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SYNTHESIS OF CONFORMATIONALLY-RESTRICTED BOROPEPTIDE THROMBIN INHIBITORS

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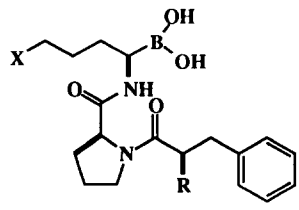
Abstract A series of boropeptide thrombin inhibitors was prepared in which the P3 residues of **2** (Ac-(*D*)-Phe-Pro-boroLys-OH · HCl) and **3** (3-Phenylpropionyl-Pro-boroLys-OH · HCl) were replaced by conformationally-restricted, benzoic acid-derived residues **4**. The potent binding affinity of the resulting inhibitors such as **10** may be due in part to a unique mode of binding in the thrombin active site.

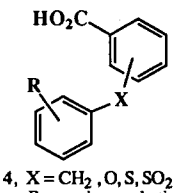
Thrombin, as the final serine protease in the blood coagulation cascade, displays a variety of biological activities that give it a crucial role in hemostasis and thrombosis. Consequently, the selective inhibition of thrombin has been vigorously pursued as a strategy for discovering potential antithrombotic agents.[1] We have previously demonstrated that boronic acid derivatives of peptides can be effective inhibitors of thrombin, having identified DuP 714 (**1**, Ac-(*D*)-Phe-Pro-boroArg-OH · HCl),[2] **2** (Ac-(*D*)-Phe-Pro-boroLys-OH · HCl)[3] and **3** (3-Phenylpropionyl-Pro-boroLys-OH · HCl)[4] as potent examples (Figure 1).

A comparison of the enzyme-bound conformations of DuP 714, **2** and **3**, determined by X-ray crystal structure analyses of their respective complexes bound to thrombin, revealed both similarities and differences in their binding modes.[3, 4] The major difference is seen in the interaction of the basic P1 side chains with Asp189 located in the S1 specificity pocket. The longer guanidino side chain of DuP 714 interacts with the Asp189 side chain directly through a bidentate hydrogen-bonding interaction, while the shorter lysine side chains of **2** and **3** interact with the Asp189 side chain indirectly through a bridging water molecule. Also, the NH of the (*D*)-Phe residues of DuP 714 and **2** participate in a hydrogen bond with the Gly216 backbone carbonyl of thrombin, an interaction that is absent in the complex of **3** with thrombin. Comparing the relative potencies of DuP 714, **2** and **3**, it appears that the guanidino P1 interaction with Asp189 accounts for nearly an order of magnitude of the greater potency of DuP 714 relative to the borolysine analogs, while the P3 hydrogen bond with Gly216 accounts for about three-fold of the greater potency of the (*D*)-Phe analogs relative to the 3-phenylpropionic acid analog **3**.

Figure 1. Lead Compounds and Benzoic Acid P3 Mimics



1, (DuP 714), R = -NHAc, X = -NHC(=NH)NH_2 · HCl
K_i = 0.04 nM
2, R = -NHAc, X = -CH_2NH_2 · HCl
K_i = 0.24 nM
3, R = H, X = -CH_2NH_2 · HCl
K_i = 0.80 nM



4, X = CH_2, O, S, SO_2
R = various substituents

In all of the complexes, the hydroxyl of the active site serine (Ser195) forms a tetrahedral complex with the boron of the inhibitor which mimics the transition state formed during hydrolysis of the natural substrate and substantially contributes to the potency of the boropeptide inhibitors.[5] Another interaction shared by DuP 714, **2** and **3** involves their P3 residues, which do not lie in their extended conformations, but rather are packed close to the P2 proline residue, allowing the aromatic ring to engage in a favorable edge-to-face interaction[6] with the side chain of Trp215 located at the base of the S3 specificity pocket.

As part of our thrombin inhibitor program, we sought to prepare compounds in which the P3 residues of **2** and **3** were replaced by novel, structurally diverse non-peptidic residues. The conformationally-restricted, benzoic acid-derived P3 mimics **4** were investigated for their novelty and for their potential, by varying the identity of the tether X and its point of substitution, for properly orienting the distal aromatic ring for participation in an edge-to-face interaction with Trp215 upon binding to thrombin. The greater rigidity of the benzoic acid P3 residues relative to the 3-phenylpropionyl residue in **2** might serve to preorganize the P3 residue and lead to more potent thrombin inhibitors.

