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The discovery of fluoropyridine-based inhibitors of the Factor VIIa/TF complex

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Abstract—The activated Factor VII/tissue factor complex (FVIIa/TF) plays a key role in the formation of blood clots. Inhibition of this complex may lead to new antithrombotic drugs. An X-ray crystal structure of a fluoropyridine-based FVIIa/TF inhibitor bound in the active site of the enzyme complex suggested that incorporation of substitution at the 5-position of the hydroxybenzoic acid side chain could lead to the formation of more potent inhibitors through interactions with the S1′/S2′ pocket.

The activated Factor VII/tissue factor complex (FVIIa/TF) is known to play a key role in the formation of blood clots.[1] Theoretically, inhibition of either the FVIIa/TF complex formation or its active site responsible for the cleavage of Factor X (FX) to activated Factor X (FXa) will block the key extrinsic coagulation pathway, but keeping intact the intrinsic pathway to sustain normal hemostasis. This may lead to enhanced antithrombotic drugs with decreased bleeding side effects.[2] There are several strategies for disruption of the FVIIa/TF complex formation and function,[3] but work still seems to be incomplete with regard to the development of small molecule, FVIIa/TF active-site inhibitors.[4] In this communication, we describe the exploration of the structure/activity relationships of a series of fluoropyridine-derived, small molecule inhibitors that appeared to utilize the S1′/S2′ binding pocket of the FVIIa/TF active site.

From a focused screening of compounds previously synthesized as potential FXa inhibitors,[5] we identified **1** (IC_{50} = 1200 nM) as a modest FVIIa/TF inhibitor with no measurable inhibition of FXa (IC_{50} > 10 μM, Fig. 1). Synthesis of a subsequent analog substituted at the 5-position of the hydroxybenzoic acid side chain of **1** led to a more potent 5-dimethylamino **2** (IC_{50} = 190 nM). This result suggested that substitution at the 5-position could be optimized further to enhance the binding affinity of a new class of FVIIa/TF inhibitors. Also in this series, a limited optimization of the 4-position of the central pyridine ring led to the potent FVIIa/TF inhibitor **3** (IC_{50} = 86 nM).

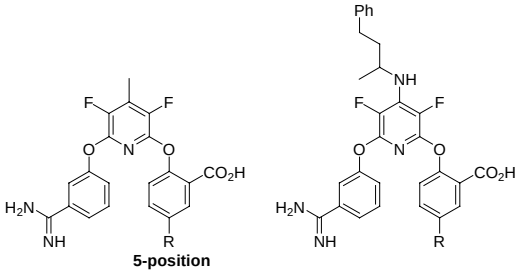

Figure 1. Lead inhibitors of FVIIa/TF.

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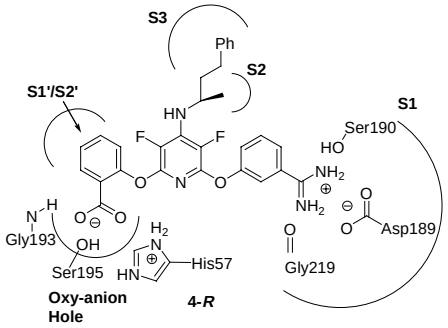
Analysis of the X-ray crystallography data of a complex of **3** (R = H) with TF/FVIIa showed that the *R*-methyl enantiomer **4-*R*** (coordinates deposited in the RCSB database as PDB ID code 2AEI) crystallized with the phenylamidine pharmacophore hydrogen bonding to the Asp189, Ser190, and Gly219 in the S1 pocket of the FVIIa active site, while the benzoic acid moiety interacted with Ser195, Gly193, and His57 in the oxy-anion hole (Fig. 2). The 1-methyl-3-phenyl-propylamine side chain was shown to be filling the S2 and S3 binding pockets through hydrophobic interactions with the (*R*)-methyl and phenyl groups, respectively.

