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Design and Synthesis of Aminophenol-Based Factor Xa Inhibitors

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Abstract—A novel potent and selective aminophenol scaffold for fXa inhibitors was developed from a previously reported benzimidazole-based naphthylamidine template. The aminophenol template is more synthetically accessible than the benzimidazole template, which simplified the introduction of carboxylic acid groups. Substitution of a propenyl-*para*-hydroxy-benzamidine group on the aminophenol template produced selective, sub-nanomolar fXa inhibitors. The potency of the inhibitors is partially explained with the aid of a trypsin complex crystal structure.

Several excellent recent reviews[1] have described the history and utility of factor Xa (fXa) inhibitors as safe and effective anticoagulants. Previously, we described the development of subnanomolar benzimidazole-based factor Xa (fXa) inhibitors,[2,3] which suffered from poor selectivity versus trypsin and a poor pharmacokinetic (PK) profile. Previous efforts to improve the selectivity and PK profile by introduction of a carboxylic acid group, which had some success in our pyridine-based fXa inhibitors,[4] or by substitution of the naphthylamidine group with either a biphenylamidine or a propenylbenzamidine group had limited success.[5] In this communication, we present fXa inhibitors in which the benzimidazole template is replaced with the more synthetically accessible aminophenol template[6] (Fig. 1).

By using the less rigid aminophenol template (Fig. 1, **2**), fXa inhibitors of similar potency and selectivity as the benzimidazole analogues (**1**, fXa $K_i = 0.3$ nM, fII $K_i > 5000$ nM, Trp $K_i = 3$ nM) were realized. Previous work towards a less rigid inhibitor molecule involved replacement of the naphthylene ring with either a biphenyl or propenylbenzene ring.[3] Replacement of naphthylamidine with either biphenylamidine or propenylbenzamidine resulted in a minor loss of fXa potency and fIIa selectivity with a slight increase in trypsin selectivity (see Table 1). Replacement of naphthylamidine with 2-methylpropenylbenzamidine afforded inhibitors with similar potency and selectivity (**2** vs **3c**).

Optimization of the aminophenol ring substituents was examined next (Table 2). When the 3-nitro group of the phenyl ring was replaced with H, F, or CO_2Et the inhibitors lost potency and selectivity (**3b** vs **4a–c**). However, replacement of the nitro group with a trifluoromethyl group resulted in only a 3-fold decrease in potency and selectivity (**3b** vs **4d**). Potency and selectivity levels were regained by exploring substitution on the aniline nitrogen. Mono- or di-acid moieties appended to the aniline afforded subnanomolar fXa inhibitors with enhanced potency and selectivity (**4d** vs **4e–f**).

With our pyridine-based fXa inhibitors, introduction of a hydroxy or an amino group *para* to the amidine enhances both inhibitor potency and selectivity. Table 3 lists inhibitors with and without the *para*-hydroxyl group. The hydroxy group improves fXa activity and selectivity to afford sub-nanomolar inhibitors (**4d** vs **5a**).

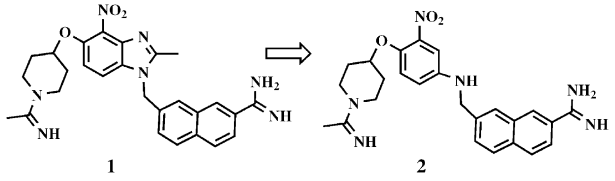

Figure 1. Replacement of benzimidazole with an aminophenol.

