HETEROCYCLES, Vol. 73, 2007, pp. 47 - 85. © The Japan Institute of Heterocyclic Chemistry
Received, 26th June, 2007, Accepted, 6th August, 2007, Published online, 7th August, 2007. REV-07-SR(U)1

CARDIOVASCULAR AGENTS: RENIN INHIBITORS AND FACTOR Xa INHIBITORS

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Abstract – Cardiovascular disease is a serious global health problem and the world's leading cause of death, with an estimated 17 million people dying from it each year. Coronary heart disease (CHD) and stroke account for 20-50% of all deaths in most countries. These two diseases are the first and third cause of death in the industrialized world, and the first and second cause in many developing countries, respectively. Hypertension is a major contributing factor to cardiovascular disease. A number of therapies like diuretics, alpha and beta-blockers, ACE inhibitors and angiotensin receptor blockers (ARBs) are available for the treatment of hypertension. Despite these therapies 35% patients do not achieve blood pressure control goals. In recent years there has been tremendous effort focused on the development of renin inhibitors as a new class of antihypertensive drugs. These inhibitors offer a novel approach to control hypertension by acting further upstream in the RAAS pathway. Thrombosis is also responsible for heart attacks and stroke. To treat thrombosis, two groups of antithrombotic drugs such as thrombolytic and anticoagulant agents, are available. Despite available treatments, there remains a critical need for a safe and orally active anticoagulants. Recently, factor Xa inhibitors have been found to be a promising approach to prevent blood coagulation. This review will highlight the recent developments of renin inhibitors as antihypertensive agents and factor Xa inhibitors as anticoagulants.

1. INTRODUCTION

Heart attacks and strokes are the leading causes of death and disability for both men and women all over

the world. Despite a number of highly effective therapies and inventions developed over the last twenty years, cardiovascular disease still remains the number one killer worldwide. According to the most recent statistics released by the American Heart Association in 2007, about 80 million people (one in three adult men and women in the United States) were diagnosed with cardiovascular disease.[1] The direct and indirect costs of cardiovascular disease for 2007 is estimated to be 431.8 billion dollars in the United States alone. These numbers are expected to rise sharply with the aging of the "Baby Boom" and post-Baby Boom generations and with the rapid escalation of metabolic diseases like diabetes and obesity.

Hypertension: Hypertension is a common disorder in which blood pressure is abnormally high, placing undue stress on the heart, blood vessels and other organs such as the kidney and brain. Hypertension is a major contributing factor to cardiovascular diseases and death. In North America and Europe alone, it is estimated that more than one third of the population over the age of 35 suffers from hypertension. It is estimated that 70 million people in the US suffer from hypertension and this number is estimated to grow to 100 million over the next decade. Consequences of hypertension include heart attack, stroke, renal disease, aneurysms, retinal vascular damage, left ventricular hypertrophy and heart failure. While global antihypertensive drug sales are forecasted to grow to $50 billion by 2009, nearly 35% patients on treatment do not achieve blood pressure goals. In fact, many patients must take three or more medicines to control their blood pressure, yet some do not respond fully to a combination of treatments. A number of drug therapies are available for treating hypertension. These medications generally fall into the categories of:

- Diuretics which increase the body's excretion of water and salt in the urine.
- Vasodilators, which relax blood vessels.
- Beta-blockers, which block adrenaline receptors, reducing the heart's pumping action.
- Alpha blockers, which block effects of stress hormones such as adrenaline that can narrow blood vessels.
- Calcium-channel blockers, which block the flow of electrolyte calcium to the cardiac muscle causing blood vessel walls to relax.
- ACE inhibitors, which reduce the body's production of angiotensin II, a peptide that causes constriction of blood vessels.
- Angiotensin receptor blocker (ARBs), which block the interaction of angiotensin II with its receptor.

Renin inhibitors represent a new class of compounds for treating hypertension and offer a novel approach because they act further "upstream" in the RAAS pathway. Renin inhibitors work through a mechanism of action that simultaneously inhibits renin and reduces plasma renin activity (PRA). Renin inhibition

may have additive benefits in blood pressure control to ARBs and ACE inhibitors, potentially resulting in superior end-organ (such as heart, kidney and brain) protection. In addition, treatment with a renin inhibitor is not believed to be associated with the bradykinin-mediated side effects seen with ACE inhibitors (cough, angioneurotic edema).

Despite intensive research in the 1980's, attempts to develop clinically useful renin inhibitors have failed. However, on March 6, 2007, Tekturna (Aliskiren, Novartis pharmaceuticals) was approved as the first orally active renin inhibitor for the treatment of hypertension. Tekturna represents the first of a new class of antihypertensive drugs to enter the market in more than a decade.[2] Based on the positive results exhibited by Tekturna (Aliskiren), interest in renin as viable drug target for the control of hypertension has intensified.[3a]

Thrombosis: Thrombosis (formation of a clot or thrombus) is responsible for heart attack, stroke and peripheral vascular disease. Venous thrombosis occurs when blood clot obstructs a vein (blood vessels carrying blood from the body back into the heart) and arterial thrombosis occurs when the blood clot obstructs an artery (blood vessels carrying oxygenated blood away from the heart to the body). Arterial thrombosis may be the result of atherosclerosis (in Greek, 'athero' means paste and 'sclerosis' means hardness). Atherosclerosis is a slow process in which deposits of fatty substances, such as cholesterol, cellular waste products, calcium and other substances build up (called plaque) in the inner lining (endothelium) of an artery. The artery's diameter shrinks and blood flow decreases, reducing the oxygen supply causing ischemia. A heart attack (also known as myocardial infarction; 'myocardium' means heart muscle; 'infarction' means tissue death due to oxygen starvation) occurs when the coronary arteries (the two arteries that coming from the aorta to provide blood to the heart muscle) become severely or totally blocked by a blood clot. When the heart muscle does not get the oxygen-rich blood that it needs, it will die, resulting in permanent heart damage or death. When the blockage occurs in the cerebral (brain) circulation, it leads to ischemic stroke. To treat these life-threatening diseases arising from the clotting cascade and blood clot formation (thrombosis), antithrombotic drugs have been used extensively in the clinic. There are two distinct groups of antithrombotic drugs:

- Thrombolytic or Fibrinolytic Drugs: Drugs like streptokinase or tissue plasminogen activator (t-PA), that break down existing blood clots.
- Anticoagulant and Antiplatelet Drugs: Drugs that prevent blood clots from forming or growing further.

The oral anticoagulant warfarin (coumadin) acts by antagonizing the effects of vitamin K and thus, inhibiting the synthesis of clotting factors. Heparin and its derivatives work by activating antithrombin III, which blocks thrombin from clotting blood. Another type of anticoagulant acts by directly inhibiting thrombin (e.g. hirudin). Antiplatelet drugs inhibit platelets from aggregating to form a plug and inhibit

thrombus formation.

The market is dominated by platelet aggregation inhibitors and heparins, which account for 58% and 28% of sales respectively. This compares with the 5% share held by vitamin K antagonists and fibrinolytics and less than a 2% share each for direct thrombin inhibitors and other products – though the latter two are growing rapidly.[3b]

Because of the poor safety profile of some of the above mentioned antithrombotic drugs, there is a critical need for development of a safe and orally active anticoagulant. Recently, factor Xa (fXa) has emerged as a particularly promising target in the blood coagulation cascade.

The present review will give an account on the recent development of renin inhibitors as antihypertensive agents and factor Xa inhibitors as anticoagulants.

2. RENIN INHIBITORS

2.1 Renin-angiotensin aldosterone system (RAAS)

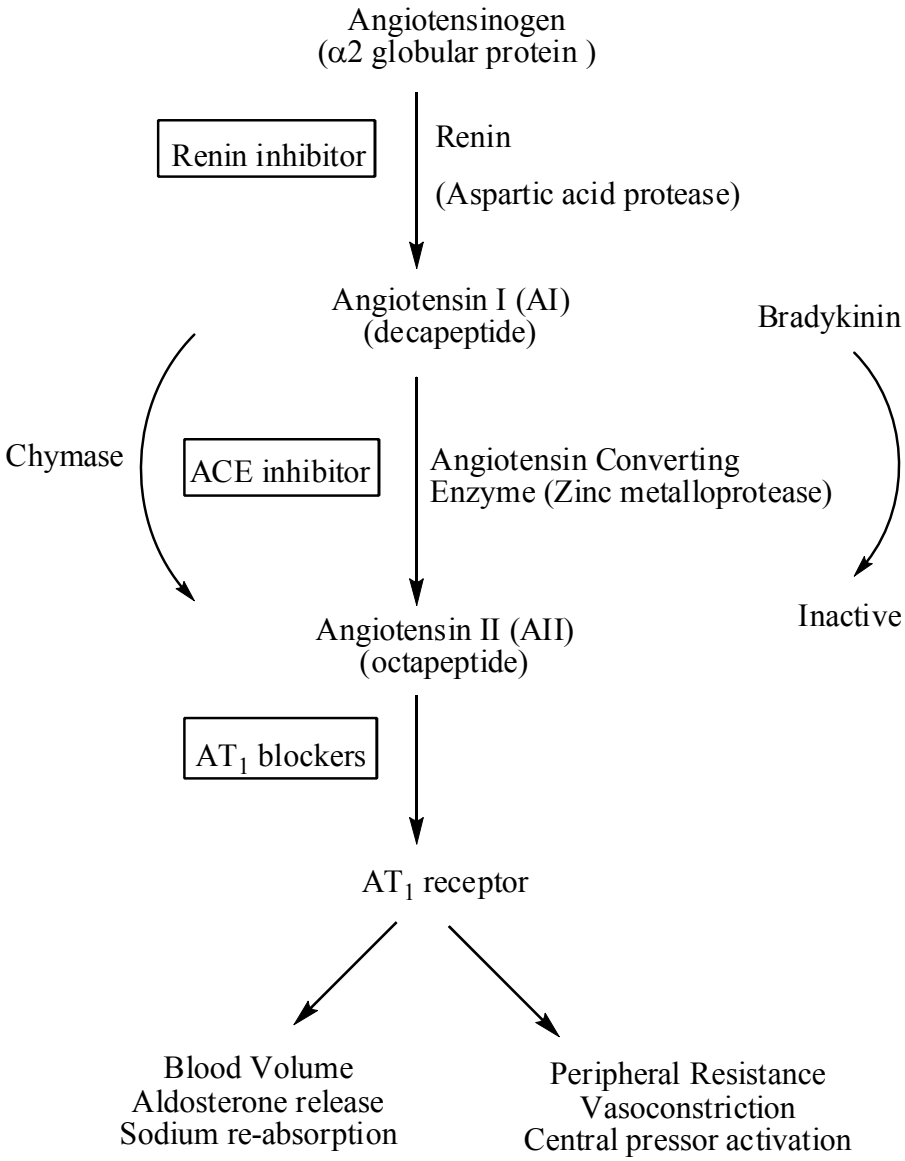
The renin-angiotensin system plays an important role in the regulation of blood pressure and volume homeostasis. Renin, a member of the aspartyl protease family, is synthesized in the juxtaglomerular apparatus of the kidney in response to a decrease in circulatory blood volume and blood pressure. Renin controls the first and rate-limiting step of the renin-angiotensin system catalyzing the cleavage of Leu_{10}-Val_{11} peptide bond of angiotensinogen (α-2 globular protein synthesized mainly in the liver) and releasing the physiologically inactive decapeptide angiotensin I. Angiotensinogen is the only known physiological substrate for renin, therefore renin is an absolutely essential and extremely specific enzyme. This is in contrast to angiotensin-converting enzyme (ACE) which can be by-passed by the serine proteinase chymase. Chymase can also cleave other peptides such as bradykinin.

Angiotensin I (AI) is converted by angiotensin converting enzyme (ACE), a zinc metalloenzyme, to form the active vasoconstrictor octapeptide angiotensin II (AII), which acts on AT_1 receptor (member of GPCR super family). The activation of the AT_1 receptor by AII triggers a number of physiological effects, such as sodium-water retention and vasoconstriction, leading to an increase in blood pressure (Figure 1.)

ACE inhibitors block the conversion of AI to AII, thus reducing circulating levels of AII. Since ACE also breaks down bradykinin and substance P, it can lead to an increase in circulating and tissue levels of these substances, which can result in side effects such as cough and angioedema. AII acts on two main receptor subtypes AT_1 and AT_2, but most functions of the RAAS are mediated through the activation of the AT_1 receptor. Activation of the AT_1 receptor leads to an increase in blood pressure, including vasoconstriction and aldosterone synthesis. AII acts on renal epithelial cells to induce retention of Na^+ and H_2O, and loss of K^+ and Mg^{2+}.

AT_1 receptor blockers specifically block the binding of AII to the AT_1 receptor, and lead to an increase in AI and AII. However AT_1 receptor blockers do not completely inhibit the RAAS since they do not block

Figure 1. The renin-angiotensin-aldosterone system



AT_2 receptor subtypes. These receptors may be stimulated by large amount of AII, during the AT_1 blockade, resulting in activation of AT_2 and AT_3 receptors.