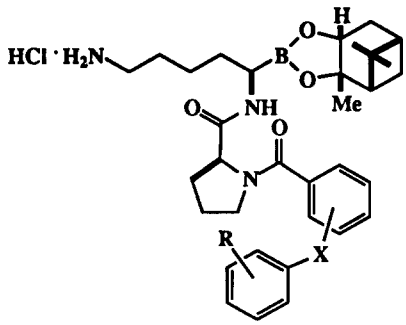
The thrombin binding results for a series of inhibitors are shown in Table 1.[7] Examples **5-9** were prepared to gain an idea of the optimal tether atom and its preferred position of substitution. In the methylene-bridged series the *ortho*- and *meta*-isomers, **5** and **6** respectively, were about an order of magnitude more potent than the *para*-isomer **7**. In the oxygen-bridged series the *ortho*- and *meta*-isomers, **8** and **9** respectively, were nearly equipotent with each other and with the methylene-bridged examples **5** and **6**. Although there was no clear preference between compounds **5**, **6**, **8** and **9**, we decided initially to focus on the *meta* methylene-bridged series because of the slightly better affinity of compound **6** and for the ease of synthesis of this series.

Representative examples of this series include compounds **10-17**, in which additional substituents were placed on the distal phenyl ring. All of these compounds are more potent than the reference compound **3**. In particular, the 2-CF_3 analog **10** and the 3,4-methylenedioxy analog **17** are about an order of magnitude more potent than **3**, and approach the potency of the boroarginine DuP 714 (K_i = 0.04 nM).

We also prepared the sulfur-bridged example **18**, which had better binding affinity than the methylene-bridged example **6**. Substitution on the distal aromatic ring in the sulfur-bridged series did not afford an increase in binding affinity as was observed for some of the methylene-bridged examples, although all of the compounds **19-21** are still more potent than **3**. Preparation of some sulfone-bridged examples, as in **22** and **23**, led to a decrease in activity relative to the corresponding sulfide, although these compounds still compare favorably to the reference compound **3**.

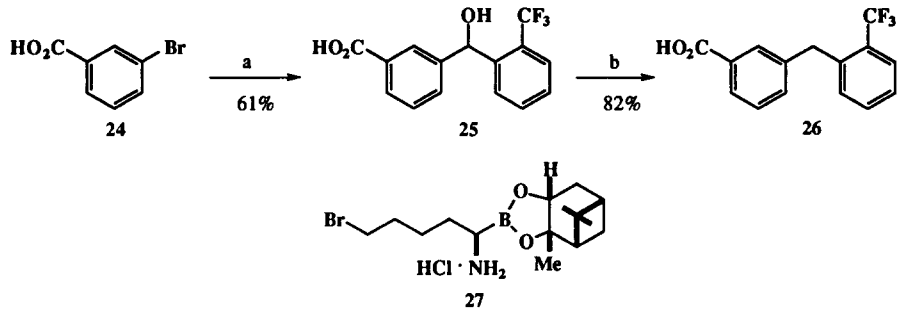
The synthesis of these inhibitors involved initial preparation of the P3 benzoic acids followed by elaboration to their corresponding boropeptides. The *meta* methylene-bridged benzoic acids were prepared as outlined in Scheme 1 for the 2-trifluoromethyl residue contained in **10**. The dianion of 3-bromobenzoic acid **24**[8] was generated with 1 eq of *n*-BuLi and 2 eq of *t*-BuLi and then 2-(trifluoromethyl)benzaldehyde was added to afford the alcohol **25**.[9] Reduction to the required methylene **26** was readily accomplished with triethylsilane and trifluoroacetic acid.[10] The elaboration of **26** to its boropeptide derivative **10** followed an established synthesis route[11] in which the source of the α-aminoboronate was optically pure (+)-pinanediol (*S*)-1-amino-5-bromopentyl boronate hydrochloride **27**, prepared from 4-bromo-1-butene by the asymmetric homologation procedure of Matteson.[12]

