Discovery of an Orally Efficacious Inhibitor of Coagulation Factor Xa Which Incorporates a Neutral P_1 Ligand

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Abstract: The discovery and SAR of ketopiperazino methylazaindole factor Xa inhibitors are described. Structure−activity data suggesting that this class of inhibitors does not bind in the canonical mode were confirmed by an X-ray crystal structure showing the neutral haloaromatic bound in the S_1 subsite. The most potent azaindole, **33** (RPR209685), is selective against related serine proteases and attains higher levels of exposure upon oral dosing than comparable benzamidines and benzamidine isosteres. Compound **33** was efficacious in the canine AV model of thrombosis.

Introduction. Thrombosis-related diseases, including myocardial infarction, deep vein thrombosis, and unstable angina, often have life-threatening consequences, and thus, considerable research is directed toward developing novel antithrombotic agents. Current therapies, using heparin[1] and warfarin,[2] are based on indirect inhibition of thrombin, the terminal enzyme in the coagulation cascade. These therapies are limited by side effects, mode of administration, stringent monitoring of drug levels, slow onset of action, and excessive bleeding. The trypsin-like serine protease factor Xa (fXa) occupies a unique position at the final convergence point of the intrinsic and extrinsic pathways,[3] thus inhibiting coagulation without directly affecting platelet function. As part of the prothrombinase complex, factor Xa is the singular enzyme responsible for thrombin activation; thus, fXa has been identified as an attractive target for antithrombotic therapy.[4]

In a previous communication, we described the optimization of azaindole P_1 and heteroaromatic P_4 groups in the pyrrolidinone series leading to double-digit nanomolar azaindole inhibitors of factor Xa.[5] Although these compounds, **1**, exhibited good affinity for fXa in vitro, only modest oral bioavailability was obtained in our canine model as determined by an ex vivo anti-fXa bioassay.[6] Concurrently, a new series[7] of factor Xa inhibitors (e.g., **2**) was identified that seemed more tolerant of changes in the putative P_1 region of the molecule. The discovery of ketopiperazines as an alternative scaffold required reoptimization of the "privileged" P_1 and P_4 ligands (i.e., fragments known to impart good potency) from the earlier pyrrolidinone series. In this communication, we describe the evolution of the azaindole pyrrolidinones represented by **1** to ketopiperazines **3** and subsequent optimization to yield orally efficacious inhibitors of fXa (Figure 1).

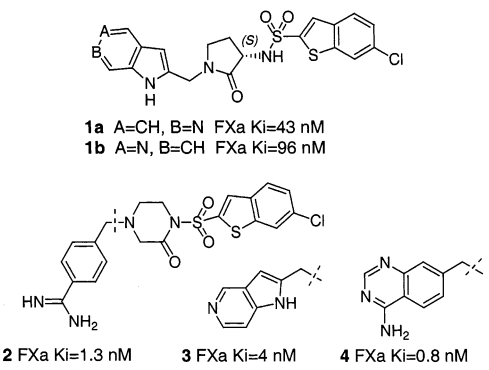
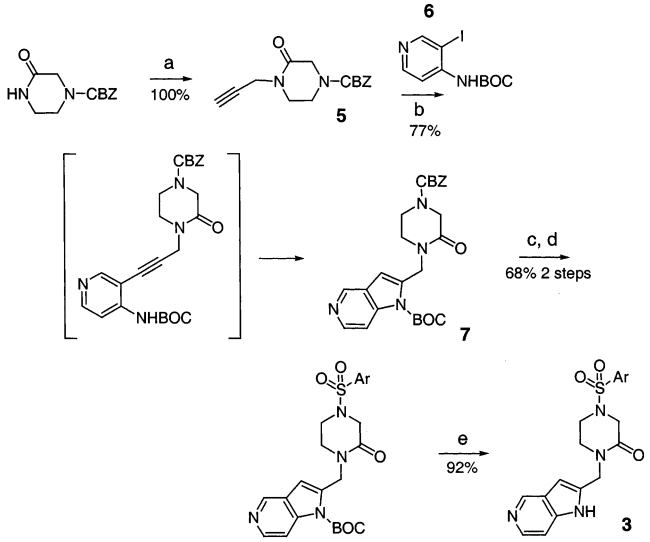


Figure 1.

