



**Trip Report:  
Discovery and Selection of Successful Drug Candidates  
Hilton Boston Back Bay, Massachusetts  
March 15– 18, 2005**

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***Abstract.** This ACS ProSpectives conference was hosted by Andrew Combs (Incyte), Gregory Roth (Abbott) and Peter Huggins (ACS). The conference included a total of 21 lectures, each 45 to 60 min long, and one evening poster sessions. The total count of attendees was limited to 200 participants.*

*Below are presentation abstracts and a list of topics presented during the conference. A copy of the program is available for review to anyone at AMRI.*

**“Discovery of Orally Active Chemokine Receptor-1 Antagonists,”***Geraldine Harriman (Millenium Pharmaceuticals).*

The chemistry and biology of a novel series of pyridobenzoxepines as antagonists of chemokine receptor-(CCR)1 and development of MLN3897, a candidate for phase I clinical trial, was presented. Chemokines belong to the superfamily of proteins whose primary function is to control leukocyte activity and trafficking through tissues. Increased expression of chemokines and their respective receptors are associated with inflammatory diseases and autoimmune and allergic reactions. CCR-1 is one of twenty seven known CC chemokine receptors and is a member of the G protein-coupled receptor (GPCR) family. This receptor is expressed on the surface of monocytes, basophils, and activated T-memory cells. Both CCR1 and its ligands have been implicated in chronic inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, and psoriasis. GPCRs as therapeutic targets have historically been important and highly druggable but chemokine receptors as large protein binders will potentially involve complex binding interactions (80-120 amino acids). Identified were three different strategies:

- Development of modified chemokines that bind but do not activate (e.g. truncated MCP-1, Met- or APO-Rantes) which would predictably lead to PK and production issues;
- Blocking of monoclonal antibodies, which historically has been difficult with GPCRs (e.g. MLN1202, a CCR2 antibody which is currently in phase II of clinical trials);
- Non-peptide, small molecule antagonists, which allow for high-throughput screening, oral administration and cheaper production costs but posed the challenge of identifying desired and sufficient interaction with CCR1 receptors.

The CCR1 receptor physiology was closely examined for sufficient scientific target rationale:

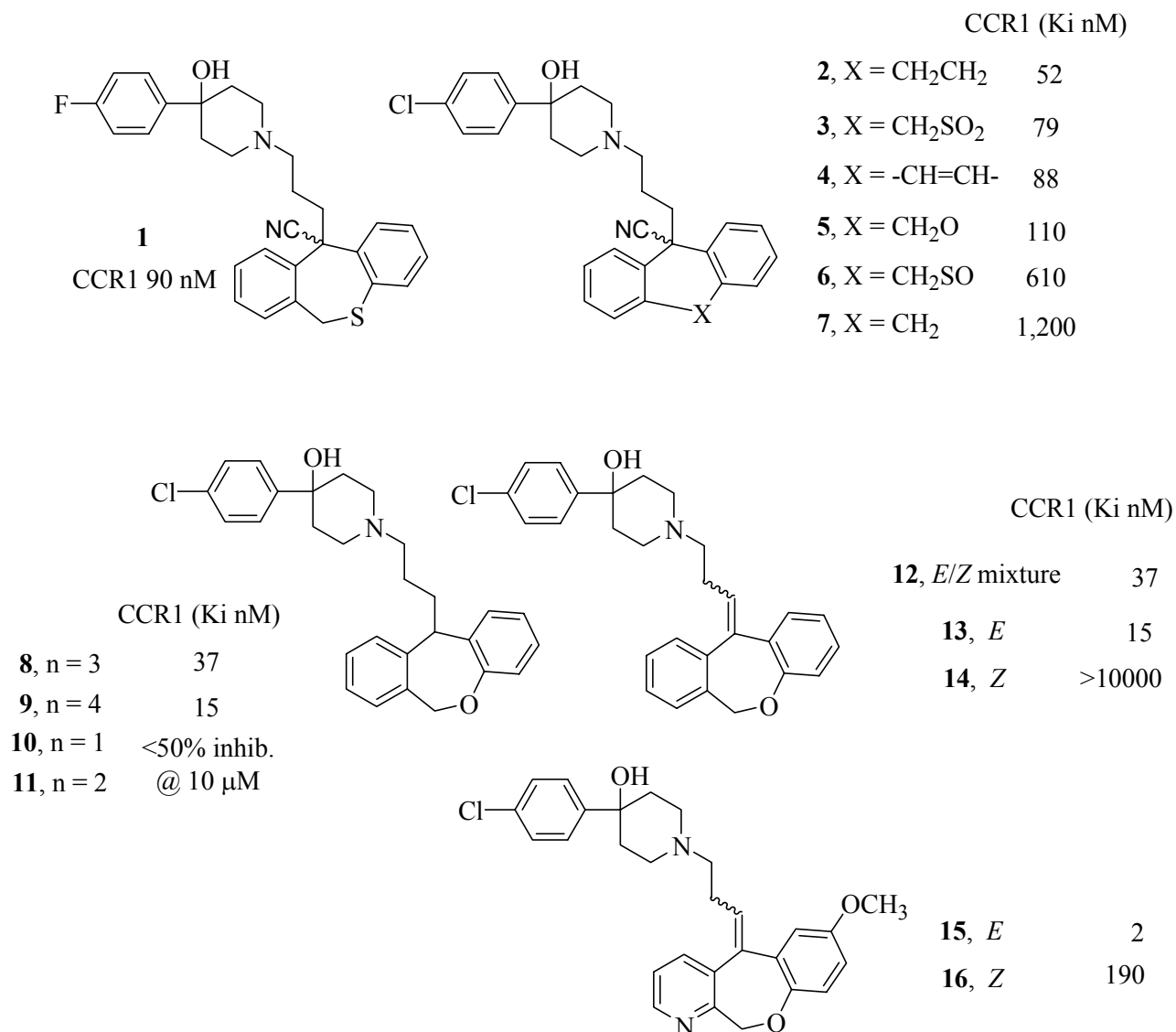
- Binding of CCR1 receptor occurs for MIP-1a and RANTES as well as for other, minor chemokines.
- CCR1 receptors are widely expressed in mononuclear cells. High levels are found on monocytes and basophils, and variable levels on eosinophiles. MIP-1a and RANTES responses are completely blocked by CCR1 mAb. Low levels of CCR1 receptors are found in CD45RO+ and CD26+ peripheral memory T cells. Human neutrophils show low basal level which can be upregulated. Human B cells and NK cells possess low to absent levels of CCR1 receptors.
- CCR1 receptors mediate chemotaxis and degranulation of monocytes and basophils, as well as migration of some activated T cells.
- The distribution of expression in rodents and some other species is not the same as in humans.

