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Design, Synthesis, and SAR of Amino Acid Derivatives as Factor Xa Inhibitors[1]

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Abstract—A series of potent and selective factor Xa inhibitors was synthesized using various readily available amino acids as central templates. The most potent compound displays IC_{50} of 3 nM.

Thrombin, a plasma protease, plays a central role in both venous and arterial thrombosis. It has been postulated that efficient regulation of thrombosis may be achieved by regulation of thrombin. The current strategies employed involve reduction of thrombin generation as well as inhibition of the proteolytic activity of thrombin. The prothrombinase complex is the sole site of thrombin formation in the vasculature[2] and factor Xa (fXa) is the key component of the complex. Once assembled into the prothrombinase complex with cofactor Va and calcium ions on a phospholipid surface, fXa converts prothrombin to thrombin. In recent years, fXa has become a major target enzyme for new therapeutic agents with potential for treatment of arterial and venous thrombosis.[3]

We have previously reported the design and synthesis of various dibasic fXa inhibitors using amino acids such as 3-OH-proline, *m*-amidinophenylalanine, and 2,3-diamino propionic acid as templates (**1** in Fig. 1).[4] To discover novel inhibitors with improved bioavailability, we focused our synthetic efforts on reducing the overall basicity and enhancing the potency of this series of compounds. Quan et al.[5] has reported isoxazoline-containing fXa inhibitors (**2** in Fig. 1) in which the biphenylsulfonamide moiety was designed to interact with the S4 aryl-binding domain of the fXa active site. In this report, the design, synthesis, and in vitro structure–activity relationships of amino acid-based monobenzamidine factor Xa inhibitors incorporating benzamidine at P1 and this novel biphenylsulfonamide at P4 will be discussed.

Compounds **5**–**21** were readily prepared as shown in Scheme 1. BOP coupling of 4′-amino[1,1′-biphenyl]-2-*tert*-butylsulfonamide **3**[5] with various *N*-Boc protected amino acids, followed by BOC removal and subsequent coupling to 3-cyanobenzoic acid afforded nitrile intermediates **4**. Two procedures for amidine formation were used. One consisted of sequential reaction of the nitrile

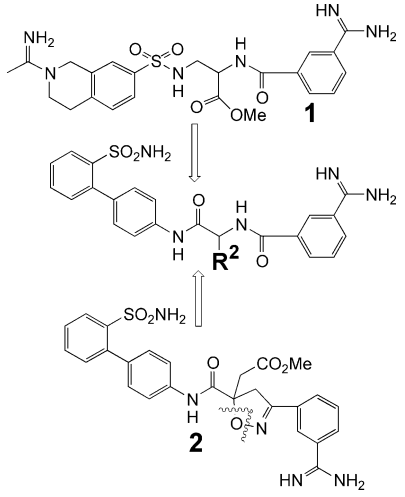

Figure 1. Design of amino acid-based inhibitors.

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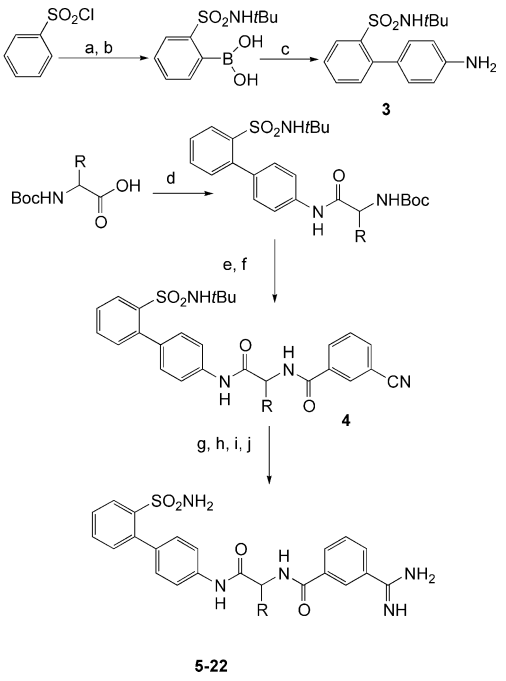
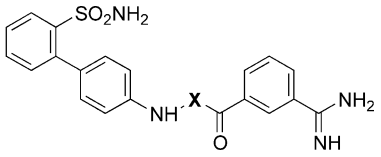


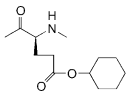
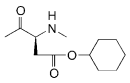
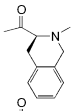
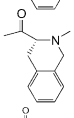
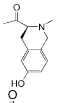
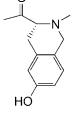
Scheme 1. Synthesis of compounds **5**–**21.** (a) NH_2tBu, TEA, DCM; (b) BuLi, $\{(CH_3)_2CHO\}_3B$, 1 N HCl, THF; (c) *p*-bromoaniline, $Pd(Ph_3P)_4$, Bu_4NBr, NaOH, toluene; (d) Biphenyl **3**, BOP, DIEA, DMF, rt; (e) 50% TFA/DCM; (f) 3-cyanobenzoic acid, BOP, DIEA, DMF; (g) H_2S, TEA/Pyr; (h) MeI, acetone, reflux; (i) NH_4OAc, MeOH, reflux; (j) Neat TFA, rt or (g) $NH_2OH.HCl$, TEA; (h) AcOH, Ac_2O; (i) 10% Pd/C, H_2; (j) Neat TFA.

