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DESIGN AND SYNTHESIS OF RING-CONSTRAINED BOROPEPTIDE THROMBIN INHIBITORS

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Abstract Ring-constrained boropeptide thrombin inhibitors were designed using information from the X-ray crystal structure of 1 (3-Phenylpropionyl-Pro-boroLys-OH \cdot HCl) bound to thrombin. The constraints utilized cyclohexane and pyrrolidine rings to preorganize an aromatic ring in an orientation allowing optimum edge-to-face interaction with the tryptophan 215 side chain located in the S3 specificity pocket of thrombin.

The serine protease thrombin catalyzes the conversion of fibrinogen to fibrin, the last enzyme-mediated step in the blood coagulation cascade. Fibrin contributes to clot formation by cross-linking platelets which aggregate at the point of blood vessel injury. Additionally, thrombin is a potent activator of platelets and also potentiates its own production by activation of factor V and factor VIII. These and other biological activities demonstrate the central role of thrombin in hemostasis and thrombosis and have led to an intense pursuit of thrombin inhibitors as potential antithrombotic agents.¹

We have previously demonstrated that boronic acid derivatives of peptides can be effective inhibitors of serine proteases.^{2a,b} Recently, the peptide boronic acids 1 (3-Phenylpropionyl-Pro-boroLys-OH · HCl) and 2 (3-[3-Trifluoromethyl]phenylpropionyl-Pro-boroLys-OH · HCl) were identified as potent, orally active inhibitors of thrombin (Figure 1).^{2c} An X-ray crystal structure analysis of the complex of 1 bound to thrombin showed the expected interaction of the amino side chain with Asp189 in the S1 specificity pocket and the tetrahedral complex between the hydroxyl of the active site serine (Ser195) and the boron of the inhibitor.³ This complex mimics the transition state formed during natural substrate hydrolysis and strongly contributes to the observed potency of this class of inhibitors. Another important interaction involves the 3-phenylpropionyl (P3) residue, which is kinked out of its extended conformation, placing the phenyl ring in the vicinity of the proline residue and allowing it to form a favorable edge-to-face interaction⁴ with Trp215 located at the base of the S3 specificity pocket.

Figure 1. Lead Compounds and Ring-Constrained 3-Arylpropionic Acid Mimics



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As part of our thrombin inhibitor program, we have used this X-ray crystal structure information to guide the design of ring-constrained inhibitors.⁵ Specifically, we sought to maximize the edge-to-face aromatic interaction with Trp215 observed in the S3 pocket. We have designed rigidized mimics for the 3-arylpropionic acid P3 residue which, when incorporated into the boropeptide, rely on a cyclohexane or pyrrolidine template to hold an aromatic ring near the proline residue in approximately the same spatial orientation as it occupies in the Xray crystal structure of the 1:thrombin complex (Figure 1). The diequatorially-substituted (1R, 2R)-2phenylcyclohexane carboxylic acid 3 appeared to allow excellent phenyl ring overlap when its minimized conformation was compared with the bound conformation of the P3 residue of 1. Similarly, the (3S, 4R)-4arylpyrrolidine-3-carboxylic acids 4 also showed good overlap between the aromatic rings, although it appeared to be less favorable due to the flatter conformation and different bond angles of the pyrrolidine ring. Potential advantages of this template relate to its greater flexibility and to the structural diversity which can be introduced onto the pyrrolidine nitrogen, both of which were expected to influence its conformation and possibly compensate for what initially looked to be a less favorable overlap between the aromatic rings. In each series it was also of interest to prepare inhibitors containing the enantiomers of each template as a test of our binding hypothesis.

The synthesis of the cyclohexane-based inhibitors begins as outlined in Scheme 1. Suzuki coupling⁶ of phenylboric acid with the enol triflate derived from the β -ketoester 5 afforded the unsaturated ester 6.⁷ Hydrogenation over 10% Pd/C catalyst gave the saturated *cis*-ester (±)-7, which underwent essentially complete epimerization and subsequent ester saponification to afford the racemic *trans*-2-phenylcyclohexane carboxylic acid (±)-8. Coupling of this racemate with proline methyl ester hydrochloride, followed by saponification, gave the expected 1:1 mixture of diastereomers 9 and 10.