A close look at rheumatoid arthritis (RA) revealed a presence of

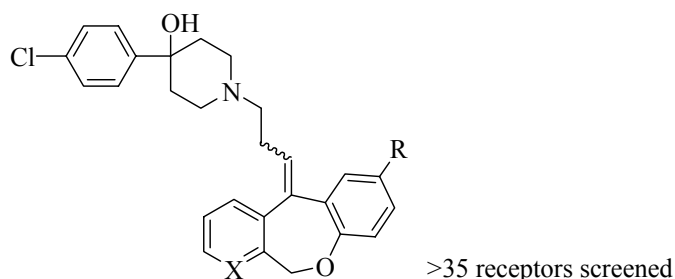
- Intensely immunoreactive mononuclear cells present within the synovial connective tissue of RA patients, morphologically consistent with monocytes/macrophages and dendritic cells
- The concentration of MIP-1a and RANTES ligands are elevated in RA synovial samples versus normal samples.
- Literature examples report that Anti-RANTES antibody reduces disease symptoms in rat AIA model.
- MTX treatment significantly lowered the serum levels of RANTES, GRO-a, and MCP-1.
- A positive significant correlation was reported between RANTES SF levels and the mononuclear migratory index in RA SF, the Ritchie Articular Index, and pain. High throughput screening identified tricyclic piperidines as hits effective in <sup>125</sup>I-MIP-1 $\alpha$  radioligand binding and CCR1 bearing THP membranes assays, FLIPR, Chemotaxis, and whole blood receptor occupancy assays as well as ADME and PK profiling, chemokine and receptor selectivity, and in vivo efficacy/recruitment models.

As initial lead a 90 nM CCR1 inhibitor was identified (**1**); in vitro SAR was optimized by variation of dibenzothiepine ring structure, olefine geometry, and introduction of azacycles (Figure 1).

**Figure 1**



Receptor selectivity and species cross-reactivity proved problematic and were optimized in high throughput screening. Substituted pyridobenzoxepines provided desired high selectivity for CCR-1 over D2 (Table 1).

**Table 1**

X	R	E/Z ratio	CCR1 Ki (nM)	D <sub>2</sub> binding Ki (nM)
CH	H	1:1	37	107
CH	CN	1:1	64	100
CH	CO <sub>2</sub> CH <sub>3</sub>	1:1	21	102
CH	CO <sub>2</sub> Bu	1:1	140	99
N	OCH <sub>3</sub>	E only	1.1	59
N	OC(CH <sub>3</sub> ) <sub>2</sub> CON(CH <sub>3</sub> ) <sub>3</sub>	E only	0.32	1100
N	OCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> OH	E only	0.43	21% @ 10 μM
N	OC(CH <sub>3</sub> ) <sub>3</sub> OH	E only	0.29	18% @ 10 μM

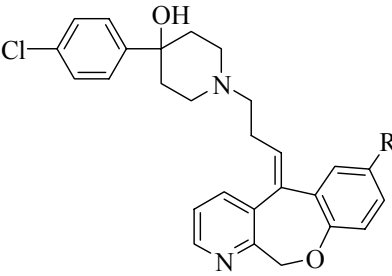
In search for an in vivo model for CCR-1 guinea pigs were identified as the species with best cross-reactivity to humans (Table 2). However, the guinea pig disease model is not predictive due to differences in the CCR-1 expression pattern between species: monocytes/macrophage subsets in humans and monocytes/macrophages and neutrophils in guinea pigs. This limits the in vivo model to a MIP-1 $\alpha$  skin recruitment model to predict efficacy.

**Table 2: IC<sub>50</sub> (nM)**

Compound	Human (THP-1)	Rat (PMN)	Pig (PMN)(PBMC)	Dog (PMN)(PBMC)	Guinea Pig (PMN)	Cyno (PBMC)	Rhesus (PBMC)
MLN013	0.5	6.128	71,000, 2.846	150, 1,520	1.1	-	32,400
MLN029	7.8	16.925	3,953, 347	110, 1,470	10.6	109,000	80,900
MLN065	0.6	12.377	11,833, 429	60, 132	9.4	5,700	12,700

The profile of a late stage antagonist for CCR-1 is shown in figure 2. Millenium announced beginning of phase I clinical trials for MLC3897 in December 2003.

