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EXPLORATORY SOLID-PHASE SYNTHESIS OF FACTOR Xa INHIBITORS: DISCOVERY AND APPLICATION OF P_3-HETEROCYCLIC AMIDES AS NOVEL TYPES OF NON-BASIC ARGININE SURROGATES[1]

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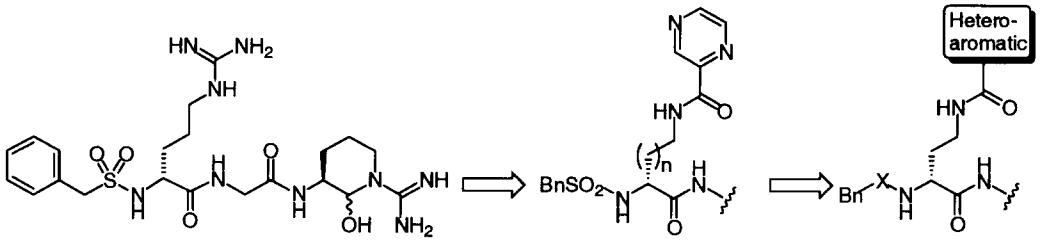
Abstract: A series of novel FXa inhibitors **2a-m** and **3a–f** was discovered that feature heterocyclic carboxamides tethered to a *d*-diaminobutyric acid sidechain. These neutral amide derivatives serve as novel P_3 *d*-arginine mimics. Pyrazine carboxamide scaffolds afforded the most potent FXa inhibitors (e.g., **2b** IC_{50} = 4.6 nM). The synthesis and biological activity of two focused libraries are reported.

Factor Xa (FXa) is a trypsin-like serine protease and holds a central position in the coagulation cascade, linking the intrinsic and extrinsic activation pathways. Interaction of FXa with cofactor FVa in a Ca^{+2}-phospholipid membrane assembly generates the prothrombinase (PTase) complex, which in turn cleaves the zymogen prothrombin to thrombin (FIIa). Thrombin is the terminal protease of the cascade, converting fibrinogen to fibrin, which ultimately combines with platelets and other components to form a blood clot. Thus, FXa, PTase and FIIa play key roles in the regulation of normal hemostasis and abnormal intravascular thrombus development (thrombosis).[2] The development of efficacious small molecule inhibitors of thrombosis would fulfill a major unmet medical need, and accordingly is an area of intensive investigation in the pharmaceutical industry.[3] Selective FXa and PTase inhibitors may have distinct therapeutic advantages over thrombin inhibitors, ultimately providing greater efficacy for both venous and arterial antithrombotic indications.[4]

We recently described the design and synthesis of a range of novel, potent and orally bioavailable thrombin inhibitors incorporating P_3-lactam[5] and P_3-heterocyclic[6] scaffolds. In the context of our evolving antithrombotic program, we have concurrently investigated a number of new strategies for small molecule FXa inhibitors. In this letter, we describe the design and discovery of potent FXa inhibitors **2a–m** and **3a–f**, which employ P_3-tethered heterocyclic amides as novel, non-basic arginine mimics (Figure 1). The design, synthesis, and biological activity of these targets will be presented.



1 (CVS 2371): Prototypical bis-cationic FXa inhibitor. IC_{50} FXa 0.7 nM, FIIa > 2500 nM, trypsin 75 nM

2a–c, n = 0-2

2d–m, X = SO_2
3a–f, X = O_2C

Figure 1. Design of FXa Inhibitors **2a–m** and **3a–f** Incorporating P_3-Heteroaromatic Carboxamide Moieties.

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Inhibitor and Library Design Strategy

The prototypical bis-cationic FXa inhibitor **1** (CVS 2371)[7] is topologically based on an endogenous peptide substrate[2,4] and expresses high in vitro potency on FXa, IC_{50} = 0.9 nM, and selectivity against thrombin, IC_{50} > 2500 nM and trypsin, IC_{50} 75 nM (Figure 1). This covalent transition-state analog inhibitor displays the usual compliment of important active-site binding interactions, including hydrophobic, electrostatic, β-sheet hydrogen bonding and van der Waals-type interactions typical of small molecule serine protease inhibitors.[8] Attenuating the high basicity of the P_3-guanidine (pKa ~12.5) unit by replacement with novel, non-basic isosteres was deemed a viable strategy for improving the oral absorption characteristics in this series of inhibitors.

