**, Pharma Algorithms Inc, 591 Indian Rd., Toronto, ON M6P 2C4, Canada*
This study presents a mechanistic QSAR and C-SAR analysis of the apparent volume of distribution (Vd) and drug binding to plasma proteins (%DBP) in humans that considers the dependence of Vd and %DBP on multiple physicochemical parameters and structural features of drugs. Values of Vd for 760 compounds and that of %DBP for 1030 compounds were compiled from original literature sources. Physicochemical parameters, charge state, hydrophobicity and H-bonding potency were used in modeling Vd and %DBP. The Vd model employed a combination of C-SAR (recursive partitioning) and linear regression. The %DBP model was built using separate non-linear regressions for acids, neutrals, bases and zwitterions. Final models were tested on external validation sets (N=90 for Vd; N=43 for %DBP). The mean fold error of prediction in the case of Vd modeling was less than 2. Predicted %DBP significantly correlated with experimental %DBP values with an R squared of 0.76.

MEDI 329

Exploring oral bioavailability and drug plasma concentration using physicochemical parameters

Alanas Petrauskas, Pranas Japertas, Remigijus Didziapetris, and ***Paulius Jurgutis****, Pharma Algorithms Inc, 591 Indian Rd., Toronto, ON M6P 2C4, Canada*

This study presents a simulation of drug plasma concentration (Cp) vs time, dose and physicochemical parameters of drugs. The simulation was based on solving differential equations characterizing drug dissolution, absorption and excretion from the gastrointestinal tract and total drug clearance from the body. Dissolution, solubility in the gastrointestinal tract, passive absorption, first-pass effect in the liver and gut, volume of distribution and total body clearance were considered. This simulation leads to an estimation of the dependency of oral bioavailability (%F) on dose and physicochemical parameters (charge state and hydrophobicity). Simulations can be useful in lead optimization and selection as these allow to model changes in pharmacokinetic parameters (%F, Cp, Cmax, AUC0-t and others) of drugs by changing the main physicochemical parameters: ionization constants (pKa) and hydrophobicity (logP). The model was validated by analyzing published Cp-time curves and predicting oral bioavailability (%F) values for a number of drugs.

MEDI 330

In silico ADME: From hit selection to lead optimization

F. Lebon[1]*, I. Ortmans*[1]*, P. Collart*[2]*, C. Genicot*[1]*, F. Moureau*[1]*, J-M. Nicolas*[2]*, L. Quéré*[1]*, and D. Smaragd*[3]*. (1) Chemical Research, UCB SA, rue du Foriest, braine l'alleud 1420, Belgium, florence.lebon@ucb-group.com, (2) Predevelopment Pharmacokinetic, UCB SA, (3) Chemical Informatics, UCB SA*

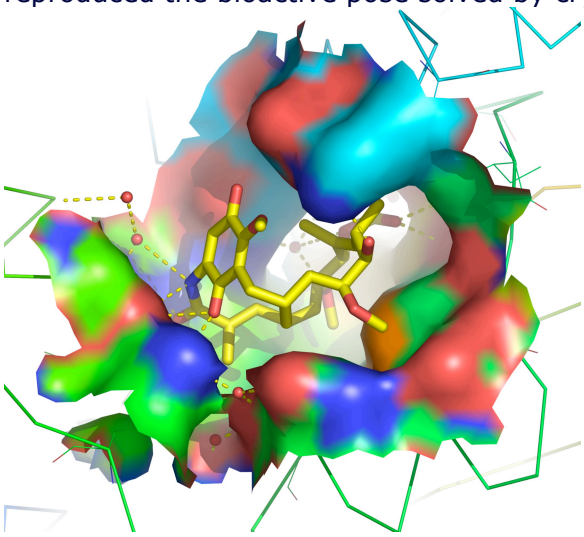
It is now widely accepted that increasing the attrition rate in less costly, early stages increases the efficiency and reduces the costs of pharmaceutical R&D. The strategy is known as the "fail fast, fail cheap" paradigm. One component is to take into account ADME parameters as early as possible in the drug discovery process. At UCB we use computational & experimental early ADME approaches in a complementary way to address hit selection and hit to lead processes. In the hit selection process we show that association of both in silico (MW, clogP, RotB, 3D PSA) and experimental (Clint & solubility) data can be used to rapidly assess the ADME risk associated to a scaffold. The work results from a project-based analysis of UCB data and is able to correctly classify 89% of the compounds based on bioavailability data in rat. The dataset used for the analysis is diverse and shows distribution of each property in agreement with the global UCB collection. The data for hit selection is made available to the chemist through a pipeline pilot web protocol. To address CYP2D6 issues in lead optimization stages of projects we use a 3D comparative model of cytochrome P450 2D6 for which we developed docking and post-processing protocols. Post-processing criteria involve 1) distances between the Glu216 or Asp301 carboxylate oxygen atoms and the basic nitrogen atom of the docked ligand: r(O216/301-Nbasic), 2) distances between the heme iron atom and the heavy atoms of the docked ligand: r(Fe-Hev) and finally 3) the accessibility to Phe120. The docking protocols provide docking solutions compatible with the known metabolic profiles of several reference and in house CYP2D6 substrates. These solutions are retrieved from the ensemble of docking poses on the basis of the post-processing criteria mentioned above.

MEDI 331

Comparative NAMFIS analysis of Geldanamycin and Radidicol

Pahk Thepchatri[1]*, Daniel O Cicero*[2]*, Tomasso Eliseo*[2]*, David C. Myles*[3]*, and James P. Snyder*[1]*. (1) Department of Chemistry, Emory University, 1515 Dickey Drive,*

Atlanta, GA 30322, Fax: 404-727-6586, pthepch@emory.edu, (2) NMR Laboratory, Faculty of Chemical Sciences and Technologies, University of Rome "Tor Vergata", (3) Department of Chemistry, Kosan Biosciences Inc
The unknown effects of a receptor's environment on a ligand's conformation presents a difficult challenge in predicting feasible bioactive conformations, particularly if the receptor is ill-defined. The primary hypothesis of this work is that a structure's conformational ensemble in solution presents viable candidates for protein binding. The NAMFIS (NMR Analysis of Molecular Flexibility In Solution) method deconvolutes the average NMR spectrum of small flexible molecules into individual contributing conformations with varying populations. Geldanamycin and radicicol are structurally different molecules determined by X-ray crystallography to bind to a common site on the cellular chaperone Heat shock protein 90 (Hsp90). Without benefit of a receptor structure, NAMFIS identified the bioactive conformer of geldanamycin in solution with a population of 4% and that of the radicicol at 21%. GLIDE docking of the NAMFIS ensemble (12 structures) and the entire conformational pool (1246 structures) reproduced the bioactive pose solved by crystallography.



MEDI 332
Synthesis of 2,5-oligoadenylate-folate conjugate: Ribonuclease L activation and folate receptor binding
Nidhi Gupta[1]*, Longhu Zhou*[1]*, Ross Molinaro*[2]*, Robert H. Silverman*[2]*, Zhanna V. Zhilina*[3]*, Scot Ebbinghaus*[3]*, and Paul F. Torrence*[1]*. (1) Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ 86011, (2) Cancer Biology Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44196, (3) Department of Medicine, Arizona Cancer Center,Tucson, AZ 85724*
The small 2',5'-linked oligoriboadenylate known as 2-5A plays a key role in interferon action and has been a key player in attempts to develop antiviral and antitumor agents.However, in vivo applications have been hampered by inefficient cellular uptake. The cell membrane folate receptors (FR) is a valuable therapeutic target highly expressed by variety of malignant cells, and is the tumor marker that selectively binds folic acid and some folate drug conjugates with high affinity (Kd = 10-10M) and shuttles these bound molecules inside cell via an endocytic mechanism without causing harm to normal tissues. As a consequence, normal tissues lacking FR are spared the toxicity that commonly limits non-targeted therapies. In the 2-5-A arm of the interferon system, 2,5-A provides an unambiguous signal to initiate RNA decay through the activation of the latent 2,5-A dependent RNase L, which degrades viral mRNA, resulting in an inhibition of the protein synthesis. To probe structural

requirements for folate receptors (FR) targeting with 2,5-A, a conjugate was synthesized by carbodiimide mediated coupling of folic acid to 1,4-bis(3-aminopropoxy)butane and then coupled with 2',5'-oligoadenylate through chemical modification of 2,5-A tetramer by periodateoxidation/schiff base formation/borohydride reduction cycle in which the ribose of the 2'-terminal nucleotide was transformed to an N-substituted morpholino (azahexapyranose). These conjugates were characterized by MALDI-TOF mass spectroscopy and HPLC analysis of enzymatic cleavage with carboxypeptidase G2. RNase L assay showed that this novel 2,5-A-folate chimeras can activate RNase L effectively; however, its binding to the folate receptor is significantly reduced compared to free folic acid. Acknowledgment: This work was supported by funding form the U.S. Army Medical Research and Materiel Command, Congresionally Directed Program on Prostate Cancer Research DAMD17-02-1-0255.

MEDI 332

Synthesis of 2,5-oligoadenylate-folate conjugate: Ribonuclease L activation and folate receptor binding

Nidhi Gupta[1]*, Longhu Zhou*[1]*, Ross Molinaro*[2]*, Robert H. Silverman*[2]*, Zhanna V. Zhilina*[3]*, Scot Ebbinghaus*[3]*, and Paul F. Torrence*[1]*. (1) Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ 86011, (2) Cancer Biology Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44196, (3) Department of Medicine, Arizona Cancer Center,Tucson, AZ 85724*

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MEDI 333

Characterization of G-Quadruplex complexes of heterocyclic diamidines using biophysical methods

Rupesh K Nanjunda[1]*, Elizabeth W. White*[1]*, Mohamed A. Ismail*[2]*, Markus W. Germann*[1]*, David W. Boykin*[2]*, and W. David Wilson*[1]*. (1) Department of Chemistry,*

Georgia State University, 50 Decatur Street, Atlanta, GA 30303, rupesh.nanjunda@gmail.com, (2) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University
Among the higher order DNA structures, G-quadruplexes have been recognized in various biological functions including telomere stabilization, anti-aging mechanisms, and cancer control. Due to the many G-rich sequences, the possible roles of G-quadruplexes as therapeutic agents and targets for anti-cancer and anti-parasitic drugs are being investigated. Aromatic dicationic compounds have emerged as an important class of small molecules due to their unique ability to recognize various DNA sequences. Our recent structural and binding studies have shown that certain heterocyclic furan-based diamidine derivatives selectively target G-quadruplexes and stabilize them. Circular dichroism and NMR studies have shown that these diamidines bind to models of human telomere sequences and stabilize a single parallel-type structure. Structural studies are in progress on the telomere complexes of these compounds.

MEDI 334
DNA template-directed oligodeoxynucleotide and PNA crosslinking by strain-promoted click chemistry
***Xuan Yue**, Huafeng Fang, and John-Stephen Taylor, Department of Chemistry, Washington University in St. Louis, One Brookings Drive, Campus Box 1134, St. Louis, MO 63130*
Huisgen's 1,3-dipolar cycloaddition of azides to multiple bonds is an old and widely used reaction, but in recent years it has aroused a lot of attention as a biocompatible click reaction for applications in biology. One of our current research interests is in the development of new antisense and antigene agents for controlling gene expression with a particular aim of enhancing heat induced radiosensitization of tumors. In this poster we will describe how we have attached phenyl azide and norbornene to the termini of short strands of natural and peptide nucleic acids and demonstrated, by polyacrylamide gel electrophoresis, that the modified nucleic acids underwent efficient DNA template-dependent crosslinking. Application of this crosslinking reaction to the development of new agents for controlling gene expression will also be described.

MEDI 335
Analysis of deadenylation followed by adduct formation of adenine-based nucleosides with 2-iodopropane, bromoethane and 2,3-dibromopropene at the physiological condition using HPLC and LC/MS/MS
***Eung-Seok Lee**, Jong Geol Kim, Jyoti Sherchan, Arjun Basnet, Jung Ki Park, Yurngdong Jahng, and Tae Cheon Jeong, College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Kyongsan 712-749, South Korea, Fax: +82-53-810-4654, eslee@yu.ac.kr*
Halogenated alkanes are widely used as the solvents, laboratory reagents and cleansing agents. However, they have noxious propensity to human body. They are absorbable in human body through skin, eye, respiratory organs etc. that results in direct damage of absorbed body region, and display toxicity action being concentrated in interior of the body. They may cause cancer, dementia, infertility etc. We have been previously reported that 1- and 2-bromopropane could form adducts with 2'-deoxyguanosine and calf-thymus DNA at the physiological condition, as well as *in vivo* experiments, which indicated the possible mechanism of toxicity of 2-bromopropane. Recently, it has been observed that most of the adenine-based nucleosides were deadenylated by the treatment with 2-bromopropane and several halogenated alkanes at the physiological condition. In this study, analysis of

deadenylation of adenine-based nucleosides with 2-iodopropane, bromoethane and 2,3-dibromopropene at the physiological condition were performed using HPLC and LC/MS/MS. In addition, time and dose response effects were also studied. As well as, formation of adenine adducts after deadenylation was also observed at the physiological condition using HPLC and LC/MS/MS, which indicated the adenine adducts formation by those compounds were not related to adducts formation on 2'-deoxyadenosine but direct adduct formation to adenine. These results may suggest that the toxicity of the 2-bromopropane and other halogenated compounds may be induced by the deadenylation of the adenine-based nucleosides.

MEDI 336

Synthesis and identification of N3-adenine adducts formed by 1- and 2-bromopropane with 2'-deoxyadenosine or calf-thymus DNA at the physiological condition by HPLC and LC/MS/MS

Jung Ki Park*, Jong Geol Kim, Byong Ki Choi, Jyoti Sherchan, Arjun Basnet, Yurngdong Jahng, Tae Cheon Jeong, and Eung-Seok Lee, College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Kyongsan 712-749, South Korea, Fax: +82 53-810-4654, rudmo1234@hanmail.net*

2-bromopropane (2-BP) has been used as a replacement for chlorofluorocarbon and 1,1,1-trichloroethane as a cleansing solvent in the electronic industry. But 2-BP was found to cause amenorrhea in female and oligospermia in male workers when exposed to it. Due to the toxicity of 2-BP, it has been increased tendency to use 1-bromopropane (1-BP) as an alternating cleansing solvent to 2-BP. However, 1-BP has also been reported to be neurotoxic in rats. Therefore, in order to elucidate mechanism of 1-BP- and 2-BP-induced toxicities in the molecular level, formation of N3-adenine adducts by 1-BP and 2-BP was investigated at the physiological condition. N3-adenine adducts of 1-BP and 2-BP were chemically synthesized and structurally characterized by ^{1}H NMR, ^{13}C NMR, UV, HPLC and ESI LC/MS/MS to utilize as a reference materials. N3-adenine adducts were detected and identified by UV, HPLC and ESI LC/MS/MS after incubation of 2'-deoxyadenosine with 1-BP and 2-BP at the physiological condition. In addition, N3-adenine adducts were also detected and identified from the reaction of calf-thymus DNA with 1-BP and 2-BP at the physiological condition by ESI LC/MS/MS. These results might explain the toxic effect of 1-BP and 2-BP by the formation of N3-adenine adducts formation.

MEDI 337

Analysis of depurination of calf-thymus DNA by the halogenated alkanes at the physiological condition by ESI LC/MS/MS

Jyoti Sherchan*, Jong Geol Kim, Arjun Basnet, Byong Ki Choi, Jung Ki Park, Yurngdong Jahng, Tae Cheon Jeong, and Eung-Seok Lee, College of Pharmacy, Yeungnam University, 214-1 Dae-dong, Kyongsan 712-749, South Korea, Fax: +82-53-810-4654, jyotisherchan@hotmail.com*

Short-chained halogenated alkanes have been used industrially as chemical intermediates, extraction solvents, degreasing compounds, and copolymer cross-linking agents. Members of this chemical class have also been employed as pesticides. Several of the compounds have been reported to be mutagenic and carcinogenic, and to cause acute toxic effects in the kidney, testis and/or liver. In Korea, 2-bromopropane (2-BP) had been widely used as the cleaning solvents in the chemical and electronic industries in place of halons, but 2-BP was found to cause reproductive and hematopoietic disorders in local workers exposed to solvents containing 2-BP. Owing to the toxicity of 2-BP, there has been a growing tendency to use 1-bromopropane (1-BP) as an alternative cleaning solvents to 2-BP. However, 1-BP also has a depressing action on the central nervous system (CNS) and is reported

to be irritating to the skin and eyes of mice. We have previously reported the detection and quantitation of the *N*-7 guanine adduct of 1- and 2-bromopropane by the treatment of 1- or 2-brompopropane to 2'-deoxyguanosine and calf thymus DNA at the physiological condition. In addition, we observed and quantitated the *N*-7 guanine adducts from *in vivo* experiments. We also observed the depurination of purine-based nucleosides by the halogenated alkanes at the physiological condition using HPLC and ESI LC/MS/MS. In this study, we observed the depurination of calf-thymus DNA by the treatment of halogenated alkanes under the physiological condition using ESI LC/MS/MS. In addition, time-response and dose-response effects of depurination of calf-thymus DNA by the halogenated alkanes at the physiological condition were investigated. Depurination of purine-based nucleosides or calf thymus DNA by small chemicals has never been reported. This result might explain that the toxic effects of the halogenated alkanes could be from the depurination as well as adducts formation of the DNA.

MEDI 338

Binding of cationic porphyrins to DNA and their X-ray irradiation

Anila F. Gill[1]*, Doyle Barrow Jr.*[1]*, Tomasz Wasowicz*[2]*, Haben O. Tekeste*[1]*, Dijana Piljak*[1]*, Yoshiko Santoso*[1]*, and Dabney W. Dixon*[1]*. (1) Department of Chemistry, Georgia State University, P.O. Box 4098, Atlanta, GA 30302-4098, Fax: 404-651-1416, agill4@student.gsu.edu, (2) Physics and Astronomy, Georgia State University*

Auger Electron Therapy (AET) involves x-ray irradiation of a metal in close proximity to DNA. The short-range secondary electrons (Auger electrons) are expected to produce localized (clustered) damaged in the DNA, which in turn inhibits replication of the cell. The current study involves cationic metalloporphyrins (derivatives of the tetracationic 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin, TMPyP4) as carriers of the metal atoms. The metals included indium, molybdenum, palladium, ruthenium, silver and zirconium. The amount of clustered DNA damage was quantitated in a plasmid assay. Experiments evaluated the effect of buffer, concentration of glycerol, irradiation time, and concentration of the porphyrin on DNA cleavage. Binding of metalloporphyrins to the DNA was also evaluated using isothermal titration calorimetry.