Figure 2

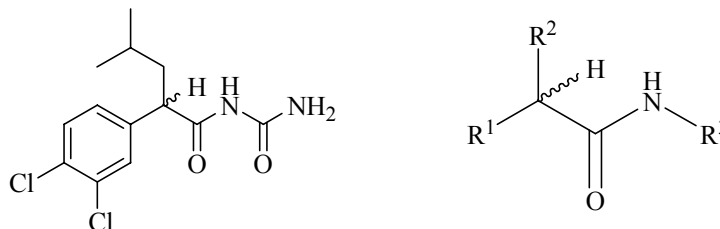
	<p>huCCR1 binding <math>K_i</math>: 0.29 nM  huPBM chemotaxis <math>IC_{50}</math>: 1.43 nM  Specificity: &gt;1,000 fold selectivity over &gt;40 receptors  In Vivo Efficacy (recruitment): Guinea pig MIP-1a skin recruitment: <math>ED_{50}</math>: 0.03 mg/kg, po; PD <math>t_{1/2}</math>: 19.8 hr; joint recruitment <math>ED_{50}</math>: 0.036 mg/kg, po  PK (rat, 10 mg/kg, po) plasma <math>t_{1/2}</math>: 3.03 h; plasma <math>C_{max}</math>: 175 ng/mL (<math>t_{max}</math>: 2h  Plasma <math>AUC_{0-\infty}</math>: 1240 ng/mL  F= 35.8% (100% in dog)  PK (rat, 1 mg/kg, iv) clearance: 2.89 L/h/kg, <math>V_{des}</math>: 3.69 L/kg  Hu Serum Protein Binding: 95.6%  Hu Microsomal Stability: 86% remaining @ 10 min.  No significant inhibition in P450 inhibition profile.</p>
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<http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/12-22-2003/0002079641&EDATE>

### “Discovery of Allosteric Activators of Glucokinase,”

Robert F. Kester (Hoffmann-La Roche Inc.).

Glucokinase (GK), also known as hexokinase IV or D, is one of for hexokinase isozymes that metabolize glucose, utilizing ATP for the phosphorylation of glucose to glucose 6-phosphate. GK has a limited cellular distribution, found predominantly in the pancreas and liver. GK acts as a molecular sensor in the pancreatic  $\beta$ -cells. Comparing to hexokinases A, B, and C it has higher  $K_m$  for glucose (7.5 mM vs. 0.04, 0.13, and 0.02 respectively). It is not inhibited with G-6-P, while other isozymes are. Glucokinase activators (GKAs) address two of the three major type 2 diabetic pathophysiology, i.e. reduced  $\beta$ -cells function and excessive hepatic glucose production. They will improve post-prandial glucose control, increase hepatic glucose uptake, and decrease hepatic glucose production thus will lead to better glucose control and reduced morbidity. Glucokinase assay has been developed and HTS of 65,000 compounds led to one hit compound (Figure 1).

**Figure 1**

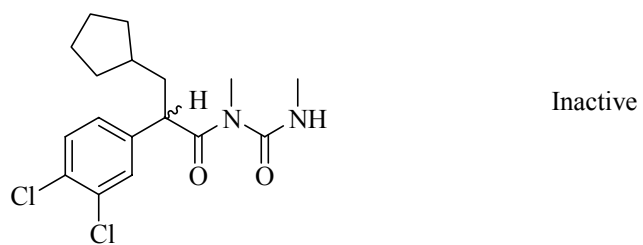
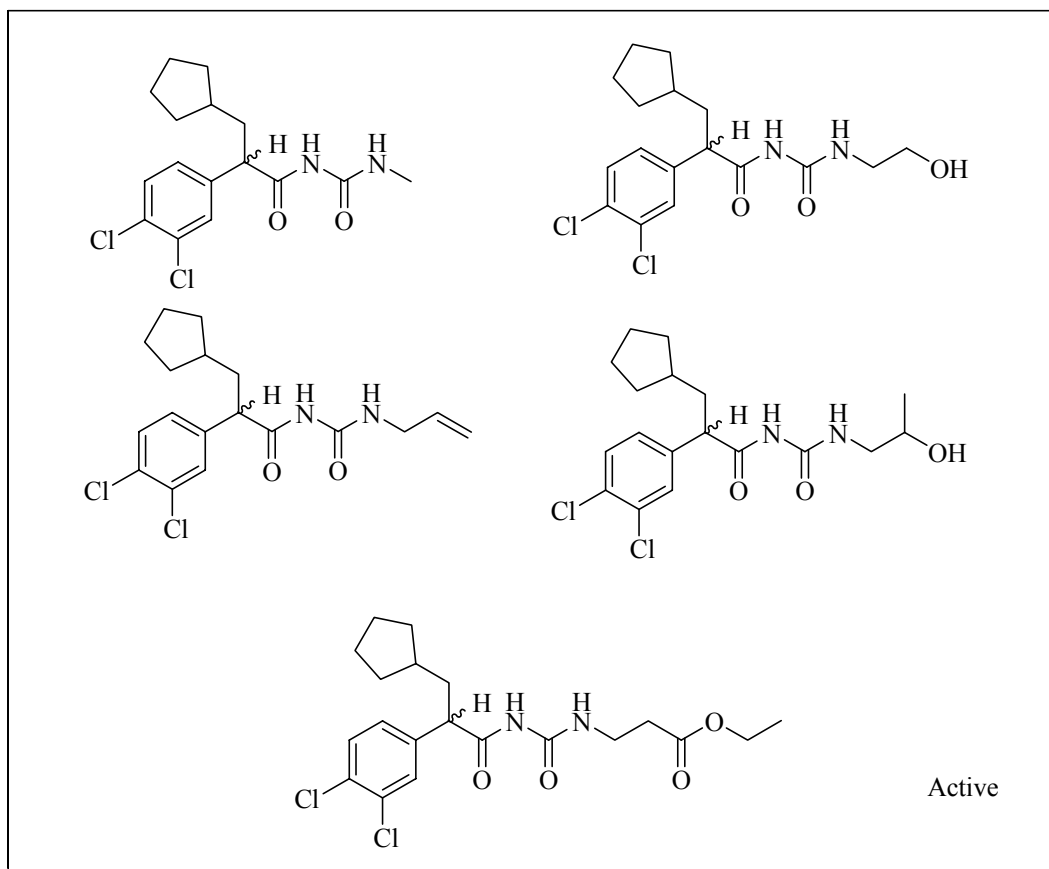
SC<sub>1.5</sub> = 21.7 μM  
MW = 303.19

