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Investigation of factor Xa inhibitors containing non-amidine S1 elements

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Abstract—Several non-amidino S1 derivatives of the 1,2-diaminobenzene-based scaffold (**4**) were synthesized and evaluated for their ability to bind to the active site and inhibit the human protease factor Xa. A subset of these compounds were also evaluated for their anticoagulant effects in human plasma as measured by prothrombin time (PT).

Human factor Xa (fXa) is a trypsin-like serine protease that plays a critical role in the blood coagulation cascade.[1] This enzyme is responsible for the generation of thrombin that, in turn, produces fibrin and ultimately leads to blood clot formation. Consequently, the discovery of a small molecule inhibitor of fXa as an oral anticoagulant has been the focus of many pharmaceutical research groups.[2–5]

Our laboratories have previously disclosed a novel series of 1,2-diaminobenzene-based fXa inhibitors (Fig. 1).[6] that display lipophilic groups into both the S1 and S4 binding pockets of this protease. For example, a computational binding model of compound **1** in the active site of factor Xa projects the 4-methoxyphenyl group into the S1 site and the 4-*t*-butylphenyl group into the S4 site (Fig. 2). Also, this model indicates that the 1,2-diamide motif interacts with the S3 backbone of the enzyme. This compound has reasonable binding affinity to fXa, but displays a prothrombin time (2× PT) activity >50 μM (Table 1). This difference may be attributed to the highly lipophilic nature of **1** and enhanced non-specific interactions with plasma proteins, which detrimentally impact the functional potency of this inhibitor. In contrast, compound **2**, which incorporates an amidine into the S1 binding element of this scaffold, displays very high binding affinity for fXa and potent anticoagulant activity in vitro[7] (Table 1). However, there is precedent

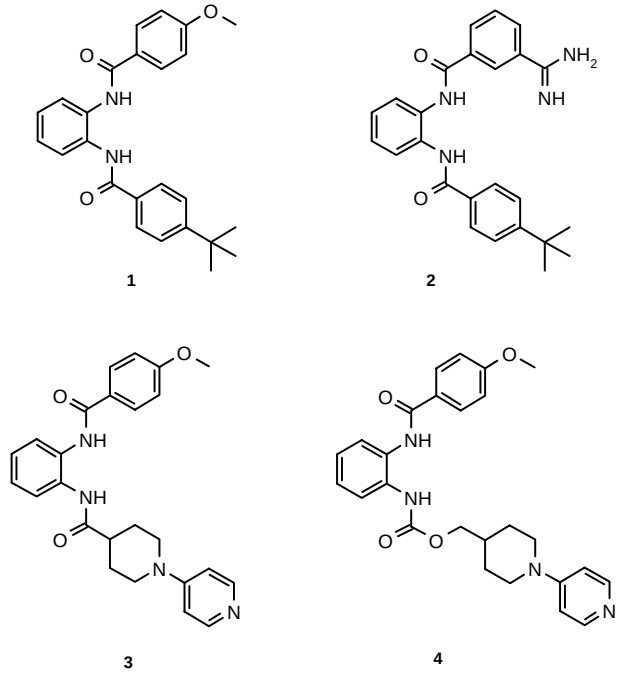

Figure 1. Structures of factor Xa inhibitors **1**–**4**.

Keywords: Factor Xa; Thrombosis; Coagulation; 1,2-Diaminobenzene.
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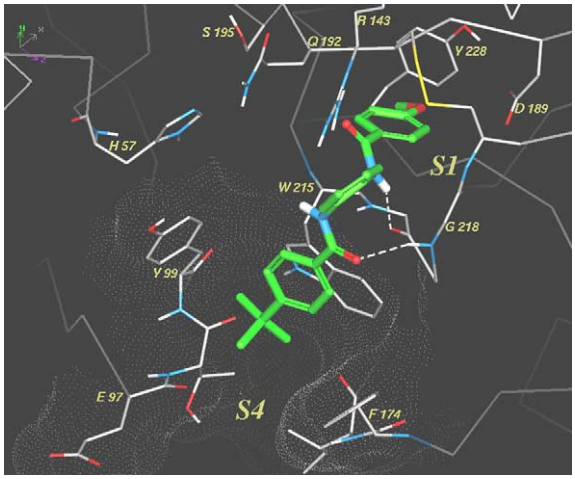


Figure 2. Proposed energy-minimized binding model of compound **1** complexed with the active site of factor Xa. The orientation is such that Asp189 appears in the upper right-hand corner, Ser195 near the top central, and Glu97 in the lower left-hand corner. Hydrogen bonds are shown as dashed lines. All aliphatic hydrogens and waters have been removed for clarity. The molecular modeling was performed, as described previously in Herron et al.[6] utilizing the X-ray structure 1hcg.pdb.[12]