MEDI 339

Syntheses and DNA interactions of new, bifunctional photosensitizers based on methylene blue

Beth Wilson[1]*, María-José Fernández*[2]*, Antonio Lorente*[2]*, and Kathryn B. Grant*[1]*. (1) Department of Chemistry, Georgia State University, P.O. Box 4098, Atlanta, GA 30302-4098, bwilson1@gsu.edu, (2) Departamento de Química Orgánica, Universidad de Alcalá de Henares*

In photodynamic therapy (PDT), visible light is utilized to selectively activate drugs in diseased tissue, thereby minimizing damage to surrounding healthy cells. Although porphyrin derivatives have been widely employed in PDT, the use of other chromophores, such as phenothiazine and cyanine dyes, remains relatively unexplored. Here we report the syntheses of new, bifunctional photosensitizers in which DNA-cleaving methylene blue (MB) rings are attached to a central, positively charged DNA-binding linker. Thermal melting studies indicate that the new compounds increase the T_m of double-helical DNA by at least 10 ≡C relative to methylene blue. When irradiated at either 676 nm, 700 nm, or 710 nm (wavelengths of light transparent to many biological tissues), our lead compound cleaves DNA more efficiently than MB under near physiological conditions of temperature and pH. To the best of our knowledge, this is the first example of an efficient, bifunctional DNA photosensitizer based on methylene blue.

MEDI 340

Interaction of Ruthenium complexes of Schiff base ligands with DNA

Bhavik Kumar*, Maha Zewail-Foote, and Gulnar Rawji, Chemistry & Biochemistry, Southwestern University, 1001 East University Ave, Georgetown, TX 78626, Fax: 512-863-1696, kumarb@southwestern.edu*

Ru(II) and Ru(III) complexes of Schiff base ligand derived from condensation of 1,2-phenylenediamine with salycaldehyde have been prepared and characterized by NMR, UV-Visible, and IR spectroscopies. The interaction of these complexes with DNA was been investigated using absorbance titrations, and gel electrophoresis. Bathochromic shifts as well as hyperchromism is observed in the UV-Vis spectra indicating interaction between the metal complex and DNA. Furthermore, these studies also reveal that the complexes are effective in cleaving double stranded DNA. In addition to these data, the results of investigations of the mechanism of interaction leading to the cleavage of DNA will be presented.

MEDI 341

Chemical synthesis and stability studies on ribo- and deoxynucleoside *α-P*-borano- and *α-P*-thio-diphosphates

Zhihong Xu*, Department of Chemistry, Duke University, Durham, NC 27708, and B. R. Shaw, Department of Chemistry, Duke university*

Dideoxynucleoside-based inhibitors of reverse transcriptase are the first drugs used in the chemotherapy of AIDS. In order to overcome their inactivity against emerging drug resistance by mutant strains, new classes of nucleoside analogs and nucleotide prodrugs are being developed, which include the modification of one oxygen atom replaced by borane or sulfur group in the *α-P* position of various nucleotides. The specific aim of our study is to synthesize and investigate the chemical stability properties of ribo- and deoxyribonucleoside (rN and dN) *α-P*-borano- and thiodiphosphates. The title compounds were synthesized by our newly developed phosphoramidite approach. Temperature and pH dependent stabilities of diastereomers were investigated in comparison with their parent nucleoside diphosphates. Ribo- and deoxynucleoside *α-P*-modified diphosphates and their parent nucleoside diphosphates exhibited different stabilities in our study. The stabilities of nucleoside *α-P*-boranodiphosphates support the rationale for making similar modifications on clinically used nucleoside drugs and for further investigations of the unique biological properties imparted by the isoelectronic substitution of borane (BH_3) for one of the nonbridging oxygens in the *α-P*-phosphate moiety.

MEDI 341

Chemical synthesis and stability studies on ribo- and deoxynucleoside *α-P*-borano- and *α-P*-thio-diphosphates

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MEDI 342

Novel synthesis of α-*P*-modified nucleoside diphosphates and affinity studies on ADPαB

Ping Li[1]*, Hongyan Liu*[1]*, Mikhail I. Dobrikov*[1]*, Charlotta K. Wennefors*[1]*, Janos Ludwig*[2]*, Zhihong Xu*[1]*, and Barbara R. Shaw*[1]*. (1) Department of Chemistry, Duke University, Box 90346, Durham, NC 27708, (2) N/A*

Nucleoside diphosphates (NDP) and triphosphates are ubiquitous biological molecules and their analogues could have important diagnostic and therapeutic applications. For example, α-*P*-modified phosphorothioate analogues have been well studied and employed to determine enzymatic mechanisms. α-*P*-Modified boranophosphate analogues have recently shown potential applications in antiviral drug research. However, preparation of these biologically active nucleotides, especially the diphosphate analogues, remains a challenge. Here we report a one-pot synthesis of α-*P*-borano- (NDPαB), α-*P*-thio- (NDPαS) and α-*P*-seleno- (NDPαSe) modified nucleoside diphosphate analogues with ethylenediamine in good yields. The absolute configurations of *P*-diastereomers were confirmed by proton NMR. Affinity studies of ADPαB with rabbit muscle creatine and pyruvate kinases are also described.

MEDI 343

Mechanism of inhibition of human neutrophil á defensin 1 by its pro peptide

Guozhang Zou*, Zhibin Wu, and Wuyuan Lu, Institute of Human Virology, University of Maryland Biotechnology Institute, 725 West Lombard Street, Baltimore, MD 21201, zou@umbi.umd.edu*

Human neutrophil á defensins (HNPs) are small, cationic and cysteine-rich antimicrobial peptides that constitute an integral part of the first line of defense against a wide range of invading microbes, including bacteria, fungi, and viruses. Initially synthesized in vivo as inactive precursor proteins (preproHNPs), defensins undergo a series of posttranslational proteolytic processing, leading to their maturation and storage in azurophilic granules. Ample evidence suggests that the 45-residue proHNP1 pro peptide is essential for correct defensin folding and trafficking. Further, the pro peptide specifically interacts with and inhibits the antimicrobial function of HNP1, although the mechanism of inhibition remains poorly understood. To elucidate the molecular basis of the interaction between the pro peptide and HNP1, we chemically synthesized a battery of pro peptide analogs, and biochemically, biophysically as well as functionally characterized their interactions with HNP1. Our findings identify the residues involved in the recognition of HNP1 by its pro peptide and point to a combination of electrostatic and hydrophobic forces dictating the inhibition of a-defensins by their pro peptides.

MEDI 344

Luminally-active, non-absorbable CFTR inhibitors as potential therapy to reduce intestinal fluid loss in cholera

Nitin D. Sonawane*, Jie Hu, Chatchai Muanprasat, and Alan S. Verkman, Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, 1246 Health Sciences East Tower, San Francisco, CA 94143-0521, Fax: 415-665-3847, ndsonawane@yahoo.com*

Enterotoxin-mediated secretory diarrheas such as cholera involve chloride secretion by enterocytes into the intestinal lumen by the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. We previously identified glycine hydrazide CFTR blockers that by electrophysiological studies appeared to block the CFTR anion pore at its lumen-facing surface. Here, we synthesize highly water soluble, non-absorbable malondihydrazides by coupling 2,4-disulfobenzaldehyde, 4-sulfophenylisothiocyante, and PEG moieties to 2-naphthalenylamino-[(3,5-dibromo-2,4-dihydroxyphenyl)methylene]propanedioic-acid-dihydrazide, and aminoacethydrazides by coupling PEG to [(N-2-naphthalenyl)-2-(2-hydroxyethyl)]-glycine-2-[(3,5-dibromo-2,4-dihydroxyphenyl)methylene]hydrazide. Compounds rapidly, fully and reversibly blocked CFTR-mediated chloride current with Ki of 2-8 μM when added to the apical surface of epithelial cell monolayers. Inhibitor-polymer conjugation produced polyvalent CFTR inhibitors with potencies in nanomolar range. Compounds did not pass across Caco-2 monolayers, and were absorbed by <2 %/hr in mouse intestine. Luminally added compounds blocked by > 90% cholera toxin-induced fluid secretion in mouse intestinal loops, without inhibiting intestinal fluid absorption. These orally administered, non-absorbable, non-toxic CFTR inhibitors may reduce intestinal fluid losses in cholera.

MEDI 345

Metal based antiamoebic chemotherapy: Modification of metronidazole by complexation with transition metal ions and biological screening against Entamoeba histolytica

Neelam Bharti *and Shailendra Singh, Department of Medicinal Chemistry, University of Florida, Collage of Pharmacy, Gainesville, FL 32610, neelambh@ufl.edu*

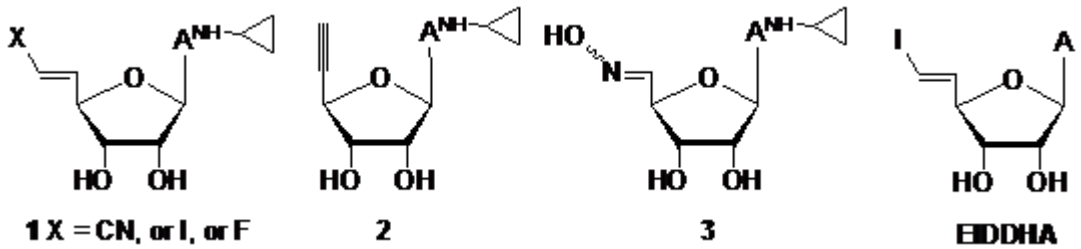
Entamoeba histolytica (E. histolytica), the cause of amoebic dysentery and amoebic liver abscesses, remains a significant threat to health in large parts of the world. More than 50 million people are affected, and responsible for 100000 deaths annully. Metronidazole, drug of choice has several side effects including immunosuppression, mutagenic in bacteria and carcinogenic to rodents. We modified metronidazole by coordination with transition metal ions. Metronidazole-metal complexes were synthesized by direct reaction of free metronidazole with appropriate metal precursors. New complexes were tested in vitro and on golden hamster animal model. These results indicate that efficacy of parent drug molecule increases upon coordination with metal ion. This study establishes the potential for developing new antiamoebic drugs that are more effective and may prove to be useful clinical alternatives to metronidazole.

MEDI 346

Synthesis and antitrypanosomal activity of 6'-(iodohomovinyl)adenosine and related 6-N-cyclopropyladenosine analogs

Magdalena Rapp*, Lucresia Montes, and Stanislaw F. Wnuk, Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, magdrapp1@op.pl*

The novel 6-*N*-cyclopropyladenosine analogues modified at carbon 5' including cyano-, fluoro-, iodo-(homovinyl) **1**, as well as acetylenic **2** and oxime **3** derivatives, which parental adenosine analogues are known inhibitors of AdoHcy hydrolase (e.g., EIDDHA), were prepared using Wittig-type homologations or related chemistry. Thus, Horner/Wittig-treatment of protected 6-*N*-cyclopropyladenosine 5'-aldehyde with a sulfone-stabilized fluorophosphonate reagent followed by stannylodesulfonylation and protiodestannylation sequence yielded the 6'-fluorohomovinyl analogue **1** (X = F). Interaction of these compounds with AdoHcy hydrolase as well as their antitrypanosomal and antiviral activity will be discussed.



MEDI 346

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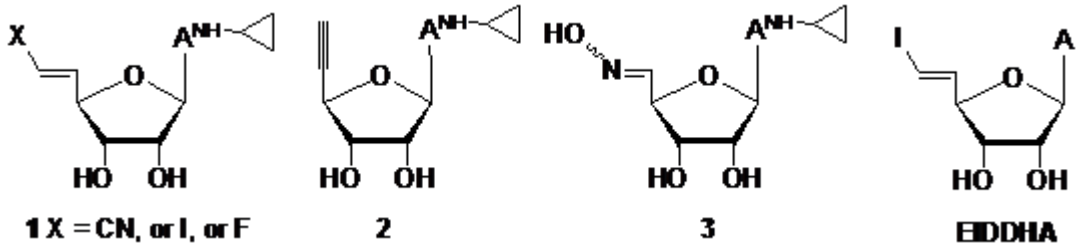


MEDI 347

Antiviral drug ribavirin is a selective inhibitor of S-adenosyl-L-homocysteine hydrolase from *Trypanosoma cruzi*

***Sumin Cai**[1], Qing-shan Li[2], Richard L. Schowen[1], Krzysztof Kuczera[3], and Ronald T. Borchardt[2]. (1) Department of Molecular Biosciences, University of Kansas, 1200 Sunnyside Avenue, Lawrence, KS 66045, sumin@ku.edu, (2) Department of Pharmaceutical Chemistry, University of Kansas, (3) Departments of Chemistry and Molecular Biosciences, University of Kansas*

Ribavirin (1,2,4-triazole-3-carboxamide riboside) is a well known antiviral drug. Ribavirin has also been reported to inhibit human S-adenosyl-L-homocysteine (AdoHcy) hydrolase. This enzyme catalyzes the conversion of AdoHcy to adenosine and Hcy. In this study, we report that ribavirin, which is structurally similar to adenosine, produces time-dependent inactivation of the human and *Trypanosoma cruzi* AdoHcy hydrolases. Ribavirin inactivates the parasite enzyme approx. 5 times faster than the human enzyme. Fluorescence experiments indicate that ribavirin binds to the adenosine-binding site of AdoHcy hydrolase and reduces the NAD^+ cofactor to NADH. Kinetic experiments showed that the human and parasite enzymes have similar affinities for ribaviran. Docking simulations with the MOE program

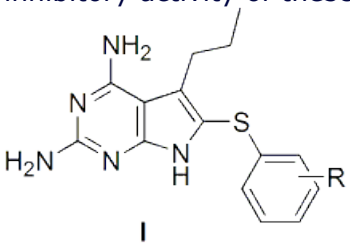
predicted that ribavirin should bind to both the human and parasite enzymes. These results indicate that ribavirin is a promising structural lead for design of even more selective inhibitors of *Trypanosoma cruzi* AdoHcy hydrolase as potential anti-parasitic drugs.

MEDI 348

Nonclassical 5-Alkyl-6-substituted arylthio-pyrrolo[2,3-*d*]pyrimidines as potent and selective Toxoplasma gondii dihydrofolate reductase inhibitors

*Aleem Gangjee[1], **Hiteshkumar D. Jain**[1], Roy L. Kisliuk[2], and Sherry F. Queener[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, hiteshjain_1978@yahoo.com, (2) Department of Chemistry, School of Medicine, Tufts University, (3) Department of Pharmacology and Toxicology, Indiana University*

Infection caused by *Toxoplasma gondii* (*T. gondii*) is responsible for morbidity and mortality in immunocompromised patients. Inhibitory effects of nonclassical 2,4-diamino-5-methyl-6-substituted pyrrolo[2,3-*d*]pyrimidine antifolates against dihydrofolate reductase (DHFR) from *Pneumocystis carinii* and *T. gondii* have been reported by Gangjee *et al*. Several of these analogues were potent and selective inhibitors of DHFR from *T. gondii*. To further explore the effect of 5-alkyl substituent on structure-activity/selectivity relationship, two series (I and II) of 2,4-diamino-5-alkyl-6-arylthiosubstituted pyrrolo[2,3-*d*]pyrimidines were synthesized as an extension of the original 5-methyl series. The synthesis and selective DHFR inhibitory activity of these compounds will be presented and discussed.



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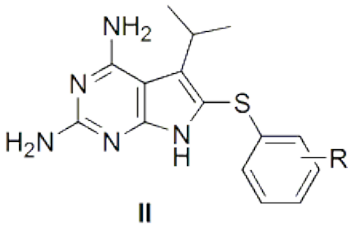

II

MEDI 349

CoMFA analysis of tgDHFR and rlDHFR based on antifolates with 6-5 fused ring system

*Aleem Gangjee and **Xin Lin**, Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, Fax: 412-396-5593, gangjee@duq.edu*

As a continuation of our previous 3D-QSAR study, we developed comparative molecular field analysis (CoMFA) models for the inhibitory activities against *Toxoplasma gondii* (tg) DHFR and rat liver (rl) DHFR, using a data set of 83 structurally diverse bicyclic 6,5-fused ring DHFR inhibitors. In addition, we identified a potential problem associated with the CoMFA cross-validated r^2 guided region selection (q^2-GRS) routine and made a modification to it. For each enzyme, four models were developed using the conventional CoMFA, the all orientation search (AOS) CoMFA, the original q^2-GRS CoMFA and the modified version of q^2-GRS CoMFA. The details of the model development and the modified CoMFA/q^2-GRS routine will be presented.

MEDI 350

Determination of basic bioavailability and other pharmacological properties of substituted benzyl nitrofuranyl amides as novel antituberculosis agents

Rajendra P Tangallapally[1], *Nageshwar R Budha*[2], *Robin E. B. Lee*[1], *Anne J. M. Lenaerts*[3], *Bernd Meibohm*[2], *and Richard E Lee*[4]. *(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 Health Science Center, (3) Department of Microbiology, Colorado State University, (4) Department of Pharmaceutical Sciences, University of Tennessee HSC*

In our efforts to address the continuously rising impact of tuberculosis and multi-drug resistant tuberculosis on the world population, a novel and potent anti-tuberculosis agents of nitrofuranyl amide series have been identified. These compounds exhibits sub micromolar level inhibition of M. tuberculosis in vitro and a detailed structure activity relationship has been successfully developed. In order to advance further, a set of potent compounds were selected for in vivo testing to determine their basic bioavailability in mice, pharmaco kinetics and pharmaco dynamics. The in vivo structure activity relation ship of these compounds and their in vivo - in vitro activities against M. tuberculosis will be presented.

MEDI 350

Determination of basic bioavailability and other pharmacological properties of substituted benzyl nitrofuranyl amides as novel antituberculosis agents

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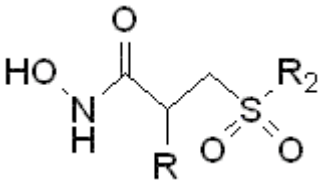
MEDI 351

γ-Sulfone Hydroxamic acid anthrax lethal factor inhibitors: Synthesis and SAR

Seongjin Kim[1], *Alan T. Johnson*[1], *Lynne Cregar*[2], *Sherri Mills*[2], *Anthony Mehok*[1], *Alan McClelland*[2], *Sean O'Malley*[1], *and Cho Tang*[1]. *(1) Department of Chemistry, Hawaii Biotech, Inc, 99-193 Aiea Heights Drive, #200, Aiea, HI 96701, ipf4alpha@yahoo.com, (2) Department of Lead Discovery, Hawaii Biotech, Inc*

Anthrax toxins are in the form of three proteins: protective antigen (PA), lethal factor (LF), and edema factor (EF). Heptamerized PA shuttles LF and EF into an intracellular, endosomal compartment, and LF is released into the cytoplasm to exert toxic effects. LF is a zinc metalloprotease that specifically cleaves mitogen-activated protein kinase kinase (MAPKK) in the macrophage. Because MAPKKs are critical signaling molecules, the cleavage by this protease results in cell death. We have found that γ-sulfone hydroxamic acids are very potent LF inhibitors. We will present

synthesis and SAR for these γ-sulfone hydroxamic acid inhibitors verses anthrax lethal factor.