The crystal structure also revealed a potential binding pocket close to the carboxylate group bound in the oxy-anion hole. This area of the active site is made up of both the S1′ and S2′ pockets (Fig. 3). Therefore, analogs that could access this S1′/S2′ pocket via incorporation of a substituent at the 5-position on the benzoic acid side chain should result in more potent FVIIa/TF inhibitors.

The synthesis of these analogs started with the 5-substituted salicylates **6** and **8** (Scheme 1). Formation of 5-alkyl substituted **6** resulted from heating a carbon tetrachloride solution of 4-alkyl-phenol **5** (R^1 = alkyl group) in the presence of copper and base, followed by acid-catalyzed esterification.[6] 5-*N*,*N*-Dialkylamino substituted **8a** was formed by reductive amination of



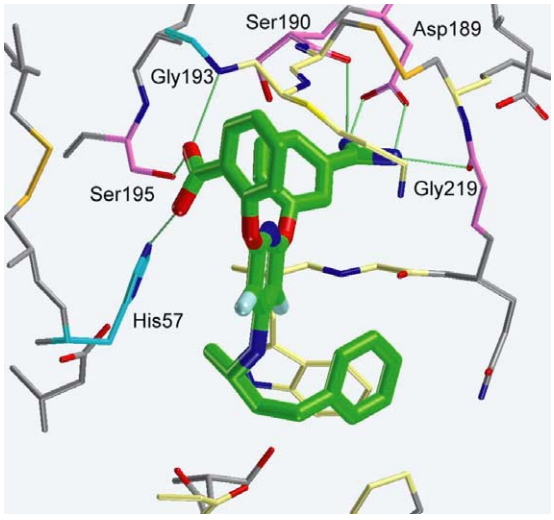


Figure 2. Crystal structure of **4-*R*** in the active site of the FVIIa/TF complex.

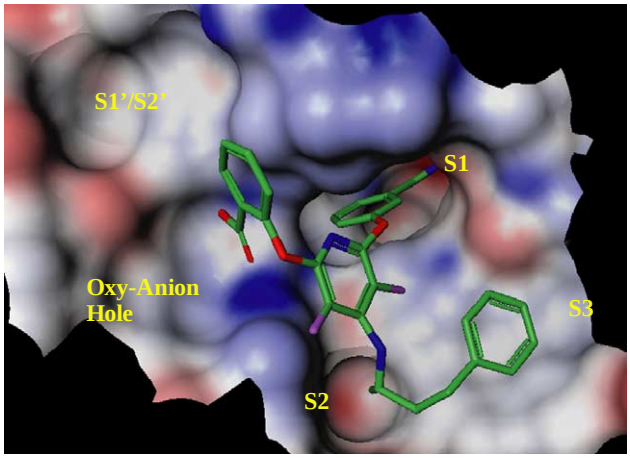
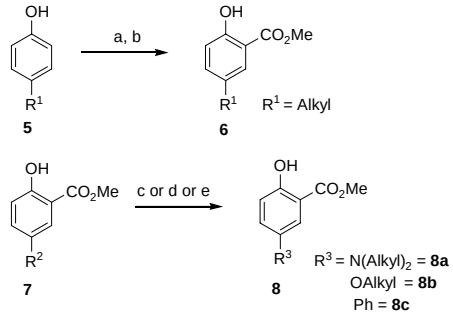


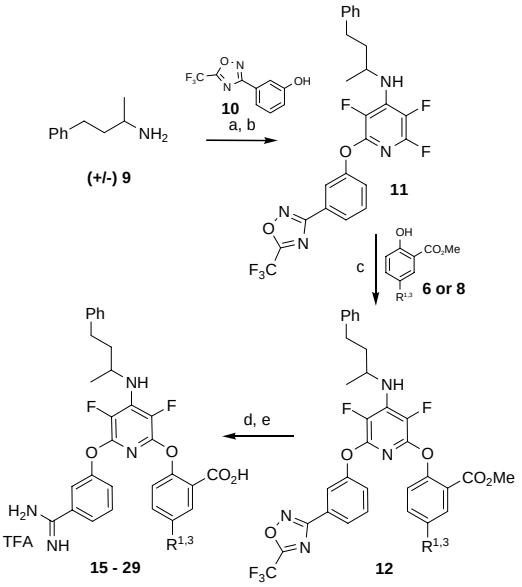
Figure 3. Connolly surface depicting the binding pockets of the FVIIa/TF active site with respect to **4-*R***. The enzyme is colored by residue electrostatic charge: red, negatively charged residue; blue, positively charged residue; white, residue of negligible charge.



