



Trip Report for

**“Residential School on Medicinal Chemistry
Chemistry and Biology in Drug Discovery”
Drew University, Madison, New Jersey
June 11-15, 2007**

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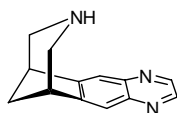
Abstract: *This week-long course is designed for chemists and biologists interested in broadening their understanding of the fundamental principals for small molecule drug discovery and development. It included 16 lectures, 3 seminars, and 5 case histories which covered topics such as target validation, assay development, hit-to-lead process, development of structure-activity relationship, pharmacokinetics, pharmacodynamics, metabolism, toxicology, and also recent successful drug discovery programs. This report highlights selected materials from the course.*

“The search for an Effective Smoking Cessation Treatment”

Dr. Jotham W. Coe, Research Fellow, Pfizer Global Research and Development

This is the story about the successful discovery and development of Varenicline (Figure 1. trade name **Chantix** in the USA and **Champix** in Europe and Canada, usually in the form of varenicline tartrate).

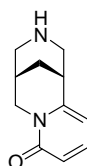
Figure 1. Varenicline Tartrate



Nicotine binds predominantly to nicotinic acetylcholine receptors (nAChRs) in the CNS, primarily the $\alpha 4\beta 2$ subtype in the Ventral Tegmental Area (VTA). After nicotine binds to the $\alpha 4\beta 2$ nicotinic receptor, it results in a release of dopamine in the Nucleus Accumbens (nAcc) which is believed to be linked to reward. When a smoker tries to quit, the absence of nicotine leads to withdrawal symptoms, which can make the smoker to start smoking cigarettes again to boost the blood level of nicotine to the level where there is no such symptoms. Thus Nicotine Replacement Therapy (NRT) may help the smoker in the course of quitting. All types of NRT deliver a measured amount of nicotine. As the nicotine dose is lowered, the tobacco user is gradually weaned off nicotine. However, the success of nicotine replacement therapy is not guaranteed. In the worst case, a tobacco user may again start smoking while he still is using NRT. In this case, the body is addicted to a higher level of nicotine, making quitting harder. Varenicline offers a mechanism of action different from NRT. It is a partial nicotine receptor agonist, which evokes a reduced level of response and antagonizes the response of a full agonist such as nicotine. The clinical studies have demonstrated favorable cessation rates with an encouraging safety profile.

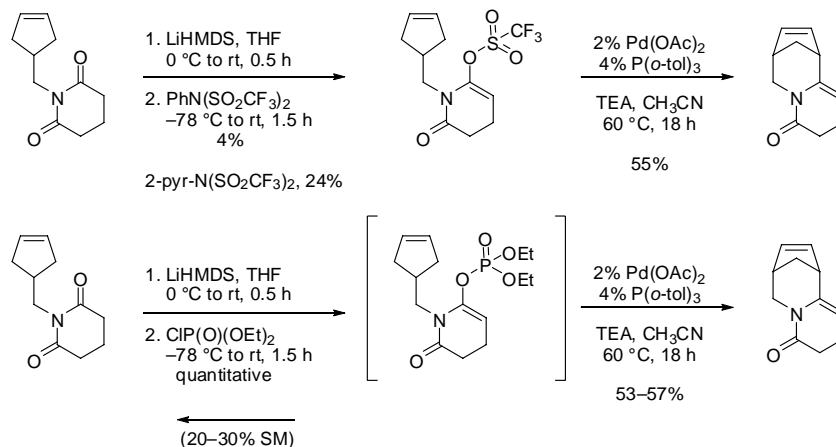
At the outset of the discovery program, (–)-cytisine (Figure. 2) was chosen as a starting point for the studies. This natural product, found in numerous plant species, was reported in 1994 to be a partial agonist of $\alpha 4\beta 2$ nAChR and antagonize the receptor response to its endogenous neurotransmitter, acetylcholine. In the 1960s an early smoking cessation trial with (–)-cytisine failed to exhibit robust efficacy, possibly due to poor absorption and limited brain penetration.

