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Discovery of novel heterocyclic factor VIIa inhibitors

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Abstract—Structure–activity relationships and binding mode of novel heterocyclic factor VIIa inhibitors will be described. In these inhibitors, a highly basic 5-amidinoindole moiety has been successfully replaced with a less basic 5-aminopyrrolo[3,2-*b*]pyridine scaffold.

We have reported the development of direct, small-molecule inhibitors of the factor VIIa-tissue factor complex as novel anticoagulants.[1–4] These and other published factor VIIa (fVIIa) inhibitors[5] contain an amidino group (the P1 element) which interacts with Asp-189 (in the S1 pocket) via a salt-bridge. The strongly basic amidino group, while providing a binding anchor to the enzyme, is also the main limitation to oral bioavailability. Herein, we describe our efforts to replace the amidine functionality of our fVIIa inhibitors with a less basic P1 element. Structure–activity relationships (SARs) establishing in vitro potency for fVIIa and selectivity against factor Xa (fXa), thrombin, and trypsin are presented.

The potencies of our previously disclosed amidine-containing fVIIa inhibitors **1** and **2**[1] are shown in Table 1. As a first step toward generating orally bioavailable fVIIa inhibitors, we aimed to replace the amidino-aryl group with a less basic heterocycle, while maintaining critical interactions in the S1 pocket. Such a strategy has been previously demonstrated with fXa and thrombin inhibitors.[6] In order to assess the binding contribution from the amidine group on scaffold **1**, we generated the des-amidino benzimidazole **3**. Not surprisingly, removal of this group results in a >700-fold loss of potency. Knowing that we needed significant binding interactions between our inhibitor and the S1 pocket, we designed and generated a series of biaryl analogs that could potentially interact with the Asp-189 and other residues within the S1 pocket. From this work, the 5-aminopyrrolo[3,2-*b*]pyridine **5** proved to be quite promising. Compound **5** had an inhibition constant of 0.3 μM for fVIIa and possessed some selectivity over the related serine proteases (fXa, thrombin, and trypsin). Surprisingly, when the imidazopyridine analog **4** was generated, it had significantly less activity toward fVIIa than its analog **5**.

In order to gain insight into the binding contributions of the 5-aminopyrrolo[3,2-*b*]pyridine heterocycle in fVIIa, we obtained a crystal structure of **5** bound to fVIIa.[8] This structure was then overlaid with the fVIIa crystal structure of an amidine analog of **5** reported earlier,[4] as depicted in Figure 1. The key H-bonding contacts between inhibitor **5** and fVIIa are represented schematically in Figure 2.

The interactions of the indole N–H and the phenol with the catalytic residues (Ser195, His57) are similar to those observed for our amidine-based fVIIa inhibitors.[1] This similarity extends to the distal aryl ring of the molecule where the 3′-phenylnitro group occupies the fVIIa S1′ pocket. Major differences between the binding modes exist in two areas: the S2 region and the S1 pocket. The indole ring of Trp215 relocates into what would have been the S2 pocket. This indole now provides an edge-to-face contact with the phenolic ring of **5**. With amidine-containing inhibitors, Trp215 is not displaced

Keywords: Factor VIIa; Inhibitor; fVIIa; Heterocyclic; 5-aminopyrrolo[3,2-*b*]pyridine; Factor VIIa crystal structure; Anticoagulant.
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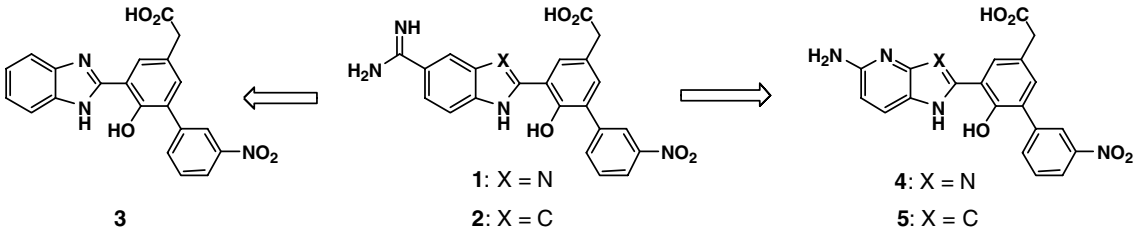
Table 1. SAR of amidine-containing compounds compared with non-amidino heterocyclic leads