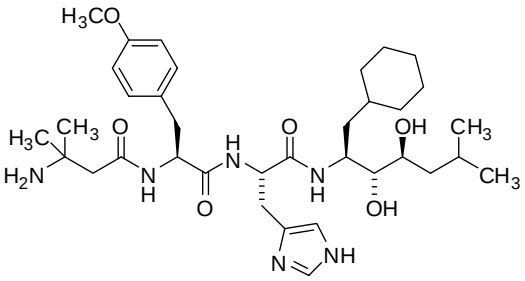
Since renin is the rate limiting step in RAAS, renin inhibition is considered to be an attractive antihypertensive strategy.

2.2 Peptidomimetic Renin Inhibitors

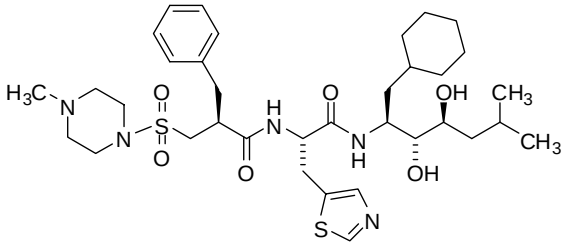
Renin has been recognized as a desirable target for antihypertensive drugs for almost four decades. Pepstatin (N-isovaleryl-Val-Val-Sta-Ala-Sta) is a naturally occurring inhibitor of renin and most aspartic proteases. Renin, being a very specific enzyme, requires an octapeptide as the minimal acceptable substrate. This minimum octapeptide substrate, His-Pro-Phe-His-Leu-Leu-Val-Tyr, is similar to the eight amino acid sequence, His_6-Pro_7-Phe_8-His_9-Leu_{10}-Val_{11}-Ile_{12}-His_{13}, which is found in angiotensinogen. In

comparing the minimum substrate with the human sequence, it is seen that some variability is tolerated at the C-terminus of the peptide. The Val_{11}-Ile_{12}-His_{13} sequence present in angiotensinogen can be replaced with other hydrophobic amino acids without the loss of substrate activity. While the goal is to develop an inhibitor of renin and not the substrate, known substrates of an enzyme are frequently used as starting points for the development of inhibitors. Intensive efforts at many pharmaceutical companies (such as, Abbott, Ciba-Geigy (now Novartis), Hoffman-La Roche, Merck and Pfizer) in 1980s led to the discovery of many potent renin inhibitors that had a molecular weight of a tetrapeptide. These molecules included (Figure 1) Enalkiren (**1**, A64662, Abbott),[3c] Zankiren (**2**, A72517, Abbott),[3c] Remikiren (**3**, Ro 42-5892, Hoffmann-La Roche),[3c] Ciprokiren (**4**, Ro 44-9375, Hoffmann-La Roche),[3c] CGP 38560 (**5**, Ciba-Geigy (now Novartis)).[3c]

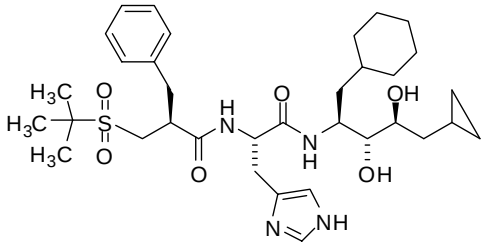
Figure 2. Structures of several potent peptide-like renin inhibitors



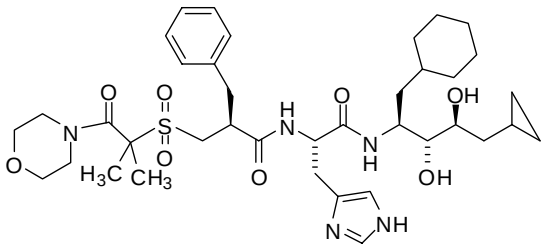
1, Enalkiren (A 64662, Abbott)



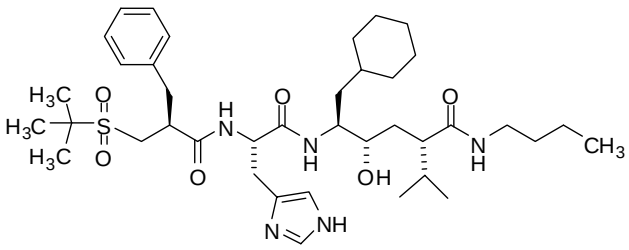
2, Zankiren (A 72517, Abbott)



3, Remikiren (Ro 42-5892, Hoffmann-La Roche)



4, Ciprokiren (Ro 44-9375, Hoffmann-La Roche)



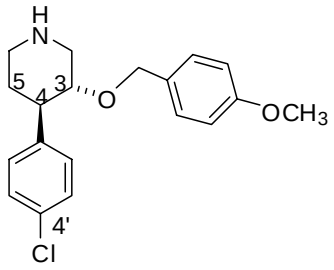
5, CGP 38560 (CIba-Geigy, now Novartis)

Clinical development of these first-generation renin inhibitors, **1**-**5** was far more challenging than originally expected. Thus, none of the above mentioned renin inhibitors successfully completed clinical trials. The decision not to develop the above compounds as clinical candidates was primarily attributed to the high cost of their synthesis, low bioavailability (attributable to poor gastrointestinal absorption), short

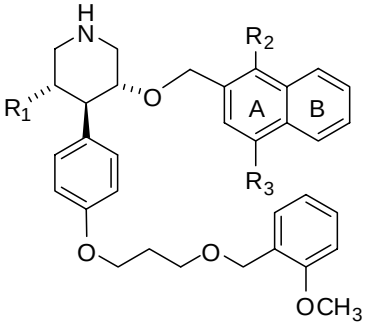
duration of action, substantial first-pass metabolism (hepatic clearance), weak blood pressure lowering activity, and the remarkably successful therapeutic results with angiotensin receptor blockers (ARBs) and angiotensin converting enzyme inhibitors (ACEs) in the 1990s. Clearly one of the major challenges in the synthesis of such structures is the control of absolute and relative stereochemistry of a minimum of three stereogenic centers bearing different functional groups.

2.3 Piperidine-based Renin Inhibitors

Although clinical efficacy was established for several of the peptidomimetic inhibitors of human renin, almost all development compounds were finally abandoned. The need for increased oral bioavailability and longer duration of action have been added challenges in the quest for a marketable antihypertensive drug based on the inhibition of the renin enzyme. Over the past decade, using the structure-based drug design approach, new classes of renin inhibitors without resemblance to the renin substrate have been discovered and some of them are likely to progress to market. Scientists from Hoffmann-La Roche discovered substituted piperidine renin inhibitor **6,** via a high through-put screen.[4]

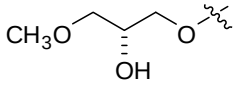


6

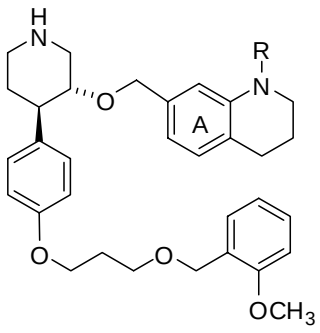


7a, $R_1 = H, R_2 = R_3 = H$

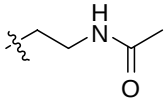
7b, $R_1 = H, R_2 = R_3 = OCH_3$

7c, $R_1 =$ 

$R_2 = R_3 = OCH_3$



8a, R = H

8b, R = 

The lead compound **6**, *trans*-4-(4-chlorophenyl)-3-(4-methoxybenzyl) piperidine was weak inhibitor of human renin (IC_{50} = 50 μM). The (R, R)-isomer with 99.4% ee was more active (IC_{50} = 26 μM) than the (S, S)-isomer with 96.4% ee which had IC_{50} = 1200 μM. Thus, compound **6** was the first representative of a new structural class of renin inhibitors. Low resolution X-ray data of renin complexed with (R, R)-isomer of compound **6** revealed the nature of interactions at the molecular level.[4-6] These interactions are:

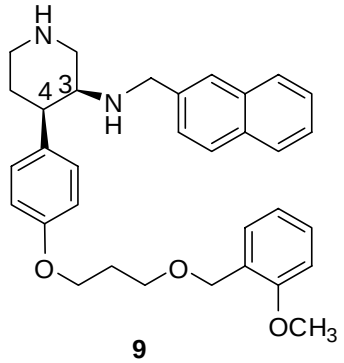
[a] The protonated nitrogen was found to be symmetrically positioned between the two catalytic aspartic acid residues (Asp32 and Asp215) forming one hydrogen bond with each of the carboxylates.

[b] The lipophilic chlorophenyl residue was directed towards the large hydrophobic subsite S1/S3

which normally accommodates Leu and Phe side chains of angiotensinogen. The chlorine atom at position 4 did not use the space available in the S1/S3 subsite of the enzyme, suggesting that structural modifications in the 4-position is possible. Replacement of the chlorine atom in the 4′-position by substituents consisting of aromatic moieties attached by chains of variable length gave compounds with equal or improved potency, e.g. **7a** (IC_{50} = 1.5 nM). More hydrophilic structural modifications at the 4′ position gave inhibitors with dramatically reduced activity. Thus, when polar functionalities like pyridine rings, amide or sulfonamide functionalities were introduced, potency was lost. Polyether links to the phenyl ring in the extended 4′-substituent was useful and propylene-dioxy benzyl ethers were clearly the most potent compounds, e.g. **7b** (IC_{50} = 0.06 nM).

[c] To improve water solubility, introduction of a polar 3-methoxy-2-hydroxy propoxy moiety at the 5-position of the piperidine ring, gave compound **7c** (Ro-0661132) with IC_{50}s of 0.067 nM and 8.9 nM against purified human renin and plasma renin, respectively. Compound **7c** displayed a potent and long-lasting blood pressure lowering effect in conscious sodium depleted marmoset monkeys. In addition, **7c** was able to normalize albuminuria and kidney tissue damage in rats when given over a period of four weeks.

[d] The lipophilic naphthyl residue of compounds **7a**, **7b**, and **7c**, occupies the large hydrophobic S1/S3 subsite of renin. The aromatic ring B is the only part of the naphthyl moiety that allows hydrophilic modifications. Thus, 3, 4-disubstituted piperidine compounds bearing a tetrahydroquinoline methoxy substituent in position 3 displayed good inhibitory potency against human renin and a reduced lipophilicity. Compound **8a**, with IC_{50}'s of 0.67 and 37 nM against purified human renin and plasma renin respectively and a log D of 1.9, displayed a potent long lasting blood pressure lowering effect after oral administration (1 and 3 mg/kg) to sodium depleted conscious marmosets. Compounds with tetrahydoquinoline nitrogen substituents of a length of four to five atoms bearing at least one non-basic H-bond acceptor function showed substantially improved inhibitory potency against human renin as compared to the non-substituted analogues. Compound **8b** with the acetylaminoethyl substituent had sub-nanomolar potency (IC_{50} = 0.039 nM). This, together with its improved physicochemical characteristics, made compound **8b,** the most promising candidate out of this series.

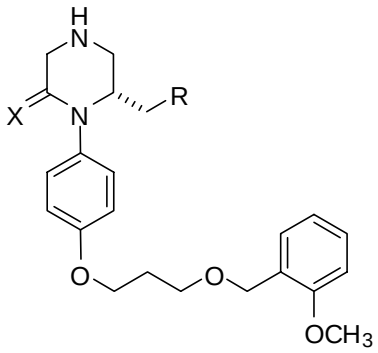


9

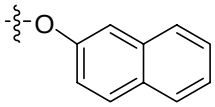
An analogue of compound **7a** in which the naphthylmethoxy substituent at the 3-position of the piperidine ring was replaced by a naphthylmethylamino group was reported by the scientists from Pfizer Global Research & Development.[7] Compound **9** from this series had good inhibitory activity (IC_{50} = 61 nM) and interestingly, in this series, the 3, 4- cis stereochemistry was preferred. These compounds also inhibited CYP enzymes and further development was dropped.

