



Trip Report for

**“Residential School on Medicinal Chemistry:
Chemistry and Biology in Drug Discovery”**

Drew University, Madison, New Jersey

June 9-13, 2008

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Discovery R&D, Chemistry and Medicinal Chemistry Departments

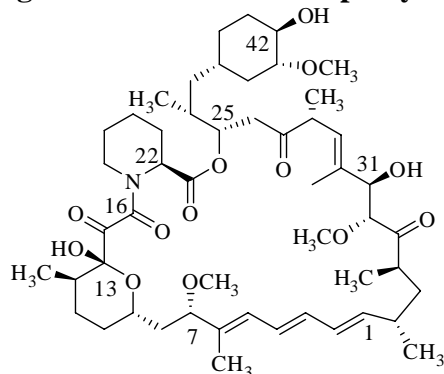
Abstract: *The 22nd Residential School on Medicinal Chemistry course was held at Drew University from June 9-13, 2008. This course is designed for chemists and biologists interested in broadening their understanding of the fundamental principles of small molecule drug discovery research and preclinical development. This year’s course was attended by over 200 scientists, representing researchers from academia and medicinal chemists from over seventy companies from around the globe. The course was comprised of sixteen lectures, five case history summaries and three seminars.*

“The Discovery of Torisel®”

Jerauld S. Skotnick, Wyeth Research, Pearl River, New York

Rapamycin (**1**, Figure 1) is a novel immunosuppressant that was first discovered in a soil sample of *Streptomyces hygroscopicus* from the Pacific island Rapa Nui (Easter Island). The unique mechanism of action of compound **1** is to bind to FKBP-12 and the resulting **1**/FKBP-12 complex directly inhibits the mammalian target of the rapamycin (mTOR) pathway by binding mTOR. Rapamycin has been marketed as Rapamune™ since 1999 by Wyeth to prevent rejection in organ transplant and offers an improved toxicity profile over available calcineurin inhibitors.

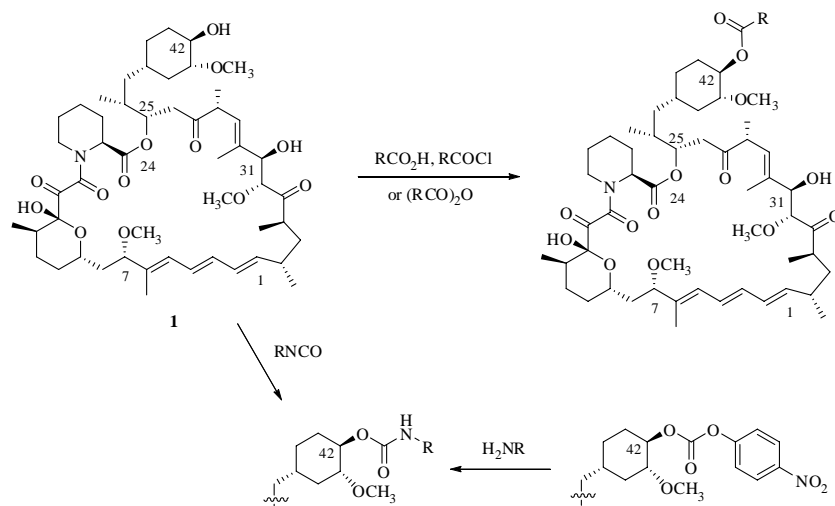
Figure 1. Structure of Rapamycin.



Wyeth initiated a program to identify analogues of **1** that could serve as rapamycin “back-ups” and identify analogues with other therapeutic applications. Rapamycin is a 31-membered ring that is both a lactone and a lactam with fifteen chiral centers, a hemiketal masked tricarbonyl, an all *trans* triene and several hydroxyl groups. It was hypothesized that manipulation of the functional groups of **1** would improve chemical and pharmaceutical properties as well as possibly lead to other therapeutic applications.

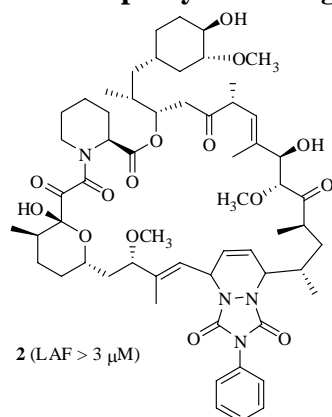
Acylation of the hydroxyl group at C-42 proved to be a facile route to introduce functionality to **1** (Scheme 1). Derivatization at C-42 is consistent with activity and a number of analogues were synthesized to try to modulate the PK profile of **1**. The selective acylation of C-42 proved trivial and only a small amount of acylation at C-31 was observed.