Compound	K_i (μM)[7]			
	fVIIa	fXa	Thrombin	Trypsin
1	0.015	0.32	>150	11
2	0.004	0.05	2.9	0.40
3	11	>150	>150	>150
4	39	>150	>150	>150
5	0.30	3.5	44	120

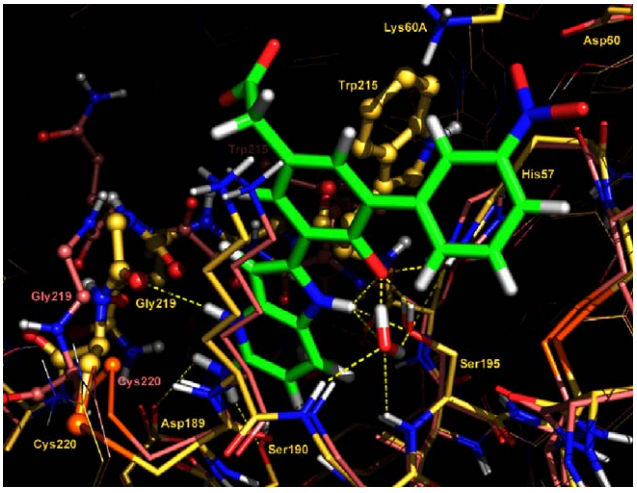


Figure 1. Crystal structure of **5** bound to fVIIa (protein shown in tan, H-bonds indicated by dotted lines). This is overlaid with a fVIIa crystal structure bound to an amidino analog (protein shown in orange, inhibitor not shown). The rearranged residues 215–220 and Trp215 are shown as ball and stick representations.

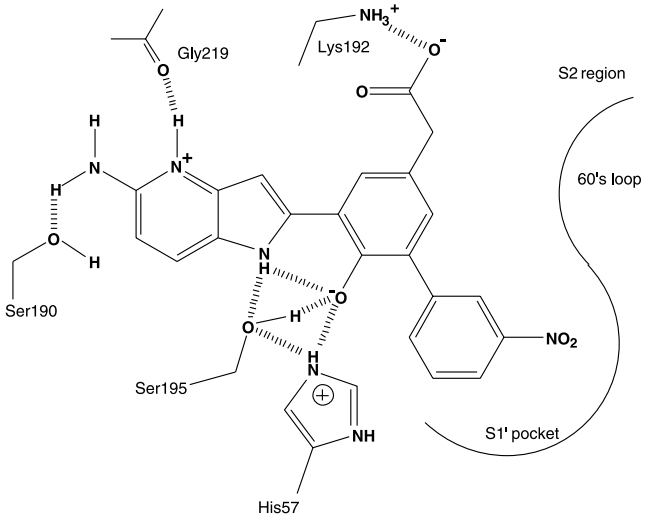
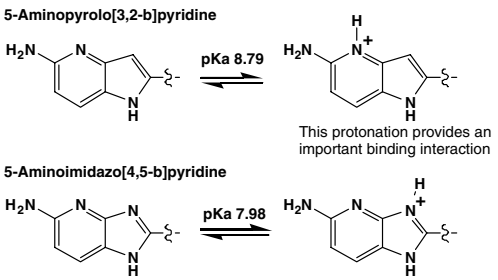


Figure 2. Representation of key interactions between **5** and fVIIa.

and an S2 pocket is clearly defined. The second difference was seen in the S1 pocket, where the 5-aminopyrrolo[3,2-*b*]pyridine interacts with the protein in a novel way. The residues from 215 to 220, which define one side of the S1 pocket, have significantly rearranged from the position observed for the amidine-based inhibitors. Some parts of this loop appear to be somewhat disordered. Whereas an amidine makes strong hydrogen bonds to Asp189, Ser190, and Gly219, the aminopyridine of **5** makes weak interactions with Gly219 and Ser190 as shown in Figure 2.