2.4 Piperazine-based Renin Inhibitors

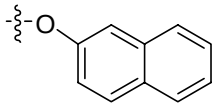
Taking the piperidine-based renin inhibitor **7a** as the lead, scientists at Pfizer Global Research & Development introduced a second nitrogen atom into the piperidine ring of **7a** and moved the ether oxygen (at 3-position) away from the piperazine ring, thus forming compound **10a** (IC_{50} = 180 nM). The corresponding 5-oxo analogue, **10b** (keto-piperazine), had IC_{50} = 54 nM.[8-11]



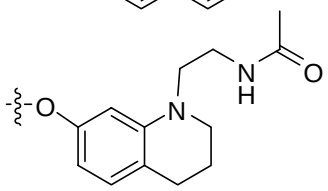
10a, X = H_2, R =



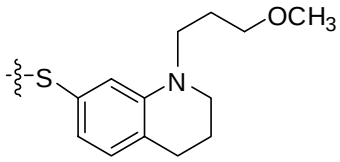
10b, X = O, R =



10c, X = O, R =

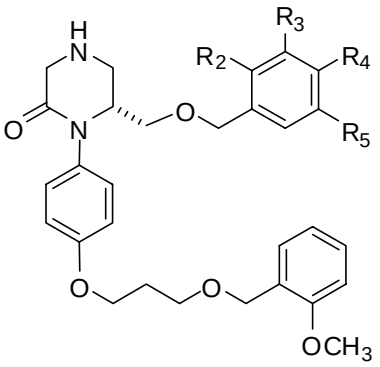


1**0d**, X = O, R =

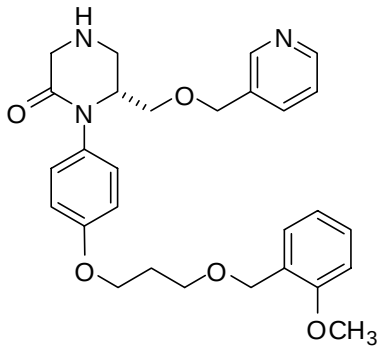


Compound **10b** at 30 mg/kg reduced blood pressure by up to 20 mm-Hg in double transgenic mice, however, blood pressure returned to baseline levels after 3h. Compounds **10c** and **10d** were the most potent renin inhibitors in this series with IC_{50}'s of 0.30 nM and 0.18 nM respectively. Although the ketopiperazine scaffold effected sub-nanomolar renin inhibition and demonstrated *in vivo* blood pressure reduction in transgenic mice, these inhibitors suffered from high molecular weights and sub-optimal PK properties. Replacement of the tetrahydroquinolinyl ring in **10c** with a benzyl ether resulted in compound **11a** which displayed a dramatic loss of renin inhibition (IC_{50} = 7000 nM).

However, substitution on the benzyl ether did lead to greatly improved renin inhibition. For example, introduction of a 4-Cl substituent led to a >20-fold improvement in renin inhibition (**11b**, IC_{50} = 300 nM). A further potency gain was achieved by adding a second electron withdrawing group at the 3-position, such as the 3,4-dichloro benzyl ether (**11c**, IC_{50} = 180 nM). The most active compounds in this series



11a, $R_2 = R_3 = R_4 = R_5 = H$
11b, $R_2 = R_3 = R_5 = H$, $R_4 = Cl$
11c, $R_2 = R_5 = H$, $R_3 = R_4 = Cl$
11d, $R_2 = R_5 = H$, $R_3 = CF_3$, $R_4 = F$
11e, $R_2 = R_5 = H$, $R_3 = CF_3$, $R_4 = Cl$



12, IC_{50} = 120 nm

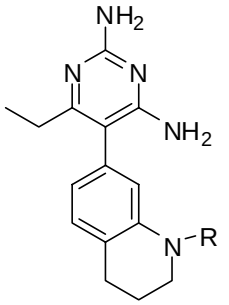
were **11d** (IC_{50} = 140 nM) and **11e** (IC_{50} = 120 nM).[11a] In this series, increased renin inhibition was driven by aryl substituents that led to a net increase in the lipophilic and electron deficient nature of the aromatic ring. Stereochemistry of the benzyl ethers attached to the 3-position of the ketopiperazine scaffold also played a significant role in the renin inhibition. Surprisingly, the benzyl ether analogs of S-configuration were found to be much less potent against renin.

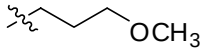
A series of pyridinyl ethers was also examined[11a] to explore the effect of basic groups in the S3 pocket. While introduction of nitrogen atoms at the 2- and 4-positions led to inactive compounds (IC_{50} = >1000 nM), the pyridinyl ether **12** inhibited renin with an IC_{50} = 120 nM. Compound **12** displayed significantly less Cytochrome P450 3A4 inhibition (44% inhibition at 3 μM), lower molecular weight (MW = 491), improved cell membrane permeability, and aqueous solubility. In general, the ketopiperazine series suffered from CYP3A4 inhibition and a poor Rat PK profile.

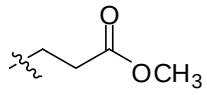
2.5 Aminopyrimidine-based Renin Inhibitors

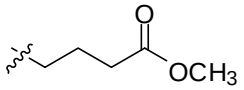
From a weakly active HTS hit, Scientists from Pfizer laboratories designed a novel, non-peptidic small molecule renin inhibitor (**13a),** that contained a 6-ethyl-5-(1, 2, 3, 4-tetrahydroquinoline-7-yl)pyrimidine -2,4-diamine ring system. This compound served as an excellent starting point for further investigation.[11b-d]

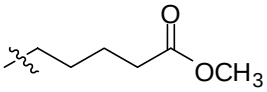
Side chains containing hydrogen bond acceptors that could possibly penetrate into $S3^{sp}$ of renin, were introduced at the N-atom of tetrahydroquinoline ring. The activity of the compounds increased as the side chain length increased. To keep the molecular weight low, the length of the side chain was limited to five carbons (**13d**, IC_{50} = 91 nM). Compound **13a** exhibited low molecular weight (341 daltons), excellent solubility (59.3 μg/mL), permeability (26.5 x 10^{-6} cm/s), low cLogP (3.34), and low Cytochrome P450 3A4 inhibition (IC_{50} = 2.8 μM)). Furthermore, compound **13a** displayed moderate *in vivo* elimination

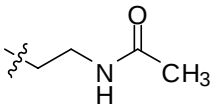


13a, R =  IC_{50} = 650 nM

13b, R =  IC_{50} = 198 nM

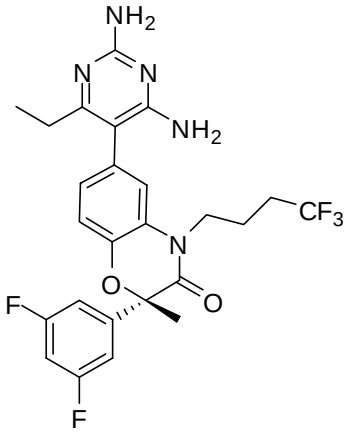
13c, R =  IC_{50} = 120 nM

13d, R =  IC_{50} = 91 nM

13e, R =  IC_{50} = 178 nM

half-life (1.11 hours), systemic plasma clearance (36.8 mL/min/Kg), and volume of distribution (1.6 L/Kg), but exhibited low oral bioavailability (<10%) in Sprague Dawley (SD) Rats.

The side chain of **13e** contains a hydrogen bond donor and acceptor and was a key side chain in the ketopiperazine series. Compound **13e**, demonstrated an increase in potency (3.5 fold) relative to **13a** with a molecular weight of 354 daltons and was stable in HLM (human liver microsome) up to 40 minutes. Compounds **13a-e**, were also devoid of activity against the aspartic peptidases Cathepsin D, Cathepsin E and Pepsin (0-5% inhibition @ 100 μM). Further manipulation of these framework led to the development of the 6-(2,4-diaminopyrimidinyl)-1,4-benzoxazinone series, represented by compound **13f**.[11b]

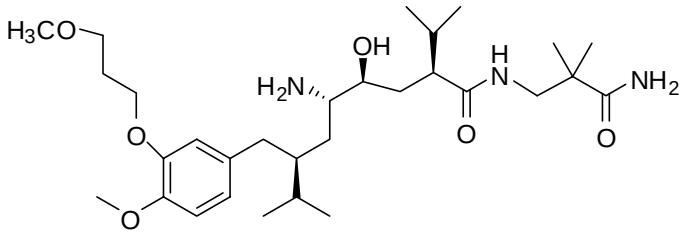


13f, IC_{50} = 48 nM

Compound **13f** is an enantiopure compound ("S" stereochemistry) and has modest potency against renin. This compound has a molecular weight of 521 daltons and the following SD rat PK parameters: CL = 26 mL/min/Kg, V_{dss} = 5.7 L/Kg, $T_{½}$ = 2.96 hours. Most impressive was the oral bioavailability (rat) of 74%. This series of renin inhibitors is quite unique and shows promise of obtaining a potent renin inhibitor with good physicochemical and pharmacokinetic properties. Enormous efforts have been conducted by Pfizer and Hoffmann-La Roche. It will be interesting to see what the future holds for the frameworks presented from these companies.

2.6 Aliskiren

Because none of the peptide-like renin inhibitors survived all stages of drug development, there was a need for new classes of nonpeptide renin inhibitors that fulfill all criteria for becoming a successful drug. To improve the unfavorable pharmacokinetic behavior of earlier peptide like renin inhibitors, a combination of molecular modeling and crystallographic structural analysis were employed by a team of Novartis chemists to design a new class of hydroxyethylene based renin inhibitors lacking the peptide backbone.[12] Out of this intensive study, the nonpeptide compound **14**, [2(*S*), 4(*S*), 5(*S*), 7(*S*)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]octanamide] successfully emerged as potent renin modulator, exhibiting sub-nanomolar



14, Aliskiren (SP100)

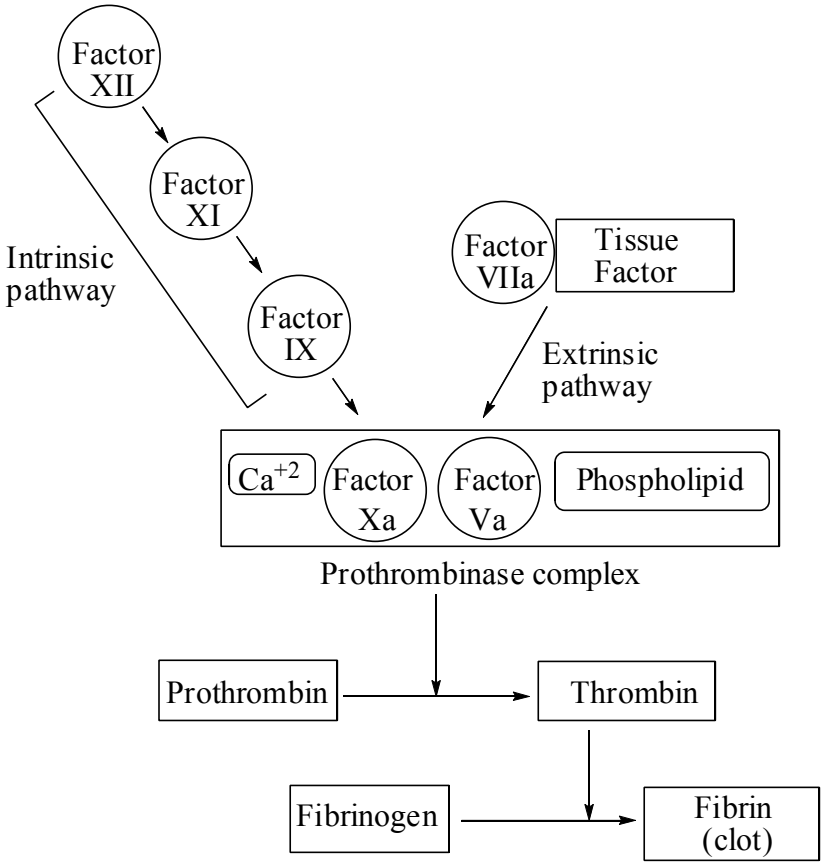
binding affinity to human renin and oral administration properties.[12,13] However, the pathway for its synthesis, (patented in 1995) consisted of many steps and was not suitable for industrial manufacture. In 1999, Aliskiren was out-licensed to Speedel AG (Basel, Switzerland) who succeeded in designing a cost effective method of production.[14] In April 2006, Novartis filed the first NDA for an inhibitor of renin for the treatment of hypertension. Tekturna (Aliskiren) was approved on March 6, 2007 and was the first of a new class of antihypertensive drugs to enter the market in more than a decade. For almost 20 years, the development of oral renin inhibitor met huge problems. The clinical development of aliskiren signifies a major breakthrough. However, the search for agents with improved oral bioavailability (Tekturna human oral bioavailability is 3%) and efficacy will continue.

3. FACTOR Xa INHIBITORS

3.1 Blood Coagulation Cascade

The blood coagulation factor Xa (fXa), a serine protease, plays a critical role in the blood coagulation cascade, serving as the point of convergence of both the intrinsic and extrinsic pathways. Factor Xa combined with the nonenzymatic cofactors Va and Ca^{+2} on the phospholipids surface of platelets or endothelial cells, form the prothombinase complex which is responsible for the proteolysis of prothrombin to form catalytically active thrombin. Thrombin, in turn, catalyzes the cleavage of fibrinopeptides from fibrinogen to form insoluble fibrin, thus initiating a process that ultimately leads to clot formation (Figure 3).

