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POTENT AND SELECTIVE BICYCLIC LACTAM INHIBITORS OF THROMBIN: PART 3: P1' MODIFICATIONS

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Abstract: The synthesis and antithrombotic activity of a series of nonpeptide bicyclic thrombin inhibitors are described. We have explored the SAR around the P1' site. Modification of the P1' site has been found to affect potency and selectivity.

Introduction

The serine protease thrombin plays a critical role in the blood coagulation cascade. Activation of both the intrinsic and extrinsic pathways leads to the cleavage of prothrombin in the blood plasma resulting in the formation of thrombin. The diverse biological functions attributed to thrombin include the conversion of fibrinogen to fibrin and the activation of platelets and other coagulation factors.[1] An imbalance in the coagulation cascade can result in thrombus formation and subsequent occlusive events including deep vein thrombosis which may lead to pulmonary embolism. Current anticoagulants and antithrombotics such as coumarin, heparin and thrombin inhibitors such as hirudin and hirulog have been assessed clinically.[2] Side effects such as excessive bleeding and difficulties of drug monitoring associated with these current therapies have led to considerable activity in the search of novel orally active small molecule thrombin inhibitors.[3]

We have designed a series of potent and novel thrombin inhibitors with a bicyclic core[4] at the P2 site. While the previous communication[5] described our efforts in exploring the SAR at the P1 site, the current publication presents our efforts in exploring the P1' site.

Chemistry

The compounds listed in Table 1 were synthesized using a convergent strategy that involved coupling the bicyclic pyrrolidine **5**[4] with the appropriate chiral keto-heterocycle, **4** (Scheme 1). In general the chiral keto-heterocycles were derived from the Weinreb amides of the appropriate amino acid derivative. The synthesis of chiral keto-heterocycle **4** utilized in the thrombin inhibitor **15** is shown in Scheme **1**.[6] Boc-3-L-pyridyl alanine **1** was treated with N,O-dimethylamine hydrochloride, DIEA and BOP reagent in DMF affording the Weinreb amide in 85% yield, which was reduced using PtO_2 in acetic acid to afford the piperidine derivative **2**. Addition of 1-H-pyrazole-1-carboxamidine hydrochloride, DIEA in DMF and subsequent protection with PmcCl/NaOH/acetone at 0 °C for 2 h afforded the separable diastereomers **3a** and **3b** in a 1.8:1 ratio respectively in 63% overall yield from the Weinreb amide. Addition of the lithiated heterocycle to Weinreb

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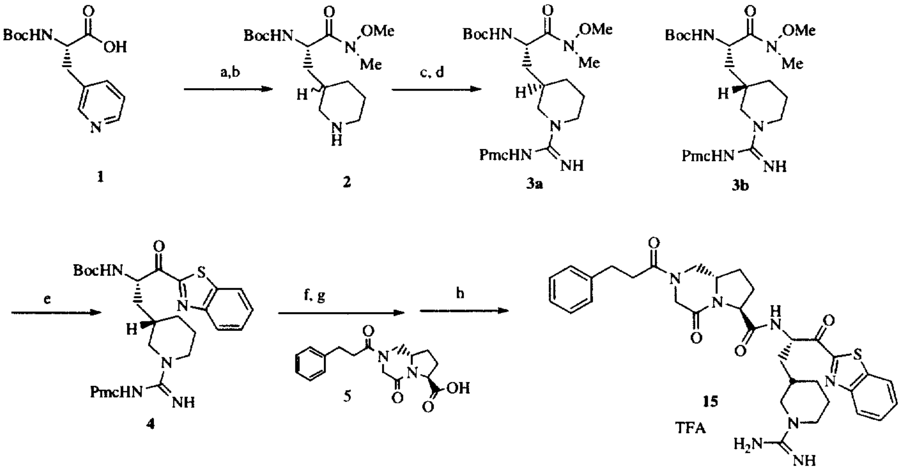
amide **3b** at -78 °C, warming to -40 °C, and quenching at -78 °C afforded **4** in 45% yield. Selective deprotection of **4** was accomplished with 4N HCl/dioxane in quantitative yield. Condensation of the amine with the bicyclic pyrrolidine derivative **5**[4] and subsequent deprotection with TFA afforded the target compounds which were purified by preparative reverse-phase HPLC. Compounds **6**- **19** (Table 1) were synthesized in a similar manner starting from the appropriate amino acid derivative and the appropriate lithioheterocycle.

