

Tuesday, January 24, 2005

Poster Session

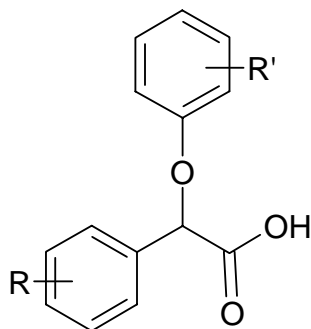
Poster Session, Bryan Norman, Eli Lilly & Company, Program Chair

Poster # 1

**SELECTIVE HUMAN DUAL PPAR AGONIST FOR THE TREATMENT OF DIABETE
MELLITUS**

Freeman, S.L.*, von Langen, D., Adams, A.D., Hu, Z., Elbrecht, A., MacNaul, K.L., Berger, J., Zhou, G., Doebber, T.W., Meurer, R., Forrest, M.J., Moller, D.E, and Jones, A.B.
Merck Research Laboratories, Merck & Co., Inc, PO Box 2000 Rahway NJ 07065
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A dual peroxisome proliferator activated receptor (PPAR) agonist has the potential for the treatment of glucose and lipid abnormalities associated with type 2 diabetes. An *O*-arylmandelic acid series of highly selective human PPAR α/γ agonist showed excellent anti-hyperglycemic efficacy in a db/db mouse model of type 2 diabetes. These PPAR agonists did not show the typical PPAR γ related side effects.



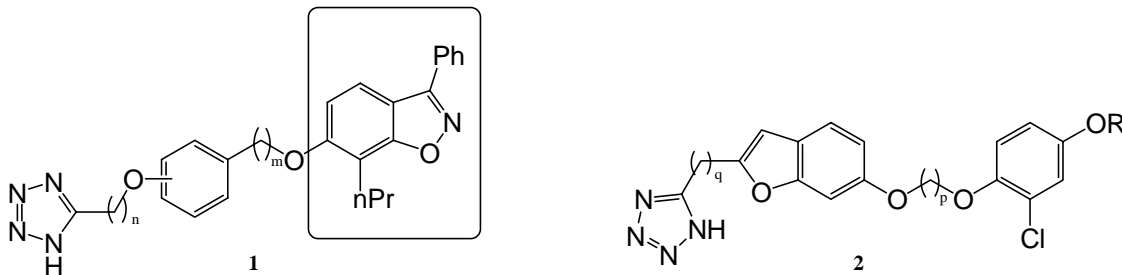
Poster # 2

DISCOVERY OF TETRAZOLE PPAR α/γ DUAL AGONISTS: SYNTHESIS AND SAR STUDIES. Hu, Z.*, Adams, A.D., Yang, G., Koyama, H., Jones, A.B., Berger, J.P., MacNaul, K.L., Doebber, T.W., Meinke, P. T., Moller, D. E. and Sahoo, S.P. Department of Medicinal Chemistry, Department of Metabolic Disorders and Department of Atherosclerosis and Endocrinology, Merck Research Laboratories P.O. Box 2000, Rahway, New Jersey 07065 USA, zao_hu@merck.com

Type 2 diabetes mellitus (Type 2 DM) is a chronic disease characterized by insulin resistance in the liver and peripheral tissues accompanied by a defect in pancreatic β -cells. Insulin resistance is a state in which the body fails to sufficiently respond to normal circulating level of insulin. In the clinic, type 2 DM is frequently associated with other features of metabolic syndrome such as obesity (70-80%), dyslipidemia (>60%), and hypertension (60%). As a result, approximately 75 to 80% of type 2 DM patients die from coronary heart disease or cerebrovascular disease. These findings clearly indicate the unmet clinical needs of lipid profile management among diabetics. With this background, we and others have postulated that a PPAR α/γ dual agonist might present a superior agent for the treatment of type 2 DM and diabetic dyslipidemia, providing glycemic control by PPAR γ activation and lipid profile improvement by PPAR α activation. Currently at least two NCEs in this class are being studied in late-stage clinical trials.

Recent structural biological studies of PPAR-ligand complexes indicated that acidic functional groups of ligands such as carboxylic acid, thiazolidine-2,4-dione (TZD), or oxazolidine-2,4-dione (OZD) bind to helix 12 of PPAR causing key conformational changes that are necessary to initiate a series of events leading to the regulation of gene expression. While most PPAR agonists reported in the literature have TZD, OZD or carboxylic acid as the key functional group in their structures, cases where tetrazole was used as a carboxylic acid replacement have been relatively rare. Since the late 1990s, two PPAR agonists have been approved by the FDA for the treatment of Type 2 DM. Both of them have TZD in their structures and have been reported to cause undesired fluid retention and weight gain.

Given the acidic functional group of PPAR ligands play an important role in the initial conformational change of the receptor and following co-factors association, we set out to investigate PPAR agonists containing a tetrazole as the key functional group. General scope of the SAR studies is depicted below. Results of a SAR studies, in vitro, and in vivo, will be presented.

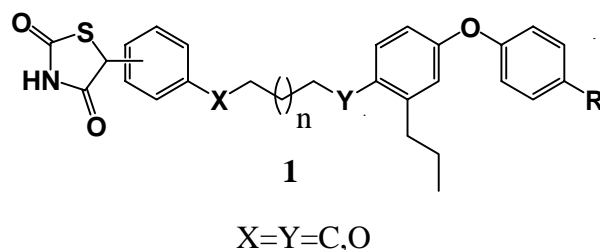


Poster # 3

NOVEL CLASS OF CARBON-TETHERED ARYL THIAZOLIDINEDIONE DERIVATIVES AS SELECTIVE PPAR γ AGONISTS FOR THE TREATMENT OF TYPE 2 DIABETES MELLITUS. Metzger, E.J.* , Lombardo, V., Desai, R.C., Berger, J.P., MacNaul, K.L., Doebber, T.W., Wright, S., Moller, D.E., and Sahoo, S.P.

(1) Department of Medicinal Chemistry, Merck & Co., 126E Lincoln Ave, Rahway, NJ, 07065 (2) Department of Molecular Endocrinology, Merck & Co., Edward_metzger@merck.com

Type II diabetes is a metabolic disease characterized by insulin resistance, hyperglycaemia, and hyperlipidemia. Untreated insulin resistance is rapidly increasing in prevalence, and is the principle cause of morbidity and mortality in industrial nations. Antidiabetic agents known as “glitazones” are among the currently available medicines, specifically PPAR γ agonists, for the treatment of this disease. Earlier, workers from this laboratory have reported on novel thiazolidinedione (TZD) antidiabetic agents as potent gamma and gamma/alpha dual PPAR agonists^{1,2}. In continuation of this work, we have expanded the SAR further and explored replacement of oxygen linked tether with a carbon of the general structure 1 with a view to enhance the metabolic stability of these class of PPAR agonists. The synthesis and SAR, including in vivo properties, for this novel series of carbon tethered aryl thiazolidinedione will be presented



¹ Desai, R.J.; Han, W.; Metzger, E.J.; Bergman, P.J.; Gratele, D.; MacNaul, K.L.; Berger, J.P.; Doebber, T.W.; Leung, K.; Moller, D.E.; Heck, V.J.; Sahoo, S.P. *Bioorg. Med. Chem. Lett.* **2003**, 13, 2795

² Koyama, H.; Boures, J.K.; Han, W.; Metzger, E.J.; Bergman, P.J.; Gratele, D.; Miller, D.J.; Tolman, R.L.; MacNaul, K.L.; Berger, J.P.; Doebber, T.W.; Leung, K.; Moller, D.E.; Heck, V.J.; Sahoo, S.P. *Bioorg. Med. Chem. Lett.* **2003**, 13, 1801-1804

Poster # 4

CONTROL OF THE PPAR RECEPTOR SUBTYPE SELECTIVITY BY MODIFICATION IN THE LIPOPHILIC PART OF THE PPAR $\alpha\gamma$ AGONIST NNC 61-0029.

