



Trip Report for
ACS ProSpectives Course
“Advances in Structure-Based Drug Design”

San Francisco, California
September 9-11, 2007

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Abstract: *The 2007 ACS ProSpectives course was held on September 9-11, 2007 in San Francisco, California. This conference brought together structural biologists, computational chemists and medicinal chemists with the shared goal of furthering structure-based drug discovery. The topics of the presentations included: structural biology in drug discovery, computational tools for structure-based design, application of structure-based design: structure, models and medicinal chemistry, and fragment-based drug design, and structure-driven medicinal chemistry. This report will highlight some interesting topics and discussions presented at the meeting.*

“Can Docking Method Be Used to Rank Order Protein-Ligand Binding Affinities: New Results Using the Glide Extra Precision Scoring Function?”

Richard Friesner (Columbia University), New York, NY 10027

Dr. Richard Friesner of Columbia University gave a talk on the development of a new empirical scoring function for calculation of the binding affinities of protein-ligand complexes. Implemented in the Glide docking program as Glide Extra Precision (XP), this new function contains novel terms, such as an explicit treatment of hydrophobic enclosure of the ligand by the protein, and identification of special hydrogen bonding regions, which provides substantially improved accuracy as compared to standard scoring functions.

Dr. Friesner demonstrated by data that, assuming accurate prediction of the structure of the protein-ligand complex, the most recent version of Glide XP is capable of ranking ordering diverse compounds, with an average error of ~1 kcal/mole, using global parameters that can be applied to an arbitrary target. This represents a significant advance as compared to scoring functions previously described in the literature.

Dr. Friesner further discussed the novel terms in the XP scoring function, or “special” hydrogen bonds. Normal protein-ligand hydrogen bonds can contribute to binding affinity because releasing water molecules into solution can yield a gain in entropy and/or enthalpy. However, there will be structures in which a water hydrogen bonded to a protein donor or acceptor has great difficulty making additional hydrogen bonds. This is most likely to occur when there is hydrophobic enclosure of the local region surrounding the water in question. Displacement of such water with the ligand can yield exceptional contributions to binding free energy. XP Glide detects such hydrogen bonding patterns and assigns additional free energy terms based on fitting to experimental data.

Recent results were preliminary but very encouraging and involved the use of inhomogeneous solvation theory to calculate binding free energy differences between pairs of factor Xa ligands.

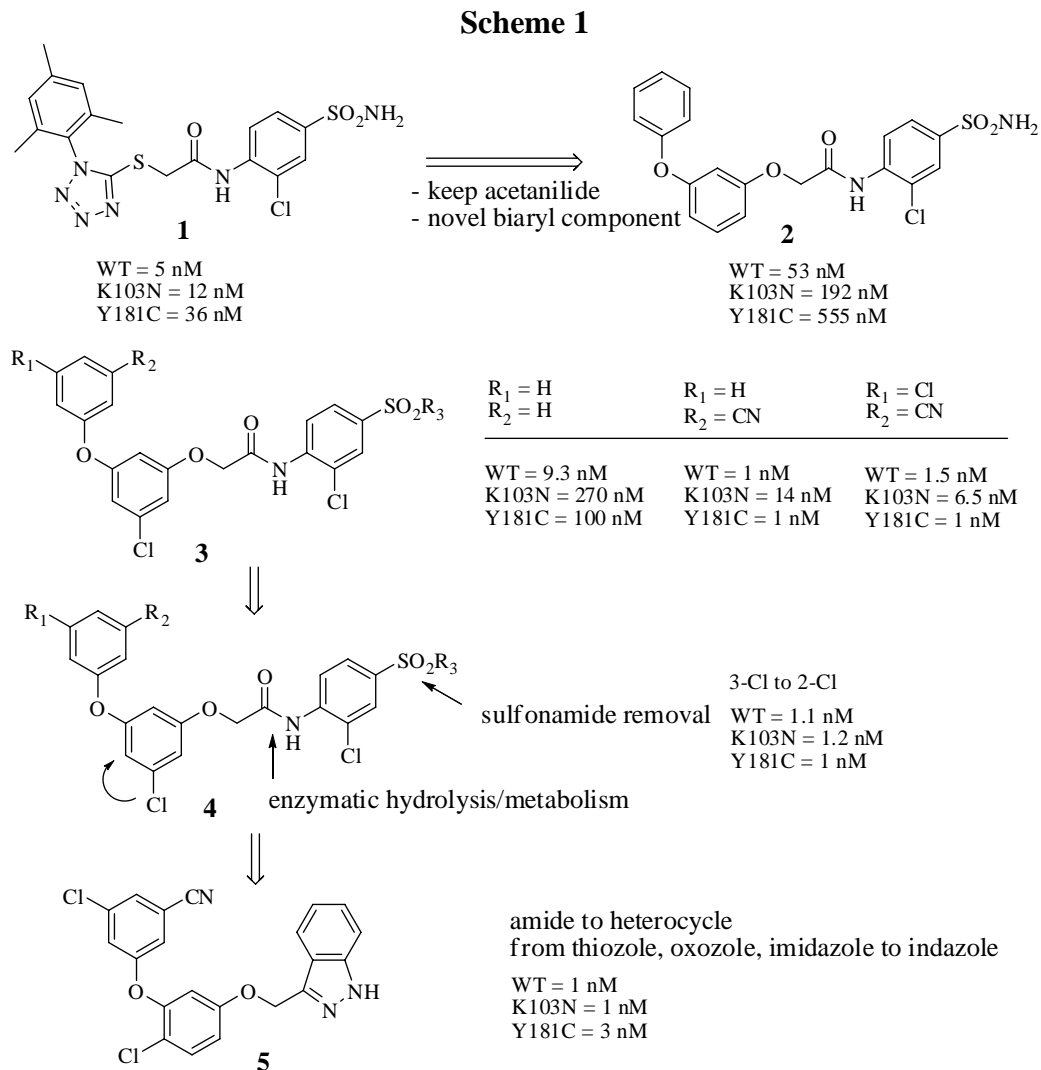
“The Design and Synthesis of Novel Second Generation HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)”

Thomas J. Tucker (Merck Research Laboratories), West Point, PA, 19454

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) have been shown to be a key component of highly active anti-retroviral therapy since the 1990's. Currently, there are three commercially available NNRTIs and their use has become part of standard combination antiviral therapies, producing clinical outcomes with efficacy comparable to other antiviral regimens.