Figure 2. (–)-cytisine



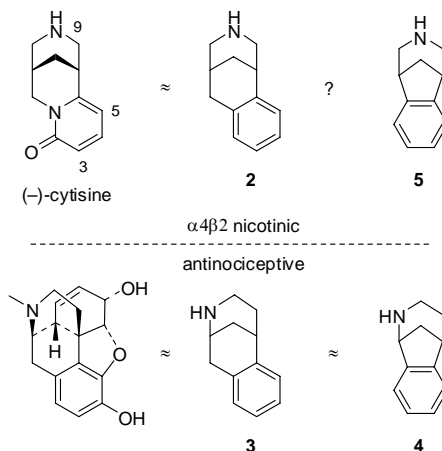
To synthesize (–)-cytisine and its analogues, an intramolecular Heck cyclization has been developed to establish the bridged tricyclic skeleton. The cyclization employed an activated enol phosphate or triflate intermediate (Figure 3), the first time such an enol phosphate intermediate has been used in metal-catalyzed cyclization.

Figure 3. Enol Triflate or Phosphonate Heck Cyclization



The initial SAR studies revealed that $\alpha 4\beta 2$ nAChR binding affinity was considerably decreased with changes at position N-9 and C-5 (Figure 4), but maintained or improved with substitution at C-3 of (–)-cytisine. These results led them to explore pyridone replacements based on **2**. However this series of analogues exhibited a weaker affinity and lower efficacy partial agonist profile relative to (–)-cytisine.

Figure 4. Related Substructures of (–)-Cytisine and Morphine



To find compounds with promising *in vitro* and *in vivo* profiles, the Pfizer team searched for alternative template. The amazing resemblance between substructures of (–)-cytisine and morphine was recognized, where their [3.3.1] bicyclic skeletons differ only by the position of nitrogen atom (cf. **2** vs **3**). In the 1970s, it was found that the simplified analogue **3** had morphine-like antinociceptive activity, as did the modified derivative **4**. However the N-positional isomer **5** lacked antinociceptive activity. Interestingly, 3,5-bicyclic aryl piperidine (BAP) **5** displayed *in vivo* pharmacology similar to natural nicotinic agents. On the basis of the resemblance between **3** and **4**, both antinociceptive compounds, it is speculated that **2** and **5** might share a similar nicotinic pharmacology. It turned out to be true that **5** is a nicotinic antagonist with a K_i of 20 nM. The symmetry in the structure makes synthesis of this series of analogues straightforward.

Potent natural nicotinic agonists, such as epibatidine, (–)-nicotine, (+)-anatoxin α , and (–)-cytisine, contain electron-deficient π -systems. Electron-withdrawing substituents therefore were introduced on the position 1, 2 and/or 3 of **5** (Figure 5). From *ortho*-dinitroderivative **6**, other types of derivatives were prepared, including quinoxalines, benzimidazoles, benzoxazoles, benzothiazoles. *In vitro* activity of these analogues is sensitive to structural modification, showing a dependence on both steric and electronic nature of substituents. Furthermore they exhibit a range of partial agonist activity and potency *in vivo*.