MEDI 352

Synthesis and biological activity study of new amine cyanoboranes

Khuloud J Takrouri[1]*, Jehoshua Katzhendler*[1]*, and Morris Srebnik*[2]*. (1) Department 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*

A new group of antifungal agents is proposed. It is based on the synthesis of amine cyanoboranes and related structures by novel methodology. A number of structure-antifungal activity relationship (SAR) series were observed according to the structure of the alkyldimethylamine cyanoboranes and carboxyboranes. A long alkyl chain attached to nitrogen of the amine cyanoboranes and carboxyboranes enhances this antifungal activity. In addition to the alkyldimethylamine cyanoboranes, beta-hydroxylalkyldimethylamine cyanoboranes, amine bromocyanoboranes, amine dibromocyanoboranes, amine carboxyboranes, amine bromocarboxyboranes, amine dibromocarboxyboranes, amine fluorocyanoboranes diamine bis-cyanoboranes and diamine bis-carboxyboranes were synthesized and tested. An enhanced activity was also obtained upon halogentaion of the amine cyanoboranes, as well as the presence of C=C double bond at the end of the N-alkyl group. The lead compound was dimethylundecylamine dibromocyanoborane [C11H23N(CH3)2BBr2CN], where MIC values ranged from 39 to 79 micromol/L.

MEDI 353

Tetracycline biosynthesis: Reconstitution of the malonamyl-specific initiation module in a heterologous host

Wenjun Zhang*, Department of Chemical and Biomolecular Engineering, University of California at Los Angeles, 5667 Boelter Hall, 420 Westwood Plaza, Los angeles, CA 90095, wjzhang@ucla.edu, and Yi Tang, Department Chemical Engineering, University of California, Los Angeles*

Engineered biosynthesis of tetracyclines using genetic engineering is an attractive route towards obtaining analogs that can overcome the current modes of antibiotic resistance. Tetracyclines are aromatic polyketides that are biosynthesized by bacterial type II polyketide synthases (PKS). We sequenced the entire gene cluster of oxytetracycline PKS from Streptomyces rimosus. Sequence analysis revealed a total of twenty-one genes that are putatively involved in oxytetracycline (oxy) biosynthesis. One of the distinguishing features of tetracyclines is the presence of an amide unit at one terminus of the polyketide backbone. We elucidated the biosynthesis and incorporation of the malonamate starter unit with a combination of in vivo and in vitro experiments. In vivo reconstitution using Streptomyces coelicolor (CH999) revealed that the asparagine synthase homolog OxyD is necessary and sufficient for the biosynthesis and incorporation of the malonamate starter unit. An amidated polyketide (WJ35) was synthesized as the major product when the oxy minimal PKS, the C9 ketoreductase (OxyJ) and OxyD are coexpressed in CH999. We are also reconstituting the cyclization steps in S. coelicolor and Steptomyces livadans by expressing the minimal oxy PKS, the initiation module and the immediate tailoring

enzymes. During these studies, additional novel, amidated polyketides have been afforded and characterized.

MEDI 354

In silico screening for the identification of new lead inhibitors of NAD synthetase

Whitney B. Moro[1], *Wayne J. Brouillette*[2], *Christie G. Brouillette*[3], *Pamela Pruett*[3], *Chi-Hao Luan*[3], *Steven R. Harville*[1], *and Lawrence J. DeLucas*[3]. *(1) Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294, Fax: 205-934-2543, whitneygail02@yahoo.com, (2) Center for Biophysical Sciences and Engineering, Department of Chemistry, The University of Alabama at Birmingham, (3) Center for Biophysical Sciences and Engineering, The University of Alabama at Birmingham*

Nicotinamide adenine dinucleotide synthetase (NADs) is found in all Gram-positive and Gram-negative bacteria and is essential for vegetative growth as well as for the outgrowth of spore-forming bacteria, like Bacillus anthracis, into the vegetative cell. The simultaneous inhibition of both spore outgrowth and vegetative growth may be particularly effective for antibacterial actions against such bacteria. Our research group has developed tethered dimer inhibitors of NADs, and some possess IC_{50}'s around 10 μM and MIC values below 1 μg/mL for Gram-positives. However, these suffer from a requirement for a permanent positive charge and are of moderately high molecular weight ($\geq$500). In order to identify new lead structural classes of inhibitors that are smaller and do not contain a positive charge, a virtual screening study was undertaken. Using the in silico screening program FlexX (BioSolve IT), over 300,000 commercial compounds were virtually screened against the NADs catalytic site, divided into two overlapping subsites for greater efficiency. Top-scoring ligands were filtered according to several criteria, and over 250 compounds were purchased and screened as both enzyme inhibitors and antibacterial agents. Three distinct structural classes with low micromolar activity were identified. Aspects of these studies involving new lead identification and/or optimization will be presented.

MEDI 355

Preliminary results in bioactivity tests of tropical fungal extracts

Anastacio Emiliano Sosa, Department of Chemistry and Physics, Universidad del Turabo, Carretera No. 189, km. 3.3, Gurabo, PR 00778, Fax: 7877445427, aemilian@suagm.edu, Sharon A. Cantrell, Department of Biology, Universidad del Turabo, ***Carmen E. Bonilla Rivera****, Chemistry and Physics, Universidad del Turabo, Carretera No. 189, km. 3.3, Gurabo, PR 00778, Fax: 7877445427, carmensaeli@yahoo.com, and Zulma Ortiz, Department of Biolofy, Universidad del Turabo*

This Project pursues the isolation of potentially bioactive metabolites from pure cultures of marine and forest tropical fungal strains. The studied fungal strains have been isolated from solar salterns located at Cabo Rojo, PR, and have been identified by morphological and molecular techniques. Selection of fungal strains for cultivation starts with a literature search on the ability of a particular species for the production of bioactive secondary metabolites; followed by a qualitative pre-screening by means of bacterial growth inhibition, using the sensitivity test, as described by Kirby & Bauer. Further screening for bioactivity of fungal strains is performed by Brine Shrimp Lethal Toxicity test (BSLT) of the ethyl acetate extracts from pure cultures. Fourteen species have been tested for bacterial growth inhibition, eight of which have been tested for BSLT. The data obtained for both tests correlates for most tested species. Some of the promising species are being cultured in relatively large amounts to pursue the molecules responsible for the shown bioactivity. Isolated

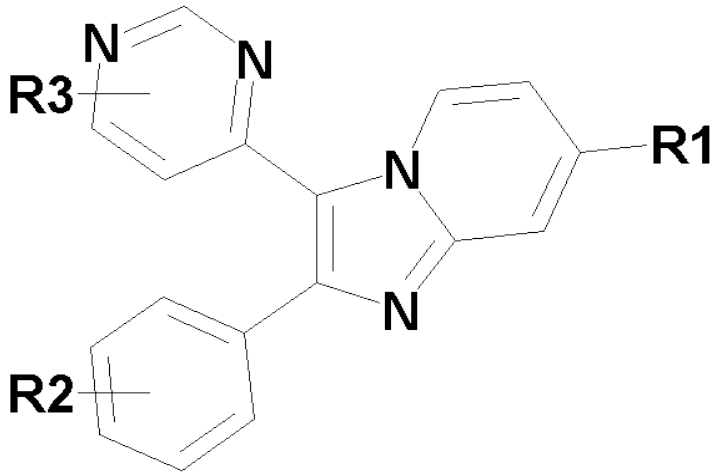
bioactive compounds in this project will be tested against menacing infectious diseases, such as Tuberculosis and Malaria. Funds for this investigation are provided by NIH/UPR though the Alliance for the Advancement of Biomedical Research Excellence in Puerto Rico (PR-AABRE) (Grant No. P20 RR03-010)

MEDI 356

Synthesis and biological activity of imidazopyridine anticoccidial agents

Andrew Scribner[1], *Tesfaye Biftu*[2], *Michael Fisher*[2], *Matthew Wyvratt*[2], *Penny Leavitt*[3], *Paul Liberator*[3], *Anne Gurnett*[3], *Chris Brown*[3], *John Mathew*[3], *Donald Thompson*[3], *Dennis Schmatz*[3], *Richard Dennis*[1], *Jean Hong*[1], *Shuliang Lee*[1], *Don McIntyre*[1], *Gilles Ouvry*[1], *and David Perrey*[1]. *(1) Discovery Chemistry, SCYNEXIS, Inc, P.O. Box 12878, Research Triangle Park, NC 27709-2878, Fax: 919-544-8697, andrew.scribner@scynexis.com, (2) Department of Medicinal Chemistry, Merck Research Laboratories, (3) Department of Human and Animal Infectious Disease Research, Merck Research Laboratories*

Coccidiosis is the major cause of morbidity and mortality in the poultry industry. Protozoan parasites of the genus Eimeria invade the intestinal lining of the avian host causing tissue pathology, poor weight gain, and in some cases mortality. Resistance to current anticoccidials has prompted the search for new therapeutic agents with novel mechanisms of action. Recently, we have reported on novel anticoccidial agents with potent in vitro and in vivo activity against Eimeria. Antiparasitic activity is due to inhibition of a parasite specific cGMP-dependent protein kinase (PKG). In this study, we have focused our efforts on a more potent class of PKG inhibitors that possess a 2-aryl-3-(pyrimidin-4-yl)-7-substituted-imidazopyridine scaffold. The synthesis and biological activity of these compounds will be described.

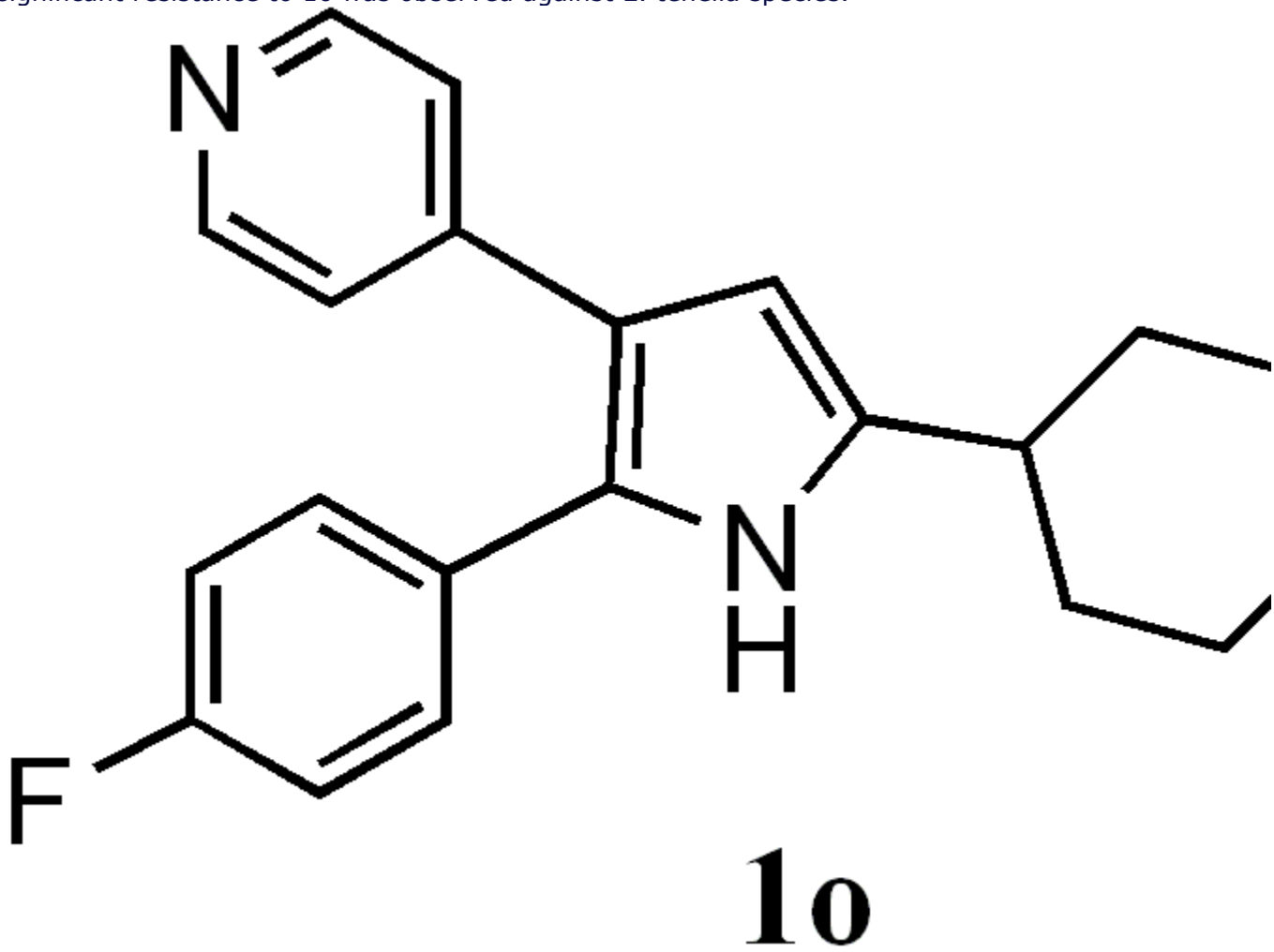


MEDI 357

Synthesis and SAR of 2-aryl 3-(4-pyridyl)-pyrrole derivatives as anticoccidial agents

Danqing D. Feng[1], *Gui-Bai Liang*[1], *Xiaoxia Qian*[1], *Anne Gurnett*[2], *Narindar Girotra*[1], *Paul Liberator*[2], *Paula Dulski*[1], *Penny Leavitt*[2], *Tami Crumley*[1], *Andrew Misura*[1], *Terence Murphy*[1], *Mitree Ponpipom*[1], *Sandra Rattray*[1], *Tamas Tamas*[1], *John Mathew*[2], *Donald Thompson*[2], *Dennis Schmatz*[1], *Michael Fisher*[1], *Mathew Wyvratt*[1], *and Tesfaye Biftu*[3]. *(1) Department of Medicinal Chemistry and Human and Animal Infectious Disease Research, Merck & Co, P.O. Box 2000, Rahway, NJ 08876, (2) Department of Human and Animal Infectious Disease Research, Merck Research Laboratories, (3) Department of Medicinal Chemistry, Merck Research Laboratories*

2-Aryl-3-(4-pyridyl)pyrrole derivatives were discovered as potent Eimeria tenella cGMP-dependent protein kinase (Et-PKG) inhibitors for use as anticoccidial agents in chickens. The 2-position of 3-(4-pyridyl)-5-(N-methylpiperidyl)pyrrole was modified with various aromatic groups to probe SAR at this position. 4-Fluorophenyl substituted pyrrole 1o is potent in the Et-PKG enzyme assay (IC50 = 0.7nM) and is fully active at 75 ppm in chickens in vivo against several Eimeria species. No significant resistance to 1o was observed against E. tenella species.



1o

MEDI 358

Development of isoflavones as antigiardial agents: Design, synthesis and 3-D quantitative structure-activity relationship of a series of substituted isoflavones using comparative molecular field analysis

Nakul S. Telang, *Tomoko Mineno, and Mitchell A. Avery, Department of Medicinal Chemistry and Laboratory for Applied Drug Design and Synthesis, University of Mississippi, 417 Faser Hall, University, MS 38677, Fax: 662-915-5638, ntelang@olemiss.edu*

Giardiasis is one of the major intestinal diseases caused by a pathogenic unicellular protozoan, Giardia lamblia. It has been identified as the major cause of the most frequent waterborne outbreaks infecting 20 – 30% of the population in developing countries and 2 - 5% in United States. At present, metronidazole and furazolidone

are used as the current first line treatment for giardiasis. Recent reports of Giardia resistance against current therapy have necessitated the need to develop new and effective drugs.
In the search to discover a novel and effective lead for the treatment of giardiasis, solution-phase syntheses of a library of isoflavone derivatives has been accomplished and were biologically evaluated for antigiardial activity. The *in vitro* results have been used as the basis for three dimensional quantitative structure-activity relationship (3D-QSAR) study utilizing comparative molecular field analysis (COMFA) procedures. The best resulting COMFA model has cross-validated q^2 and conventional r^2 values of 0.696 and 0.864 respectively with a predicted r^2 value of 0.819 and thus offers important structural insight into designing novel antigiardial agents prior to their synthesis.

MEDI 359
Synthesis and antiprotozoal activity of 1-(4-amidinophenyl)-4-(4-amdinobenzyl) benzenes
*Mohamed A. Ismail[1], Reem K. Arafa[1], **Anuradha Illendula**[1], Reto Brun[2], Tanja Wenzler[2], and David W. Boykin[1]. (1) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303, Fax: 404-651-1416, anuradha@gsu.edu, (2) Swiss Tropical Institute*
Parasitic diseases are a threat to half the world's population. Trypanosomiasis and malaria diseases are of great concern. As part of a program of synthesis of novel aryl diamidines we found that 1-(4-amidinophenyl)-4-(4-amdinobenzyl)benzene exhibited promising in vitro activity against *Plasmodium falciparum* and *Trypanosoma brucei rhodesience* and consequently have under taken a study to expand this lead. The diamindines were obtained from the respective dinitriles through the bis-O-acetoxyamidoxime analogues followed by hydrogenation. Direct coupling of 4-bromobenzyl bromides with two equivalents of 4-cyanophenyl boronic acid under Suzuki conditions gave the dinitriles. N-hydroxyamidine and N-methoxyamidine, potential prodrugs, were also prepared. The N-hydroxyamidines were obtained directly from the dinitriles on reaction with hydroxylamine. Reaction of the N-hydroxyamidines with dimethylsulfate in DMF/LiOH gave the N-methoxyamidines. Dicationic compounds of this class of diamidines demonstrated promising anti-trypanosomal and anti-malarial efficacies *in vitro* giving nanomolar IC_{50} values and providing cures in a *Trypanosoma* mouse model at low dosage.