**Chemistry strategy for lead optimization included SAR around R<sup>1</sup>, R<sup>2</sup>, and R<sup>3</sup>, and clarifying role of the chiral center.**

Lead optimization around R<sup>2</sup> substituent showed SC<sub>1.5</sub> < 30 μM for R<sup>2</sup> = *i*-Bu, methylene-cyclopropyl-, -cyclobutyl-, -cyclopentyl-, -cyclohexyl-, and -cycloheptyl-substituents. Benzyl- and phenethyl-derivatives were less active.

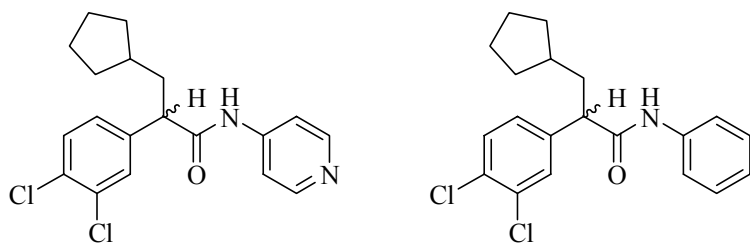
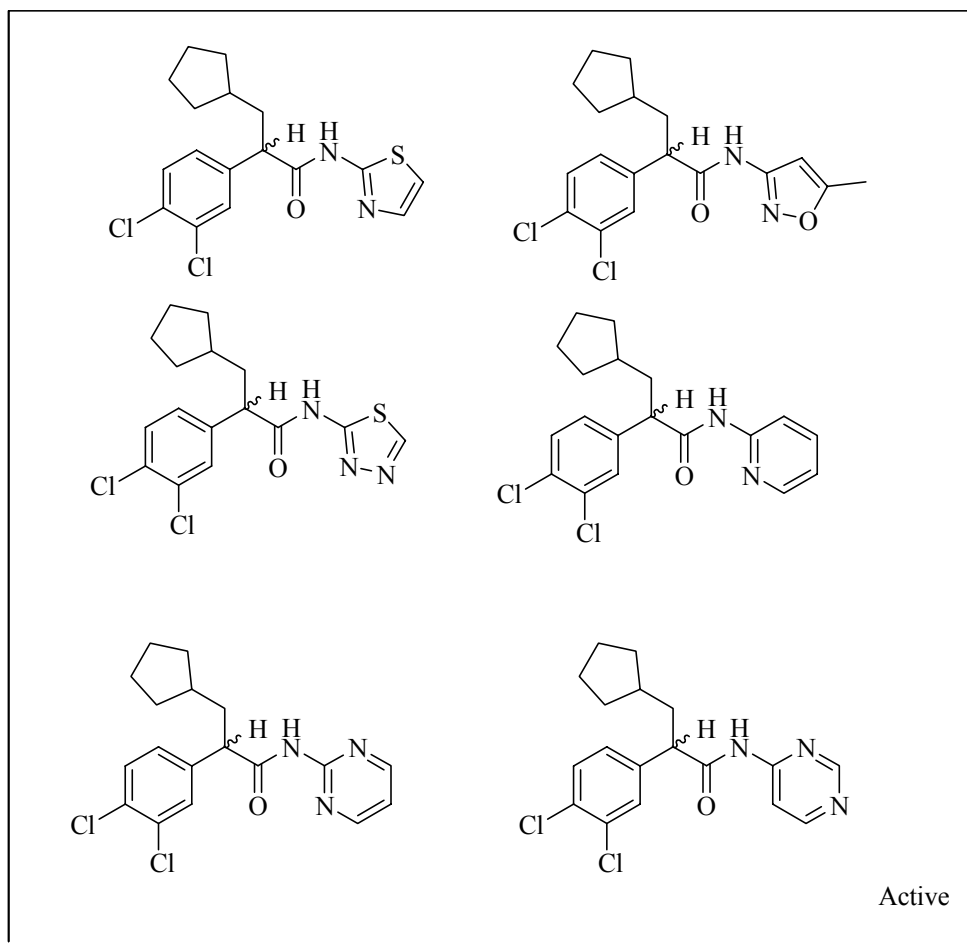
Examples of active R<sup>3</sup> urea type compounds are depicted in Figure 2.

Figure 2



Further optimization included synthesis of heterocyclic compounds isosteric to ureas (Figure 3).