However, clinical resistance has become a critical issue and therefore novel NNRTIs with a broad spectrum of activity against key HIV-1 RT mutations are in demand. Dr. Tucker presented a topic regarding the NNRTIs program at Merck that is directed toward finding novel NNRTIs. The presentation described the design and development of the new series

of compounds from early lead structures to mature second generation NNRTIs, using a combination of medicinal chemistry/SAR analysis, crystallography, and molecular modeling. Scheme 1 illustrates such efforts guided by the tools listed above, from the early lead **1** to the next generation structure **5** for further optimization.



“Ligand Efficiency and Fragments: Trends and Physical Interpretation”

Charles H. Reynolds (Johnson & Johnson Pharmaceutical R&D), Spring House, PA, 19477

Ligand efficiency (LE) has become an important concept in drug discovery. For any ligand of a target protein, its efficiency can be roughly represented as the ratio of potency to size, measured by the number of heavy atoms present in the ligand.

Dr. Reynolds presented a work that studied a large number of protein-ligand complexes (>8000) in order to develop representative statistics for ligand efficiencies across a variety of targets and molecular weight ranges. In his talk, the following points were discussed:

- LE used as a metric to measure whether a ligand is potent simply because it has many contacts with a protein, or because of a more optimal fit.
- If all substituents contribute equally to binding, the relationship between potency and size should be linear.
- Study showed nonlinear relationship; LE drops markedly as the size increases.

The decline of LE with increasing ligand size can be explained by examining a variety of physical phenomena. It might be expected that with size increase greater rotational entropy cost, more positive enthalpy due to suboptimal fits, and inherent nonlinearity in ligand surface area available for binding play a more and more important role.

To address the issue of size dependency, adjustment can be done in two approaches: first, simply use plots to adjust expectations; secondly, adopt Fit Quality (FQ). FQ score has the following properties:

- Provides a consistent metric for small and large molecules;
- Average fit FQ = 0.68;
- FQ > 0.95 are exceptionally efficient;
- Can filter out inefficient (nonspecific) binders;
- Provide a more level comparison for fragments and large ligands;
- Also good for lead optimization.

Dr. Reynolds lastly concluded that smaller ligands have the potential for inherently great binding efficiency than large ligands and the size-independent FQ score can be useful in evaluating how optimally small fragments, leads, and drug candidates bind.

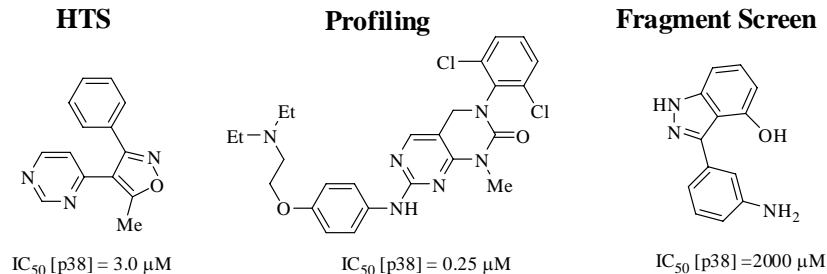
“p38 Kinase Inhibitors: From Multiple Leads to Two Drug Candidates”

Andreas Kugstatter, Roche, Palo Alto, CA

This presentation described the development of two clinical candidates for the inhibition of p38 kinase. The inhibition of p38 kinase targets rheumatoid arthritis, which is a chronic inflammation condition causing pain and ultimately joint destruction. Current therapeutic treatments block TNF (Tumor-Necrosis Factor) and IL-1 (Interleukin-1) which serve to slow disease progression. Both TNF and IL-1 are key inflammatory mediators and their production is controlled by p38 kinase. Therefore, the goal of this program was to develop a small molecule p38 inhibitor.

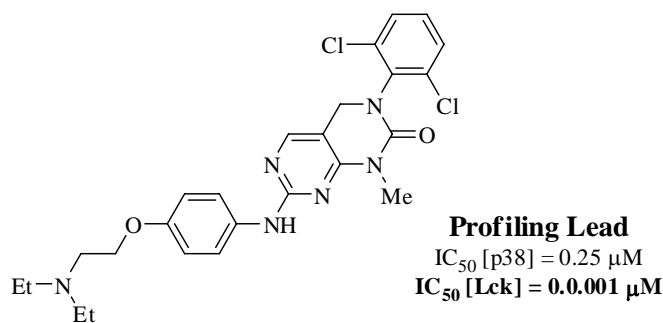
Three diverse p38 kinase inhibitors were identified from HTS (High-Throughput Screening), kinase library profiling and fragment screening as shown in Scheme 2.

Scheme 2



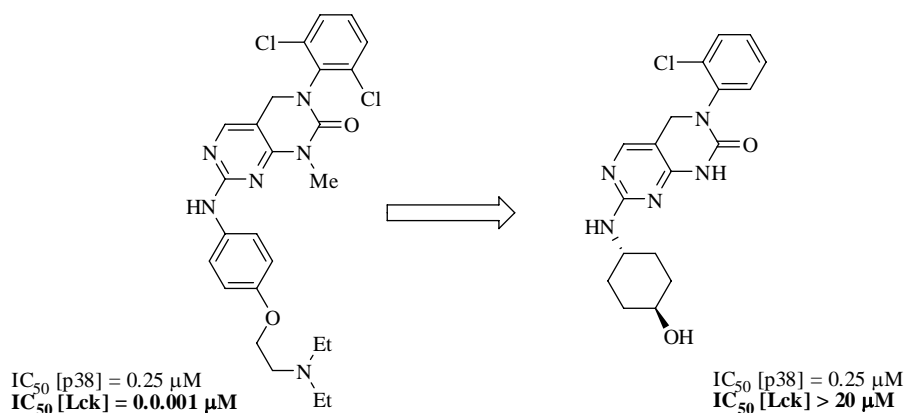
The p38 profiling lead compound (Scheme 3) was crystallized in the enzyme, which showed that the compound was bound to the ATP binding site. The crystal structure also revealed a two-point 'hinged' interaction along with a partially filled 'back pocket'. However, the profiling lead was not selective, as it showed inhibition of Lck and other tyrosine kinases.

