

5-Amidinoindoles as dual inhibitors of coagulation factors IXa and Xa

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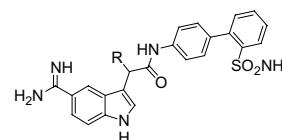
Abstract—Structural features of a 5-amidinoindole inhibitor of factor Xa, which displayed modest inhibition of factor IXa were varied to increase potency and improve selectivity for factor IXa.

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Thrombotic diseases, such as myocardial infarction, stroke, deep vein thrombosis and pulmonary embolism, are leading causes of mortality. Currently these diseases are most often treated with heparin or warfarin, both of which carry significant risks for bleeding complications. Enormous effort has been expended in the search for safer agents, mostly aimed at inhibition of thrombin or coagulation factor Xa (fXa).¹ Factor Xa, which converts prothrombin to thrombin in the penultimate step in the coagulation cascade, can be generated from the zymogen (factor X) through proteolysis by either factor VIIa via the extrinsic pathway² or by factor IXa (fIXa) via the intrinsic pathway.³ Selective blockade of only the intrinsic pathway by inhibiting fIXa could provide a drug with less liability for problem bleeding. The viability of fIXa inhibition as an approach to selective anti-thrombosis is supported by studies with anti-IXa monoclonal antibodies^{4,5} and with active-site covalently inhibited fIXa.⁶

Since the active sites of fIXa and fXa are very similar,⁷ screening of our proprietary fXa inhibitors in an assay for fIXa inhibition⁸ was undertaken to seek an initial lead. Among the compounds revealed by this procedure were the 3-substituted 5-amidinoindoles⁹ **1** and **2** (Table 1). Although **1** was only a weak inhibitor of fIXa, and was 2000-fold selective for fXa, incorporation of the

Table 1. Screening leads



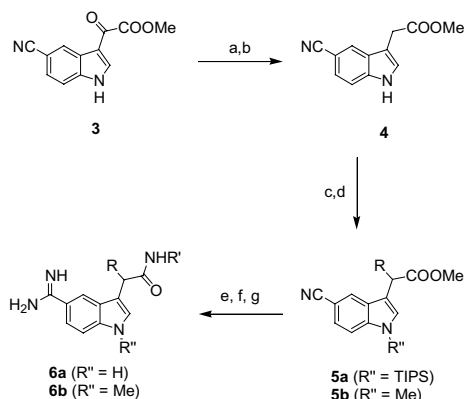
Compound	R	fIXa K_i , nM	fXa K_i , nM	fIXa/fXa
1	H	6700	3.2	2100
2	CH ₂ Ph	660	0.63	1050

benzyl substituent of **2** improved activity 10-fold. Compound **2** was selected for structure–activity studies in an attempt to increase potency for fIXa inhibition, and to seek selectivity with respect to inhibition of fXa.

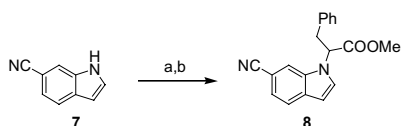
The synthetic approach to compounds **13–24**, **26**, and **29–32** is shown in Scheme 1. The ketoester **3**⁹ was reduced to the indoleacetate ester **4** with triethylsilane. Over-reduction always produced about 30% of the corresponding indoline, which was difficult to remove chromatographically; it was more expedient to subject the mixture to re-oxidation using a published procedure¹⁰ followed by a much easier chromatographic purification to provide **4** in 76% overall yield. After methylation or protection of the ring nitrogen, benzylic alkylation of the derived anion generally provided **5** in good yields. Anilides were prepared directly from the esters by treatment with the corresponding aniline and trimethylaluminum; benzylamides were prepared from the derived carboxylic acid using coupling reagents. Pinner reaction

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Scheme 1. Reagents and conditions: (a) Et_3SiH , CF_3COOH , rt; (b) $(\text{PhSeO})_2\text{O}$, indole, THF, 60°C , 76% (2 steps); (c) for **5a**: $\text{KN}(\text{TMS})_2$, THF, 0°C ; $i\text{-Pr}_3\text{SiCl}$, to rt (75%); for **5b**: NaH , DMF, 0°C ; MeI , to rt (99%); (d) $\text{LiN}(\text{TMS})_2$, THF, -78°C ; RBr or RI , to rt (30–80%); (e) Me_3Al , $\text{R}'\text{NH}_2$, CH_2Cl_2 , or LiOH , then DIC , pyridine/DMSO, $\text{R}'\text{NH}_2$, $60\text{--}80\%$; (f) EtOH , HCl , rt; (g) NH_3 , MeOH , rt; or NH_4OAc , MeOH , rt, 50–80% (2 steps).