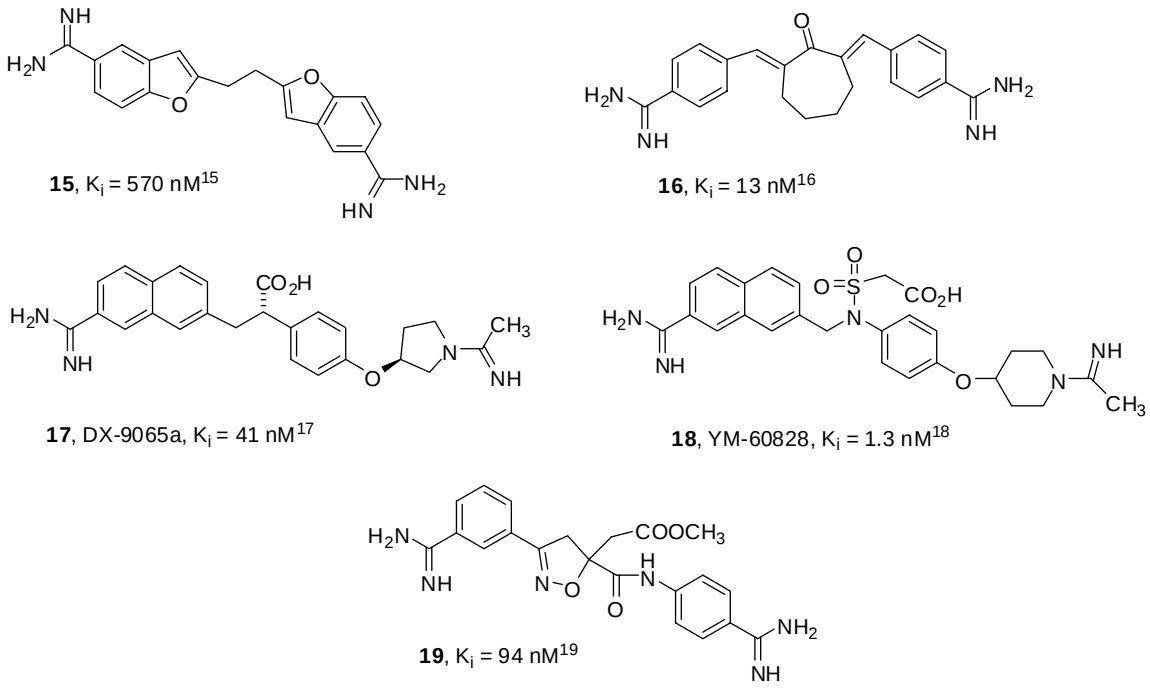
Figure 3: Blood coagulation cascade



3.2 Dibasic Factor Xa Inhibitors

Between the early 1980s and late 1990s, a number of dibasic fXa inhibitors containing highly charged,

Figure 4: Representative examples of dibasic fXa inhibitors.

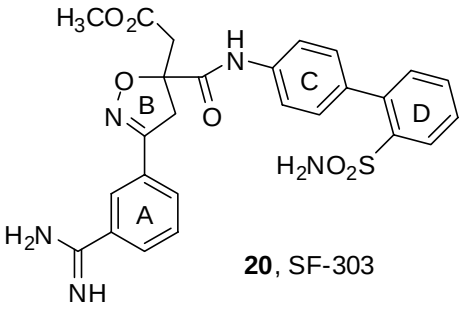


strongly basic, phenylguanidine and phenylamidine moieties were reported in the literature. These basic

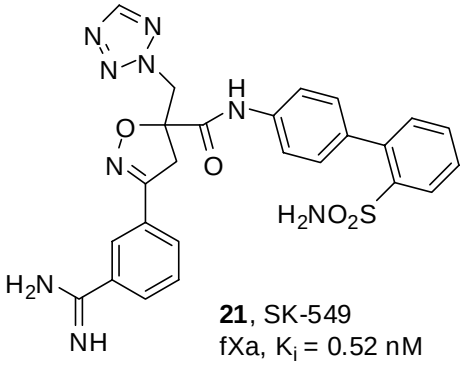
pharmacophores anchored the inhibitor in the key S1 sub-pocket of fXa through hydrogen bonding with the carboxylic acid of Asp189. Inspite of being potent fXa inhibitors, undesirable pharmacokinetic properties such as poor oral absorption and short duration of action was observed-presumably due to the strongly basic moieties present. Representative examples are shown below (Figure 4).[15-19]

3.3 Monobasic Factor Xa Inhibitors

To reduce the basicity of the above mentioned dibasic compounds, the scientists from DuPont modified compound **19** to create the first monobasic fXa inhibitor (**20**, SF-303). The biaryl moiety of **20** was designed to interact with the S4 aryl binding domain of the fXa active site.[20]



20, SF-303

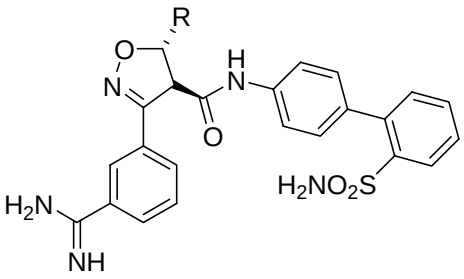


21, SK-549
fXa, K_i = 0.52 nM
Thrombin, K_i = 400 nM
Trypsin, K_i = 45 nM

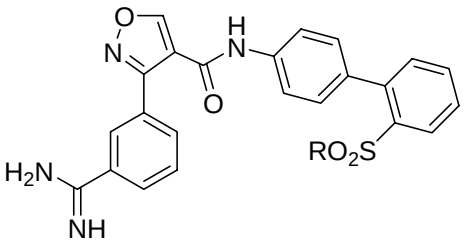
Different substitutions (e.g. F, CH_3) were introduced in the C-ring of **20**. Furthermore, the C-ring was replaced with either a pyridine or pyrimidine ring. These compounds (not shown) exhibited good selectivity for fXa compared to thrombin and trypsin and exhibited a potent antithrombotic effect in the rabbit arterio-venous shunt thrombosis model. Since stability of the ester side chain of **20** (SF-303) *in vivo* was a concern, compound **21** (SK-549) was made that had improved pharmacokinetic properties. SK-549 had a relatively low clearance in both rabbits and dogs with β-phase half-lives of 0.6 and 1.6 h respectively. To discover what aspects of the isoxazoline core were necessary for activity and to improve the activity by changing the point of attachment of the amide moiety of compound **21**, regioisomeric isoxazolines (3,4,5-trisubstituted), **22** were prepared.[21]

Furthermore, to eliminate all the chiral centers of compounds **21** and **22**, the isoxazoline ring was subsequently aromatized to a planar isoxazole core providing compound **23a** (SA-862) with improved potency. Interestingly, replacement of the sulfonamide moiety in compound **23a** with a methyl sulfone resulted in compound **23b** with similar potency.

The lack of chirality of the isoxazole and high affinity for fXa made it an attractive template for further optimization. To mimic the isoxazole, a pyrazole ring was installed. This modification ultimately led to the discovery of the novel pyrazole analogue **24** (SN-429, fXa K_i = 13 pM).[22] Unfortunately, poor oral

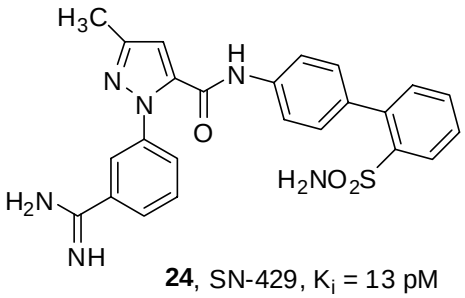


22a, R = CH_3
22b, R = CH_2OCH_3
22c, R = CH_2-tetrazole

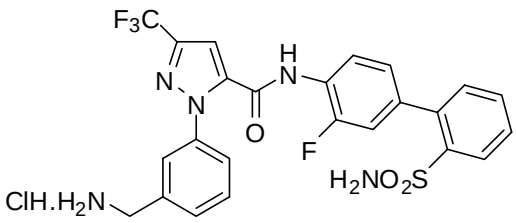


23a, SA-862 (R = NH_2), K_i = 0.15 nM
23b, (R = CH_3), K_i = 0.10 nM

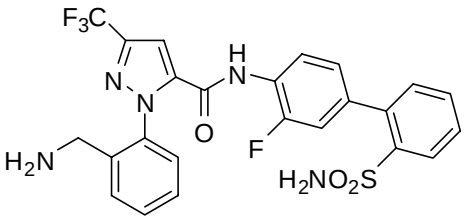
bioavailability and relatively short duration of action precluded further development of the amidine containing compounds (Table 1).



24, SN-429, K_i = 13 pM



25, DPC-423, K_i = 0.15 nM



26, DPC-602, K_i = 0.91 nM

Efforts to improve the oral bioavailability and pharmacokinetic profile of **24** while maintaining subnanomolar potency and *in vitro* selectivity, was accomplished by replacing the amidine moiety with the less basic benzylamine (pKa ~ 8.8) group . This work culminated in the synthesis of compound **25** (DPC-423), a highly potent, selective and orally active fXa inhibitor which was selected for clinical development.[23]

Further optimization of **25** by moving the aminomethyl group around the benzene ring attached to the N-1 atom of the pyrazole core led to a potent, selective o-aminomethyl phenylpyrazole analogue, **26** (DPC-602).[24] The best overall PK profile (Table 1) was achieved with **26**, which exhibited excellent oral bioavailability (F% = 100%) in dog.

Furthermore, when compared (Table 2) with **25**, the selectivity profile of **26** was excellent. Both **25** and **26** inhibited plasma kallikrein (>60 fold) equally.

From another ongoing effort for the development of orally active fXa inhibitors, scientists from

Table 1: In vivo dog pharmacokinetics and cell permeability of aminomethyl P1 analogues

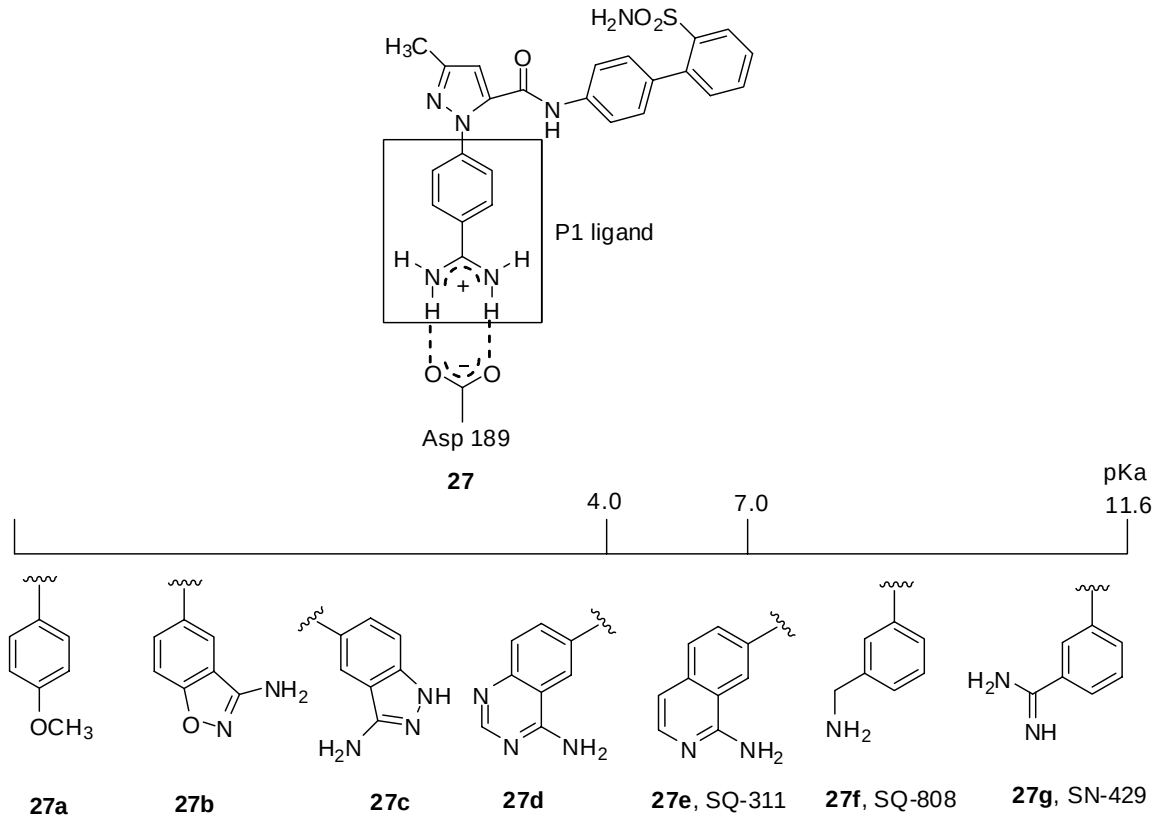
Compound	CL (L/h/kg) iv	V_{dss} (L/kg) iv	$T_{1/2}$ (h) iv	F% po	Caco-2 P_{app} X 10^{-6} cm/s	Human Protein Binding (% bound)
SN-429	0.67	0.29	0.82	4	0.30	-
DPC-423	0.24	0.9	7.5	57	4.9± 0.3	89
DPC-602	0.14	1.2	7.1	100	6.5± 0.4	89