Scheme 3



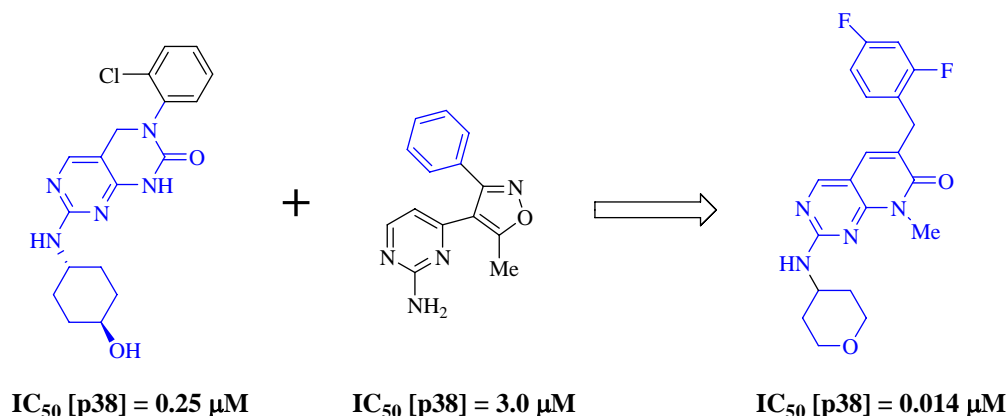
This selectivity was achieved through a structure guided approach, where saturation of the southern ring by introduction of an sp^3 center at the point of attachment enabled the ring to rotate out of the plane of the fused bicyclic system and led to improved selectivity (Scheme 4).

Scheme 4



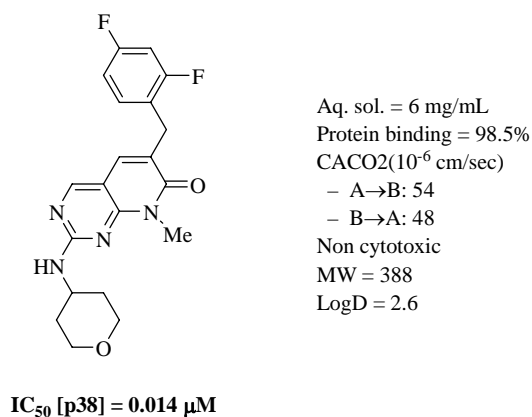
Structural features of the HTS lead were used to improve potency. The incorporation of the 2,4-difluorophenoxy fragment provided an extension of the molecule into the ‘back pocket’ of the binding site (Scheme 5).

Scheme 5



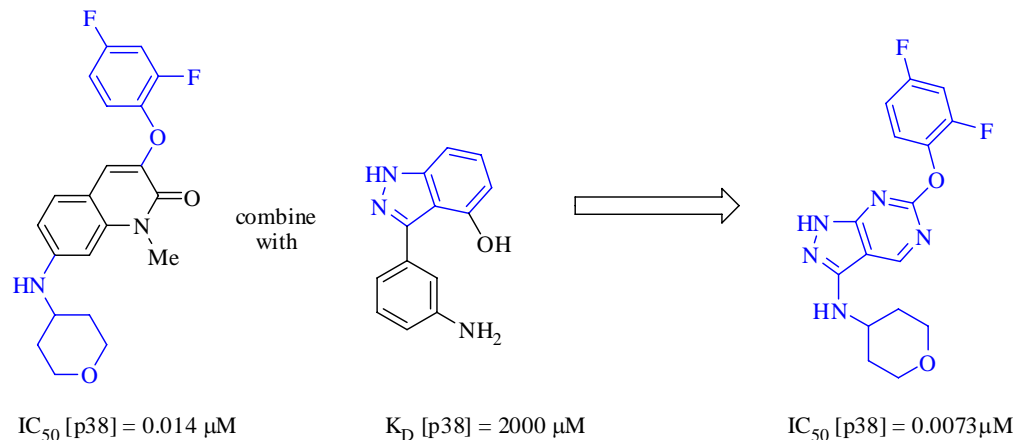
This led to the development of the first drug candidate which was both potent and selective. Clinical studies showed that there was up to 80% IL-1 inhibition in healthy volunteers after a single dose of 150 mg (Scheme 6).

Scheme 6



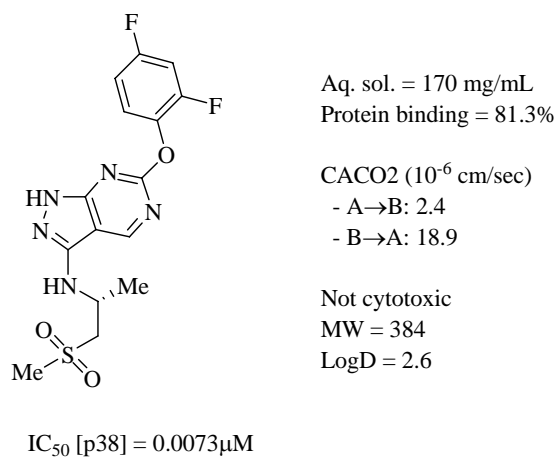
In order to develop a backup candidate, a ‘‘Scaffold Hopping’’ strategy was employed, which required the development of the Fragment screen lead compound as shown in Scheme 7. This led to an inhibitor with improvement in potency and when the X-ray structure of the inhibitor in the binding site was solved, it was shown to exhibit a three-point hinge interaction. However it also displayed suboptimal molecular properties.

Scheme 7



The aqueous solubility of the lead compound was increased by replacing the tetrahydropyran ring with a methanesulfonyl propane unit. This led to a reduction in lipophilicity and crystallinity. However, the initial studies were performed on the racemate and the enantiomers were separated but chirality not determined. The identity of the potent enantiomer was uncovered by protein crystallography which showed that the (*R*)-enantiomer was the desired one. Consequently, the second candidate was chosen with excellent kinase selectivity (Scheme 8).

Scheme 8

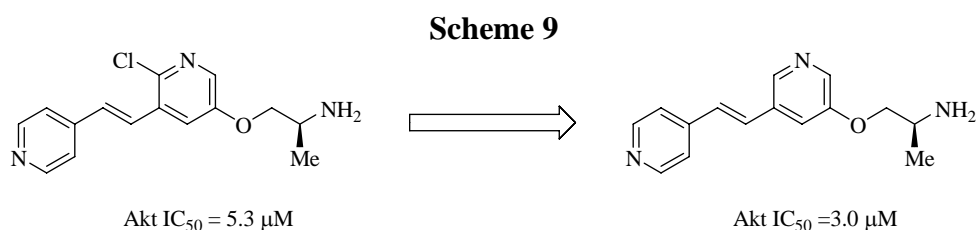


This case study showed how multiple technologies can be applied for lead generation. The structural data was integrated by morphing compounds from different leads to identify two novel p38 inhibitors for clinical studies.

