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Novel Bicyclic Lactam Inhibitors of Thrombin: Potency and Selectivity Optimization through P1 Residues

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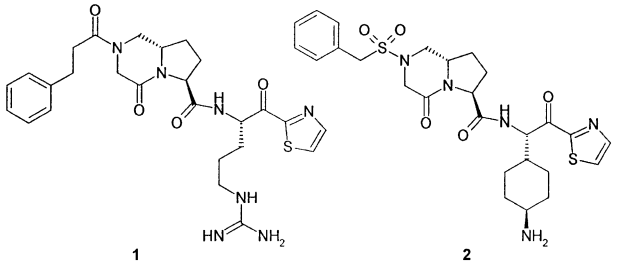
Abstract—Peptidomimetic inhibitors of thrombin lacking the important Ser195–carbonyl interaction have been prepared. The binding energy lost after the removal of the activated carbonyl was recaptured through a series of modifications of the P1 residues of the bicyclic lactam inhibitors. Selected substituted compounds displayed useful pharmacological profiles both in vitro and in vivo.

We have recently published the preparation and biological activity of **1**[1] and **2**,[2] which are examples of bicyclic lactam inhibitors of thrombin with high potency and in vivo activity. Most of the inhibitors prepared in the early studies leading to **1** and **2** were designed to capture the interactions of the catalytic triad of thrombin with the inhibitors. However, due to the reactive nature of the carbonyl function of the P1 residue, an improvement in inhibitor design was envisaged by excising the ketothiazole functions of inhibitors **1** and **2**.[3] As a result of this removal, we expected a dramatic reduction in thrombin binding affinities.



1 **2**

We planned to compensate for this reduction in potency by incorporating additional chemical features in the inhibitors, primarily through introduction of various P1 residues. In this paper, we will describe our efforts in this direction.

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The synthesis of the bicyclic template with numerous P3 hydrophobes has been already described.[1] The preparations of the different P1 moieties of the inhibitors are exemplified in Schemes 1–4. The preparation of the suitably protected *trans*-4-aminomethyl-cyclohexylamine (entry **28**, Table 1) has been reported elsewhere.[4]

A common intermediate was used for the preparation of the different 4-aminomethyl-piperidines based inhibitors **7** and **8**, as illustrated in Scheme 1. Thus, the amino group of isonipecotic acid **3** was first protected as its *t*-butyl carbamate. The acid group was then reduced using BH_3THF, to give the protected aminoalcohol **4**. Mesylation of **4**, followed by displacement with NaN_3 in anhydrous DMF afforded an azide, which was submitted to hydrogenolysis to give amine **5**. This amine was coupled with the suitable bicyclic template using conventional peptide coupling reagents (HATU, collidine, and DMF) and the BOC protecting group was removed by treatment with 4 N HCl in dioxane. Piperidine salts **6** were converted to guanidines **7** using the appropriate guanylating agent, followed by deprotection and reverse phase HPLC purification. Intermediates **6** could also be converted to a cyanamide, which in turn was transformed into *N*-hydroxyguanidine **8**.[5]

The synthesis of the benzimidazolyl–methylamine is described in Scheme 2. Commercially available acid **9** was first esterified, after which the amine was tritylated. Ester **10** was then reduced ($LiAlH_4$, THF) and the