Table 2: Selectivity profile of DPC-602 Versus DPC-423

Human enzymes	K_i (nM) DPC-423	DPC-602
fXa	0.15	0.91
Trypsin	60	3500
Thrombin	6000	3600
Plasma Kallikrein	61	58

Bristol-Myers Squibb[25] used structure-based drug design techniques and molecular recognition principles to improve the PK profile of SN-429. It was demonstrated that lowering the pKa of the P1 ligand (by

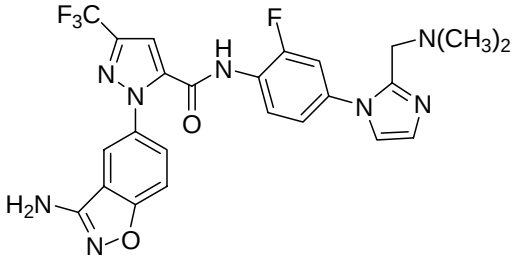
Figure 5: Design of guanidine/ benzamidine mimics



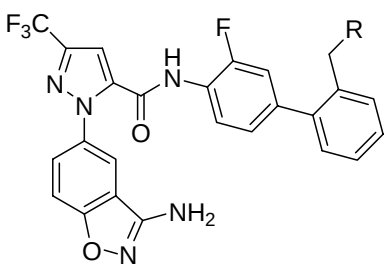
replacing the benzamidine in the P1 position with less basic benzamidine mimics or neutral residues) resulted in compounds (Figure 5) with improved pharmacokinetic features mainly as a result of (a)

decreased clearance (b) increased volume of distribution (V_{dss}) and enhanced oral absorption. Among these benzamidine mimics and non-benzamidines, 1- aminoisoquinoline **27e** (SQ-311) was shown to be a highly potent and selective, orally bioavailable inhibitor of fXa. Although incorporation of an aminobenzisoxazole (e.g. compound **27b**) as the P1 ligand resulted in compounds with improved selectivity for fXa relative to trypsin and plasma kallikrein, the resulting compounds were poorly soluble, poorly permeable, and highly protein bound. Employing solubilizing and less lipophilic heterocycles at the terminal ring of the P4 moiety led to the identification of the phenylimidazole analogue **28** (DPC-906, BMS-561389).

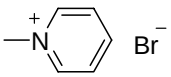
Substitution of the 2-position of the imidazole with a dimethylaminomethyl moiety reduced protein binding and provided the best balance of potency, selectivity, and pharmacokinetic parameters. Compound **28** exhibited antithrombotic efficacy in the rabbit arterio-venous shunt thrombosis model and the HCl salt of this compound emerged as a potential candidate for clinical development, as Razaxaban (in phase II).[26]

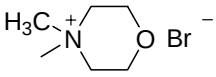


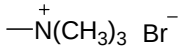
28, DPC-906, BMS-561389
(Razaxaban)



29a, R = $N(CH_3)_2$

29b, R = 

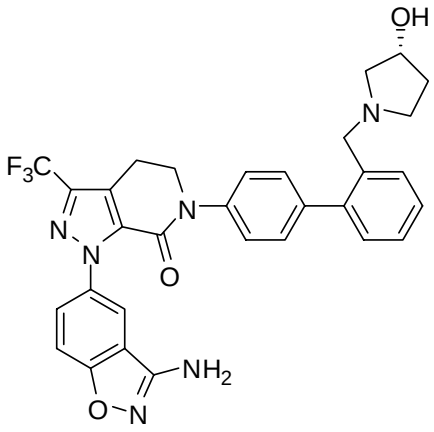
29c, R = 

29d, R = 

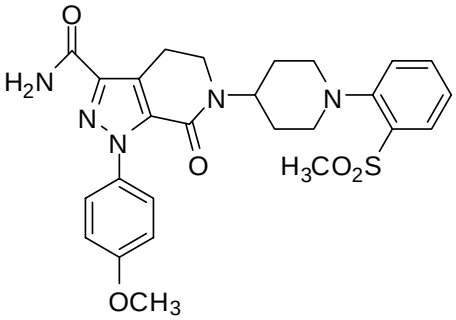
In an attempt to have a follow up compound of razaxaban, the 2-dimethylaminomethyl substituted imidazole ring of compound **28** was replaced with a variety of 2-aminomethyl substituted benzenes (e.g. **29a**-**29d**). Overall, all the compounds were very comparable to razaxaban in terms of the pharmacokinetic and selectivity profile.[27] Although the quaternary amines were the most potent fXa inhibitors, they were also less permeable in Caco-2 assay. This series of compounds, however, did not provide any added advantages over razaxaban.

The above mentioned monocyclic pyrazoles carrying an acyclic amide bond at the C-5 position can undergo *in vivo* hydrolysis to release a biarylaniline fragment, which could be potentially mutagenic. To circumvent this, two strategies were employed in an effort to prevent cleavage of the carboxamide linker to form the corresponding aminobiaryls.

Strategy I: Cyclization of the amide NH by tying back to the C-4 of the pyrazole ring to generate a bicyclic pyrazole scaffold (such as pyrazolo-pyridinone, pyrazolo-azepinone and pyrazolo-pyrimidinone).[28] An example of this is, BMS-740808 (compound **30**)[28b] which emerged as a preclinical candidate. Metabolic studies showed no cleavage of the carboxamide moiety.



30, fXa, K_i = 0.03 nM
BMS-740808

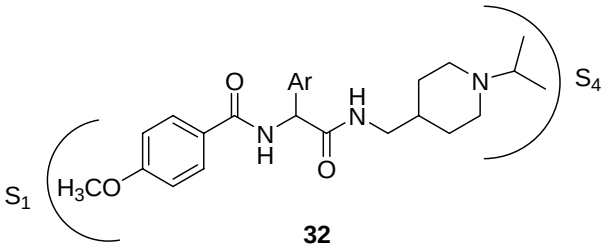


31, fXa, K_i = 0.35 nM

Strategy II: A non-aromatic ring such as 4-aminopiperidine can be used in place of the aniline moiety.[29] This fused pyrazole analogue **31** containing a neutral p-methoxyphenyl P1 group showed improved fXa inhibitory activity relative to their monocyclic counterparts. Furthermore, compound **31** had an improved selectivity profile compared with DPC-423 (compound **25**) with good oral bioavailability in dogs with %F = 78%. However, **31** had a shorter $T_{1/2}$ (0.8 h) compared to DPC-423 ($T_{1/2}$ = 7.5 h). Studies of possible metabolites of **31**, and search for structural modifications to block or reduce the metabolic pathways may improve the pharmacokinetic profile of this series of compounds.

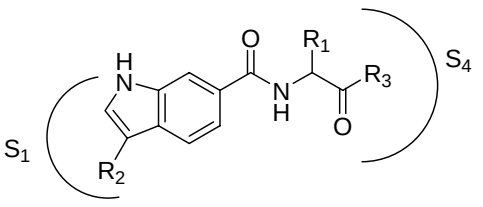
3.4 Amino-acid Based Factor Xa Inhibitors

Sheehan *et al*[30] prepared a series of non-covalent fXa inhibitors derived from D-phenylglycinamide and studied the effect of various ortho-substitutions (electron-donating, electron withdrawing, steric) on the central phenyl ring. Replacement of the central benzene ring with mono or bicyclic heteroaromatic rings did not provide any additional binding affinity toward fXa. Using the Ugi four component coupling (4CC) reaction, the scientists from the same laboratory, reported fXa inhibitors containing the more potent 3-chloroindole S1 binding element as shown in compound **33a**.[30]



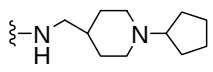
32

Ar = C_6H_5, 2-furan, 3-furan, 2-thiophene, 3-thiophene, 2-imidazole, 4-quinoline, 3-quinoline



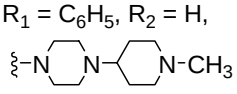
33a, R_1 = 1-naphthalene, 2-naphthalene 3-quinoline, 2-thiazole, R_2 = Cl,

R_3 =



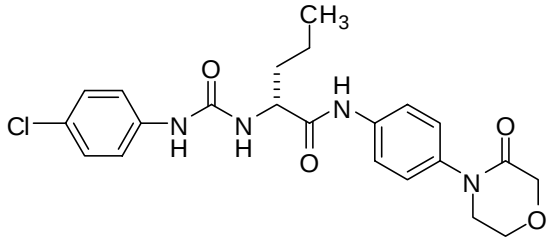
33b, R_1 = C_6H_5, R_2 = H,

R_3 =

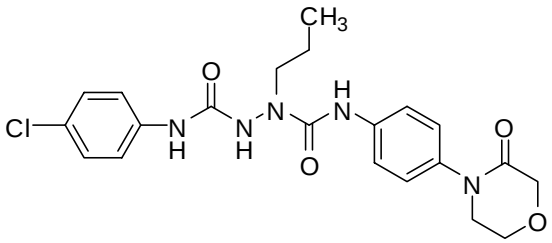


Using D-amino acids as the central template, scientists from Eli Lilly & Co., prepared a class of fXa inhibitors that are structurally related to **33a**. Work in this area led to the identification of a clinical candidate, **33b** (LY-517717) that is currently in phase II studies.[31]

In an effort to develop fXa inhibitors with a favorable PK profile Mederski *et al*[32] prepared a series of amino acid derivatives. The lead compound from this series was the D-norvaline analogue **34**.



34, fXa IC_{50} = 5.6 nM



35

The key features of this class of compounds are:

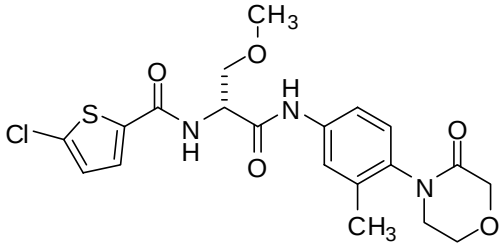
[a] the chlorophenyl serves as a surrogate for benzamidine in the S1 binding pocket.

[b] D-amino acids are the central scaffold.

[c] phenylmorpholine serves as the P4 ligand.

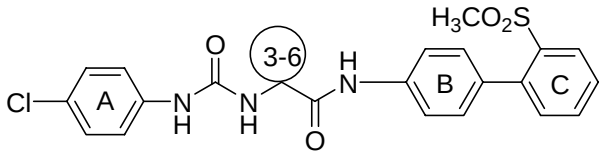
In order to extend the scope of this class of compounds, structurally related semicarbazide derivative **35** with moderate fXa activity was prepared. The replacement of C(2) tetrahedral atoms of compound **34** with a trivalent nitrogen atom resulted in symmetry loss and a configuration midway between the corresponding D- and L- amino acids.[32a]

A chlorothiophene residue can be considered as a good isoster for the replacement of the chlorophenyl ring in the S1 pocket of compound **34**. The urea moiety of **34** was not the optimal linking element between the P1 residue and amino acid portion. Computer modeling studies showed that a methanone group could be a good replacement for the carbamoyl part within the chlorothiophene derivatives giving rise to chlorothiophene carboxamides. This variation, along with the variations on the amino acid core, led to the discovery of **36** (EMD 495235).[32b]

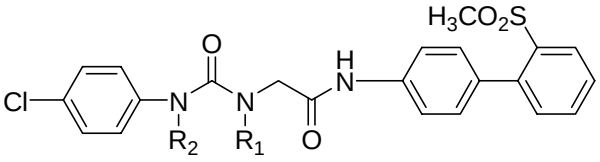


36, EMD-495235, K_i = 6.8 nM

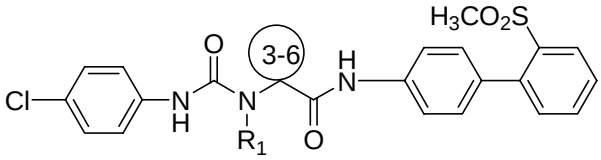
Using non-chiral amino acid as the core, scientists from Pfizer disclosed three-series of glycine-based fXa inhibitors (cycloalkylglycine series, N-alkylglycine series, and N-alkylcycloalkyl glycine series) where a neutral chlorophenyl P1 side chain was incorporated.[33]



37, cycloalkylglycine series



38, *N*-alkylglycine series



39, *N*-alkylcycloalkylglycine series

In the cycloalkylglycine series **37**:

[a] Replacement of the cyclopropyl ring with larger rings led to changes in enzyme inhibition. The cyclohexyl analogue was the most potent, while cyclopentyl or cyclopentenyl ring led to inhibitors with decreased potency.

[b] Incorporation of Sulfur or Oxygen into the cyclohexyl ring led a substantial decrease in potency.

[c] Replacement of the cyclic glycine core in **37** with a dialkyl amino acid gave compounds with activities similar to those of the corresponding cyclic compounds.

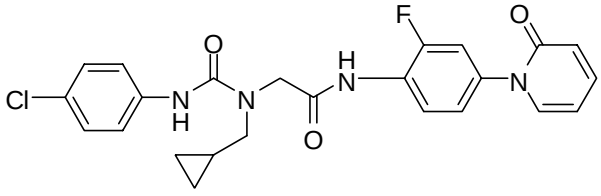
[d] Replacing the A-ring with pyridine led to an inhibitor with diminished potency.