“Inhibitors of Akt Kinase as Antitumor Agents”

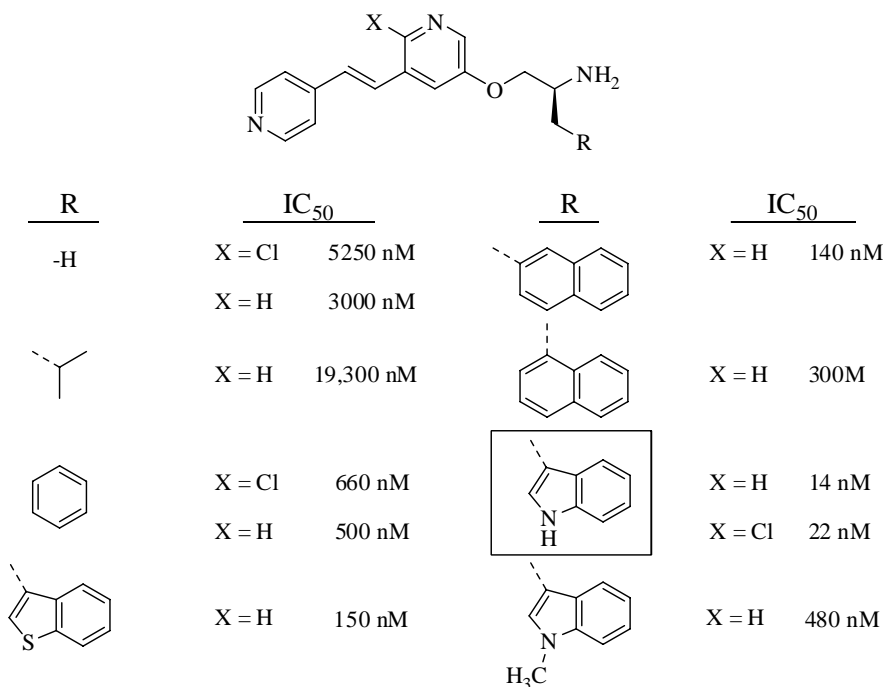
Keith W. Woods, Abbott Laboratories, Abbott Park, IL

Akt kinase, also known as Protein Kinase B (PKB), is a serine/threonine kinase. It is a member of the AGC kinase family of which there are three family members: Akt1, Akt2 and Akt3. It plays a central role in cancer cell growth and survival pathways and is overexpressed in a variety of human tumors which include lung, breast, prostate, ovarian, gastric and pancreatic. An increase expression of Akt correlates with disease progression. Akt is involved in cell survival by blocking apoptosis so the inhibition of Akt should be pro-apoptotic and lead to cell death. Therefore Akt provided an attractive target for the therapeutic treatment of a variety of cancers. Initial screening provided two hits as shown in Scheme 9.



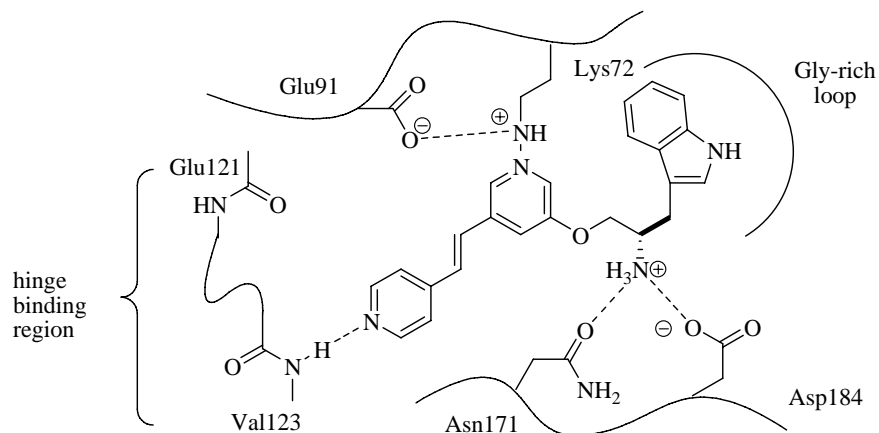
The side chain of each hit was investigated and the SAR showed that the unprotected 3-indolyl unit proved to be the most potent for both series (Scheme 10).

Scheme 10



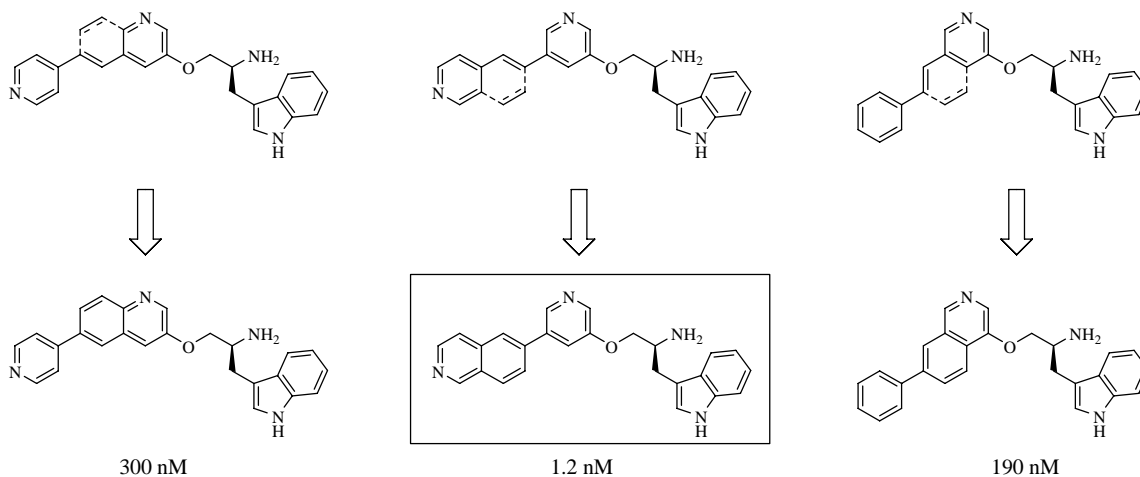
A crystal structure of compound A in the active site (PKA) showed that the glycine enriched loop had moved to incorporate the indole fragment to improve the potency. This is illustrated in Scheme 11.