Figure 5. Bicyclic Aryl Piperidines and Other Analogue Derivatives

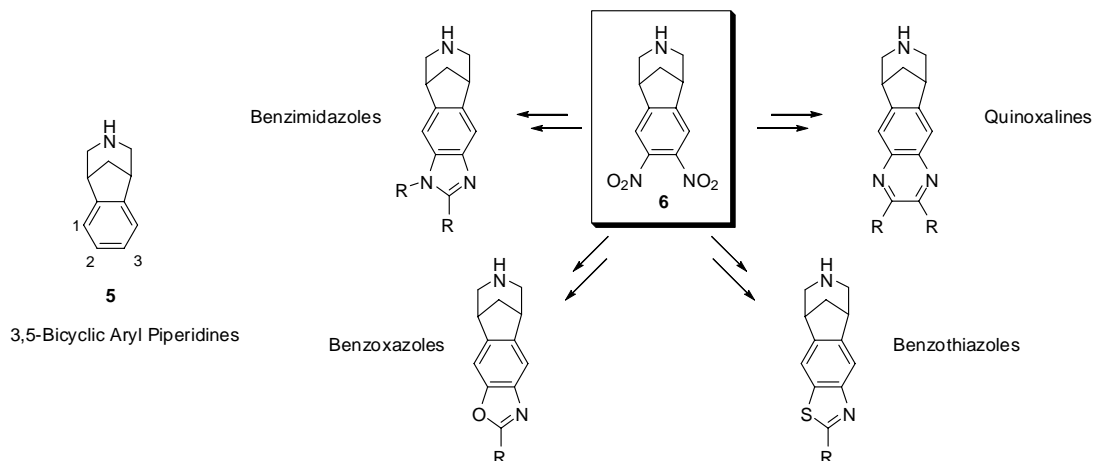


Table 1. ADME Properties in Human

Absorption

Small (MW=211), very water soluble
 Good membrane penetration (passive diffusion), highly absorbed
 99% of recovered ^{14}C material excreted in urine
 Not a substrate for the P-glycoprotein efflux transporter

Distribution

Low protein binding ($f_u \approx 0.8$)
 Moderate volume of distribution (1.9 L/kg)

Metabolism

Excreted >90% as unchanged drug in the urine
 Minor hydroxyl- and N-carbamoylglucuronide metabolites
 Parent drug represents 90% of circulating drug-related material
 Does not inhibit cytochrome P450 enzymes

Excretion

Renal clearance (mainly passive diffusion): 2.4 mL/min/kg
 Long half life: $T_{1/2} \sim 24$ hr (accumulated data from multiple clinical studies)

Among these analogues, varenicline (quinoxaline R = H) displays high affinity, selectivity, and a desirable *in vitro* partial agonist profile. It also has low affinity for other receptors, channels, uptake sites and second messengers. Varenicline is partial agonist alone and fully block nicotine's effect *in vivo*. In human clinic trial, the metabolism and disposition of varenicline displayed a straightforward profile (Table 1), which should simplify its use in the clinic as an aid in smoking cessation.

Varenicline is the first approved nicotinic receptor partial agonist, and is available as a prescription medication used to treat smoking addiction. In May 2006, it was approved for sale in the United States and in September 2006 in the European Union.

Reference:

1. Coe, J. W.; Brooks, P. R.; Wirtz, M. C.; and *et al.* *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4889-4897.
 2. Coe, J. W.; Brooks, P. R.; Vetelino, M. G.; and *et al.* *J. Med. Chem.* **2005**, *48*, 3474-3477.
 3. Obach, R. S., Reed-Hagen, A. E.; Krueger, S. S. and *et al.* *Drug. Metab. Dispos.* **2006**, *34*, 121-130.
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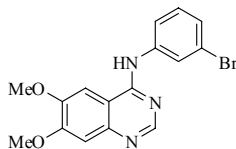
Case Study: “Discovery of Dasatinib”

Joel Barrish, Bistol-Myers Squibb Pharmaceutical Research Institute

The presentation of this case study began with an introduction to kinases and their role in disease progression and treatment. Kinases play key roles in relaying messages from the cell into biochemical events and thus deregulation leads to cell malfunction (often cancer). Kinases were first recognized as possible targets in the late 1970's but efforts to convert this knowledge to small molecule therapeutics were hampered by poor selectivity, off-target toxicity, and poor PK of identified active compounds. These challenges have been addressed by many pharmaceutical companies and positive results in the form of marketed drugs have resulted in the last several years. The case study presented traced the evolution of Dasatinib from hit to lead to drug.

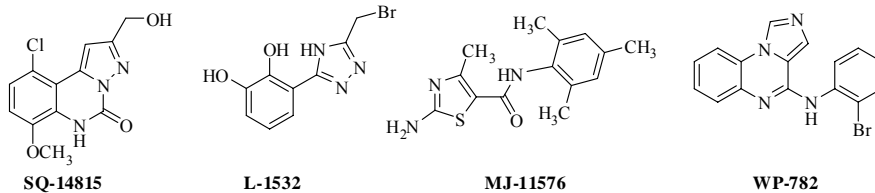
An early hit that was thought to be a selective kinase inhibitor was PD 153035 (Figure 1). It was very active against EGFR with a K_i of 5 pM, while it had very low activity ($>50 \mu\text{M}$) against PDGF, FGF, CSF-1, IR, and Src.