[e] Addition of an Fluorine-atom to the B-ring improved the potency of the inhibitor.

In the N-alkylglycine series **38** and **39**:

[a] Substitution (R_2) on the N-atom of the aniline portion of the urea greatly diminished potency as a result of a loss of critical H-bond interaction with the protein.

[b] Compounds where R_1 is a butyl or cyclopropylmethyl group were the most potent inhibitors. Increasing alkyl branching or incorporating a heteroatom into the side chain reduced potency. Inhibitors with larger cycloalkyl rings such as cyclobutyl, cyclopentyl, and cyclohexyl analogs showed a decrease in potency. In general, the N-heteroaromatic methyl analogs were more potent than the N-benzylic analogs.



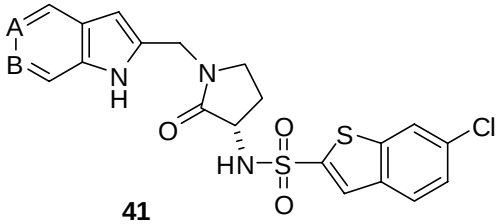
40, IC_{50} = 0.005 μM

Further optimization of the N-alkylglycine series by replacing the phenylsulfone ring with a pyridone ring led to an inhibitor (**40**) that displayed good anti-fXa activity and high plasma exposure indicating increase

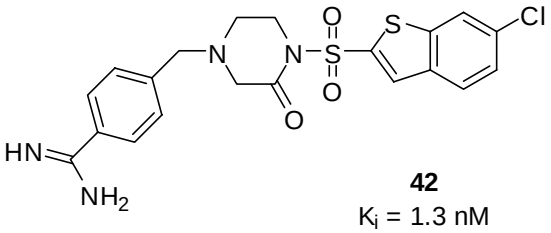
in absorption.

3.5 Arylsulfonyl Piperazinone-based Factor Xa Inhibitors

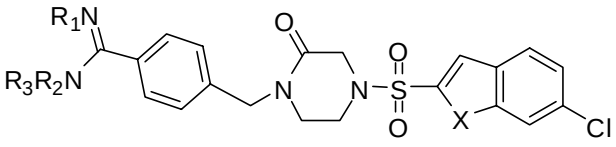
Based on an early lead compound **41**,[34] the scientists from Aventis disclosed an alternative ketopiperazinone **42** scaffold.[35] Jia *et al*[36] disclosed two closely related series **43a** and **43b** that contained 6-chlorobenzo[b]thiophene and 5-chloroindole groups as optimal S1 binding elements. Use of a substituted amidine (cyclic or acyclic) as the S4 group improved both the potency and physicochemical properties. Unfortunately, both classes of fXa inhibitors **43a** and **43b** suffered from poor oral bioavailability, thus they became less attractive for further development. Subsequent optimization of compound **42** gave the 5-azaindole derivative **44** as an orally efficacious inhibitor of fXa. After extensive modifications of the chlorine-containing sulfonamides, the most potent azaindole **45** containing a central sulfonylpiperazinone scaffold with favorable *in vitro* and PK profile was identified (RPR 209685).



41
A = CH, B = N, K_i = 43 nM
A = N, B = CH, K_i = 96 nM

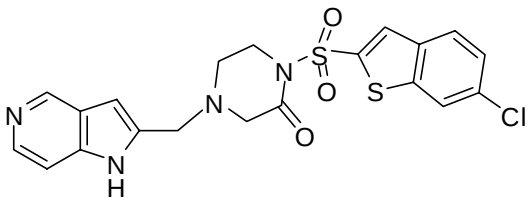


42
K_i = 1.3 nM

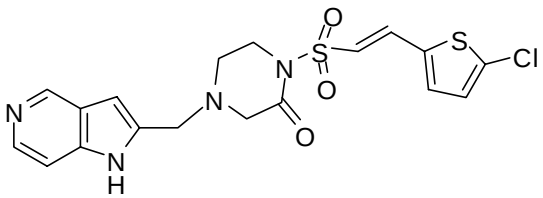


43a, X = S
43b, X = NH

Although RPR 209685 (**45**) proved efficacious in a canine arterio-venous thrombosis model, its short half life ($T_{1/2}$ in dog = 52 min) and requirement for a relatively high dose in the animal model precluded its advancement into clinical trials.[37]



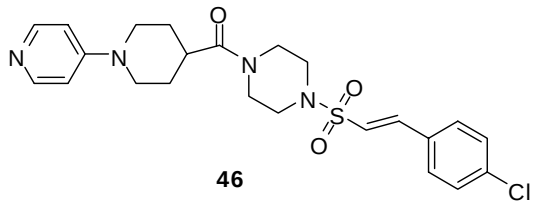
44



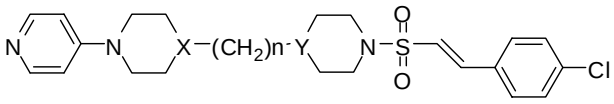
45 (RPR 209685), K_i = 1 nM

Taking the lead compound **46** reported by Zeneca Co.[38], the scientists from Mochida Pharmaceuticals, Japan, replaced the central carbonyl group with a methylene group and prepared a series of fXa inhibitors

(**47a**-**47c**).[39] To appreciate the effect of the arylsulfonyl group in compound **47c**, various naphthyl and benzothienyl groups were introduced. From this exercise a series of 1-arylsulfonyl-3-piperazinone derivatives, M55113 were identified as potent fXa inhibitors. A representative example, **48a** (R = H), exhibited high selectivity for fXa over trypsin and thrombin.

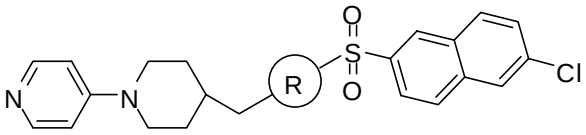


46

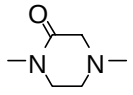
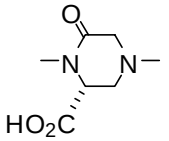
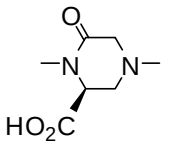
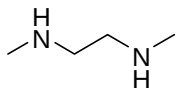
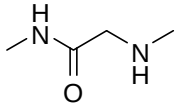
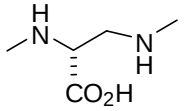
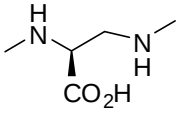


47a, n = 0, X = N, Y = CH
47b, n = 1, X = N, Y = CH
47c, n = 1, X = CH, Y = N

Table 3: Role of central piperazinone moiety on fXa activity

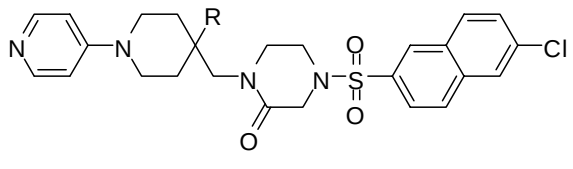


50

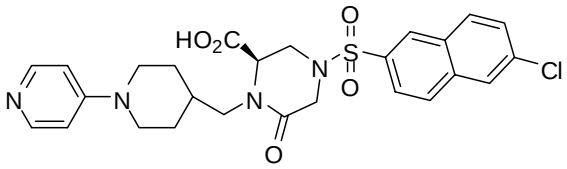
Compound	R	IC_{50} (μM)
48a		0.060
49		0.006
50a		0.079
50b		3.27
50c		0.80
50d		0.60
50e		1.76

In the continuing search for potent fXa inhibitors, introduction of a substituent either at the piperidine ring or piperazine ring of compound **48a** was conceived.[40] Introduction of a substituent with H-bond donating

ability (e.g. OH) in compound **48a** was not very helpful. However, when the 6-position of the piperazine moiety was substituted, higher inhibitory activity was observed. Thus, compound **49** (M55551) was ten times more potent than **48a** (M55113) and was selective for fXa over related serine proteases, in particular, 16000-fold more selective for fXa than for thrombin (IC_{50} > 100 μM) and trypsin (IC_{50} > 100 μM).[40, 41]



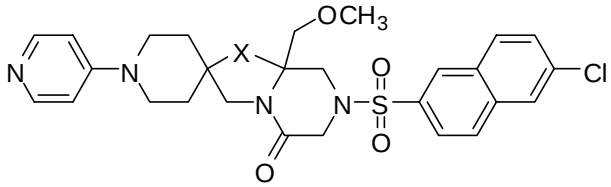
48a, R = H, M55113, IC_{50} = 0.060 μM
48b, R = OH, M55196, IC_{50} = 0.031 μM



49, M55551, IC_{50} = 0.006 μM

The role of the central piperazinone moiety (**50**) of **48** and **49** on fXa activity was investigated and the structures and data listed in Table 3.

It was shown that (*R*)-isomers have higher activity than (*S*)-isomers in both piperazine and ethylenediamine skeletons (**49a** and **50a**, **50d** and **50e**). Compounds containing a rigid and unique spiro structure (**51a**-**51e)** in the central part of the molecule were also disclosed.[42]

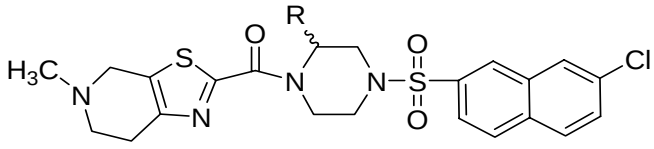


51a, M55529, X = O, IC_{50} = 5 nM
51b, M58163, X = NH, IC_{50} = 1.5 nM
51c, M58169, X = N-CH_3, IC_{50} = 1.6 nM
51d, X = S, IC_{50} = 1.3 nM
51e, X = SO_2, IC_{50} = 2.3 nM

In all instances, the (R)-isomer had stronger activity than (S)-isomer. The N,S-and the N,SO_2-spiro acetal compounds **51d** and **51e** (with a methoxymethyl moiety as a side chain) were equivalent when compared with N,N-acetal compounds **51b** and **51c**, but was superior to N,O-acetal compound **51a**.

3.6 Arylsulfonyl Piperazine-based Factor Xa Inhibitors

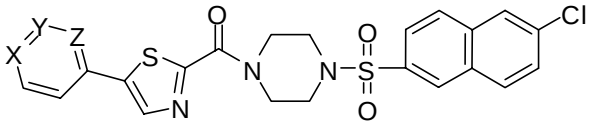
Haginoya *et al*[43] evaluated a series of N-(6-chloronaphthalen-2-yl) sulfonylpiperazine derivatives incorporating various fused-bicyclic rings as a novel S4 binding element.

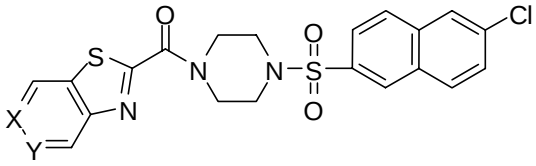


52a, R = $CONH_2$, IC_{50} = 24 nM
52b, R = $CONHCH_3$, IC_{50} = 27 nM
52c, R = H, IC_{50} = 22 nM

A SAR study of this series indicated that both the S and N atoms in the heterocyclic ring and the appropriate position of the tertiary amino group were required for fXa activity for the 5-6 fused system. It was found that 5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-c]pyridine was the most preferred S4 binding element. Among the compounds, **52a** and **52b** possessing a carbamoyl or N-methylcarbamoyl moiety, showed potent inhibitory activities when administered orally to rats.[43a]

Haginoya *et al*[44] installed a pyridine N-oxide unit to the 2-carbamoyl thiazole as shown in structures **53a**–**53c** and **54a** and **54b** respectively. The pyridine-N-oxide is expected to interact with the aromatic S4 pocket via a "cation-π interaction".





53a, X = $\overset{+}{N}$-$\overset{-}{O}$, Y = Z = CH, IC_{50} = 8.6 nM

53b, Y = $\overset{+}{N}$-$\overset{-}{O}$, X = Z = CH, IC_{50} = 37 nM

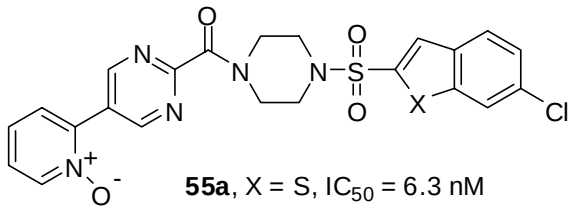
53c, Z = $\overset{+}{N}$-$\overset{-}{O}$, X = Y = CH, IC_{50} = 160 nM

54a, X = $\overset{+}{N}$-$\overset{-}{O}$, Y = CH

54b, Y = $\overset{+}{N}$-$\overset{-}{O}$, X = CH

N-oxidation of thiazole-5-yl pyridine increased the inhibition of fXa more than 10-fold independent of the position of N-oxide, when compared with the tetrahydrothiazolopyridine analogs, **52a**, **52b**, and **52c.** This finding suggested the possibility of a H-bond between the oxide anion in the 'cation hole' and the cationic pyridine ring in the S4 subsite. Surprisingly, thiazolopyridine N-oxides **54a** and **54b** were totally inactive with IC_{50} values greater than 10,000 nM.

