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POTENT BICYCLIC LACTAM INHIBITORS OF THROMBIN: PART I: P3 MODIFICATIONS

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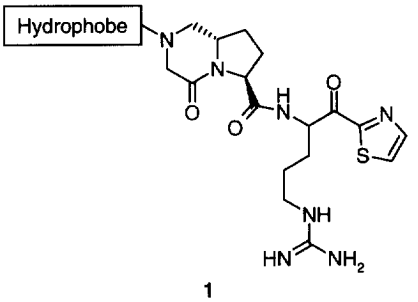
Abstract: Peptidomimetic inhibitors of general structure **1** have been prepared. Optimization of the binding affinities of these compounds through variation of the P3 hydrophobic residue is described. Selected substituted bicylic lactams displayed interesting pharmacological profiles both in vitro and in vivo.

Thrombin is a serine protease that plays a central role in the blood coagulation cascade.[1] It is responsible for the conversion of fibrinogen to fibrin and is a potent agonist of platelets and endothelial cells.[2] A number of direct thrombin inhibitors including hirudin and hirulog[3] have been evaluated for their clinical efficacy, however, undesirable side effects of such agents are excessive bleeding and difficulties of drug monitoring. As a part of our program to develop orally active thrombin inhibitors, we recently identified a series of compounds of general structure **1** that effectively inhibit thrombin.[4,5]

In order to improve both the thrombin affinity (K_i) and the in vivo efficacy (effect of drug on coagulation parameters and antithrombotic effect in vivo), we have extensively explored the P3 hydrophobic interactions of the inhibitors with thrombin's catalytic site using a large variety of hydrophobes, while keeping the rest of the molecule constant.



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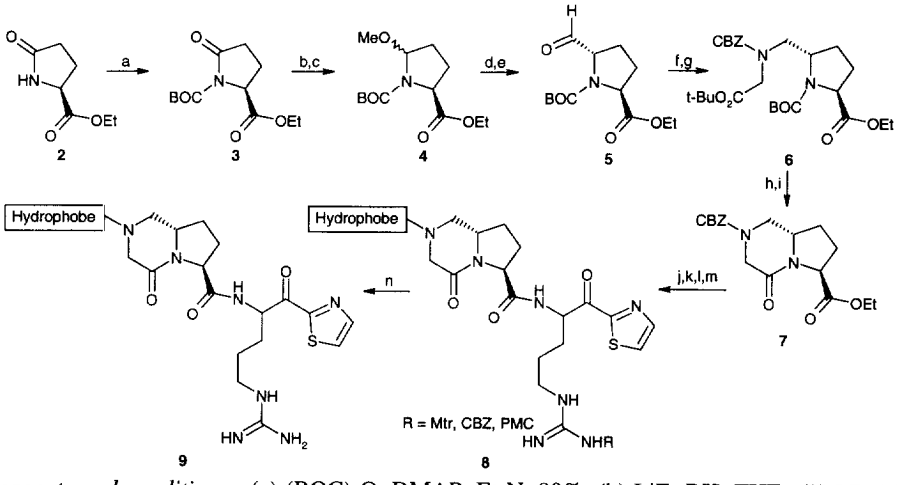
Chemistry

The preparation of the bicyclic lactam is described in the Scheme.[6] Ethyl pyroglutamate **2** was protected as its *t*-butyl carbamate **3**. Formation of the functionalized proline derivative was accomplished using a modification of an already published procedure.[7] Thus, selective reduction of the lactam group of **3** with $LiEt_3BH$ in THF at -78 °C and subsequent reaction of the resulting hemiaminal with MeOH in the presence of PTSA afforded the corresponding aminal **4** in excellent yield. Compound **4** was reacted with $(CH_3)_2C{=}CHCu{\cdot}MgBr_2$, generated in situ from the freshly prepared Grignard reagent,[8] and $CuBr{\cdot}SMe_2$ complex in the presence of $BF_3{\cdot}OEt_2$ in THF, at -78 °C, to give the corresponding 5-substituted vinylproline derivative in a highly diastereoselective manner. Oxidative cleavage of the alkene with O_3 afforded aldehyde **5** in 89% yield as a ~95:5 (*trans:cis*) mixture of isomers, which were separated by flash chromatography. Reductive amination of aldehyde **5** with the HCl salt of glycine *t*-butyl ester in the presence of $NaBH_3CN$ and subsequent protection of the amine gave dicarbamate **6**. The deprotection of both the *t*-butyl ester and the BOC-carbamate was done with 4 N HCl in dioxane, and the amino acid salt thus obtained was cyclized using BOP reagent to give a 95% yield of bicyclic template **7**. The CBZ group of the protected bicyclic lactam **7** was first hydrogenolyzed with H_2 using 10% Pd/C as a catalyst. The amine thus obtained was coupled with the desired hydrophobe (see different conditions in Scheme, condition k), which upon ester hydrolysis with $LiOH{\cdot}H_2O$, followed by coupling with the protected