Figure 1



However, upon further investigation the selectivity was not as great as originally thought and this compound was abandoned. High throughput screening was used in the mid 1990's to identify several early hits (Figure 2).

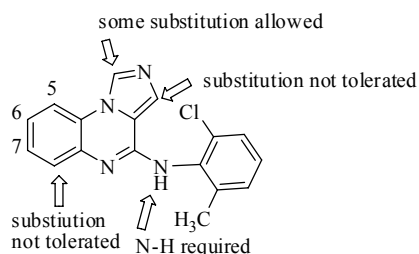
Figure 2



While active, SQ-14815 and L-1532 could not be progressed due to poor selectivity among kinases. MJ-11576 and WP-782 were more promising and the focus for development narrowed to the kinase Lck, a member of the Src-family of non-receptor tyrosine kinases. It is found in T cells, thymocytes and NK cells and is required for both T cell development and signaling. Inhibition of Lck possibly would provide a treatment for both chronic and acute T cell-mediated autoimmune and inflammatory diseases. Potential therapeutic areas included rheumatoid arthritis, multiple sclerosis and psoriasis, among others.

With a Lck IC_{50} of 170 nM, WP-782 was chosen as the lead for further investigation. Two years of SAR exploration evaluated differing substitutions around the molecule as shown in Figure 3. Aryl groups were generally required, small substituents at C-5 were tolerated while substitutions at C-8 were not. Substitution with electron-donating groups at C-6,7 were preferred and some heterocycles at these positions were tolerated. The N-H aniline was found to be required as well as the 1,5-imidazole. Bis-ortho substitution of the aniline ring was highly preferred and substitution around the imidazole ring was allowed in one position while not in the other as indicated in Figure 3.

Figure 3



The SAR results from binding assays were evaluated in conjunction with structural data available from models developed from published coordinates of activated Lck kinase domain bound to ANP, a non-hydrolyzable ATP mimic. From this it was determined that the bis-ortho substitution on the aniline (Figure 3) provides for a more favorable orientation and thus better binding. They were never able to get crystals of inhibitors in the active site, however, so all structural information was based on modeling alone. Additional compounds were evaluated in the models as the program progressed and information about the active site, such as the presence of a deep hydrophobic pocket, was used to guide the continuing SAR.

The structure of the optimized lead to this point is shown in Figure 4. BMS-238497 showed very high activity with Lck IC_{50} of 2 nM and T Cell proliferation IC_{50} of 670 nM. It had >10,000 fold selectivity versus a large number of other kinases but was unselective versus Src-family kinases. It was then evaluated in an oncology cell line panel and Bcr-Abl activity was found. Analogs of this type had previously been characterized in models of delayed hypersensitivity (DTH) and

asthma as part of an immunology program. Also in oncology activity had been shown in a PC3 xenograft model. With these studies some issues had been identified, namely that BMS 238497 had only modest potency and that it had poor PK, specifically there was high clearance and rapid CYP450-mediated oxidative metabolism.

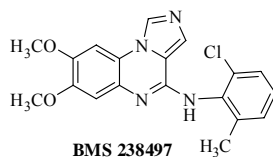
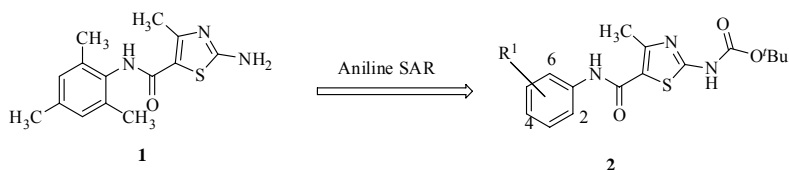


Figure 4

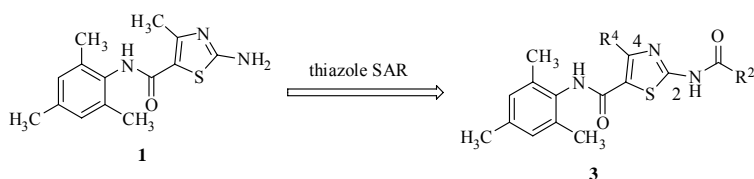
At this point the project shift to a different lead from HTS. The thiazole **1** (Figure 5) had been found to have modest activity with mouse Lck IC₅₀ of 6.6 μM and human Lck IC₅₀ of 5.0 μM. SAR was initiated around the aniline ring. It was determined that small *ortho*-substituents were preferred, *meta*-substitution was less preferred and that *para*-substitution was flexible.