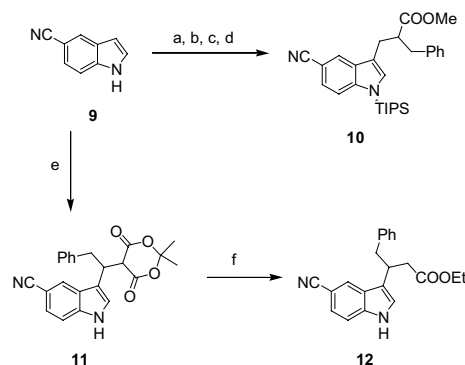
Scheme 2. Reagents and conditions: (a) NaH , DMF, 0°C ; $\text{BrCH}_2\text{COOEt}$, to rt (72%); (b) $\text{LiN}(\text{TMS})_2$, THF, -78°C ; PhCH_2Br , to rt (30%).

followed by ammonolysis was accompanied by removal of the TIPS protecting group (if present) to provide the desired compounds (represented by structures **6a** and **6b**) in 40–60% overall yield.

The alternative 1,6-disubstituted indole derivative **25** was prepared similarly, from the known 6-cyanoindole **7**,¹¹ as shown in Scheme 2. Alkylation of the sodium salt of **7** with ethyl bromoacetate proceeded in good yield, but alkylation of the derived anion provided **8** in only low yield. Amide formation and amidine elaboration then proceeded as shown in Scheme 1.

Two different approaches were used to prepare compounds with longer chains (Scheme 3). 5-Cyanoindole **9** was alkylated with acrylic acid¹² in low yield, followed by conversion to the methyl ester, nitrogen protection and alkylation to provide **10**. Several approaches to intermediate **12** were explored with limited success. Finally, following a literature method,¹³ **9** was treated with phenylacetaldehyde and Meldrum's acid in the presence of proline to provide, in moderate yield, **11**, which underwent ester exchange and decarboxylation to provide useable quantities of **12**. Intermediates **10** and **12** were elaborated to **27** and **28** (Table 5), respectively, as described above.

Inhibitory activities against fIXa and fXa were assayed using published methods, involving enzymatic hydrolysis of small peptidic chromogenic substrates.^{8,14} Meas-

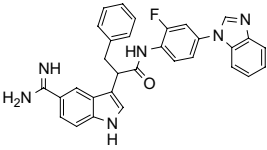


Scheme 3. Reagents and conditions: (a) $\text{CH}=\text{CHCOOH}$, HOAc , Ac_2O , reflux, 15%; (b) MeOH , conc. HCl , rt, 81%; (c) NaH , DMF, 0°C ; $i\text{-Pr}_3\text{SiCl}$, to rt (39%); (d) $\text{LiN}(\text{TMS})_2$, THF, -78°C ; PhCH_2Br , to rt, 52%; (e) PhCH_2CHO , Meldrum's acid, L-proline, MeCN , rt, 32%; (f) pyridine, EtOH , Cu powder, reflux, 79%.

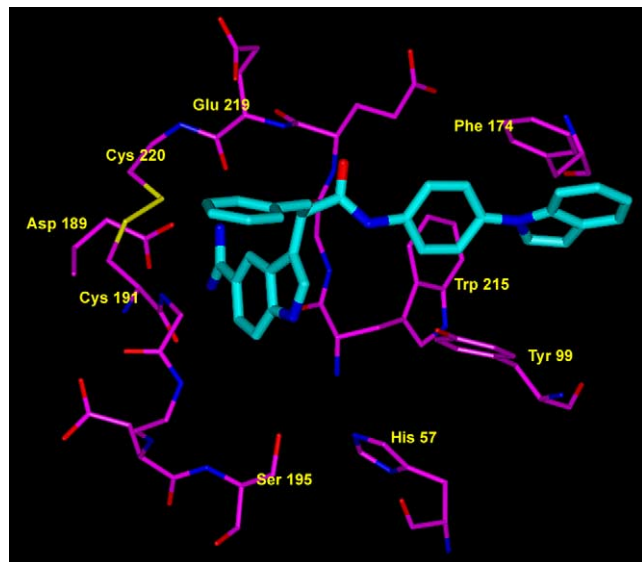
urement of inhibition of fIXa in particular is difficult since the enzyme shows poor activity against small chromogenic substrates,¹⁵ requiring the addition of substances such as ethylene glycol to obtain useful enzymatic activity.⁸ (A more physiologically relevant assay of fIXa activity, involving activation of factor X followed by monitoring the generated fXa activity via hydrolysis of a chromogenic substrate,¹⁶ was not used because the strong fXa inhibition would make dissection of the inhibition of fIXa from the overall inhibition difficult or impossible.) Since the assays for fIXa and fXa are performed under different conditions, the actual magnitudes of the two K_i values are not directly comparable, but the ratio of the two K_i values should be useful for comparing the selectivities of analogs within a series of compounds. (In results not shown, compounds **13–32** were generally 10–50 fold less potent against thrombin¹⁴ than against fIXa.)