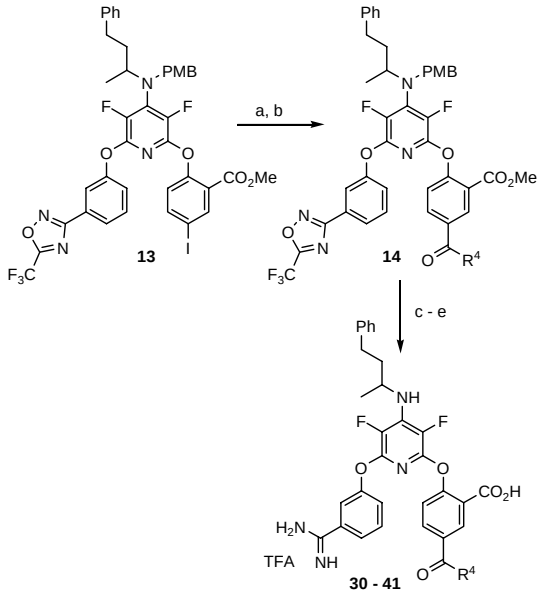
Scheme 1. Reagents and conditions: (a) R^1 = alkyl group; CCl_4, Cu, KOH, H_2O, reflux; (b) H_2SO_4, MeOH, reflux; (c) $R^2 = NH_2$; R^3CHO, HOAc, $NaBH(OAc)_3$, DCE, RT; (d) R^2 = OH; Alkyl-I, K_2CO_3, acetone, reflux; (e) R^2 = I; $PhB(OH)_2$, K_3PO_4, $Pd(PPh_3)_4$, DMF, 110 °C.

an alkylaldehyde, and **7** ($R^2 = NH_2$), while 5-alkoxy substituted **8b** was synthesized by heating **7** (R^2 = OH), an alkylhalide, and dimethylformamide in the presence of sodium hydride.[7] Finally, formation of the 5-phenyl analog of **8c** was dependent on the palladium-catalyzed Suzuki coupling of **7** (R^2 = I) and phenylboronic acid.[8]

(±)-1-Methyl-3-phenyl-propylamine **9** was added to pentafluoropyridine in the presence of triethylamine to provide a 4-amino-substituted pyridine (Scheme 2). Addition of the salicylate side chains was effected in a two-step procedure by first adding the trifluoromethyloxadiazole-phenol **10**[4c] in the presence of potassium carbonate to give the aryl ether **11**. Subsequent addition of a 5-substituted salicylate **6** or **8** in the presence of cesium carbonate produced the trisubstituted pyridine **12**. Hydrogenation of the oxadiazole over Raney nickel revealed the amidine.[4c] Final hydrolysis of the methyl ester with lithium hydroxide produced analogs **15**–**29**. All inhibitors were isolated by reverse-phase preparative HPLC as TFA salts.



Scheme 2. Reagents and conditions: (a) pentafluoropyridine, Et_3N, CH_2Cl_2, RT; (b) **10**, K_2CO_3, DMSO, 70 °C; (c) **6** or **8**, Cs_2CO_3, DMSO, 80 °C; (d) Raney nickel, H_2, Et_3N, MeOH, RT; (e) LiOH, water, dioxane, RT.