Figure 5



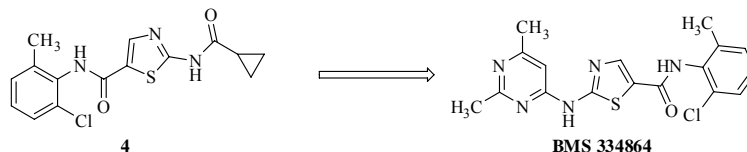
The 2,4,6-trimethyl and the 4-Br, 2,6-di-methyl substituted compounds **2** were active in the mouse Lck assay at 3.0 and 2.4 μM levels respectively. The next modification was to substitutions on the thiazole (Figure 6).

Figure 6



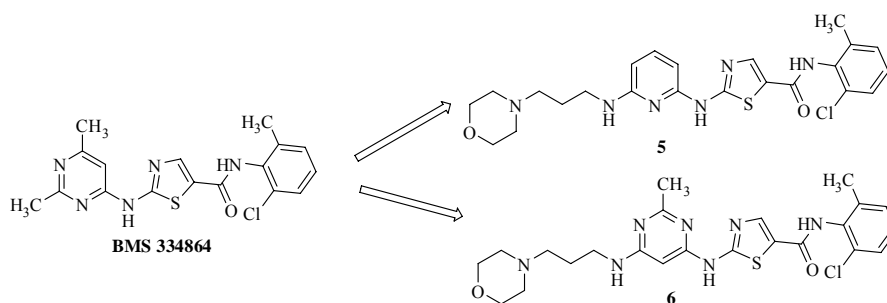
Modifications were made at positions 2 and 4 of compound **3** as shown. A series of carbamates, ureas and amides were made at position 2 while R⁴ was most often methyl though an ethyl group and a proton were also substituted. From this it was found that methyl substitution at position was highly favored and the different groups at position were well tolerated. However, no appreciable increase in potency was obtained. Through additional studies compound **4** (Figure 7) was identified as the new best lead with a human Lck IC₅₀ of 18 nM. From here a series of replacements for the amide attached to the thiazole were made which yielded BMS 334864, a very potent compound whose Lck K_i was 0.13nM and the Lck T Cell Pro IC₅₀ was 84 nM.

Figure 7



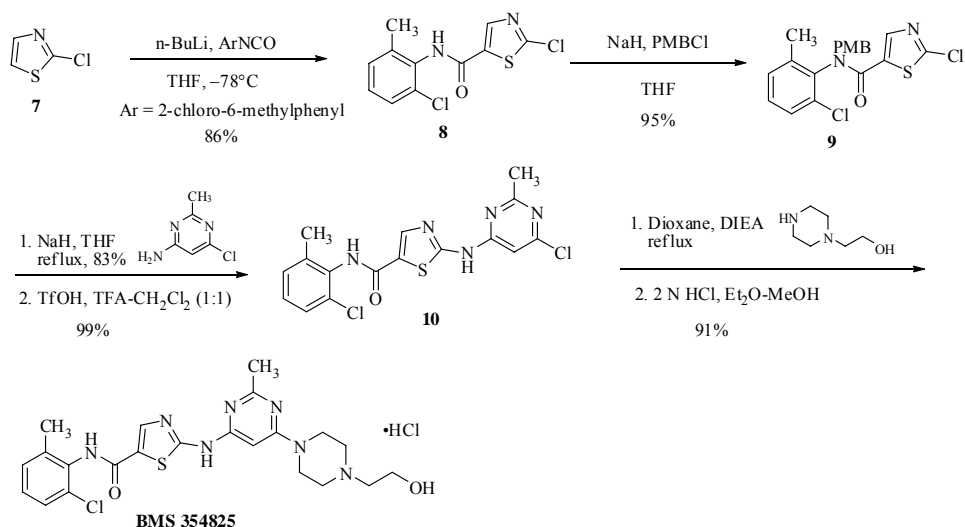
While BMS 334864 is selective versus a number of other kinase families it was unselective versus the Src-family. It did show Bcr-Abl \ll 1 nM and good results in an oncology cell line panel but also had poor solubility properties. With the promise shown, further analog work was carried out, with the investigations split into two classes (Figure 8). In both cases the aniline and thiazole portions were held constant; in one case additional pyridimines were made while in the other case pyridines were investigated. These studies yielded compounds **5** and **6** which both showed significant improvements in cell potency with IC_{50} values of <0.7 nM and <0.5 nM respectively along with T-cell proliferation IC_{50} values of 7 and 8 nM, respectively. These analogs showed potent inhibition of Lck/Scr as well as Bcr-Abl kinases and K562, a human blast phase CML line.