Reagents: (a) NaH, PhN(SO₂CF₃)₂, THF/DMSO, 0^o C to 25^o C (b) PhB(OH)₂, 3 mol% Pd(PPh₃)₄, 2M aq Na₂CO₃, C₆H₆/EtOH, 80^o C (c) H₂ (1 atm), 10% Pd/C, EtOH (d) NaOEt, EtOH, reflux (e) KOH, MeOH/H₂O, reflux (f) Pro-OMe hydrochloride, DCC, HOBT, Et₃N, THF (g) LiOH \cdot H₂O, THF/H₂O (h) (*R*)- α -phenylethylamine, MeOH (i) filter solid (j) H₃O⁺

Scheme 1

Stereochemical assignment required the resolution of the enantiomers of (\pm) -8, which was accomplished using (R)- α -phenylethylamine, giving the desired (1R, 2R) enantiomer 3 with good optical purity.⁸ Coupling of 3 with proline methyl ester hydrochloride and saponification cleanly produced diastereomer 10, allowing for the definitive stereochemical assignments of 9 and 10. The elaboration of 9 and 10 to their respective boropeptide derivatives 14 and 13 followed an established synthesis route⁹ involving initial mixed anhydride coupling with optically pure (+)-pinanediol (S)-1-amino-5-bromopentyl boronate \cdot hydrochloride 12, which was prepared from 4-bromo-1-butene by the asymmetric homologation procedure of Matteson.¹⁰ Figure 2 shows 13 and 14 along with a comparison of their inhibitory activities relative to 1.





*The (+)-pinanediol ester of 14 is hydrolyzed to the free boronic acid under the assay conditions. Throughout this manuscript, the inhibitory constant (K_i) assays were performed as described in reference 2a and the K_i values reported are averages from multiple experiments.

It was gratifying that the desired (1R, 2R) isomer 13 is about two orders of magnitude more potent in its affinity for thrombin than the (1S, 2S) isomer 14. Presumably, the P3 aromatic ring of 14 is locked into a conformation that does not allow effective edge-to-face interaction with Trp215 and, given the magnitude of the potency difference, may be locked into a conformation which results in destabilizing steric interactions with the enzyme. The desired (1R, 2R) isomer 13 showed a modest increase in binding affinity relative to 1. We were able to obtain an X-ray crystal structure of 13 bound to thrombin which confirmed our proposed binding hypothesis. This is shown in Figure 3, where the thrombin-bound conformations of 1 and 13 are superimposed.¹¹ The figure shows the excellent overlap of the P3 phenyl rings between the two inhibitors and also how they are engaged in an edge-to-face interaction with Trp215. We might have expected a greater increase in affinity for 13, given the apparent flexibility of the 3-phenylpropionyl P3 residue in 1. However, recent NMR evidence of related boropeptides which contain (D)-Phe-Pro as the P2P3 residue indicate that these inhibitors adopt a solution conformation that is similar to the thrombin-bound conformation.¹² This preorganization appears to involve stacking between the phenyl and proline rings which is stabilized by π - π interactions between the phenyl ring and the (D)-Phe-Pro amide bond. It is conceivable that 1 may be preorganized in the same manner, resulting in the observed small difference in binding potency between 13 and 1.

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Figure 3. Superposition of the thrombin binding conformations of 1 (yellow) and 13 (red). Highlighted residues of thrombin, clockwise from upper right, are Ser195, Asp189 and Trp215.

