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Novel 5-azaindole factor VIIa inhibitors

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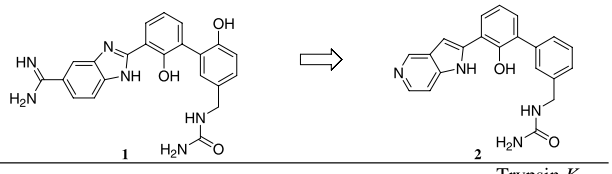
Abstract—The discovery and development of 5-azaindole factor VIIa inhibitors will be described.

In an effort to develop novel anti-thrombotic agents, we have been actively pursuing inhibition of the trypsin-like serine protease, factor VIIa (fVIIa). Although inhibition of related proteases, such as fIIa and fXa, has proven efficacious, inhibition earlier in the coagulation cascade, such as factor VIIa, may provide a safety advantage.[1–5] Our previous reports focus on the discovery and optimization of potent and selective amidine-containing P1 fVIIa inhibitors.[6,7]

Earlier, we reported on the development of compounds such as **1** as an injectable agent for the treatment of thromboembolic disorders (Fig. 1).[7] These biaryl amidine-containing compounds suffer from low oral bioavailability.[8] In an attempt to apply the knowledge we have gained from our amidine fVIIa inhibitor parenteral program to an oral program, we began with our 5-amidinobenzimidazole (**1**) scaffold and replaced the amidine with a 5-azaindole (1*H*-pyrrolo[3,2-*c*]pyridine, **2**) in conjunction with removal of the distal phenol to decrease the overall polar surface area. The amidine-containing analog has good potency for fVIIa (0.013 μM) and >200-fold selectivity versus the primary anti-targets fXa, fIIa (thrombin), and trypsin. The 5-azaindole retains respectable potency at 0.80 μM and >50-fold selectivity for the anti-targets. Overall, this non-amidino scaffold represented a promising lead with a less basic P1 and moderate potency and selectivity.

Toward our goal of a potent and selective fVIIa inhibitor, we sought fVIIa potency <0.100 μM with selectivity >250-fold against the three main anti-targets fXa, thrombin, and trypsin. To gain potency and selectivity in our 5-azaindole series, we began to optimize for the S1′ pocket. This region offers variety within the trypsin-like serine proteases affording the opportunity for selectivity and affinity.

To exploit the S1′ pocket for potency and selectivity, a number of analogs based on compound **2** were generated. Of these, 33 selected compounds are depicted in Table 1. Of the 33 compounds generated, a range of potencies (fVIIa K_i 0.028–130 μM) and selectivities versus the anti-targets (1–5000) were achieved including 24 compounds superior to the initial urea lead **2**.



Cpd	fVIIa K_i (μM)	fXa K_i (μM)	FIIa K_i (μM)	Trypsin K_i (μM)
1	0.013	2.7	90	3.6
2	0.80	125	71.5	46.5

Figure 1. Potency for fVIIa and selected anti-targets in an amidino and non-amidino scaffold.[9,10]

Keywords: Factor VIIa; Anti-coagulant; Serine protease; Amidine; Amidine replacement; Azaindole; Pyrrolopyridine.
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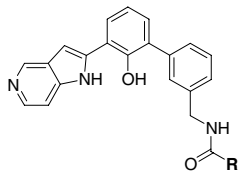
Table 1. Inhibition data for compounds **2**–**34**