Table 1. Human factor Xa binding affinity and prothrombin time activity of inhibitors **1**–**3**

Compounds	K_{ass}[a] (in 10^6 L/mol)	PT[b] (μM)
1	7.0	>50
2	242	0.9
3	2.0	9.7
4	19	1.9

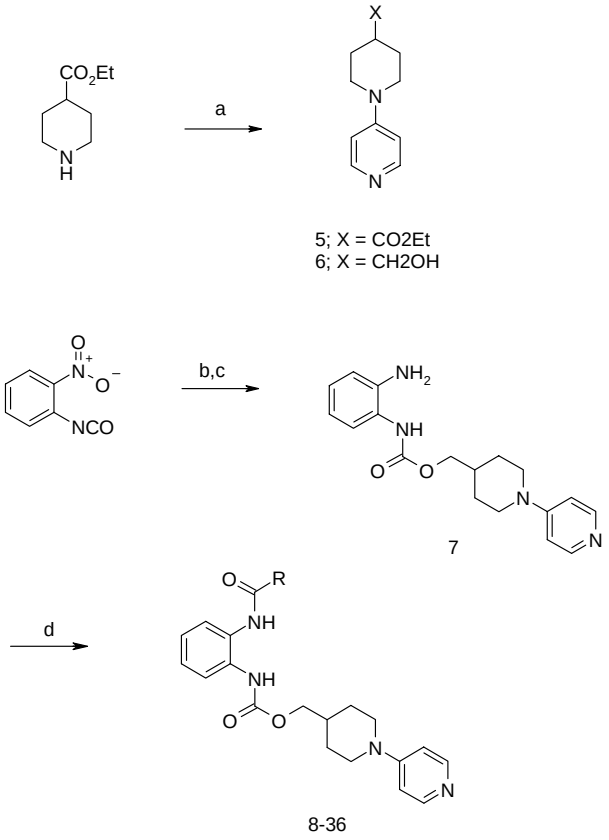
[a] K_{ass} represents the apparent association constant, as measured by the methods of Smith et al.[13]

[b] PT is defined as the concentration of compound required to double the time to clot formation in the prothrombin time assay.

that amidine containing molecules, in general, lack desirable pharmacokinetic properties upon oral administration.[9,10] Alternative structure–activity relationships (SAR) on the 1,2-diaminobenzene scaffold have indicated, the 4-*t*-butylphenyl S4 binding element can be replaced with a cationic/basic S4 group (e.g., compound **3**).[8] Alternately, the 1-(4-pyridyl)piperidine moiety may be positioned in the S4 site by the carbamate of compound **4**.[11] This compound is of particular interest on account of its improved binding affinity for fXa, but more importantly, for its enhanced anticoagulant potency in vitro within this select group of molecules. The good translation of binding affinity of the more hydrophilic compounds **3** and **4** into functional anticoagulant activity is consistent with reduced non-specific plasma protein binding.

In this paper, we report the effects of varying the S1 binding portion of the carbamate scaffold with respect to binding affinity and anticoagulant potency.

The syntheses of compounds **8**–**36** are shown in Scheme 1. Intermediate **6** was prepared by first heating ethyl isonipecotate with 4-chloropyridine hydrochloride and

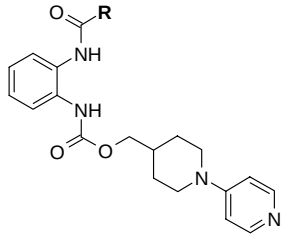


Scheme 1. Reagents and conditions: (a) EtOH, NEt_3, 120 °C, 2 days, then $LiAlH_4$, THF; (b) **6**, $CHCl_3$/THF; (c) H_2, 10% Pd/C, EtOH; (d) RCOCl, pyridine, $CHCl_3$ or RCO_2H, DCC, DMF.

triethylamine in EtOH at 120 °C. Reduction of ester **5** with $LiAlH_4$ afforded the primary carbinol **6**. Treatment of 2-nitrophenylisocyanate with **6** in $CHCl_3$/THF, followed by reduction with H_2 and Pd/C, afforded compound **7**. Acylation of aniline **7** was carried out by treating with the corresponding acid chloride or by carbodiimide-mediated coupling of the appropriate carboxylic acid to obtain the final products.

The SAR findings in the carbamate-linked series are given in Table 2. The unsubstituted phenyl compound **8** exhibits an association constant (K_{ass}) of 1.6×10^6 L/mol. Saturation of the aromatic ring affords a 40-fold reduction in fXa binding affinity (**9**). Partial unsaturation of the cyclohexyl construct (**10** and **11**) results in a 5- and 22-fold gain in activity-relative to the completely saturated version **9**. The mono-unsaturated enones, **10** and **12** display similar binding affinity as **8**. In general, substitution at the 4 position enhances the binding affinity to fXa compared to the unsubstituted phenyl ring. For example, the 4-methyl, 4-methoxy, and 4-$OCHF_2$ display binding affinities of 6.3, 19, and 5.0×10^6 L/mol, respectively. Similarly, the 4-halogen-substituted phenyl rings show increases in binding affinity of 3- to 24-fold versus compound **8**. Functionalization of the 3 position is also tolerated (**21**–**26**), but the enhancement in binding affinity is only modest when compared to those the 4-position. The 2-methoxy and 2-chloro derivatives (**27** and **28**) display 0.02 and 0.30×10^6 L/mol binding affinities, respectively.