Scheme 1



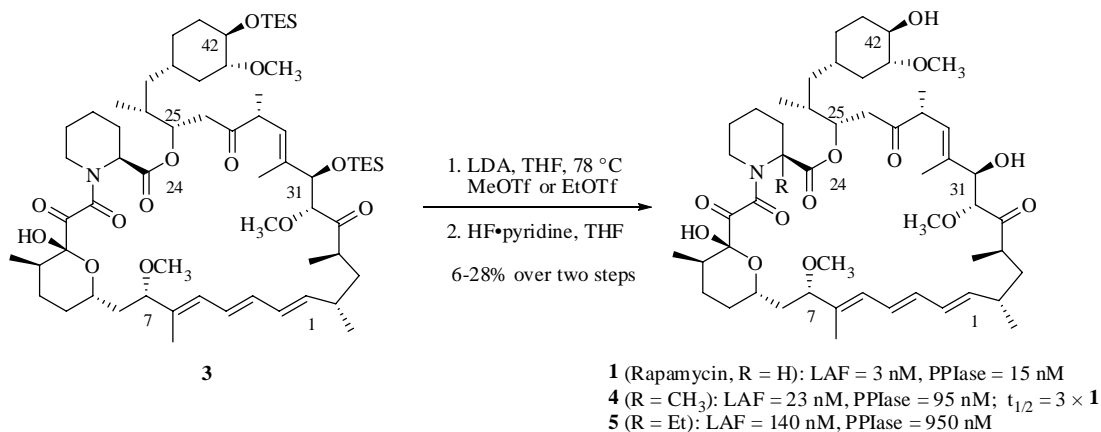
Circular dichroism analyses of the rapamycin analogue-FKBP complex versus non-bound rapamycin analogue (acylated at C-42 and C-31) indicated that there was a conformational change in the triene when FKBP was bound. Derivatization of the triene afforded rapamycin analogue **2** (Figure 2). Analogue **2** lost potency in suppression of T-cell proliferation ($\text{LAF} > 3 \mu\text{M}$ vs. $3 \mu\text{M}$ for rapamycin) and antagonizes effects of **1** on thymocyte proliferation. The perturbation of the triene does not fully compromise binding of **2** with FKBP, but underscores the importance of the triene region to mediate antiproliferative effects.

Figure 2. Rapamycin analogue 2.



Incubation of **1** in rat bile at 37°C or in NH_4OAc at 37°C led to hydrolysis of the macrolactone. In light of this observation, it was postulated that increasing the steric bulk around the carbonyl of the macrolactone would lead to enhanced metabolic stability. Deprotonation of analogue **3** with LDA, subsequent trapping of the enolate with either MeOTf or EtOTf and deprotection of the alcohols with $\text{HF}\cdot\text{pyridine}$ afforded analogues **4** and **5** in 6–28% yield over the two steps. While the binding potency of **4** and **5** decreased significantly relative to **1**, the $t_{1/2}$ of **4** increased three-fold, indicating that the added steric bulk retarded the hydrolysis of the macrolactone.

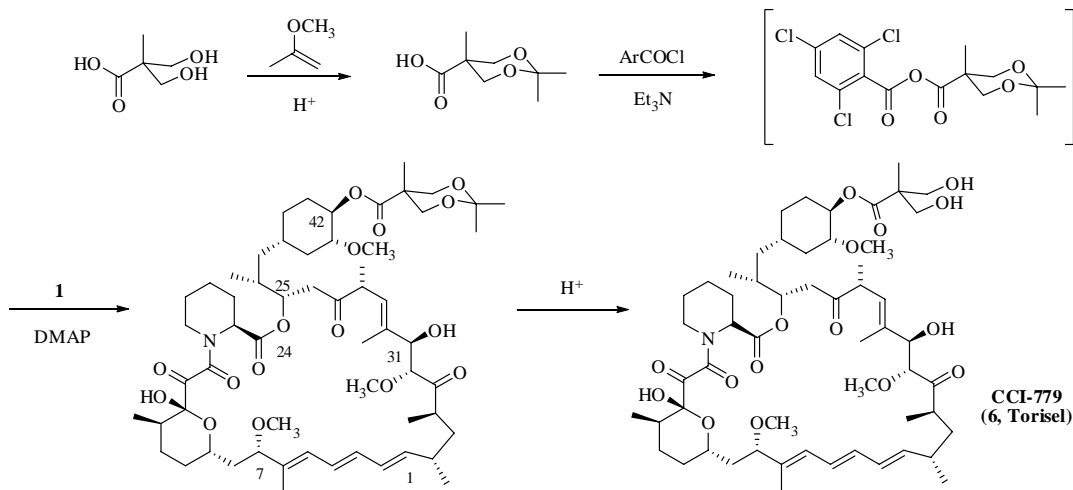
Scheme 2



A broad array of rapamycin analogues were synthesized (>600) and tested for *in vitro* potency and *in vivo* efficacy (ip and po). Among these, 31 compounds were selected for later-stage *in vivo* efficacy studies. This led to the identification of three advanced leads out of which CCI-779 (**6**) was nominated as a candidate for clinical trials (Scheme 3). CCI-779 possessed good chemical and pharmaceutical properties and was very active in murine transplantation and oncology models (iv, ip and po). CCI-779 also offers improved solubility and enhanced crystallinity relative to **1** while maintaining LAF and FKBP-12 binding efficacy.

CCI-779 (**6**) was synthesized from rapamycin in two steps as shown in Scheme 3. It has a hydrophilic diol ester tethered to C-42 bringing in additional solubility, added H-bonds to FKBP and crystallinity. The hindered nature of the C-42 distal ester retarded the hydrolysis and thereby eliminated the β -elimination problem.

Scheme 3



Compound CCI-779 was found to inhibit mTOR signaling via CCI/FKBP/mTOR complex formation *in vitro*, resulting in arrest of treated tumor cells in the G1 phase. *In vivo* testing with compound **6** showed sustained inhibition of human tumor growth (U87

glioblastoma) in nude mice dosing daily for 5 days every two weeks. *In vivo* T-cell response to dinitrofluorobenzene recovers 1 day after 5 daily doses of **6** indicating that **6** has no lingering immunosuppressive effects. In 2007, compound **6** was the first mTOR inhibitor approved for the treatment of advanced renal cell carcinoma and is marketed under the name Torisel[®].