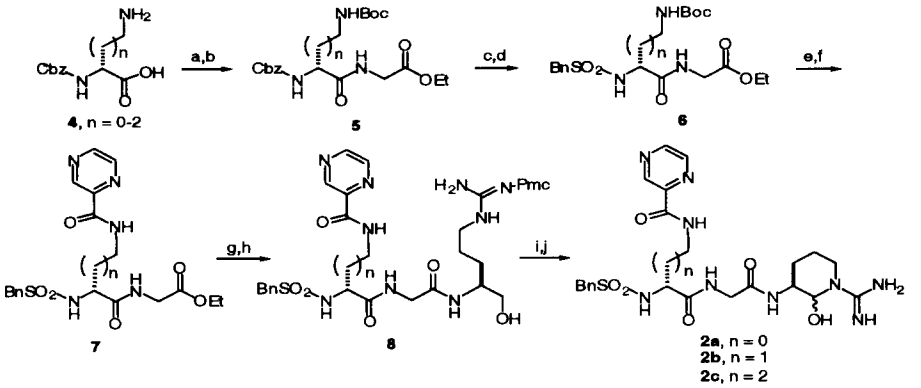
Our approach to minimizing P_3-basicity was to survey representative heteroaromatic ring systems possessing a range of size, basicity and ability to participate in edge-to-face and cation-π interactions at the S_4 pocket of FXa.[2,3,8a] Such heterocycles could potentially serve as novel types of guanidine surrogates. Using inhibitor **1** as a template, we initially designed and prepared three non-basic (pKa ~ 0.5) pyrazinecarboxamides **2a–c** that differed only in their P_3-sidechain tether. With an IC_{50} = 4.9 nM against FXa, the target **2b** (n = 1, prepared from P_3-*d*-diaminobutyric acid, DABA), expressed the highest in vitro potency of this series (Table 1). **2b** was essentially inactive on thrombin and showed 33-fold selectivity towards trypsin. Notable structural features of **2b** included an electrophilic arginine aldehyde at P_1, a simple P_2-glycine unit, a P_3-DABA-pyrazine carboxamide moiety and a tetrahedral P_4-benzylsulfonamide capping group.

In order to develop SAR around the lead **2b** and to probe the S_3-S_4 pockets of the fXa active site, two virtual libraries of P_3-heterocyclic arginine surrogates were generated which led to the subject molecules **2** and **3**. Based on SAR knowledge of small molecule FXa and PTase inhibitors, we maintained the P_1-argininal moiety, since it confers good OBA,[5,6] and the P_2-glycine unit. We investigated new P_3-heterocycles that featured either tetrahedral benzylsulfonamides or trigonal benzylcarbamates (Cbz) as the P_4-capping groups. Several representative members **2d–m** and **3a–f** were then prepared by combinations of solution and parallel solid-phase synthesis methods. Except for **2a–c**, all new compounds listed in Tables 1 and 2 were efficiently assembled using our new solid phase argininal technology that employs an aqueous TFA-cleavable linker.[9]

Chemistry[10]

A general synthetic route to the P_3-pyrazinecarboxamide homologs **2a–c** is outlined in Scheme 1. Commercially available *N*-α-Cbz-*d*-diaminocarboxylic acids **4** (n = 0–2) were protected as the *N*-ω-Boc derivatives, and then coupled to glycine ethyl ester to produce dipeptide **5**. Hydrogenolysis of **5** followed by treatment with α-toluenesulfonyl chloride gave intermediate **6** in high overall yield from **4**. Side-chain deprotection and coupling with 2-pyrazinecarboxylic acid proceeded smoothly to afford **7**. Saponification of **7** with lithium hydroxide followed by coupling of the resultant acid with a protected argininol synthon provided the advanced intermediate **8** in high overall yield. Finally, cleavage of the Pmc-moiety with TFA, oxidation of the primary alcohol function and preparative reverse-phase-HPLC delivered the desired targets **2a–c** in 59–65% overall yield from **8**.

