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Benzimidazole-Based fXa Inhibitors with Improved Thrombin and Trypsin Selectivity

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Abstract—Optimization of the benzimidazole-based fXa inhibitors for selectivity versus thrombin and trypsin was achieved by substitution on the benzimidazole ring and replacement of the naphthylamidine group. Substitution of a nitro group at the 4-position on the benzimidazole improves both potency against fXa and selectivity versus thrombin. Alternatively, replacement of the naphthylamidine with either a biphenylamidine or propenylbenzamidine not only improves fXa potency and selectivity versus thrombin, but selectivity versus trypsin as well.

As part of our effort to identify potent and selective anticoagulants, which target factor Xa (fXa),[1] we have designed a novel series of inhibitors based on our benzimidazole scaffold.[2] Although the previously-reported benzimidazole-based fXa inhibitors were potent against fXa, they are only moderately selective against thrombin (fIIa) and trypsin (Trp) and have a poor pharmacokinetic (PK) profile. Efforts to incorporate carboxylic acid functionality onto the benzimidazole template improved the PK profile, as in our pyridine based fXa inhibitors,[3] but gave little improvement in selectivity against other serine proteases.[4] In this communication we describe our efforts towards improving the selectivity profile by substitution on the benzimidazole ring and replacement of the naphthylamidine group.

Chemistry

Compounds (**8b–h** in Table 1) substituted at the 4-position of the benzimidazole ring were prepared according to Scheme 1. Nitration of 5-hydroxyl-benzimidazole (**1**) afforded a mixture of the 4- and 6-nitro isomers that were readily separated by chromatography. Mitsunobu reaction of the 4-nitrobenzimidazole with *N*-*t*-butoxycarbonyl-4-hydroxyl-piperidine afforded **2**. In contrast to alkylations without the 4-nitro group,[2] alkylation of **2** with 7-cyano-2-bromomethylnaphthalene (**3**) afforded **4** as a single regioisomer. The selective alkylation of the benzimidazole nitrogen *meta* to the nitro group may be due to the steric hindrance of the *ortho* position. Specific analogues could be prepared by reduction of **4** to the corresponding amine **5**, followed by acylation or alkylation to give intermediate **6**. A two-step process of arylamidine formation using Pinner conditions yielded **7** and *N*-alkylation gave **8** as described previously.[2,3]

A similar procedure was used to prepare benzimidazoles bearing a biphenyl or propenylphenyl substituent at C-1 instead of the naphthyl group. As shown in Scheme 2A, the biphenylmethylbromide **12** was prepared by Stille coupling between phenyl bromides **9** and **10** followed by bromination of toluene intermediate **11**. Allylbromide **19** was prepared by a three step synthesis starting with aldehyde **17** and Wittig reagent **16** (Scheme 2B). Reduction of the resulting unsaturated aldehyde **18** and replacement of the corresponding alcohol with bromine gave allyl bromide **19** (Scheme 2B). Alkylation of benzimidazole **13** with either **12** or **19** followed by the amidine-forming procedures outlined above afforded a separable regioisomeric mixture of **14a–e**/**15a–e** (Table 2) or **20a–e**/**21a–e** (Table 3), respectively. The phenolic group in **20e** and **21e** was uncovered by removal of the *p*-methoxybenzyl protecting group during amidine formation. The regiochemistry of the inhibitors in Tables 1–3 was established by NMR NOE studies.

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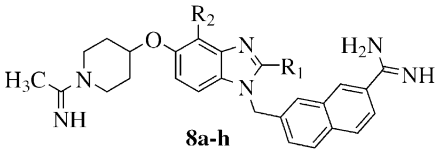
Table 1. In vitro inhibitory activities of 1,4,5-substituted benzimidazoles



8a-h

No.	R_1	R_2	fK_i (nM)[a] fXa	fIIa	Trp
8a	Et	H	4	2000	5
8b	Et	NH_2	8	3500	24
8c	Et	NMe_2	5	>5000	10
8d	Et	NHCOMe	50	>5000	25
8e	Et	$N(CH_2CO_2H)_2$	50	>5000	15
8f	Et	NO_2	0.1	3000	5
8g	Me	NO_2	0.3	>5000	3
8h	*i*-Pr	NO_2	0.2	>5000	3

