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Non-Peptidic Phenyl-Based Thrombin Inhibitors: Exploring Structural Requirements of the S1 Specificity Pocket with Amidines

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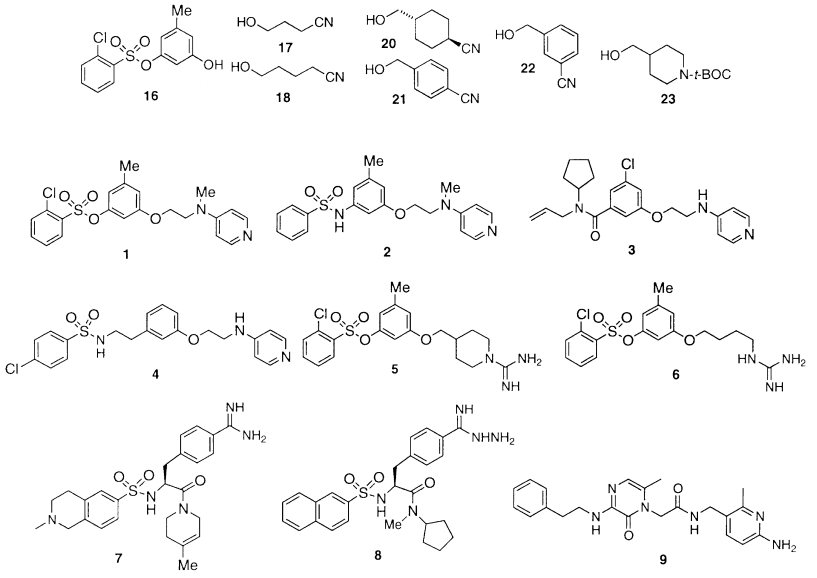
Abstract—We expand the structural requirements and structure–activity relationship of a novel class of non-peptidic aryl-based thrombin inhibitors through exploration of the S1 specificity pocket of thrombin using flexible and constrained amidines. The most active compound of this class is **11** with $K_i = 69$ nM, which is ca. 15-fold less potent than constrained guanidine **5**.

A growing body of achiral, non-peptidic thrombin inhibitors (e.g. **1**–**6**) has emerged recently in which an aromatic scaffold has replaced the polyamide- or peptide-like backbone of typical thrombin inhibitors.[1–10] In contrast to scaffolds such as those found in UK 156406 (**7**), LB30057 (**8**), and L-375378 (**9**) many of these peptide replacements, such as **1**, **5**, and **6** lack hydrogen bonding interactions with the enzyme, aside from basic group interactions with Asp 189 of the S1 specificity pocket. Given their structural simplicity, drug-like features, low molecular weight, potency, and selectivity, inhibitors based on aryl scaffolds may ultimately offer advantages in antithrombotic therapy in comparison to aminoacid-based or polyamide systems.



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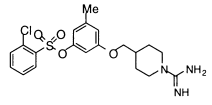
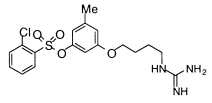
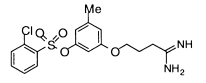
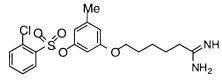
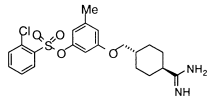
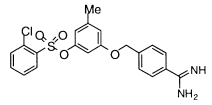
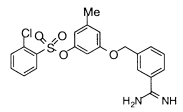
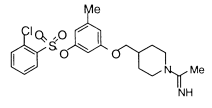
We recently reported analogues of **6**, which developed the SAR of allowable aryl groups projecting into the distal aromatic binding pocket.[6] We now extend these structure–activity studies to explore requirements of amidino-based moieties in the S1 specificity pocket of the enzyme. In appreciation of factors which may ultimately govern overall in vivo performance,[11] this study was undertaken to explore guanidino group replacement with a group of lower p*K*a but still capable of maintaining key hydrogen bonding interactions with Asp189 of the S1 pocket.

Compounds **10**–**14** were prepared in 2 steps from **16**:[3] etherification of **16** with corresponding alcohols **17**–**22** using Mitsunobu conditions (76–93% yield) followed by amidination ((a) 37% HCl in EtOH, 0 °C, 2 days; (b) $(NH_4)_2CO_3$, 0 °C, 2 days; 50–75% overall yield). Compound **15** was similarly synthesized by coupling **16** with alcohol **23** using Mitsunobu conditions[12] (90%), deprotection (4 N HCl in dioxane, 95%), and conversion to the amidine (ethyl acetimidate hydrochloride, TEA, DMF, 86%).

Compounds **10**–**15** were assayed for thrombin inhibition as well as for selectivity against a panel of other related serine proteases[3] as summarized in Table 1. Overall the amidine were weaker thrombin inhibitors in comparison to their guanidino analogues. Compound **11**, the most potent of the amidines, was about 15-fold less potent than the constrained guanidine (**5**) and about 5-fold less potent than unconstained **6**. Surprisingly, restricting the P1 chain into rigid, hydrophobic backbone isosteric with **5** failed to increase potency in contrast to the guanidino analogues. Thus, benzamidine derivative **13** was slightly less active than **11** while *trans*-cyclohexyl (**12**) was 30-fold less potent than **11**, 160-fold less potent than **6**, and more than 450-fold less potent than the constrained guanidine **5**. The benzamidine regioisomers **13** and **14** were nearly equipotent with respect to thrombin inhibition. Replacing the terminal amino group of **5** with a methyl group was detrimental to activity, as expected based on crystallographic analysis of **5**.[3] With respect to selectivity, no trypsin activity was detected as screening doses with amidine **11**, whereas the selectivity for thrombin versus trypsin decreased for benzamidines **13** and **14**.

Table 1. Inhibition of proteases: expressed as K_i (μM) or % inhibition at (μM)

	Compound	Thrombin	FXa	Chymotrypsin	Trypsin
5[3]		0.0046 ± 0.001	> 15	> 15	> 15
6[3]		0.013 ± 0.0016	110 ± 56.5	> 49	42 ± 0.78
10		0.46 ± 0.045	0% (7.5 μM)	0% (15 μM)	0% (15 μM)
11		0.069 ± 0.0032	0% (1.5 μM)	2.4 ± 0.37	0% (15 μM)
12		2.1 ± 0.33	0% (1.6 μM)	2.9 ± 0.37	0% (1.6 μM)
13		0.11 ± 0.0056	17 ± 3.8	0% (1.7 μM)	2.7 ± 0.4
14		0.099 ± 0.0068	2.7 ± 0.163	0% (1.3 μM)	1.5 ± 0.10
15		1.3 ± 0.066	0% at 1.0 μM	0% at 1.0 μM	0% at 1.0 μM

In summary, we have expanded further the SAR of a non-amide-based series of thrombin inhibitors with respect to S1 specificity pocket of thrombin. Despite potential advantages of lower p*K*a, the amidino based analogues in this series were less effective inhibitors of thrombin than isosteric guanidino counterpart. Various modes of rigidification of the flexible amidines in the hydrophobic S1 pocket failed to maintain thrombin inhibition potency. Nevertheless, given the low molecular weight and the prospects for achieving low nM thrombin inhibition, further studies of this series of achiral, aryl-based systems are in progress and will be reported in due course.

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