Scheme: *Reagents and conditions*: (a) $(BOC)_2O$, DMAP, Et_3N, 80%; (b) $LiEt_3BH$, THF, -78 °C; (c) MeOH, cat. pTsOH, 89%; (d) $(CH_3)_2C{=}CHMgBr$, $BF_3{\cdot}OEt_2$, $CuBr{\cdot}SMe_2$, Et_2O, THF, 89%; (e) *i* O_3, CH_2Cl_2, -78 °C, *ii* Me_2S, 89%; (f) H-Gly-O*t*-Bu·HCl, $NaBH_3CN$, MeOH, 0°C→rt, 90%; (g) CBZ-Cl, NMM, THF, -78 °C→rt, 90%; (h) 4 N HCl/dioxane, 100%; (i) BOP, NMM, DMF, 95%; (j) H_2, Pd/C 10%, MeOH, 100%; (k) if hydrophobe is acid: hydrophobe, HATU, NMM, DMF; if hydrophobe is acyl chloride, or chloroformate: hydrophobe, NMM, CH_2Cl_2 or pyridine; (l) $LiOH{\cdot}H_2O$, THF/H_2O, 0 °C→rt; (m) Protected-Arg-NH_3Cl, HATU, 2,4,6-collidine, DMF or i-Pr_2NEt, CH_2Cl_2; (n) if protection is CBZ: BBr_3 or HF deprotection; if protection is Mtr: MeSPh, TFA; if protection is PMC: TFA.

ketothiazoloarginine[9] produced the corresponding protected inhibitors **8** (Mtr: 4-methoxy-2,3,6-trimethylbenzene-sulfonamide; CBZ: benzyloxycarbonyl; PMC: 2,2,5,7,8-pentamethylchroman-6-sulfonamide). Deprotection of **8** (see different conditions in Scheme) afforded final inhibitors **9**, which were purified by reverse-phase HPLC.

Biology

Inhibition of the amidolytic activity of thrombin (K_i) and in vivo coagulation parameters and antithrombotic effect in the rat arterial thrombosis model, as assessed by the determination of the mean occlusion time (MOT), the activated partial thromboplastin time (aPTT) and the thrombin time (TT), were measured according to already published procedures.[10]

Results and Discussion

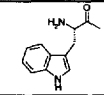
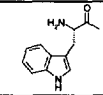
The twenty-seven inhibitors presented here are divided into two categories (see Tables 1 and 2). The inhibitors having no polar functionalities (hydrocarbons, halides, ethers) are listed in Table 1. In order to probe the optimum length of the P3 hydrophobe, a series of four inhibitors were designed where the number of carbon atoms of the chain linking the hydrophobic ring to the bicyclic template was varied (see entries **10** - **13**). The optimum length was found to be of three carbons (entry **12**). Replacement of the aromatic ring of **12** with the more hydrophobic cyclohexyl group (entry **14**) did not improve binding, indicating preference for aromatic rings. X-ray analysis suggests that the aromatic hydrophobe interacts with Trp215 through π-stacking interactions. Disruption of this interaction may account for the reduction in affinity.

It appears important that the chain be composed of carbons only, as substitutions to oxygen or nitrogen decreased the affinity for thrombin (entries **16**, **18** and **22** compared to entry **12**). The effect of the heteroatom varies depending on its position in the chain. As it penetrates further into the hydrophobic pocket, the heteroatoms have less negative effects on the binding affinity (entry **15** and **16**). When the heteroatom is next to the carbonyl, the negative effect is greater (entries **18** and **22**). This may result from conformational variations, as carbamates and ureas do not have the same rotational barriers as amides do. Otherwise, the aromatic portion of the hydrophobe tolerates substitutions at the *para*, *meta*, or *ortho* positions (entries **19**, **20**, **21**, **23**, **24**, **25**, and **26**) and more than one substitution is possible (entry **24**). It is not clear whether the improved efficacy of inhibitors with hydrophobes substituted with electron donating or withdrawing groups is due to electronic or hydrophobic factors (entries **21**, **23**, **24** and **25**).