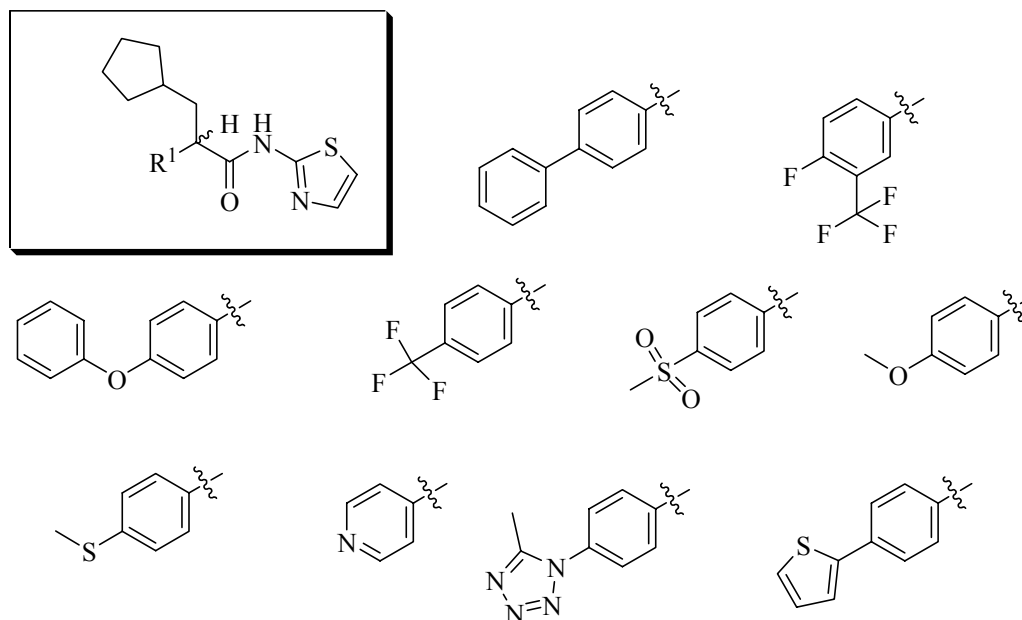
Figure 3



Inactive

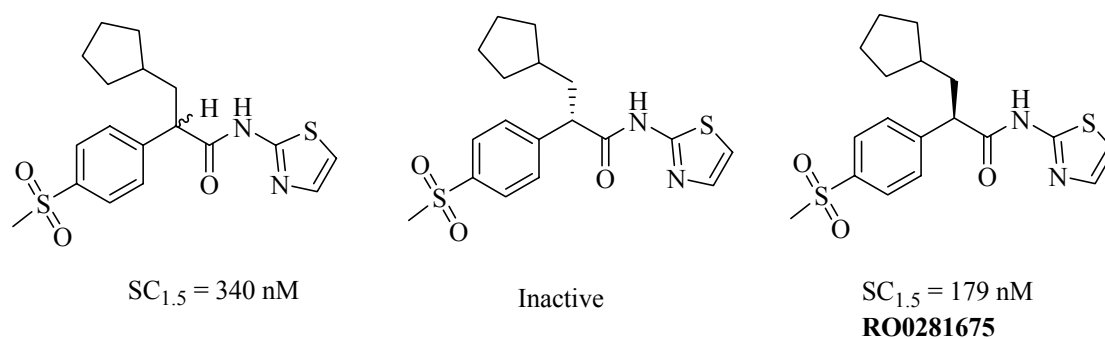
Lead optimization on site R<sup>1</sup> included the following substituents, which appeared to enhance activity (Figure 4).

Figure 4



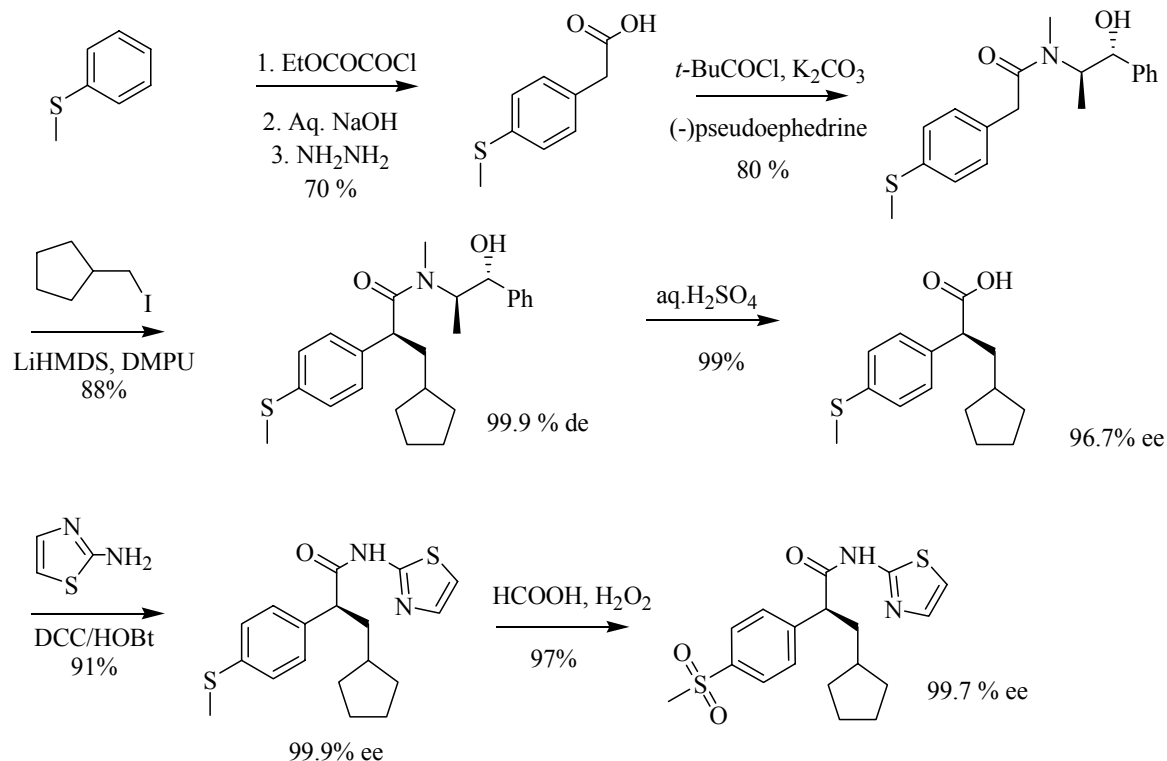
Stereoselective activation of GK provided strong evidence for the specific binding site (Figure 5).

