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AMINOISOQUINOLINES: DESIGN AND SYNTHESIS OF AN ORALLY ACTIVE BENZAMIDINE ISOSTERE FOR THE INHIBITION OF FACTOR XA.

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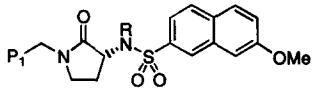
Abstract: The design, synthesis and SAR of sulfonamidopyrrolidinone fXa inhibitors incorporating a new benzamidine isostere, namely aminoisoquinolines, is described. These inhibitors have higher Caco-2 cell permeability than comparable benzamidines and attain higher levels of exposure upon oral dosing. The most potent member **14b** (fXa Ki=6 nM) is selective against other serine proteases of interest (>600 fold).

The serine protease component of the prothrombinase complex, factor Xa (fXa), is responsible for the the conversion of prothrombin to thrombin (fIIa), the final enzyme of the coagulation cascade[1]. In recent years, the inhibition of fXa in the prothrombinase complex has emerged as an alternative antithrombotic approach to direct thrombin inhibition[2]. A variety of factor Xa inhibitors have now been described and a recurring structural feature is the benzamidine unit[3]. A classic approach to the inhibition of fXa utilizes two amidine groups[4], however, more recently [5] potent inhibitors of factor Xa have been discovered in which only one arylamidine unit is present. The sulfonamidopyrrolidinones **1a-c**[6], from this laboratory, fall into the latter category; we have shown that inhibition can be enhanced by replacing simple benzamidines **1a**, with aryl amidines **1b** and **1c**.



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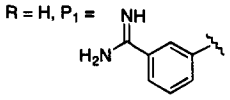
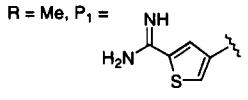
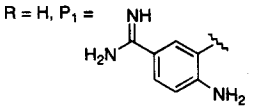


1a $fXaK_i$ = 47 nM



1b $fXaK_i$ = 7 nM



1c $fXaK_i$ = 6 nM

Independent of the aryl system, it is generally assumed that the amidine group forms a salt bridge with the carboxylate found in the specificity pocket of trypsin-like serine proteases[7]; this contributes significant binding energy to the ligand-enzyme interaction. However, inhibitors containing highly basic functions such as arylamidines are often poorly absorbed[8] and/or are associated with undesirable side effects[9]. To improve the oral activity of fIIa inhibitors, benzamidines and alkyl guanidines (pKa ~ 11-13) have been replaced with weakly basic heterocycles such as imidazoles and substituted pyridines[10]. Given the structural similarities between these trypsin-like serine proteases the strategy used for fIIa provided a starting point for our fXa work.

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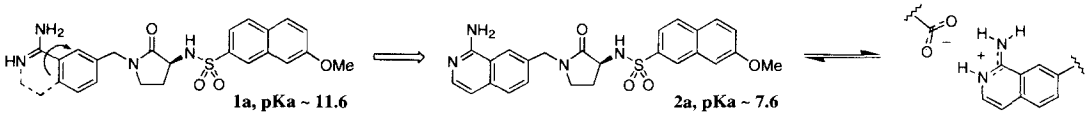
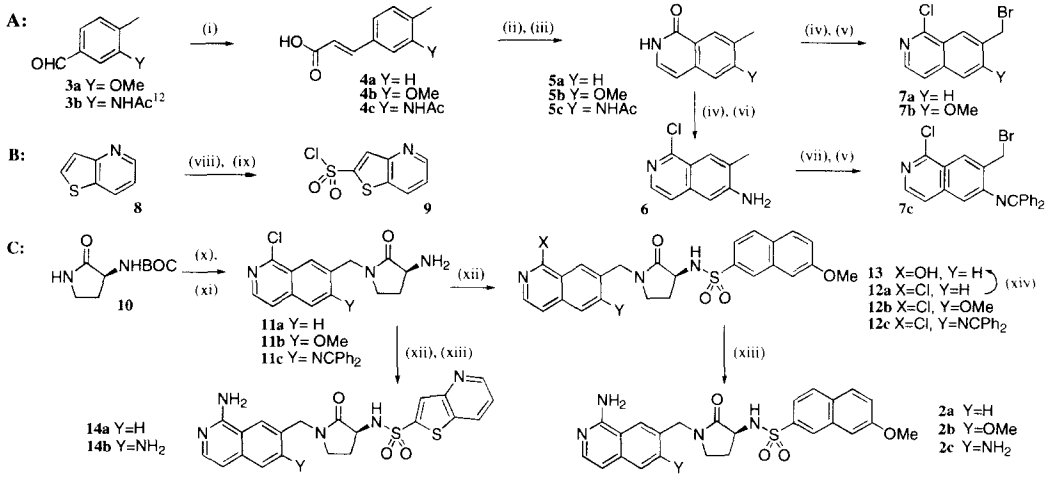
Sulfonamidopyrrolidinones incorporating imidazole and pyridine P_1 moieties were prepared, however, anti-fXa activity was not maintained.