Scheme 1



Reagents and Conditions: a. HN(OMe)Me · HCl, DIEA, BOP reagent, CH_2Cl_2 b. PtO_2, H_2, AcOH c. 1H-Pyrazole-1-carboxamidine · HCl, DIEA, DMF d. PmcCl, NaOH, Acetone,(resolved by chromatography) e. *n*-BuLi, benzothiazole, TMEDA, THF, -78 °C f. 4 N HCl/dioxane g. BOP reagent, DIEA, DMF h. TFA
note: PmcCl (2,2,5,7,8-Pentamethylchroman-6-sulfonyl chloride);
BOP reagent (Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate)

Biological Assays

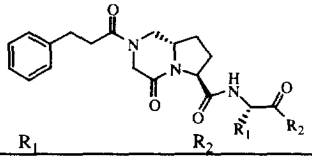
The Biological assays were performed as described in the previous paper.[5]

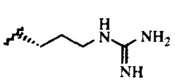
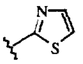
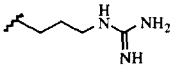
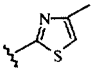
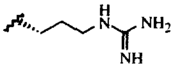
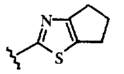
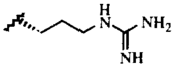
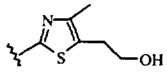
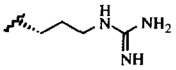
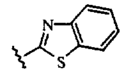
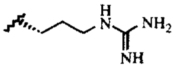
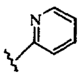
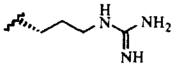
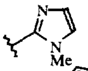
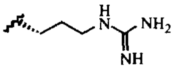
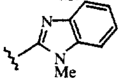
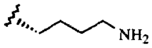
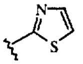
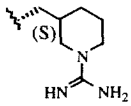
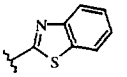
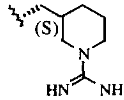
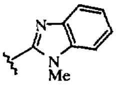
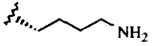
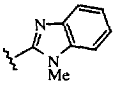
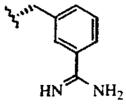
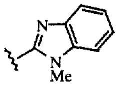
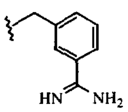
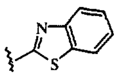
Results and Discussion

P1' Modifications

The parent compound in this series, **6**, showed excellent inhibition of thrombin with an IC_{50} of 5 nM but lacked selectivity with respect to trypsin. Our goal was to prepare potent and selective thrombin inhibitors. While evaluating a variety of P1 arginine mimetics, we concurrently explored P1' modifications of **6** to improve the selectivity over trypsin. Incorporation of alkyl groups on the thiazole (**7** and **8**) resulted in reduced inhibition of both thrombin and trypsin. Molecular modeling predicted that elaboration of the alkyl substituent as a

Table 1. In Vitro IC_{50}[a] Values (nM) and Selectivity Ratios of Compounds **6** -**19**[b] against Thrombin (FIIa)[c] and Trypsin[d]



Compound #	R1	R2	FIIa	trypsin	trypsin/FIIa
6			5	<1	.2
7			44	24	.5
8			18	2	.1
9			15	12	1
10			<1	<1	1
11			340	160	.5
12			2370	27,400	12
13			51	3360	65
14			77	14	.2
15			<1	590	590
16			750	40%100 μM	
17			2050	8490	4
18			450	18,680	42
19			57	970	17

a. Concentration (nM) of **6**-**19** necessary to inhibit enzymatic cleavage of the chromogenic substrates described in ref 5 by 50 %. b. All new target compounds were characterized by ^{1}H NMR, RP-HPLC, and mass spectroscopy c. Human Thrombin. d. Human Trypsin.

hydroxy ethyl group, **9**, might form a new hydrogen bond with the Lys60F[7] side chain. This lysine is absent in trypsin, and therefore selectivity in binding to thrombin over trypsin was anticipated. Ultimately little selectivity was observed, indicating the expected hydrogen bond may not form. However, molecular modeling also suggested that a benzothiazole ring in P1' would stack in a $\pi-\pi$ face-to-edge manner with the thrombin specific indole ring of Trp-60D, thereby improving the potency for thrombin.[8] Indeed, replacement of the thiazole group with a benzothiazole group, compound **10**, greatly enhanced activity against thrombin. However this modification had little effect on thrombin/trypsin selectivity.

At this point we turned our attention to other heterocycles which included 2-pyridyl, **11**, N-methyl imidazole **12**, and N-methylbenzimidazole **13**. Of these compounds, only the N-methylbenzimidazole derivative, **13**, displayed potency for thrombin with good selectivity over trypsin. Similar selectivity has been reported with P1-argininoyl heterocycles.[8,9] The origin of this selectivity can be attributed to several factors, one being the attenuated electrophilicity of the carbonyl of **13** vs that of **10**. The crystal structures of inhibitor **13** complexed with both thrombin and trypsin (Figure 1) reveals other sources of selectivity. In thrombin, favorable Van der Waals interactions exists between the phenyl of the benzimidazole and the indole ring of Trp60D[8] as well as the N-methyl group of the inhibitor with the hydrocarbon side chain of Glu192 (Figure 1a). In addition there is a pi-cationic interaction between the phenyl ring of the benzimidazole and Lys60F. These interactions are significant and occur with residues that are unique to thrombin.