Basic hydrolysis of intermediates **5b** and **6b** produced the corresponding carboxylic acids **9** and **10**, respectively, which were utilized as intermediates as described below in Scheme 2. For the efficient construction of the P_3-heterocyclic amide library members **2d–m**, we utilized the novel P_1-argininal synthon **11**, which features an orthogonally protected argininal aminal derivative linked to AM resin via a 6-carbon tethering unit. Key intermediate **11** was synthesized from commercial *N*-α-Boc-*l*-Arg(NO_2)-OH in 7 steps according to our recently published procedure.[9] Selective acid-catalyzed removal of the Boc group of **11** using



Scheme 1: *Reagents and Conditions:* (a) $(Boc)_2O$, K_2CO_3, dioxane, H_2O, ~quant.; (b) HCl•H-Gly-OEt, EDC•HCl, HOBt, DIEA, DMF, 87-96%; (c) H_2, Pd/C, 1N HCl, EtOH, ~quant.; (d) $BnSO_2Cl$, NMM, CH_3CN, 64-84%; (e) EtOH, HCl, ~quant.; (f) 2-Pyrazinecarboxylic acid, EDC, HOBt, DIEA, DMF, 81-85%; (g) EtOH, 1N LiOH, 90-99%; (h) H-Arg(Pmc)-ol, EDC, HOBt, DIEA, CH_3CN, 65-75%; (i) TFA, DCM, ~quant.; (j) EDC•HCl, DCA, DMSO, toluene, 59-65%, RP-HPLC purification.

freshly prepared *anhydrous* 5 M HCl in ethyl acetate cleanly afforded **12**. Use of HCl sources containing any traces of moisture led to competitive cleavage of the aminal linker residue. Subsequent block coupling of **10** to **12** (loading capacity of 0.62 mmol/g) using EDC, HOBt in the presence of excess Hunig's base afforded advanced resin-bound intermediate **13**. Acidic cleavage of the P_3-Boc group of **13** followed by acylation with a range of heteroaromatic carboxylic acids under standard amidation conditions generated **14**. P_1-Alloc removal with a Pd(0) catalyst was followed by exposure to a TFA, dichloromethane, water cocktail and RP-HPLC purification[9] to deliver the P_4-sulfonamide library targets **2d–m** in 43–89% overall yields. In a similar manner, intermediates **9** and **12** were coupled to provide resin-bound intermediate **15**, which underwent an analogous series of transformations and provided the corresponding intermediates **16** that were then converted to the final P_4-carbamate products **3a–f** in 41-88% overall yields.

Biological Activity

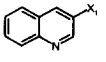
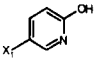
The in vitro biological activity of the P_4-benzylsulfonamide targets **2a–m** is shown in Table 1, while the P_4-benzyl carbamate series **3a–f** is found in Table 2. The assays were carried out using several important human serine proteases including the digestive enzyme trypsin, the procoagulant factor Xa (fXa) and thrombin (FIIa), and the thrombolytic protease plasmin. The targets were moderately to highly selective against both thrombin and plasmin. Top candidates from the sulfonamide series **2** generally showed classical slow binding kinetics of inhibition, reported as IC_{50} values, and higher FXa potency relative to their carbamate counterparts **3**, which demonstrated fast binding kinetics (reported as ~K_i values).[11]

Improved potency in series **2** may be due to enhanced interactions at each of the active-site P_1-P_4 regions. The tetrahedral P_4-sulfonamide linker confers optimal geometry and greater NH proton acidity which in turn improves antiparallel β-sheet hydrogen bonding to the active site Gly 216 residue.[7a] SAR trends in **2a–c** suggest the optimum P_3-side chain length to be two sp^3 methylene spacers between the peptide backbone and heterocyclic amide moieties. Deletion of the proximal nitrogen atom in the pyrazine ring, as in **2h**, resulted in ca. 16-fold loss of potency against FXa, while removal of the distal nitrogen, as in **2m**, resulted in only a 4-fold loss of potency. Little change in activity was observed with the fused heterocycles **2e**, **2f**, and **2i**. The disubstituted pyridines **2d** and **2g** also expressed interesting potency, however the hydroxyquinazoline **2k** was inactive. The N-benzoyl analog **2l** was essentially devoid of inhibitory activity, with FXa IC_{50} > 1mM.

