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Structure-based design of novel groups for use in the P1 position of thrombin inhibitor scaffolds. Part 1: Weakly basic azoles

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Abstract—Despite their relatively weak basicity, simple azoles, specifically imidazoles and aminothiazoles, can function as potent surrogates for the more basic amines (e.g., alkyl amines, amidines, guanidines, etc.) which are most often employed as the P1 ligand in the design of noncovalent small molecule inhibitors of thrombin.

Structure-based design approaches to the development of novel noncovalent small molecule inhibitors of the serine protease thrombin have involved designing templates capable of binding to the enzyme's three principal binding pockets (the specificity pocket S1, the proximal pocket S2, and the distal pocket S3).[1] A sampling of the wide variety of structures which have been developed highlights the fact that most of the structural diversity in the field has come from designing novel scaffolds to fill S3 and S2.[1] The S1 specificity pocket is much less accommodating of structural variety than either the S3 or S2 pockets. Generally, to produce potent inhibitors, the P1 ligand features a strongly basic functional group such as an alkylamine, amidine, benzamidine or a 4-aminopyridine. These basic groups are capable of making a significant contribution to overall binding energy because of their ability to form strong ionic interactions with the carboxyl group of the Asp189 residue at the bottom of the thrombin S1 pocket. As such, these basic groups can often be combined with a wide variety of P3P2 scaffolds to yield inhibitors with good inhibitory potency and a broad spectrum of chemical, physical and pharmacokinetic properties.[1] Nevertheless, such strongly basic amines have the disadvantage that they tend to impart poor oral absorption properties and in some instances unacceptable off-target activity.[2] For example, thrombin inhibitor **1**[3] is potent (K_i = 0.10 nM) but not orally bioavailable in rats or dogs (Fig. 1). Furthermore, some inhibitors with this P1 aminocyclohexane group displayed in vivo toxicity. Detailed SAR studies indicated that the observed toxicity was a function of the pK_a of the aminocyclohexane. Modifications to the aminocyclohexane, which lowered its pK_a (for example, incorporation of electron-withdrawing groups or heteroatoms), attenuated the observed toxicity. To try to address these issues, we decided to investigate the design of inhibitors in which the S1 binding element is considerably less basic.[4,5] As expected, for a given P3P2 template, an initial change from a highly basic P1 group to one of lower basicity results in a significant drop in binding potency. However, we reasoned that following such a modification, the ready availability of X-ray structural data for thrombin-inhibitor complexes and the use of molecular modeling could guide the design of structural modifications which would either result in the establishment of other compensatory binding interactions or maximize the interaction between the weak base and the carboxylic acid of Asp189. In this paper, we will describe how we used this approach to demonstrate the utility of simple azoles, specifically imidazoles and aminothiazoles, as P1 ligands in the design of noncovalent small molecule thrombin inhibitors.

Replacement of the basic P1 aminocyclohexane moiety in the potent dipeptide thrombin inhibitor **1**

Keywords: Thrombin; Anticoagulant.
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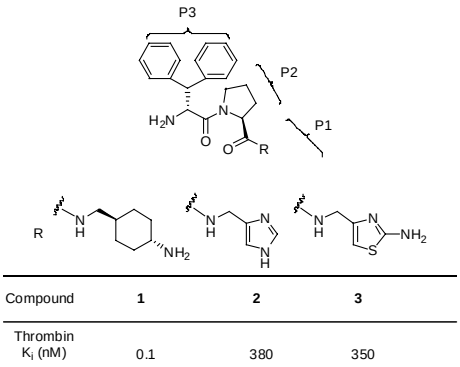


Compound	1	2	3
Thrombin K_i (nM)	0.1	380	350

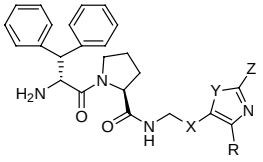
Figure 1. Effect on thrombin inhibitory potency of directly replacing a basic aminoalkyl P1 ligand in a key dipeptide inhibitor **1** with weakly basic heterocycles, imidazole and aminothiazole.

