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Design, synthesis, and biological evaluation of pyrazinones containing novel P1 needles as inhibitors of TF/VIIa

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Abstract—Herein is described the design, synthesis, and enzymatic activity of a series of substituted pyrazinones as inhibitors of the TF/VIIa complex. These inhibitors were designed to explore replacement and variation of the P1 amidine described previously [*J. Med. Chem.* **2003**, *46*, 4050]. The P1 needle replacements were selected based upon their reduced basicity compared to the parent phenyl amidine (p*K*a ~ 12). A contributing factor towards the oral bioavailability of a compound is the ionization state of the compound in the intestinal tract. The desired outcome of the study was to identify an orally bioavailable TF–VIIa inhibitor.

Cardiovascular disease is perhaps the most common cause for mortality in the western world.[1] The current therapies of heparin or warfarin are limited due to their slow onset of action and lack of selectivity, which can lead to bleeding side effects, thus requiring close patient monitoring.[2] The goal of current research in the area of antithrombotics is the development of more efficacious drugs that possess an improved safety profile and pharmacodynamics over the existing treatments.[3] Recent research in the area of anticoagulants has targeted almost every step in the coagulation pathway, with the most research being pursued around the development of selective thrombin or factor Xa inhibitors.[4]

Of the enzymes in the coagulation cascade, TF/VIIa has most recently drawn significant attention.[5a] The inhibition of the coagulation cascade at this stage of cascade has been shown to have significant advantages. For example the inhibitors act locally at the site injury, thus lessening unwanted side effects. In addition, the inhibitors have been shown to have an effect on neointimal formation and restenosis after vascular intervention, an advantage especially in the management of atherosclerotic complications.

Many approaches have been described by investigators for antagonizing the effect of the TF/VIIa complex. For example, active site inhibition of factor VIIa (VIIai), tissue factor mutants, antibodies directed to TF or VIIa, naturally occurring protein-based inhibitors, peptide-based exosite inhibitors, and small molecule active site inhibitors have appeared in the literature.[5]

Recent findings by our laboratories and others have highlighted the advantages of inhibiting the TFVIIa complex, in particular a lower risk of bleeding side effects versus other coagulation cascade targets.[6] We have detailed our work in a series of publications that described the design and synthesis of compounds that inhibit the TF/VIIa complex. The articles discuss the optimization of the P1, P2, and P3 sites, with the subsequent identification of a low nanomolar, pyrazinone-based inhibitor (see Fig. 1).[7]

An inspection of the compound reveals a highly basic amidine bound in the S1 pocket of TF/FVIIa in contact with Asp 189.[7b] In general, compounds with highly basic functionality, such as an amidine

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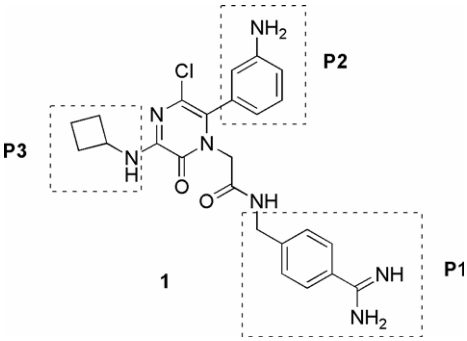


Figure 1. Structure of TF–VIIa inhibitor.

(p*K*a ~ 12), have difficulty in being orally absorbed.[8] Indeed, when **1** was dosed orally into rats at 10 mpk, very little absorption into the systemic circulation was observed. A common strategy used to enhance the absorption of such compounds is to identify a substitute for the amidine moiety that is less basic, while still being capable of making the requisite interaction with the enzyme.[8a,b] In addition, to the replacement of the amidine an alternative is the preparation of pro-drugs which can be absorbed and then converted in vivo to the active compound.[9] In this article, we wish to disclose our efforts toward the identification of a suitable P1 amidine substitute.