John P. Mogensen*, Ingrid Pettersson, Lone Jeppesen, Jan Fleckner, Erik M. Wulff, Jan Nehlin, and Per Sauerberg

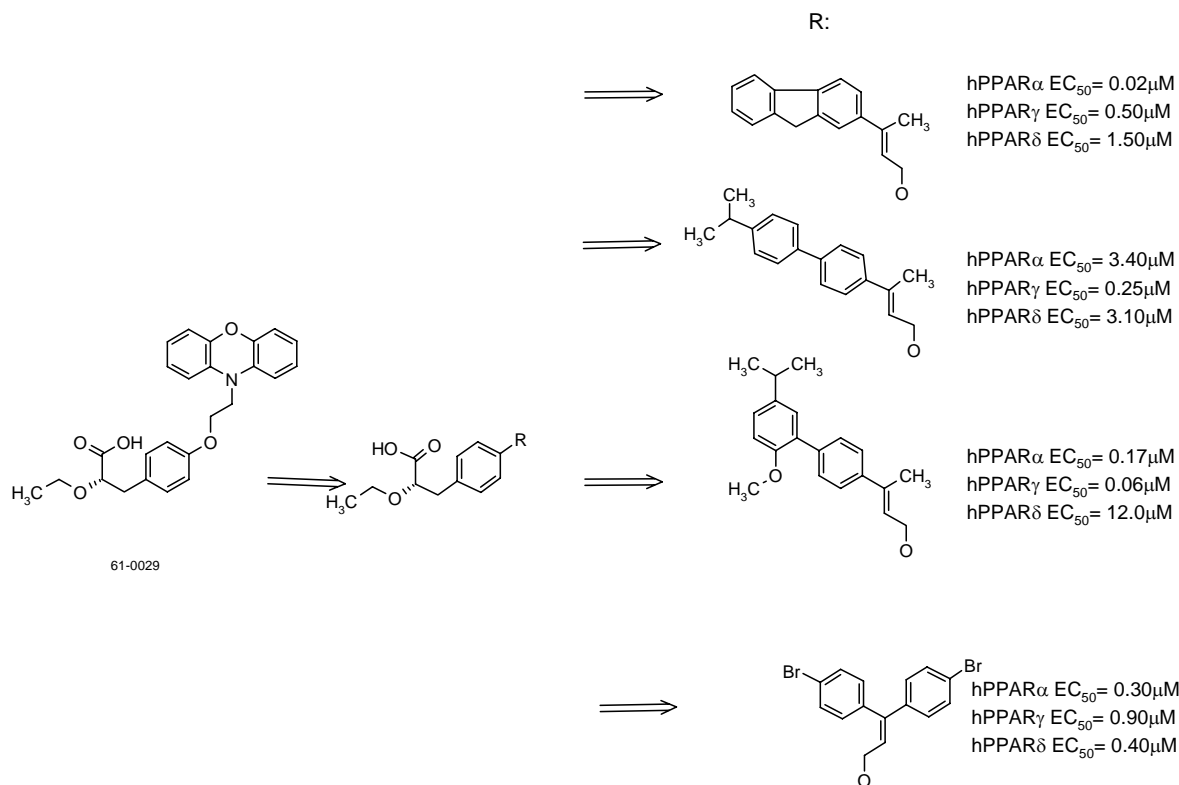
Novo Nordisk A/S, Novo Nordisk Park, 2760 Måløv, Denmark. JPM@novonordisk.com

The peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor super family. The marketed insulin sensitizers (rosiglitazone and pioglitazone) and the lipid lowering fibrates (e.g. fenofibrate and clofibrate) are PPAR γ and α agonists, respectively. There is no drug available targeting the third PPAR δ receptor, but several reports suggest PPAR δ to be involved in lipid transport and metabolism.

In spite of the high sequence homology of the 3 PPAR receptor subtypes, we here show that subtype selectivity can be achieved by only modifying the lipophilic part of the ligands.

Our structural starting point was to use the alpha-ethoxy-phenylpropionic acid as used in the dual PPAR $\alpha\gamma$ agonist NNC 61-0029 described in J. Med. Chem. 2002, 45, 789.

Systematic structural changes in the lipophilic part of NNC 61-0029 in combination with docking into the ligand binding domain (LBD) of the PPAR α , PPAR γ and PPAR δ receptors led to the identification of PPAR α , PPAR γ , PPAR $\alpha\gamma$ and PPAR $\alpha\gamma\delta$ agonists.

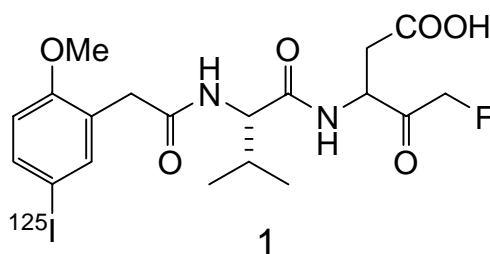


Poster # 5

The Synthesis and Properties of a Novel, Potent, Irreversible Caspase Probe. **Colucci, J**; Methot, N; Vaillancourt, J; Huang, J; Han, Y; Menard, S; Zamboni, R; Toulmond, S; Nicholson, D; Roy, S. Merck Frosst Centre for Therapeutic Research, Merck Research Laboratories, 16711 Trans Canada Highway, Kirkland, Quebec H9H 3L1, Canada. john_colucci@merck.com

Caspases are members of a group of cysteine proteases of which many (7 of the known 12) are currently thought to be involved in programmed cell death or apoptosis. It is believed that excessive caspase activity is the main cause of many pathologies found in ischemic diseases (such as stroke and myocardial infarction), alcohol-induced hepatitis, sepsis, Alzheimer's and Huntington's disease. Since caspase-3 is a major effector for the apoptotic process, much of our efforts have been directed in identifying a potent and selective inhibitor of this enzyme. During our work regarding the inhibition of caspase-3, we became interested in obtaining an active site probe to capture activated caspase-3 *in vivo*, determining the enzyme occupancy of certain inhibitors and thus correlating the level of caspase-3 inhibition with *in vivo* efficacy in animal models.

The synthesis of such a novel, potent, irreversible radiolabeled caspase inhibitor, **1**, will be described.¹ The synthesis of **1**, consisted of 10 chemical steps including introduction of the ¹²⁵I radiolabel through the intermediacy of an aryl tin precursor. In addition to the synthesis, we will describe the efficacy of the probe in capturing active caspase-3 from tissue extracts from several commonly used animal models of cellular injury.²



- 1 Patent WO2004069773, "Irreversible Caspase-3 Inhibitors as Active Site Probes"; Colucci, J. et al.
- 2 Methot, N. et al; (2004) *J. Biol. Chem.*, **279.27**, 27905-27914. "A Caspase Active Site Probe Reveals High Fractional Inhibition Needed to Block DNA Fragmentation."

Poster # 6

DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF A SERIES OF POTENT XANTHINE-BASED INHIBITORS OF THE ENZYME DPP-IV.

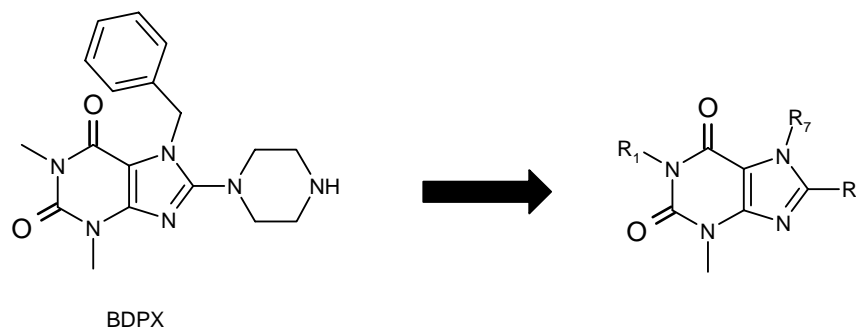
Kanstrup, A.*; Bjelke, J. R.; Bowler, A. N.; Branner, S.; Carr, R. D.; Christensen, I. T.; Christiansen, L. B.; Daugaard, J. R.; Kilburn, J. P.; Kristiansen, M.; Lundbeck, J. M.; Rasmussen, H. B.; Rolin, B.; Sams, C. G.

