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Potent 4-amino-5-azaindole factor VIIa inhibitors

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Abstract—The 4-amino-5-azaindole as an amidino-benzimidazole replacement is described. A series of potent and selective analogs were discovered and showed desirable *ex vivo* efficacy as measured by PT.

The development of novel anticoagulants with improved therapeutic indices is an attractive goal in the pharmaceutical industry. The activated factor VIIa-tissue factor complex (fVIIa·TF), which exists in the extrinsic pathway of the coagulation cascade, has been an appealing molecular target for the treatment of various thrombosis related disorders.[1–4]

We have previously reported on the discovery and development of small molecule fVIIa·TF complex inhibitors.[5–7] Many of these described inhibitors contain an amidino moiety, which forms a salt bridge with Asp 189 in the S1 pocket of fVIIa providing increased bonding and potency. The amidino compound **1** (Fig. 1) has a 0.013 μM inhibition K_i for fVIIa·TF and good selectivity (>200-fold) versus primary antitargets fXa, thrombin, and trypsin. The strongly basic amidino group, however, is considered to be a major limitation to oral bioavailability. We have studied the oral bioavailability of various amidine and amidine prodrugs in our biaryl scaffold.[8] These compounds suffer from low oral bioavailability due to low absorption or low conversion of prodrug to parent amidine. In an effort to replace the 5-amidino-benzimidazole, we explored less basic P1 elements including 5-azaindoles[9,10] and 4-chloro-5-azaindoles[10] as well as others.[9] The 5-azaindole compound **2** (Fig. 1) has a potency of 0.22 μM for fVIIa and >10-fold selectivity against the antitargets. Most recently, we identified the 4-amino-5-azaindole (1*H*-pyrrolo[3,2-*c*]pyridin-4-ylamine) moiety as another viable amidine replacement. Compound **3**, which was made as a direct comparison of **2**, has further improved potency (fVIIa $K_i = 0.081$ μM) and selectivity (>400-fold against antitargets). The 4-amino-5-azaindole analogs retained desirable potency[10] and *ex vivo* efficacy in human plasma as monitored by the coagulation assays PT (prothrombin time) and aPTT (activated partial thromboplastin time).[11] In this communication, we disclose our discovery of analogs with good *ex vivo* efficacy based on the 4-amino-5-azaindole.

We have discovered in our 5-amidino-benzimidazole analogs that substituents bearing an acid moiety at the C5′ position of the central aryl ring improved potency against fVIIa by interacting with Lys 192 of the enzyme.[5] Starting from **3**, we installed various acid substitutions at the C5′ position in order to achieve more desirable potency (compounds **4** and **5**, Table 1). The C5′ benzoic acid **4** gave the best potency against fVIIa, as well as *ex vivo* efficacy, as measured by the fVIIa·TF dependent clotting assay PT. However, compound **4** was also quite active in the fVIIa·TF independent clotting assay aPTT, possibly by inhibiting proteases along the intrinsic coagulation pathway. Compound **5**, although less potent compared to **4**, has moderately differentiated the PT and aPTT. To target more specifically the fVIIa·TF complex, which is associated with the extrinsic and common coagulation pathway, we choose the potent compound **5** as a starting point to further optimize our analogs for an improved PT profile.

Keywords: Factor VIIa; Tissue factor; Anticoagulant; Serine protease; Amidine; Amidine replacement; PT; aPTT; 4-amino-5-azaindole; 1*H*-pyrrolo[3,2-*c*]pyridin-4-ylamine.
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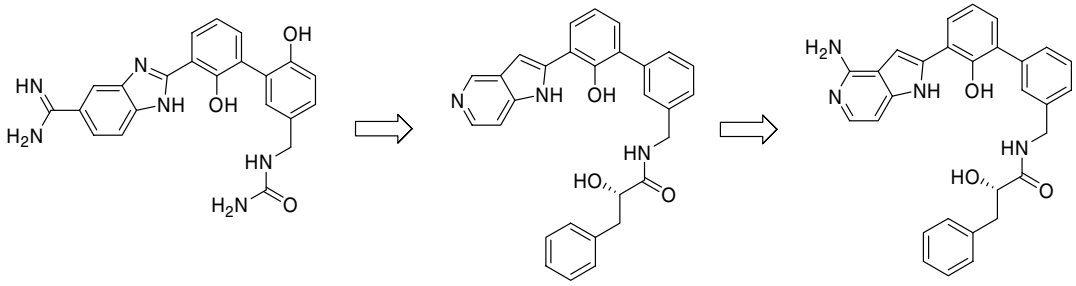