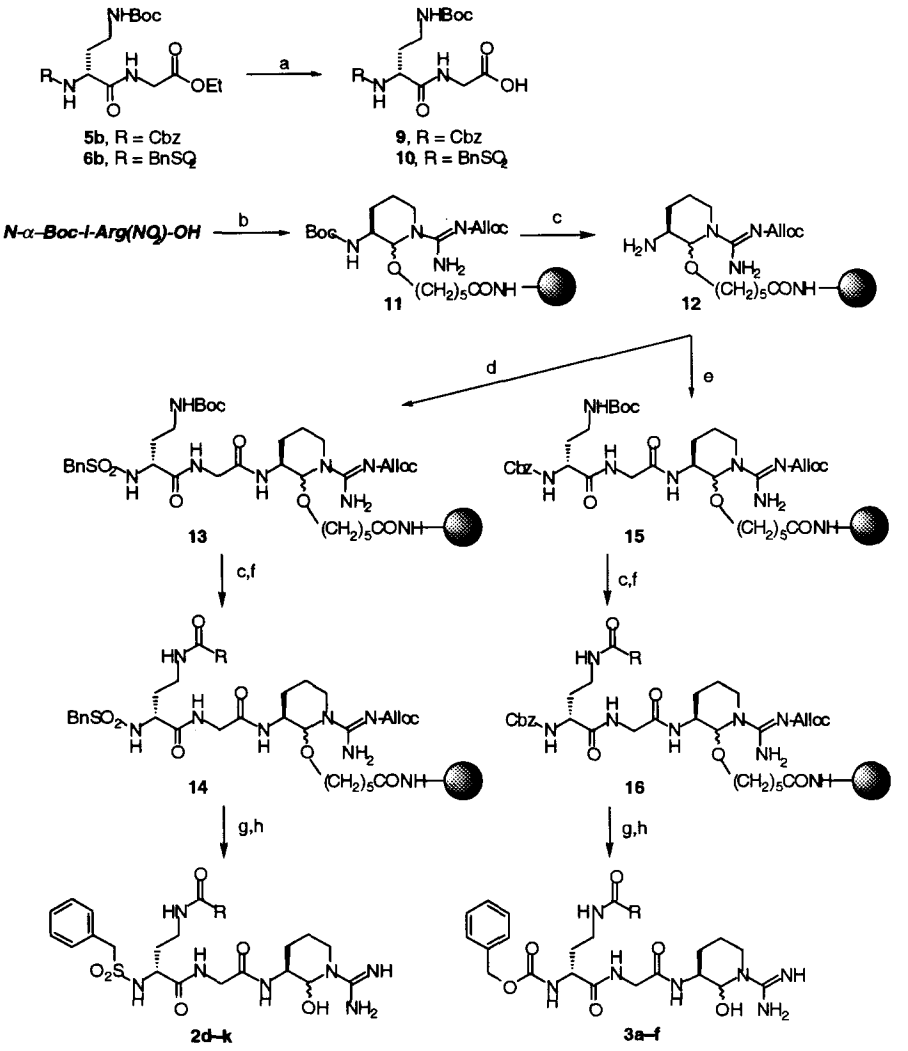
The S_4 subsite of FXa topologically resembles a cylinder whose wall is comprised of the Phe174, Trp215, and Tyr 99 aromatic residues. It's presence has been proposed as the basis for a cation-π interaction site

unique to this protease.[2,3c] Based on our SAR studies and modeling considerations, **2b** and related inhibitors probably bind to FXa in a normal substrate-like mode, with the P_3-heterocyclic ring participating in edge-to-face and/or cation-π interactions at the S_4 pocket. Additional energetically favorable salt-bridge interactions with Glu97, which appears at the distal terminus of the S_4-subsite, may also be possible via appropriate positioning of the P_3-heterocycle nitrogen atoms. With a K_i = 81.3 nM against FXa, the pyrazinecarboxamide target **3a** was an order of magnitude less active than the corresponding sulfonamide **2b**, but was the most potent P_4-carbamate in this series. Similar SAR trends are observed in the P_4-carbamate series **3**, which were kinetically fast inhibitors. Due to active-site homology and other structural similarities between FXa and trypsin,[7a] the acquisition of superior trypsin selectivity was difficult in this series of inhibitors. The trypsin selectivity of our most potent FXa inhibitors ranged from poor to satisfactory.