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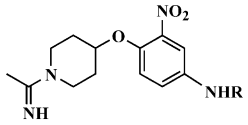
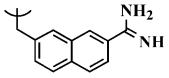
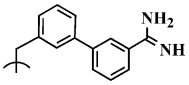
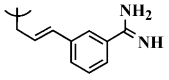
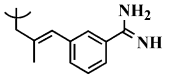
Similar effects are also observed with acylated aniline analogues such as the ester (**5b** vs **5c**) or the corresponding acid (**4e** vs **5d**) which showed 51,000-fold selectivity against thrombin and 2800-fold selectivity against trypsin. The binding of the aminophenol template is described by the X-ray crystal structure of **5a** in bovine trypsin (Fig. 2).[7] The propenylbenzamidine moiety binds to the aspartic acid residue (D189) in the S1 pocket and the alkylamidine group binds to the S4 pocket; a binding mode analogous to DX-9065a on both trypsin and fXa. The *para*-hydroxy group forms a hydrogen bond to the O^{γ} of Ser195 of the catalytic triad through a bridging water molecule.

In general, dibasic fXa inhibitors have demonstrated poor oral availability. Methods to improve the oral availability of aminophenol-based fXa inhibitors by

Table 1. Replacement of naphthylamidine



	R	fXa K_i (nM)[a]	fIIa K_i (nM)	Trp K_i (nM)
2		0.08	1960	7
3a		0.17	400	42
3b		0.27	2900	27
3c		0.07	1550	5

[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are $<30\%$ of the mean.

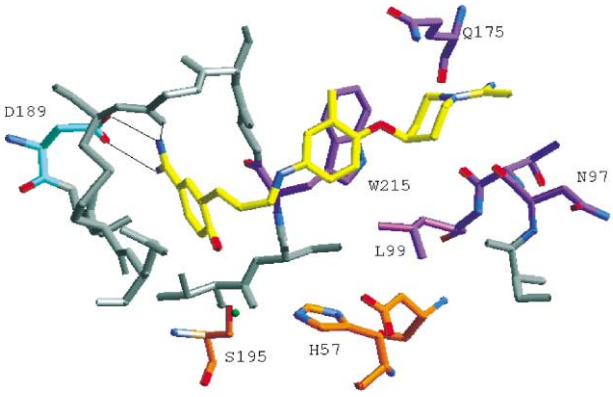
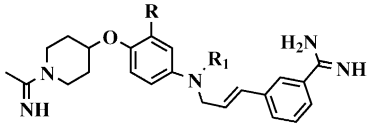


Figure 2. X-ray structure of **5a** in trypsin.

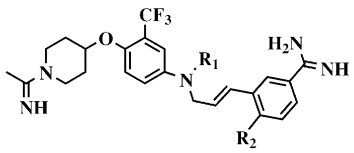
Table 2. Bioassay results for substitution effect on the phenyl ring and the aniline nitrogen



No.	R	R_1	fXa K_i (nM)[a]	fIIa K_i (nM)	Trp K_i (nM)	PT (μM)[b]	TT (μM)[c]
4a	H	H	7.5	5000	80	ND[d]	ND
4b	F	H	4.1	4700	70	0.73	240
4c	CO_2Et	H	6.8	480	40	2.6	15
4d	CF_3	H	0.88	2140	50	0.80	220
4e	CF_3	$COCH_2CH_2CO_2H$	0.28	5000	110	0.75	540
4f	CF_3	COPh-3,4-di-CO_2H	0.56	3260	10	0.52	200

[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are $<30\%$ of the mean.[8]
[b]Concentration of inhibitor required to double the prothrombin based clotting time in human plasma.
[c]Two-fold increase in thrombin clotting time.
[d]Not determined.

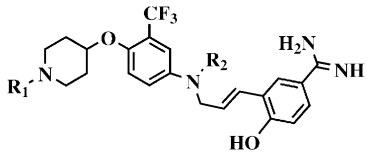
Table 3. Bioassay results on hydroxy substitution effect on the aryl amidine ring



Entry	R_1	R_2	fXa K_i (nM)[a]	fIIa K_i (nM)[a]	Trp K_i (nM)[a]	Trp/fXa selectivity	PT[b](μM)	TT[c] (μM)
5a	H	OH	0.10	1100	200	2000	1.05	210
5b	$COCH_2CH_2CO_2Et$	H	0.55	1000	76	140	0.87	32
5c	$COCH_2CH_2CO_2Et$	OH	0.07	2280	230	3300	0.8	265
5d	$COCH_2CH_2CO_2H$	OH	0.10	5140	280	2800	0.54	174