Figure 5



Further development of RO0281675, which demonstrated “drug-like” properties and could be further formulated to obtain stable and bioavailable product, included route towards its asymmetric synthesis (Scheme 1).

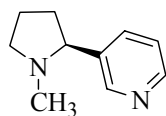
## Scheme 1



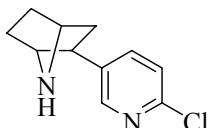
### “The Search for Subtype-Selective Neuronal Nicotinic Receptor Agonists for the Treatment of Chronic Pain,”

*Michael R. Schrimpf (Abbott Laboratories).*

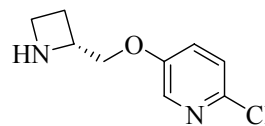
A significant and growing area of unmet medical need for therapeutics for chronic pain was identified in this presentation. Over 50 million people suffer from chronic pain in the U.S. alone and nearly 10 million from neuropathic pain patients worldwide. Existing pain medication, e.g. AEDs, Tricyclics, Tramadol, Babapentin, Pregabalin, and Duloxetine, show limited efficacy and, with exception of Tramadol and Duloxetine, poor tolerability. Opioid-like efficacy without debilitating side-effects is the ultimate goal. Discovery that the potent analgesic effects of natural product epibatidine are mediated by neuronal nicotinic receptors (NNRs) has stimulated intense interest in chronic pain research. A first generation NNR analgesic, ABT-594, exhibits broad-spectrum, opioid-like analgesic activity in animal models and was modeled after nicotine and epibatidine. Epibatidine is 200fold more potent than morphine in acute pain models and not inhibited by naloxone, an opioid antagonist (Table 1).

**Table 1**

Nicotine



Epibatidine



ABT-594

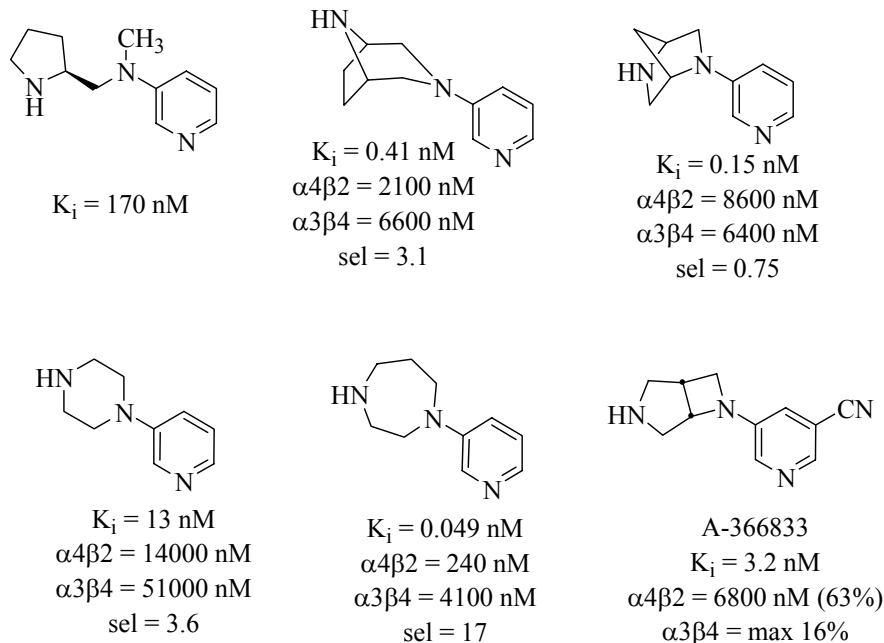
	Inflammatory Pain		Moderate to Severe		Neuropathic Pain	
	Chronic (CFA)	Acute (Carrageenan)	Persistent (Formalin)	Skin Incision	Chung	Bennett
ABT-594	+++	+++	+++	+++	+++	++
Morphine	+++	+++	+++	+++	+++	+++
Ibuprofen	+++	+++	++	0	+	++
Celecoxib	+++	+++	+	0	+	++
Gabapentin	+++	0	++	++	+++	+++

+++ is >70% efficacy; ++ is 30-70% efficacy; + is <30% efficacy; 0 is no activity

ABT-594 demonstrated efficacy in non-neuropathic pain states and robust, dose dependent efficacy in diabetic neuropathic pain participants in phase II clinical trial. Limited tolerability through gastrointestinal side effects, such as nausea, emesis, and dizziness led to high discontinuation rate at higher doses and an unacceptable therapeutic index. However ABT-594 is the first drug to have established clinical confirmation of a nicotinic mechanism for neuropathic pain. It also validated the use of the Chung model for prediction of neuropathic pain and initiated the development of a strategy for better separation between analgesia and GI side-effects.

Experiments in knockout mice implicate  $\alpha 4$  and  $\beta 2$  nicotinic receptor subunits in acute thermal pain and  $\alpha 3$  and  $\beta 4$  subunits as mediators of adverse side effects. Second generation drugs were designed to exhibit decrease in  $\alpha 3\beta 4$  NNR agonist activity and evaluated in a ferret model. Bicyclic, bridged diamines were identified as potent NNRs with poor  $\alpha 4\beta 2/\alpha 3\beta 4$  selectivity and evolved into mono- and bicyclic diamines and with good selectivity and high potency (Figure 1).