(K_i = 0.10 nM) with either an imidazole as in **2** (K_i = 380 nM) or an aminothiazole as in **3** (K_i = 350 nM) resulted in more than a 3000-fold loss in binding potency (Fig. 1). While these potency decreases could be partly rationalized in terms of reduced P1 ligand basicity (aminocyclohexane pK_a 9.8, imidazole pK_a 7.2, and aminothiazole pK_a 5.4) and the concomitant weakening of the electrostatic interaction with the carboxyl group of Asp189 in the thrombin specificity pocket, structural considerations, specifically the trajectory of the Asp189 to P1 ligand interaction was also assumed to be a contributing factor. Azoles have one ring atom less than a cyclohexane and therefore the exocyclic nitrogen atom of the aminothiazole in **3** cannot occupy a similar position in proximity to Asp189 in the thrombin S1 specificity pocket as the nitrogen of the aminocyclohexane in **1** without potentially perturbing key P3P2 enzyme–inhibitor binding interactions. In the case of the imidazole group in **2**, neither of the two ring nitrogens can be positioned to interact directly with Asp189 without likewise potentially disrupting beneficial P3P2 binding interactions. Based on this analysis, we concluded that one approach to compensating for these inherent structural restrictions would be to lengthen and thereby increase the flexibility of the chain connecting the azole to the proline portion of the inhibitor. The effects of these modifications on thrombin binding potency are summarized below in Table 1.

Addition of an extra methylene spacer to the imidazole in **2** (K_i = 380 nM) and aminothiazole in **3** (K_i = 350 nM) provided only modest potency increases [**5** (K_i = 200 nM) and **6** (K_i = 193 nM), respectively]. However, insertion of an additional methylene group in **5** gave **7** (K_i = 17 nM) which is 12-fold more potent. With the azole to Asp189 binding interaction presumably enhanced by their increased proximity and relaxed trajectory of interaction, we explored the effect of introducing conformational constraints along the three-carbon tether in imidazole containing inhibitor **7**. Replacement of the ethylene spacer by an acetylene gave **8** (K_i = 13 nM), whereas a *trans*-alkene gave **10** (K_i = 2 nM). This lengthened and *trans*-constrained imidazole containing inhibitor **10** is 190-fold more potent than the original imidazole containing inhibitor **2**, presumably reflecting both geometric optimization of the imidazole interaction with Asp189 and simultaneous maintenance of P3P2 interactions of the inhibitor with the enzyme.

Addition of a methyl group to an imidazole generally results in a modest increase in basicity. The effect of this type of substitution was investigated as a potential means of increasing binding potency while still limiting gross increases in basicity. In the case of the aminoethyl imidazole compound **2** (K_i = 380 nM), addition of a methyl group caused a 6-fold increase in potency (**4**, K_i = 65 nM). A similar substitution on the imidazole in alkyne **8** (K_i = 13 nM) and *trans*-alkene **10** (K_i = 2 nM) resulted in a 2-fold improvement in potency (**9**, K_i = 6 nM and **11**, K_i = 1 nM, respectively). It is noteworthy that the optimized imidazole containing inhibitor **11** is only 10-fold less potent than the original lead aminocyclohexane containing inhibitor **1**, despite a roughly 2 log unit pK_a difference in basicity of the two

Table 1. Thrombin and trypsin inhibition constants for dipeptide compounds **2**–**13**



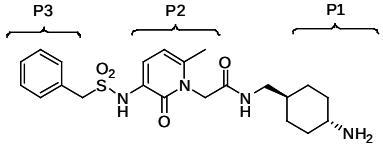
Compound	X	Y	Z	R	Thrombin K_i (nM)	Trypsin K_i (μM)
2	None	NH	H	H	380	36
3	None	S	NH_2	H	350	59
4	None	NH	H	CH_3	65	9.6
5	CH_2	NH	H	H	200	2.3
6	CH_2	S	NH_2	H	193	59
7	CH_2CH_2	NH	H	H	17	0.24
8	C≡C	NH	H	H	13	6
9	C≡C	NH	H	CH_3	6	7.2
10	*trans*-CH=CH	NH	H	H	2	0.14
11	*trans*-CH=CH	NH	H	CH_3	1	0.1
12	CH_2CH_2	S	NH_2	H	198	32
13	*trans*-CH=CH	S	NH_2	H	30	178

P1 groups. Similar P2P1 tether modifications in the aminothiazole series, while not achieving comparable levels of potency restoration, did further validate our original hypothesis by demonstrating a general increase in binding potency as a function of increased proximity of the aminothiazole to the carboxylate of Asp189 (cf. compounds **12** and **13**).