Scheme 11



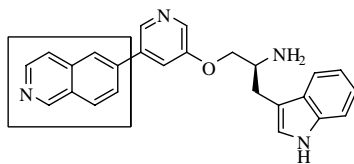
Attempts to improve the hinge interactions by replacing the 4-pyridyl styrene fragment were not successful. However, restricting the number of rotatable bonds between the pyridine rings did lead to an increase in potency Scheme 12.

Scheme 12



A study on the replacement of the isoquinoline fragment showed that the indazole unit showed similar potency and also provided a two hinge interaction in the active site (Scheme 13).

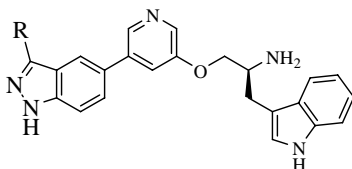
Scheme 13



	<u>IC₅₀</u>		<u>IC₅₀</u>		<u>IC₅₀</u>
	1.2 nM		1200 nM		1.2 nM
	1400 nM		230 nM		290 nM
	380 nM		580 nM		1 nM
	260 nM		2100 nM		800 nM
	400 nM		18 nM		110 nM
	7 nM				

Further optimization was achieved by incorporating indazole substitutions which showed that the 3-methyl substituent was the most effective (Scheme 14).

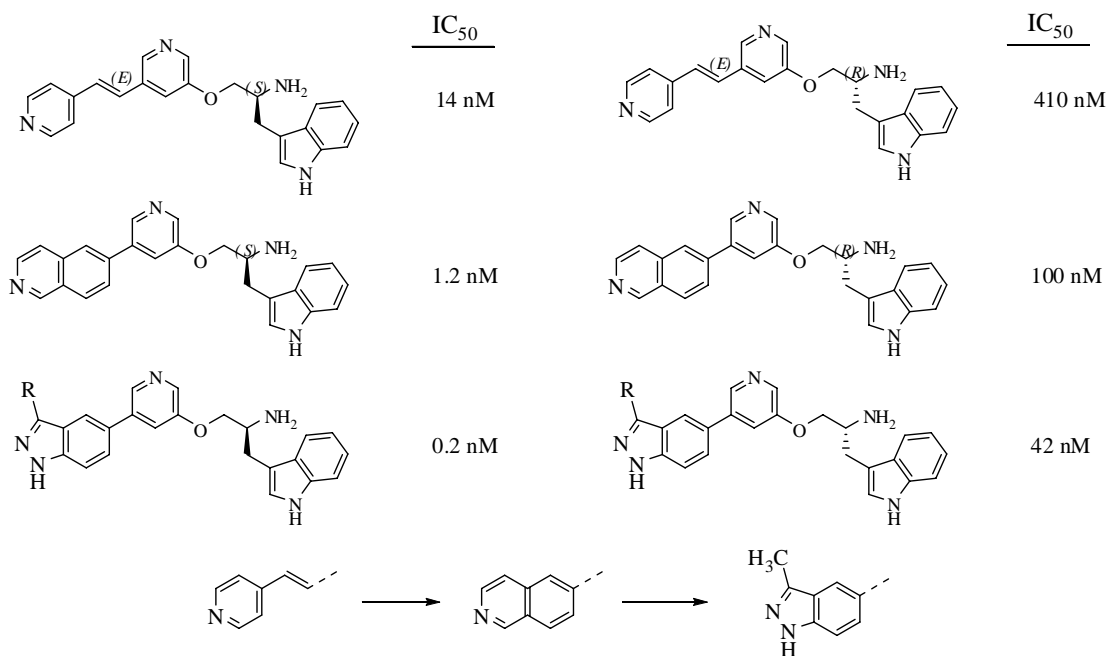
Scheme 14



<u>R</u>	<u>IC₅₀</u>	<u>R</u>	<u>IC₅₀</u>
H	1.2 nM		4.8 nM
CH₃	0.2 nM		1.6 nM
CH ₃ (and 1-CH ₃)	830 nM		1.5 nM
Et	1.4 nM		12 nM
NH ₂	3.6 nM		12 nM
N(CH ₃) ₂	22 nM		12 nM
	3.2 nM		

Further SAR studies show that the central pyridine ring was necessary for strong potency as was the primary amine functionality. It was also determined that the *S*-enantiomer was responsible for activity Scheme 15.

Scheme 15



A series of potent and selective Akt inhibitors were identified. The following key binding interactions were identified from the structural information: Hinge-binding. Central pyridine-The pyridine-lys interaction is essential. The replacement of the pyridine nitrogen resulted in loss of activity. Indole side chain-the indole side chain resides in a glycine rich pocket. Primary amine-the amine fits into the space normally occupied by Mg²⁺. Significant delay in tumor growth has been demonstrated in a mouse xenograft model.

”Discovery of Taranbant, a Potent and Selective CB1R Inverse Agonist, for the Treatment of Obesity”

Linus Lin, Merck Research Laboratories, Rahway, NJ 07090

The treatment of obesity has become a focus of attention due to the serious and chronic nature of this medical condition and it is rapidly growing worldwide. In the majority of cases, the net result has led to excessive weight gain which provides the root cause for a number of morbidities such as diabetes, hypertension, cardiovascular disease, cancer and arthritis. Despite much endeavour there are only two therapeutic agents to date with modest efficacy which have been approved for chronic use.

Clinical and animal studies have shown that the cannabinoid receptor system is involved in regulating feeding behavior. Several selective CB1R inverse agonists which include

SR141716 (rimonabant) and SLV319 have been reported to show efficacy in various models of feeding behavior and rimonabant which has been recently approved in the EU for the treatment of obesity.

This seminar provided an overview of the optimization efforts leading to the identification of taranabant which is a structurally distinct, potent, selective and highly efficacious CB1R inverse agonist. This compound is currently undergoing clinical evaluations for the treatment of obesity.