Figure 1



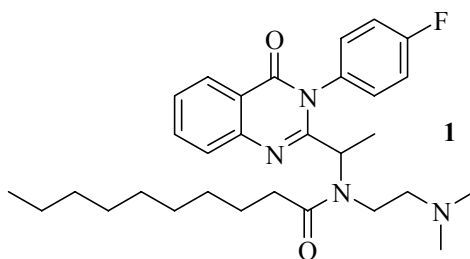
A-366833 evolved as the prototype subtype-selective NNR: a selective partial agonist with good PK, low metabolism, high bioavailability, broad analgesic efficacy and an overall 20-fold improved therapeutic index vs. ABT-594.

### “Discovery of AMG 487: A Potent and Selective CXCR3 Antagonist”

*Julio C. Medina (Amgen).*

CXCR3 chemokine receptors were identified in 1996 and are expressed in activated T cells, such as CD4+ or CD8+ predominantly in the Th1 phenotype. But also activated B cells, NK cells, malignant T (NHL) and B (CLL) cells, plasmacytoid monocytes, DC11c+ dendritic cells, GMCSF-stimulated CD34+ progenitors, endothelial cells, neurons and astrocytes are sources of CXCR3 receptors. CXCR3 receptors are known to bind the following chemokines: MIG, IP-10, and ITAC. These chemokines are selective agonists of CXCR3, produced in inflamed tissues and unregulated by Th1 cytokines. CXCR3 receptors are over expressed in Th1-associated human diseases, e.g. psoriasis, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. Animal models provide evidence that CXCR3 receptors mediate lymphocyte trafficking to diseased tissue: blockage of the receptor reduces cellular recruitment and disease severity. As neutralizing antibodies were identified: anti-IP-10, anti-MIG, and anti-CXCR3 mAb from various murine and cardiac allograft models. Knockout mice models are available, e.g. cardiac allograft, viral-induced autoimmune diabetes, graft-versus-host disease, minor MHC class I mismatch. The dihydro-quinazoline **1** (Figure 1) was identified as a potent CXCR3 antagonist and early chemical lead compound.

Figure 1



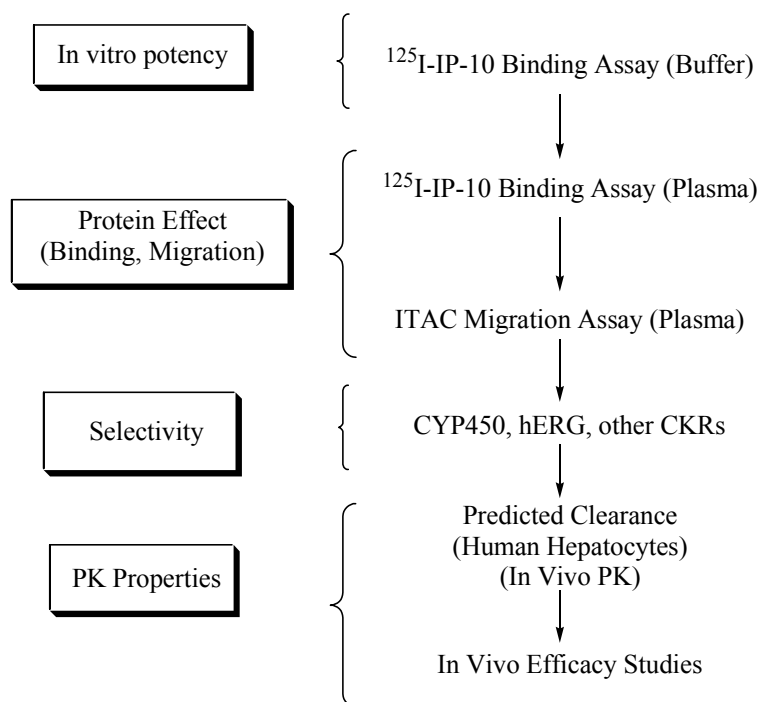
IC<sub>50</sub> (binding, <sup>125</sup>I-IP-10) = 610 nM