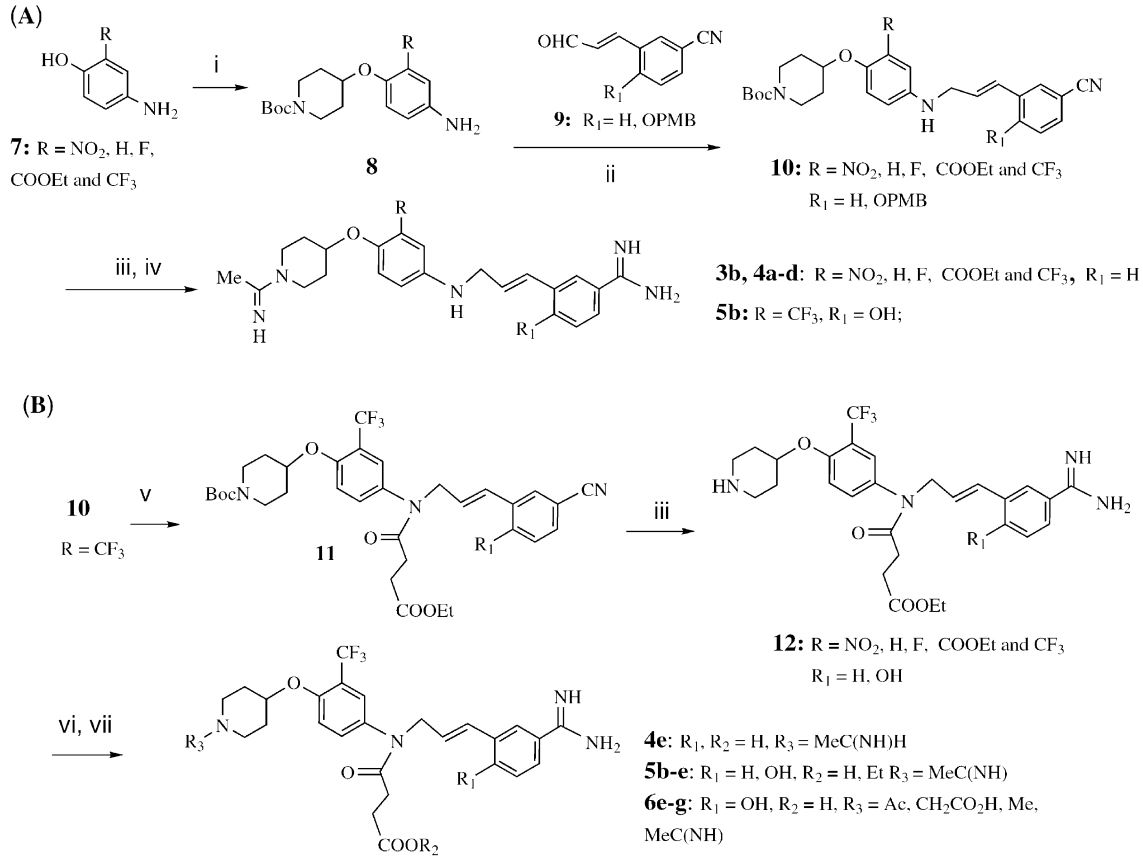
[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are $<30\%$ of the mean.[8]
[b]Concentration of inhibitor required to double the prothrombin based clotting time in human plasma.
[c]Two-fold increase in thrombin clotting time.