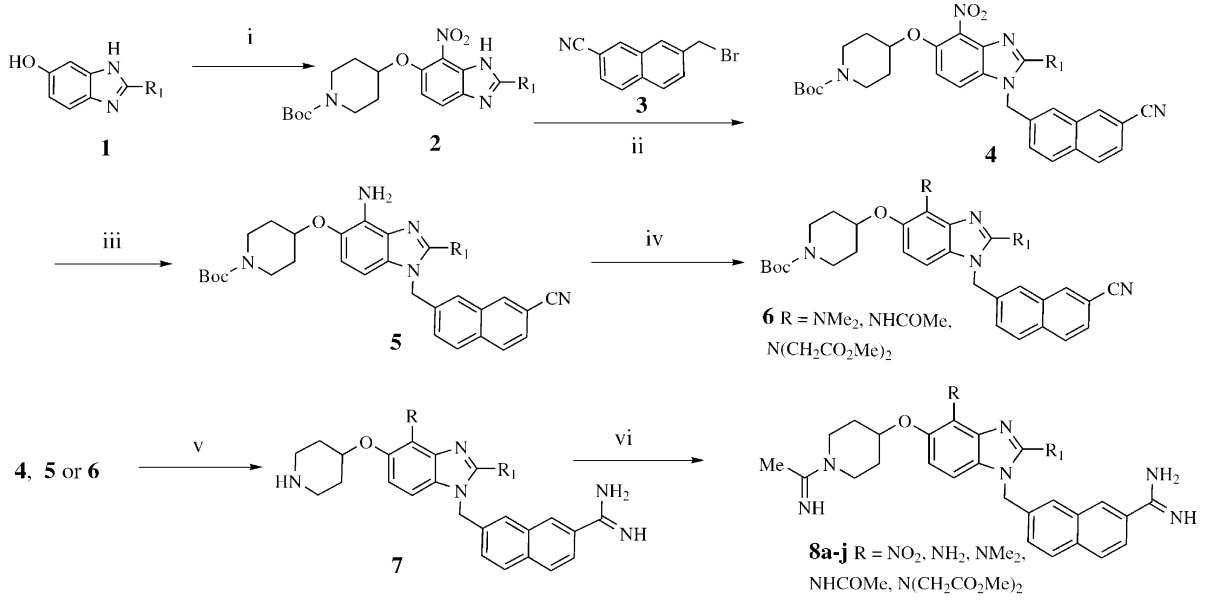
[a]K_i values for these competitive inhibitors are averaged from multiple determinations ($n \geq 2$) and the standard deviations are <30% of the mean.