The crystal structure of trypsin complexed with **13** (Figure 1b) reveals the lack of contact between the protein and the N-methylbenzimidazole in the S1' pocket. Also the catalytic serine does not appear to covalently interact with the inhibitor as is seen in the thrombin structure. In fact, there is a lack of electron density corresponding to the N-methylbenzimidazole of **13** in the trypsin crystal structure suggesting multiple conformations for this part of the inhibitor.

The crystallographic data suggests that the inadequate interactions with the N-methylbenzimidazole group, coupled with the unfavorable steric interactions of the N-methyl with Gln192, which is hydrogen-bonded to the inhibitor, disallows the stable electrostatically favorable conformation needed for the covalent intermediate to form with trypsin. These observations may explain the selectivity of **13** for thrombin versus trypsin.

P1' with P1 modification

In the previous paper,[5] it was demonstrated that P1 substituents can impart good thrombin/trypsin selectivity. We coupled these modifications with alternative P1' heterocycles. As expected, incorporation of the benzothiazole at P1' with 3-amidinopiperidine alanine in P1, compound **15**, and 3-amidinophenyl alanine in P1, compound **19**, led to increases in potency over their respective thiazole derivatives while maintaining selectivity.[5] Most notably, **15**, remained an extremely potent thrombin inhibitor and showed remarkable selectivity (590-fold) over trypsin. Replacing the basic guanidine group with a lysine resulted in compound **14**, which was 25-fold less potent thrombin inhibitor versus the parent **6**. An analog of **14** that incorporates the N-methylbenzimidazole in P1' led to a very weak thrombin inhibitor, **17**, which had little selectivity over trypsin. The loss in potency was expected, however, we anticipated better selectivity over trypsin based on the arginine derivative **13** which had a trypsin/thrombin selectivity of 65. Clearly the effects of the groups placed in P1' and P1 are not simply additive. This became more evident when we combine the selective P1 3-amidino piperidine and the 3-amidinophenyl alanine groups with the selective P1' N-methylbenzimidazole, the resulting compounds **16** and **18** which show little activity against trypsin and are weak inhibitors of thrombin.

A possible explanation for the poor thrombin inhibition could be the steric interaction of the N-methylbenzimidazole with the ethylene units of the amidino piperidine in compound **16** and the phenyl group of compound **18**. In the structure of **13** with thrombin (Figure 1a) the Glu 192 side chain has Van Der Waals interaction with the N-methyl of the benzimidazole. In the thrombin structure of compounds which contain P1 groups such as 3-amidino piperdine or 3-amidino phenyl alanine, the Glu 192 is positioned away from the