Although it is well precedented that arylamidines generally lead to potent inhibitors, molecules containing such highly basic functions are often poorly absorbed[8b] and/or are associated with undesirable side effects.[8b] During the course of this work, it became clear that not any benzamidine replacement[8a] is sufficient to impart good oral bioavailability. This was exemplified by aminoquinazoline **4**,[8a] which is highly potent against fXa but displays poor pharmacokinetic properties. In the endeavor to improve PK parameters and retain potency, X-ray structural work[9] was essential in understanding the binding modes and interpreting the SAR of the ketopiperazine fXa inhibitors.

Chemistry. A representative synthesis of the azaindole inhibitor is described in Scheme 1. Alkylation of

Scheme 1[a]



[a] (a) NaH, THF/DMF, propargyl bromide, 0 °C; (b) (i) $Pd(PPh_3)_2Cl_2$, Et_3N, CuI, DMF, 100 °C, (ii) DBU, 50 °C; (c) Pd black, H_2O, HCO_2H, MeOH; (d) $ArSO_2Cl$, Et_3N, CH_3CN; (e) 30% TFA, CH_2Cl_2.

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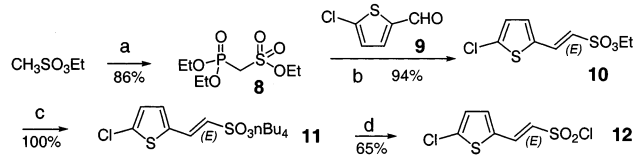
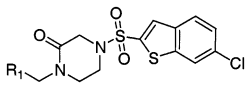
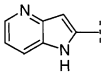
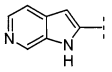
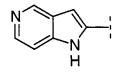
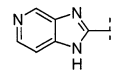
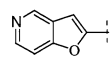
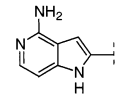
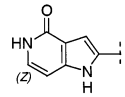
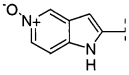
Scheme 2[a]



[a] (a) nBuLi, THF, $(EtO)_2POCl$; (b) nBuLi, THF; (c) nBu_4NI, acetone; (d) PPh_3, SO_2Cl_2, DCM.

Table 1. In Vitro Activity of 5,6-Fused Biaryl Benzamidine Replacements

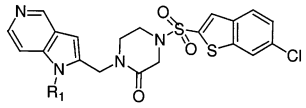


		Ki (nM)			
	R_1	factor Xa	thrombin	trypsin	pKa[10]
13		>1200	>3900	>2900	6.9
14		45	3900	>2900	8.0
3		4	2900	>2900	8.3
15		>1200	>3900	>2900	6.1
16		1219	>3900	>2900	5.8
17		1022	>3900	>2900	9.3
18		>1200	>3900	>2900	ND
19		38	>3900	>2900	ND

the commercially available CBZ-ketopiperazine introduces the alkyne **5** functionality that undergoes the key Pd-catalyzed crossing-coupling reaction with (3-iodopyridin-4-yl)carbamic acid *tert*-butyl ester **6**, followed by base-induced cyclization to yield the protected 5-azaindole **7** in a one-pot process. Sequential deprotection of CBZ under transfer hydrogenation conditions, formation of the sulfonamide with the corresponding sulfonyl chloride, and then acid-catalyzed removal of the BOC group yield the desired compound **3**. The requisite alkenylsulfonyl chloride was prepared by treatment of the Wittig reagent **8** with aldehyde **9** to afford the ethyl sulfonate ester **10**, which was subsequently converted to the tetrabutylammonium salt (Scheme 2). Treatment of the salt **11** with sulfuryl chloride and triphenylphosphine yields **12**.