Compound	R	fVIIa K_i (μM)	Xa	IIa	Trypsin
2	NH_2	0.80	156	89	58
3	Pentylamine	0.335	388	343	181
4	Phenethylamine	0.27	417	174	96
5	2-Thiophen-2-yl-ethylamine	0.35	429	169	120
6	Phenylamine	0.0615	1008	2114	2033
7	2-Fluoro-phenylamine	0.057	1930	2456	2632
8	3-Fluoro-phenylamine	0.13	923	1154	1154
9	4-Fluoro-phenylamine	0.11	382	1364	1364
10	2,6-Difluoro-phenylamine	0.033	2697	3939	2182
11	2,4-Difluoro-phenylamine	0.10	1700	1200	1500
12	3,4-Difluoro-phenylamine	0.33	455	455	455
13	3,5-Difluoro-phenylamine	0.83	181	181	181
14	2-Chloro-phenylamine	0.057	2632	2632	2632
15	2-Methoxy-phenylamine	0.047	1723	3191	3191
16	3-Methoxy-phenylamine	0.10	1600	1500	1500
17	4-Methoxy-phenylamine	0.088	705	1023	1591
18	2,4-Dimethoxy-phenylamine	0.028	2643	3179	5000
19	1-(3-Amino-phenyl)-ethanone	0.06	2167	2000	2167
20	1-(4-Amino-phenyl)-ethanone	0.19	789	305	495
21	4-Amino-benzoic acid	0.058	966	2241	1103
22	Thiophen-2-ylamine	0.16	600	938	538
23	Thiophen-3-ylamine	0.13	623	769	1154
24	Pyridin-2-ylamine	2.7	67	56	56
25	Pyridin-3-ylamine	0.155	635	968	935
26	Pyrrolidine	17	3	5	9
27	1-Methylpiperazine	130	1	1	1
28	Toluene	2.0	46	24	26
29	1,3-Difluoro-2-methyl- benzene	9.5	10	16	16
30	2-Phenyl-ethanol	0.22	136	470	57
31	2-Pyridin-3-yl-ethanol	0.61	149	107	34
32	Phenyl-methanol	0.85	80	68	57
33	Propyl-benzene	2.8	61	28	10
34	*N*-Benzyl-hydroxylamine	0.072	653	750	375

Data shown are factor VIIa K_i and fold-selective ratios (anti-target K_i/fVIIa K_i) for coagulation factors Xa, IIa (thrombin), and trypsin.[9,10]

Incorporation of an alkyl moiety on the urea as in **3** yielded a moderate increase in potency and selectivity versus **2**. Remarkably, addition of a phenyl group on the urea as in **6** offered a 10-fold increase in potency and a 6- to 35-fold increase in selectivity as compared to **2**. To further develop **6**, substituted phenylurea analogs (**7**–**21**) were produced. Interestingly, *ortho*-substitution seems to be well tolerated often increasing potency and selectivity, while *meta*- and *para*-substitution often leads to decreased potency and selectivity (e.g., compounds **7**–**9** and **15**–**17**). Following this trend, 2,6-substituted fluoro compound **10** showed a 2-fold increase in potency and selectivity over the phenyl analog **6**. Substitution of the terminal aryl ring with either electron-donating or electron-withdrawing groups was tolerated, suggesting that the advantage of substitution may be to disrupt the planarity between the urea and the phenyl group. Phenyl isosteres such as thiophenes and heterocycles such as pyridines were not well tolerated (compounds **22**–**25**). Finally, cyclic ureas such as compounds **26** and **27** lost potency for all the serine proteases tested, emphasizing the importance of the distal urea N–H.

To diversify away from ureas, a number of amides were completed based on the same base scaffold (Table 1). The amide analog **28** was found to be 30-fold less potent and >20-fold less selective than the homologous urea counterpart **6**. This trend continues as the 2,6-difluoromethyl amide **29** is far less potent and selective than the corresponding urea **10**. Substitution of the phenethyl chain with an α-hydroxyl group (**30**) via coupling of the amine with L-phenyllactic acid surprisingly retrieves some potency and selectivity. As might be predicted, the *N*-hydroxy urea

34 retains intermediate values of potency and selectivity between urea **6** and the phenyllactic amide **30** through a possible H-bonding interaction similar to the N–H of the urea.