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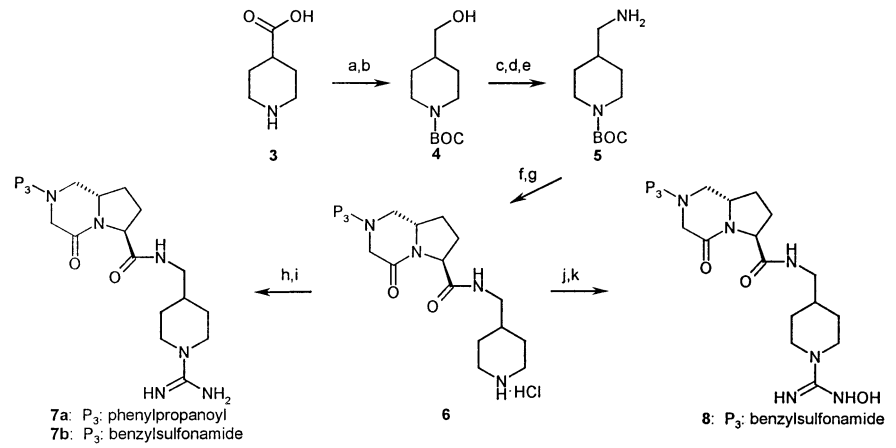
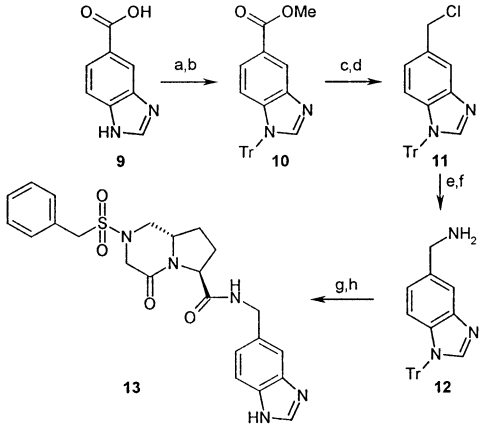
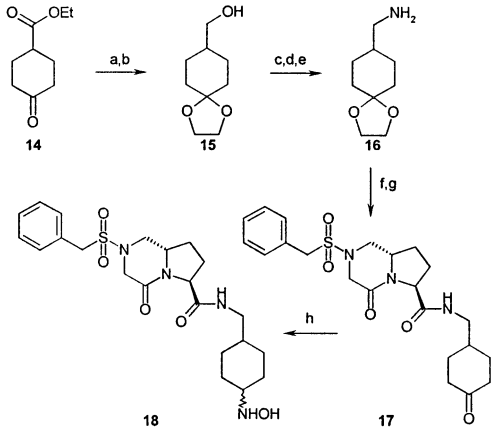


Scheme 1. (a) $(BOC)_2O$, CH_2Cl_2, 100%; (b) BH_3·THF, THF, 100%; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C; (d) NaN_3, DMF, Δ, 95% (two steps); (e) H_2, Pd/C 10%, MeOH, 100%; (f) bicyclic-CO_2H, HATU, 2,4,6-collidine, 95%; (g) HCl in dioxane, 100%; (h) *N*,*N*-(*t*-butoxycarbonyl)-1*H*-pyrazole-carboxamidine, *i*Pr_2NEt, DMF, 82%; (i) 4 N HCl in dioxane, 70%; (j) BrCN, CH_3CO_2Na, MeOH, 80%; (k) NH_2OH·HCl, Na_2CO_3, MeOH, 87%.



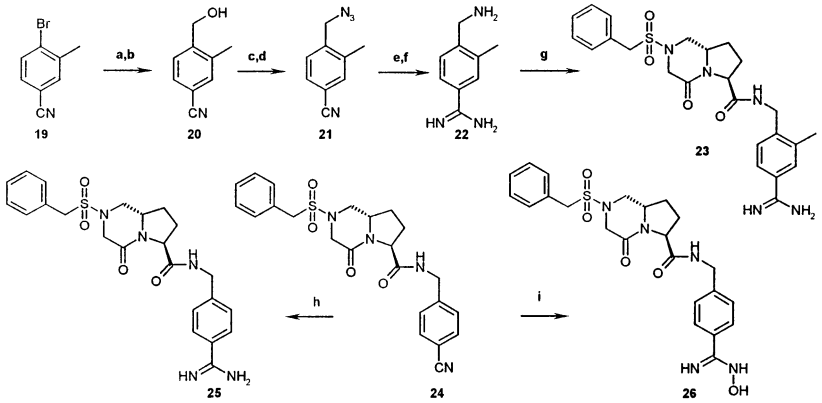
Scheme 2. (a) EDC, DMAP, MeOH, 26%; (b) Tr–Cl, TEA, CH_2Cl_2, 77%; (c) $LiAlH_4$, THF, 72%; (d) $SOCl_2$, CH_2Cl_2, 0 °C, 84%; (e) NaN_3, DMF, Δ, 77%; (f) H_2, Pd/C 10%, MeOH, 100%; (g) Bicyclic-CO_2H, HATU, 2,4,6-collidine, DMF, 65%; (h) TFA/MeOH/$CHCl_3$, 51%.