Results and Discussion

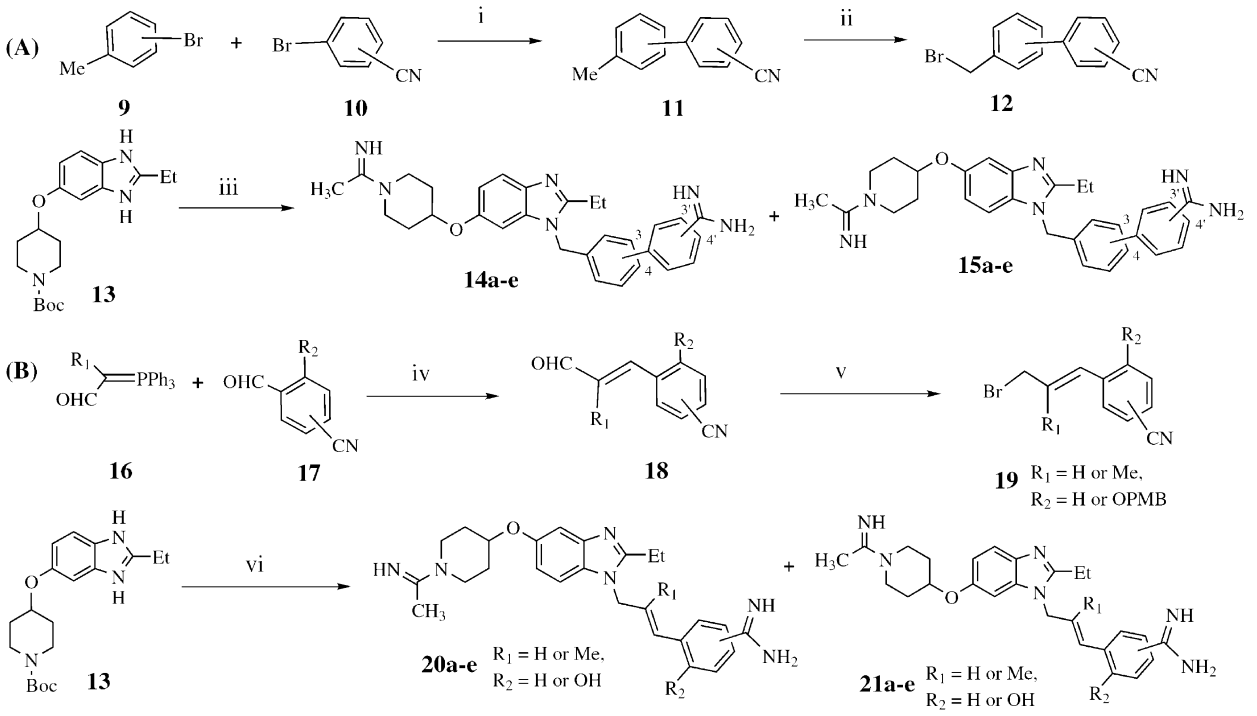
The effect of varying the substitution at the 4-position on the benzimidazole ring on potency against fXa and selectivity versus fIIa and trypsin is shown in Table 1. Substitution of an amino group at C-4 (**8b**, **8c**) increased trypsin and thrombin selectivity up to a 2-fold when compared with unsubstituted analogue **8a** with minimal impact on the inhibition of fXa. Substitution with acetamide (**8d**) or iminodiacetic acid (**8e**) decreased the fXa inhibitory activity more than 10-fold versus **8a**. However, substitution with a nitro group enhanced fXa potency about 40-fold (**8f** vs **8a**) and enhanced selectivity against thrombin 60- and 40-fold versus trypsin. The increase in potency and selectivity was independent of the substituent at C-2 as seen in the 2-methyl (**8g**) and 2-isopropyl analogues (**8h**). Thus, substitution of a nitro group at the 4-position on the 1,5-benzimidazole template affords potent fXa inhibitors with excellent thrombin selectivity (**8f**, **8g**, and **8h**) and improved trypsin selectivity.

To evaluate the importance of the naphthylamidine group to the biological activity of the benzimidazole template, we replaced the naphthylamidine with different substituted biphenylamidines. Table 2 shows the disappointing in vitro profile for the four *meta* and *para* substituted isomers in both the 1,5-disubstituted and 1,6-disubstituted regioisomers. Little difference was seen in the activity between the 1,5- (**15a–b**) and 1,6-disubstituted (**14a–b**) regioisomers in the 3,4′ and 4,4′ series of analogues. The 3,3′ (**14d–15d**) and 4,3′ (**14c–15c**) series of analogues were more potent and selective than the other biphenyl analogues, with **15d** affording a subnanomolar fXa inhibitor with over 1000-fold thrombin and 200-fold trypsin selectivity.

Simplification of the naphthylamidine group was accomplished by removal of carbon atoms from the naphthalene ring to yield a propenylbenzene group, which we hoped would have a similar binding mode to trypsin as the 1,5-substituted analogue of **8a**.[5] The propenylbenzene series of analogues (**20b–c** and **21b–c**) showed dramatically improved potency and selectivity (Table 3) over the corresponding unsubstituted naphthalene analogue. In particular, propenylbenzene analogue **21b** shows a 10-, 4-, and 64-fold increase in fXa potency, thrombin selectivity, and trypsin selectivity, respectively, over the corresponding naphthalene analogue **8a**. Methyl substitution on the propenyl group in the 1,6-series as in **20c** decreased the potency 40-fold

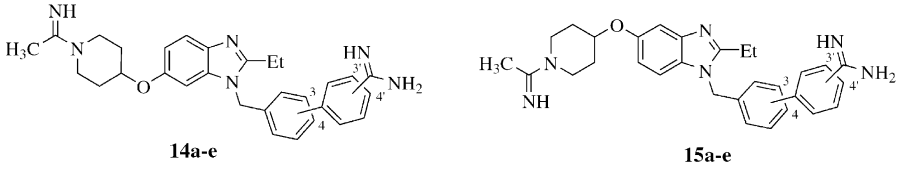


Scheme 1. Reagents and conditions: (i) (a) HNO_3, TFA, separate isomers; (b) *N*-*t*-butoxycarbonyl-4-hydroxyl-piperidine, DEAD, PPh_3; (ii) NaH, DMF; (iii) $SnCl_2$, pyr; (iv) MeOCOCl, Et_3N, CH_2Cl_2 or RBr, K_2CO_3, DMF; (v) (a) HCl, EtOH; (b) NH_3, EtOH; (vi) (a) LiOH or aq HCl; (b) MeCNHOEt·HCl, Et_3N, MeOH.