Screening results for other proprietary fXa inhibitors suggested that replacement of the 4-biphenyl moiety (which is bound in the S4 region of trypsin,¹⁷ and presumably binds similarly in both fXa and fIXa) with 4-(1-benzimidazolyl)phenyl¹⁸ should boost fIXa activity. Incorporation of this substituent into **2** provided **13a**, which enhanced fIXa potency by an order of magnitude and, importantly, failed to produce a similar increase in fXa potency, thus lowering the selectivity ratio to 25 (Table 2). This racemic compound was resolved by chiral HPLC, and better potency and selectivity were displayed by the levorotatory isomer (**13b**). The carboxylic acid obtained by hydrolysis of **5a** ($\text{R} = \text{CH}_2\text{Ph}$) was also resolved by chiral HPLC, and a single crystal X-ray structure of the cinchonidine salt of the (+) isomer proved this to have the (*S*) configuration. The (–) isomer of the acid was converted to **13b**, by coupling the acid directly with the appropriate $\text{R}'\text{NH}_2$ using DIC followed by amidine elaboration as in Scheme 1, establishing the configuration of the more active isomer as (*R*).

This stereochemical finding matches the results of modeling des-fluoro **13** in the active site of fIXa, derived from

Table 2. Effect of absolute configuration


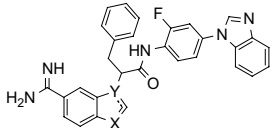
Compound	Config.	fIXa K_i , nM	fXa K_i , nM	fIXa/fXa
13a	(<i>RS</i>)	63	2.5	25
13b	(<i>R</i>)-(–)	19	1.3	15
13c	(<i>S</i>)-(+)	820	26	32

**Figure 1.** Model of Compound **13b** in the active site of IXa. The inhibitor, shown in blue, has the amidine positioned near Asp 189 in the back of the S1 pocket, with the S4 pocket to the right.

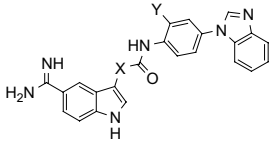
a published X-ray structure, as shown in Figure 1.¹⁹ In this model, the indole lies in the S1 pocket, with the amidine interacting with Asp 189 at the bottom of this pocket. The amide carbonyl of **13** lies within hydrogen

bonding distance (3.1 Å) of the backbone nitrogen of Glu 219 at the edge of the S1 pocket. The benzimidazolylphenyl group lies in the S4 region, interacting with Tyr 99, Phe 174, and Trp 215. The (*R*) benzyl substituent could engage in a hydrophobic interaction with the Cys191-Cys220 disulfide bond near the edge of the S1 pocket, whereas such interactions would not be possible for the (*S*) enantiomer; in fact, the (*S*) benzyl would clash with the edge of the S1 pocket if the rest of the molecule bound as shown in Figure 1.

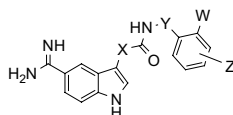
Variation of the benzyl substituent showed no strong structure–activity trends, as the examples in Table 3 demonstrate. (The presence or absence of the 2-fluoro substituent on the aniline ring had a minimal effect on either potency or selectivity.)¹⁸ The presence of at least a 2-carbon chain seemed required for better selectivity (compare **14** and **15** with **16**), although longer groups did not help significantly (**18** vs **13a**). Larger lipophilic groups (**19**, **24**) seemed in general to provide slightly better selectivity, due to their negative impact on fXa activity. The naphthyl derivative **19** showed the weakest fXa activity of the substituents explored. This could suggest unfavorable steric interactions with fXa, although no such interactions were apparent from computer modeling. The cyclopropylmethyl compound **17** was anomalously weak against fIXa. Since a major difference

Table 4. Effect of indole core changes


Compound	X	Y	fIXa K_i , nM	fXa K_i , nM	fIXa/fXa
13a	NH	C	63	2.5	25
25	CH	N	300	5.6	54
26	NMe	C	360	160	2

Table 3. Effect of chain substitution


Compound	X	Y	fIXa K_i , nM	fXa K_i , nM	fIXa/fXa
13a	CH(CH ₂ Ph)	F	63	2.5	25
14	CH(Me)	H	480	3	160
15	C(Me) ₂	H	750	2.2	340
16	CH(Et)	H	160	2.8	57
17	CH(CH ₂ -cyclopropyl)	H	3800	14	270
18	CH(CH ₂ CH ₂ Ph)	F	240	6.1	39
19	CH(CH ₂ -2-naphthyl)	F	98	28	4
20	CH(CH ₂ -Ph-3-COOH)	F	59	1.8	33
21	CH(CH ₂ -Ph-4-NH ₂)	H	75	4	19
22	CH(CH ₂ -3-pyridyl)	H	1200	7	170
23	CH(CH ₂ -Ph-4-NHCOPh)	H	270	18	15
24	CH(CH ₂ -Ph-4-NHSO ₂ Ph)	H	130	19	7