Our initial approach to the design of a suitable amidine replacement was to lower the basicity of the amidine (p*K*a) by incorporating substituents into the aryl ring. The designed compounds all possess a reduced p*K*a relative to compound **1** (see Table 1).[10]

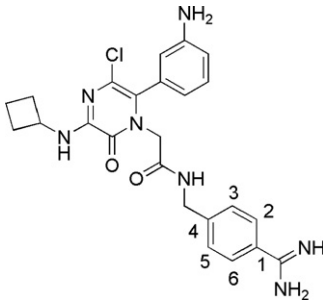
The compounds were assayed against VIIa, Xa, IIa (thrombin), and trypsin. Most of the compounds retained their activity against VIIa with the exception of compounds **5**, **6**, and **7**, which lost >10-fold activity. Interestingly, compound **3** retained TF–VIIa activity as well as displaying selectivity over Xa and thrombin (>100-fold). Compound **3** was dosed orally into rats at 10 mpk, but disappointingly very little oral exposure was observed.

The syntheses of the P1 needles for compounds **1**–**7** are described in Scheme 1. For compounds **2**, **3**, **5**, and **7** the P1 needle was coupled to the acid as previously reported,[7c] followed by reductive removal of the Cbz group. For compounds **4** and **6** the P1 needle was coupled followed by reduction of N—O bond to give the final product (see Scheme 2).

In addition to the incorporation of substitutions into the aryl ring of the amidine other variations were explored. Previous work by others in the Xa area had demonstrated success in the replacement of an amidine with a less basic functionality.[9] Given the success of the strategy, we sought to apply a similar strategy toward the identification of a replacement P1 needle for our TF–VIIa inhibitor **1**. The compounds designed, synthesized, and assayed are presented in Table 2. Unlike the F-substituted amidine derivatives, all of the analogs failed to show any activity less than 3 μM against TF–VIIa.

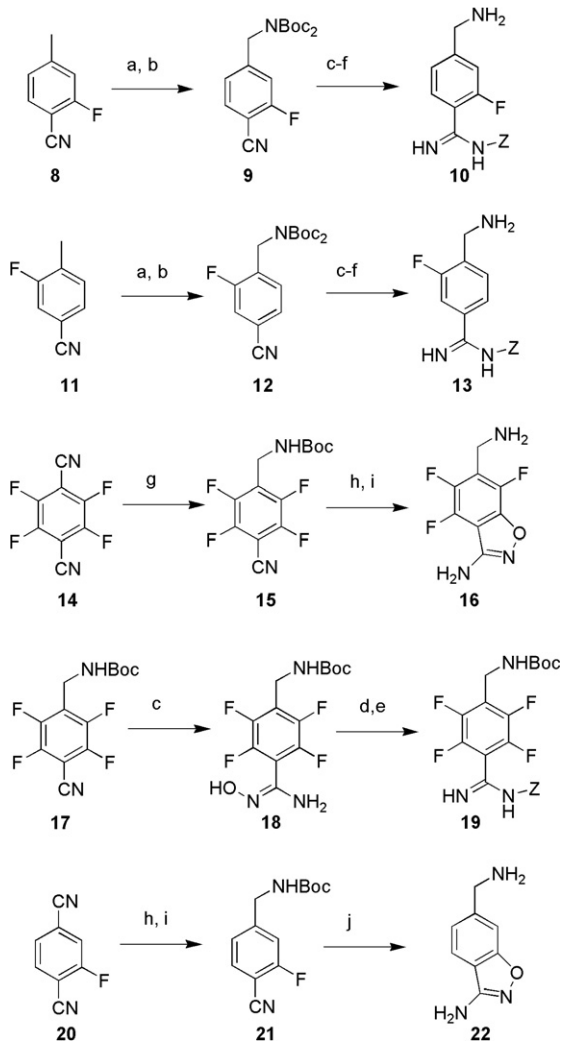
These results suggest an essential requirement for the amidine moiety for a compound to possess TF–VIIa inhibitory activity. The syntheses of the P1 needles for compounds **54**, **55**, and **57**–**59** are described in Scheme 3, for compound **56** the corresponding P1 needle was obtained from commercial sources.