Scheme 3. (a) Ethylene glycol, pTSA, benzene, Δ, 85%; (b) Super-hydride, THF, −78→0 °C; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C; (d) NaN_3, DMF, Δ; 62% (three steps); (e) H_2, Pd/C 10%, MeOH, 22%; (f) bicyclic-CO_2H, HATU, 2,4,6-collidine, DMF, 100%; (g) PPTS, acetone/H_2O, 57%; (h) (i) NH_2OH, MeOH; (ii) $NaBH_3CN$, AcOH, 26%.

obtained alcohol transformed into chloride **11**. Displacement of the chloride with NaN_3 in DMF, followed by hydrogenolysis of the azide afforded amine **12**, which was coupled with the bicyclic template. Removal of the trityl protecting group gave the benzimidazole compound **13**.

Scheme 3 describes the synthesis of the aminomethyl–cyclohexyl–hydroxylamine. Commercially available ketone **14** was first protected as an acetal, and the ester group was reduced to give alcohol **15**. The alcohol was then transformed into amine **16** as in Scheme 1.

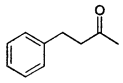
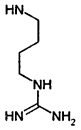
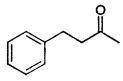
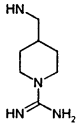
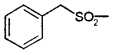
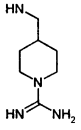
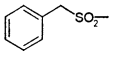
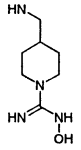
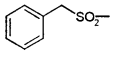
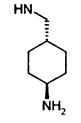
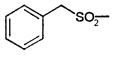
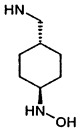
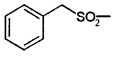
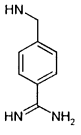
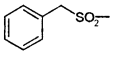
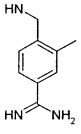
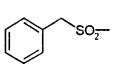
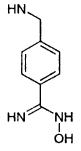
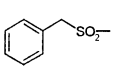
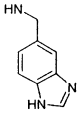


Scheme 4. (a) BuLi, DMF, THF, −100 °C, 86%; (b) $NaBH_4$, EtOH, 96%; (c) MsCl, DIPEA, CH_2Cl_2; (d) NaN_3, DMF, Δ, 95% (two steps); (e) (i) HCl in EtOH, 4 °C; (ii) NH_3 in EtOH; (f) H_2, Pd/C 10%, MeOH; (g) bicyclic-CO_2H, HATU, NMM, DMF, 36% (three steps); (h) (i) HCl in EtOH, 4 °C; (ii) NH_3 in EtOH, 70%; (i) NH_2OH·HCl, Na_2CO_3, MeOH, 26%.

Coupling of amine **16** with the bicyclic template followed by hydrolysis of the acetal lead to ketone **17**, which was transformed into hydroxylamine **18** by reductive amination.

Finally, the preparation of the phenylamidines is described in Scheme 4. Bromide **19** was first lithiated, and the anion formed was formylated with DMF. The aldehyde produced was then reduced with $NaBH_4$ to give alcohol **20**. Transformation of the cyano group of **21** into the amidine group was done according to a published procedure.[6] Formation of amine **22** and coupling with the bicyclic template was accomplished as in Scheme 1. Cyanobenzyl amine **24** from the coupling of the bicyclic template with commercially available 4-cyanobenzyl amine, was transformed into amidine **25** and amidoxime **26** in modest to good yield.

Table 1. In vivo potency and selectivity

Compd	P3	P1	$K_{i\ thr}$ (nM)	Sel[b]
27[a]			900	—
7a			180	—
7b			78	51
8			14	296
28			100	250
18			1100	1
25			8	5
23			7	11
26			>10,000	—
13			1000	16

[a]All new target compounds were characterized by ^1H NMR, reverse-phase HPLC and mass spectroscopy.
[b]$K_{i\ trypsin}/K_{i\ thrombin}$.

Inhibition of the amidolytic activity of thrombin (K_i) and in vivo coagulation parameters in the rat arterial thrombosis model such as the mean occlusion time (MOT), the activated partial thromboplastin time (aPTT) and the thrombin time (TT) were measured according to an already published procedure.[7]