Table 5. Effect of S4 substituent

Compd	X	Y	Z ^a	W	fIXa <i>K</i> _i , nM	fXa <i>K</i> _i , nM	fIXa/fXa
13a	CH(CH ₂ Ph)	Bond	4-(1-bzim)	F	63	2.5	25
27	CH ₂ CH(CH ₂ Ph)	Bond	4-(1-bzim)	H	130	45	3
28	CH(CH ₂ Ph)CH ₂	Bond	4-(1-bzim)	H	250	36	7
29	CH(CH ₂ Ph)	CH ₂	4-(1-bzim)	H	310	72	4
30	CH(CH ₂ Ph)	CH ₂	4-(CONH-2-bzim)	H	930	810	1
31	CH(CH ₂ Ph)	CH ₂	3-(CONH-2-bzim)	H	1900	2600	0.7
32	CH(CH ₂ Ph)	Bond	4-(4-Phenylimidazolyl)	H	270	79	3
32	CH(CH ₂ Ph)	CH ₂	4-(CONH-6-MeSO ₂ -2-bzim)	H	660	1100	0.6

^a bzim = benzimidazolyl.

between fXa and fIXa is the replacement of Gly 219 with Glu 219,⁷ the activity of basic and acidic groups, which might interact with Glu 219 was of special interest.¹⁸ However, neither acidic (**20**) nor basic (**21**) groups on the benzyl substituent had much effect, while basic heterocycles (**22**, for example) lost significant fIXa potency. The broad, shallow nature of the SAR may suggest that a major role for this substituent could be to provide a conformational bias for the flexible side-chain, favoring the modeled disposition of the S4-binding group relative to the amidinoindole, with added potency arising from nonobvious interactions of lipophilic groups with the enzyme.

Modeling of compound **13b** in the active site of fIXa (Fig. 1) positioned the indole NH near the catalytic serine 195. Although the N–O distance in this model (4.3 Å) is long for a hydrogen bond, the possibility of an interaction clearly exists. Two compounds were prepared to explore the importance of any such interaction (Table 4). Compound **25**, lacking the NH but isosteric with **13b**, showed a five-fold loss of potency against fIXa, suggesting the possibility of an interaction with serine 195. A two-fold loss against fXa was seen. The *N*-methyl analog **26**, interestingly, had about the same potency against fIXa as **25**, yet showed a very dramatic loss of potency against fXa. This suggests that the increased size of **26** does not further damage the binding with fIXa, but that fXa is much more intolerant of increased size in this region, possibly due to greater rigidity in the catalytic triad of the enzyme. Examination of published X-ray structures of the two enzymes⁷ shows that in fXa, the ‘60’s loop’ lies closer to serine 195 than in fIXa, possibly providing a buttressing effect, which could limit the ability of fXa to accommodate a larger inhibitor.

Published X-ray data for these enzymes also indicate that the ‘99 loop’, which forms part of the S4 binding pocket, is significantly altered in fIXa relative to fXa, due in large part to a two-residue insertion in fIXa.⁷ Hypothetical reorganization of this loop in fIXa following binding to cofactors and macromolecular substrates could be important in affecting the catalytic efficiency of the enzyme.^{20,21} We hypothesized that extending the S4 substituent deeper into this region of the enzyme might

enhance selectivity through a detrimental effect on fXa potency. This was found to be the case, as shown in Table 5. Adding a carbon atom between the indole and amide group, on either side of the benzyl substituent (**27**, **28**), caused a 2- to 4-fold decrease in fIXa activity, but a 10- to 20-fold decrease in fXa activity, leading to an improved selectivity ratio. Replacement of the aniline moiety of the amide with longer groups weakened fIXa activity further, but led to dramatic increases in selectivity, with two analogs (**31**, **33**) representing the first examples of compounds, which are equipotent to slightly more potent against fIXa than fXa.

In summary, a screening lead with half-micromolar fIXa potency but 1000-fold fXa selectivity was examined for structural variations, which would favor fIXa inhibition over that of fXa. An alpha-substituent favors fIXa more than Xa, with SAR suggesting beneficial effects from lipophilicity or a possible conformational biasing effect. Steric interference in the region of the catalytic triad is poorly tolerated by fXa, while fIXa seems to prefer the indole NH more than does fXa. Larger, more extended groups expected to bind in the S4 pocket of the enzymes also seem to differentiate between the two, possibly due to steric interference with a less flexible region of fXa. Thus, several analogs of compound **2** with greatly diminished fXa selectivity were prepared, and structural features favorable to fIXa potency were identified, setting the stage for further optimization of this series.²²

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