“Aliskiren: Overcoming Challenges of Designing and Developing the First Direct Renin Inhibitor”

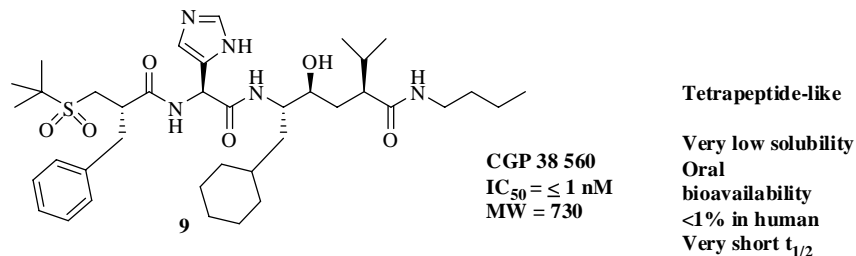
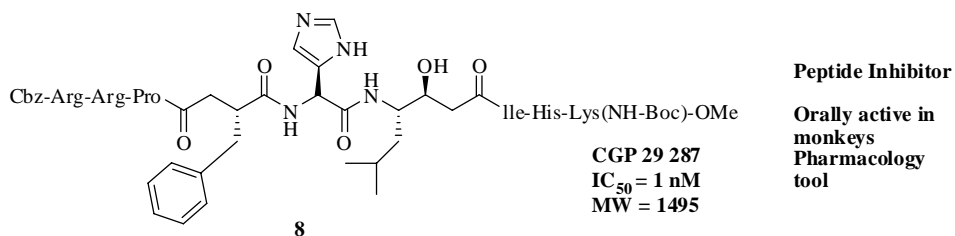
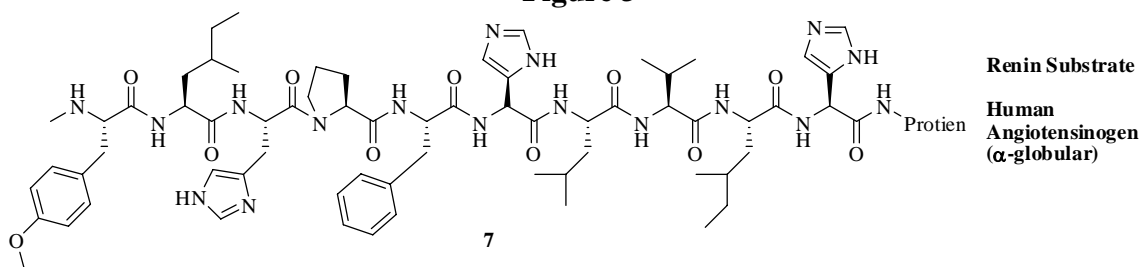
Pascal Rigollier, Novartis Institute for Biomedical Research, Basel, Switzerland

Hypertension is one of the major health challenges facing the world today. Worldwide, it is estimated that one billion people suffer from hypertension with >50 million United States citizens requiring treatment. There are more than 7 million deaths per year attributed to hypertension and the global liability is predicted to increase despite treatment. The blood pressure relationship to risk in cardiovascular disease is consistent, continuous and independent of other factors. The risk of cardiovascular disease doubles with each 20/10 mm Hg across the entire blood pressure range starting from 115/75 mm Hg. The majority of patients being treated for hypertension are not being treated to goal.

The Renin Angiotensin System (RAS) is the hormone equilibrium mechanism within the human body that controls blood pressure and water balance. Over the years there have been many drug discovery programs that have tried to affect different steps in this pathway to lower overall blood pressure. Losartan is an angiotensin II receptor type 1 (AT1) antagonist that was approved in 1995 and has been shown to increase vasodilation and reduce the secretion of aldosterone and vasopressin. The inhibition of renin would block the RAS entirely at the first rate determining step of the process and prevent the formation of angiotensin I and angiotensin II even at high circulating renin levels.

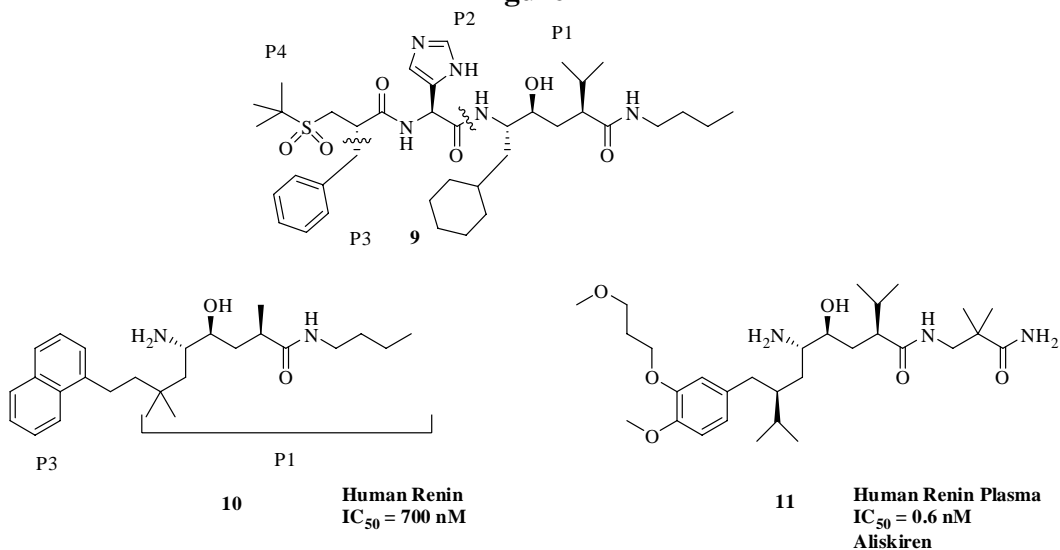
The first and second generation renin inhibitors were modeled after the natural human renin substrate angiotensinogen (**7**, α -globular) as shown in Figure 3. The first generation renin inhibitors were large peptide renin substrate analogues with very high molecular weights. Peptide inhibitor CGP 29 287 (**8**) was the first orally-active direct renin inhibitor in monkeys with an IC₅₀ of 1 nM. The first generation inhibitors were abandoned because of their “un-drug-like” characteristics, while the smaller tetrapeptide-like second generation renin inhibitors were abandoned because of low oral bioavailability, insufficient efficacy or duration of action.