Table 1. Binding results of Bridged Benzoic Acid-containing Inhibitors



CMPD#	X	Position	R	K_i (nM)
2	-	-	-	0.24
3	-	-	-	0.80
5	CH_2	*ortho*	H	0.29
6	CH_2	*meta*	H	0.19
7	CH_2	*para*	H	1.8
8	O	*ortho*	H	0.27
9	O	*meta*	H	0.36
10	CH_2	*meta*	2-CF_3	0.07
11	CH_2	*meta*	2-CH_3	0.25
12	CH_2	*meta*	2-SCH_3	0.50
13	CH_2	*meta*	2-Br	0.23
14	CH_2	*meta*	3-F	0.43
15	CH_2	*meta*	3-CF_3	0.16
16	CH_2	*meta*	4-CF_3	0.22
17	CH_2	*meta*	3,4-(-OCH_2O-)	0.09
18	S	*meta*	H	<0.10
19	S	*meta*	2-CF_3	0.45
20	S	*meta*	2-OCH_3	0.19
21	S	*meta*	4-OCH_3	0.42
22	SO_2	*meta*	H	0.85
23	SO_2	*meta*	2-OCH_3	0.58

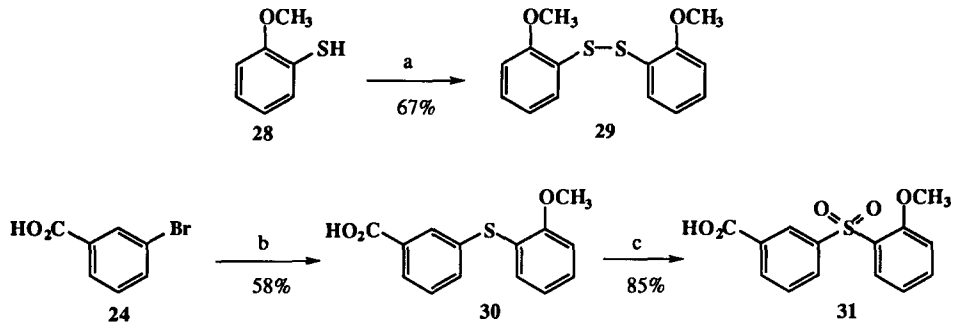
Scheme 1. Synthesis of *meta* Methylene-bridged Benzoic Acids



Reagents: (a) i) *n*-BuLi (1 eq), THF, -100 °C ii) *t*-BuLi (2 eq) iii) 2-(F_3C)C_6H_4CHO (b) Et_3SiH (1.5 eq), TFA, 25 °C

The sulfur and sulfone-bridged benzoic acids were prepared as outlined in Scheme 2 for the 2-methoxy analog contained in **20** and **23**. The thiol **28** was air oxidized to its disulfide **29**,[13] which was then added to the dianion of **24** to give the diaryl sulfide **30**. Oxidation to the sulfone **31** was conveniently carried out using oxone.[14] Both the sulfide **30** and the sulfone **31** were elaborated to their respective boropeptide analogs as described for the methylene-bridged series.

Scheme 2. Synthesis of *meta* Sulfur-bridged Benzoic Acids



Reagents: (a) Air, Al_2O_3, toluene (b) i) *n*-BuLi (1 eq), THF, -100 °C ii) *t*-BuLi (2 eq) iii) **29** (c) oxone (4 eq), MeOH/H_2O, 0 °C to 25 °C

The inhibitors listed in Table 1 are noteworthy for their extremely potent binding affinity for thrombin. Many of the inhibitors are more potent than compound **2**, which has Ac-(*D*)-Phe as its P3 residue. Ac-(*D*)-Phe has been shown repeatedly to be a very effective P3 residue for binding to thrombin.[1] Indeed, recent NMR evidence indicates that inhibitors that contain (*D*)-Phe-Pro residues in P2P3, like DuP 714, have a solution conformation that is similar to their thrombin-bound conformation, so that they are essentially preorganized for enzyme binding.[15] The P3 residues described above result in compounds that are up to three-fold more potent than compound **2**, despite the absence of an important hydrogen bond to Gly216 in the S3 pocket. These results could not be rationalized by assuming a similar binding conformation to **2**, as the bridged biaryls, in light of the NMR results described above and the results described in our accompanying paper,[4] would not be expected to account for the dramatic increases in potency even if the P3 residue is preorganized in a conformation allowing an effective edge-to-face interaction with Trp215. We believed that these compounds either had an overall different binding conformation than **2** or that these novel P3 residues were picking up other favorable interactions in the S3 pocket that are not possible with the Ac-(*D*)-Phe residue. We turned to X-ray crystallography to attempt to explain these results.