Scheme 3. Reagents and conditions: (a) CO, $Pd(OAc)_2$, K_2CO_3, DMF/H_2O (4:1), 100 °C; (b) amine, HOAt, HATU, Et_3N, DMF, RT; (c) H_2, Raney nickel, MeOH, Et_3N, RT; (d) LiOH, MeCN, H_2O, RT; (e) TFA, CH_2Cl_2, RT.

Synthesis of the isophthalamic acid derivatives **30**–**41** was achieved via a Pd(0)-catalyzed carboxylation of 5-iodo **13** to yield carboxylic acid **14** (R^4 = OH).[9] Using *p*-methoxybenzyl (PMB) protected **9**, formed via reductive amination with 4-methoxy-benzaldehyde and sodium triacetoxyborohydride, the 5-iodo **13** was prepared in a similar manner to **12**. The use of this protecting group was necessary to obtain good yield in the subsequent carboxylation step. The amides were formed with amines via a HOAt/HATU mediated coupling reaction.[4c] Raney nickel reduction of the oxadiazole to the amidine and LiOH hydrolysis of the ester were followed by TFA-mediated deprotection of the PMB protecting group to reveal the analogs **30**–**41** (Scheme 3).

To enhance the binding affinity of this series of inhibitors, investigation of analogs with substitution at the 5-position of the hydroxybenzoic acid side chain was undertaken (Table 1). Substitution of the 5-hydrogen with a 5-methyl group on the hydroxybenzoic acid ring resulted in **16** with a 4-fold increase in binding (IC_{50} = 20 nM) from our lead **3**. Compared to **16**, addition of a larger *tert*-butyl group **17** (21 nM), phenyl group **29** (23 nM), or isopropyl group **15** (15 nM) did not substantially increase the binding. Unsubstituted hydrogen-bond-donating groups, such as the 5-amino analog **18** and 5-hydroxy analog **23**, decreased the binding from **16** by 3.5- and 2.5-fold respectively, but alkylation of the respective nitrogen or oxygen atom regained potency. For example, mono- or dialkylation of 5-amino **18** produced analogs **19**–**22** with an increased potency of approximately 2- to 5-fold over **18**. Similarly, alkylation of 5-hydroxy **23** produced alkoxy analogs **24**–**27** with increased potency. Finally, incorporation of a 4-substituent on the hydroxybenzoic acid ring did not increase the binding. For example, analog **28** (15 nM) substituted by a 4-methyl substituent showed potency similar to the 4-H analog **19** (13 nM). In all cases, these inhibitors maintained selectivity for the inhibition of FVIIa/TF over that of FXa.

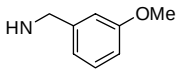
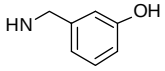
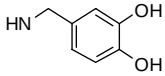
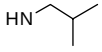
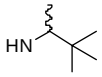
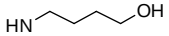
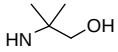
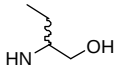
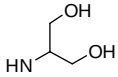
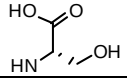
Table 1. 5-Substituted FVIIa/TF inhibitors[a]

FVIIa/TF inhibitors	$R^{1,3}$	FVIIa/TF IC_{50}[11] (μM)	2× PT (μM)	FXa IC_{50}[12] (μM)
3	H	0.086	35	55
4-*R*	H	0.045	29	51
15	iPr	0.015	28	4.8
16	CH_3	0.020	ND	29
17	*t*-Bu	0.021	24	3.3
18	NH_2	0.079	ND	11
19	NMe_2	0.013	49	6.8
20	NEt_2	0.016	83	9.6
21	NHMe	0.017	41	8.0
22	NBu_2	0.036	ND	ND
23	OH	0.056	ND	11
24	OiPr	0.017	52	12
25	OPr	0.019	95	26
26	OEt	0.029	94	17
27	OMe	0.039	ND	11
28	NMe_2 4-Me	0.015	16	4.5
29	Ph	0.023	112	17

[a] All compounds tested as racemic mixtures except where noted.