Table 1 Non polar hydrophobes

#	Structure Hydrophobe	K_i (nM)	Mean occlusion time (min)[c]	Activated partial thromboplastin time (s)[d]	Thrombin time (s)[e]
10[a]		25	45.8 ± 14.2	25.5 ± 3.0	144 ± 10.2
11		2.5	>60	79.2 ± 7.4	387.1 ± 33.5
12		0.6	42.25 ± 11.9	44.3 ± 3.4	381.3 ± 42.9
13		1.2	>60	120	494
14		24	25.4 ± 3.8	52.4 ± 4	320 ± 30
15		5	47.7 ± 15.2	45.2 ± 2.6	276 ± 7.3
16		16	37.8 ± 13.6	31.5 ± 6.8	248 ± 26
17		17	28.3 ± 2.1	30.5 ± 2.1	122 ± 28
18		50	n/d[f]	n/d	n/d
19		0.35 (S)[g]	33.2 ± 1.5	217.4 ± 140	>600
20		0.5	n/d insolubility	n/d	n/d
21		0.5	>60	55.2 ± 2.5	632.8 ± 58.6
22		58	n/d	n/d	n/d
23		0.5 (S)	>60	88 ± 12	>999
24		0.106 (S)	n/d	n/d	n/d
25		0.5 (S)	>60	90 ± 2.6	>999
26		0.92	n/d	n/d	n/d

Table 2 Hydrophobes containing polar groups

#	Structure Hydrophobe	K_i (nM)	Mean occlusion time (min)[c]	Activated partial thromboplastin time (s)[d]	Thrombin time (s)[e]
27		32	n/d	n/d	n/d
28 fm[h]		84 (S)	n/d	n/d	n/d
29 sm[i]		16	>60	49.1 ± 9.3	476.1 ± 1.4
30		3	47.7 ± 8.9	44.2 ± 5.4	556.9 ± 19.5
31 fm		160	n/d	n/d	n/d
32 sm		18	53 ± 8.6	76 ± 6	433 ± 85
33 fm		75.3 (S)	n/d	n/d	n/d
34 sm		0.96	>60	188 ± 24	929 ±86
35 fm		3	47.6	44.2	556.9
36		4.4	>60	463 ± 8.6	678.6 ± 82

[a]Dose: intravenous bolus dose (0.75 mg/kg) followed by an infusion (50 μg/kg/min); [b]All new target compounds were characterized by ^{1}H NMR, reverse-phase HPLC and mass spectroscopy. Unless otherwise noted, the inhibitors are a mixture of two isomers at the α-carbon of arginine; [c]Mean occlusion time in minutes (control: 17-19 min); [d]Activated partial thromboplastin time in seconds (control 20 - 22 s); [e]Thrombin time in seconds (control: 40 - 45 s); [f]Not determined; [g]Slow binder kinetics; [h]Fast moving isomer by HPLC; [i]Slow moving isomer by HPLC.

As can be seen in the Tables 1 and 2, K_i values below 20 nM will typically lead to a doubling or even tripling of the mean occlusion time, a measure of the antithrombotic efficacy in vivo. The only exception seems to be the α-naphthyl compound (entry **19**).

In Table 2, the chain length has been kept constant (except for entries **28** and **29**). Polar groups such as alcohols and amines have been added in order to see if additional interactions could be obtained to further improve the affinity and possibly obtain selectivity through interactions with Tyr60A. These new functionalities allowed for the separation of the two isomers at the α-carbon of arginine present in the mixture, which was not possible with the inhibitors shown in Table 1. As can be seen in Table 2, the slow moving isomers (by RP-HPLC) are always more potent, and it was thought that the arginines of these isomers had the natural *S* configuration. Indeed, when both the *S* and *R* isomers of **12** were prepared separately, it was found that the natural *S* isomer was more potent.[11] This was also verified by X-ray crystallography (Figure 1). The

incorporation of the polar functionalities did not improve to a great extent the binding affinities of the inhibitors. Since the molecules of this category were more difficult to synthesize, they were not pursued further.

Most of the compounds were tested for selectivity for thrombin versus trypsin. None of them were found to be selective.