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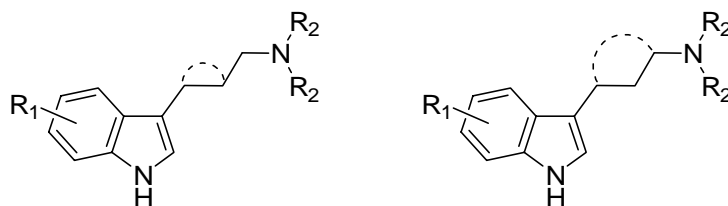
The use of inhibitors of the enzyme Dipeptidyl peptidase IV (DPP-IV) is undergoing clinical evaluation for the treatment of diabetes. The DPP-IV enzyme cleaves peptide substrates with a proline or alanine residue at the penultimate position from the N-terminus, and DPP-IV catalysed cleavage is responsible for the very short half-life of the insulinotropic gut hormones GLP-1 and GIP. The major part of the currently known DPP-IV inhibitors consists of an alpha amino acid pyrrolidine core, to which substituents have been added to optimise potency, selectivity, bioavailability, and duration of action.

We have found that 7-Benzyl-1,3-dimethyl-8-piperazinoxanthine (BDPX) is a competitive inhibitor of DPP-IV with a K_i of 5.4 μM . Based on this lead structure we have prepared libraries of analogs aimed mainly at optimising the substituents at the R_1 , R_7 , and R_8 positions. This has resulted in inhibitors with low nanomolar affinity, favourable pharmacokinetic properties, and high selectivity for DPP-IV. The inhibitor design and rationalisation of the SAR has been guided by molecular modeling and co-crystallization of selected inhibitors with hDPP-IV.



Poster # 7

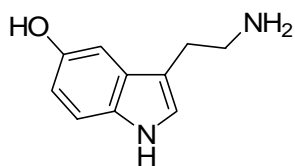
A NEW CLASS OF POTENT SSRIs BASED ON A CONFORMATIONALLY-RESTRICTED HOMOTRYPTAMINE BACKBONE. King, H.D.*; Denhart, D.J.; Meng, Z.; Ditta, J.; Deskus, J.; Mattson, R.J.; Kimura, R.; Wu, D.; Gao, Q.; Lodge, N.J.; Mattson, G.K.; Molski, T.; and Macor, J.E. Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492. dalton.king@bms.com



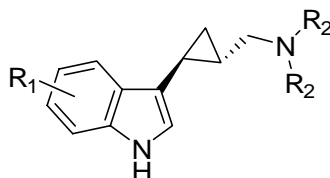
Homotryptamines have been shown to be potent selective serotonin reuptake inhibitors. In order to enhance potency and PK properties, we synthesized several novel series of homotryptamines in which the side chain is incorporated into a carbocyclic structure. Such conformationally restricted analogs are indeed highly potent SSRIs and possess desirable PK properties. In addition to discussing the evolution of these chemotypes, we will also present novel synthetic chemistry which was developed to access these analogs.

Poster # 8

AN EFFICIENT METHOD FOR THE ASYMMETRIC SYNTHESIS OF CYCLOPROPANE-DERIVED, HOMOTRYPTAMINE-LIKE ANALOGS. Marcin, L. R.;* Denhart, D. J.; Deskus, J.; Lodge, N. J.; Mattson, G. K.; Mattson, R. J.; Macor, J. E. Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492. lawrence.marcin@bms.com



5-hydroxytryptamine



homotryptamine-like analogs

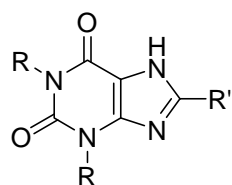
This poster details synthetic chemistry efforts that have enabled rapid and efficient access to an interesting class of cyclopropane-derived, homotryptamine-like compounds. In particular, a catalytic asymmetric synthesis of *trans*-2-(indol-3-yl)-1-cyclopropanecarboxylic esters has been developed. Application of this method to the preparation of a potent SSRI will be presented.

HIGH AFFINITY AND SELECTIVE A_{2B} ADENOSINE RECEPTOR ANTAGONISTS: 8-(PYRAZOL-4YL) XANTHINES.

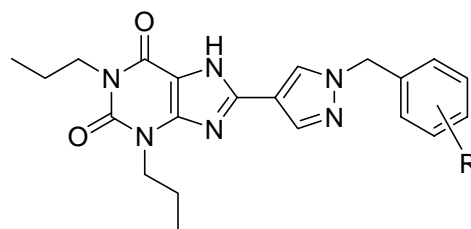
Rao Kalla,^{a*} Elfatih Elzein,^a Thao Perry,^a Xiaofen Li,^a Venkata Palle,^a Vaibahv Varkhedkar,^a Tenning Maa,^b Arthur Gimbel,^b Victoria Maydanik,^c David Lustig,^c Kwan Leung,^c Dewan Zeng,^b and Jeff Zablocki^a

^a Department of Bioorganic Chemistry, ^b Department of Drug Research and Pharmacological Sciences, ^c Department of Pre-Clinical Development, CV Therapeutics Inc., 3172 Porter Drive, Palo Alto, CA 94304, USA; rao.kalla@cvt.com

Adenosine is an endogenous agonist that can activate all four adenosine receptor subtypes – A₁, A_{2A}, A_{2B}, and A₃. Adenosine has been implicated to play a role in inflammatory airway diseases such as asthma through activation of the A_{2B} adenosine receptor (AdoR) on mast cells, that may lead to mast cell degranulation and the release of inflammatory cytokines (e.g. interleukin-4, 8 and 13). Theophylline **1**, is used for the treatment of asthma in both IV rescue therapy for acute asthma attacks and chronic oral treatment. Although the mechanism of action of theophylline is not completely understood, it is known to be a non-selective inhibitor for phosphodiesterases (PDE) and a non-selective AdoR antagonist (A_{2B} AdoR K_i = 7059 nM, selectivity A₁ 0.6: A_{2A} 0.6: A₃ 14). We hypothesize that the low therapeutic index of theophylline, due to both CNS and cardiac side effects, may be the result of its poor selectivity (for both PDEs and AdoRs). Based on our understanding of the role of A_{2B} AdoRs in asthma, we hypothesize that a potent and selective A_{2B} AdoR would provide a better therapeutic effect with fewer side effects.



- 1** R = Me, R' = H; Theophylline
2 R = Pr, R' = 4-pyrazole



- 3** R = H
4 R = m-F
5 R = m-CF₃
6 R = 2-Cl-5-CF₃

In order to discover potent and selective A_{2B} adenosine receptor (AdoR) antagonists we explored 8-pyrazol-4-yl xanthine derivatives, which represent a new class of xanthine based AdoR antagonists. Compound **2**, 8-(1*H*-pyrazol-4-yl)-1,3-dipropyl xanthine, is a N-1 unsubstituted pyrazole derivative that has favorable binding affinity (K_i = 9 nM) for the A_{2B} AdoR, but it is non-selective. Introduction of a benzyl group at the N-1-pyrazole position of **2** resulted in **3** that had moderate selectivity. The corresponding N-1-phenyl, phenethyl and phenylpropyl 8-pyrazolyl derivatives show a decrease in A_{2B} affinity and selectivity relative to **2**. Therefore, we focused on the benzyl substituted members of 8-(C-4-pyrazolyl) xanthine derivatives. The SAR for substitution on the benzyl suggests that an electron-withdrawing group on the phenyl ring specifically F (**4**), and CF₃ (**5**) at the *m*-position, increased the selectivity. Exploring the

disubstitution on the phenyl ring of derivatives 4 and 5 led to the 2-chloro-5-trifluoromethyl-phenyl derivative 6 [K_i (A_{2B})= 22 nM; selectivity A_1 54; A_{2A} 63; A_3 129] that retained the A_{2B} AdoR affinity, but enhanced the selectivity relative to 5. After optimization of the C-8 substituent on the xanthine core, symmetrical 1,3-disubstitution of xanthines 3, 4 and 5 with methyl, ethyl, butyl and isobutyl groups was explored. The smaller groups like methyl, and ethyl increased the A_{2B} selectivity while retaining the affinity compared to propyl groups whereas larger groups like butyl and isobutyl decreased the A_{2B} affinity and selectivity. Key members of the 8-(pyrazol-4-yl) xanthine class were used in a training set to generate a pharmacophore model using Hypogen in Catalyst™.

The design, synthesis, and SAR of substituted 8-pyrazol-4-yl xanthine derivatives and the details of the Hypogen model will be presented.