Figure 3



Utilizing computed-aided molecular modeling based on a published renin homology model (T. Blundell 1984), a third generation of renin inhibitors was pursued. The medicinal chemistry challenge was to reduce the lipophilicity and molecular size and shape. In order to reduce molecular weight and peptidic character P2 and P4 were removed and P1 and P3 were linked (Figure 4).

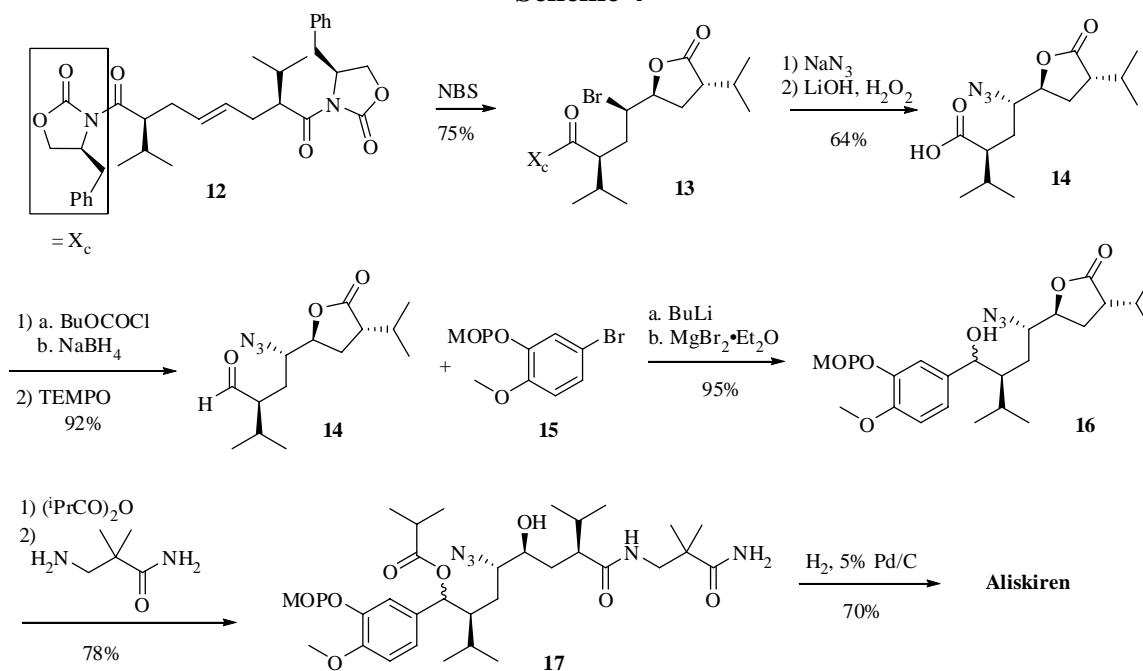
Figure 4



Extensive SAR studies afforded aliskiren (**11**) which exhibited an IC₅₀ of 0.6 nM (substrates usually have a lower binding affinity for human renin from the plasma than the purified human protein). Aliskiren showed excellent pharmacokinetic and ADME profiles and *in vivo* efficacy equivalent or superior to other blood pressure lowering agents, with no blood pressure rebound after drug discontinuation.

The first convergent kilogram-scale synthesis of aliskiren was complete in nine linear steps (Scheme 4). No chromatography was required and the overall yield was 23% from the chiral precursor **12**. All four stereocenters were set in four steps, highlighted by a bromolactonization reaction. In 2007, aliskiren became the first direct renin inhibitor approved for treatment of hypertension and is marketed under the name Tefturna[®].

Scheme 4

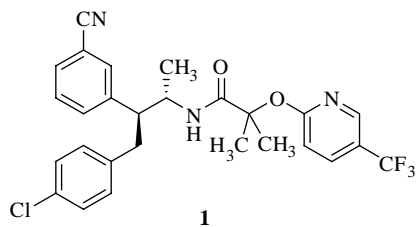


“Cannabinoid-1 Receptor Blockade for the Treatment of Obesity: Discovery of Taranabant”

William K. Hagmann, Merck Research Laboratories

The speaker presented the case history of Taranabant (**1**, Figure 5), currently in Phase III clinical trials for the treatment of obesity. Obesity has continued to grow as a worldwide health problem. In the United States, the incidence of obesity is currently estimated to be greater than 60%. Being overweight has a significant correlation with poor health conditions such as cardiovascular disease, diabetes, hypertension, cancer and arthritis.