1 **2** **3**

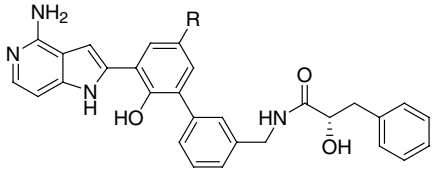
Cmpd	fVIIa·TF K_i(M)	fXa K_i(M)	Thrombin K_i(M)	Trypsin K_i(M)
1	0.013	2.7	90	3.6
2	0.22	30	1.0×10^2	12
3	0.081	54	99	38

Figure 1. Potency for fVIIa·TF and selected antitargets in amidino and non-amidino scaffolds.

In our effort to improve the potency and selectivity of the 5-azaindole series, we had identified substituted phenylureas as the optimal S1′ substitution.[9] In the 5-azaindole series, the *para*-benzoic acid-substituted phenylurea gave desirable potency and decreased lipophilicity, which is believed to lower binding to serum albumin and therefore improve the PT. The same SAR transfers to the current series where the corresponding compound **6** (Table 2) retained potency against fVIIa and *ex vivo* efficacy with a greater differentiation between PT and aPTT. The 2,6-difluorophenylurea moiety was also found to provide further improvement to fVIIa potency and selectivity in 5-azaindole series.[9] Accordingly, compound **7** showed an increase in potency against fVIIa by ~10-fold as well as selectivity against other trypsin family enzymes. The PT profile was further improved to 2.4 μM, while aPTT was greater than 20 μM. This 4-amino-5-azaindole series marks an improvement in the PT and the differentiation between the intrinsic and extrinsic coagulation pathways when compared to the 5-azaindole series.

Synthesis of analog **7** is outlined in Scheme 1). Methylation of commercially available 4-methoxyphenylacetonitrile **8** followed by hydrolysis of the nitrile, deprotection of the methyl ether, and finally esterification provided ester **9**. Compound **9** was selectively *ortho*-formylated with paraformaldehyde and $MgCl_2$.[13] The resulting salicylaldehyde was brominated with *N*-bromosuccinamide in DMF, followed by benzyl protection of the phenol to afford ether **10**. Suzuki coupling between bromide **10** and the boronic acid **11** led to biaryl compound **12**. Subsequent treatment of aldehyde **12** with the diazophosphonate Ohira reagent[14,15] generated alkyne **13**. The 4-chloro-5-azaindole ring was established via a Sonagashira reaction between **13** and mesylate **14**[16] to form a transient biaryl alkyne, which cyclized spontaneously to form the *N*-mesyl indole. The indole was subsequently treated with NaOH/MeOH to induce cleavage of the sulfonamide and hydrolysis of the ester providing compound **15**. The chloro-substituted indole **15** was treated with NH_4OAc in melting phenol to displace the 4-chloro group providing the 4-amino-5-azaindole. The boc and benzyl protecting groups were removed upon treatment with 6 N HCl(aq) followed by hydrogenation with Pearlman's catalyst to afford **16**. The amine **16** was treated with 2,6-difluoroisocyanate **17** under basic conditions followed by purification on reverse phase HPLC provided **7** as a mono HCl salt.

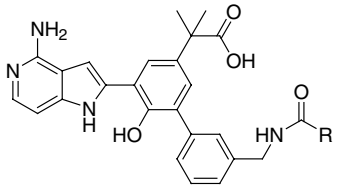
Table 1. SAR at the C5′-position of central aryl ring[12]

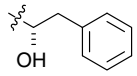
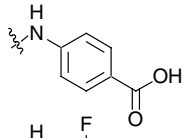
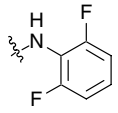