Poster # 10

Synthesis and Biological Evaluation of Azabicyclic NK₁ Receptor Ligands

Raubo P.*^a, Huscroft I.T.^a, Kulagowski J.J.^a, London C.^b,
Swain C.J.^a, Carlson E.J.^a, Chicchi G.G.^b

a) Merck Sharp & Dohme Research Laboratories, The Neuroscience Research Centre, Terlings Park, Harlow, CM20 2QR, UK; b) Merck Research Laboratories, 126 E. Lincoln Avenue, Rahway, NJ, USA. 07065
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The piperidine ring is a feature of a number of selective NK₁ receptor ligands¹. In the search for conformationally restricted piperidine derivatives of **1**, we were interested in replacing the piperidine ring with an azabicyclic as in **2** and **3** (Figure 1).

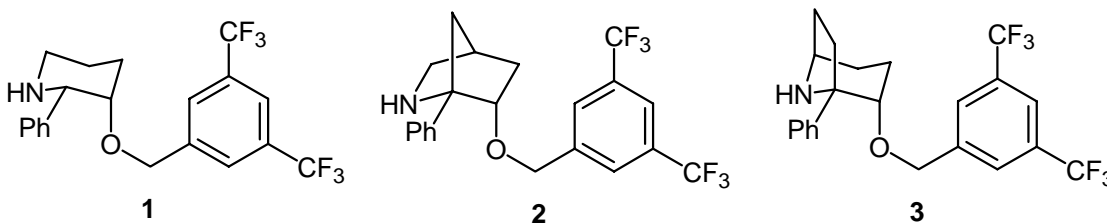


Figure 1

In the forthcoming poster, the development of new azabicyclic NK₁ receptor antagonists will be presented.

Literature:

[1] Seward E. M.; Swain C.J. *Expert Opin. Ther. Pat.* **1999**, 9, 571.

Poster # 11

SUBSTITUTED ACETOPHENONES AS SELECTIVE AND POTENT ALLOSTERIC POTENTIATORS OF THE METABOTROPIC GLUTAMATE RECEPTOR 2 (MGLUR2)

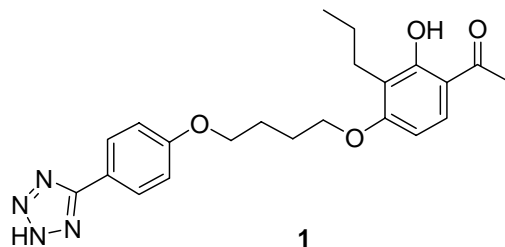
Pinkerton, A. B.*; Cube, R. V.; Hutchinson, J. H.; Gardner, M. F.; James, J. K.; Rowe, B. A.; Schaffhauser, H.; Rodriguez, D. E.; Campbell, U. C.; Baccei, C. S.; Lorrain, D. S.; Daggett, L. P.; and Vernier, J.-M.

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Metabotropic glutamate receptors (mGluRs) have been implicated in a number of CNS disorders including schizophrenia and anxiety. Eight subtypes of mGluRs have been identified, which fall into three main groups. Group II consists of two subtypes, mGluR2 and mGluR3, for which a number of non selective agonists and antagonists have been developed. Recently, non selective group II mGluR agonists, which are generally rigid glutamate analogs, have been shown to be efficacious in human clinical trials for anxiety. Due to the high degree of sequence homology between group II mGlu receptors, especially at the glutamate binding site, selective agonists for mGlu2 over mGlu3 have not, as yet, been discovered. Therefore, another strategy for selectivity involves the discovery of allosteric modulators that do not bind at the glutamate binding site

Herein we disclose the discovery of a new class of positive allosteric potentiators of the metabotropic glutamate receptor 2 (mGlu2), substituted acetophenones, e.g. 1-(2-hydroxy-3-propyl-4-{4-[4-(2H-tetrazol-5-yl)phenoxy]butoxy}phenyl) ethanone (**1**). These potentiators were shown to have no effect in the absence of glutamate as well as no effect at mGlu3 or the other mGlu receptors. The compounds were also evaluated in rodent models with potential relevance for schizophrenia and **1** was shown to have activity in the inhibition of ketamine induced norepinephrine release and ketamine induced hyperactivity. This represents the first example of the efficacy of mGlu2 receptor potentiators in these models.



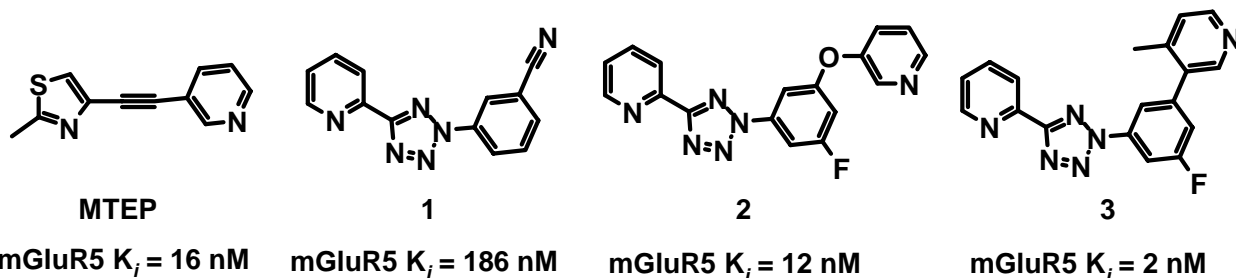
NOVEL, HIGHLY POTENT, ORALLY ACTIVE AND BRAIN PENETRANT HETEROARYL AZOLE METABOTROPIC GLUTAMATE (MGLU5) RECEPTOR ANTAGONISTS

Nicholas D. Smith,* Dehua Huang, Steve F. Poon, Jeffrey R. Roppe, Lida Tehrani, Jeffrey Anderson, Jesse Brodtkin, Deborah P. Chapman, Janice Chung, Merryl Cramer, Brian Eastman, Michael Gardener, Mitchell Green, Gregory Holtz, Xiaohui Jiang, Christopher King, Benito Munoz, Thomas S. Reger, Grace Reyes-Manalo, Bowei Wang, Mark A. Varney, Peptiboon Prasit and Nicholas D.P. Cosford. e-mail: Nicholas_Smith@merck.com

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Excessive activation of the G protein-coupled metabotropic glutamate subtype-5 (mGlu5) receptor in the CNS has been implicated in a number of disease states. Selective mGlu5 receptor antagonists, therefore, may be of therapeutic benefit in the treatment of pain, anxiety and depression, drug dependence or mental retardation.^[1]

Recent publications from this laboratory have described the discovery of **MTEP**, a potent and selective mGlu5 receptor antagonist.^[2] In our search for further structural classes, we examined the replacement of the alkyne linker in **MTEP** with a series of nitrogen-linked heteroaromatic azoles.



The discovery of tetrazole **1** (mGluR5 K_i = 186 nM) and its further development into the more potent derivatives **2** (mGluR5 K_i = 12 nM) and **3** (mGluR5 K_i = 2 nM) will be described in this poster.^(3, 4, 5) Specifically, *in-vitro* potencies against the mGlu5 receptor, pharmacokinetics, brain penetration and *in-vivo* rat receptor occupancy will be discussed.

- [1] Brodtkin, J.; Busse, C.; Sukoff, S. J.; Varney, M. *Pharmacol., Biochem. Behav.* **2002**, *73*, 359-366. Varney, M. A.; Gereau, R. W. I. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 283-296. Chiamulera, C.; Epping-Jordan, M. P.; Zocchi, A.; Marcon, C.; Cottiny, C.; et al. *Nat. Neurosci.* **2001**, *4*, 873-874.
- [2] Cosford, N.D.P.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N.D. *J. Med. Chem.* **2003**, *46*, 204-206.
- [3] Roppe, J.; Smith, N. D.; Huang, D.; Tehrani, L.; Wang, B.; Anderson, J.; Brodtkin, J.; Chung, J.; Jiang, X.; King, C.; King, C.; Munoz, B.; Varney, M. A.; Prasit, P.; Cosford, N. D. P. *J. Med. Chem.* **2004**, *47*, 4645-4648.
- [4] Poon, S.F.; Eastman, B.; Chapman, D.F.; Chung, J.; Cramer, M.; Reger, T.; Roppe, J.; Tehrani, L.; Cosford, N.D.P.; Smith, N.D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5477-5480.
- [5] Huang, D.; Poon, S.; Chapman, D.P.; Chung, J.; Cramer, M.; Reger, T.; Roppe, J.; Tehrani, L.; Cosford, N.; D. P.; Smith, N. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5473-5476.