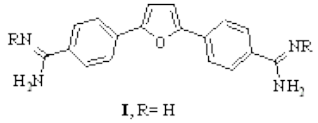
MEDI 360
Efficient synthesis and anti-protozoal evaluation of 2,2'-bichalcophene analogues of furamidine
*Mohamed A. Ismail[1], Ekaterina M. Paliakov[2], David W. Boykin[1], **Chad E. Stephens**[2], Karl A. Werbovetz[3], and Reto Brun[4]. (1) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303, (2) Department of Chemistry, Georgia State University, Atlanta, GA 30303, Fax: 404*

651 1416, cstephe7@aug.edu, (3) College of Pharmacy, Division of Medicinal Chemistry, The Ohio State University, (4) Swiss Tropical Institute
Furamidine (DB75) is a furan-based diamidine with potent acitivity against a number of microorganisms. The methoxime prodrug of furamidine (i.e., DB289) is currently in clinical trials for the treatment of Pneumocystis carinii pneumonia, African sleeping sickness and malaria. As part of an ongoing effort to develop other diamidines with even better antimicrobial activity, we have recently become interested in preparing and studying the bifuran analogues of furamidine, as well as some of the analogous bithiophenes and biselenophenes. In this paper, we describe a new, highly efficient synthesis of such bichalcophene derivatives which involves the homocoupling of 2-bromochalcophene precursors using hexabutylditin and a palladium catalyst. We also report on the preliminary biological results of these new diamidines, which have shown that some of the compounds are more active than furamidine against Leishmania donovani. In addition, some of these compounds also show promising activity against Trypanosoma and Plasmodium species.

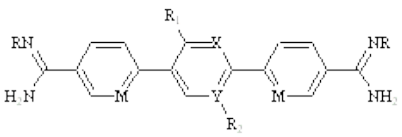
MEDI 361

Synthesis, DNA affinity, and antiprotozoal activity of linear dications: Terphenyl diamidines

*Mohamed A. Ismail[1], **Reem K. Arafa**[2], Reto Brun[3], Tanja Wenzler[3], Yi Miao[2], David Wilson[2], and David W. Boykin[1]. (1) Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303, (2) Department of Chemistry, Georgia State University, Atlanta, GA 30303, Fax: 404-651-1416, (3) Swiss Tropical Institute*
Parasitic diseases continue to pose a great health hazard to people all around the world. Of these, trypanosomiasis and malaria diseases are caused by etiologic micro-organisms belonging to *Trypanosoma* and *Plasmodium* species, respectively. Furamidine (DB75) (**I**), a dicationic diamidine, has shown potent antiparasitic activity against the afore-mentioned species. DB289 (**II**), the methoxyamidine prodrug of DB75, is currently in phase III clinical trials. In a search for novel anti-parasitic agents, a series of linear-terphenyl diamidines and their analogues (**I**) was obtained from the respective dinitriles either by direct reaction using lithium trimethylsilylamide or through the bis-*O*-acetoxyamidoxime followed by hydrogenation in glacial acetic acid. Some dinitriles were obtained from the respective dialdehyde precursors via the oxime intermediates followed by acetic anhydride induced dehydration. Other dinitriles were prepared *via* a Suzuki coupling reaction either employing bis-1,4-phenyleneboronic acid with 4-bromobenzonitrile/or 6-chloronicotinonitrile, or employing 4-cyanophenylboronic acid with 1,4-dibromobenzene and its derivatives. On the other hand the potential methoxy-amidine prodrugs were prepared *via* methylation of the respective diamidoximes with dimethylsulfate in DMF solution and using Li(OH) as a base. Dicationic compounds belonging to this class of linear diamidines have demonstrated promising anti-trypanosomal and anti-malarial efficacies with *in vitro* IC50 values of <5nm and with 4/4 cures of infected mice upon *in vivo* testing in *Trypanosma* mouse model.



I, R= H
II, R= OMe

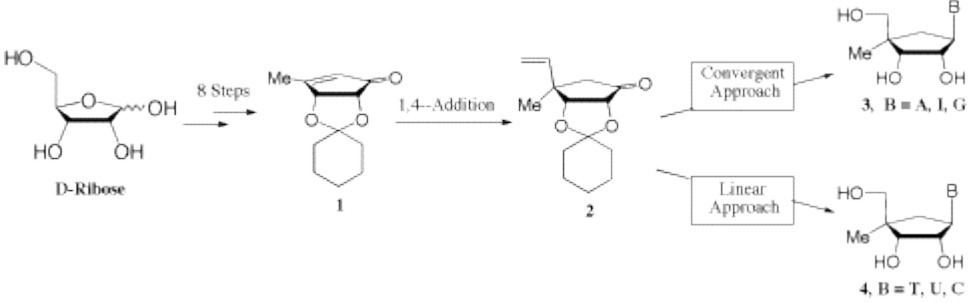


III

R= H, OH, OMe
R_1= H, F, CF_3, OMe
R_2= nil, OMe
X, Y, M= CH, N

MEDI 362
Synthesis of 8-azapurine analogs
*P. Brad Poole, Kevin M. Barley, and **Erland P. Stevens**, Department of Chemistry, Davidson College, PO Box 7120, Davidson, NC 28035, Fax: 704-892-2709, brpoole@davidson.edu, erstevens@davidson.edu*
A novel route to 8-azapurine nucleoside analogs has been developed. This method relies on cycloaddition of an alkyl azide with an enol ether. The resulting 1,2,3-triazole forms the core of the nucleoside. Variation of the alkyl groups on the azide and substituents on the triazole allow access to the 8-azapurine analogs. Our efforts to determine the scope of this chemistry will be discussed.

MEDI 363
Synthesis of 4'-C-methylcarbocyclic nucleosides via conjugate addition of D-4-methylcyclopentenone
***Peng Liu**, Pharmaceutical Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA 30605, Fax: 706-542-5381, lpeng@rx.uga.edu, Raymond F. Schinazi, Medical research 151H, Emory University School of Medicine/VA Medical center, and C. K. Chu, College of Pharmacy, The University of Georgia*
C4'-Substituted nucleosides have shown interesting antiviral activity. Thus, it was of interest to synthesize 4'-substituted carbocyclic nucleoside analogs such as aristeromycin analogs 3, and 4 (Scheme 1). D-4-methyl cyclopentenone 1 was prepared in 8 steps via an oxidative-rearrangement as the key step. Conjugate addition of an organocopper (I) reagent (CH2=CHMgBr/CuBr.Me2S/TMSCl/HMPA) to a,b-unsaturated ketone 1 provided the addition product 2 bearing a quaternary chiral carbon at the C4-position. The intermediate 2 was subsequently condensed with 6-chloropurine or 2-amino-6-chloropurine via SN2 reaction followed a series of functional group transformation and deprotection to furnish the target compound 3. Through a linear approach, pyrimidine bases were built up on the carbocyclic sugar moiety 2, and thus furnished target compound 4. Biological evaluation of the synthesized compounds is in progress. (Supported by NIH AI 56540 and VA).



MEDI 363
Synthesis of 4'-C-methylcarbocyclic nucleosides via conjugate addition of D-4-methylcyclopentenone
***Peng Liu**, Pharmaceutical Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA 30605, Fax: 706-542-5381, lpeng@rx.uga.edu, Raymond F. Schinazi, Medical research 151H, Emory University School of Medicine/VA Medical center, and C. K. Chu, College of Pharmacy, The University of Georgia*
C4'-Substituted nucleosides have shown interesting antiviral activity. Thus, it was of interest to synthesize 4'-substituted carbocyclic nucleoside analogs such as aristeromycin analogs 3, and 4 (Scheme 1). D-4-methyl cyclopentenone 1 was prepared in 8 steps via an oxidative-rearrangement as the key step. Conjugate addition of an organocopper (I) reagent (CH2=CHMgBr/CuBr.Me2S/TMSCl/HMPA) to

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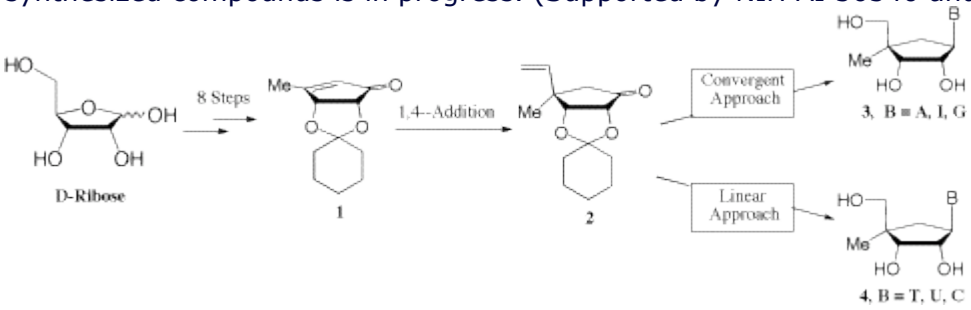


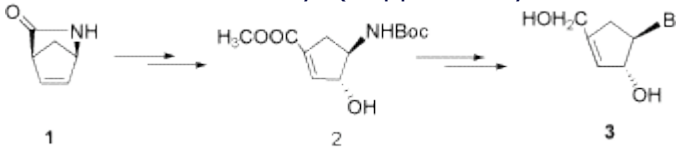
MEDI 364

3'-Deoxy-3',4'-unsaturated carbocyclic nucleosides: Synthesis of neplanocin F analogs

Hongwang Zhang[1]*, Raymond F. Schinazi*[2]*, and C. K. Chu*[1]*. (1) College of Pharmacy, The University of Georgia, Brooks Drive, Athens, GA 30602, Fax: 706-542-5381, hzhang@rx.uga.edu, (2) Emory University School of Medicine/Veterans Affairs, Atlanta, GA, 30033*

A natural carbocyclic nucleoside, neplanocin A exhibits potent antiviral and antitumor activity, however, it is also toxic to normal cells. Through modification, a number of analogues have been synthesized and some of them showed interesting biological activity. Carbovir and its prodrug, abacavir exhibit potent anti-HIV activity and abacavir is being used for the treatment of HIV. Another carbocyclic nucleoside, entecavir has recently been approved by the FDA for the treatment of chronic HBV infection.

As parts of our ongoing drug discovery program for antiviral agents, neplanocin F and its analogs were synthesized. The intermediate **2** was synthesized from the commercially available (±)-**1** as the key building block. From which various target compounds **3** were synthesized. Adenine analogue 3 has moderate activity against HIV-1 with less toxicity. (Supported by NIH AI 32351, AI 056540 and VA).



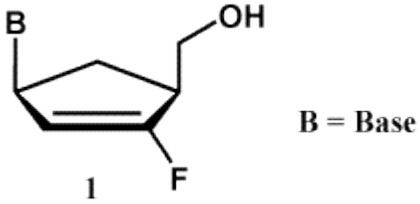
MEDI 365

Synthesis and antiviral activity of L-3'-fluoro-2', 3'-unsaturated carbocyclic nucleosides

Jianing Wang[1]*, Yunho Jin*[1]*, Raymond F. Schinazi*[2]*, and C. K. Chu*[1]*. (1) College of Pharmacy, The University of Georgia, Brooks Drive, Athens, GA 30602, Fax: 706-542-5381, wangj@rx.uga.edu, (2) Emory University School of Medicine/Veterans Affairs, Atlanta, GA, 30033*

Introducing 2'-F substitution on the 2', 3'-unsaturated moiety in carbocyclic nucleosides has provided nucleosides with interesting anti-HIV activity. Hence, it was of interest to synthesize the 3'-fluorine counterparts and evaluate their antiviral activity to further understand the structure-activity relationships (SAR) of these

classes of molecules. Based on this rationale, various pyrimidine and purine L-3'-F-2', 3'-unsaturated carbocyclic nucleosides were synthesized. Although having the similar structure feature, the target compounds **1** could not be obtained using the same synthesis scheme as for the 2'-F isomers. Due to the instability of 3'-F-2',3'-unsaturated elimination products, 3',3'-difluoro-cyclopentanyl alcohols were directly condensed by the Mitsunobu reaction for the purine derivatives. However, pyrimidine derivatives were synthesized by building the heterocyclic moieties. Elimination reactions were conducted in the last step to obtain the target compounds **1**. Antiviral evaluation of the synthesized nucleosides will be presented (Supported by NIH AI 32351 and VA).

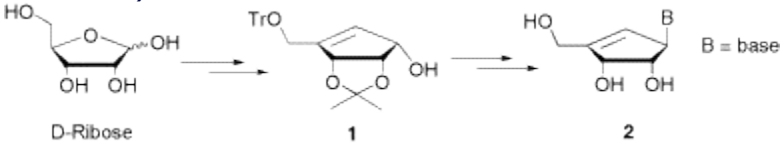


MEDI 366

Enantioselective syntheses and antiviral activity of purine and pyrimidine cyclopentenyl carbocyclic C-nucleosides

Jagadeeshwar R. Rao *and C. K. Chu, College of Pharmacy, The University of Georgia, Brooks Drive, Athens, GA 30602, Fax: 706-542-5381, jrao@rx.uga.edu*

Carbocyclic C-nucleosides possessing the structural features of both carbocyclic nucleosides and C-nucleosides have received relatively little attention, although they are chemically challenging and may possess interesting biological activity. Furthermore, most of the carbocyclic C-nucleosides have been synthesized as racemic mixtures. Therefore, it was of interest to synthesize optically active carbocyclic C-nucleosides, possessing cyclopentenyl moiety as potential analogs of neplanocin. The enantiomerically pure carbocyclic purine and pyrimidine C-nucleosides 2 were synthesized via the key intermediate, 2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-ol (1), which was prepared from D-ribose in 8 steps. Biological screenings are in progress and will be presented (Supported by NIH AI056540).

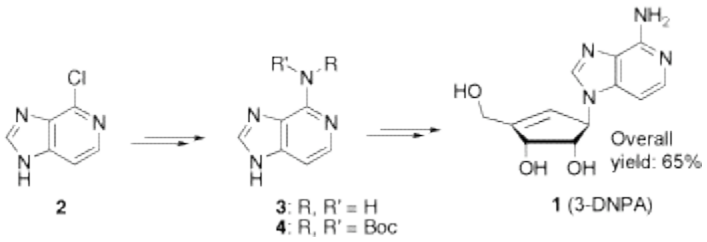


MEDI 367

Development of an efficient synthetic method for 3-deazaadenine using ionic liquid and a practical synthesis of (-)-3-deazaneplanocin A

Jong-Hyun Cho*, Department of Pharmaceutical and Biomedical Sciences, The University of Georgia, College of pharmacy, Athens, GA 30602, Fax: 706-542-5381, jcho@rx.uga.edu, and C. K. Chu, College of Pharmacy, The University of Georgia*

An efficient synthetic methodology for a gram-scale synthesis of 3-deazaneplanocin A (3-DNPA, 1) has been developed. The key intermediate, 3-deazaadenine (3), was prepared from 4-chloroimidazo[4,5-c]pyridine (2) with LiN3 and DMF-[emim]BF4, followed by hydrogenolysis. The ionic liquid was readily recovered by simple filtration and recycled. The practical synthesis of (-)-3-deazaneplanocin A was accomplished by the Mitsunobu reaction of a chiral cyclopentenyl derivative with N6,N6-di-Boc-3-deazaadenine (4) in 65% overall yield from 2 (Supported by NIH AI 056540).

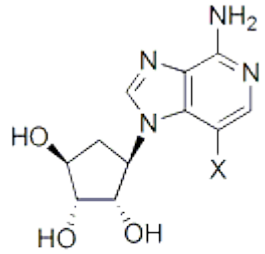


MEDI 368

3-Deaza-3-halo-5'-noraristeromycin

Chong Liu, *Minmin Yang, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, 179 Chemistry Building, Auburn, AL 36849, Fax: 334-844-0239, liuchon@auburn.edu*

3-Deazapurine nucleoside analogs display a wide variety of biological properties. As part of the program investigating 3-deazapurine carbocyclic nucleosides with various substituents at the C-3 position, 3-deaza-5'-noraristromycin derivatives possessing a halo atom (**1**-**3**) at this site have been considered. The syntheses using N-halosuccinimides and protected forms of 5'-noraristeromycin have been accomplished. This and the antiviral properties of these compounds will be reported. This research was supported by funds from DHHS (AI 56540).



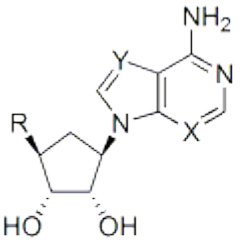
1, X=Cl
2, X=Br
3, X=I

MEDI 369

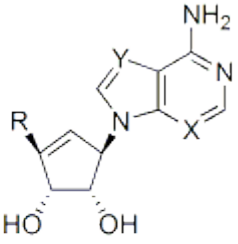
Syntheses and antiviral properties of novel 3,7-dideazanucleoside analogs

Xueqiang Yin, *Department of Chemistry, Auburn university, Chemistry building, auburn, AL 36849, Weikuan Li, Auburn University, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University*

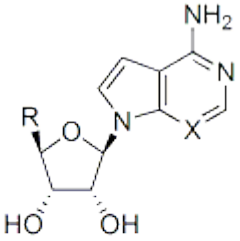
Deazapurine nucleoside analogs have provided significant usefulness in antiviral agent design and biomedical investigations. Both 3-deazaaristeromycin (**1**) and 3-deazaneplanocin (**2**) possess broad-spectrum antiviral properties. Meanwhile, 7-deazaadenosine (**3**, tubercidin) shows potent anti-tumor and antibiotic activities. By combining the structural features of these two structural prototypes, we have investigated several 3,7-dideazapurine carbocyclic nucleosides (**4**-**8**). The syntheses and antiviral properties of these compounds will be reported. This research was supported by funds from DHHS (AI 56540).



1, R=CH_2OH; X=CH;Y=N
4, R=H; X, Y=CH
5, R=CH2OH, X, Y=CH



2, R=CH_2OH; X=CH; Y=N
6, R=CH_2OH; X,Y=CH
7, R=H,; X,Y=CH



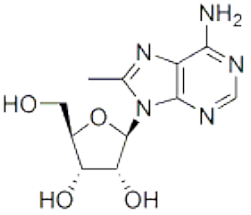
3, R=CH_2OH, X=N, tubercidin
8, R= CH_2OH, X=CH

MEDI 370

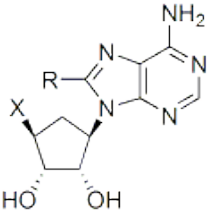
Design and syntheses of 8-substitued carbocyclic nucleoside analogs

Xueqiang Yin[1]*, Wei Ye*[2]*, and Stewart W. Schneller*[2]*. (1) Chemistry, auburn, chemistry Building, auburn, AL 36849, Fax: 334-8440239, (2) Department of Chemistry and Biochemistry, Auburn University*

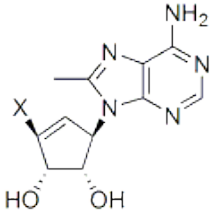
The orientation of the heterocyclic base relative to the sugar moiety, the glycosidic torsion angle, is one of the most important conformational properties of nucleosides. By locking the base in either of the extreme anti or syn conformations, the biological properties of nucleosides are dramatically different. One such example is "syn" 8-methyladenosine (**1**), which has displayed potent anti-poxvirus activity by interfering with DNA processing. Driven by our investigations to find therapeutically useful antiviral agents, a series of 8-substituted carbocyclic nucleoside analogs (**2**-**8** and ent-**3**) were sought. The syntheses and antiviral properties of these compounds will be reported. This research was supported by funds from DHHS (AI 56540).