Although thrombin inhibitory potency was optimized in the dipeptide azole series, the reduced basicity of these compounds did not result in improved animal pharmacokinetics. For example, following oral dosing of inhibitor **11** in dogs at 5 mpk, the observed C_{max} was only 550 nM and the compound was very rapidly cleared. However, no in vivo toxicity was observed. More generally, the toxicity observed with aminocyclohexane containing inhibitors was absent with imidazole and aminothiazole containing inhibitors.

Compound **11** displayed excellent functional in vitro anticoagulant potency. The concentration of inhibitor required to double the activated partial thromboplastin time (APTT) in human plasma was 0.32 μM. Excellent in vivo antithrombotic efficacy was observed in the rat ferric chloride arterial thrombosis assay.[6] At an iv infusion rate of 10 μg/kg/min and $n = 6$, only one animal occluded.

Several of the imidazole and aminothazole P1 ligands which had been optimized for potency in the peptide series of thrombin inhibitors were also evaluated in our nonpeptide 3-aminopyridinone series (Table 2).[7a] Historically, we have found that these nonpeptide compounds have the advantage of improved in vivo pharmacokinetic properties relative to their corresponding dipeptide analogs.[7b] Compound **14** ($K_i = 4.6$ nM) was the baseline aminocyclohexane compound targeted for P1 substitution studies.



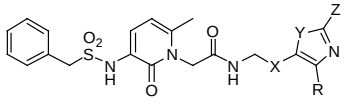
14 K_i 4.6 nM

As was the case in the peptide series, for both imidazole and aminothiazole P1 ligands, increasing the P2P1 tether from one carbon to three improved binding affinity. Thus, imidazole compound **16** ($K_i = 34$ nM) was 5-fold more potent than imidazole compound **15** ($K_i = 170$ nM) and aminothiazole compound **20** ($K_i = 450$ nM) was 2.5-fold more potent than aminothiazole compound **19** ($K_i = 1100$ nM). In contrast to the observation in the peptide series, the *trans*-alkenylimidazole and aminothiazole P1 ligands did not furnish potency enhancement in the nonpeptide series.

Methyl imidazole compound **18** ($K_i = 8$ nM) was the most potent imidazole analog in the pyridinone series. The 20-fold increase in potency relative to des-methylimidazole compound **15** is inconsistent with merely an increase in ligand basicity but more likely stems from an additional lipophilic binding interaction between the methyl group and the aliphatic side chain of Val-213 as previously observed in the X-ray structure of a methylaminopyridine P1 analog.[7b] In the absence of any X-ray structural data for methylaminothiazole P1 inhibitor **22** ($K_i = 10$ nM), we also speculate that the methyl group plays a similar role in enhancing potency relative to aminothiazole inhibitor **19** ($K_i = 1.1$ μM). It should also be noted that based on our initial structure–activity analysis, it was anticipated that the imidazole group in **18** would be incapable of reaching sufficiently far into the S1 pocket to interact directly with Asp189 without potentially disrupting beneficial P3P2 binding interactions with the enzyme. We speculate that compound **18** likely binds to Asp189 via the intermediacy of an ordered water molecule. Although we do not have an X-ray structure of compound **18** (Y = NH) bound to thrombin to confirm this, we do have a structure of a related analog (Y = NCH_2CONH^tBu) in which such a water mediated binding interaction is observed.[8]

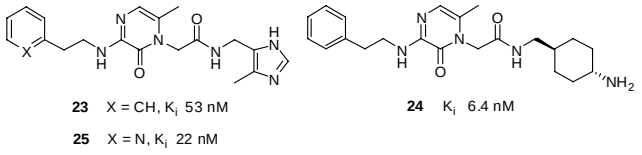
None of the pyridinone azole analogs were orally bioavailable. The optimized aminomethylimidazole P1 ligand in compound **18** was next evaluated in our 3-aminopyrazinone series of thrombin inhibitors.[9] The corresponding pyrazinone compound **23** ($K_i = 53$ nM) was found to be about 7-fold less potent. By contrast, the corresponding aminocyclohexane P1 containing pyrazinone compound **24** ($K_i = 6.4$ nM) was equipotent