Diverse range of P1 residues explored are presented in Table 1. The starting point of the SAR was inhibitor **27** ($K_i = 900$ nM), where the ketothiazole moiety of **1** ($K_i = 0.6$ nM) has been removed completely.[8] As expected, the binding affinity of this inhibitor was rather low, as one of the major interactions with thrombin has been nullified, namely, the interaction between the electrophilic carbonyl and Ser195. The inhibitor **7a** displayed ~5-fold improvement in potency. This improved binding affinity of the inhibitor could be due to the higher rigidity of the P1 group, and hence better alignment of the guanidine group, and also to the additional hydrophobic interactions introduced by the piperidine group. Additional improvement in potency was attainable by replacement of the phenylpropanoyl group with the benzyl sulfonyl group (**7b**). Replacement of the amidine fragment of the P1 residue by amidoxime significantly improved the potency (compare **8** and **7b**). In order to explain this unexpected improvement in potency, compound **8** was co-crystallized with thrombin (Fig. 1).[9] We can clearly see in Figure 1, that the benzylsulfonamide group penetrates deeply in the S3 pocket of thrombin and also that the Ser195 is no more implicated in the binding of the inhibitor. The amidine fragment makes a salt bridge with Asp189 and the presence of the amidoxime OH group forms an hydrogen bond with Tyr22B in the S1 pocket. This additional and unprecedented interaction may explain the ~5- to 6-fold improved potency of inhibitor **8** compared to inhibitor **7b**.

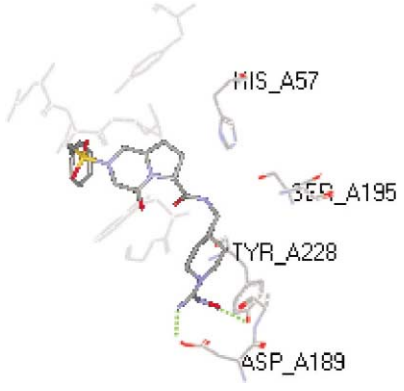


Figure 1.

Table 2. In vivo parameters

Compd	Rat arterial thrombosis model[a]		
	MOT[b]	APTT[c]	TT[d]
8	48±13	38±5	231±29
25	>60	58±11	455±64
23	46±10	54±14	393±102

[a]Dose: intravenous bolus dose (0.75 mg/kg) followed by an infusion (50 μg/kg/min).
[b]Mean occlusion time in min (control: 17/19 min).
[c]Activated partial thromboplastin time in seconds (control: 20/22 s).
[d]Thrombin time in seconds (control: 40/45 s).

The cyclohexylamine and piperidylamidine moieties were comparable P1 residues in terms of thrombin affinities. However, the cyclohexylamine residue displayed an improved selectivity index (**28** vs **7b**). The introduction of phenyl amidines improved the binding affinities of inhibitors **25** and **23**. However, there was a significant loss of selectivity, presumably due to the reduced lipophilicity of the aromatic ring compared to the piperidyl and cyclohexyl rings. Inhibitors **13**, **18**, and **26** were all inactive, suggesting the highly specific nature of the P1 subsite.

The three inhibitors having the optimal in vitro potencies were then tested in the rat arterial thrombosis model (see Table 2, analogues **8**, **25**, and **23**). All of them displayed greater than 2-fold increase in mean occlusion time. No occlusion was observed with inhibitor **25** for the whole duration of the experiment (60 min).

We have demonstrated that the activated carbonyl of compounds **1** and **2** was not necessary for high potency and selectivity. By modifying P1 residues of these inhibitors, we could capture additional interactions in order to improve the binding affinity and selectivity for thrombin. Selected compounds were administered via intravenous infusion in the rat arterial thrombosis model and were shown to have high in vivo efficacy.

Acknowledgements

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References and Notes

1. St-Denis, Y.; Augelli-Szafran, C. E.; Bachand, B.; Berryman, K. A.; DiMaio, J.; Doherty, A. M.; Edmunds, J. J.; Leblond, L.; Lévesque, S.; Narasimhan, L. S.; Penvose-Yi, J. R.; Rubin, J.; Tarazi, M.; Winocour, P. D.; Siddiqui, M. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3193.
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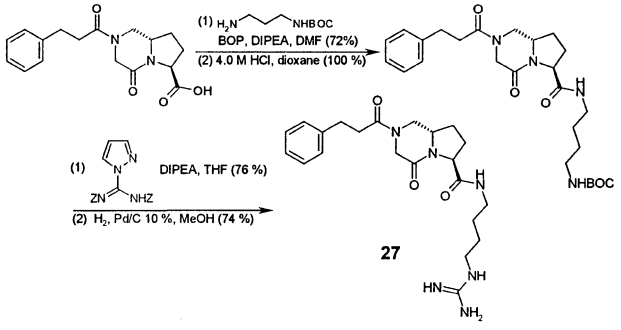
8. Compound **27** was prepared in a straightforward manner as shown below:



9. The authors have deposited X-ray crystallographic data with the Brookhaven Protein Data Bank. Deposition code 1JWT.