1



2, X=OH, R=Me
3, X=CH_2OH, R=Me
4, X=H, R=Me
5, X=H, R=CF_3
6, X=H, R=CH_2CH_3



7, X=CH_2OH
8, X=H

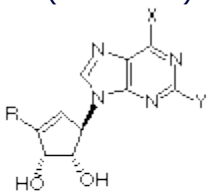
MEDI 371

Highly efficient synthesis and antiviral properties of novel 2-fluoro-5', 5', 5'-trifluoro-5'-deoxyneplanocin A and its guanine analog

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*

The remarkable biological activity displayed by 2-fluoro neplanocin A (**1**) is due to its apparent inhibition of S-adenosyl-L-homocysteine hydrolase (SAHase), but 2-fluoro neplanocin A (**1**) itself is cytotoxic to the host cells because of phosphorylation of its 5' primary hydroxyl group by adenosine kinase and subsequent metabolism by cellular enzymes. In seeking new nucleoside analogs endowed with potent antiviral activity yet with no or much less toxicity, modification at the C-5' position became

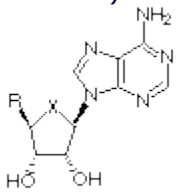
relevant. The trifluoromethyl group is a representative change for consideration. Introducing this to compound **1** brings forth **2**. The synthesis and antiviral activity of **2** and its guanine analog **3** will be presented. This research has been supported by DHHS (AI 56540).



1: R = CH_2OH, X = NH_2, Y = F
2: R = CF_3, X = NH_2, Y = F
3: R = CF_3, X = OH, Y = NH_2

Figure 1

MEDI 372
An expeditious synthesis and biological activity studies of 5'-deoxy-5'-fluoro- and 5'-deoxy-5', 5'-difluoro aristeromycin
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*
Aristeromycin (**1**) has shown broad-spectrum antiviral properties as a consequence of inhibiting S-Adenosyl-L-homocysteine hydrolase (SAHase). However, clinical application of **1** as an antiviral agent has been limited because of its significant cytotoxicity, which has arisen due to the ease of phosphorylation of its 5'-hydroxyl group by adenosine kinase. Fluorinated analogs 5'-deoxy-5'-fluoroadenosine (**2**) and 5'-deoxy-5'-gemdifluoroadenosine (**3**) are also very potent inhibitors of SAHase. In seeking new non-toxic carbocyclic nucleoside analogs based on **1**, **2** and **3** while retaining their potent antiviral properties, fluoro analog **4** and difluoro analog **5** were sought as surrogate compounds for aristeromycin **1**. The preparation and antiviral capabilities of **4** and **5** will be described. This research has been supported by DHHS (AI 56540).

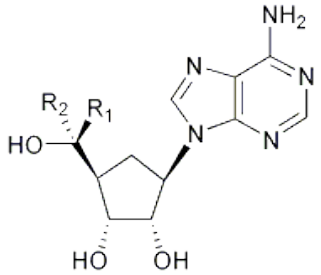


1 : X = CH_2, R = CH_2OH
2 : X = O, R = CH_2F
3 : X = O, R = CHF_2
4 : X = CH_2, R = CH_2F
5 : X = CH_2, R = CHF_2

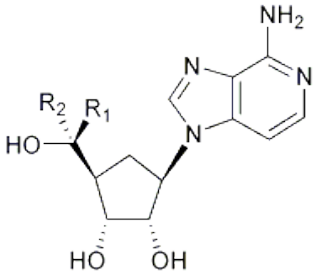
Figure 1

MEDI 373
Synthesis and biological properties of 5′-methyl modified 3-deazaaristeromycin analogs
Wei Ye*, Chong Liu, and Stewart W. Schneller, Department of Chemistry and Biochemistry, Auburn University, Auburn University, AL 36849, yewei01@auburn.edu*
The antiviral potential of aristeromycin (**1**) is limited by its toxicity as a result of metabolism to the 5′-nucleotide derivatives. In seeking ways to limit these undesirable transformations, the 5′-modified analogue 5′-methylaristeromycin, was

considered as a potential AdoHcy hydrolase inhibitor that, because of the steric hindrance at the 5′-center, might not be susceptible to nucleotide formation while retaining the biological properties of aristeromycin. (5′R)-5′-methylaristeromycin (**2**) has shown non-toxic antiviral properties against EBV, HCMV and HCV. Combining the features of 2 and the well known 3-deazaaristeromycin (**3**) led to the desire to prepare and evaluate 5′-methylhomoaristeromycin (**4** and **5**). The synthesis and antiviral activities of enantiopure 5′-methyl-3-deazaaristeromycin (**4**, **5**) will be described. This research was supported by funds from the Department of Health and Human Services (AI56540).



1, $R_1=R_2=H$
2, $R_1=CH_3$; $R_2=H$



3, $R_1=R_2=H$
4 $R_1=CH_3$; $R_2=H$
5 $R_1=H$; $R_2=CH_3$

MEDI 374
Quantitative structure activity relationships (QSAR) of antibacterial fatty acids
Ezekeil H. Hudson II[1]*, Brooke Woodard*[1]*, and Shavon Clark*[2]*. (1) Chemistry, Texas Southern University, 3100 Cleburne, Houston, TX 77004, hudsoneh@tsu.edu, (2) Department of Chemistry, Texas Southern University*
Networking bacteria essentially is the basis of gram-positive bacteria conclusively transmitting communication mechanisms cell-to-cell. This signaling phenomenon is now indisputably known as quorum sensing. In initiating the emergence of bacteria upon rigid surfaces, a quorum must be reached in order to form biofilm. Biofilim will not be achieved until a desired quorum population at large has unanimously reached a critical level. Many chemicals present in bacterial entities will act as signaling derivatives for bacterium. In gram-negative bacteria acylated homoserine lactones (acyl-HSL) are the active signaling derivative. However, in gram-positive bacteria, it is typically found to be fatty acids. A group of fatty acids known to be active in that respect will be used to create a table for structure activity relation ships. SYBYL 7.1 (Tripos, Inc. St. Louis, MO) software will then be used to build structures, achieve energy minimizations, and construct conformational analogs. Comparative molecular field analysis (CoMFA), a module of SYBYL 7.1, will then be used to derive models correlating structure and activity relationships of fatty acids. The outcome of this research will enable us to elucidate the chemical nature and structural requirements for designing novel chemicals that exhibit much more potent activity and selectivity towards bacterial quorum sensing thereby providing a new tool for controlling undesirable biofilm formation.

MEDI 375
One versatile approach towards the Ansamycin antibiotics
Weimin Peng *and Brian S. J. Blagg, Department of Medicinal Chemistry and The Center for Chemical Methodology and Library Development, University of Kansas,*

1251 Wescoe Hall Drive, Malott Hall 4070, Lawrence, KS 66045-7562, wmp100@ku.edu
The ansamycin antibiotics are metacyclophanic macrolactams that possess potent anti-tumor activity. One of the members of this family, geldanamycin, is a lead compound for which more than 20 clinic trials have been initiated. Because total synthesis of this class of compounds is difficult and modifications to improve potency have not been described, we have pursued the development of the combinatorial methodology as a useful approach toward the synthesis of these natural products. The synthesis is highlighted by the preparation of resin-bound triphenyl phosphonium salts, which serve as both a reagent and a traceless linker to afford olefinic products that undergo RCM to afford the macrocyclic skeleton. The synthesis of these compounds will be presented.

MEDI 376

Moronic acid derivatives as novel potent anti-HIV agents

*Yojiro Sakurai[1], **Donglei Yu**[1], Chin-Ho Chen[2], Fang-Rong Chang[1], and Kuo-Hsiung Lee[3]. (1) Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill, 302 Beard Hall, chapel hill, NC 27599-7360, dyu@email.unc.edu, (2) Medical Center, Duke University Medical Center, (3) School of Pharmacy, University of North Carolina at Chapel Hill*
It has been found that the betulinic acid derivative 3-O-(3',3'-dimethylsuccinyl)-betulinic acid (DSB, PA-457, 1) inhibits HIV-1 maturation by interfering with HIV-1 P24/P2 processing, which results in a noninfectious HIV-1 particle. Recently, DSB completed Phase IIa clinical trial and has been classified as the first in a new class of antiretroviral drugs called Maturation Inhibitors (MIs), directed against a novel viral target. In our prior papers, we also reported that the structurally related moronic acid (2), isolated from Brazilian propolis, exhibited significant anti-HIV activity (EC50 <0.1 microgram/mL, TI >186) in H9 lymphocytes. In addition, moronic acid derivatives showed less cytotoxicity than betulinic acid derivatives. This result implies that the toxicity profile could be altered without impairing the anti-HIV potency by changing the betulinic acid core to a moronic acid skeleton. Therefore, in our continuing design, 2 was strategically modified based on the SAR of betulinic acid derivatives. Two moronic acid derivatives 3 and 4 were synthesized to target two critical steps, entry and maturation, in the HIV-1 replication cycle. They were evaluated for inhibitory activity against HIV-1 NL4-3, PI-R and FHR-2 strains in the MT-4 cell line. Compound 4 showed significant anti-HIV activity with EC50 values of 0.0057 micromolar against NL4-3 (a T-cell adapted HIV-1 strain), 0.021 micromolar against PI-R (a multiple protease inhibitor resistant strain), and 0.13 micromolar against FHR-2 (an HIV strain resistant to DSB). Both 3 and 4 showed better antiviral profiles against these strains than DSB. Moreover, introduction of a leucine moiety potentially increases water solubility, and possibly results in better pharmaceutical properties. Compound 4 is a promising a new lead for modification, and further development of 4-related compounds as clinical trial candidates is warranted. [This investigation was supported by Grant AI-33066 from the National Institute of Allergy and Infectious Diseases (NIAID) awarded to K. H. Lee.]

MEDI 377

Structural identification of unknown low level degradants in Reyataz market life stability sample using a multidisciplinary approach

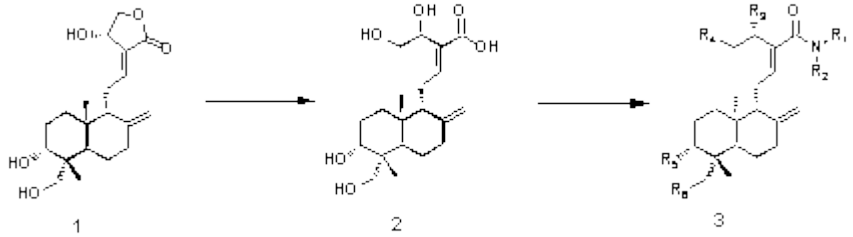
***Qingmei Ye**[1], Yande Huang[1], Amy Bu[1], Richard Gedamke[1], and Venkatapuram Palaniswamy[2]. (1) Analytical Reseach and Development, Bristol-Myers Squibb*

Company, One Squibb Drive, New Brunswick, NJ 08903, qingmei.ye@bms.com, (2) Bristol-Myers Squibb Co
Reyataz (atazanavir sulfate) is the first "once-daily" protease inhibitor approved by the FDA. During the analysis of the 15-month stability samples of the drug product capsules, three unknown degradants, with relative retention times (RRT) 0.75, 1.06 and 1.07 present at levels of 0.11, 0.09 and 0.16 area %, respectively, were observed in the HPLC impurity profile. A multidisciplinary approach using LC/MS, MS/MS, isolation and advanced two-dimensional NMR techniques was used to identify these unknown degradants. Each degradant was isolated using preparative HPLC for full structural characterization. The degradant at RRT 0.75 was identified as a known compound by matching its LC-PDA, LC/MS and LC/MS/MS data with those of the authentic sample. Comprehensive analysis of 1D and 2D NMR, and LC/MS/MS data led to the assignments of two new structures for RRT 1.06 and 1.07 degradants. In this presentation we report the identification of RRT 0.75 degradant, and the isolation and comprehensive spectral characterization of the RRT 1.06 and 1.07 degradants using HPLC, LC/MS, MS/MS, and one and two-dimensional NMR techniques such as 1H, 2D-COSY, 1H-13 C HMQC and 1H-13C HMBC. Mechanism for the formation of these degradants will also be presented.

MEDI 378

Synthesis and structure-activity relationships of novel andrographolide analogs as potent anti-HIV agents

Srinivas Nanduri[1]*, Vijay Kumar Nyavanandi*[1]*, Kiran Kumar Chetluru*[2]*, Sriram Rajagopal*[3]*, Ajaya Kumar Reka*[4]*, Anand K Kondapi*[5]*, Rajagopalan Ramanujam*[4]*, and Javed Iqbal*[2]*. (1) Discovery Chemistry, Dr Reddy's Laboratories Ltd– Discovery Research, Bollaram Road, Miyapur, Hyderabad – 500 050, India, Fax: 91-40-3045438, nandurisrinivas@drreddys.com, (2) Discovery Chemistry, Discovery Research, Dr. Reddy's Laboratories Ltd, (3) Biology, Orchid Chemicals & Pharmaceuticals Ltd, (4) Discovery Biology, Dr Reddy's Laboratories Ltd– Discovery Research, (5) Department of Biochemistry, University of Hyderabad*
Andrographolide (1) is the major diterpenoidal constituent of the medicinal plant Andrographis paniculata (Family: Acanthaceae). The plant extracts and its various constituents are reported to possess a wide array of biological properties. Potent anti-HIV activity was reported for a number of succinoyl esters of 1. In our endeavor to generate a number of novel, structurally diverse and biologically active andrographoliode analogues, we have converted 1 into andrographolic acid 2 quantitatively. Further, 2 was transformed into a number of novel amide derivatives 3 by a series of semi-synthetic conversions. The synthesized analogues were found to possess potent anti-HIV activity. Synthesis and SAR derived will be presented.



MEDI 379

Flap structure and dynamics in HIV-1 protease simulations

Viktor Hornak[1]*, Asim Okur*[2]*, Robert C. Rizzo*[3]*, and Carlos L. Simmerling*[2]*. (1) Center for Structural Biology, Stony Brook University, Stony Brook, NY 11794, Fax: 631-632-1555, viktor.hornak@sunysb.edu, (2) Department of Chemistry, Stony*

Brook University, (3) Department of Applied Mathematics and Statistics, Stony Brook University
We report unrestrained, all-atom molecular dynamics simulations of HIV-1 protease that sample large conformational changes of the active site flaps. In particular, the unliganded protease undergoes multiple conversions between the "closed" and "semi-open" forms observed in crystal structures of inhibitor-bound and unliganded protease, respectively, including reversal of flap "handedness". Simulations in the presence of a cyclic urea inhibitor yield stable closed flaps. Furthermore, we observe several events in which the flaps of the unliganded protease open to a much greater degree than observed in crystal structures and subsequently return to the semi-open state. Our data strongly support the hypothesis that the unliganded protease predominantly populates the semi-open conformation, with closed and fully open structures being a minor component of the overall ensemble. The results also provide a model for the flap opening and closing that is considered to be essential to enzyme function.

MEDI 380

Application of a fuzzy-neural network to predict IC50 values for potential HIV-1 protease inhibitors

Nicholas Salim[1]*, Sarah Abdul-Wahid*[2]*, Catharine J. Collar*[1]*, Levente Fabry-Asztalos*[1]*, and Razvan Andonie*[2]*. (1) Department of Chemistry, Central Washington University, 400 East University Way, Ellensburg, WA 98926-7539, (2) Department of Computer Science, Central Washington University*
Most QSAR studies employ Multiple Linear Regression (MLR) for predicting biological activities for known and novel compounds. Few studies investigated the capability of neural networks for QSAR. We implemented and studied the efficiency of a Fuzzy-Neural Network (FNN) to predict for IC50 values for potential HIV-1 protease inhibitors. One advantage of using FNN is that the knowledge database can be extracted in the form of IF/THEN statements. These statements were analyzed to correlate physicochemical properties of known and potential inhibitory structures and their corresponding biological activities.

MEDI 381

Neuro-fuzzy prediction of biological activity and IF/THEN rule extraction for HIV-1 protease inhibitors

Levente Fabry-Asztalos[1]*, Razvan Andonie*[2]*, Catharine J. Collar*[1]*, Sarah Abdul-Wahid*[2]*, and Nicholas Salim*[1]*. (1) Department of Chemistry, Central Washington University, 400 East University Way, Ellensburg, WA 98926-7539, Fax: 509-963-1050, fabryl@cwu.edu, (2) Department of Computer Science, Central Washington University*
A fuzzy neural network (FNN) and multiple linear regression (MLR) were used to predict biological activities of newly designed HIV-1 protease potential inhibitory compounds. Molecular descriptors of known inhibitors were used to train and test the FNN and to develop MLR models. The predictive ability of these two models was investigated and compared. We found the predictive ability of the FNN to be comparable with that of MLR. The fuzzy IF/THEN rules were extracted from the trained network. These rules map chemical structure descriptors to predicted inhibitory values. The obtained rules can be used to analyze the influence of descriptors. Our results indicate that FNN and fuzzy IF/THEN rules are powerful modeling tools for QSAR studies.

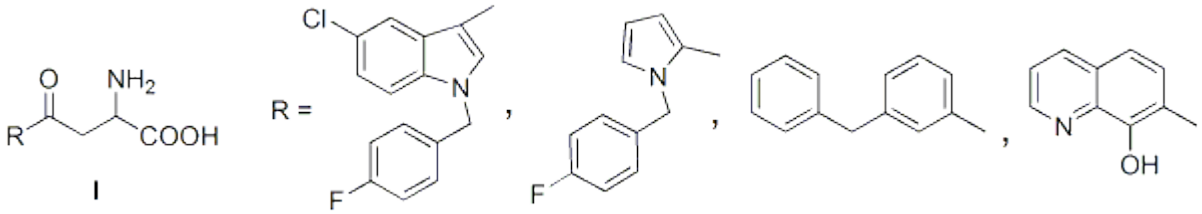
MEDI 382

Design and synthesis of γ-keto-α-amino acid derivatives as Interfacial inhibitors of HIV-1 Integrase

***Srikanth Kolluru**, Department of Medicinal Chemistry and Center for Drug Design, University of Minnesota, 8-125 WDH, 308 Harvard St. SE, Minneapolis, MN 55455, Fax: 612-624-0139, kollu001@umn.edu, and Robert Vince, Department of Medicinal Chemistry, University of Minnesota*

HIV-Integrase (IN) is responsible for the integration of pro-viral DNA into the host genome which is essential step in the viral life cycle. The unique properties of IN and its lack of a cellular homologue makes it an attractive target for drug design. From the various crystal structures of IN, it is evident that DDE motif in the catalytic core domain is coordinated by two divalent metal (Mg^{2+}) ions, rendering its preferential binding to DNA. The X-ray crystal structure IN-Inhibitor (5-ClTEP) complex has also provided a platform for drug design.