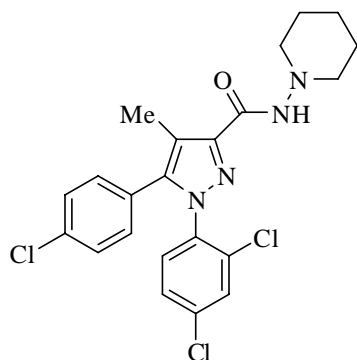
The iterative process of structure-based design relies heavily on being able to obtain a crystal structure of the receptor/ligand complex in order to initiate drug design of target compounds by organic synthesis for biological evaluation. However, in the case when a receptor/ligand crystal is not available there are other means to achieving this process. The shape of the binding pocket can be determined from the crystal structure of the ligand, a solution structure of the ligand and by mechanical calculations. The nature of interactions can be derived from the nature of the substituents on the binding pocket.

The validation of CB1R inverse agonism for the treatment of obesity was summarized as follows:

- Cannaboid Receptors are G-protein coupled receptors (Gi) with no crystal structure available. They are comprised of 1) CB1R which occurs primarily in the CNS; 2) CB2R which is found primarily in the immune cells.
- CB1R agonist stimulates appetite. THC (tetrahydrocannabinol more commonly known as Marinol) has been approved for AIDS-associated anorexia. Agonists can increase intake in rodents.
- CB1R inverse agonist reduce food intake and weight gain as shown by Rimonabant (SR141716A).
- CB1R-deficient mice are resistant to diet induced obesity.

Rimonabant is a first in class CB1R inverse agonist (Scheme 16).

Scheme 16



CB1R inverse agonist (SR141716A)

- $hCB1R_{IC_{50}} = 6 \text{ nM}$

Approved in the EU in June 06

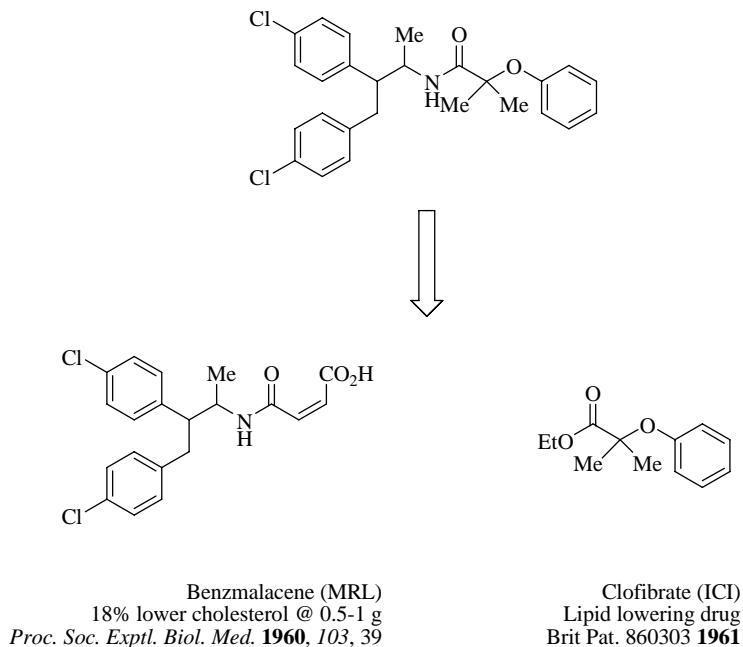
- indicated for patients with BMI > 30 kg/m²
or BMI > 27 kg/m² with associated risk factors

FDA recommended against approval in June 07

Sanofi-Aventis withdrew the application and would re-submit in the future

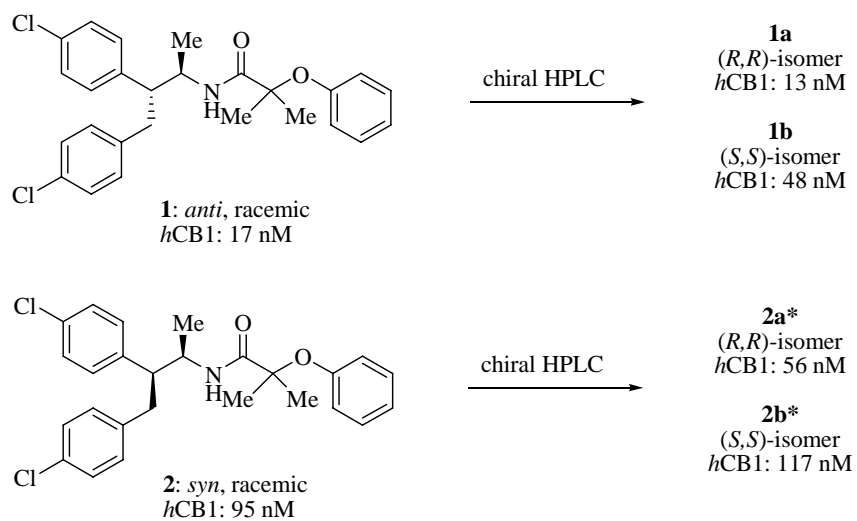
A structurally unique lead from Merck is shown in Scheme 17, which is comprised of features from Benzmalacene (Merck) and Clofibrate (ICI).

Scheme 17



Stereoisomers of the acyclic lead compound were determined by chiral HPLC separation. The absolute stereochemistry for the *syn* isomers **2a** and **2b** were tentatively assigned (Scheme 18).

Scheme 28



*Absolute stereochemistry tentative

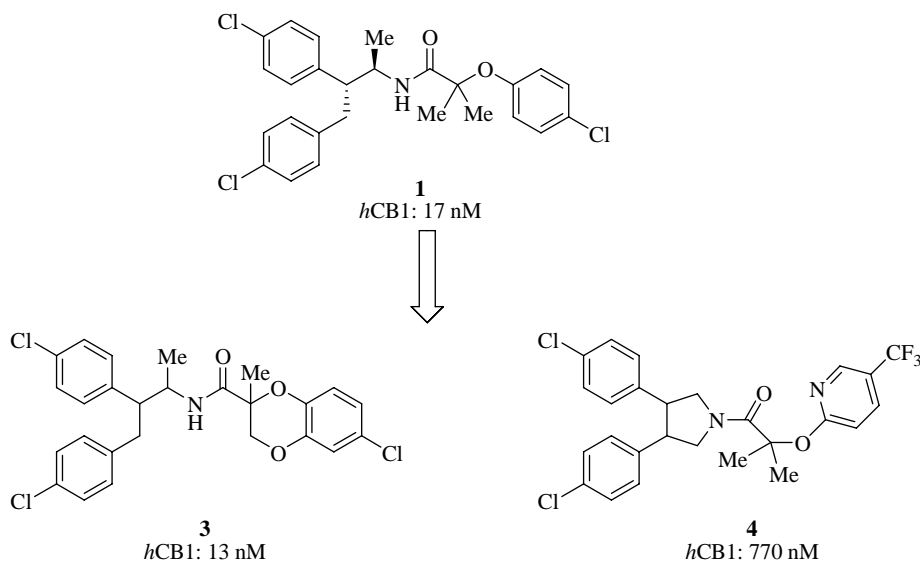
A profile of 1a vs Rimonabant highlighted areas for improvement in order to develop a candidate:

- To improve *in vitro* potency: A better understanding was required of the 3D structure of the lead class. Also needed to probe the nature of interactions of aryl substituents.
- To reduce lipophilicity/non-specific binding: Polar substituents need to be introduced which can be achieved by replacing the phenyl ring with heterocycles.
- Establish receptor occupancy / efficacy relationship.