In Vitro Optimization. Using our preferred 6-chlorobenzothiophene sulfonamide group as the invariant, we examined a number of 5,6-fused biaryls (Table 1) as replacements for the undesired benzamidine. In contrast to the pyrrolidinone scaffold, in which the 6-azaindole **1a** yielded the more potent analogue,[5] the 5-azaindole compound **3** in the ketopiperazine series afforded the more potent inhibitor with a K_i of 4 nM. The 6-azaindole

Table 2. 5-Azaindole Modifications



		K_i (nM)				
	R1	fXa	thrombin	trypsin	CaCo-2 (% absorbed)[a]	metabolism[a,b]
3	H	4	2900	>2900	6.0 (mod)	high (64%)
20	Me	3	>3900	>2900	3.0 (mod)	high (60%)
21	$-CH_2CO_2Me$	18	>3900	>2900	47.6 (high)	high (99%)
22	$-CH_2CO_2H$	154	>3900	>2900	ND	ND
23	$-CH_2CONH_2$	8	>3900	>2900	7.0 (mod)	mod (40%)
24	$-CH_2CH_2OH$	3	>3900	>2900	15.3 (mod)	mod (47%)

[a] mod = moderate. [b] Rat liver microsomes.

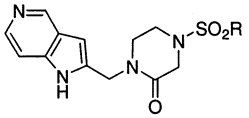
derivative **14** was 11-fold less active, while the 4-azaindole **13**, paralleling the pyrrolidinone result,[5] was dramatically less active. The activities of these analogues appear to loosely correlate with the basicity of the azarene group except in the case of the 4-amino-5-azaindole analogue **17**. This derivative is 1 log more basic than the simple 5-azaindole and was designed to interact with D189 in the S_1 active site in a bidentate fashion; thus, it was surprising that the potency dropped 255-fold versus the parent compound **3**. A significant loss in activity was also observed with the oxo analogue **18**, whereas the neutral *N*-oxide **19** was a modest inhibitor with a K_i of 38 nM.

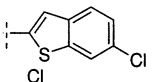
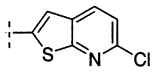
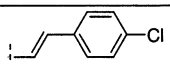
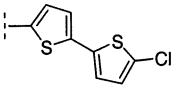
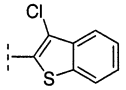
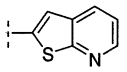
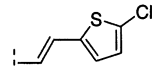
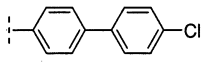
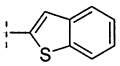
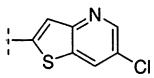
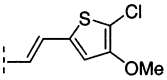
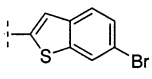
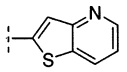
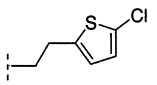
Table 2 summarizes attempts to optimize the series by substitution at the N-1 position of the 5-azaindole ring. This position offers a chemical handle to append moieties that could modify physicochemical properties, improve potency by additional interaction with the enzyme, and improve pharmacokinetic properties by blocking a possible site of metabolism. Two in vitro measures of metabolism and permeability, i.e., microsomal stability and CaCo-2 cell monolayer absorption,[11] respectively, were monitored as a function of N-1 substitution.

Incorporation of a negative charge, e.g., compound **22**, had a detrimental effect on inhibitor potency, although in general, substitution at the N-1 position had little effect on K_i. Note that carboxamide **23** and hydroxyethyl **24** analogues, both capable of H-bonding interactions, were no more potent than the parent compound **3**. These results are difficult to reconcile with a binding mode that places the azaindole system in the congested S_1 specificity pocket and suggest that the appended chains are not in intimate contact with the enzyme. Permeability was significantly improved in only one case, ester **21**, which was rapidly degraded presumably to acid **22**. The rate of microsomal metabolic turnover for carboximide **23** and hydroxyethyl **24** analogues was lower than for the parent **3**, but these modifications had only a moderate effect on reducing metabolism. In general, this approach maintained in vitro potency, had modest effects on absorption, but could not concurrently reduce microsomal metabolism.