Recently, major emphasis by many researchers, is being made in the design of inhibitors which can chelate two metal ions at the active site. We have designed and synthesized γ-keto-α-aminoacid derivatives (I) with two metal binding sites which could act as interfacial inhibitors. Since Magnesium is reported to have selective affinity towards amine functionality compared with other divalent metals, we proposed compounds having free amine group. in vitro screening of these compounds against HIV integrase have shown impressive activities. From the IN inhibition results, potential leads generated are being taken for further optimization.



MEDI 383

Design and analysis of HIV RRE RNA targeting zinc finger proteins

***Subrata H. Mishra**[1], Markus W. Germann[1], and Martyn K. Darby[2]. (1) Department of Chemistry, Georgia State University, 38 Peachtree Ctr Ave, 540 GCB, GSU, Atlanta, GA 30303, Fax: 404-651-1416, smishra3@gsu.edu, (2) National Institute of Environmental Health Sciences*

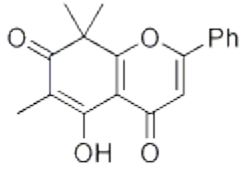
In HIV infected cells, the interaction of Rev Responsive Element (RRE) with the viral protein Rev facilitates nucleo-cytoplasmic viral RNA transport. Successful prevention of this transport can disrupt the viral life cycle. Using phage display techniques, zinc finger proteins were generated to bind specifically to RREIIB, the high affinity Rev binding site. Mutations were designed on these zinc finger proteins to determine contribution of individual amino acid side chains by evaluating their affinities to the RNA. Binding of the zinc finger proteins was localized to the same region on the RNA that the Rev protein utilizes. The RRE-zinc finger binding was found dependent on the zinc finger structure as chelation of the zinc resulted in their dissociation from the RNA. The structure of the RNA-protein complex has been studied by conventional NMR methodology to provide further insight into RNA-protein interactions and refine RNA binding activity of these proteins.

MEDI 384

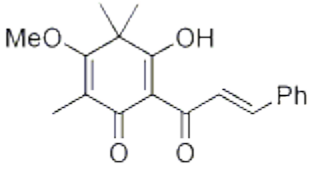
Design, syntheses, and SAR of flavonoids from *Desmos dumosus*: Exploration of potent anti-tumor and anti-HIV agents

Kyoko N. Goto[1]*, Chieh-Yu Peng*[1]*, Tzu-Hsuan Chen*[1]*, Kenneth F. Bastow*[1]*, Chin-Chung Wu*[1]*, Jin-Hong Wu*[2]*, and Kuo-Hsiung Lee*[1]*. (1) School of Pharmacy, University of North Carolina at Chapel Hill, CB# 7360, Beard Hall, Chapel Hill, NC 27599, goto@email.unc.edu, (2) Department of Pharmacy, Hospital of PLA*

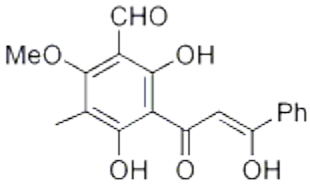
Three flavonoids, desmosdumotin B (**1**), desmosdumotin C (**2**) and 2-methoxy-3-methyl-4,6-dihydroxy-5-(3'-hydroxy)cinnamoylbenzaldehyde (**3**), which were isolated from *Desmos* spp, showed unique *in vitro* cytotoxic activity or anti-HIV effects. To verify structural characterization, we accomplished the first total syntheses of these compounds. To explore structure activity relationships (SAR) and optimize activity, related derivatives were also synthesized and evaluated as in vitro inhibitors of human cancer cell growth or HIV-1 replication in H9 lymphocytes. Notably, synthetic **1** showed significant and selective cytotoxic activity against a multi-drug resistant cell line, and the 4-bromophenyl analog of **2** displayed potent cytotoxic activity against four different tumor cell lines. We will discuss the synthetic methodology as well as the anti-tumor and anti-HIV SAR findings. (Aided by NIH grants CA17625 and AI33066 awarded to KHL)



1: Desmosdumotin B



2: Desmosdumotin C



3: 2-Methoxy-3-methyl-4,6-dihydroxy-5-(3'-hydroxy)cinnamoylbenzaldehyde

MEDI 385

The discovery of a novel orally available CCR5 antagonist, as a therapeutic for HIV-1 infection

Rena Nishizawa Nogi[1]*, Toshihiko Nishiyama*[1]*, Katsuya Hisaichi*[1]*, Chiaki Minamoto*[1]*, Naoki Matsunaga*[1]*, Keisuke Hirai*[1]*, Hiromu Habashita*[1]*, Yoshikazu Takaoka*[1]*, Masaaki Toda*[1]*, Eiji Takahashi*[2]*, Haruo Imawaka*[2]*, Kenji Sagawa*[3]*, Shiro Shibayama*[3]*, Daikichi Fukushima*[3]*, Kenji Maeda*[4]*, and Hiroaki Mitsuya*[4]*. (1) Department of Medicinal Chemistry, ONO PHARMACEUTICAL CO.,LTD, 3-1-1 Sakurai, Shimamoto, Mishima, Osaka 618-8585, Japan, (2) Pharmacokinetic Research Laboratories, ONO PHARMACEUTICAL CO.,LTD, (3) Exploratory Research Laboratories, ONO PHARMACEUTICAL CO.,LTD, (4) Experimental Retrovirology Section, Medicine Branch, National Cancer Institute*

CCR5 represents a member of G protein-coupled, seven-transmembrane segment receptors (GPCRs), which comprise the largest superfamily of proteins in the body. In 1996, it was revealed that CCR5 serves as one of the two essential co-receptors for HIV-1 entry to human CD4+ cells, thereby serving as an attractive target for possible intervention of HIV-1 infection. We have attempted to develop low molecular weight CCR5 antagonists, which blocked HIV-1 from binding to CCR5 and exerted potent anti-HIV-1 activity. Here, we illustrate our optimization efforts to improve both anti-HIV-1 activity and pharmacokinetics, starting from a structurally novel chemical lead “spirodiketopiperazine” which was discovered among the newly designed combinatorial library targeting GPCRs. In our optimization process, we achieved two substantial improvements. First, we conducted hydroxyl group introduction, based on the nature of the metabolites of the lead compound, resulting in enhanced antiviral activity to an unanticipated extent. Secondly, we introduced acidic functionality into the other part of the lead compound, which greatly improved oral bioavailability. Combination of these two means conferred potent anti-HIV-1 activity and favorable oral bioavailability upon the lead compound. Thus generated

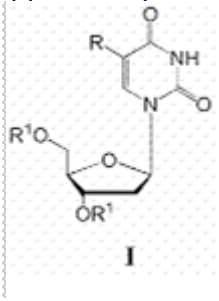
ONO-4128/873140 proved to non-covalently bind to CCR5 highly tightly, a nature with which one can expect its persistent CCR5 occupancy following its clearance from plasma. This biological profile is expected to explain a long-lasting in vivo effect after its disappearance from plasma.

MEDI 386

A novel orthopoxvirus antiviral: 5-(dimethoxymethyl)-2'-deoxyuridine

Xuesen Fan[1]*, Xinying Zhang*[1]*, Longhu Zhou*[1]*, Kathy A. Keith*[2]*, Earl R. Kern*[2]*, and Paul F. Torrence*[1]*. (1) Department of Chemistry & Biochemistry, Northern Arizona University, Building 20, Room 125, Humphreys & Dupont, Flagstaff, AZ 86011, Xuesen.fan@nau.edu, (2) Department of Pediatrics, University of Alabama School of Medicine*

That undeclared stocks of variola (smallpox) virus might be used as a bioterrorist weapon have made it imperative that antivirals be developed against this threat. We report a novel 5-substituted pyrimidine nucleoside with potent in vitro activity against two representative orthopoxviruses, vaccinia virus (VV) and cowpox virus (CV). Acetals of 5-formyl-2'-deoxyuridine (**I**, R: -CHO, R^1: -H) and corresponding 3', 5'-diacetates were prepared from the 3', 5'-diacetate of 5- formyl -2'-deoxyuridine (**I**, R: -CHO, R^1: -$COCH_3$) which was converted to the corresponding protected acetals (R: -$CH(OCH_3)_2$, -$CH(OC_2H_5)_2$, -$CH(OCH_2CH_2O)$, -$CH(OCH_2CH_2CH_2O)$; R^1: -$COCH_3$) by reacting with the appropriate alcohols (CH_3OH, C_2H_5OH, $HOCH_2CH_2OH$, $HOCH_2CH_2CH_2OH$). Each of these was deprotected to the free acetal by treating with NH_3/CH_3OH. In plaque reduction assays, compound **I** (R = -$CH(OCH_3)_2$, R^1= H) reduced VV replication by 50% at 8.9 μM and reduced CV replication by 50% at 4.4 μM whereas Cidofovir effected 50% reduction at 27 and 41 μM, respectively. (Supported by USAMRAA DAMD17-03-C-0081.)



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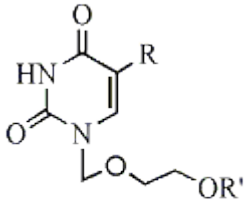
MEDI 387

Synthesis and biological activity of an acyclic analog of a novel orthopoxvirus antiviral

Xinying Zhang[1]*, Xuesen Fan*[1]*, Kathy A. Keith*[2]*, Earl R. Kern*[2]*, and Paul F. Torrence*[1]*. (1) Department of Chemistry & Biochemistry, Northern Arizona University, Building 20, Room 125, Humphreys & Dupont, Flagstaff, AZ 86011, xinying.zhang@nau.edu, (2) Department of Pediatrics, University of Alabama School of Medicine*

The bioterrorist threat of reintroduction of variola (smallpox) virus mandates the discovery of novel anti-orthpoxvirus agents. Our recent discovery of potent in vitro anti-orthopoxvirus activity of 5-(dimethoxymethyl)-2'-deoxyuridine prompted us to synthesize an acyclic analogue for antiviral evaluation. Compound **I** (R= -CH_3, R'= -$COCH_3$) was prepared by condensing trimethylsilylated thymine and (2-acetoxyethoxy)methyl bromide. The resulting 1-[(2-acetoxyethoxy)methyl]-thymine was oxidized by $K_2S_2O_8$ in the presence of $CuSO_4$ and 2, 6-lutidine in aqueous CH_3CN to give the 1-[(2-acetoxyethoxy)methyl]-5-formyluracil **I** (R= -CHO, R'= -$COCH_3$) in 28% yield. In turn, the formyl compound was converted to the acetylated

dimethylacetal in 98% yield by reacting with methanol. The resulting compound **I** (R= -$CH(OCH_3)_2$, R'= -$COCH_3$) was then deprotected by NH_3/CH_3OH to the final product 1-(2-hydroxyethoxymethyl)-5-(dimethoxymethyl)uracil (I, R= -$CH(OCH_3)_2$, R'= -OH) in 97% yield. Compound **I** (R = -$CH(OCH_3)_2$, R'= -OH) proved inactive against both vaccinia virus and cowpox viruses. (Supported by USAMRAA DAMD 17-



I

03-C-0081.)

MEDI 388
Antiviral activity of nucleoside analogs against SARS-Coronavirus (SARS-CoV)
*C. K. Chu[1], **S. Gadthula**[1], Xin Chen[1], H. Choo[1], D. L. Barnard[2], and R. W. Sidwell[2]. (1) College of Pharmacy, The University of Georgia, Brooks Drive, Athens, GA 30602, Fax: 706-542-5381, dchu@rx.uga.edu, sgadthul@rx.uga.edu, (2) Institute for antiviral research, Utah State University*
Severe acute respiratory syndrome (SARS) is a new form of non-typical pneumonia, which is caused by a new member of the coronaviridae family, the SARS-coronavirus (SARS-Cov). To date, there are no effective therapies for the treatment of SARS. Therefore, intensive efforts are being made throughout the world to discover clinically effective antiviral agents. Although corticosteroids, antibiotics and antiviral agents have been used empirically for the treatment of this disease, these agents have not been demonstrated with reasonable assurance to have clinical efficacy. As a synthetic nucleoside, ribavirin has been studied in combination with corticosteroids and interferon-b for the treatment of SARS. It was of interest to evaluate the antiviral activity of a series of nucleoside analogues against SARS-CoV in vitro. We have evaluated a wide variety of such analogs that have been synthesized in our laboratory against this virus. Among the compounds we evaluated, some nucleosides displayed moderate anti-SARS activity. Structure-activity relationships will be presented (Supported by NIH UO19AI056540 and NO1-AI-30048).

MEDI 389
Design and synthesis of dipeptidyl glutaminyl fluoromethylketones as potent SARS-CoV inhibitors
*Han Zhong Zhang[1], William Kemnitzer[2], Hong Zhang[2], Ben Tseng[2], Jindrich Cinatl Jr[3], Martin Michaelis[3], Hans Wilhelm Doerr[3], and **Sui Xiong Cai**[1]. (1) Maxim Pharmaceuticals inc, 6650 Nancy Ridge Drive, San Diego, CA 92121, Fax: 858-202-4000, hzhang@maxim.com, scai@maxim.com, (2) Maxim Pharmaceuticals Inc, (3) Institute for Medical Virology, Clinics of the Johann Wolfgang Goethe-University*
The causative pathogen of Severe Acute Respiratory Syndrome (SARS) has been identified as a novel coronavirus (SARS-CoV), which is known to encode a chymotrypsin-like main protease Mpro that is essential for viral replication. We have been working in the design and synthesis of inhibitors of caspases, which is a class of cysteine proteases that play a critical role in apoptosis. Since SARS-CoV Mpro is also a cysteine protease, we have applied our knowledge from our studies of caspase inhibitors for the discovery of SARS-CoV inhibitors. We have identified several

compounds, such as Z-Leu-Gln(NMe2)-fmk (6a), as potent inhibitors of SARS-CoV, protecting cells against SARS-CoV induced cell death in Vero cells with EC50 value of ~2 mM, and exhibiting a selectivity index (SI) of > 40. Herein we will report the design, synthesis, and SAR of a series of dipeptidyl glutaminyl fluoromethylketones as novel inhibitors of SARS-CoV.

MEDI 390

Identification of pyrazolo[3,4-d]pyrimidines as inhibitors of a hepatitis C virus replicon system

Michael C Barnes[1], *Dagmar Alber*[2], *Richard M Angell*[1], *G Stuart Cockerill*[3], *Emma C Goulding*[2], *Stephen J Griffiths*[2], *Amanda J Hallott*[2], *Raquel Lazaro*[2], *James A Lumley*[1], *Neil Mathews*[1], *Ken Powell*[2], *and Karen Reynolds*[1]. *(1) Department of Chemistry, Arrow Therapeutics Ltd, 7 Trinity Street, London SE1 1DB, United Kingdom, Fax: +44 (0)207 0151020, mbarnes@arrowt.co.uk, (2) Virology, Arrow Therapeutics, (3) Research Director, Arrow Therapeutics*

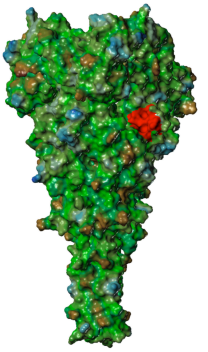
Hepatitis C Virus (HCV) infection is a major cause of chronic liver disease, and acute hepatitis. An estimated 170 million people are infected globally, with 3 to 4 million newly infected each year. There is as yet no vaccine available, and current treatment consists of subcutaneous alpha-interferon or PEGylated alpha-interferon alone or in combination with oral ribavirin. In this presentation we give an insight into the structure-activity relationship of a novel series of HCV replicon inhibitors, 1-aryl-4-aminopyrazolopyrimidines and their derivatives. We took initial screening hits from our in-house library and diversified the substitution around the 1-aryl ring. We demonstrated improved activity from our initial hits and devised a shortened synthetic route from those previously published. Of the compounds presented, A-890 showed the best activity-toxicity profile with a low micromolar IC50 and a TD50 >50 micromolar. Activity of the compounds was determined in Huh7 cells containing a 1b replicon construct.

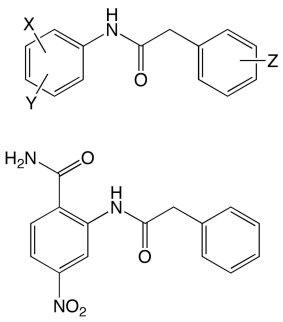
MEDI 391

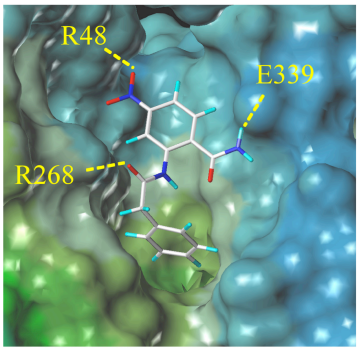
Rational design of measles virus entry inhibitors and mechanisms of viral resistance

Andrew J. Prussia[1], *Richard K. Plemper*[2], *Aiming Sun*[1], *Joshua Doyle*[2], *Laura K. White*[2], *Li-Ting Cheng*[2], *Dennis C. Liotta*[1], *James P. Snyder*[1], *and Richard W. Compans*[2]. *(1) Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, aprussi@emory.edu, (2) Dept. of Microbiology & Immunology, School of Medicine, Emory University*

We have previously reported a panel of small-molecule measles virus (MV) entry inhibitors which show therapeutic promise, although the lead compound was found unstable under physiological conditions. Using the lead molecule as a template, we have designed and tested analogs predicted to favorably interact with the proposed binding site on MV fusion protein (MV-F) resulting in a stable entry-inhibitor of MV (1.0μM IC50). Exposure of this stable inhibitor to MV caused resistance-conferring double mutations in MV-F at residues 94 and 462 located at the proposed binding site and the distal six helix bundle (6HB), respectively. These mutations decrease the transport competence of MV-F. By molecular dynamics and peptide inhibition, we found these mutations destabilize the 6HB. Transport competence can be restored by incubation at lower temperatures or in the presence of inhibitor, suggesting inhibition is due to over-stabilization of the pre-fusion form of MV-F.