Table 1. Calculated p*K*a for amidine analogs **1**–**7**



Compound	P1-substitution	pK_a[a]	IC_{50} (μM)			
			VIIa	Xa	IIa	Try
1	H	11.6	0.02	0.04	10.6	>42
2	3-F	10.8	0.04	0.05	12.8	>30
3	2-F	9.84	0.06	>30	>30	0.10
4	2-OH, 3,5,6-F	10.8	0.11	>100	>100	34.4
5	2,3,5,6-F	6.60	5.48	>30	>100	4.7
6	2-OH	—	0.19	>100	>100	7.55
7	3-CF_3	10.6	0.79	>30	>30	0.23

[a] Values calculated using the ACD/Physchem Batch 5.0 Program.



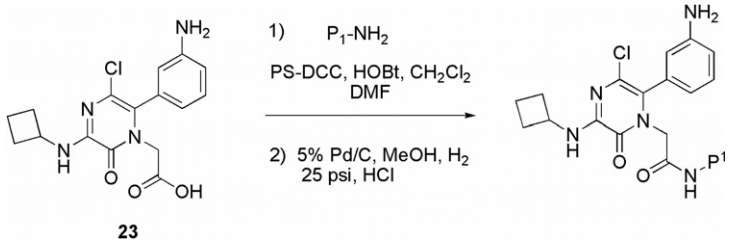
Scheme 1. Preparation of P1 needles for compounds **2**–**7**. Reagents and conditions: (a) NBS, CCl_4, reflux; (b) NaH, THF, $HNBoc_2$; (c) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH/H_2O (2:1), reflux; (d) AcOH, Ac_2O, Pd/C, H_2 (40 psi); (e) NaOH, Cbz–Cl, THF; (f) 4 N HCl in dioxane; (g) Boc_2O, 10% Pd/C, EtOH, H_2 (70 psi); (h) ^{t}BuoK, THF $(CH_3)_2C{=}NOH$; (i) EtOH:H_2O, concd HCl, reflux; (j) 5 N HCl, EtOH, 5% Pd/C, H_2 (60 psi).

Following the synthesis of the P1 needles, the amines were coupled to the acid **23** followed by removal of the Cbz group via hydrogenation as shown in Scheme 2 to give the compounds **54**–**59**. For the synthesis of compounds **43** and **44** the fluoronitrile derivative was treated with acetohydroxamic acid in the presence of potassium *tert*-butoxide in DMF to provide the oxazole derivative **43**. On the other hand, the thiazole derivative was prepared by treating the fluoronitrile derivative with sodium sulfide in DMSO at 70 °C followed by cooling and treatment with aqueous ammonia and sodium hypochlorite to give the compound **44**.

The synthesis of compounds **47** and **48** required modification of the pendant acid in the core template. This was accomplished by a four-step protocol to give the primary amine **46**, which served as the key intermediate. For **47**, the amine was treated with 4-cyanobenzene-1-sulfonyl chloride followed by conversion of the nitrile to the amidine with LiHMDS,[11] which was protected in situ as the Cbz derivative to facilitate isolation. The Cbz and nitro group were then reduced simultaneously to give the desired product. Compound **48** was prepared by coupling 4-(*N*-(benzyloxycarbonyl) carbamimidoyl) benzoic acid[12] to the amine under standard conditions, followed by reduction and removal of the nitro and Cbz group, respectively (Scheme 4).

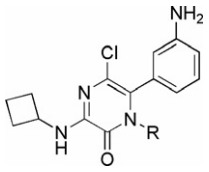
The syntheses of compounds **52** and **53** are described in Scheme 5. The Cl-isoquinoline **51** was prepared from 6-methylisoquinoline in five steps. The Cl-isoquinoline was then coupled to **44**, followed by Cl displacement and reduction of the nitro group to give the final compound. Compound **53** was prepared through the sequence described (Scheme 5, steps i–n) to give the final product **53**.