In an attempt to understand the binding of this structural series, we obtained the crystal structure of compound **2** in fVIIa (Fig. 2). An array of hydrogen bonds to the catalytic residues are observed including the short interactions between Ser195, the hydroxypyrazole oxygen, and a water molecule in the oxyanion hole. The residues from 215 to 220 which define one side of the S1 pocket are significantly rearranged from the position observed for the amidine-based inhibitors such that the indole ring of Trp215 is relocated into the position of the S2 pocket. This ring now provides an edge to face contact with the phenol ring of the inhibitor. The P1 pyridine ring does not interact directly with the enzyme but is likely protonated and binds through a water bridge to Asp189. The substituent on the distal ring of **2** does not extend into the S1′ pocket. The distal phenyl ring on the urea likely binds in an edge to face interaction with the rearranged Trp215.

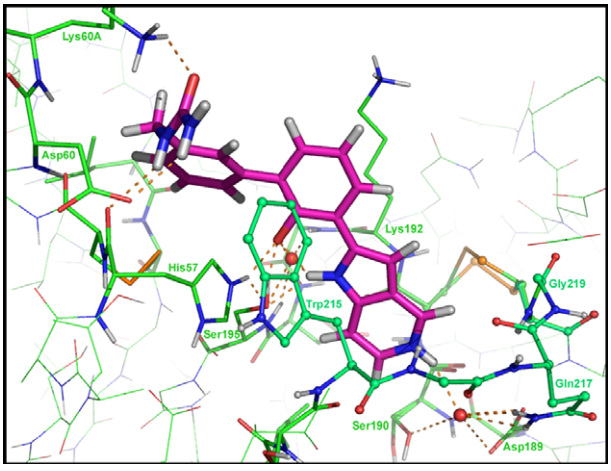


Figure 2. Crystal structure of **2** bound to fVIIa.[11]

Synthesis of the urea S1′ analogs began with the commercially available 2-bromophenol **35** (Scheme 1). Selective *ortho*-formylation using paraformaldehyde and $MgCl_2$ followed by benzyl protection provided salicylaldehyde **36**.[12] Palladium-catalyzed Suzuki coupling between bromide **36** and 3-cyanophenylboronic acid followed by conversion of the aldehyde to the alkyne via the diazophosphonate Ohira reagent gave intermediate **37**. Construction of the indole ring was achieved using a Sonogashira coupling of the alkyne **37** and mesylate **38**.[13] Reduction of the nitrile and benzyl ether of **39** was achieved via hydrogenation with Pearlman's catalyst followed by addition of phenylisocyanate to provide the target urea **6**. Urea compounds **3**–**25** were constructed following the above procedure. Amides **28**–**33** were completed using standard amide coupling procedures with the deprotected benzylamine of **39**.

The replacement of the amidino P1 group in trypsin-family serine protease inhibitors has been a major focus of research in the pharmaceutical industry. Herein, we have demonstrated that the 5-azaindole is a viable replacement for the 5-amidinobenzimidazole for fVIIa inhibition. Furthermore, the P1′-phenylurea **6** provides a substantial increase in potency and selectivity from our original azaindole lead **2**. A systematic SAR study on phenylurea **6** revealed that *ortho*-substitution was optimal with 2,6-difluoro compound **10** increasing potency 25-fold and selectivity over 15-fold for all anti-targets compared to the initial lead **2**. Crystallographic data support the potency achieved by the 5-azaindole urea **2** and phenylurea **6** through a combination of hydrogen-bonding effects and phenyl edge to face interactions. These potent and selective non-amidino fVIIa inhibitors are undergoing pharmacokinetic evaluation and will be presented in a future publication.