Table 1. In vitro activity for compounds **5**–**21**



Compd	X	IC_{50}, μM		
		fXa	Thrombin	Trypsin
5		0.851	> 10	> 10
6		0.676	> 10	6.07
7		0.635	> 10	7.93
8		0.337	> 10	5.17
9		0.0762	> 10	> 10
10		1.44	> 10	> 10
11		0.644	> 10	5.64

Table 1 (*continued*)

12		0.842	> 10	> 10
13		0.055	> 10	4.81
14		0.0348	> 10	> 10
15		0.0348	> 10	1.77
16		0.0313	> 10	> 10
17		> 10	> 10	> 10
18		0.003	> 10	2.15
19		0.0187	> 10	4.39
20		0.0967	> 10	0.888
21		1.78	> 10	> 10

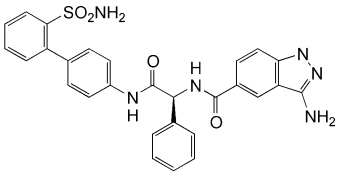
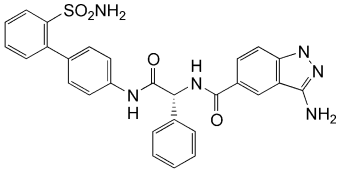
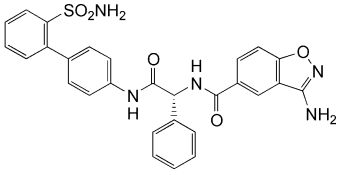
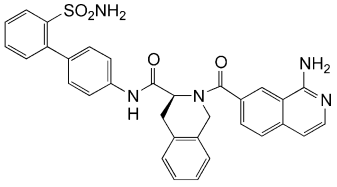
with hydrogen sulfide, methyl iodide, and ammonium acetate.[6] The second method consisted of reaction of the nitrile with hydroxyamine, acetic anhydride and hydrogenation. Final removal of the *t*-butyl group afforded free sulfonamides **5**–**21**.

We also prepared aminoindazole, aminobenzisoxazole, and aminoisoquinoline analogues (**22**–**25**) using the published procedures.[7]

The enzyme inhibition constants for compounds **5**–**25** toward fXa and the structurally related serine proteases thrombin and trypsin are summarized in Tables 1 and 2.[8,9]

The initial compound (**5**) prepared in this series contained a glycyl residue, which displayed only modest fXa inhibitory activity. Replacement of the glycyl residue with isoleucyl or β-cyclohexylalanyl residue afforded **6** and **7**, which were comparable to **5** in fXa inhibitory activity. Replacement of the glycyl residue with more hydrophobic phenylglycyl residue afforded **8** with 2.5-fold enhancement in the potency. Its homologue phenyl alanyl-containing analogue **9** exhibited 5-fold increase in activity when comparing with **8**. While the glutamic acid analogue **10** had diminished inhibitory potency relative to **5**, its benzyl and cyclohexyl esters **11** and **12** had

Table 2. In vitro activity for compounds **22**–**25**: P1 modifications

Compd		Factor Xa IC_{50}, μM[a]	Thrombin IC_{50}, μM[a]
22		>10	>10
23		3.45	>10
24		>10	>10
25		2.77	>10

similar activity compared to **5**. These data suggest that a hydrophobic side chain is preferred and acidic side chain is not preferred at the central position. Aspartic acid cyclohexyl ester-containing analogue **13** was significantly more potent (15-fold) than its homologue **12**.

To examine the stereochemical effect of the amino acid residues employed, several D-amino acids were incorporated into the series. D-isoleucine-containing analogue **14** is 19-fold more potent than L-isoleucine-containing analogue **6**, D-phenylglycine-containing analogue **15** is 10-fold more potent than L-phenylglycine-containing analogue **8** and D-phenylalanine-containing analogue **16** is 2.5-fold more potent than L-phenylalanine-containing analogue **9**. These data suggest an enzyme preference for the *R*-configuration at the α-position of these amino acids. Very recently, Jones et al.[10] published a series of potent phenylglycine containing benzamidine carboxamides fXa inhibitors based on a lead compound they selected using their proprietary PRO-SELECT module. In that module, they predicted that lipophilic D-amino acids can pick up an additional interaction with 'disulfide' pocket adjacent to S1, comprising residues Gln192, Cys191, Cys220 and Gly218. The lipophilic D-amino acid derivatives were synthesized and found to be the preferred central unit in their series. Molecular modeling of our two phenylglycine enantiomers (**8** and **15**) (Fig. 2) in the active site of fXa shows the benzamidine in the S1 pocket with a bidentate interaction with Asp189 while the biphenylsulfonamide moiety projects towards the S4 pocket bordered by Phe174, Trp215, and Tyr99. Hydrogen bonding between this sulfonamide moiety and the carboxylate side chain of Glu97 helps hold the ligand in the active site. Additional hydrogen bonding interactions can be observed between the backbone carbonyl of Gly216 and the amide hydrogens in the central position of the ligand. In our model, the benzene ring of the D-phenylglycine (**15**) is accommodated in the lipophilic 'disulfide' pocket as described by Jones et al.[10] This interaction is not prefered with the enantiomer derived from L-phenylglycine (**8**). In this case, the