Further optimization of compound **53a** was carried out by replacing the thiazole ring with phenyl and pyrimidine, and replacing the chloronaphthyl moiety with chloro-substituted indole, benzothiophene, benzofuran, benzthiazole, and benzimidazole groups.[45] The optimized compounds **55a** and **55b,** showed excellent fXa inhibition when orally administered to rats with 1 mg/ kg of compound.



55a, X = S, IC_{50} = 6.3 nM

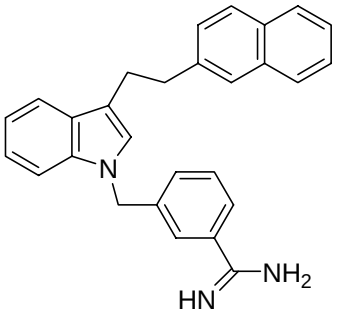
55b, X = NH, IC_{50} = 4.7 nM

3.7 Arylsulfonyl Pyrrolidinone-based Factor Xa Inhibitors

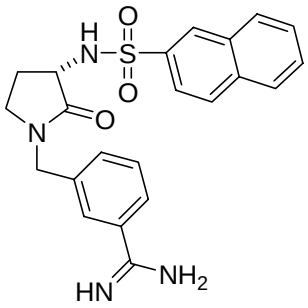
From the initially identified 1,3-disubstituted indole **56** (K_i = 0.9 μM), Ewing *et al*[46] designed a series of non-peptide fXa inhibitors having a 3-(*S*)-amino-2-pyrrolidinone as the central scaffold (**57)**.

Several structural modifications of **57** that led to compound **58** (RPR-120884) with enhanced fXa activity and good selectivity include:

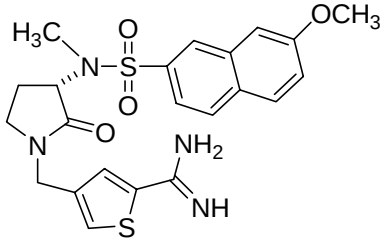
[a] Replacement of the indole scaffold with 3-(*S*)-sulfonamide-2-pyrrolidinone resulted in a more potent fXa inhibitor with good selectivity.



56, K_i = 0.9 μM



57, K_i = 0.23 μM



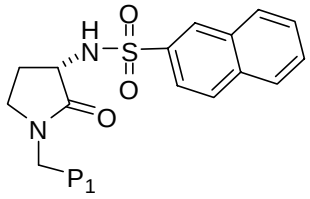
58, RPR120884, K_i = 7 nM

[b] The 7-methoxynaphthalene group was found to bind optimally in the S4 binding pocket.

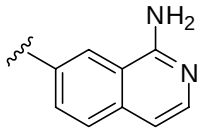
[c] N-methylation of the sulfonamide nitrogen improved potency for analogs containing the (S)-enantiomer of the scaffold.

[d] Factor Xa activity was further enhanced by replacing the m-benzamidine with an appropriately substituted thiophene amidine.

In a subsequent publication, Gong *et al*[47] reported several non-basic fXa inhibitors. Since, the highly basic benzamidine moiety (pKa = 11.6) imparts poor physicochemical properties for oral administration, 4-aminopyridine (pKa = 9.2), aminoisoquinoline (pKa = 7.6), 4-azaindole (pKa = 6.9), 5-azaindole (pKa = 8.3), and 6-azaindole (pKa = 8.0) were investigated. From this effort, several new fXa inhibitors were prepared (**59a**-**59e**), however the compounds were found to be weak fXa inhibitors.



59a, P_1 =



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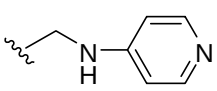
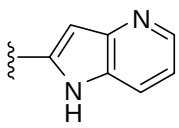
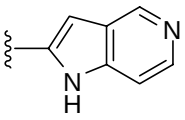


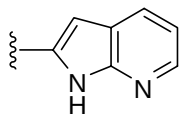
59c, P_1 =



59d, P_1 =

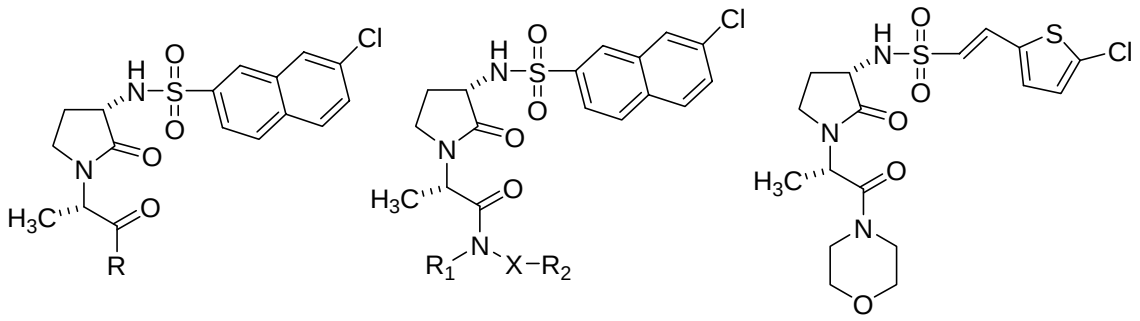


59e, P_1 =

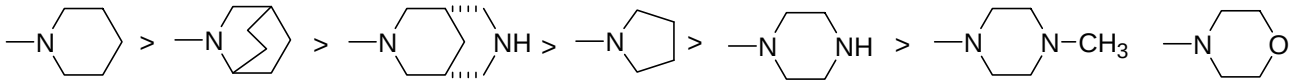


Over the past several years GlaxoSmithKline put a major effort in developing non-amidine based fXa inhibitors containing N-substituted pyrrolidine-2-one motif, **60**.[48]

This work demonstrated that the α- methyl (alanyl) group (e.g. compound **60**) conferred potent fXa inhibitory activity. Interestingly, the related α-ethyl and α,α-dimethyl analogs showed a significant reduction in potency. When the R group of **60** was substituted with cyclic amides, the following was the order of activity:



60 **61** **62**



60a **60b** **60c** **60d** **60e** **60f** **60g**

fXa K_i (nM)	1	3	13	50	320	2060	6

A major breakthrough was achieved when the piperidine ring was replaced with a morpholine ring. Among the four possible stereoisomers, the (1*S*,3*S*)-isomer conferred the greatest potency. Furthermore, the morpholino compound **60g** (R = morpholine-1-yl, fXa K_i = 6 nM) possessed an excellent PK profile in the dog [($T_{1/2}$ = 2.1 h), plasma clearance (CLp = 2.7 mL/min/Kg), volume of distribution at steady state (V_{dss} = 0.44 L/kg)] with excellent bioavailability (F% = 100%). In a related series, when an acyclic alanyl amide P4 motif was incorporated[49], the resulting compound (**61)** revealed poor pharmacokinetic properties characterized by increased plasma clearance and reduced oral exposure as compared with related cyclic morpholine-based analogs.

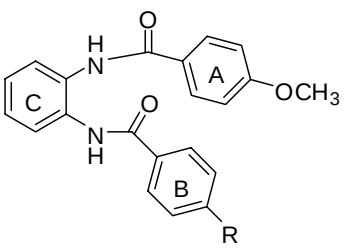
Further modifications to the aromatic ring distal to the chlorine atom (i.e. directly attached to SO_2 of the sulfonamido group) were undertaken using a variety of 5,6-fused, 5,5-fused aromatic systems and biaryl systems.[50] This systematic investigation led to identification of compound **62** as a potential candidate for further evaluation. Compound **62** showed good pharmacokinetic profiles in the rat and dog with high oral bioavailability (Table 5).

Table 5: PK parameters of **62** in male rats and dogs following intravenous and oral administration at doses of 1 mg/kg i.v. and 2.5 mg/kg p.o.

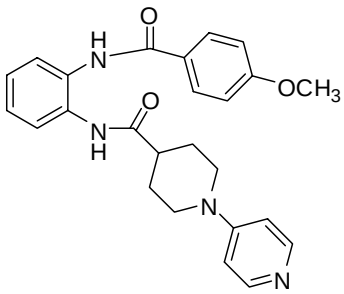
Species	$T_{1/2}$ (h)	Cl_p (mL/min/kg)	V_{dss} (L/kg)	F %
Sprague Dawley rats	0.7± 0.1	8.0 ±1.7	0.29 ± 0.02	75 ± 23
Beagle dogs	1.2± 0.3	4.6 ± 1.7	0.42 ± 0.03	53 ± 9

3.8 Anthranilamide-based Factor Xa Inhibitors

Scientists from Lilly Research Laboratories[51] disclosed a series of 1,2-dibenzamidobenzenes **63** (R = OCH_3, ^tBu, NMe_2) with fXa inhibitory activity.

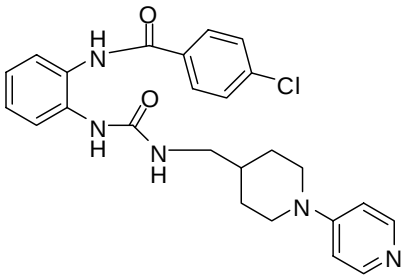


63



64

K_{ass} = 2.3 X 10^6 L/ mol

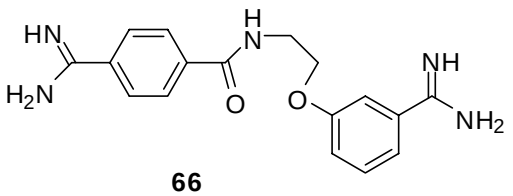


65

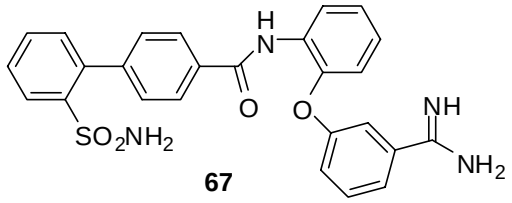
K_{ass} = 100 X 10^6 L/mol

To increase the hydrophilicity of **63**, the R-group was replaced with a 4-pyridyl group.[52] Furthermore, the 1-(4-pyridyl)piperidine containing derivative **64** was prepared with the hypothesis that 1-(4-pyridyl)piperidine is significantly more basic (pKa ~ 9) than the 4-phenylpyridine derivative, **63** (R = 4-pyridyl, pKa ~ 5). Thus, compound **64** should carry a positive charge at plasma pH, further reducing undesirable interactions with plasma proteins. Variation of the linkage connecting the central ring of **64** to the S4 binding group, 1-(4-pyridyl)piperidine, produced several potent fXa inhibitors. One interesting compound from this series (**65**) was 43-fold more active than **64** in fXa binding assay.

In 1998, the Ajinomoto Company patented a series of fXa inhibitors carrying an ethanolamine linker between two benzamidine moieties (**66**).[53]

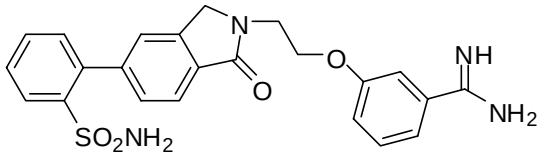


66



67

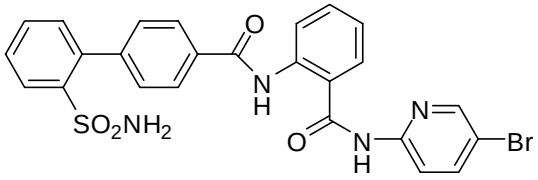
IC_{50} = 3.6 nM



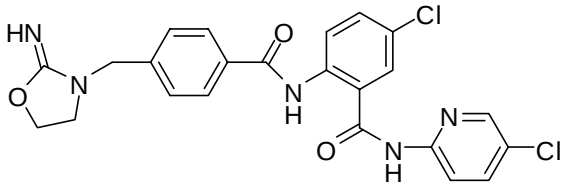
68

IC_{50} = 3.6 nM

Using this scaffold, Zhang *et al*[54] prepared two series of novel conformationally constrained monoamidine fXa inhibitors by installing substitution on the ethanolamine template of compound **66**. In the rabbit deep vein thrombosis model, the diaryl ether **67** inhibited thrombosis by 41% at 0.8 μM plasma concentration after i.v. dose of 2 mg/kg. The benzopyrrolidinone derivative **68** was also highly potent against fXa. Because of insufficient oral bioavailability, these compounds were not pursued further. In order to improve the low oral bioavailability (%F = <5%) of compound **67,** a 5-bromo-2-aminopyridine moiety was incorporated (**69**).[55]



69
IC_{50} = 3.4 nM



70
IC_{50} = 0.2 nM

Compound **69** was very selective for fXa over other serine proteases and displayed oral bioavailability of 31% in Sprague-Dawley rats at 6 mg/Kg. However, high lipophilicity of **69** led to extensive plasma protein binding (99.8%). To enhance hydrophilicity of this type of compound, several strategies were employed:

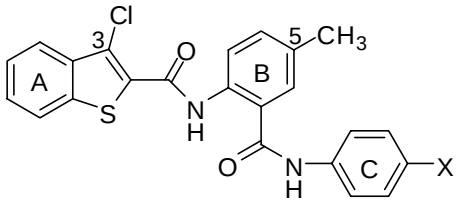
[a] Attaching an amino group to the central phenyl ring.