Consequently, we explored azarenes which had not been previously reported as benzamidine replacements. Conformational restriction of the benzamidine group by a hydrocarbon bridge leads to the aminoisoquinoline, **2a**. This moiety has a pKa of 7.6[11] suggesting that an equilibrium of neutral and protonated forms exists at physiological pH. The neutral species should be membrane permeable, while the protonated form could interact productively with D189 of the fXa specificity pocket (S_1). Herein, we describe the synthesis, SAR and preliminary *in vivo* results of our aminoisoquinoline inhibitors.

Scheme 1: Synthesis of Aminoisoquinolines



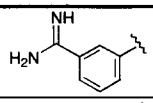
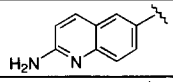
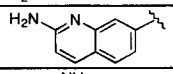
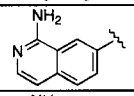
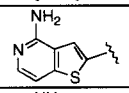
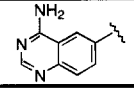
(i) malonic acid, piperidine, pyridine, 100 °C (90-95%) (ii) EtOCOCl, TEA, acetone; NaN_3, H_2O (90-96%) (iii) Bu_3N, Ph_2O, 210°C (37-72%) (iv) $POCl_3$, reflux (70-75%) (v) NBS, $(PhCO_2)_2$, CCl_4, reflux (52-86%) (vi) conc. HCl, EtOH, reflux (vii) HCl(g), MeOH then benzophenone imine, 1,2-dichloroethane (57% for 2 steps(vi) and (vii)) (viii) nBuLi, THF; SO_2 (ix) SO_2Cl_2, hexane (64% for 2 steps) (x) NaH, 7, THF, 0°C (72- 96%) (xi) HCl, EtOAc, 0 °C (100%) (xii) $ArSO_2Cl$, Et_3N, CH_3CN (60-85%) (xiii) NH_4OAc, PhOH, 100°C (38-45%) (xiv) 10% NaOH, dioxane (60%).

Aminoisoquinolines **2** and **14** were prepared by alkylating the pyrrolidinone template **10**[6] with arylbromide **7**, followed by BOC deprotection and coupling with the appropriate sulfonyl chloride (Scheme 1C). A one-pot ammonolysis was developed by modification of Nuvole's procedure[13] to give **2** and **14** from the penultimate chloroisoquinoline **12**. 7-Methyl-1-chloroisoquinoline was synthesized by adapting literature procedures[14] (Scheme 1A). Yields for the cyclization step are variable (~37 % for **4c**); subsequent benzylic

bromination was lower yielding for the simple case. Sulfonyl chloride **9** was prepared by sulfonation (SO_2) of thieno[3,2-b]pyridine **8**[15] followed by oxidation with sulfuryl chloride (Scheme 1B).