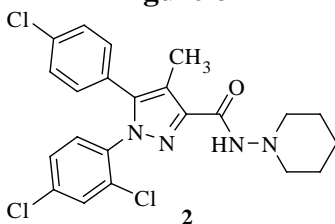
Figure 5



CB-1 IC_{50} = 0.3 nM

The endocannabinoid receptor system has been demonstrated in both animal and clinical studies to be involved in regulating feeding behavior and in weight loss. Both endogenous and exogenous agonists of the cannabinoid-1 (CB-1) receptor have been demonstrated to enhance food intake and body weight. Blockade of the CB-1 receptor function with selective antagonists or inverse agonists have been demonstrated to be effective in suppression of food intake resulting in the reduction of body weight. Rimonabant (**2**, Figure 6), a selective CB-1 receptor inverse agonist, has been approved in Europe for the treatment of obesity in humans.

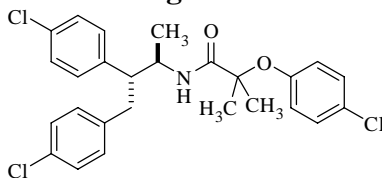
Figure 6



CB-1 IC_{50} = 6 nM

Utilizing a Merck sample collection containing compounds that shared the rimonabant pharmacophore, compounds were screened for binding to the CB-1 receptor in a competition assay using radiolabeled agonist CP55490. Eventually compound **3a** (originally prepared prior to 1967 during a program to enhance cholesterol secretion) was identified as a potential lead compound (Figure 7). This lead has three hydrophobic domains surrounding a polar core, similar to rimonabant. Examination of the more active (*R,R*) enantiomer **3b** showed good *in vitro* data, but poor *in vivo* efficacy.

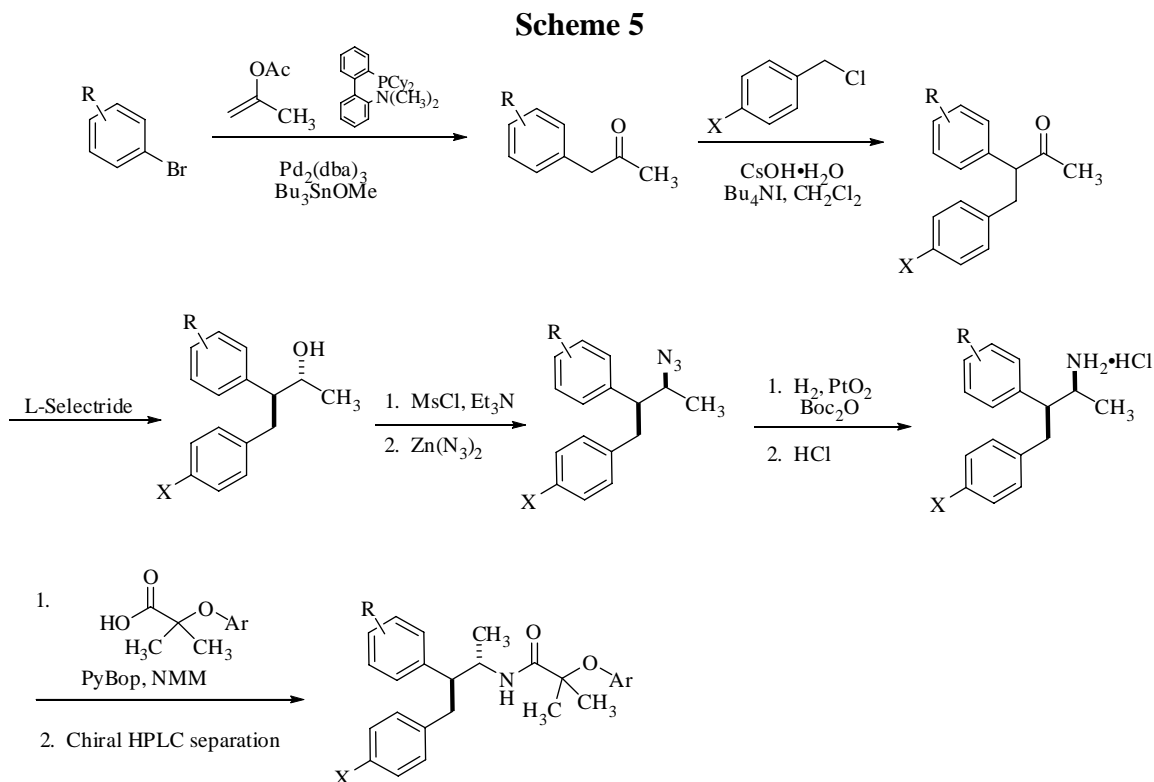
Figure 7



3a CB-1 IC_{50} = 17 nM
(racemic)

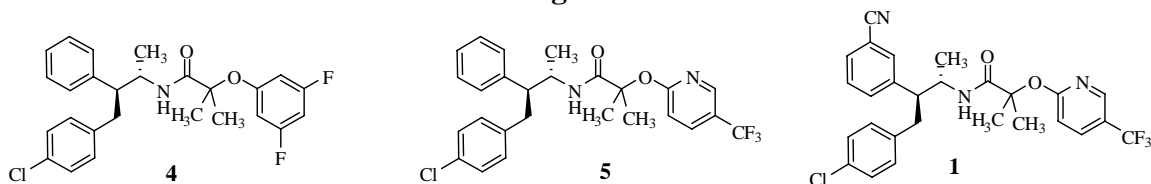
3b: (*R,R*) enantiomer
 IC_{50} = 13 nM

Exploring this lead compound, a series of compounds were synthesized following the general procedure shown in Scheme 5. Following this route, taranabant could be synthesized in nine steps in 12% yield. Additionally, two stereoselective process syntheses were presented.