MEDI 392

Synthesis of acyclic and cyclic nucleosides and bis-intercalating agents with potential anticancer or antiviral activity

*Cyril Parkanyi and **Yuxiang Zhao**, Department of Chemistry and Biochemistry, Florida Atlantic University, 777 Glades Road, P.O. Box 3091, Boca Raton, FL 33431-0991, Fax: 561-297-2759, parkanyi@fau.edu, yzha5292@yahoo.com*

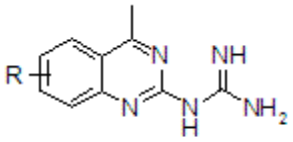
Within the framework of our continuing studies of heterocyclic anticancer and antiviral agents, three groups of compounds will be discussed. The first four compounds, 5-amino-2H-1,2,4-thiadiazol-3-one, 5-amino-3H-1,3,4-thiadiazol-2-one, 2H,4H-1,2,4-thiadiazole-3,5-dione, and 3H,4H-1,3,4-thiadiazole-2,5-dione can be considered as potential antimetabolites and the thiadiazole analogs of cytosine and uracil, respectively. This comparison is based on the well-known analogy between a -CH=CH- group in aromatic hydrocarbons and heterocycles and a bivalent sulfur, -S-, in sulfur-containing aromatic heterocycles. The second group to be discussed are the acyclic and cyclic nucleosides of the above thiadiazoles. The acyclic nucleoside analogs contain an aliphatic chain with two hydroxy groups, the cyclic nucleosides a 2-tetrahydrofuryl group. The last group are bis-intercalating compounds with an aminochloropyrimidine ring at each end and a flexible connecting chain consisting of a varying number of methylene groups. Two of the bis-intercalating agents displayed a significant in vitro activity in tests carried out at the National Cancer Institute and further testing is in progress.

MEDI 393

2-(Quinazolin-2-ylamino)-1,4,5,6-tetrahydro-pyrimidines as bacterial DNA primase inhibitors and potential novel antibacterial agents

***Xicheng Sun**, Sarah Strong, Urs Ochsner, Teresa Hoang, Kimberley Stone, Casey Young, Ian Critchley, Jennifer Bertino, Louis Green, Glenn Sanders, Thale Jarvis, Nebojsa Janjic, and Joseph Guiles, Replidyne, Inc, 1450 Infinite Dr, Louisville, CO 80027, xsun@replidyne.com*

Bacterial DNA primase is an enzyme that catalyses the synthesis of RNA primers on a DNA template during replication and it is proven to be essential in all bacteria. In addition, the significant structural differences between mammalian and bacterial primases make it a good target for discovery of novel antibacterials. During our high throughput screening campaign, a number of 2-(quinazolin-2-ylamino)-guanidines (I) have been identified as potent primase inhibitors, but demonstrated lack of specificity with respect to the eukaryotic enzyme. Optimization of these hits has lead to a series of cyclic guanidines, 2-(quinazolin-2-ylamino)-1,4,5,6-tetrahydro-pyrimidines (II) as potent primase inhibitors with antibacterial activity of MIC = 4 - 64 mcg/ml.



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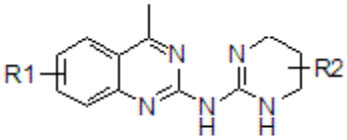


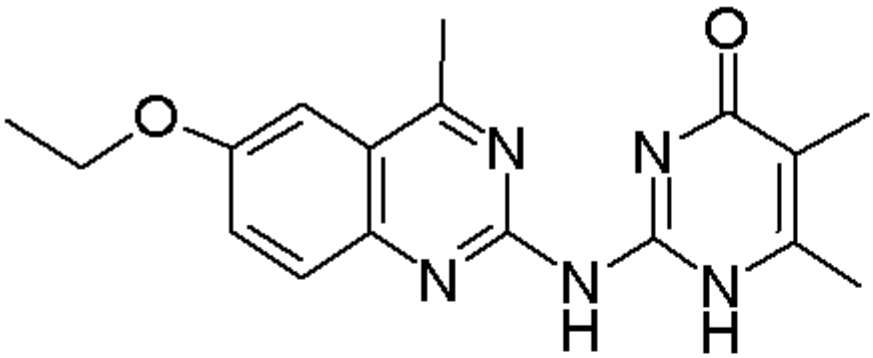
II

MEDI 394

2-(Quinazolin-2-ylamino)-pyrimidinones as bacterial DNA polymerase inhibitors and potential novel antibacterial agents

Sarah Strong*, Xicheng Sun, Urs Ochsner, Casey Young, Kimberley Stone, Teresa Hoang, Ian Critchley, Jennifer Bertino, Louis Green, Thale Jarvis, Nebojsa Janjic, and Joseph Guiles, Replidyne, Inc, 1450 Infinite Drive, Louisville, CO 80027, sstrong@replidyne.com*

DNA replication is among the most vital of cellular processes and has been proven to be essential to all bacteria. Replication of DNA requires the highly coordinated action of multiple proteins that assemble into integrated enzyme systems (the DNA polymerases). In addition, significant differences exist between mammalian and bacterial replication proteins. These factors make the bacterial DNA polymerase "machine" a good target for the discovery of novel antibacterials. During our high throughput screening campaign, 2-(quinazolin-2-ylamino)-pyrimidinone (I) was identified as a polymerase inhibitor. Optimization of this hit has led to a set of potent polymerase inhibitors (II) with MIC values of 1-64 µg/ml in relevant pathogens. The synthesis, structure-activity-relationship and biological activities of this series will be presented.



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MEDI 395

Tracking bacterial growth in a bryostatin microbial broth

*Jason Geddings[1], Tucker Irwin[1], **Thomas Manning**[1], Giso Abadi[2], Dennis Phillips[3], Jim Nienow[1], Lyn Noble[2], and Paul Groundwater[2]. (1) Department of Chemistry, Valdosta State University, 1500 Patterson, Valdosta, GA 31698, Fax: 229-333-7389, jmgeddin@valdosta.edu, tmanning@valdosta.edu, (2) Chemistry and Pharmacy, Sunderland University, (3) Chemistry, University of Georgia*

It is believed that a symbiotic bacterium produces the marine natural product bryostatin. Marine bacteria are notoriously difficult/impossible to grow in a lab setting. The symbiotic bacteria that are believed to produce bryostatin has, to the best of our knowledge, been raised or cultured in a lab setting. In this presentation we have developed a novel approach to raising these bacteria in a lab setting. We monitor the bacterial growth with a Scanning electron MICROSCOPE and bryostatin production via UV/VIs and various types of mass spectrometry. Results to date will be presented on using this approach as a method of mass production for bryostatin. Below is a SEM image of a bacterial colony that has produced bryostatin.

MEDI 396

Therapeutic opportunities for targeting biofilms: Computational studies of the EPS matrix

Hoda Abdel-Aal *and Richard A Bryce, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom, hoda.abdel-aal@manchester.ac.uk*

A more detailed understanding of the complex interactions and properties of the extracellular polymeric substances (EPS) matrix of microorganisms in biofilms offers new opportunities for anti-infective design. Here, we develop a computational model of alginate components of the EPS. We perform parametrization of a molecular mechanical force field against high level quantum mechanical calculations. The model is subsequently validated using available experimental data. Based on molecular simulations, we then obtain structure-property relationships for the alginate components, considering implications for protein-carbohydrate interactions and enzyme activity in the EPS.

MEDI 397

Inhibitors of host-pathogen interactions

Cristina Fernandez-Perez *and Robert J. Kerns, Division of Medicinal and Natural Products Chemistry, University of Iowa, College of Pharmacy, 115 S. Grand Ave., Iowa City, IA 52242, Fax: 319-335-8766*

Heparan sulfate (HS) is a highly charged polyanionic polysaccharide located on the surface and in the extracellular matrix (ECM) of mammalian cells. HS plays a critical role in binding and/or invasion of host cells by many pathogens by serving as cell surface receptors or co-receptors for pathogenic surface proteins, thus aiding in host cell invasion. The identification of molecules, including polyanionic saccharides that inhibit HS-mediated host-pathogen interactions has revealed the enormous therapeutic potential of blocking HS-mediated host pathogen interactions. In the work presented here, a library of structurally diverse *N*-desulfonated/*N*-acylated and carboxyl-modified non-anticoagulant heparin derivatives was evaluated for binding to the HS binding sites of HIV gp120, HIV Tat and *Plasmodium falciparum* circumsporozoite protein. The structural requirements for certain library members to possess increased affinity and selectivity for these pathogen proteins over parent heparin and other polyanionic saccharides will be discussed.

MEDI 398

2,5-Dideoxystreptamine derivatives: The first synthetic small molecule inhibitors of furin

Guan-Sheng Jiao[1]*, Cho Tang*[1]*, Sean O'Malley*[1]*, Jason Larson*[2]*, Gary Thomas*[2]*, and Alan T. Johnson*[1]*. (1) Department of Chemistry, Hawaii Biotech, Inc, 99-193 Aiea Heights Drive, #200, Aiea, HI 96701, gjiao@hibiotech.com, (2) The Vollum Institute, Oregon Health Sciences University*

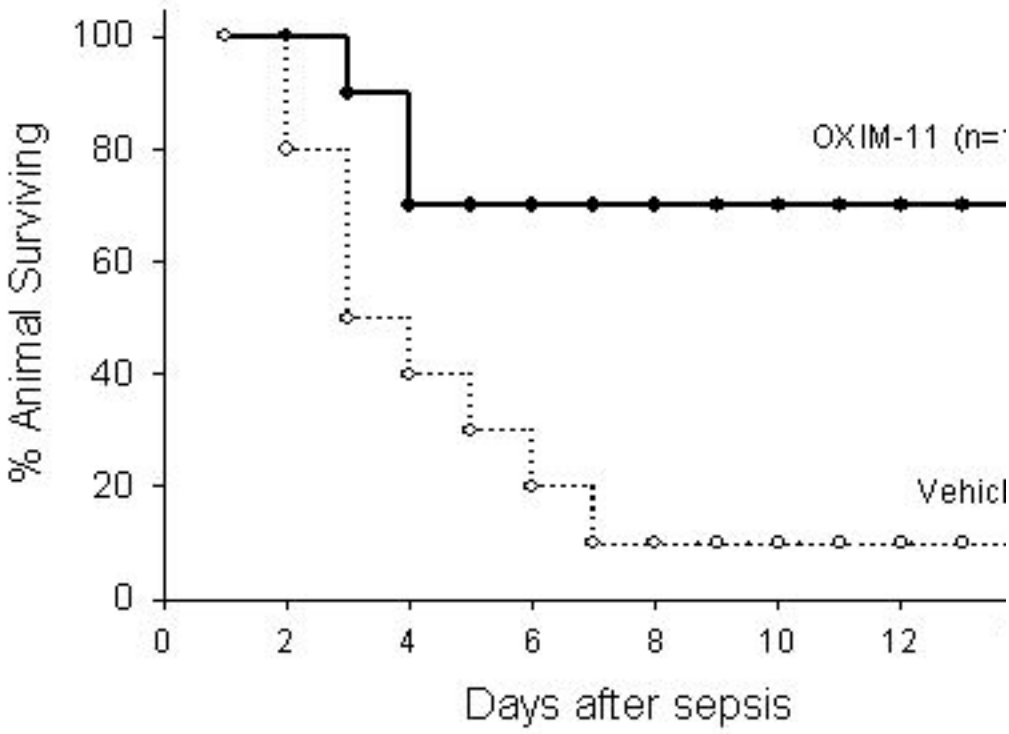
Furin plays a crucial role in human diseases such as Alzheinmer's diseases, cancer, and viral and bacterial infections. Furin inhibitors thus hold great promise as potential therapeutic agents for treating furin-mediated diseases, particularly for short-term therapy. A novel class of small molecule furin inhibitors based on 2,5-dideoxystreptamine were synthesized, some of which show strong inhibitory activity against furin. Structure-activity relationships of these molecules are discussed.

MEDI 399

Development of potent inhibitors of a critical mediator of sepsis

Yousef Al-Abed*, Kai Fan Cheng, Darrin Dabideen, Bayan Aljabari, Valentin Pavlov, and Edmund Miller, Laboratory of Medicinal Chemistry, The Feinstein Institute for*

Medical Research, 350 Community Drive, Manhasset, NY 11030, Fax: 1-516-365-5090, yalabed@nshs.edu
Sepsis, the progressive injurious systemic response of the host to an infection, is a major cause of morbidity and mortality and the leading cause of death in non-cardiac intensive care units. With the exception of activated protein C, which was recently approved for treatment of severe sepsis, no other agents are approved by the FDA for its treatment. The pathogenesis of this inflammatory disorder remains to be clearly defined. However, sepsis is mediated, at least in part, by soluble factors and among these the proinflammatory cytokine macrophage migration inhibitory factor (MIF), has been shown to play a central role(1, 2). Recently, we showed that, during sepsis, abolition of MIF activity by antibodies, or our specific inhibitor ISO-1 improves cardio-circulatory efficiency and prevents the lethality associated with sepsis(1). We designed ISO-1 to fit into the hydrophobic active site of MIF, an interaction confirmed by the crystal structure of the MIF complex with ISO-1(3). Administration of ISO-1 in a clinically relevant model of sepsis confers moderate protection (%80 survival in ISO-1 treatment versus 40% in control). These results identify ISO-1 as the first small molecule inhibitor of MIF proinflammatory activities with therapeutic implications and indicate the potential of the MIF active site as a novel target for therapeutic interventions in human sepsis. To improve the potency of ISO-1, we explored the SAR of ISO-1 and designed new scaffolds, e.g. OXIM-11. Our studies generated several new potent inhibitors of MIF with IC50s as low as 200 nM, i.e. 55-fold more potent than ISO-1. We will present our medicinal chemistry approach to inhibit MIF proinflammatory activity in vivo. References: 1. Y. Al-Abed et al., J Biol Chem (Aug 22, 2005). 2. X. Lin et al., Shock In press (2005). 3. J. B. Lubetsky et al., J Biol Chem 277, 24976-82 (Jul 12, 2002).



OXIM-11 *is protective when administered 24 h after CLP:* Mice were inje
intraperitoneally with OXIM-11 (3.5 mg/kg) (n= 13, **P< 0.01) or vehicle
CLP (n=13). Two additional injections were given on each days 2 and 3.

MEDI 400

Orotidine monophosphate decarboxylase: Novel inhibitors with new twists and turns

Lakshmi P. Kotra[1], *Emil F. Pai*[2], *Angelica M. Bello*[3], *Masahiro Fujihashi*[2], *and Ewa Poduch*[4]. *(1) Departments of Pharmaceutical Sciences, Chemistry and Division of Cell & Molecular Biology, University of Toronto and Toronto General Research Institute,*

Molecular Design and Information Technology Centre, 19 Russell Street, Toronto, ON M5S 2S2, Canada, Fax: 416-978-8511, p.kotra@utoronto.ca, (2) Departments of Medical Biophysics, Biochemistry and Molecular & Medical Genetics and Division of Molecular & Structural Biology, University of Toronto & Ontario Cancer Institute, (3) Faculty of Pharmacy, University of Toronto, (4) Leslie Dan Faculty of Pharmacy, University of Toronto

Orotidine monophosphate decarboxylase (ODCase) is one of the most proficient members of the enzymic world. Inhibitors of orotidine monophosphate decarboxylase (ODCase) have applications in RNA viral and other infectious diseases. Novel inhibitors, 6-amino-UMP and 6-cyano-UMP were designed using the bioisosteric principle of volume, based on the substructure volumes in the substrate, OMP, and a potent inhibitor of ODCase, BMP. Competitive inhibition of the enzyme (M. thermoautotrophicum) was observed and the inhibition constants (Ki) were determined to be 12.4 uM and 29 uM for 6-aza-UMP and 6-cyano-UMP, respectively. 6-Amino-UMP was found to be among the potent inhibitors of ODCase, having an inhibition constant of 840 nM. Additionally, 6-cyano-UMP was found to undergo hydrolysis into BMP inside the active site of ODCase over several hours to days, inactivating the enzyme. Details of the inhibitions and the new potential of these novel inhibitors, and the mechanistic issues will be discussed.

MEDI 401

Syntheses and biological activities of small molecules at thyrotropin-releasing hormone (TRH) receptors

Jian-Kang Jiang[1]*, Craig J. Thomas*[1]*, Susanne Neumann*[2]*, Xinping Lu*[2]*, John Childress*[2]*, and Marvin C. Gershengorn*[2]*. (1) Chemical Biology Core Facility, NIDDK, National Institutes of Health, National Institutes of Health, Bethesda, MD 20892, Fax: 301-402-2639, jiankangj@niddk.nih.gov, (2) NIDDK Clinical Endocrinology Branch, National Institutes of Health*

Thyrotropine-releasing hormone (TRH) is a tripeptide and synthesized in hypothalamus. It primary acts on the pituitary and regulates the synthesis of prolactin and thyroid-stimulating hormone (TSH). The action of TRH in rodents is mediated by two receptor subtypes TRH-R1 and TRH-R2, which belong to the family of seven transmembrane-spanning receptors. There are few non-peptide small molecules that have binding affinity for these two receptor subtypes. A small molecule designated PK 11195 has low binding affinity for TRH-R1 and TRH-R2. Based upon the 1-(2-chlorophenyl)isoquinoline scaffold of PK 11195, we designed and synthesized two generations of PK 11195 analogues, in hopes of amplifying the affinity and generating selectivity for TRH-R1 and TRH-R2. Several analogues displayed not only higher binding affinity but also moderate selectivity for the two TRH receptor subtypes.

MEDI 402

Novel tyrosine phosphatase inhibitors for treatment of Yersinia infections

Lutz Tautz*, Shane Bruckner, Sina Sareth, Andres Alonso, Jori Bogetz, Nunzio Bottini, Maurizio Pellecchia, and Tomas Mustelin, Infectious and Inflammatory Disease Center, Burnham Institute for Medical Research, 10901 N Torrey Pines Rd, La Jolla, CA 92037, tautz@burnham.org*

To avoid detection and targeting by the immune system, the plague-causing bacterium *Yersinia pestis* uses a type III secretion system to deliver a set of inhibitory proteins into the cytoplasm of immune cells. One of these proteins is an exceptionally active tyrosine phosphatase termed YopH, which paralyzes lymphocytes and macrophages by dephosphorylating critical tyrosine kinases and signal transduction molecules. Because *Yersinia pestis* strains lacking YopH are

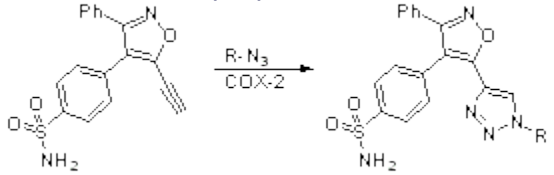
avirulent, we set out to develop small molecule inhibitors for YopH. Chemical library screening, structure-activity-relationship studies and *in silico* docking resulted in the identification of a series of novel YopH inhibitors with nanomolar Ki values, as well as the structural basis for inhibition. Our inhibitors readily entered live cells and rescued them from YopH-induced tyrosine dephosphorylation, signaling paralysis, and cell death. These inhibitors may become useful for treating the lethal infection by *Yersinia pestis*.