Compound	R	fVIIa·TF K_i (μM)	2× PT (μM)	2× aPTT (μM)	Selectivity for fVIIa versus		
					Thrombin[a]	fXa[b]	Trypsin[c]
3	H	0.081	12	22	1.2×10^3	6.7×10^2	4.7×10^2
4	COOH	0.0013	4.5	3.7	4.0×10^4	5.0×10^3	3.5×10^3
5	$C(CH_3)_2CO_2H$	0.015	6.4	17	$>1.0 \times 10^4$	2.8×10^3	3.2×10^3

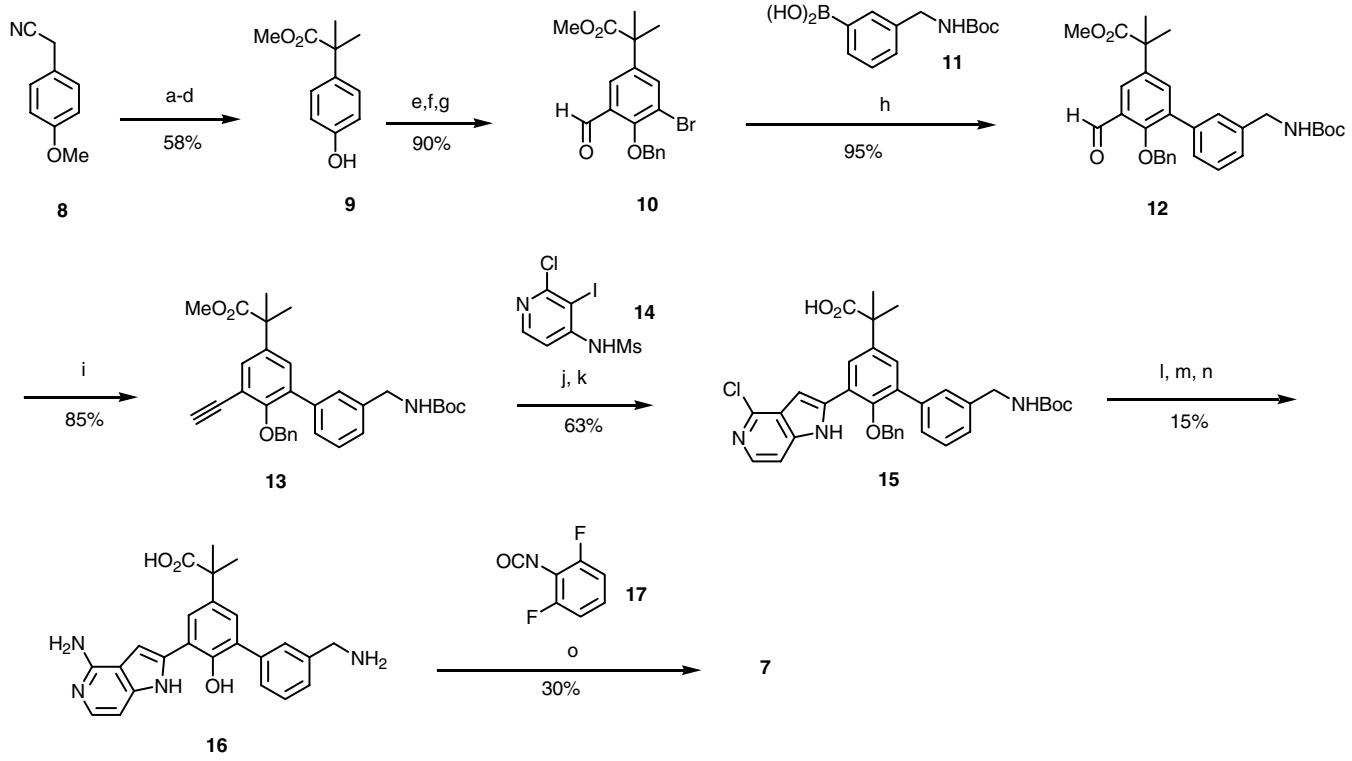
[a] Thrombin K_i (μM)/fVIIa·TF K_i (μM) = fold selectivity.
[b] fXa K_i (μM)/fVIIa·TF K_i (μM) = fold selectivity.
[c] Trypsin K_i (μM)/fVIIa·TF K_i (μM) = fold selectivity.