The aryl sulfonamide portion of the molecule was subsequently investigated using privileged ligands[12] (Table 3), which had imparted good activity in our pyrrolidinone series of inhibitors. The pairs **26**, **27** and **28**, **29** exemplify the necessity of the halogen atom for good potency. A 240-fold drop in potency was observed by removing the halogen atom, **26**, and an even greater loss was observed by placing the chlorine at the 3-posi-

Table 3. Optimization of Sulfonamides



	R	fXa Ki (nM)		R	fXa Ki (nM)		R	fXa Ki (nM)		R	fXa Ki (nM)
3		4	**28**		4	**32**		48	**36**		23
25		>1200	**29**		>1200	**33**		1.1	**37**		>1200
26		959	**30**		1200	**34**		>1200			
27		3	**31**		>1200	**35**		128			

tion, **25**. On the basis of the crystal structure of the 6-azaindole pyrrolidine **1a** with human fXa[13] and assuming that the aryl sulfonamide was engaged in lipophilic interactions in the S_4 site, weakly basic azarenes (**28**−**31**) were examined as a means to improve potency by accessing the cation hole region of the enzyme. Compound **30** was designed to retain the empirically observed key chloro interaction while positioning the nitrogen toward the side chain of E97. To our surprise, this compound showed poor fXa inhibitory activity. Regioisomer **28** maintained good activity; however, the presence of nitrogen did not enhance its potency against fXa. Divergent SAR trends between the pyrrolidinone and ketopiperazine scaffolds were particularly apparent with the thienopyridine sulfonamide containing analogues (e.g., **29** and **31**). The thienopyridine group, which ordinarily imparted good anti-fXa activity in combination with benzamidine[12] or its isosteric replacement[14] in the pyrrolidinone series, was a poor inhibitor in the latter series. X-ray crystallographic data clarified the reasons we were unable to improve inhibitory activity using this approach (vide infra).

In a survey of chlorine-containing aryl sulfonamides, it became clear that the trajectory of the halogenated aryl was critical for optimal fXa binding. The chlorostyryl derivative **32**, which can be viewed as a ring-opened derivative of compound **3**, was 12-fold less potent. The activity was regained by substituting the phenyl ring with thiophene **33**, resulting in the most potent inhibitor identified in this series (fXa, $K_i = 1$ nM). None of the other entries in Table 3 resulted in potency enhancements. In particular, inhibitor **35**, the saturated, more conformationally mobile derivative of **33**, was 2 orders of magnitude less active. The aryl sulfonamide SAR within the ketopiperazine series underscores the importance of the aryl halogen interaction with the enzyme for optimum potency. The SAR trends diverge from those established with canonical pyrrolidinone inhibitors, suggesting an alternative binding mode for this series.

X-ray Crystallography. The X-ray crystal structure of **33** in human fXa, obtained at a resolution of 2.1 Å,[9] clarified the stringent requirements for good potency that was observed within the ketopiperazine series. Our working hypothesis had assumed that the azaindole was located in the S_1 site and the halogenated aromatic group was making hydrophobic contact in the S_4 region. To our surprise, the chlorothiophene of **33** was bound in the S_1 pocket; closest contact to D189 was ca. 3 Å. The chlorine atom was located in an area normally occupied by a water molecule in canonical benzamidine/factor Xa co-complexes.[13] The chlorine atom is engaged in direct contact with the centroid of the aromatic ring of Y228 located on the back wall of the S_1 binding pocket. Reminiscent of pyrrolidinone binding, the carbonyl oxygen of piperazine forms an H bond with the NH of G218. The azaindole group fills the S_4 site with the indole NH directed toward the solvent, while the pyridine nitrogen forms an H bond with the structural water molecule bridging I175 and T28 at the entrance of the hydrophobic pocket. This binding mode explains the preference for the 5-azaindole in this series and the tolerance to substitution at N-1.