Table 4. Bioassay results upon replacement of the alkylamidine group



Entry	R_1	R_2	fXa K_i (nM)[a]	FIIa K_i (nM)	Trp K_i (nM)	PT[b] (μM)	TT[c] (μM)
6a	MeCO	H	18.5	480	830	21	151
6b	NH_2CO	H	7.3	250	520	ND[d]	ND
6c	Ms	H	44	890	1250	ND	ND
6d	Me	H	0.58	1820	630	0.98	156
6e	MeCO	$COCH_2CH_2CO_2H$	3.0	>5500	891	3.0	173
6f	CH_2CO_2H	$COCH_2CH_2CO_2H$	0.34	>5000	870	ND	ND
6g	Me	$COCH_2CH_2CO_2H$	0.28	>5000	765	ND	ND

[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geqslant 2$) and the standard deviations are <30% of the mean.[8]
[b]Concentration of inhibitor required to double the prothrombin based clotting time in human plasma.
[c]Two-fold increase in thrombin clotting time.
[d]Not determined.



Scheme 1. Reagents and conditions: (i) *N*-*t*-butoxycarbonyl-4-hydroxyl-piperidine, DEAD, PPh_3; (ii) $NaCNBH_3$; (iii) (a) HCl(g), EtOH; (b) NH_3, EtOH; (iv) MeC(NH)OEt, Et_3N, MeOH; (v) $EtOOCCH_2CH_2COCl$, Et_3N; (vi) (a) LiOH, MeOH, H_2O; (vii) AcCl, Et_3N, CH_2Cl_2 or $BrCH_2COOH$, CH_2Cl_2, Et_3N or MeI, Et_3N, CH_2Cl_2 or HCO_2H, $NaCNBH_3$.

reducing the overall basicity of the molecule include addition of a carboxylic acid group, as done with our pyridine-based fXa inhibitors, or elimination of a basic group. The alkyl amidine non-basic and less-basic replacements we explored are listed in Table 4. Acetamide, urea, or methane-sulfonamide groups afforded inhibitors 10- to 400-fold less potent than the alkyl amidine (**5a** vs **6a**–**c** and **4e** vs **6e**). *N*-Methyl or *N*-carboxymethyl substituents had minimal effect on potency and selectivity (**5a** vs **6d** and **5d** vs **6f**, **6g**). Unfortunately, all of the analogues in the aminophenol template exhibited poor oral availability when dosed in dogs.

All compounds listed in Tables 2–4 were prepared by the general routes shown in Scheme 1. 2-Substituted

aminophenol **7** was coupled with *N*-*t*-butoxycarbonyl-4-hydroxypiperidine under Mitsunobu conditions to afford intermediate **8**. (route **A**). Reaction of aniline **8** with aldehyde **9**, under reductive amination conditions, affords **10**, which could be converted to the alkyl amidine using previously described conditions[2] to obtain **4a–d** and **5b**. The synthesis of carboxylate-containing inhibitors is shown in Scheme 1, route **B**. Acylation of aniline **10** with either ethyl succinyl chloride or trimellitic anhydride chloride (intermediate to prepare **4f**, not shown) gave intermediate **11**. Conversion of the nitrile to the corresponding amidine with concomitant loss of the *para*-methoxylbenzyl- and the Boc-group gave intermediate **12**. The piperidine nitrogen of intermediate **12** can be selectively alkylated with either ethyl acetimidate, iodomethane, or methyl bromoacetate to prepare analogues shown in Tables 2–4. Alternatively intermediate **12** can be acylated with acetyl chloride or mesyl chloride to afford the acylated inhibitors in Table 4.

In summary, the aminophenol template is an easily accessible scaffold for the development of potent and selective fXa inhibitors. The aniline nitrogen can be substituted with either a naphthylamidine, biphenylamidine or propenyl-benzamidine group with minimal change in vitro activity. A *para*-hydroxy substituent on the propenylbenzamidine ring yields subnanomolar fXa inhibitors with 50,000- and 3000-fold selectivity against thrombin and trypsin, respectively. The potency and selectivity of the aminophenol-based inhibitors can be modulated with substituents on the piperidine nitrogen. The potency of the inhibitors can be partially explained with the aid of a trypsin complex crystal structure.

References and Notes

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