Figure 8



The analogs were screened in solid tumor cell proliferation assays and 4 hour oral exposure studies in mice were done. Through further analoging and testing it was determined that BMS 354825 (Scheme 1) had the desired *in vivo* activity as well as a favorable PK profile. This compound was chosen for clinical development and ultimately became Dasatinib. The synthesis of BMS 354825 is shown in Scheme 1 and begins with thiazole **7**. Deprotonation and coupling with the arylisocyanate gave compound **8** in good yield. It was necessary to protect the nitrogen of **8**, which was subsequently followed by coupling with the pyrimide fragment Removal of the protecting group on **9** gave **10** in excellent yield. All that remained was the coupling of the piperazine piece and subsequent salt formation to give BMS 354825.

Scheme 1



As is required for all clinical candidates extensive PK, efficacy and toxicology studies were carried out on BMS 354825. In the rat it was found to have moderate bioavailability of $F_{po} = 27\%$. Protein binding was determined to be 92% in the rat and there was low systemic clearance of 26.4 mL/min/kg. The aqueous solubility was acceptable at 13 $\mu\text{g/mL}$. Several other parameters such as C_{max} , T_{max} , AUC_{tot} , $t_{1/2}$ and MRT showed acceptable values as well. Examination of 354825 in kinase profile assays found it have good selectivity outside the Src family.

It had been noted that compounds of the same structural class as BMS 354825 had activity in the inhibition of Bcr-Abl kinase. Inhibition of this kinase has been shown to be an effective treatment for Chronic Myelogenous Leukemia (CML). There are three stages of CML: chronic, accelerated, and blast crisis. Most cases are in the chronic phase during which the disease can be controlled by medications such as imatinib (Gleevec), IFN- α , hydroxyurea, or busulfan. The accelerated phase is a transient phase which leads to the final stage of blast crisis, which is terminal. Good response is often achieved from front line treatments but development of drug resistance over the long term is often observed. The activity of BMS 354825 versus that of Imatinib in several Bcr-Abl *in vitro* assays examined was found to be an improvement of almost three orders of magnitude. The *in vivo* activity of BMS 354825 was also greatly enhanced versus Imatinib.

Scientists at BMS were able to obtain the crystal structure of BMS 354825 bound in the active site of the Abl complex. This showed that the molecule was bound to the activated conformation in the ATP-binding site. This represents a different binding mode than that for Imatinib, which is known to bind to the inactive conformation. Several key binding interactions of BMS 354825 in the active site were noted, including three H-bonding interactions with Met 318 and Thr 315.

As mentioned previously the problem of drug resistance, specifically to Imatinib, has been noted in patients. BMS 354825 was tested in the Imatinib-resistant K562 cell line with excellent results. The compound was then screened against 17 Imatinib-resistant cell lines and found to be active against all but one of them. Further testing *in vivo* showed positive results and the compound was progressed into the clinic. Phase I data were positive and further clinical trials were carried out on CML patients resistant or intolerant to imatinib. The NDA was filed in 2005 and Dasatinib is currently on the market.