Molecular modeling did not provide clues as to additional interactions that led to higher binding affinities observed for the 5-substituted inhibitors, but it did indicate that the S1′/S2′ pocket was potentially a deeper binding site than was assumed initially. Additional attempts to fully exploit this binding pocket to gain more potent FVIIa/TF inhibitors were explored through the synthesis of analogs containing an amide at the 5-position of the hydroxybenzoic acid side chain (Table 2).[4c,10]

Table 2. 5-Benzamide FVIIa/TF inhibitors

FVIIa/TF inhibitors	R^4	FVIIa/TF IC_{50} (μM)	2× PT (μM)	FXa IC_{50} (μM)
30		0.008	53	9.5
31		0.039	49	4.1
32		0.140	ND	ND
33		0.010	22	9.6
34		0.014	15	4.3
35		0.067	16	20
36		0.071	12	ND
37		0.016	11	5.9
38		0.023	10	5.7
39		0.046	9.0	13
40	OH	0.200	ND	ND
41		0.730	ND	ND

A substantial increase in FVIIa/TF binding affinity was obtained by the formation of 3-methoxybenzyl-amide **30** (8 nM). All other substituted benzyl-amides showed decreased binding affinity from **30**. Interestingly, the isobutyl-amide **33** (10 nM) was of similar potency to analog **30**, while the trimethylpropyl-amide **34** (14 nM) showed only a small loss in activity. Reduced binding was also observed in the hydroxyalkyl-amides **35** and **36**, but the alkyl-hydroxyalkyl-amides **37**–**39** helped to regain some binding activity. The serine-based amide **41** (730 nM) was an exception displaying significantly reduced activity potentially as a result of the presence of the free carboxylic acid. Likewise, the incorporation of a carboxylic acid **40** (200 nM) directly at the 5-position also significantly decreased binding.

Testing of these inhibitors in the in vitro assay for prolonging prothrombin time (PT), which is a measure of the anticoagulant effect of a drug on the extrinsic coagulation pathway, showed that the parent 5-H analog **3** had a doubling of PT (2× PT) at 34 μM (Table 1).[13] Inhibitors 5-*O*-isopropyl **24** (52 μM), 5-isopropyl **15** (28 μM), and 5-*N*,*N*-dimethylaniline **19** (49 μM) all had 2× PT values similar to or higher than that of the parent **3**, despite their higher binding affinities. Only the 4-methyl substituted **28** (16 μM) had a significant lowering of the 2× PT value from the parent **3**.

While it appeared that the amide series of inhibitors favored branched alkyl and benzyl-amides for a modest increase in binding affinity, a more significant relationship was apparent in regard to the lowering of 2× PT potency (Table 2). Branched alkyl-amides had a superior doubling of PT than the benzyl-amides. While both the isobutyl amide **33** and 3-methoxybenzyl-amide **30** had similar binding affinities, the former analog was 2.5-fold more potent for the doubling of PT. Another 2-fold improvement in 2× PT potency was gained in the alkyl-hydroxyalkyl-amide series. For example, weaker binding inhibitors, such as diol **39** (IC_{50} = 46 nM), offered a better 2× PT profile (9.0 μM) than the stronger binding 3-methoxybenzyl-amide **30** (IC_{50} = 8 nM, 2× PT = 53 μM). While we are uncertain as to the reasons for the variations in the observed 2× PT data, it has been suggested that the changes in pharmacological parameters (c log *P*, solubility, permeability, protein binding, etc.) resulting from the hydroxyl groups are accounting for some of the differences.[14]

Examination of 5-substituted hydroxybenzoic acid side chains of a fluoropyridine-based series of FVIIa/TF inhibitors showed a modest improvement in binding affinity for this enzyme complex. Enhancement of the interactions needed for increased binding affinity that can lead to inhibitors that also display a more potent 2× PT profile has not yet been fully optimized. Further investigations into the synthesis of analogs that continue to probe the S1′/S2′ pocket are in progress.

Acknowledgment

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