Unfortunately, attempted X-ray crystallography of the complex of **10** bound to thrombin failed to produce a solution with continuous density in the P3 region of the inhibitor. The P1 region, however, showed continuous electron density extending deep into the S1 pocket and suggested a rationale for the high affinity of this series of inhibitors. This rationale is illustrated in cartoon form in Figure 2. The direct interaction of boroarginine side chains with Asp189 depicted in **I** is in contrast to the weaker interaction of borolysine side chains generally

observed which occurs through a bridging water molecule, as depicted in **II**.[3] The P1 region of compound **10** showed the borolysine side chain extending deeper into the S1 specificity pocket than has been observed for other borolysine inhibitors. Continuous density was observed for the side chain, with no evidence of a bridging water molecule between the amino group and the Asp189 side chain. These results are consistent with the idea that the amino residue has displaced the bridging water molecule and interacts directly with the Asp189 side chain, as shown by **III**. This interaction is entropically favorable relative to **II** and is thought to account for the increased binding affinity of this class of inhibitors relative to inhibitors such as **2** and **3**. The steric bulk of the bridged biaryl P3 residues is thought to account for this change in the P1 binding mode.

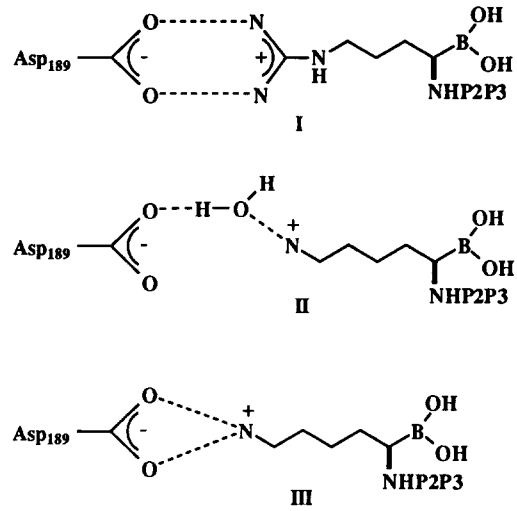


Figure 2. Cartoon rendition of the P1 binding modes of DuP 714 (I), borolysine **2** (II) and borolysine **10** (III). Dashed lines indicate hydrogen bonds and charge-charge interactions. Hydrogens not shown for clarity. The figure is meant to provide information about the basic side chains only and not about the binding of the boronic acid residue.

Further evidence for a different P1 binding mode was obtained when the boroarginine analog of compound **10** was prepared and found to have a thrombin K_i of 0.4 nM. The weaker affinity of the boroarginine analog relative to the borolysine **10** is in direct contrast to our previous observations that boroarginine analogs are generally about an order of magnitude more potent toward thrombin than the corresponding borolysine analogs. If the bulky P2P3 portion of these inhibitors are forcing the P1 residue deeper into the S1 pocket, as we have proposed above, the longer boroarginine P1 side chain may be too close to the Asp189 residue to allow for optimum interaction, while the shorter borolysine side chain may be able to interact effectively as shown in Figure 2 (complex **III**) above.

Also of note is the contrast between the thrombin binding affinity of this series of inhibitors and the homolysine analog of **2**, which has a thrombin K_i of 8.1 nM.[3] The X-ray crystal structure of the complex of the homolysine bound to thrombin reveals that the P1 amino side chain interacts directly with the Asp189 side chain, as we have observed with compound **10**. The 30-fold weaker binding affinity of the homolysine analog relative

to **2** is thought to be due to the increased flexibility of the homolysine side chain. Its binding requires the immobilization of an additional methylene unit which, apparently, more than offsets the advantage gained by displacement of water from the S1 pocket and direct binding to Asp189. The inhibitors described here have the advantage of direct binding to Asp189 without the compromise of an additional methylene unit to be immobilized.

In summary, we have prepared a series of thrombin inhibitors in which the Ac-(*D*)-Phe P3 residue present in **2** has been replaced by a variety of benzoic acid-derived residues **4**. The resulting compounds are extremely potent thrombin inhibitors, with K_i values up to three-fold more potent than **2** and approaching the potency of the boroarginine **1**. The data regarding these novel P3 residues is inconsistent with their dramatic effects on potency being due only to effective preorganization of the P3 aromatic ring or only to better complimentarity in the S3 pocket. Rather, the data support the hypothesis that inhibitors containing these P3 residues adopt a unique binding mode which allows for the direct interaction of the P1 amino side chain with the Asp189 side chain in the S1 specificity pocket. Further X-ray crystallographic work to confirm this hypothesis is ongoing.

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References and Notes

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