Table 2. SAR on distal aryl ring[12]



Compound	R	fVIIa·TF K_i (μM)	2× PT (μM)	2× aPTT (μM)	Selectivity for fVIIa versus		
					Thrombin	fXa	Trypsin
5		0.015	6.4	17	$>1.0\times10^4$	2.8×10^3	3.2×10^3
6		0.020	5.8	>20	$>7.5\times10^3$	5.5×10^3	5.5×10^3
7		0.0026	2.4	>20	$>5.8\times10^4$	2.8×10^4	2.9×10^4

In conclusion, we have identified potent and selective fVIIa·TF inhibitors in a novel non-amidino scaffold which differentiates between the intrinsic and extrinsic coagulation pathways. This series was dropped in lieu of pursuing a more attractive series and absorption data were never obtained. We would like to suggest that from a potency standpoint, the 4-amino-5-azaindole is a suitable surrogate for the benzamidine and that others con-



Scheme 1. Reagents and conditions: (a) *t*-BuOK, MeI, THF, rt; (b) KOH, ethylene glycol/water (5:1), 150 °C; (c) Py·HCl, 180 °C; (d) $SOCl_2$, MeOH; (e) $MgCl_2$, anhydrous paraformaldehyde, Et_3N, CH_3CN, reflux; (f) NBS, DMF; (g) BnBr, DIEA, CH_3CN; (h) **11**, $Pd(PPh_3)_4$, K_2CO_3, DME, reflux; (i) Ohira's reagent, K_2CO_3, MeOH; (j) **14**, $PdCl_2(PPh_3)_2$, CuI, Et_3N, CH_3CN, 80 °C; (k) 50% NaOH, MeOH, 60 °C; (l) NH_4OAc, PhOH, 105 °C; (m) 6 N HCl, reflux; (n) 20% $Pd(OH)_2$ on carbon (Pearlman's catalyst)/H_2, EtOH; (o) **17**, Et_3N, DMF.

sider such a moiety in their drug development programs, especially in the challenging arena of developing fVIIa inhibitors.

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10. The 5-azaindole, which has a calculated pK_a ~7.8 (ACD Labs/pKa DB), is thought to be partially protonated in the assays (pH 7.4) and forms water bridged hydrogen bonds with Asp 189 of the enzyme to afford desired nanomolar potency against fVIIa. The 4-chloro-5-azaindole P1, which has a calculated pK_a ~5.7, is not protonated and therefore has little interaction with Asp 189, which is consistent with the loss of potency against fVIIa (micro-molar K_i for fVIIa.TF). The 4-amino-5-azaindole P1, which has a calculated pK_a ~9.9, is mostly protonated and forms a stronger interaction with Asp 189 through water bridged hydrogen bonds. In addition, the 4-amino group is also thought to interact with G219 of the enzyme. Both interactions are considered to contribute to the improved potency.
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12. 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). Coagulation assays (PT and aPTT) were carried out at 37 °C in human plasma using a Beckman Coulter ACL100 in accordance with the manufacturer's instructions.
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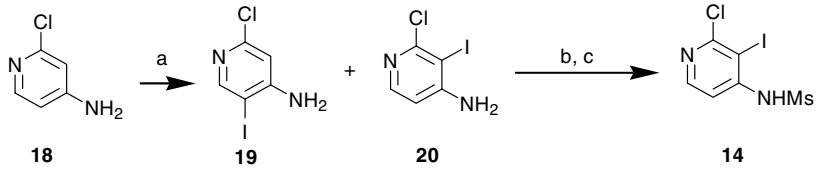
16. The synthesis of intermediate **14** is shown in Scheme 2. Commercially available 4-amino-2-chloropyridine **18** was iodinated to afford a mixture of iodopyridines **19** and **20** (~6:4). Compound **20** can be easily isolated by chromatography on silica. Treatment of **20** with methanesulfonyl chloride led to a mixture of monomesylate **14** and bis-*N*-mesylated byproduct. Subsequent treatment with NaOH(aq)/THF converted the bismesylate to monomesylate **14**.



Scheme 2. Reagents and conditions: (a) ICl, KOAc/HOAc, 60–70 °C; (b) MsCl, Et_3N, DCM, 0 °C; (c) 10% NaOH, THF.