Table 2. Thrombin and trypsin inhibition constants for 3-aminopyridinone imidazole and aminothiazole containing compounds **15**–**22**



Compound	X	Y	Z	R	Thrombin K_i (nM)	Trypsin K_i (μM)
15	None	NH	H	H	170	314
16	CH_2CH_2	NH	H	H	34	17
17	*trans*-CH=CH	NH	H	H	143	21
18	None	NH	H	CH_3	8	43
19	None	S	NH_2	H	1100	>1000
20	CH_2CH_2	S	NH_2	H	450	124
21	*trans*-CH=CH	S	NH_2	H	1800	>1000
22	None	S	NH_2	CH_3	10	6.5

with pyridinone analog **14** (K_i = 4.6 nM). The P3 group could be adjusted to compensate for these differences. Thus, replacing the phenyl group in **23** by a 2-pyridyl group gave **25** with 2-fold improvement in potency. With respect to animal pharmacokinetics, compound **23** was the first azole compound to display improved results. Following oral dosing in dogs at 4 mpk, the observed C_{max} was 1.4 μM and the half-life was 2 h.



The aminoalkylimidazoles **31** and **36** used in the preparation of inhibitors **11** and **18**, respectively, were prepared from similar starting materials (Fig. 2).[10] Commercially available 5-methylimidazole-4-carboxaldehyde **26** was first protected as its trityl derivative **27**. Wittig homologation gave ester **28**, which was reduced to the allylic alcohol **29** using DIBAL. Alcohol **29** was converted to an azide **30**, which was then reduced with triphenylphosphine to give the aminoalkylimidazole **31**. EDC mediated amide coupling to Boc–D-diphenylalanine–L-proline[3] followed by simultaneous acid promoted removal of the Boc- and trityl-protecting groups gave dipeptide imidazole inhibitor **11**. Commercially available acohol **33** was converted to amino alkylimidazole **36** by the same sequence of steps as described for aminoalkylimidazole **31**. Amide coupling with the requisite benzylsulfonamidopyridone acid[7a] followed by deprotection gave pyridinone imidazole inhibitor **18**. The aminothiazole analogs were prepared using similar functional group interconversions of suitably protected aminothiazole derivatives.[10]

We have shown that simple azoles, in particular imidazoles and aminothiazoles, can be incorporated into the design of noncovalent small molecule thrombin inhibitors as the P1 ligand. To compensate for the modest basicity of such groups, the ionic interaction with the carboxylic acid of Asp189 in the S1 specificity pocket of thrombin can be optimized by varying the distance and the geometric disposition of the ring nitrogen atoms relative to the carboxylate of Asp189. With relatively few exceptions, the azole compounds displayed very good selectivity for thrombin versus trypsin. The azoles furnished inhibitors free of the in vivo toxicity observed with more basic aminocyclohexane analogs. Improved oral bioavailability was observed only in the pyrazinone series. Antithrombotic efficacy was demonstrated in the pyridinone series. Further structure-driven optimization of these compounds with respect to thrombin binding affinity, functional in vitro anticoagulant potency, and in vivo antithrombotic efficacy will be presented in a subsequent manuscript.

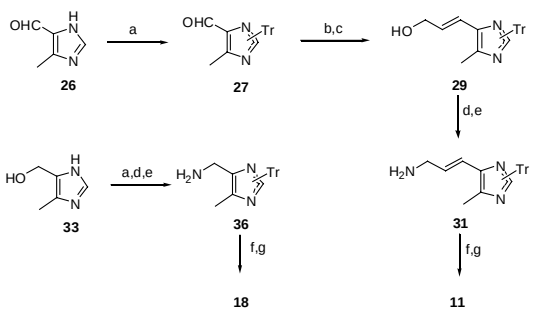


Figure 2. Synthesis of analogs **11** and **18**. Reagents and conditions: (a) TrCl, Et_3N, CH_2Cl_2; (b) $Ph_3P{=}CHCO_2Me$, toluene, reflux; (c) DIBAL, CH_2Cl_2, −78 °C; (d) $(PhO)_2PON_3$, DBU, THF; (e) Ph_3P, THF, water, 60 °C; (f) RCO_2H, EDC, HOBt, Et_3N, DMF; (g) Et_3SiH, TFA, CH_2Cl_2.

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