Poster # 13

PREPARATION AND BIOLOGICAL EVALUATION OF INDOLE AND BENZIMIDAZOLE CARBOXAMIDES: POTENT HUMAN HISTAMINE H₄ ANTAGONISTS. Jennifer D. Venable*, Hui Cai, Wenying Chai, Curt A. Dvorak, Cheryl A. Grice, Jill A. Jablonowski, Annette K. Kwok, Kiev S. Ly, Barbara Pio, Jianmei Wei, Pragnya J. Desai, Wen Jiang, Steven Nguyen, Sandy J. Wilson, Paul J. Dunford, Robin L. Thurmond, Timothy W. Lovenberg, Lars Karlsson, Nicholas I. Carruthers, and James P. Edwards. Johnson & Johnson Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Row San Diego, CA 92121. jvenable@prdus.jnj.com.

The human histamine H₄ receptor was recently discovered and cloned by several groups. The expression profile includes eosinophils, mast cells, dendritic cells, and other leukocytes, implicating H₄ in inflammation and regulation of the immune system. A significant medicinal chemistry effort has been undertaken to discover and develop potent antagonists of the histamine H₄ receptor. Following a high throughput screen of our corporate compound collection, an indolylpiperazine was chosen as a lead compound. The synthesis, structure activity relationships (SAR), and initial biological evaluation of this series as well as a related series of benzimidazolylpiperazines will be discussed.

Poster # 14

DESIGN & SYNTHESIS OF SMALL MOLECULE LIBRARY OF PIPERAZINYALKYLISOXAZOLES AND IDENTIFICATION OF LIGANDS FOR GPCR AND T-TYPE CA CHANNEL.

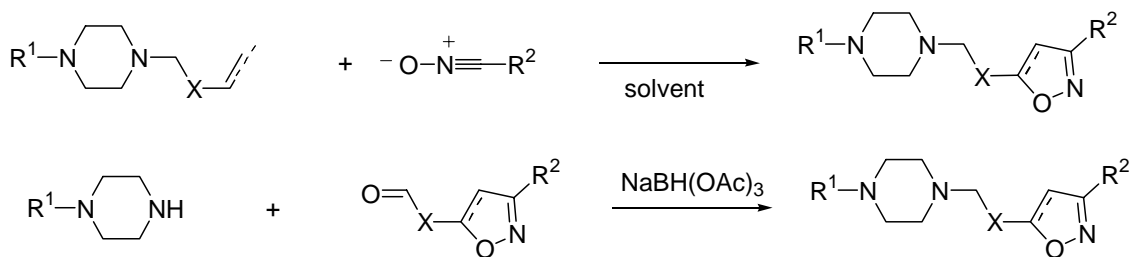
Hee-Yoon Lee*, Yongsik Jung, and Tae Gyu Chun

Department of Chemistry, School of Molecular Science(BK21) , KAIST, Daejeon 305-701, Korea.

MuniKumar R. Doddareddy, Ae Nim Pae and Hun Yeong Koh*.

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Small molecule libraries of piperazilyalkylisoxazole derivatives were developed using on the [2+3] cycloaddition reaction of nitrile oxides with unsaturated compounds or reductive amination of piperazine compounds with aldehydes. Both strategies did not require extensive work-up or purification process and produced large sets of compounds in a short period of time.



The piperazinyalkylisoxazoles were designed based on the privileged structures for G-Protein Coupled Receptor (GPCR) ligands and channel blockers. Through this design and preparation of libraries, we were able to identify compounds for Dopamine receptors and Serotonin receptors with excellent subtype selectivity.

Next we used these libraries and known T-type calcium channel blocker, mibefradil to identify new channel blockers using CoMFA and CoMSIA. Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were performed on these compounds. A set of 24 structurally similar compounds served to establish the model. Four different conformations of the most active compound that were obtained from Catalyst pharmacophore modeling and SYBYL random search option were used as the template structures for the alignment. All CoMFA and CoMSIA models gave cross-validated r² (q²) value of more than 0.5 and conventional r² value of more than 0.85. The predictive ability of the models was validated by an external test set of 10 compounds. They gave satisfactory r² values ranging from 0.577 to 0.866 for all models. CoMFA and CoMSIA contour maps were used to analyze the structural features of the ligands accounting for the activity in terms of positively contributing physicochemical properties. Based on this analysis and validation process, we were able to define a three dimensional pharmacophore model for the channel and then to identify several new potential channel blockers. The key to the success of this analysis and pharmacophore search turned out to be the careful selection of reasonable structural features among the selected compounds through CoMFA and CoMSIA analysis.

Alkyl- and Aralkylpolyaminoguanidines and -Polyamino(bis)guanidine Analogues as Potential Antitrypanosomal Agents

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²Haskins Laboratories, Pace University, 41 Park Row, New York, NY 10012

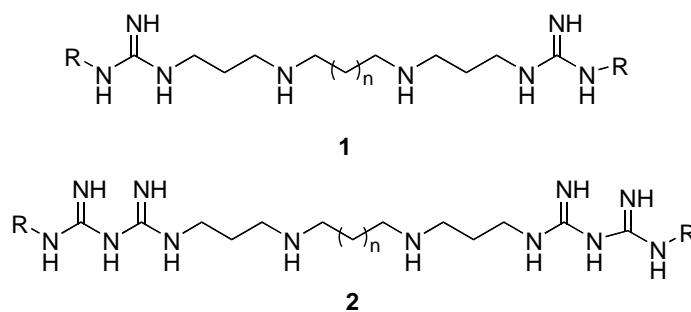


Figure 1. Structures of alkyl- and aralkylpolyaminoguanidines (**1**) and polyamino(bis)guanidines (**2**) with potent antitrypanosomal activity in vitro

We have previously reported that terminally alkylated polyamine analogues synthesized in our laboratories possess significant antitrypanosomal activity in vitro and in vivo,^{1,2} and that these compounds also show excellent activity against a variety of human parasitic diseases. More recently, a series of novel polyamine analogues containing terminal guanidiny and bis-guanidiny groups were synthesized (Figure 1) and found to exhibit significant inhibition against the parasitic enzyme trypanothione reductase (TR) in vitro, with IC₅₀ values ranging between 1 to 5 μM. In vitro cellular evaluation against *Trypanosoma brucei brucei* was also conducted, and potent trypanocidal activity was observed for these compounds, with the most effective compound showing an IC₅₀ value of 0.09 μM. No significant inhibition was observed when these analogues were evaluated against the related human enzyme glutathione reductase (GR). The remarkable selectivity of these compounds for TR over GR, combined with their significant antitrypanosomal activity, make them promising leads for the development of antiparasitic agents. In this presentation, the synthesis and preliminary biological evaluation of these agents will be described.

40. Woster, P.M.; Casero, Jr., R.A.: Perspective: Alkylpolyamine Analogues as Chemotherapeutic Agents, *J. Med. Chem.*, **2001**, *44*, 1-26.

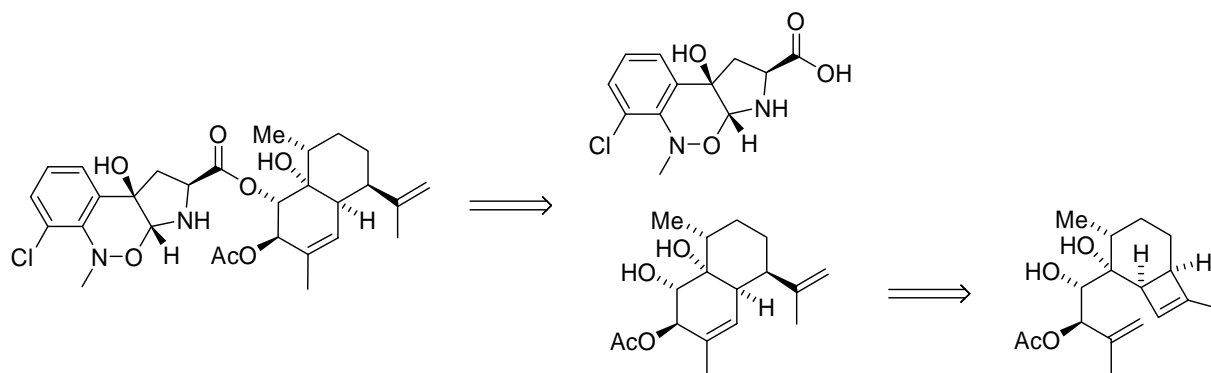
43. Zou, Y., Wu, Z.Q., Sirisoma, N., Casero, R.A., Weiss, L.M., Lane, S., Bacchi, C.J. and Woster, P.M. Novel Alkylpolyamine Analogues That Possess Both Antiparasitic and Antimicrosporidial Activity. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1613-1617.