Changes to the amide backbone were unproductive. Investigation into aromatic ring substitutions revealed the difluorophenoxy analogue **4** (Figure 8) to be potent, selective and to have a good pharmacokinetic profile ($\text{hCB-1 IC}_{50} = 1.1 \text{ nM}$, $\text{hCB-2 IC}_{50} = 170 \text{ nM}$, rat: $F = 75\%$, $t_{1/2} = 2.7 \text{ hr}$, $\text{Cl}_p = 33 \text{ mL/min/kg}$) and worked well in the overnight food intake/body weight reduction in diet induced obesity (DIO) rats. Examination of the metabolism of **4** revealed a high level of glutathione incorporation into the electron rich phenoxy ring along with a high level of covalent protein binding. Synthesis of the 5-trifluoromethyl pyridine analogue **5** nearly eliminated both the glutathione adduct formation and covalent protein binding. Incorporation of the electron withdrawing cyano group into the unsubstituted phenyl group afforded **1** (taranabant), which reduced covalent binding below targeted levels.

Figure 8



Selected preclinical data for Taranabant was presented:

Receptor Binding: human CB-1 $K_i = 0.13$ nM; human CB-2 $K_i = 170$ nM;
Inverse Agonism: human CB-1 $EC_{50} = 2$ nM; human CB-2 $EC_{50} = 230$ nM;
Overnight efficacy on food intake (FI) in mice: FI = 28% reduction;
Overnight efficacy on body weight (BW) gains in mice: BW = 73% reduction at 3 mg/kg po (no effect on CB-1 receptor deficient mice);
Overnight efficacy on FI in DIO mice: FI = 49% reduction;
Overnight efficacy on BW gains in DIO mice: BW = -10 grams at 3 mg/kg po (vehicle = + 10 grams);
14-Day efficacy on food intake in DIO rats: FI = 10-25% reduction;
14-Day efficacy on body weight gains in DIO rats: BW = -19 grams at 3 mg/kg po (vehicle = +15 grams);
Rat PK: F = 74%; $AUCN_{po} = 0.75$ uM•h/mg; $Cl_p = 33$ mL/min/kg; $t_{1/2} = 2.7$ h.

In a 12-week clinical trial weight loss study, taranabant affected significant weight loss of 2.9-5.3 kg at single daily doses of 0.5-6 mg. Decreased food consumption and increased energy expenditure and fat oxidation were suggested as reasons for the observed weight loss. A dose-related increase in adverse events was observed, including mild to moderate gastrointestinal and psychiatric effects. Additionally, weight loss was correlated with the extent of receptor occupancy as assessed by a selective CB-1 receptor PET ligand.

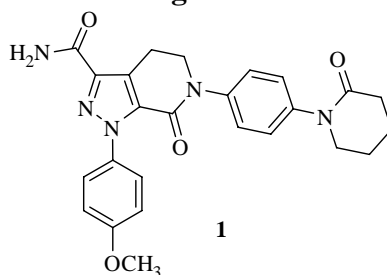
“The Discovery of Apixaban (BMS-562247), a Potent, Selective and Orally Bioavailable Coagulation Factor Xa Inhibitor”

Ruth Wexler, Bristol-Myers Squibb Company

Thrombotic diseases are the leading cause of death in developed countries despite the availability of anticoagulants such as warfarin, aspirin, clopidogrel (all orally available) and heparin and low molecular weight heparins (injectable). The standard oral anticoagulant warfarin inhibits the post-translational maturation of coagulation factors, such as factor Xa and prothrombin, and has proven effective in both venous and arterial thrombosis. However, warfarin's usage is limited due to a narrow therapeutic index (limited by bleeding), slow onset of therapeutic effect, numerous dietary and drug interactions and the need for monitoring and dose adjustment. Therefore, the development of safe and efficacious oral anticoagulants for the prevention and treatment of thrombotic diseases remains an important unmet medical need.

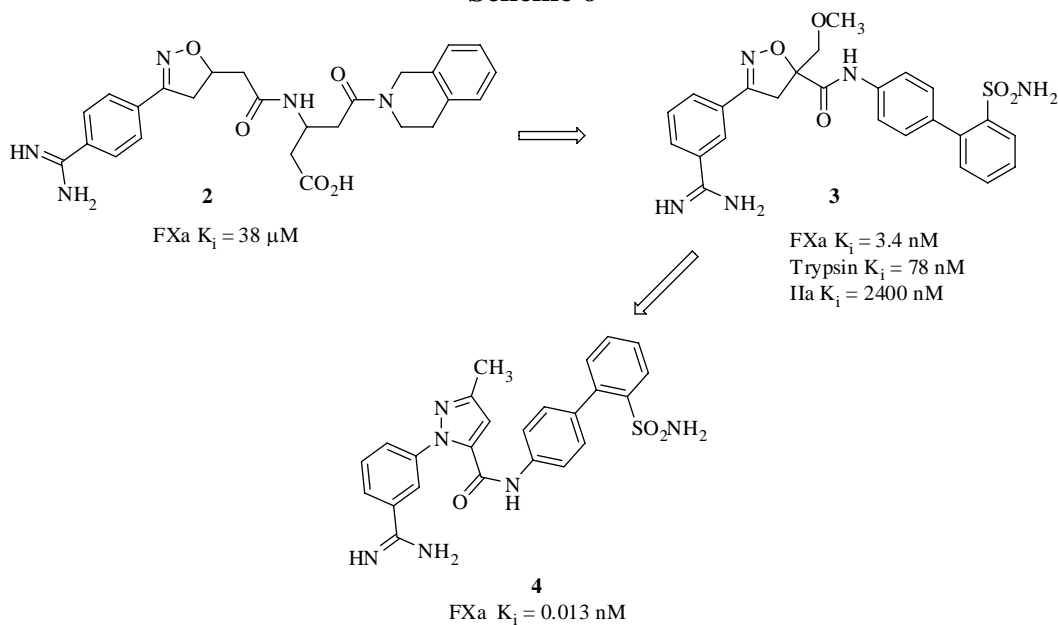
The researchers developed apixaban (**1**, Figure 9) as a high affinity, selective inhibitor of factor Xa with good oral bioavailability and excellent safety. Factor Xa is a trypsin-like serine protease required for the conversion of prothrombin to thrombin, the final enzyme in the coagulation cascade that is responsible for fibrin clot formation and platelet activation. Preclinical animal models have suggested that inhibiting factor Xa has the potential for providing excellent antithrombotic efficacy with minimal bleeding risk compared to direct thrombin inhibitors. Recent disclosures from clinical studies with direct factor Xa inhibitors have confirmed the preclinical findings.