With this binding model in hand, we can now offer a rationale as to why inhibitor **4** was significantly less potent than **5**. We attribute this loss in potency to an unfavorable entropic contribution of the imidazole tautomers combined with a change in pK_a (Scheme 1). The calculated pK_a[9] for the pyridine nitrogen of a 5-aminopyrrolo[3,2-*b*]pyridine is 8.79, while a 5-amino imidazo[4,5-*b*]pyridine is predicted to protonate the second imidazole ring nitrogen at 7.98. This pyridine protonation allows for an important interaction of **5** with the rearranged Gly219. Amidino inhibitors **1** and **2** have similar potencies since the positive charge is localized at the amidine in both these compounds.

Our next goal was to improve the potency and selectivity profile of this novel 5-aminopyrrolo[3,2-*b*]pyridine



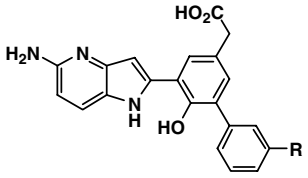
Scheme 1. Difference in pK_a and site of protonation of 5-aminopyrolo[3,2-*b*]pyridine and 5-aminoimidazo[4,5-*b*]pyridine P1 groups.

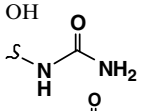
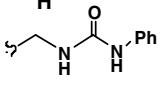
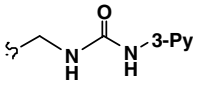
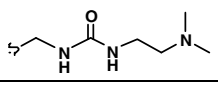
scaffold. We noted that the 60s loop, which forms the interface between the S2 and S1′ pockets of fVIIa, is significantly different from that of fXa and thrombin.[4] Building off the 3′-aryl group of **5** should extend into this region and might offer some selectivity gains for fVIIa. Initial attempts to improve binding by incorporation of 1- and 2-naphthyls as well as ortho- and para-substituted benzene moieties at the 3′ position resulted in reduced potency. A series of meta- substituted 3′-phenyl ring analogs were examined, several of which proved to be quite successful. Selected examples are shown in Table 2. Initially, we found that incorporation of small groups at the meta position such as a methylsulfone (as in **7**), hydroxyl (as in **8**), and a urea (as in **9**) did not offer any potency gains over the initial lead **5**. Next we chose to generate analog **10**, whereby the urea was extended out by one methylene and a hydrophobic aryl group was incorporated on the distal nitrogen of the urea. Analog **10** proved to have a 15-fold improvement in binding to fVIIa (K_i = 0.02 μM) as compared to **5**. Crystal structures of inhibitors in our amidino series show that a methyl urea can participate in a specific H-bond with the carbonyl of His57.[4] While His57 is conserved in all serine proteases, the His57 carbonyl in fVIIa appears to have a conformation that is ideal for this H-bond. In addition, it is the only enzyme in the coagulation cascade with a Lys60a that can hydrogen bond to the urea carbonyl and thereby improve this interaction. The terminal phenyl substitution on the urea may provide added thrombin selectivity due to negative steric interactions with the 60s loop of thrombin. Replacing the distal phenyl ring with a polar pyridine or dimethylamino group, as in **11** and **12**, respectively, results in loss of potency, as compared to **10**, perhaps due to a loss in hydrophobic interactions or an unfavorable entropy.