Poster # 16

STUDIES TOWARD THE SYNTHESIS OF THE POTENT ANTHELMINTIC CJ-12662, AND SEMI-SYNTHETIC STUDIES TOWARD THE STEREOCHEMICAL ASSIGNMENT OF THE NEMATICIDAL ANTIBIOTIC PAECILOXAZINE.

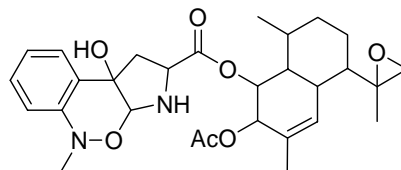
Barrett, A. G. M., and Brewer, A. R. E.* Imperial College London, SE1 5XN, UK. alice.brewer@imperial.ac.uk.

A novel natural product was recently isolated from *Aspergillus fischeri* var *thermomutatis*.¹ This new compound, CJ-12662, is a member of a select group of only four terpenoid pyrrolobenzoxazine natural products, and possesses broad-range antiparasitic activity. Our interest lies in the synthesis of the eastern terpenoid *cis*-decalin entity, the key step of which is a novel “ring-closing ring-opening” metathesis.



We have conducted the interesting metathesis reaction on a simplified model system, and the details of these studies, and those of our studies toward the synthesis of the metathesis precursor will be described.

The fourth member of the terpenoid pyrrolobenzoxazine natural product family was isolated recently from a *Paecilomyces* BAUA3058 strain.² This nematocidal natural product, designated Paeciloxazine, has yet to be stereochemically defined. The clear resemblance of this natural product to CJ-12662 has led to our semi-synthetic studies to confirm the stereochemical assignment.



Again, our interest lies in the eastern terpenoid moiety, and our studies into the deoxygenation and epoxidation of the terpene fragment of CJ-12662 will be described.

- (1) Kojima, Y.; Yamauchi, Y.; Kojima, N.; Bishop, B. *WO 95/19363* **1995**, A1.
- (2) Kanai, Y.; Fujimaki, T.; Kochi, S.-I.; Konno, H.; Kanazawa, S.; Tokumasu, S. *The Journal of Antibiotics* **2004**, *57*, 24-28.

Poster # 17

BIOPHYSICAL TOOLS TO ASSIST IN LEAD DISCOVERY AND OPTIMIZATION.

Brouillette, C. G. *, McDonald, H., Yang, Z., Teale, M., and Protasevich, I.
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A collection of short examples will be presented that illustrate how biophysical tools such as calorimetry and light scattering (dynamic and static), can be integrated into the process of drug target structure determination, lead discovery, and optimization. We have learned that these techniques can provide useful mechanistic information on ligand binding and assist in trouble-shooting when target crystallization conditions are not easily identified.

Differential scanning calorimetry (DSC) is used to analyze the domain substructure and folded state of a drug target. This is especially helpful when a convenient functional assay does not exist. We have used it to verify the folded state and the presence of functional dimers versus nonfunctional monomers of both soluble and integral membrane proteins (detergent or lipid reconstituted). A related thermal protein unfolding microassay is used to verify the folded state and has also been used to screen a small compound library which led to the identification of inhibitors of osteoclast formation. A DSC analysis was performed on a protein that was otherwise homogeneous and monodisperse protein but failed to crystallize in over 700 select conditions. DSC showed that it was apparently only partially folded, a reasonable explanation for the lack of crystals.

From determination of the osmotic second virial coefficient (B_{22}), conditions were obtained to crystallize another protein drug target, a homodimeric enzyme that was otherwise monodisperse by dynamic light scattering, homogeneous by SDS-PAGE and mass spectrometry. The B_{22} is a dilute solution parameter usually determined by static light scattering and shown to correlate with solution conditions favorable for crystallization. DSC indicated a folded protein but crystals failed to grow until optimized crystallization conditions were predicted from B_{22} measurements. Crystals obtained at the predicted conditions diffracted to better than 2 angstroms.

Lastly, examples from two publications will show that isothermal titration calorimetry, especially in conjunction with DSC, can be used to identify (1) key functional groups on the protein target and ligand that make significant contributions to binding, (2) ligand binding cooperativity in oligomeric receptors, (3) non-functional target binding sites, and (4) the relative and absolute contributions of noncovalent bonding and solvation to affinity.

Poster # 18

THE TOTAL SYNTHESIS OF TUBULYSIN D. Steele, J.C.* and Fecik R.A. Department of Medicinal Chemistry, University of Minnesota, 308 Harvard St. S.E., 8-101 WDH, Minneapolis, MN 55455, jcsteele00@hotmail.com

Tubulysin D (**1**) is a new bioactive peptide produced by myxobacteria (*Angiococcus discoformis* An d48) that was recently isolated by Höfle and co-workers. Interest in this novel tetrapeptide is due to its potent activity in a variety of cancer cell lines. Tubulysins are so-named due to their ability to inhibit tubulin polymerization by interacting at the peptide binding site and are more potent ($IC_{50} = 30$ pg/mL) than either dolastatin 10 ($IC_{50} = 110$ pg/mL) or vinblastine ($IC_{50} = 2000$ pg/mL). Unlike the Vinca alkaloids, the tubulysins have shown to be very potent in cancer cell lines that express P-glycoprotein, thus permitting a new treatment option for multidrug resistant cancers. The limited quantities of tubulysin D available by fermentation (< 1 mg/L) have hindered further biological evaluation. We have designed a modular and efficient synthetic route to the tubulysins that is readily amenable to the synthesis of analogs and is capable of readily providing gram quantities for further biological testing. Additionally, we plan to design photoaffinity labels and fluorescent labeled compounds to characterize the peptide binding site of tubulin.

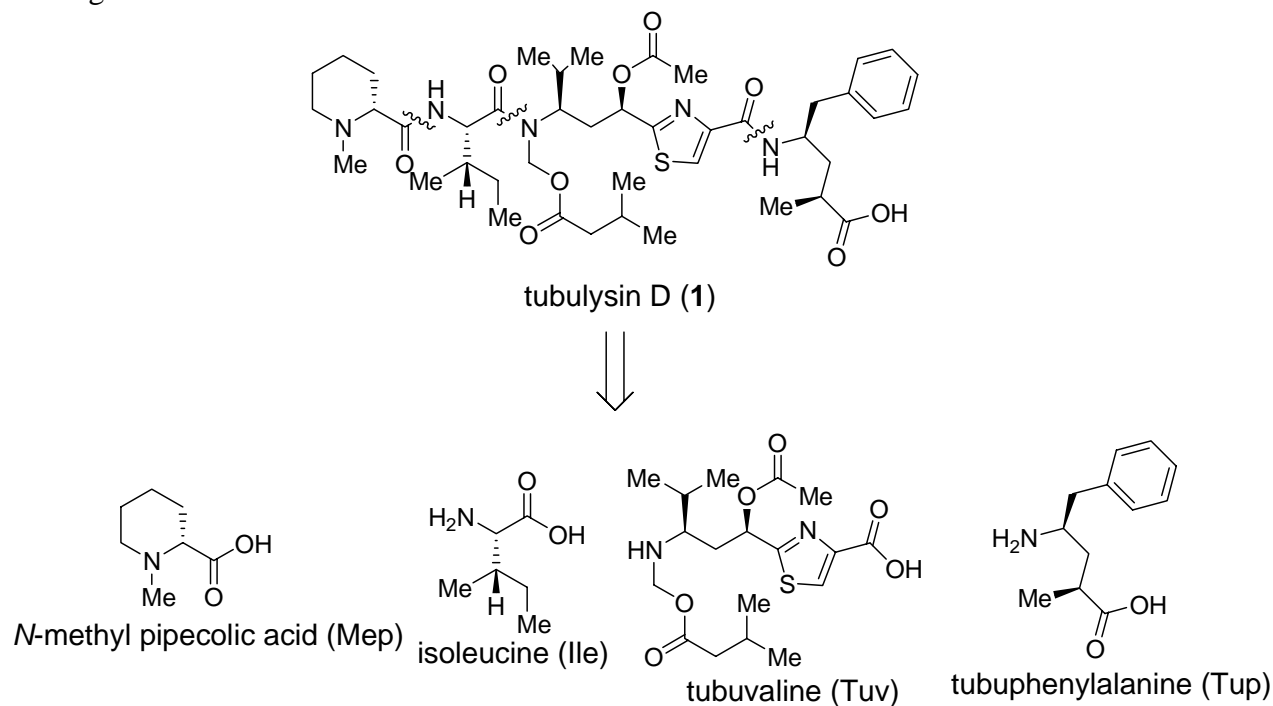


Figure 1. Retrosynthesis of Tubulysin D.