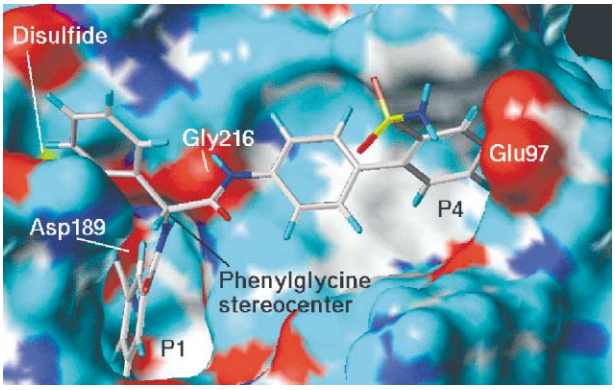


Figure 2. Proposed binding mode of compound **15** in the factor Xa active site. The Connelly surface coloring represents proximal atoms (red, blue, white, and teal identify oxygen, nitrogen, carbon and hydrogen, respectively). The P1 and P4 binding pockets are labeled, as are critical residues on the protein.

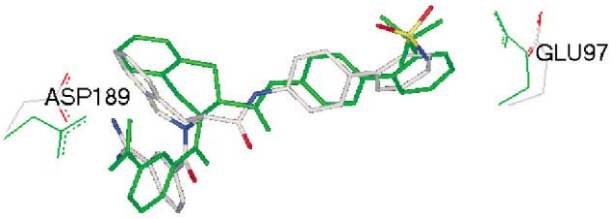


Figure 3. Overlap of the lowest energy docked conformations of compound **18** and compound **19** (green) in the factor Xa active site. The side chains of residues Asp189 and Glu97 are shown for perspective, and residues associated with compound **19** are colored green. Docked conformations of compound **18** were consistently more stable than the most favorable binding conformations of compound **19**.

hydrogen binding to the backbone carbonyl is greatly inhibited by the change in conformation necessary to place the phenyl group in the lipophilic 'disulfide' pocket.[11]

To determine whether the conformational restriction within the central unit would modulate activity, the glycyl residue was replaced by several cyclic amino acids. The modifications, which included the incorporation of a proline, afforded analogue **17** with loss of activity. However, incorporation of the tetrahydroisoquinoline-3-carboxyl group generated this series' most potent compound **18**, with 283-fold enhanced activity in the fXa inhibitory assay relative to the corresponding glycine-containing analogue **5**. Interestingly, the D-isomer of this bulky amino acid **19** is less potent than the corresponding L-isomer **18**. Molecular modeling of these two enantiomers suggests that favorable P1 region binding factors are somewhat disrupted by inverting the stereocenter from D- (**18**) to L- (**19**) (Fig. 3). Introduction of an H-bonding hydroxy group to the tetrahydroisoquionline ring resulted in analogues **20** and **21** with significantly reduced activity for both isomers.

As shown in Table 1, all compounds displayed good selectivity for fXa over thrombin and trypsin. High selectivity against a panel of serine proteases including tissue plasminogen activator (tPA), activated protein C (APC) and plasmin, was also observed for these compounds (data not shown). For example, compound **15** has IC_{50} values of 0.0348 μM for Xa, >180 μM for tPA, 6.56 μM for APC and 18.1 μM for plasmin. **18** has IC_{50} values of 0.003 μM for Xa, >180 μM for tPA, 73 μM for APC and 77.9 μM for plasmin. The pharmacokinetic properties of the most potent analogues **15** and **18** were evaluated in Sprague–Dawley rats. The oral bioavailability of **15** and **18** was found to be less than 5%. In order to increase the bioavailability of these monobenzamidine leads, amidine groups in **8**, **15**, and **18** were replaced by aminoindazole (**22** and **23**), aminobenzisoxazole (**24**) and aminoisoquinoline (**25**). Similar approaches have been used by other laboratories[12] where potent compounds have been reported. However, the replacement within our series resulted in a more than 100 to 1000-fold drop in fXa inhibitory potency.

In summary, we have synthesized a series of potent and selective fXa inhibitors using various amino acids as central templates. These readily available amino acids gave us rapid access to the critical structure–activity information within this class of compounds. Hydrophobic interactions between the side chain of the amino acid residue and fXa and the orientation of the side chain played important role for enhancing the potency of this series of inhibitors. Further exploration of amino acid templates aimed at improving pharmacokinetic properties will be the subject of additional communications from our laboratories.

References and Notes

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