Table 1. In Vitro IC_{50} Values (nM) of Targets **2a–m** against Factor Xa, Thrombin, Plasmin, and Trypsin[a]

Cmpd.	n	R	FXa	FIIa	Plasmin	Human Trypsin	MW	HPLC % purity	% overall yield
2a	0		311	>2500	1480	152	561.6	95	61
2b	1		5.0	>2500	521	166	575.6	99	66
2c	2		15	2500	673	324	589.7	90	65
2d	1		10.6	>2500	1060	160	653.6	92	61
2e	1		4.9	>2500	730	97.8	624.7	94	64
2f	1		10.6	537	250	111	624.7	96	49
2g	1		6.1	>2500	367	26.6	590.7	94	67
2h	1		79.1	>2500	2500	561	574.7	90	89
2i	1		5.6	1770	700	4.21	625.7	93	62
2j	1		44.4	1270	1440	59.2	624.7	95	43
2k	1		>2500	>2500	Inact.	Inact.	641.7	92	71
2l	1		Inact.	Inact.	Inact.	Inact.	573.7	99	88
2m	1		20.5	>2500	1140	223	574.7	96	55

[a]Concentration of compounds **2a–m** necessary to inhibit Factor Xa (FXa), thrombin (FIIa), plasmin, and human trypsin cleavage of the chromogenic substrates described in ref 5 by 50%. Reported value for each compound is from a single IC_{50} determination which confirmed initial range values.

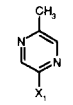
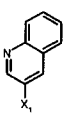


Scheme 2. *Reagents and conditions:* (a) 3M LiOH, MeOH, 0 °C to rt, 82-99%; (b) 7 steps, see ref. 9; (c) 5 M HCl in EtOAc, 0 °C to rt, 0.5 h, ~quant.; (d) **10**, EDC, HOBt, DIEA, DMF, 12 h, ~quant. by Kaiser test; (e) **9**, EDC, HOBt, DIEA, DMF, 12 h, ~quant. by Kaiser test; (f) Heteroaryl-CO_2H, EDC, HOBt, DIEA, DMF, 12 h; (g) $Pd(Ph_3P)_4$, CH_2Cl_2, 14 h; (h) TFA, CH_2Cl_2, H_2O: 6,3,1; 2 h; HPLC purification.

Conclusion

Two series of FXa inhibitors **2a–m** and **3a–f** incorporating P_3-heterocyclic amide moieties were designed and synthesized. Based upon protease active-site considerations and in vitro activity profiles, these neutral P_3-*d*-DABA-heteroaromatic amide units may be regarded as novel types of *d*-arginine surrogates. Application of our recently described solid phase P_1-argininal linker technology expedited the assembly of these targets and facilitated rapid SAR development. The top inhibitors emerged from series **2** and showed FXa inhibitory potencies in the 5–15 nM range. Selectivity against human trypsin ranged from poor to satisfactory. Subtle variations of the heterocyclic ring affected both the potency and selectivity profiles of these targets.

Table 2. In Vitro K_i Values (nM) of Inhibitors **3a–f** Against Factor Xa, Thrombin, Plasmin, and Trypsin[a]

Cmpd.	R	FXa	FIIa	Plasmin	Human Trypsin	MW	HPLC % purity	% overall yield
3a		106	2500	2030	30	555.6	98	63
3b		232.5	>2500	>2500	40.3	569.6	95	59
3c		346.3	>2500	3270	74.5	543.6	95	42
3d		312.5	>2500	>2500	>31.2	633.5	95	64
3e		312.5	>2500	>2500	>2500	604.7	93	89
3f		168.8	>2500	1140	20.375	604.7	94	41

[a] K_i values for kinetically fast inhibiton of Factor Xa (FXa), thrombin (FIIa), plasmin and human trypsin were determined from the IC_{50} values as reported in Table 1. Reported value for each compound is from a single determination which confirmed initial range values.

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References and Notes

1. (a) JZH dedicates this paper with genuine affection and respect to Professor Robert M. Coates, Department of Chemistry, University of Illinois at Urbana, on the occasion of his 60th birthday. (b) Portions of this work were presented at the 218th National American Chemical Society Meeting, New Orleans, LA, August 22-26, 1999. MEDI.31.
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