Disassembly of Tubulysin D across the three amide bond reveals four amino acids: D-*N*-methylpipecolic acid (D-Mep), L-isoleucine (Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup). We envisioned a convergent assembly of the *N*-terminal dipeptide Mep-Ile to the *C*-terminal dipeptide Tuv-Tup to prepare the fully functionalized tetrapeptide. The greatest challenge posed was in the synthesis of the tubuvaline fragment since this contains the delicate *N,O*-acetal function, a thiazole, as well as two stereocenters. Our synthesis of this key fragment features a magnesium-halogen exchange of bromothiazole **2** in the presence of an ester and subsequent Grignard addition to β -bis-Boc-homovalinal to provide **3**, which contains the entire carbon scaffold of tubuvaline (Figure 2). This extremely efficient synthesis rapidly assembles the carbon skeleton in 4 steps. We will present the results of our synthesis of tubulysin analogs as well as preliminary results from both *in vitro* tubulin polymerization assays and data in selected cell lines. Our efforts will help facilitate the development and design the tubulysins as potential new anticancer agents targeting tubulin.

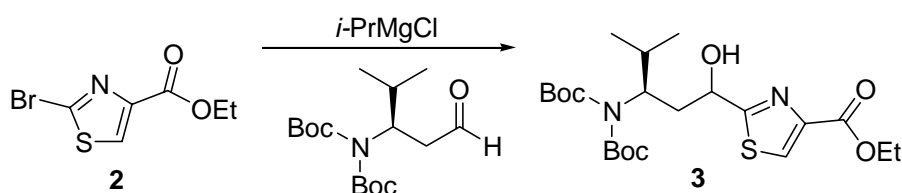
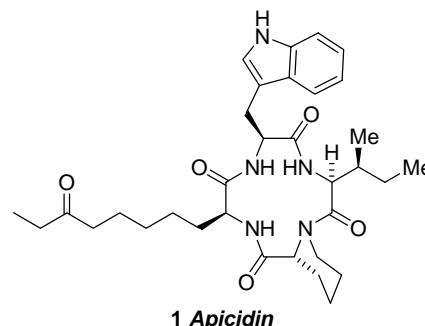


Figure 2. Synthesis of Tubuvaline

Alkene Metathesis in the Synthesis of Novel Cyclic Tetrapeptide Pharmacophores: Towards the Synthesis of Apicidin

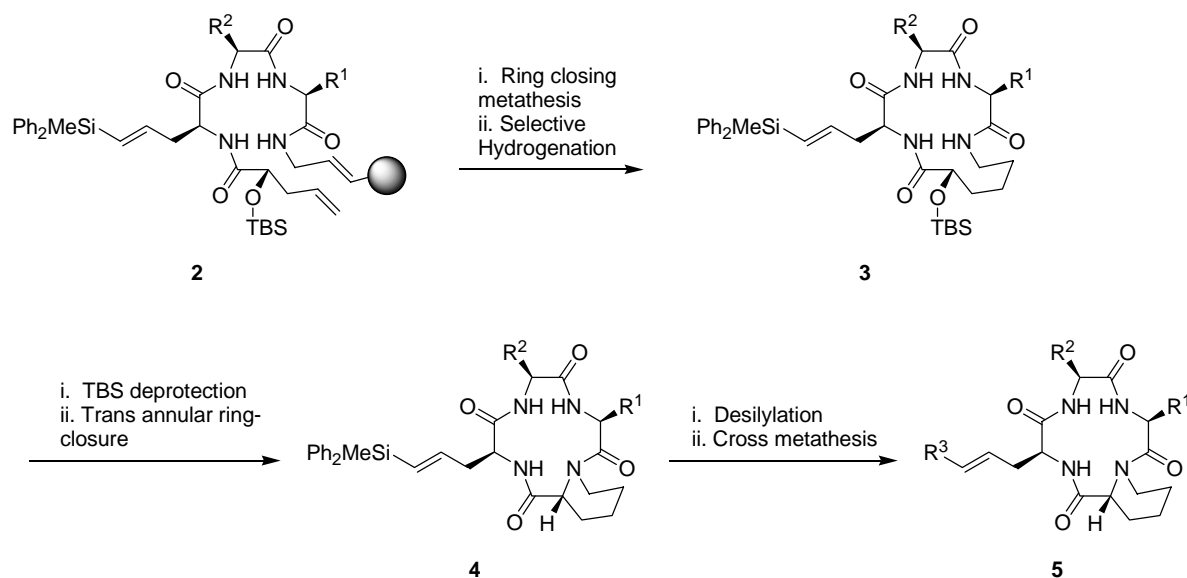
Prashant H. Deshmukh, Imperial College London, Exhibition Road, London SW7 2AY
Supervisors : Prof. Anthony G. M. Barrett (Imperial College London) and Dr Panayiotis A. Procopiou (GSK)

Apicidin (**1**), a potent, reversible inhibitor of histone deacetylase (HDAC), is an antimalarial and anticoccidiostatic agent, isolated from *fusarium pallidoroseum*.¹ Cyclic tetrapeptides such as these offer a useful scaffold for drug development due to their conformational rigidity and resistance to proteases, and as such have been the focus of several synthetic studies. Additionally, the 2*S*-amino-8-oxodecanoic acid residue has been shown to be crucial to the biological activity of apicidin.



In our novel, general approach to **1**, selective ring-closing metathesis of **2** leads to cyclorelease from the resin, and precedes hydrogenation to give **3**. In a series of model studies, we found that vinyl silanes were inert to metathesis and hydrogenation conditions, allowing for selective manipulation of double bonds.² TBS deprotection and transannular ring closure of **3** gives the bicyclic tetrapeptide **4**. Desilylation and cross metathesis of **4** affords analogues of **5** bearing a range of functionality at R³ in a late stage diversification strategy. Incorporation of different R¹ and R² sidechains into the tetrapeptide synthesis could provide variation of the scaffold. It is envisaged that this route should circumvent side reactions inherent in macrolactam bond formation, a strategy used in previous syntheses of **1** and its analogues.

General strategy for the synthesis of apicidin (**1**) and its analogues



- (a) Darkin-Rattray, S. J.; Gurnett, A. M.; Myers, R. W.; Dulski, P. M.; Crumley, T. M.; Allocco, J. J.; Cannova, C.; Meinke, P. T.; Coletti, S. L.; Bednarek, M. A.; Singh, S. B.; Goetz, M. A.; Dombrowski, A. W.; Polishook, J. D.; Schmatz, D. M. *Proc. Natl. Acad. Sci.* **1996**, *93*, 13143.; (b) Singh, S. B.; Zink, D. L.; Polishook, J. D.; Dombrowski, A. W.; Darkin-Rattray, S. J.; Schmatz, D. M.; Goetz, M. A. *Tetrahedron Lett.* **1996**, *37*, 8077.
- Schultz-Fademrecht, C; Deshmukh, P. H.; Malagu, K.; Procopiou, P. A.; Barrett, A. G. M. *Tetrahedron* **2004**, *60*, 7515.