The pyrrolidine-based inhibitors were prepared as shown for the 3-trifluoromethyl analog in Scheme 2. The key step involved the [3+2] cyclization of the azomethine ylide derived from 16^{13} with the methyl cinnamate 15 to establish the racemic 3,4-*trans*-disubstituted pyrrolidine (±)-17. N-protecting group exchange and saponification led to the acid (±)-18. Coupling with proline methyl ester hydrochloride and saponification produced the expected 1:1 mixture of diastereomers 19 and 20. These diastereomers were separately elaborated to their respective borolysine derivatives as described for the cyclohexane series.



Reagents: (a) TFA, CH_2Cl_2 (b) H_2 (1 atm), 10% Pd/C, HCl, MeOH (c) (BOC)₂O, diisopropylethylamine, CH_2Cl_2 (d) LiOH \cdot H₂O, THF/H₂O (e) Pro-OMe hydrochloride, HBTU, diisopropylethylamine, DMF

Stereochemical assignment of the diastereomers 19 and 20 was accomplished as outlined in Scheme 3. Cuprous chloride-mediated¹⁴ reaction of (1S)-(-)-2,10-camphorsultam 21¹⁵ with *trans*-3-trifluoromethylcinnamoyl chloride afforded the sulfonamide 22, which underwent [3 + 2] azomethine ylide cyclization to produce an unoptimized 7:1 mixture of readily separable diastereomers 23 and 24. An X-ray crystal structure analysis of 23 confirmed the desired (3S, 4R) stereochemistry.^{16,17} Subsequently, 23 was easily converted into 20, which allowed for the definitive stereochemical assignments of diastereomers 19 and 20.



Reagents: (a) i) NaH, toluene ii) *trans*-3-(trifluoromethyl)cinnamoyl chloride, CuCl (b) 16, TFA, CH₂Cl₂ (c) H₂ (1 atm), 10% Pd/C, (BOC)₂O, MeOH (d) LiOH \cdot H₂O, THF/H₂O (e) Pro-OMe hydrochloride, EDC, HOBT, Et₃N, THF

The binding results for the pyrrolidine-constrained inhibitors are presented in Table 1. The desired (3S, 4R) isomers 27 and 29 were more active than the (3R, 4S) isomers 26 and 28, although in this series the binding difference between isomers was not as dramatic as in the cyclohexane series (one order of magnitude vs. two orders of magnitude). These results may reflect the greater flexibility of the pyrrolidine series in that the undesired

Table 1. Binding Results of Pyrrolidine-constrained Inhibitors vs. 2

н	CMPD#	isomer	R	Ar	K _i (nM)
	2	-	-	-	0.78
\sim \uparrow \circ \rightarrow \downarrow \circ \rightarrow \downarrow \circ \circ \rightarrow \downarrow \circ	26	(3 R ,4S)	BOC	3-CF ₃ C ₆ H ₄	3.1
	27	(3S,4R)	BOC	3-CF ₃ C ₆ H ₄	0.32
	28	(3 <i>R</i> ,4 <i>S</i>)	H HCI	3-CF ₃ C ₆ H ₄	5.6
	29	(3 <i>S</i> ,4 <i>R</i>)	H · HCl	3-CF ₃ C ₆ H ₄	0.5

isomers may be better able to adopt a conformation which minimizes severe steric interactions. Both the N-BOCprotected 27 and the pyrrolidine 29 are about two-fold more potent than the reference compound 2. Given the opposite polarities of the added functionality in the pyrrolidine 29 and the cyclohexane 13, that both constraints result in a two-fold increase in potency relative to their unconstrained counterparts suggests that the potency increase is a preorganization effect and is not due to interactions of the added functionality in the S3 pocket.

In summary, we have prepared ring-constrained boropeptide thrombin inhibitors which were designed to optimize an aromatic edge-to-face interaction observed in the X-ray crystal structure of 1 bound to thrombin. The results are an illustration of the effectiveness of using X-ray crystallography data in drug design, as the targeted compounds showed improved binding potency relative to the unconstrained lead compounds. This increase in binding affinity is notable because of the evidence that compounds like 1 and 2 may already be preorganized for binding as a result of the stacking between the P3 aromatic moiety and the P2 proline residue.

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