Acknowledgments

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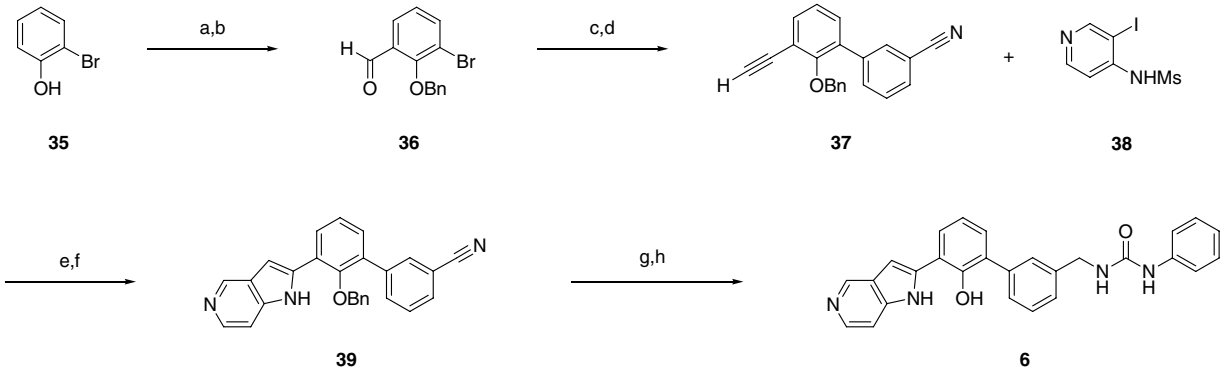

Scheme 1. Synthesis of **6**. Reagents and conditions: (a) $MgCl_2$, TEA, paraformaldehyde, 95%; (b) BnBr, K_2CO_3, 95%; (c) 3-benzonitrile boronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME, reflux, 80%; (d) (1-diazo-2-oxo-propyl)-phosphonic acid dimethyl ester (Ohira reagent), K_2CO_3, MeOH, 80%; (e) $PdCl_2(PPh_3)_2$, TEA, CuI; (f) NaOH, MeOH, reflux, 60% over 2 steps; (g) $Pd(OH)_2$, H_2, MeOH, trace of HCl, 95%; (h) phenylisocyanate, TEA, DMF, 30–50%.

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9. General inhibition assays inhibitor potency measurements were performed at room temperature using either a Molecular Device's SpectraMax 250 (absorbance assays) or fMax (fluorescence assays) 96-well kinetic plate readers. Reaction velocities were monitored at varying inhibitor concentrations by following the hydrolysis of either *p*-nitroanalide (A_{405nm}) or aminomethylcoumarin substrates (ex_{355}, em_{460}). All substrates were added at concentrations equal to or near their K_m. All reactions were performed in a total volume of 100 μLs. Control reactions in the absence of inhibitor were performed in parallel. Factor VIIa: factor VII (Enzyme Research) was incubated at 7 nM together with recombinant soluble human tissue factor (11 nM) with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 5.0 mM $CaCl_2$, 1.5 mM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with the addition of substrate, CH_3SO_2-D-CHA-But-Arg-pNA (Centerchem), supplied at the K_m (500 μM). The change in absorbance as a function of time was monitored at 405 nm. Factor IIa (thrombin): thrombin (Calbiochem) was incubated at 12.7 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 5.0 mM $CaCl_2$, 1 mM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with the addition of substrate, Tosyl-Gly-Pro-Lys-pNA (Centerchem), supplied at the K_m (25 μM). The change in absorbance as a function of time was monitored at 405 nm. Factor Xa: factor Xa (Haematologic Technologies) was incubated at 2 nM with variable concentrations of inhibitor in 50 mM Tris (pH 7.4), 150 mM NaCl, 5.0 mM $CaCl_2$, 1.5 mM EDTA, 0.05% Tween 20, and 10% DMSO. The reaction was initiated with the addition of substrate, CH_3OCO-D-Cha-Gly-Arg-pNA (Centerchem), supplied at the K_m (1.0 mM). The change in absorbance as a function of time was monitored at 405 nm. Trypsin: 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). The change in absorbance as a function of time was monitored at 405 nm.
10. The K_i apparent (K_i') values were determined by a non-linear least-squares regression fit of the experimentally derived data to the Morrison equation for tight-binding inhibitors as described Kuzmic, P. et al. *Anal. Biochem.* **2000**, *281*, 62, Enzyme and inhibitor were incubated 30 min prior to initiation of reaction with the addition of substrate.
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