Structural evidence for the halogen−S_1 tyrosine interaction, although unprecedented in factor Xa, has been observed in thrombin/inhibitor X-ray structures.[15] Compound **33** represents a parallel example in fXa inhibition wherein a basic group thought to be necessary to form the crucial salt bridge with D189 can be eliminated and replaced by a neutral group capable of contributing enough favorable binding energy to maintain good affinity for the enzyme. The SAR necessitating the presence of halogen suggests that ketopiperazines are binding in the "reversed" mode in all cases, unlike the pyrrolidinone series where multiple binding modes are possible and divergent SAR trends are observed among the isomeric azaindoles.

Biological Activity. Key compounds in this series showing excellent selectivity (>1000-fold) against related serine proteases were evaluated for anticoagulant properties in different species; APTT prolongation[16] in plasma was in the low micromolar range (Table 4). An ex vivo anti-fXa bioassay[6] was used to measure and compare bioavailability, half-life, and plasma levels of the inhibitors (Table 5). In contrast to the aminoquinazoline inhibitor **4**, which exhibits negligible plasma levels when administered orally at 10 mg/kg in dogs, the analogous azaindole **3** achieves plasma levels of 660 nM at the same dose. The N-alkylated azaindole **24** exhibits plasma levels similar to **3** but has a superior half-life (2 h). At half the dose, **33** reaches micromolar plasma levels with a concomitant increase in half-life;

Table 4. Selectivity Profile and APTTs for Selected fXa Inhibitors[a]

	K_i(fXa)	K_i(APC)/ K_i(fXa)	K_i(plasmin)/ K_i(fXa)	K_i(tPA)/ K_i(fXa)	2(APTT),[b] human (μM)	2(APTT),[b] dog (μM)
3	4.0	>4600	>1800	>2200	2.0	5.0
24	3.0	>6100	>2400	>2900	1.7	ND
33	1.1	>17000	>17000	>8000	0.59	2.2

[a] Source of enzyme: human fXa, human APC, human plasmin, recombinant tPA. [b] 2 × APTT is defined as the concentration of inhibitor required to double the activated partial thromboplastin time.

Table 5. Pharmacokinetic Parameters after Oral Dosing in Dogs

compd	K_i(fXa) (nM)	dose (mpk, po)	F (%)	$T_{1/2}$ (min)	C_{max} (nM)
4	0.8	10	<5%	ND	10
3	4	10	99	36	658
24	3	10	11	139	783
33	1	5	97	52	1638

the estimated bioavailability for this inhibitor is 97%. These results represent a substantial improvement over previous inhibitors in terms of their pharmacokinetic properties.

On the basis of the favorable in vitro and PK profile of inhibitor **33**, efficacy studies were conducted in a canine arteriovenous thrombosis model.[17] Upon oral dosing (20 mg/kg), the time-to-occlusion was prolonged 1.9-fold (145 vs 77 min) on the venous side and 1.8-fold (147 vs 80 min for vehicle) on the arterial side. A concomitant reduction in venous thrombus weight (34%) and a smaller reduction in arterial thrombus weight (13%) were observed.

Conclusion. In summary, a series of ketopiperazine inhibitors with an azaindole P_4 group and nonbasic chloroaryl groups have been shown to be potent and selective inhibitors of factor Xa. The X-ray crystal structure of **33** and fXa reveal that the key interaction in the S_1 pocket does not involve interaction with D189 as is typical for basic inhibitors. The critical interaction between chlorine and the aromatic ring of Y228 in the S_1 pocket may be a general phenomenon, which may be exploited with other serine proteases containing this residue. Consequently, additional opportunities to obtain orally active inhibitors will be possible with the elimination of a highly basic charged group in the molecule. The properties of our inhibitors are such that good exposure is obtained upon oral dosing, and in the case of **33** (RPR209685), antithrombotic efficacy is observed. The arylsulfonamido ketopiperazine azaindoles represent an important milestone in the development of orally efficacious factor Xa inhibitors.

Supporting Information Available: Experimental details for **6**, **33**, **3**, **20**, **17**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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