Figure 9



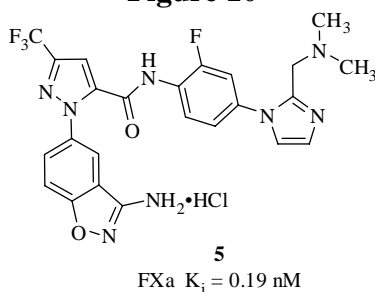
Screening of a DuPont collection of non-peptide IIb/IIIa antagonists in a factor Xa assay identified a few 30-40 μM inhibitors such as **2** (Scheme 6). Exploration of the SAR around the appendages on the heterocyclic core resulted in **3** as a 3.4 nM inhibitor of factor Xa. Further exploration of the SAR varying the heterocyclic core itself resulted in pyrazole derivative **4** as a 13 pM inhibitor of factor Xa.

Scheme 6



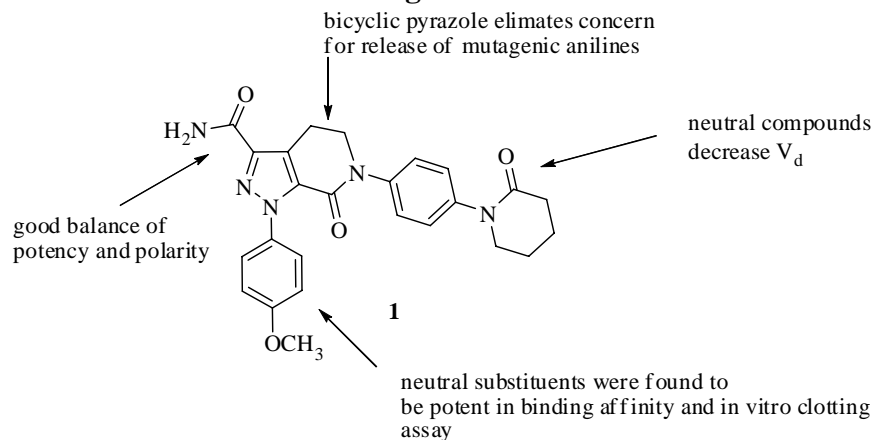
However, benzamidines such as **4** were found to have short *in vivo* half-lives and low oral absorption due to high basicity. Compound **4** was also found to be less selective over some serine proteases such as trypsin. A series of first generation non-benzamidines were developed with reduced basicity showing significant improvement in oral absorption, however with reduced selectivity over trypsin. A second generation of non-benzamidine compounds were developed resulting in razaxaban (**5**, Figure 10), which was well absorbed and tolerated after oral administration and more effective than enoxaparin (low molecular weight heparin) in a clinical deep-vein thrombosis trial. Due to an increase in steric bulk, selectivity for factor Xa over trypsin (>40,000-fold) was achieved.

Figure 10



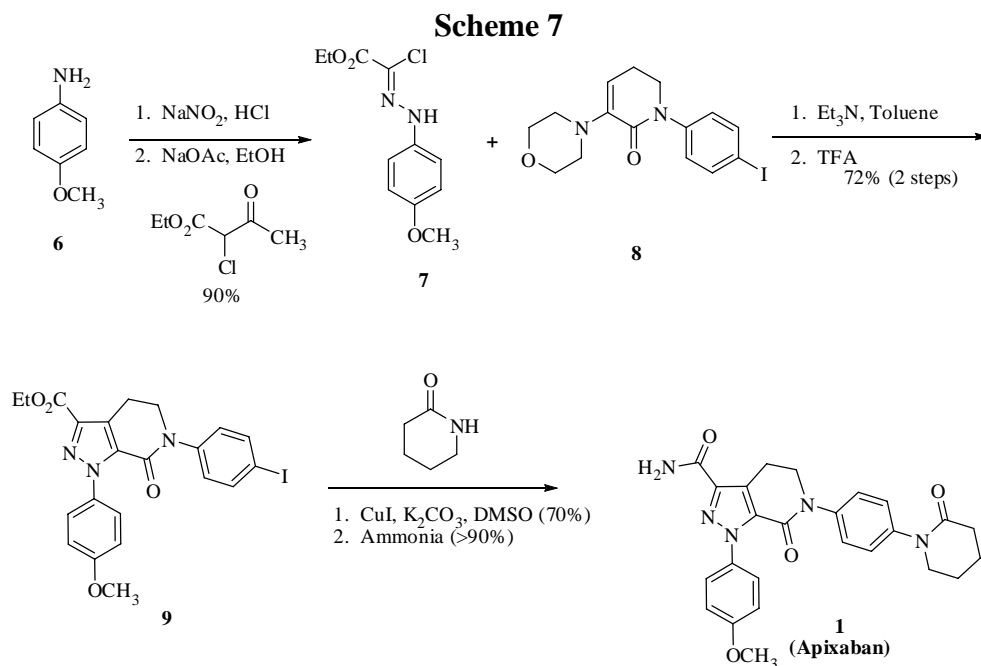
Concerned over the release of potentially mutagenic anilines via amide hydrolysis, bicyclic pyrazoles were synthesized and found to retain excellent factor Xa potency (Figure 11, apixaban). Examination of the pyrazole C-3 SAR showed that the carboxamide demonstrates a good balance between potency and polarity. Examination of the SAR around the aryl pyrazole substituent revealed the neutral *para*-methoxy phenyl group to be a potent binder of factor Xa as well as potent in the *in vitro* clotting assay. Finally, neutral substituents such as the aryl piperidinone were found to decrease the volume of distribution of the drug, allowing for more interaction of the drug in the vascular compartment as opposed to extravascular compartments.