Poster # 20

STUDIES TOWARD THE TOTAL SYNTHESIS OF LACTONAMYCIN. Pavé, G.*, Henderson, D.; Collier, P., Barrett, A. G. M. Imperial College London, South Kensington, SW7 2AZ, London, UK. g.pave@imperial.ac.uk

In the continual fight against bacterial infection, the natural product lactonamycin **1** (Figure 1) shows appreciable activity against a number of Gram-positive bacteria, in particular methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE).¹ Its novel structure, incorporating a densely oxygenated west side containing a 2-deoxy sugar bound through a tertiary glycosidic bond and a naphtho[*e*]isoindole ring to the east side, coupled with its biological activity makes lactonamycin a perfect candidate for a total synthesis programme.

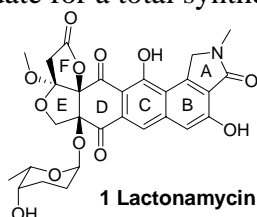
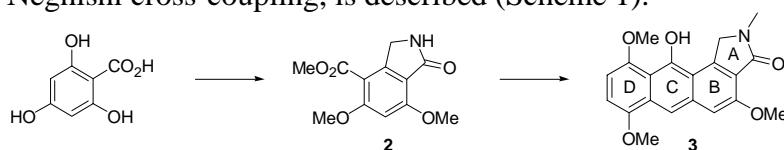


Figure 1

Synthesis of the ABCD ring system

In the quest to construct the Eastern tetracyclic portion (ABCD ring system) of lactonamycin a number of model studies have been carried out. The current, most successful approach utilising Negishi cross-coupling, is described (Scheme 1).

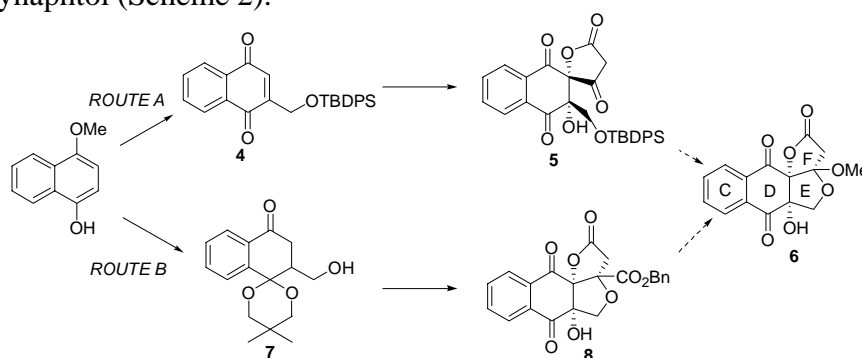


Scheme 1

Starting from 2,4,6-trihydroxybenzoic acid, lactam **2** was generated, which could be converted to the tetracyclic system **3**.

Synthesis of the CDEF ring system

Concerning the Western part (CDEF ring system), two routes were envisioned starting from 4-methoxynaphthol (Scheme 2).



Scheme 2

Following route A, the naphthol was converted to the protected alcohol **4** which could be converted to the spirocyclic lactam **5**. A simple deprotection should afford the CDEF ring system **6**. On the other hand, route B shows the synthesis of alcohol **7**, which was transformed into ester **8**. This last lactone is a good candidate to obtain the expected tetracycle **6**.

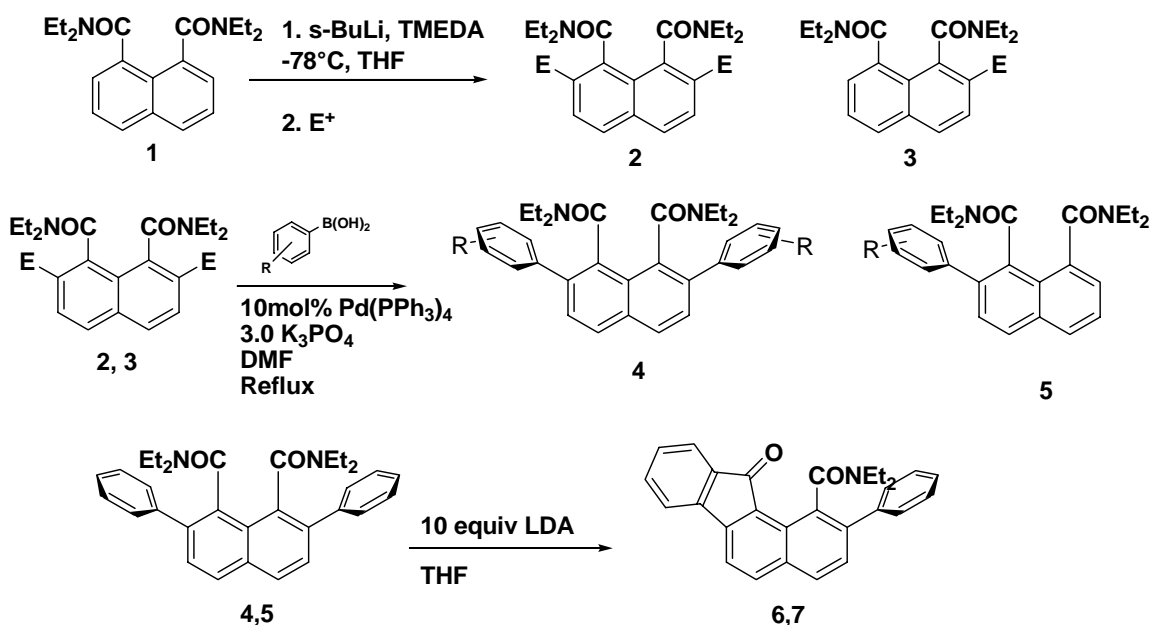
The strategy and synthesis of each compound will be discussed.

¹ Matsumoto, N.; Tsuchida, T.; Nakamura, H.; Sawa, R.; Takahashi, Y.; Naganawa, H.; Iinuma, H.; Sawa, T.; Takeuchi, T.; Shiro, M. *J. Antibiot.* **1999**, 52, 276

Poster # 21

DIRECTED *ortho* METALATION – CROSS COUPLING METHODOLOGY OF NAPHTHALENE 1,8-BIS-DIETHYLAMIDE Jones, C.C.V. * Snieckus, V.A. Queen's University, Department of Chemistry, 90 Bader Lane, Kingston, Canada K7L 3N6 chrisj@chem.queensu.ca

Metalation of the readily prepared naphthalene 1,8-bis-diethylamide **1** leads, after electrophilic quench, to mono- and bis- substituted products **2**, **3** as a function of conditions. The scope and limitations of this new methodology and cross coupling chemistry of **2** and **3** and subsequent Directed remote Metalation (DreM) of the cross coupled products **4**, **5** will be described. Mechanistic studies concerning mono-and/or di-anion formation will be presented. Rotational barriers studies leading to the synthesis of potentially chiral 1,8-disubstituted naphthalenes will also be described.



Poster # 22

IMPROVED SOLUTION AND SOLID PHASE PREPARATION OF HYDROXAMIC ACIDS FROM ESTERS. Eric Strobel*, Chih Y. Ho, Janet Ralbovsky and Robert A Galemmo, Jr., Johnson & Johnson Pharmaceutical Research and Development, Welsh and McKean Roads, Spring House, Pennsylvania, 19446-0776, estrobe1@prdus.jnj.com

The addition of small amounts of solid KCN to solution and solid phase esters in THF: MeOH: 50% aqueous NH_2OH increases the efficiency of their transformation to the corresponding hydroxamic acids.

EVALUATION OF THE *N,N*-DIETHYLSULFAMATE AS A NEW DIRECTED *ORTHO* METALATION GROUP. Macklin, T. K.* and Snieckus, V. *Queen's University, Kingston, ON, Canada, K7L 3N6*, tmack@chem.queensu.ca

The prototype *N,N*-diethylphenylsulfamate **1** has been established as a new directed metalation group (DMG). Its similarity to the established *O*-carbamate DMG (OCONEt₂) has prompted investigation of the scope of the look-both-ways metalation (**2**), its potential as a partner in transition metal catalyzed cross coupling reactions with aryl organometallics to develop a new route to contiguously substituted aromatics (**3**). Furthermore, aryne formation of *ortho*-lithiated and -magnesiated *O*-sulfamates at higher temperatures allow trapping with furan to yield adducts **4** which lead to functionalized naphthalenes. Most recent results aimed at adding the *N,N*-diethylsulfamate to the DMG family will be presented.