Scheme 2. Reagents and conditions: (i) $Sn_2(Bu)_6$, $Pd(PPh_3)_4$; (ii) NBS, AIBN, CCl_4; (iii) (a) **12**, NaH, DMF; (b) HCl, EtOH; (c) NH_3, EtOH; (d) MeC (NH)OEt·HCl, Et_3N, MeOH and HPLC separation; (iv) MeOH, rt; (v) (a) NaBH4; (b) PPh_3, Br_2; (vi) (a) **18**, NaH, DMF; (b) HCl, EtOH; (c) NH_3, EtOH; (d) MeC(NH)OEt·HCl, Et_3N, MeOH and HPLC separation.

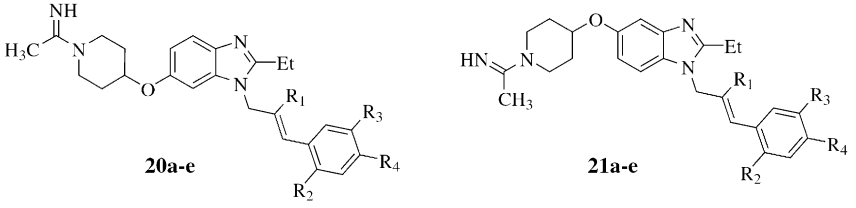
Table 2. In vitro inhibitory activities of biphenylamidino benzimidazole regioisomers



Biphenyl	No.	1,6-Regioisomer, K_i (nM)[a]			No.	1,5-Regioisomer, K_i (nM)		
		fXa	fIIa	Trp		fXa	fIIa	Trp
3,4′	**14a**	150	3600	430	**15a**	260	>5000	690
4,4′	**14b**	180	22	91	**15b**	670	1500	150
4,3′	**14c**	2.2	770	73	**15c**	17	210	140
3,3′	**14d**	34	190	190	**15d**	0.46	1120	67

[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.

Table 3. In vitro inhibitory activities of propenylbenzamidino benzimidazole regioisomers



R_1	R_2	R_3	R_4	No.	1,6-Regioisomer, K_i (nM)[a]			No.	1,5-Regioisomer, K_i (nM)[a]		
					fXa	fIIa	Trp		fXa	fIIa	Trp
H	H	H	Am	**20a**	64	880	320	**21a**	74	2500	350
H	H	Am	H	**20b**	0.028	200	21	**21b**	0.40	870	32
Me	H	Am	H	**20c**	1.3	380	73	**21c**	0.31	>5000	6.9
H	OMe	Am	H	**20d**	13	2600	130	**21d**	58	5000	410
H	OH	Am	H	**20e**	0.10	75	43	**21e**	0.10	610	120

[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.

and selectivity about 20-fold versus **20b**, but had a more positive effect in the 1,5-regioisomer series (**21b** vs **21c**). The importance of the substitution pattern on the benzamidine ring is demonstrated by the over 100-fold increase in potency and selective difference between the the propenyl-*para*-benzamidine analogues (**20a** and **21a**) and the corresponding propenyl-*meta*-benzamidine analogues (**20b** and **21b**). Saturation of the double bond in the alkyl chain resulted in a 10-fold loss of activity versus the propenyl analogues, **20b** and **21b** (data not shown). Introduction of a methoxy group on the benzamidine ring causes over a 100-fold decrease in the fXa inhibitory activity in both the 1,5- and 1,6-regioisomer series (**20d** and **21d**). The *para*-hydroxyl group on the benzamidine ring had minimal effect on the potency and selectivity over the corresponding unsubstituted compound in both the 1,5-series (**21e** vs **21b**) and the 1,6-regioisomer series (**20b** vs **20e**).

In summary, selective, subnanomolar fXa inhibitors in the benzimidazole series can be prepared by placement of a nitro group at the 4-position on 1,5-benzimidazole template or by replacement of the naphthylamidine with either a biphenylamidine or a propenylbenzamidine group.

References and Notes

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