Figure 11



Apixaban is a potent inhibitor of factor Xa ($K_i = 0.08$ nM) and shows a high degree of selectivity versus other proteases. The compound shows weak activity against various P_{450} isozymes ($IC_{50} > 25$ μ M) and weak activity against the hERG potassium channel ($IC_{50} > 25$ μ M). Apixaban has a half-life of >100 minutes in the human liver microsome assay. The compound demonstrated moderate permeability in the Caco-2 assay and good aqueous solubility (43 μ g/mL). The dog pharmacokinetics for apixaban were excellent, showing very low clearance (0.02 L/Kg/h), and low volume of distribution (0.2 L/kg). In dog, the compound had a moderate half-life (5.8 hours) and good oral bioavailability (58%). In the rabbit AV Shunt thrombosis model, the compound inhibited thrombus formation in a dose-dependent manner. Apixaban is currently in phase III clinical trials for the prevention and treatment of venous and arterial thrombosis.

The synthesis of apixaban is shown in Scheme 7. Diazotization of **6** followed by condensation with ethyl 2-chloroacetoacetate afforded **7**. Treatment of **7** with compound **8** in the presence of excess Et₃N, followed by treatment with TFA afforded intermediate **9**. Subsequent treatment of **9** with δ -valerolactam under Ullmann conditions followed by aminolysis afforded apixaban.



Pharmacokinetics and ADME

Cheryl L. Zimmerman, Department of Pharmaceutics, University of Minnesota

An excellent seminar on pharmacokinetics and ADME properties was delivered by Dr. Zimmerman. Pharmacokinetics was defined as “a study of the time course of drug absorption, distribution, metabolism and excretion (ADME) in the body”. The rate and extent of absorption have consequences for pharmacological and toxicological action of a drug. The process by which a drug proceeds from the site of administration (*e.g.* into the intestine or muscle) to site of measurement (in blood) in the body is called absorption. This step is particularly important in extra-vascular drug administration (*e.g.* oral, pulmonary, intranasal, intramuscular and transdermal applications). Passive diffusion, measured mathematically by Fick’s law, plays a major role in transmembrane movement of molecules.

Membrane structure influences drug absorption through interaction with drug molecules. Since membranes are made up of phospholipid bilayers, molecules need to have some lipid-like characteristics to be able to cross the membrane. As a consequence, lipophilicity and ionization play a major role in absorption of the drug. According to the pH-partition hypothesis, the unionized form of the drug is more lipophilic than the ionized fraction. Among two compounds whose unionized forms have an equal

lipophilic character, the one which has a higher percentage of the unionized form will be more rapidly absorbed.

The oral route of administration is the most commonly-used delivery system for therapeutic agents. However, it is also the most complex form of administration since it is greatly affected by the anatomy and physiology of the average gastrointestinal (GI) tract. The small intestine is the most important absorptive organ for the majority of drugs and dosage forms.

The absorption rate constant (K_a) helps to describe the rate of absorption. The extent of absorption is measured by bioavailability (%F). It is a measure of the extent of absorption into the systemic circulation from any route of exposure. Oral bioavailability is a function of absorption from lumen (F_a), and escape from first-pass by intestinal wall (F_G) and liver (F_H).

The concentration and disposition of drug at the target site is influenced by its distribution. The rate and extent of distribution are determined by how well each tissue is perfused with blood. This also depends on binding of drug to plasma proteins and tissue components. Permeability of tissue membranes to the drug also effects distribution. Distribution is a reversible process of transfer of a drug into and out of the bloodstream, the compartment from which assay measurements are made. Any drug leaving the site of measurement which does not return has undergone elimination. Extent of distribution is measured by volume of distribution (V). Although not directly measurable, tissue binding has a significant influence on volume of distribution. An increase in tissue binding will increase the extent of drug distribution in the body.

Elimination can be defined as irreversible loss from site of measurement. Metabolism and excretion are the two main processes involved in elimination. A drug must be carried to organs of elimination by blood. Even if drug distributes into non-eliminating tissues, it must re-enter blood and be carried into organs of elimination in order to be completely removed from the body. Elimination processes are quantitatively described by a measure of clearance. Clearance is a proportionality constant relating the rate of elimination (dx/dt) to the plasma concentration (cp).