The results summarized in Table 1 underscore the importance of the nitrogen atom location, pKa and the type of heteroaromatic ring in achieving optimal activity. Compounds which incorporate two nitrogens in essentially the same relationship as an amidine function, but are much less basic, include imidazoles, aminopyridines, aminoquinolines, and aminoisoquinolines. However, in the pyrrolidinone series, amino-thienopyridine **1f** and aminoisoquinoline **2a** were the most active fXa inhibitors. Modeling studies with these systems (Figure 2) indicate that the diaza moiety of the aminoisoquinoline is optimally positioned to interact with the S_1 carboxylate. Compound **2a** was less potent against trypsin perhaps reflecting the more polar nature of the trypsin S_1 subsite. Incorporation of a second nitrogen in the aromatic system yields the less basic quinazoline system **1g** and results in a loss in binding affinity. This observation was interpreted to mean that reducing the population of protonated species results in a less active inhibitor.

Table 1. *In Vitro* Activity of Benzamidine Surrogates in Sulfonamidopyrrolidinone Inhibitors **1**

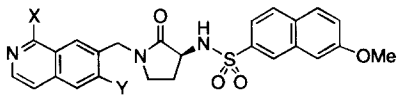
Compound[16]	P_1	R	pKa*	fXa K_i+ (μM)	fIIa K_i+ (μM)	Tryp. K_i+ (μM)
1a		H	11.6	0.047	1.4	0.85
1d		H	7.3	1.2	>4.0	>2.9
1e		H	7.3	0.37	~4	>2.9
2a		H	7.6	0.18	1.35	>2.9
1f		H	ND	0.25	~4	>2.9
1g		Me	5.3	1.6	>4.0	>2.9

*Literature values of parent heterocycle[11] ND: Not determined +*In vitro* assays as described in Ref. 17

To quickly gauge the potential of aminoisoquinolines for *in vivo* absorption, the permeability of inhibitor **2a** across Caco-2 cell monolayers was measured[18]. Comparing structurally related systems, the aminoisoquinoline inhibitor **2a** exhibited a higher apparent permeability coefficient (P_{app}=710 nm/s) relative to benzamidine **1a** (P_{app}=17 nm/s). This result suggested that aminoisoquinolines were an advantageous substitute for arylamidines.

In an effort to understand the factors important for *in vitro* activity, a number of modifications were examined (Figure 1). Clearly, the amino group was necessary for optimal potency; replacing this group with hydroxyl **13** or chlorine **12a** abated activity. We have previously shown how heteroatoms positioned *para* to the amidine attachment point can result in potency increases for the sulfonamidopyrrolidinone class of fXa inhibitors[6]. Similarly, the addition of an amino function at the 6-position of the isoquinoline increased potency (2-3 fold) for **2c** over **2a**. In contrast, the methoxy analog **2b** was less effective than its unsubstituted parent **2a**.

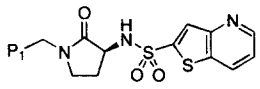
Figure 1. Isoquinoline Analogs:

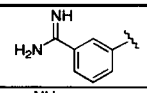
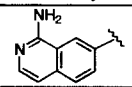
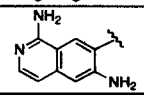


2a, X = NH_2, Y = H, fXa K_i = 0.18 μM
13, X = OH, Y = H, fXa K_i > 1.2 μM
12a, X = Cl, Y = H, fXa K_i > 1.2 μM
2b, X = NH_2, Y = OMe, fXa K_i = 0.89 μM
2c, X = NH_2, Y = NH_2, fXa K_i = 0.08 μM

Concurrent research with benzamidine derived pyrrolidinones had identified thieno[3,2-b]pyridyl sulfonamides **15** as desirable P_4 motifs; we applied these findings to the aminoisoquinolines[19]. The amino-isoquinoline **14a** was equipotent to its benzamidine analog **15** (Table 2) and had improved activity *versus* the methoxynaphthalene lead **2a** (~8-fold). Hybrid **14b**, which combines the most effective P_1 ligand with the thieno[3,2-b]pyridine sulfonamide P_4, ranks as the most potent inhibitor of this limited series (fXa K_i = 6 nM).