IC<sub>50</sub> (Ca<sup>2+</sup> mobil., ITAC) = 500 nM

Rat Cl = 3.6 L/kg/h, F = 1.5%

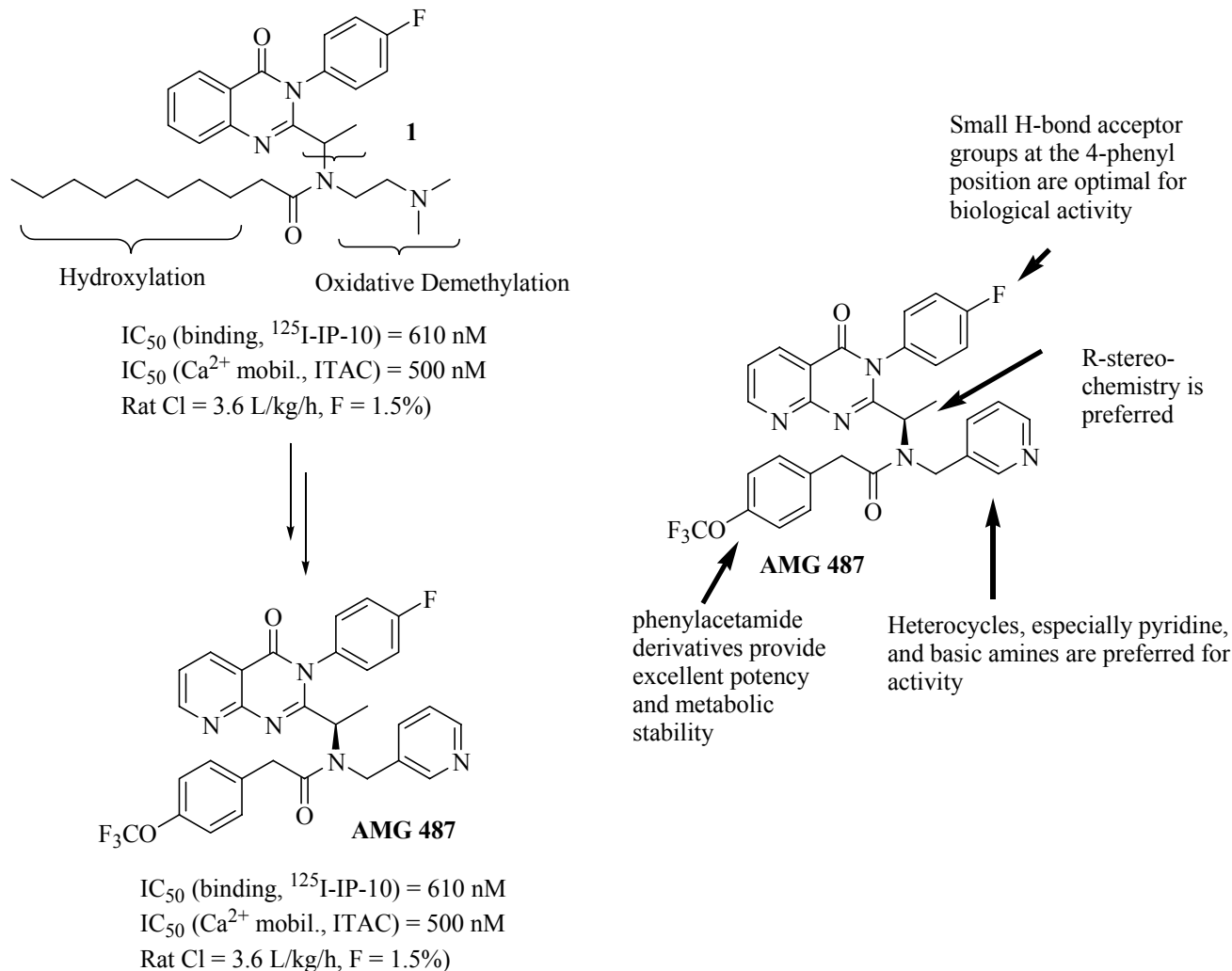
Selection of lead compounds proceeded through a regimen of evaluation: in vitro potency, protein effect (binding, migration), selectivity and PD property (Figure 2).

**Figure 2**



In vitro metabolism of compound 1 by S9 human liver digestive enzymes indicated the following chemical processes: hydroxylation of the carbon side chain and oxidative dealkylation of the amid and amine. SAR of follow up compounds designed AMG 487 as potent CXCR3 antagonist (Figure 3) which was entered in phase 1 clinical study in 2002 and phase 2a psoriasis trial in 2003.

Figure 3



### Lecture Topics:

1. Drug Discovery; Where Have We Been and Where Are We Going: Paul S. Anderson (Consultant)
2. Discovery of Orally Active Chemokine Receptor-1 Antagonists: Geraldine Harriman (Millennium Pharmaceuticals)
3. The Identification of Leads for Drug Discovery: John W. Ellingboe (Wyeth Research)
4. Technology Enabled Synthesis: A New Paradigm for Lead Optimization: Craig Lindsley (Merck & Co., Inc.)
5. Design, Synthesis and Optimization of Privileged Structure Based Ligands: John P. Mayer (Eli Lilly & Company)

6. Drug Design Circa 2005: Some Lessons Learned: Mark Murcko (Vertex Pharmaceuticals)
7. Discovery of Allosteric Activators of Glukokinase: Robert F. Kester (Hoffmann-La Roche Inc.)
8. Structure-Based Design of Serine Protease Inhibitors: Discovery of Novel Cathepsin G and Chymase Inhibitors: Michael N. Greco (Johnson & Johnson)
9. Lead Selection Criteria in Advancing Novel Bicyclic and Tricyclic  $\beta$ -Lactamase Inhibitors: Terek Mansour (Wyeth)
10. An Evaluation of the [Phe out] Binding Mode for Kinase Inhibitors: Neil Moss (Boehringer Ingelheim Pharmaceuticals, Inc.)
11. The Search for Subtype-Selective Neuronal Nicotine Receptor Agonists for the Treatment of Chronic Pain: Michael R. Schrimpf (Abbott Laboratories)
12. Establishing and Maintaining COX-2 Selective Inhibition While Addressing ADME Challenges: Lawrence Black (Abbott Laboratories)
13. hERG Modeling and Nyktuoaraneter optimization: Roy Vaz (Sanofi-Aventis Pharmaceuticals)
14. Decreasing ADME/Tox Attrition in Drug Discovery: Scott J. Grossman (Bristol-Myers Squibb Co.)
15. Discovery of AMG 487: a Potent and Selective CXCR3 Antagonist: Julio C. Medina (Amgen)
16. Discovery of Novel ( $\alpha/\gamma$ ) Dual PPAR Activators: Peter T.W. Cheng (Bristol-Myers Squibb Co.)
17. Discovery of Telavancin, a Rapidly Bactericidal Antibiotic for the Treatment of Serious Gram-Positive Infections: Michael R. Leadbetter (Theravance, Inc.)
18. "Design" of Zetia<sup>TM</sup> (Ezetimbe<sup>TM</sup>) a Novel Inhibitor of cholesterol Absorption: Stuart B. Rosenblum (Schering-Plough Research Institute)
19. Successful Discovery and Development of New Drugs in Cancer and Virology: Carl P. Decicco (Bristol-Myers Squibb Co.)
20. Case Study of Marketed Drugs & Strategies: Robert W. Armstrong (Eli Lilly & Company)
21. The Discovery and Development of LIPITOR<sup>®</sup> (Atorvastatin calcium): Bruce D. Roth (Pfizer Global Research and Development)