No suitable crystals of 1a could be obtained, but an x-ray structure of the tartrate salt of the amine portion of 1a showed that the amine is conformationally rigid. Solution NMR studies supported this observation and also showed that this was enforced by non-bonded interactions, but the acid fragment was highly flexible. Molecular modeling studies showed that there was good overlay with Rimonabant. Strategies implemented would have to take into consideration the conformation constraints on the acid fragment only. Therefore the focus was to vary the nature of the substituents.

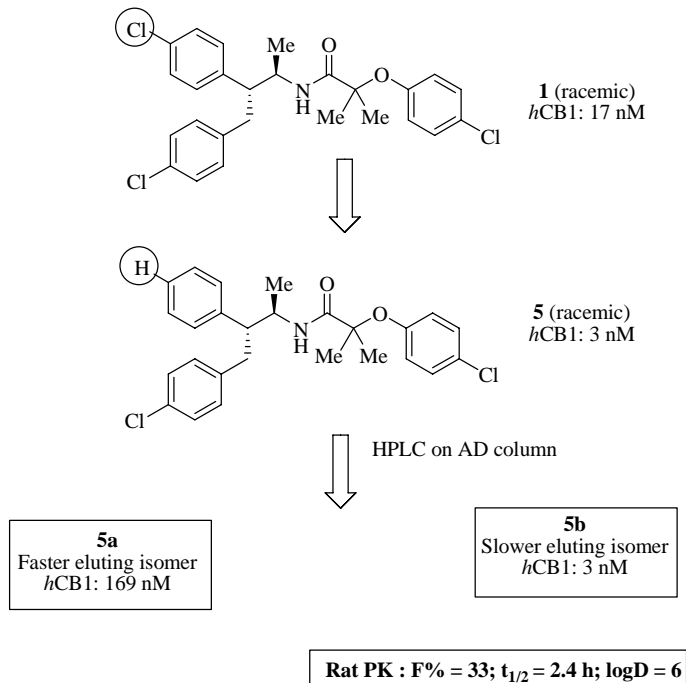
A study on the conformationally restricted analogues led to little improvement in potency as shown in Scheme 19.

Scheme 19



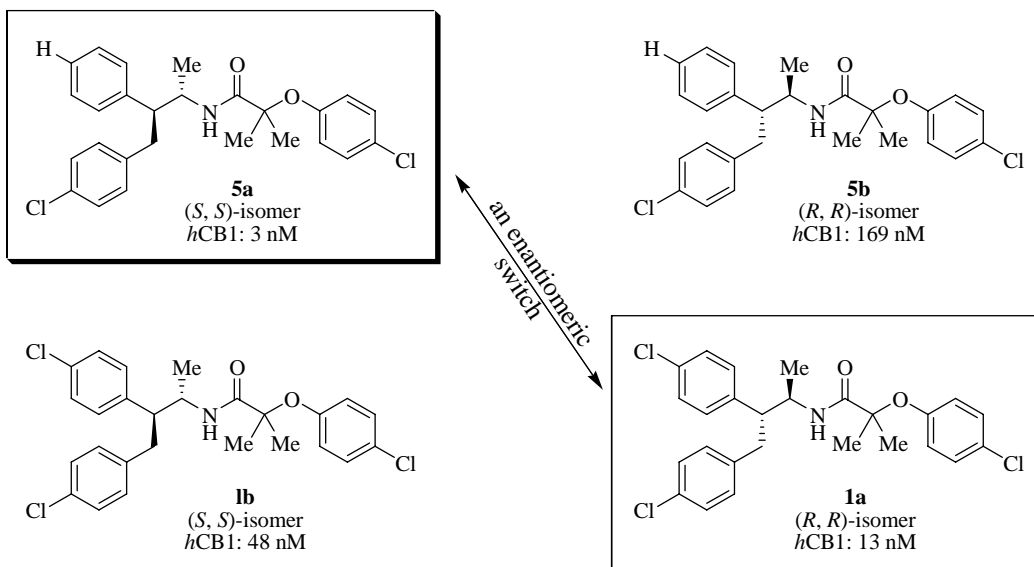
However, investigation into varying the substituents on the aryl ring led to the first breakthrough (Scheme 20).

Scheme 20



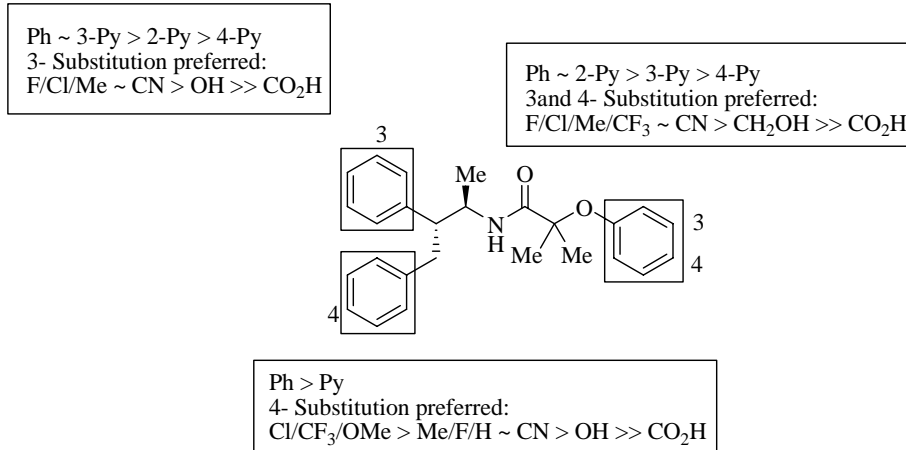
The absolute stereochemistry of compound **5a** was determined by stereospecific synthesis which showed that there was an enantiomeric switch with **1a** as shown in Scheme 21.