[b] Substituting the central phenyl ring with a pyridyl moiety.

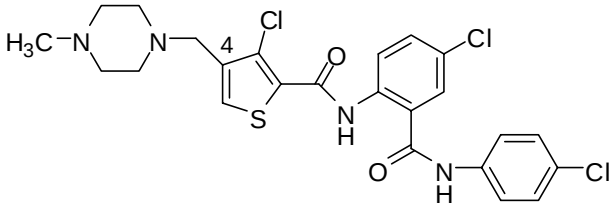
[c] Replacing the sulfonamide moiety that extends into the P4 region with a substituted aminomethyl, amidino or guanidino group.

By fine tuning the physical and chemical properties of the anthranilamide-based fXa inhibitor, **69**, scientists from Millennium Pharmaceuticals synthesized compound **70**, which not only showed strong antithrombic activity in rabbit deep vein thrombosis model, but also had good oral bioavailability (F% = 44%) and long half life ($T_{1/2}$ = 8.5 h) in rat.[56]

From high throughput screening, another anthranilamide based non-amidine fXa inhibitor with good selectivity against thrombin and trypsin was identified (**71**).[57]



71a, X = F, $K_{i,app}$ = 11 nM
71b, X = Cl, $K_{i,app}$ = 0.32 nM
71c, X = Br, $K_{i,app}$ = 0.46 nM



72
fXa, $K_{i,app}$ = 1.0 nM

Key features of compound **71** are:

[a] Small hydrophobic substituents were found to be optimal at C-3 in the benzothiophene ring.

[b] On the central ring (B), halogen or methyl substitution at C-5 is critical for high fXa potency.

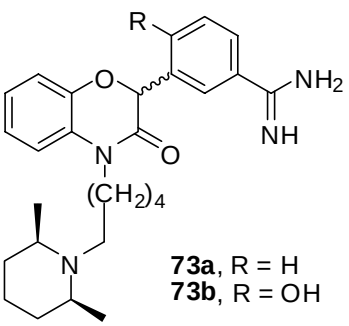
[c] The only significant increase in potency was obtained by adding a Cl or Br atom at the 4-position of the aniline (ring C). These substitutions resulted in subnanomolar non-amidine fXa inhibitors (**71b** and **71c**).

By replacing the benzothiophene core of compound **71** with a thiophene possessing a basic substituent at C-4, Kochanny *et al*[58] discovered a series of potent non-amidine, thiophene substituted anthranilamides with improved anticoagulant activity. From extensive chemical modifications, the thiophene substituted

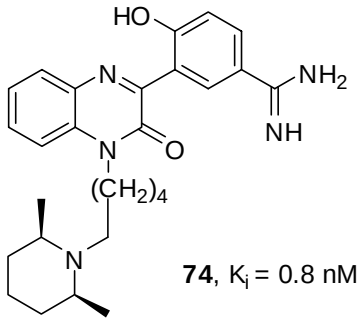
anthranilamide **72,** displayed low micromolar anticoagulant activity. After i.v. dosing in dog, compound **72** had a half life of 2 h, clearance of 0.3 L/h/Kg, and a volume of distribution at steady state of 0.7 L/Kg. The peak plasma level after oral dosing was 6.3 μM, and the oral bioavailability was estimated to be 41%.

3.9 Miscellaneous Factor Xa Inhibitors

From high-throughput screening, scientists at Pfizer disclosed a new class of fXa inhibitors having benzoxazinone skeleton (**73a**).[59] Studies on the benzoxazinone template resulted in incorporation of a p-hydroxyphenylamidine moiety which improved potency, **73b**.[60]



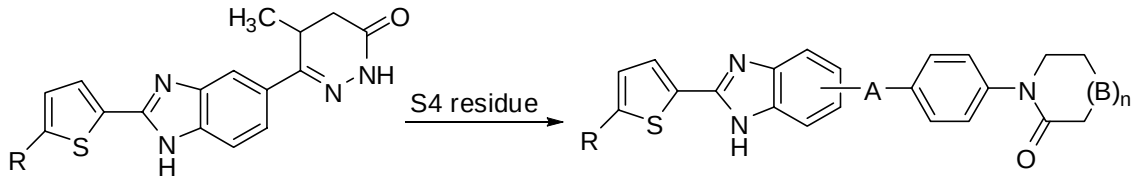
73a, R = H
73b, R = OH



74, K_i = 0.8 nM

Molecular modeling suggested that the high potency of the series could be retained if the oxygen atom of the benzoxazinone nucleus could be replaced with sulfur, carbon or nitrogen. To avoid the troublesome asymmetric center, many chemical modifications were carried out and from extensive SAR studies, the quinoxalinone derivative **74** emerged as a clinical candidate.[61]

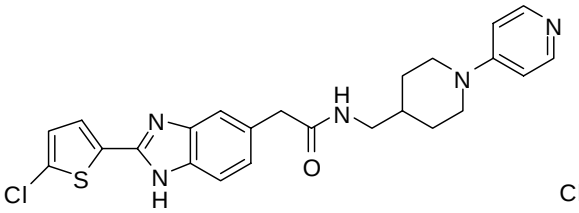
Through high-throughput screening, Mederski *et al*[62] identified two achiral and non-basic weak fXa inhibitors, **75a** and **75b**. Both of these compounds contain a halothiophene benzimidazole moiety as the P1 residue. An X-ray study of compound **75a** with human fXa was employed to optimize the interactions



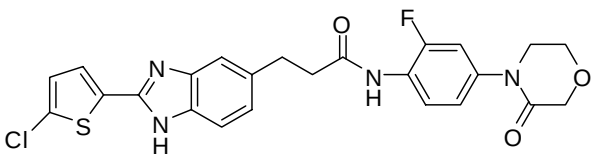
75a, R = Cl, IC_{50} = 1100 nM
75b, R = Br, IC_{50} = 980 nM

75c
A = linkage, e.g. amide bond
B = CH_2, O
n = 0,1

with the S4 pocket. A tethered amide linkage was introduced between 5(6)-position of the benzimidazole and the P4 residue. Potency depends on the length and nature of the linker between benzimidazole and P4 residue. Among the many S4 residues, substituted anilines were the preferred ligands. Modifications of these parameters led to the identification of the low nanomolar fXa inhibitors **76** and **77**. Although this class of compounds displayed good *in vitro* properties, physicochemical parameters such as solubility and oral pharmacokinetic profiles were not sufficient for further development.



76, IC_{50} = 5.2 nM

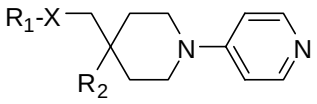


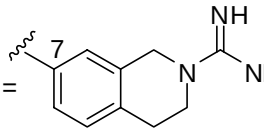
77, IC_{50} = 5.9 nM

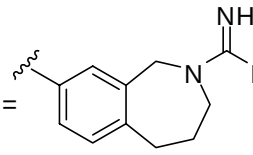
Recently, a series of 2,7-disubstituted tetrahydroisoquinoline derivatives (**78a**-**78e**) were reported as fXa inhibitors.[63] The design of the inhibitors was based on the following:

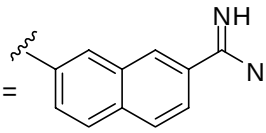
[a] The N-amidinotetrahydroisoquinoline ring is expected to completely fill the S1 pocket of fXa and interact with Asp189.

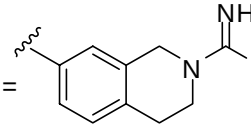
[b] A 4-piperidinylmethyloxy group was selected for position 7 of the tetrahydroisoquinoline ring as a spacer for the introduction of basic substituents because the N-atom of the piperidinyl group could be oriented towards the S4 site.

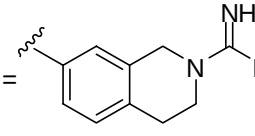


78a R_1 =  NH_2 , $R_2 = CO_2H$, X = O, IC_{50} = 0.026 ± 0.003 μM

78b R_1 =  NH_2 , $R_2 = CO_2H$, X = O, IC_{50} >10 μM

78c R_1 =  NH_2 , $R_2 = CO_2H$, X = O, IC_{50} = 0.51 ± 0.01 μM

78d R_1 =  NH_2 , $R_2 = CO_2H$, X = S, IC_{50} = 0.39 ± 0.01 μM

78e R_1 =  NH_2 , $R_2 = CO_2H$, X = SO_2, IC_{50} = 5.44 ± 0.12 μM

From the extensive SAR studies of compounds **78a** to **78e**, several key points can be mentioned:

[a] Expansion of the piperidine ring of **78a** to give N-amidino-2-benzazepine **78b**, did not inhibit fXa.

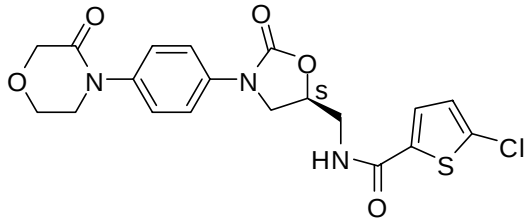
[b] Naphthalene derivative **78c** had weak activity.

[c] Modifications or extensions of the middle spacer from OCH_2 to OCH_2CH_2, SCH_2, SO_2CH_2 or SO_2NH did not provide a compound with significant activity.

[d] When R_2 = H, the resulting compounds exhibited fatal acute toxicity.

Compound **78a** exhibited potent fXa inhibitory activity after i.v. and p.o. administration to cynomolgus monkeys, displayed a dose-dependent antithrombotic effect at 0.1, 0.3 and 1 mg $Kg^{-1}h^{-1}$ in a rat model of venous thrombosis and significantly reduced the size of brain infarction in a middle cerebral artery occlusion model at a dose of 0.1 mg $Kg^{-1}h^{-1}$, and emerged as candidate for clinical studies.[63b]

Roehrig *et al*[64a] discovered a new class of fXa inhibitors that had an oxazolidinone core **79** (BAY 59-7939). The (*S*)-Configuration at the oxazolidinone core was preferred.



79 (Bay 59-7939), Rivaroxaban
IC_{50} = 0.7 nM

Table 6: PK parameters of **79** in male rats and female dogs following intravenous and oral administration at various doses.

Species	$T_{1/2}$ (h)	Cl (L/h/kg)	V_{ss} (L/kg)	F %
Male Wistar rats	0.9[a]	0.4[a]	0.3[a]	60[b]
Female Beagle Dogs	0.9[c]	0.3[c]	0.4[c]	60-86[d]

[a] mean value derived by administration of 1 and 3 mg/kg, i.v.[b] mean value calculated by dividing the AUC_{norm} after 1, 3 and 10 mg/kg, p.o. [c] mean value derived from administration of 0.3 and 1 mg/kg, i.v. [d] range calculated by dividing the AUC_{norm} after 0.3, 1, and 3 mg/kg p.o.

The compound displayed excellent *in vitro* and *in vivo* efficacy and had a good pharmacokinetic profile (Table 6). Compound **79** doubled the prothrombin time (PT) at a concentration of 0.23 μM in the rat plasma and in the arterio-venous (AV) shunt model in anesthetized rats, after i.v. administration, demonstrated a lower ED_{50} value of 1 mg/kg. Compound **79** was identified as a lead candidate for clinical development.[64b,c]

4. CONCLUSION

Cardiovascular disease remains the leading cause of death and disability not only in the established western societies, where it has been a long standing health-care concern, but also now in countries undergoing rapid economic change such as India and China. Worldwide the burden of disease is well over one billion people, and increasing. The 20th century bore witness to great strides in the understanding, diagnosis, and treatment of cardiovascular disease. Impressive developments in therapeutics, from technological and surgical interventions to innovative pharmaceuticals, have prevented the deaths and

eased the suffering of many. Yet there is still much to be done. The impact of cardiovascular disease on humanity remains a significant factor in driving up the rates of mortality, morbidity and the ever growing cost of health care. To tackle the growing public health crisis of cardiovascular disease, many biotechnology and pharmaceutical companies have created dedicated R&D groups to identify and develop drugs to slow, halt or reverse pathologies. These groups engage in drug development by evaluating new chemical entities against the existing targets or by discovering new therapeutic targets, and evaluating the new or existing drugs against these new therapeutic targets. We hope, the discovery and development of new drugs in the near future will ultimately begin to reduce the world's leading cause of death and suffering.

ACKNOWLEDGEMENTS

The authors thank Naeja Pharmaceutical Inc for the facilities and support.

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   (b) Antithrombotics – Current Market and Future Outlook, *IMS Therapy Reports*, July 1, 2005.
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