Structure and X-ray Analysis

Figure 1 illustrates the X-ray structures of compound **12** co-crystallized with thrombin. The important interactions of the inhibitor with the enzyme are schematically depicted in Figure 2. One can clearly see how the phenylpropionyl group penetrates deep into the S3 pocket of the enzyme, whereas the arginine's side chain forms a salt bridge at the bottom of the S1 pocket. The ethylene portion of the five membered ring of the bicyclic template seems to insert itself between Trp60D and Tyr60A of the specificity pocket (S2/P pocket, or insertion loop) forming hydrophobic interactions with these two residues. Finally, Ser195 attacks the activated carbonyl (ketothiazole) of the arginine mimic, forming a tetrahedral intermediate. This intermediate is stabilized through hydrogen bonding with the amino groups of Ser195 and Gly193. X-ray crystal structure of peptidic D-Phe-Pro-Arg-5-methylketothiazole with thrombin (Ki = 0.1 nM) was also obtained (not shown). Parallel interactions with thrombin were seen in both peptidic and peptidomimetic inhibitor **12** with an exception of additional hydrogen bonding interaction of amino group of D-Phe with the amide carbonyl of Gly 216.

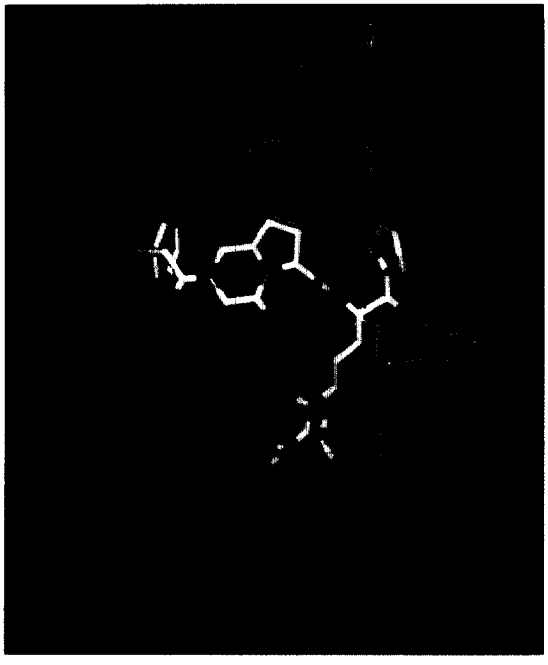


Figure 1

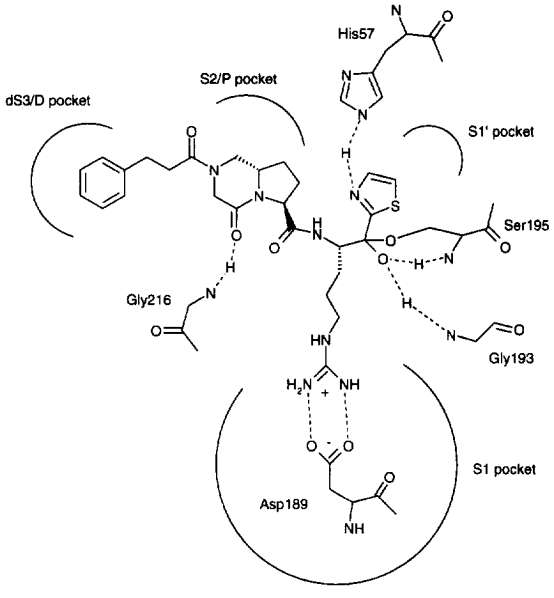


Figure 2

The rigidity of the bicyclic template may account for the excellent affinity of **12** by directing the P3 hydrophobic side chain more efficiently into the S3 subsite of thrombin. One can also notice the bending of the linker between the bicyclic template and the hydrophobe. The plane of the phenyl ring of the hydrophobe is almost perpendicular to the plane of the bicyclic template. This may in part explain why compounds, such as **27**, with a rigid hydrophobic group in P3, bind less efficiently than **12**.

Conclusion

We have shown in this SAR study that the S3 subsite of the active site of thrombin can accommodate different types of hydrophobes. The simple hydrocarbons or halogen-substituted hydrocarbons clearly stand out as they offer both high affinity and ease of preparation (Table 1). Introduction of polar groups such as amines or alcohols in the hydrophobes (Table 2) did not offer any advantage over the all-carbon ones. In vivo efficacy of the most potent inhibitors was demonstrated by intravenous infusion in the rat arterial thrombosis model.

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References and Notes

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11. Compound **12**: IC_{50} (R isomer) = 16 nM; IC_{50} (S isomer) = 4 nM.