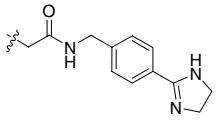
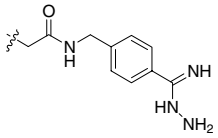
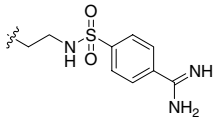
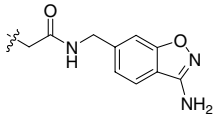
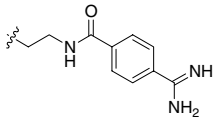
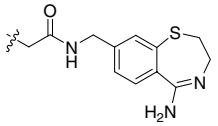
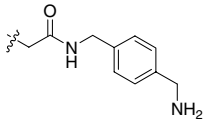
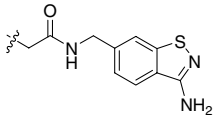
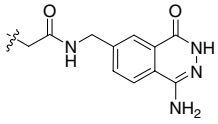
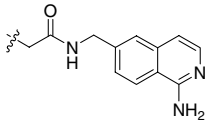
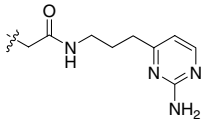
In conclusion, a series of analogs with various P1 needles were prepared to replace the benzamidine functionality present in the TF–VIIa inhibitor **1**.[7] Despite the synthesis and biological evaluation of a variety of less basic P1 needles a suitable replacement was not identified that possessed the desired enzymatic activity against the panel of VIIa, Xa, IIa (thrombin) and trypsin enzymes. Due to the lack of activity of the compounds in the primary screen, none were further evaluated in rat oral PK models to determine if indeed an improvement in oral absorption was achieved. Current research is being pursued around a pro-drug strategy for the future development of a TF–VIIa inhibitor and will be reported in due course.[13]



Scheme 2. Coupling of P1 needles to acid core.

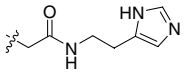
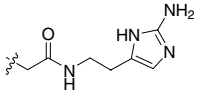
Table 2. Structure and biological activity of compounds **44–60**



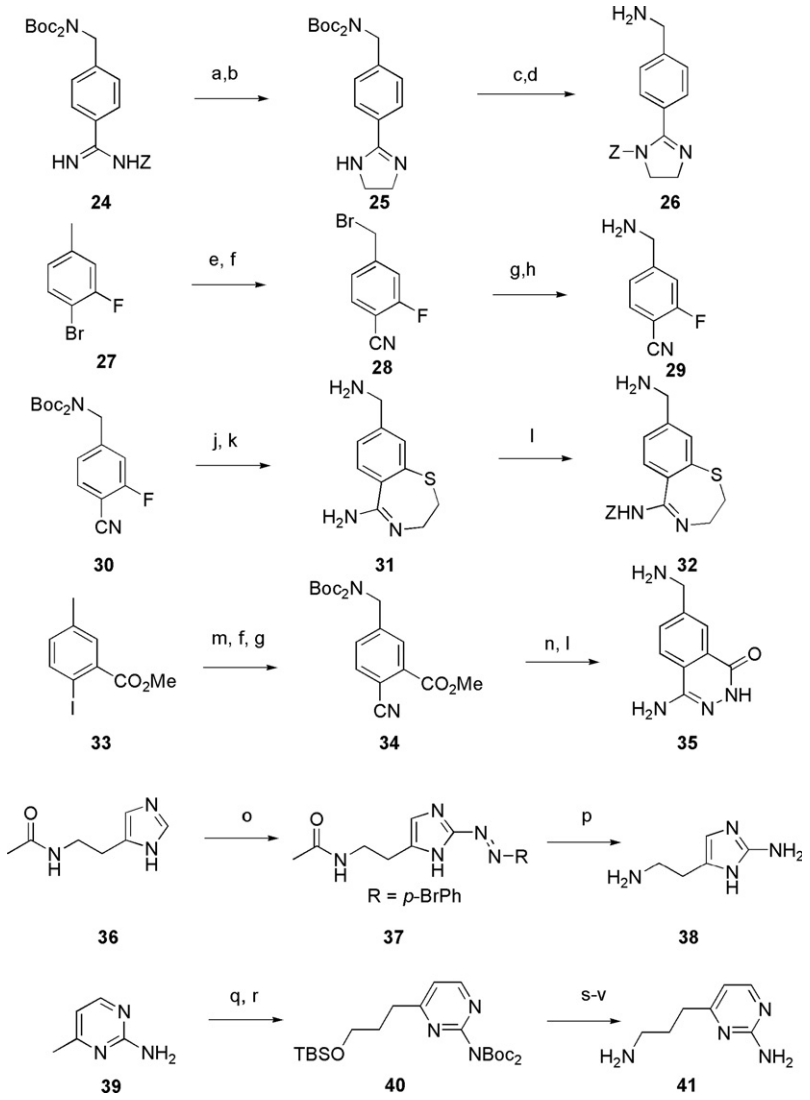
Compound	Substitution	pK_a [a]	IC_{50} (μM)			
			VIIa	Xa	IIa	Try
54		9.92	12.5	>30	>30	16
53		8.59	7.02	>100	>100	>3
47		10.9	30	>30	>30	9.1
43		4.62	12.0	>30	>30	>30
48		10.8	10.7	>30	>30	2.53
55		6.32	>30	>30	>30	>30
56		9.31	3.00	>30	>30	0.23
44		6.89	26.8	>30	>30	>30
57		6.12	>30	>30	>30	>30
52		7.50	>30	>30	>30	>30
58		4.6	>30	>30	>30	