Scheme 21



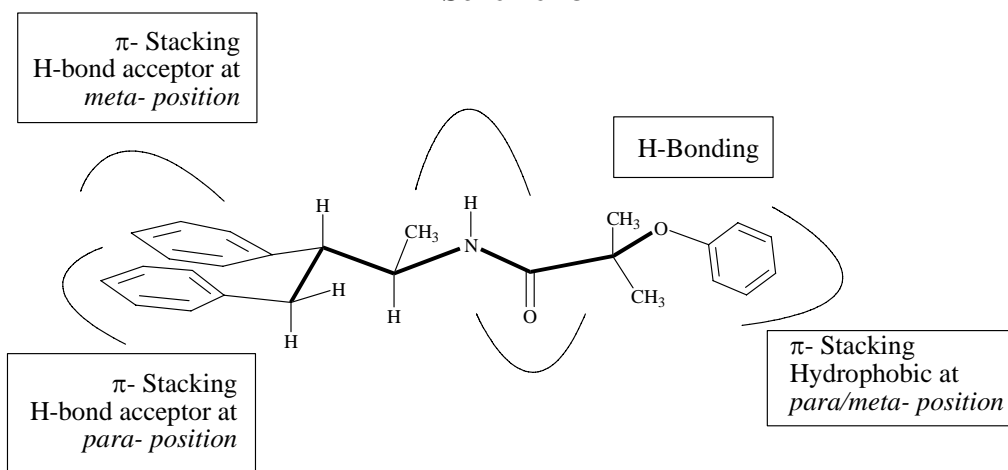
A summary of the SAR around the aryl substituents is shown in Scheme 22.

Scheme 22



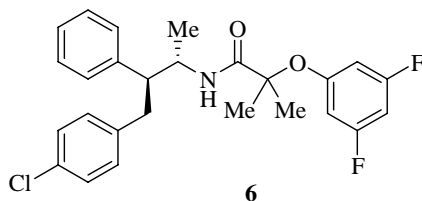
Based on the results of these SAR studies a pharmacophore of CB1R was proposed (Scheme 23).

Scheme 23



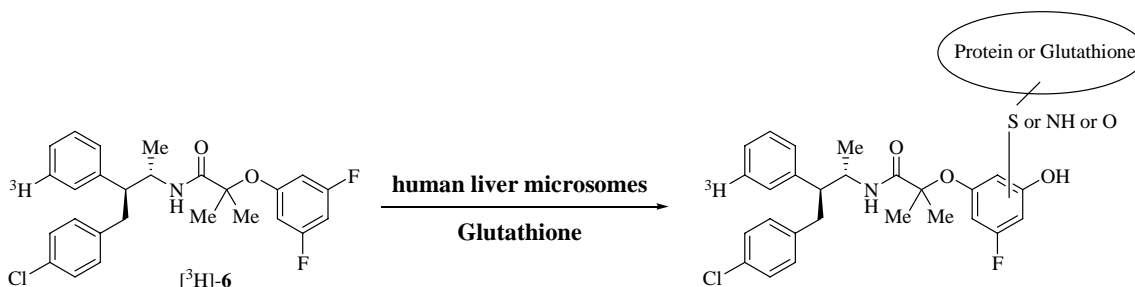
The first lead candidate chosen was compound 6 which showed better potency and comparable pharmacokinetics compared with rimonabant, but suffered from chronic efficacy in 14 day toxicology studies (Scheme 24).

Scheme 24



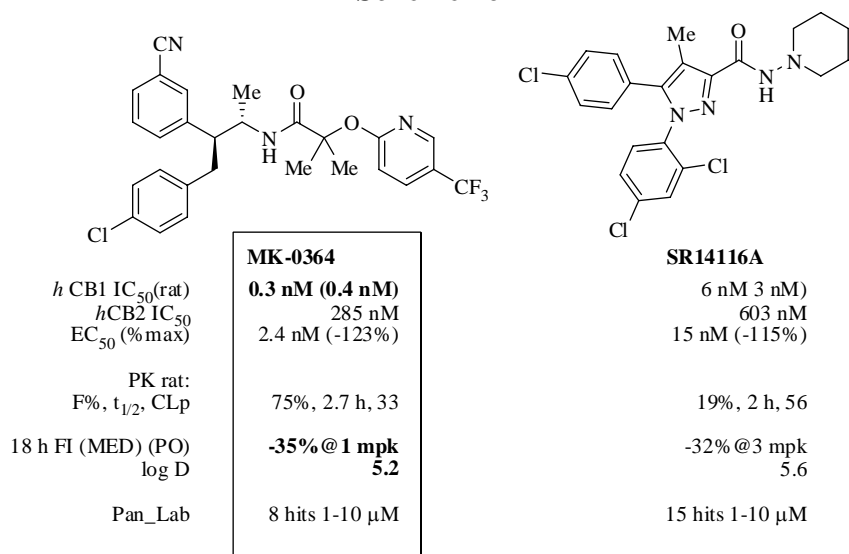
This was attributed to the formation of a reactive metabolite with occurred through the covalent binding of compound 6 with microsomal proteins. By following the metabolism of tritium labeled 6, it was determined that in the absence of GSH (glutathione) there was extensive irreversible covalent binding to microsomal proteins (1,690 pmol-eq/mg protein @ 1 h). Furthermore, in the presence of GSH, there was extensive conjugation with GSH observed through the phenoxy ring which was determined by MS (Scheme 25).

Scheme 15



In order to minimize the covalent binding of compound 6, the electron density of phenoxy ring was reduced by the substitution of a 2-trifluoromethyl-2-pyridyloxy ring and by incorporating a 2-cyano substituent on the upper phenyl ring which reduced HLM covalent binding to 27 pmol-eq/mg protein @ 1 h, which led to compound MK-0364 being selected as a clinical candidate. Merck has targeted MK-0364 for Phase III program for the treatment of obesity (Scheme 26).

Scheme 26



This case study showed that if conventional structure-based design is not feasible due to lack of crystal structure of receptor then an alternative approach is to determine the 3D structure of ligands early in the program by X-ray crystallography, solution NMR studies long with mechanical calculations. This can be combined with the systematic study of the nature of interactions and thus build a pharmacophore.