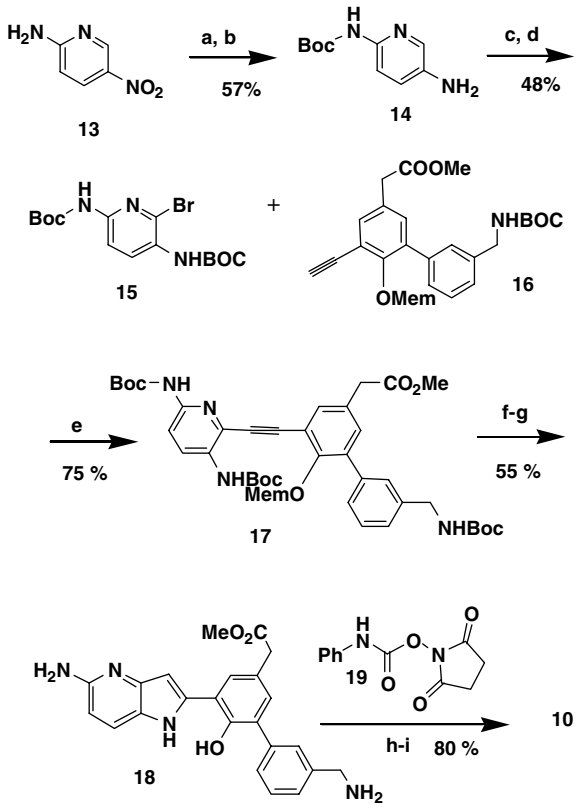
Scheme 2 describes a synthetic route to compound **10** which can be generalized to apply to other compounds described in Table 2. Protection of the commercially available 2-amino-5-nitropyridine (**13**) with BOC-anhydride followed by reduction of the nitro group under hydrogenation conditions gave **14**. Bromination of **14** using *N*-bromosuccinimide in DMF followed by subsequent treatment with BOC-anhydride affords **15** in high yield. Bromide **15** was then coupled with **16**[10] producing alkyne **17**. Alkyne **17** is cyclized with TBAF and subsequent removal of protecting groups affords **18**. Amine **18** is then treated with **19**, the succinimidyl ester of phenylcarbamic acid. The crude reaction mixture is then purified by reverse-phase chromatography to afford compound **10**.

We have described the synthesis and SAR of novel fVIIa inhibitors, wherein the amidinoindole group has been successfully replaced with a less basic 5-aminopyrolo[3,2-*b*]pyridine moiety. A comparison of the fVIIa crystal structures of these two classes of compounds reveals that although the S1 pocket alters its conformation to accommodate this change, the S1′ region of the protein remains virtually unchanged. We have therefore been

Table 2. SAR of 5-aminopyrrolo[3,2-*b*]pyridine analogs



Compound	R	Selectivity for fVIIa versus			
		fVII K_i (μM)	Xa	fIIa	Trypsin
5	NO_2	0.30	12	147	400
6	H	2.4	6	83	67
7	SO_2Me	7.4	2.5	13	16
8	OH	2.5	6	116	84
9		12	2.8	>13	>13
10		0.02	336	>7000	>7000
11		0.062	90	>2000	>7000
12		1.4	11	>100	>100



Scheme 2. Synthesis of **10**. Reagents and conditions: (a) NaHDMS, THF, Boc-anhydride, 0 °C to rt; (b) 10% Pd–C, THF, MeOH, H_2 (1 atm); (c) NBS, DMF 0 °C to rt; (d) BOC-anhydride, ^{t}BuOH; (e) $Pd(PPh_3)_2Cl_2$, triethylamine, CuI, acetonitrile, 80 °C; (f) TBAF, THF; (g) HCl (4 N in dioxane), MeOH $_{anhyd}$, (h) **19**, triethylamine, DMF; (i) NaOH (10% aq), MeOH, 50 °C.

able to adapt key elements of our amidino series of compounds to this new class of fVIIa inhibitors.

Acknowledgments

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7. Inhibition assays for factor Xa and thrombin were performed as described Cregar, L.; Elrod, K. C.; Putnam, D.; Moore, W. R. *Arch. Biochem. Biophys.* **1999**, *366*, 125, with the pH adjusted to 7.4. The trypsin and fVIIa assays were performed and analyzed as in the above reference with the following additional details. Factor VIIa (Enzyme Research) was incubated at 7 nM and CH_3SO_2-D-CHA-But-Arg-pNA (Centerchem) was used as the substrate. The buffer for the factor VIIa assay was supplemented with 11 nM relipidated tissue factor and 5 mM $CaCl_2$. Trypsin (Athens Research Institute) was incubated at 10 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 1.5 mM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with substrate, Tosyl-Gly-Pro-Lys-pNA (Centerchem), supplied at the K_m (25 μM).
8. PDB Entry Code 2F9B.
9. Calculations done using ACD Labs Product version 8.07.
10. Alkyne **16** was prepared from the corresponding aldehyde by its reaction with dimethyl-1-diazo-2-oxopropylphosphonate and K_2CO_3 in methanol. The 3-aryl-substituted aldehyde was prepared from previously reported [3-bromo-5-formyl-4-(2-methoxy-ethoxymethoxy)-phenyl]-acetic acid methyl ester[1] via a Suzuki reaction with BOC-protected aminomethyl 3-boronic acid.