(*continued on next page*)

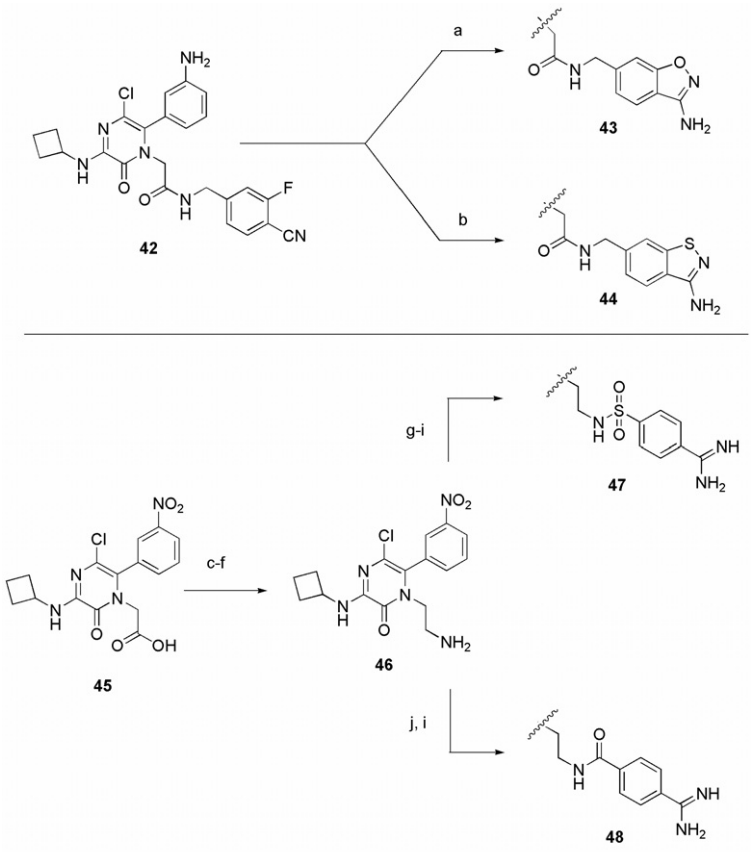
Table 2 (*continued*)

Compound	Substitution	pK_a[a]	IC_{50} (μM)			
			VIIa	Xa	IIa	Try
59		7.16	>30	>30	>30	>30
60		8.61	>30	>30	>30	6.20

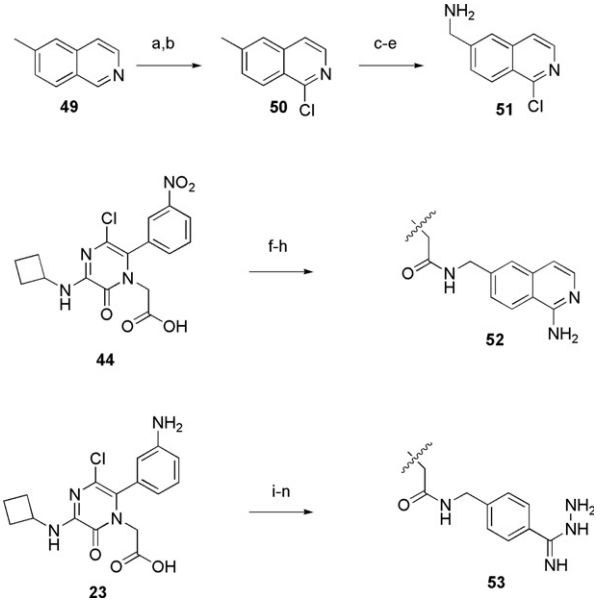
[a] Values calculated using the ACD/Physchem Batch 5.0 Program.