MEDI 403

Application of Click Chemistry to the development of COX-2 and CA-II inhibitors

Hartmuth Kolb[1], *Vani P. Mocharla*[1], *Joseph C. Walsh*[1], *Henry C. Padgett*[1], *Rajendra T. Tanpure*[2], *Tatsushi Toyokuni*[3], *Helen Su*[2], *Wolfgang A. Weber*[2], *Johannes Czernin*[2], *Naveen Jain*[2], *Tomo-o Ishikawa*[2], *and Harvey Herschman*[4]. *(1) Siemens Biomarker Solutions, 6140 Bristol Parkway, Culver City, CA 90230, Fax: 310-670-8428, hartmuth.kolb@ctimi.com, (2) Department of Molecular and Medical Pharmacology, University of California, Los Angeles, (3) Department of Molecular & Medical Pharmacology, UCLA School of Medicine, (4) Molecular and Medical Pharmacology, UCLA School of Medicine*

The in situ click chemistry approach to lead discovery uses the biological target itself for synthesizing inhibitors. Equilibrium-controlled sampling of bio-orthogonal, click chemistry enabled fragments by the biological target eventually leads to an irreversible reaction that essentially 'freezes' the fragment pair that best fits the protein's binding pockets. The products usually show high affinity for the target, since they simultaneously engage in multiple binding interactions with it. New cyclooxgenase-2 (COX-2) and carbonic anhydrase II (CA-II) inhibitors were developed using this target guided synthesis (TGS) approach. Acetylene-bearing fragments that exhibited relatively low binding affinities for the respective targets were incubated with the proteins and a library of azide-bearing fragments, leading to the formation of several hit compounds in situ. Traditional enzyme assays revealed these in situ generated hits to be potent inhibitors of the enzymes that generated them. In case of COX-2, the best inhibitor displayed an IC50 value of 20 nM, and in case of CA-II, IC50 values of 0.5 nM were achieved. Fluorine-18 versions of these inhibitors were prepared and used for PET imaging in mice.



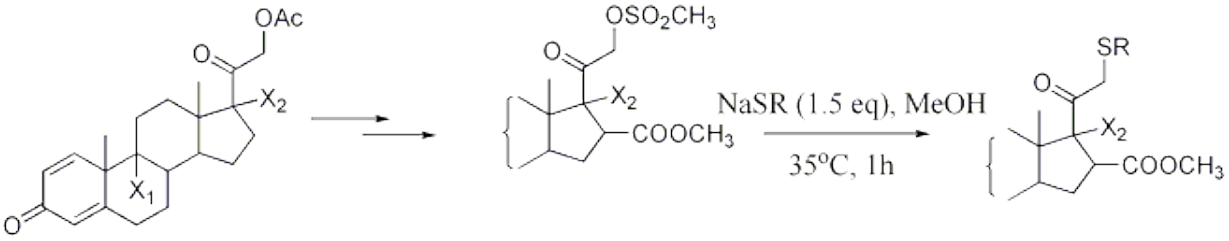
MEDI 404

Synthesis and biological evaluation of 21-thioalkylether derivatives of methyl 16-prednisolone carboxylates as a new class of anti-inflammatory steroidal antedrugs

Md. Omar F. Khan[1], *Kwan-K. Park*[1], *and Henry J Lee*[2]. *(1) Basic Sciences, College of Pharmacy and Pharmaceutical Sciences, Florida A & M University, Tallahassee, FL 32307, Fax: 850-599-3323, omar.khan@famu.edu, (2) Basic Sciences, College of Pharmacy and Pharmaceutical Sciences, Florida A&M University*

Antedrug is a designed locally active drug that undergoes a predictable metabolic inactivation upon entry into the systemic circulation from the applied site. Thus antedrrugs act locally and are devoid of systemic toxicities. The continued effort to synthesize non-systemic steroids have led to develope several chemical classes of

the anti-inflammatory steroidal antedrugs and the effort is extended further to improve their potency. It deemed rational to increase the lipophilicity by substituting the 21-hydroxy with thioalkyl functions to have better receptor binding and/or more localized effects in vivo and thus reduced systemic side effects. The 21-mesylate of the methyl-16-perdnisolonecarboxylate and 9-fluoro-17-dehydro methyl 16-prednisolonecarboxylate were reacted with Na-thioalkoxides to furnish the desired thioethers. The preliminary in vitro metabolic study of the compounds has shown to retain their original antedrug property. The glucocorticoid receptor binding affinity is weaker than that of prednisolone. Synthesis of newer derivatives and more pharmacological studies are in progress.

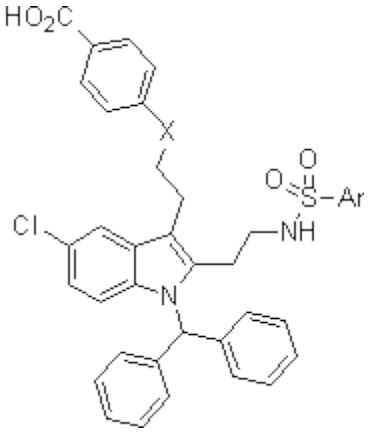


MEDI 405

Indole phenyl sulfonamide cPLA$_2$α inhibitors for the treatment of inflammation

Katherine L. Lee[1]*, Mark L. Behnke*[2]*, Lihren Chen*[1]*, Megan A. Foley*[3]*, Jill Nunez*[1]*, Richard Vargas*[1]*, Weiheng Wang*[1]*, Steve Tam*[1]*, Marina WH. Shen*[4]*, Nevena Mollova*[5]*, Xin Xu*[6]*, Debra G. Goodwin*[4]*, Cheryl Nickerson-Nutter*[4]*, James D. Clark*[4]*, and John C. McKew*[1]*. (1) Department of Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5682, klee@wyeth.com, (2) Department of Chemistry, Infinity Pharmaceuticals, Inc, (3) Department of Chemistry, University of Pennsylvania, (4) Department of Inflammation, Wyeth Research, (5) Department of Pre-Clinical Development, CV Therapeutics, Inc, (6) Department of Drug Safety and Metabolism, Wyeth Research*

Cytosolic phospholipase A$_2$α (cPLA$_2$α) selectively cleaves the *sn-2* position of arachidonyl-glycerophospholipids to generate free arachidonic acid. This arachidonic acid is in turn metabolized to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can be acetylated to form yet another inflammatory mediator, platelet activating factor (PAF). Selective inhibition of cPLA$_2$α would provide a novel therapeutic with applications in many disease states including rheumatoid arthritis, osteoarthritis, and asthma. The development of a class of novel and selective cPLA$_2$αinhibitors containing a phenyl sulfonamide substituent at C2 will be discussed.

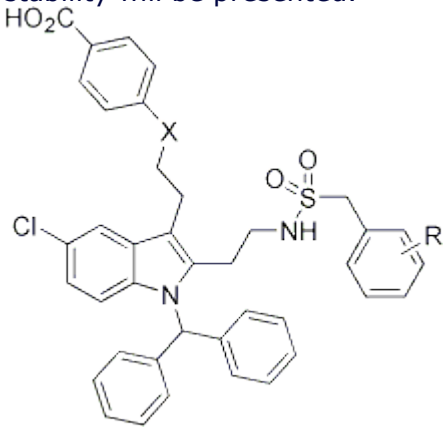


MEDI 406

Indole benzyl sulfonamide cPLA$_2$α inhibitors: Optimization and efficacy in inflammatory models

John C. McKew[1], *Katherine L. Lee*[1], *Lihren Chen*[1], *Richard Vargas*[1], *Jill Nunez*[1], *Steve Tam*[1], *Marina WH. Shen*[2], *Nevana Mollova*[3], *Xu Xin*[3], *Debra G. Goodwin*[2], *Cheryl Nickerson-Nutter*[2], *Martin Hegen*[2], *Cara Williams*[2], *Louis Leung*[3], *and James D. Clark*[2]. *(1) Department of Chemical and Screening Sciences, Wyeth Research, 200 CambridgePark Drive, Cambridge, MA 02140, Fax: 617-665-5685, (2) Department of Inflammation, Wyeth Research, (3) Department of Drug Safety and Metabolism, Wyeth Research*

Cytosolic phospholipase A$_2$α (cPLA$_2$α) selectively cleaves the sn-2 position of arachidonyl-glycerophospholipids to generate free arachidonic acid. This arachidonic acid is in turn metabolized to a variety of inflammatory mediators including leukotrienes, prostaglandins and thromboxanes. The lysophospholipid remaining after arachidonic acid cleavage can be acetylated to form yet another inflammatory mediator, platelet activating factor (PAF). Selective inhibition of cPLA$_2$α would provide a novel therapeutic with applications in many disease states including rheumatoid arthritis, osteoarthritis, and asthma. Efforts to optimize the indole benzyl sulfonamide class of inhibitors with respect to potency, in vivo efficacy and metabolic stability will be presented.



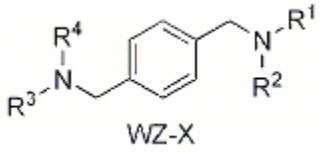
MEDI 407

Discovery of substituted spiropiperidines as a novel chemotype of potent and selective GlyT1 inhibitors

E. Pinard[1], *D Alberati*[2], *E Borroni*[2], *S Ceccarelli*[1], *H Fischer*[1], *D Hainzl*[2], *S Jolidon*[1], *J L Moreau*[2], *H Stalder*[1], *and A W Thomas*[1]. *(1) Pharmaceutical Research Basel,*

Discovery Chemistry, Hoffmann-La Roche, Basel CH-4070, Switzerland, Fax: 41 61 688 87 14, emmanuel.pinard@roche.com, (2) Pharmaceutical Research Basel, Discovery Biology Neuroscience, Hoffmann-La Roche
NMDA receptor hypofunction is suggested to be involved in the pathophysiology of schizophrenia. Thus, therapeutic intervention aimed at increasing NMDA synaptic tone is expected to show beneficial effect in schizophrenic patients. As glycine is an obligatory co-agonist at the NMDA receptor complex, one strategy to enhance NMDA receptor activity is to elevate extracellular levels of glycine in the local microenvironment of synaptic NMDA receptor. Glycine elevation can be achieved by inhibition of the glycine transporter 1 (GlyT1) which is co-expressed in the brain with the NMDA receptor and is responsible for glycine removal from the synaptic cleft. As a consequence, GlyT1 transporter inhibition has emerged as an attractive therapeutic strategy for the treatment of Shizophrenia. We have recently identified substituted spiropiperidines as a novel class of GlyT1 inhibitors. We describe here synthetic and SAR studies as well as the Multi Dimentional Optimisation program that led, in this series, to the identification of selective and in vivo active GlyT1 inhibitors.

MEDI 408
Discovery of novel nonpeptide CXCR4 antagonists
Weiqiang Zhan[1]*, Zhongxing Liang*[2]*, James P. Snyder*[1]*, Dennis C. Liotta*[1]*, and Hyunsuk Shim*[2]*. (1) Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, wzhan@emory.edu, (2) Winship Cancer Institute, Emory University*
CXCR4 is a G-protein coupled 7-transmembrane receptor which first drew attention as a major co-receptor for the entry of HIV. Compelling evidence is accumulating that the interaction of CXCR4 and its only natural ligand SDF-1 plays a unique and important role in cancer/tumor metastasis, regulation of stem cell trafficking and tumor vascularization. Consequently, therapeutic strategies to block the binding between CXCR4 and SDF-1 with CXCR4 antagonists could have important applications in the clinic. In view of aspects of the molecular mechanism of the CXCR4 antagonists T140 and AMD3100, we designed a template with the general structure WZ-X, identified the initial screening hit 6-18-10 as a lead through an affinity binding assay against T140 analog, TN14003 whose IC50 is less than 1 nM, and followed with the design and synthesis of a series of novel small molecular CXCR4 antagonists. This led to the discovery of WZ811S, which shows excellent water solubility and IC50 is about 1 nM in affinity binding assay. WZ811S was then subjected to four functional assays: Akt activation, calcium mobilization, martrigel invasion, and tubular formation (anti-angiogenic). These data further support the role of CXCR4 in chemotaxis, motility, invasion, and angiogenesis and reinforces its value as a point for therapeutic intervention where these play key roles.



WZ-X

MEDI 409
Engineered biosynthesis: A strategy for drug development and the NIH Roadmap
John M. Schwab*, Bldg. 45, Rm. 2AS.43A, National Institutes of Health, NIGMS, 45 Center Drive, Bethesda, MD 20892, Fax: (301) 480-2802*
Natural products (NPs) have evolved for their bioactivities and for the ability to enhance the survival of the producing organisms. Not surprisingly, a very substantial

fraction of marketed drugs--for a plethora of clinical indications--has been derived from NPs. However, NPs have lost favor as drug leads, due largely to supply issues and the challenge of making analogs, and for the past dozen years the predominant industrial model for drug discovery has been high-throughput screening of libraries of synthetic compounds. Thus far, this model hasn't met the ambitious expectations. Through the NIH Molecular Libraries Roadmap, a number of grants were made recently for the development of new methodologies for NP chemistry. These projects--most of which deal with the engineering of secondary metabolic pathways--may facilitate the discovery of new bioactivities, which will lead to the development of new drugs as well as probes of biological function--the goal of the Molecular Libraries Roadmap.

MEDI 410

Halogenation in the cyanobacteria: Novel natural products and their biosynthesis

***William H. Gerwick**, Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, MC 0212, La Jolla, CA 92093, Fax: 858-534-0529, wgerwick@ucsd.edu*

Macrophytic marine algae have long been known to produce natural products which incorporate halogen atoms (chlorine, bromine and iodine) into their covalent organic structures. In the last few years, a growing trend observed in the metabolites of marine cyanobacteria (blue-green algae) is the incorporation of halogen atoms into a rich and unparalleled diversity of organic functional groups. Our drug discovery investigations using marine cyanobacteria have yielded several new examples of halogenated natural products. We have investigated the biosynthesis of several using stable isotope labeling techniques in concert with laboratory cultures of producing strains and NMR analysis. Additionally, in three cases we have cloned the putative biosynthetic gene clusters which code for the production of halogenated natural products, and we infer the functioning of novel gene products to produce brominated and chlorinated secondary metabolites in these prokaryotes. It appears that some of these metabolites are halogenated by novel radical-based mechanisms.

MEDI 411

Chemical diversity and enzymatic versatility of microbial natural product systems

***David H. Sherman**, Department of Medicinal Chemistry, Chemistry, Microbiology and Immunology, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, MI 48109-2216, davidhs@umich.edu*

We have synthesized the natural pikromycin pathway pentaketide and hexaketide chain elongation intermediates as N-acetyl cysteamine (NAC) thioesters and have used them as substrates for in vitro conversions with engineered PikAIII+TE, and in combination with native PikAIII (module 5) and PikAIV (module 6) multifunctional proteins from Streptomyces venezuelae. This investigation demonstrates directly the remarkable ability of these monomodules to catalyze one or two chain extension reactions, keto group processing steps, acyl-ACP release and cyclization to generate 10-deoxymethynolide and narbonolide. The results reveal the enormous preference of Pik monomodules for their natural polyketide substrates, and provide an important comparative analysis with previous studies using unnatural diketide NAC thioester substrates. To complement our work with Pik monomodules, we initiated analysis of the Pik TE domain as an effective macrolactonization catalyst. This was done using a synthetic hexaketide-SNAC ester mimic of the natural chain elongation intermediate and unnatural structural variants as substrates. We expanded our analysis of microbial systems by investigating the TE domain from the cryptophycin biosynthetic

pathway from Nostoc sp. ATCC 53789. This catalytic domain was isolated, purified and analyzed with a series of synthetic linear chain elongation intermediates to develop a novel chemoenzymatic synthesis of the cryptophycin/arenastatin class of anti-tumor agents. The results show high efficiency of the thioesterase to generate the 16-membered depsipeptide ring system. Moreover, analysis of selected substrates revealed considerable tolerance for structural variation within the seco-cryptophycin Unit C beta-alanine residue, but strict structural requirements at the phenyl group position of the Unit A delta-hydroxy octanoate chain elongation intermediates.

MEDI 412
Combinatorial biosynthesis: New opportunities for natural product drug discovery
***Ben Shen**, Division of Pharmaceutical Sciences and Department of Chemistry, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705, Fax: 608-262-5345, bshen@pharmacy.wisc.edu*
Microorganisms produce a large variety of biologically active metabolites representing a vast diversity of fascinating molecular architecture not available in any other systems. Combinatorial biosynthesis offers a promising alternative to preparing complex natural products and their analogs biosynthetically. The success of this approach depends critically on (1) the development of novel strategies for combinatorial manipulation of secondary metabolite biosynthesis gene clusters and (2) the continuous discovery and characterization of biosynthetic machinery that catalyzes novel chemistry. Examples from our current study on biosynthesis of various antitumor antibiotics and engineering of the various biosynthetic machinery will be presented to highlight the progress in this field, with the emphasis on (1) novel chemistry and biology involved in natural product biosynthesis and (2) application of combinatorial biosynthesis methods to microbial biosynthetic machinery to generate natural structural diversity for drug discovery and development.

MEDI 413
Expanding the role of genetically engineered polyketides in drug discovery
***Gary W. Ashley**, Kosan Biosciences, Inc, 3832 Bay Center Place, Hayward, CA 94545, Fax: 510-732-8401, ashley@kosan.com*
The use of genetic engineering of polyketide biosynthetic pathways to produce analogs of natural polyketides too complex for efficient total synthesis has been amply demonstrated by several groups. Engineering of these complex pathways to produce particular molecules is not always feasible, however: access to the genes encoding the biosynthetic enzymes of a complex natural product is not always assured; engineering of a pathway may not result in formation of the desired compound; and biosynthetic machinery having the necessary activities to produce a desired compound may not be known. This talk will focus on two examples where we have combined the powers of genetic engineering and organic synthesis to overcome limitations of the individual techniques. In one example, genetic engineering is used to introduce unique functional groups into a natural polyketide scaffold. In a second example, engineered polyketides are used as synthons to assemble into more complex structures.