Table 2. Human factor Xa binding affinity and prothrombin time activity of compounds **8**–**36**



Compounds	R	K_{ass} [a] (in 10^6 L/mol)	PT (μM)
8	Phenyl	1.6	—
9	Cyclohexyl	0.04	—
10	1-Cyclohexenyl	0.90	—
11	2-Cyclohexenyl	0.20	—
12	1-Cyclopentenyl	0.70	—
13	Phenyl(4-Me)	6.3	8
14	Phenyl(4-OMe)	19	1.9
15	Phenyl(4-$OCHF_2$)	5.0	6
16	Phenyl(4-F)	4.6	—
17	Phenyl(4-Cl)	35	1.9
18	Phenyl(4-Br)	38	1.8
19	Phenyl(4-I)	14	6
20	Phenyl(4-CN)	0.60	—
21	Phenyl(3-Me)	5.9	—
22	Phenyl(3-OMe)	3.3	10
23	Phenyl(3-F)	2.5	17
24	Phenyl(3-Cl)	4.1	12
25	Phenyl(3-OH)	1.0	—
26	Phenyl(3-NH_2)	5.1	8
27	Phenyl(2-OMe)	0.02	—
28	Phenyl(2-Cl)	0.30	—
29	Phenyl(3-F, 4-OMe)	46	1.1
30	Phenyl(3-F, 4-Me)	5.8	7
31	Phenyl(3-Cl, 4-Me)	2.3	22
32	Phenyl(2,3-diCl)	19	5
33	2,3-[Dihydro]benzofuran-5-yl	0.60	—
34	6-Indoyl	54	1.6
35	5-Cl-furan-2-yl	0.70	—
36	5-Cl-thiophen-2-yl	33	2.6

PT is defined as the concentration of compound required to double the time to clot formation in the prothrombin time assay.

[a] K_{ass} represents the apparent association constant as measured by the methods of Smith et al.[13]

These data suggest that substitution in the ortho position negatively impacts binding to fXa. The 3,4-disubstituted compounds display different results when compared to their 4-substituted counterparts. The 3-fluoro-4-methoxy analog (**29**) has a more than 2-fold higher binding affinity than the 4-methoxy compound (**14**), whereas the 3-fluoro-4-methyl analog (**30**) has a binding affinity similar to the mono-substituted 4-methyl compound (**13**). Bicyclic groups display mixed results. The 6-indoyl derivative (**34**) shows the highest binding affinity for fXa of 54×10^6 L/mol and the 2,3-[dihydro]benzofuran-5-yl compound (**33**) has one of the lowest affinities of 0.6×10^6 L/mol. Heterocyclic replacements for the phenyl group display very different effects on fXa binding. For example, the 5-chloro-furan-2-yl compound (**35**) has a binding affinity of 0.7×10^6 L/mol and the 5-chloro-thiophen-2-yl compound (**36**) has a binding constant of 33×10^6 L/mol. These data suggest that the specific placement of the substituent in the S1 pocket is critical. There exists a general correlation between fXa binding affinity and enhanced plasma anticoagulant potency (PT) within this set of inhibitors ($R^2 = 0.54$). In addition, the most active compounds show a high degree of selectivity versus similar proteases. For example, compound **17** showed minimal binding to serine proteases involved in hemostasis (fIIa (thrombin), t-PA, plasmin, and urokinase), and to bovine trypsin (Table 3).

Table 3. Binding affinity of compound **17** for various enzymes

Enzyme	K_{ass} [a] (in 10^6 L/mol) for **17**
fXa	35
fIIa	0.02
t-PA	<0.01
Plasmin	<0.01
Urokinase	<0.01
Trypsin (bovine)	<0.01

[a] K_{ass} represents the apparent association constant as measured by the methods of Smith et al.[13]

In summary, structural modification of the S1 binding element in a carbamate-based series affords human factor Xa inhibitors that do not contain an amidino functionality, but display both high binding affinity and anticoagulant potency in human plasma. The most potent derivatives contain some type of substitution at the 4 position of the phenyl S1 binding element. These derivatives also display a high degree of specificity against similar enzymes. In general, within this series of inhibitors, there is a correlation between binding affinity for factor Xa and anticoagulant potency as measured by prothrombin time (PT).

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