Scheme 3. Preparation of P1 needles for compounds **54**, **55**, **57**, **58**, and **59**. Reagents and conditions: (a) 5% Pd/C, EtOH:THF, H_2 (40 psi); (b) $H_2N(CH_2)_2NH_2$, MeOH, reflux; (c) 4 N HCl in dioxane; (d) Cbz–Cl, pyridine, CH_2Cl_2; (e) CuCN, CMF; (f) NBS, CCl_4, benzoyl peroxide, reflux; (g) NaH, $HNBoc_2$, THF, 0 °C; (h) TFA, CH_2Cl_2, rt; (i) Boc_2O, TEA, CH_2Cl_2, 0 °C; (j) $HS(CH_2)_2NH_2{\cdot}HCl$, KOH, ^{n}BuOH, 100 °C; (k) NaOH, CbzCl, THF, 0 °C; (l) TFA, CH_2Cl_2, 0 °C; (m) $Pd_2(dba)_3$, dppf, $Zn(CN)_2$, DMF, 120 °C; (n) H_2NNH_2, MeOH, 50 °C; (o) $NaNO_2$, *p*-$BrPhNH_2$, H_2O, 0 °C; (p) PtO_2, H_2 (35 psi), EtOH, rt; (q) BuLi, TMEDA, $Br(CH_2)_2OTBS$, THF, 0 °C; (r) Boc_2O, TEA, DMAP, DCM, rt; (s) TBAF, THF, rt; (t) MsCl, Et_3N, PhMe, 0 °C; (u) NaN_3, TBAl, PhMe:H_2O, 60 °C; (v) 10% Pd/C, H_2 (10 psi), EtOH.



Scheme 4. Preparation of compounds **43**, **44** and **47**, **48**. Reagents and conditions: (a) MeCONHOH, DMF, KO^{t}Bu, rt; (b) Na_2S, DMSO, 70 °C then $NH_{3(aq)}$, 5% $NaOCL_{(aq)}$, −5 °C → 0 °C; (c) BH_3–THF, 0 °C; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C; (e) NaN_3, DMF, 60 °C; (f) PPh_3, THF:H_2O, 50 °C; (g) $ClSO_2PhCN$, Et_3N, CH_2Cl_2, 0 °C; (h) LiHMDS, THF, 0 °C, then CbzCl, Na_2CO_3, THF:H_2O; (i) 10% Pd/C, H_2 (40 psi), EtOH, HCl; (j) $HO_2CPh(C{=}NH)NHCbz$, EDC, HOBt, Et_3N, DMF, 0 °C.



Scheme 5. Preparation of compounds **51**–**53**. Reagents and conditions: (a) mCPBA, CH_2Cl_2, rt then HCl/MeOH, 0 °C; (b) $POCl_3$, 90 °C; (c) NBS, CCl_4, Bz_2O; (d) PhtNH, NaH, THF; (e) H_2NNH_2, MeOH, 0 °C; (f) PS–DCC, HOBt, Et_3N, CH_2Cl_2/DMF; (g) NH_4OAc, DMF, reflux; (h) 5% Pd/C, MeOH, H_2 (25 psi); (i) 4-(aminomethyl)benzonitrile, EDC, HOBt, Et_3N, DMF, 0 °C; (j) Boc_2O, Et_3N, DMAP, THF; (k) H_2S, Et_3N, pyridine, 0 °C; (l) Mel, acetone, rt; (m) H_2NNH_2, MeOH, rt; (n) TFA, CH_2Cl_2, rt.

Acknowledgment

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