Table 2. Selectivity of Thieno[3,2-b]pyridyl-sulfonamidopyrrolidinone Inhibitors



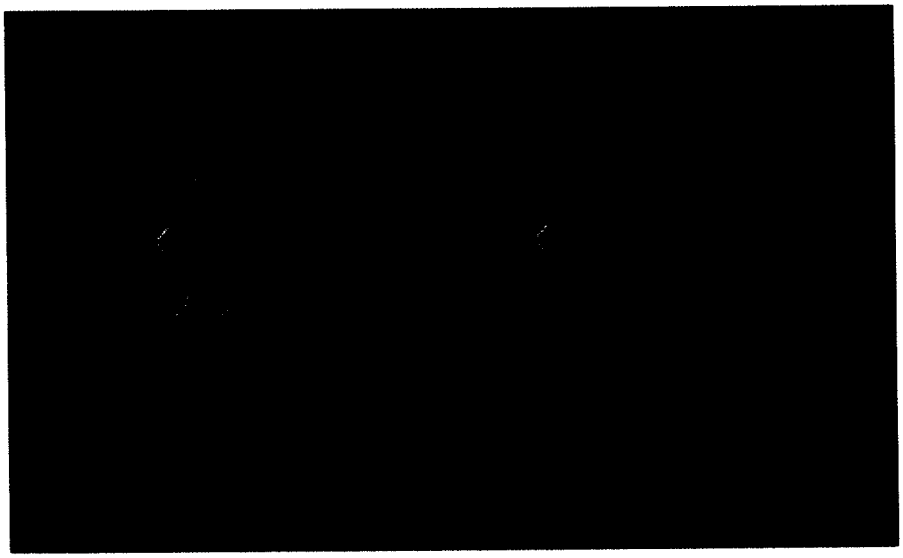
Compound	P_1	fXa K_i (nM)	fIIa K_i / fXa K_i	Tryp. K_i/ fXa K_i	APC K_i[17]/ fXa K_i	Plasm. K_i[17]/ fXa K_i	tPA K_i[17]/ fXa K_i
15		26	>150	>110	440	>280	>335
14a		22	>181	>131	>841	>330	>395
14b		6	>660	>480	>3080	>1200	>1450

Compound **14b** did not significantly inhibit the related serine proteases thrombin and trypsin. More importantly, **14b** is selective against anticoagulant enzymes such as activated protein C (APC) and plasmin and has no

activity against the fibrinolytic serine protease tissue plasminogen activator (t-PA). Inhibitor **14a**, is likewise selective (Table 2) and was chosen for *in vivo* studies.

Inhibitor **14a** (fXa K_i = 22 nM) was dosed orally in beagle dogs at 10 mg/kg[20]. This compound achieved high plasma levels (Cpmax = 2.7 μM @ 2 h) and showed *ex vivo* anti-fXa activity of 60% out to 4 hours post dose. This compares to the amidinothiophene inhibitor **1b** (fXa K_i = 7 nM) which required a dose of 50 mg/kg to achieve similar anti-fXa activity in the *ex vivo* assay (Cpmax = 3.1 μM @ 0.5 h). Furthermore, estimated oral bioavailability for **14a** (determined by pharmacodynamic analysis of *ex vivo* anti-fXa activity[21]) was 33% as compared to negligible oral bioavailability for **1b** in the dog.

Figure 2: Stereoview of **2a** Binding Model in the Factor Xa Active Site.



A model of compound **2a** in the active site (Figure 2) revealed several interactions which are thought to be important for pyrrolidinone binding to fXa[22]. The pyrrolidinone carbonyl makes a weak interaction with the NH of G218 whereas the methoxynaphthalene moiety is inserted in the aryl binding pocket making extensive hydrophobic contacts. Typical of sulfonamidopyrrolidinones[6], the sulfonamide group is solvent exposed making no direct interaction with the enzyme. The aminoisoquinoline moiety fills the S_1 subsite; the protonated isoquinoline forms an H-bond to D189. The amino function makes two hydrogen bonds, one to D189 and one to the carbonyl of G218, much like benzamidine inhibitors.

In summary, aminoisoquinolines have been identified as viable P_1 ligands for factor Xa. Optimization studies yielded selective, nanomolar fXa inhibitors which are superior to comparable benzamidines in their

Caco-2 cell permeability and oral bioavailability. The aminoisoquinoline may serve as a general bioisosteric replacement for benzamidines in inhibitors of trypsin-like serine proteases [23].

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