Figure 1

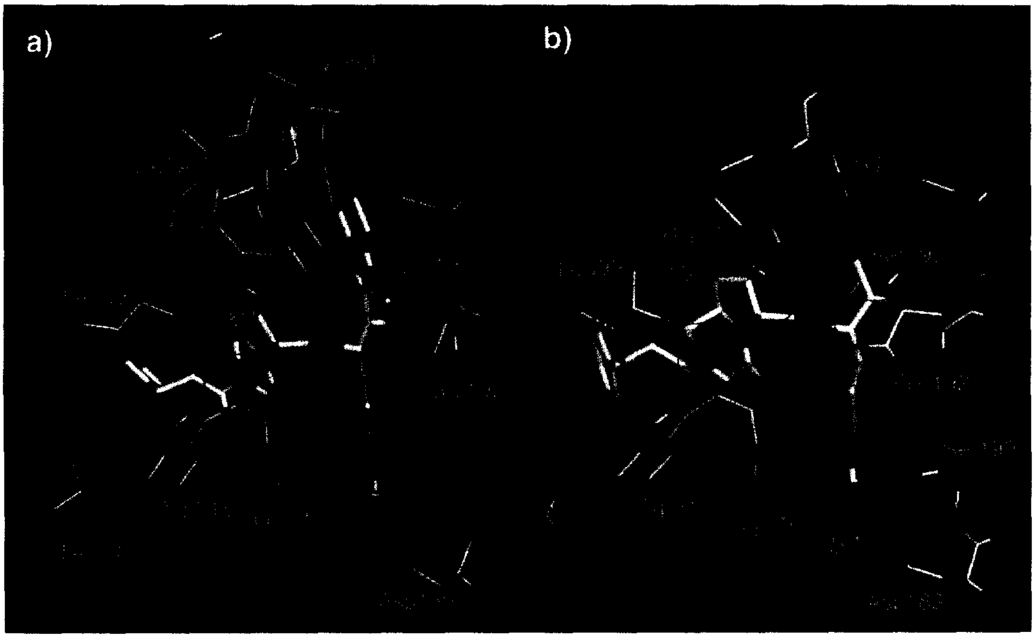


Figure 1. Stick rendition of compound **13** complexed to the active site of (a) thrombin and (b) trypsin. Inhibitor is shown by thick lines; nearby protein residues are shown by thin lines. Color coding for atoms is: *red*, oxygen, *blue*, nitrogen, *yellow*, sulfur, *white*, carbon. *Green dashed* arcs denote van der Waals contacts, *purple dashed* lines denote electrostatic interactions between the proteinand inhibitor. *Grey circled regions* denote P1, P1', P2, and dP3 binding pockets of enzyme. *Dark blue hashed oval* in panel (b) highlights missing N-methyl benzimidizole group (not seen in trypsin crystal structure) Crystals of bovine trypsin complexed with benzamidine were grown by hanging drop method from 2.4 M NH_4SO_4, 10 mM $CaCl_2$, pH 7.0 and then soaked in a saturated solution of **13** for 3 days. X-ray data were collected on a MAR 300 with a rotating anode to 1.9 Å, R_{merge} = 8% (26 % in 2.0 – 1.9 Å shell), completeness = 90% (89 % in 2.0 – 1.9 Å shell). The refined structure has good geometry, R_{merge} = 18.2 % using 8.0 – 1.9 Å data.

inhibitor, resulting in no Van Der Waals interaction between the N-methyl and the Glu 192 side chain thereby yeiding a poorer thrombin inhibitor.

In Vivo Results

Two selective inhibitors (**13** and **15**) were evaluated for their antithrombotic activity in the rat arterial thrombosis model. Compound **13** was not effective in this model and was not tested for oral bioavalibility. Compound **15** demonstrated good antithrombotic activity with a 3.5 fold shift in mean occusion time. Further evaluation of **15** for oral (30 mg/kg) bioavailability in the rat, unfortunately showed that the compound lacked significant activity when dosed in this manner.[10]

Conclusions

We have demonstrated that the incorporation of heterocycles at the P1' position of these inhibitors has beneficial affects toward not only the intrinsic activity but also the selectivity for the serine proteases thrombin and trypsin. The benzothiazole substituent greatly improves potency in the thrombin inhibitors presented here whereas the N-methylbenzimidazole group at P1' coupled with an arginine at P1, imparts selectivity of thrombin over trypsin.

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References

1. (a) Lowe, G. D. O.; Prentice, C. R. M. *Hemostasis and Thrombosis*; Bowie, E. J. W. Sharp, A. A., Eds.; Butterworths: London, 1985, Chapter 10. (b) Davie, E. W.; Fujikawa, K.; Kisiel, W. *Biochemistry* **1991**, *30,* 10363.
2. (a) Maraganore, J. M. *Perspect. Drug Discovery Des.* **1994**, *1*, 461. (b) Lefkovits, J.; Topot, E. J. *Circulation* **1994**, *90*, 1522.
3. (a) Trapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N.S. *Trends Pharmacol. Sci.* **1993**, *18*, 366.(b) Lyle, T. A. *Perspect. Drug Discovery Des.* **1994**, *1*, 453.
4. (a) DiMaio, J.; Siddiqui, M. A.; Gillard, J. W.; St-Denis, Y.; Tarazi, M.; Preville, P.; Levesque, S.; Bachand,B. WO 9619483A, 1996; *Chem Abstr.* **1996**, *125*, 167970. (b) Fobian, Y. M.; d'Avignon, D. A.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 315..
5. Plummer, J. S.; Berryman, K. A.; Cai, C.; Cody, W. L.; DiMaio, J.; Doherty, A. M.; Edmunds, J. J.; He, J. X.; Holland, D. R.; Kent, D.; Lafleur, D.; Levesque, S.; Narasimhan, L.; Rubin, R.; Rapundalo, S. T.; Siddiqui, M. A.; Susser, A.; St.-Denis, Y.; Winocour, P. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 3409.
6. Berryman, K. A.; Doherty, A. M.; Edmunds, J. J.; Plummer, J. S. WO 9748687, 1997; *Chem Abstr.* **1997**, *128*, 115227.
7. Rezaie, A. R.; Olson, S. T.; *Biochemistry* **1997**, 36, 1026-1033.
8. Costanzo, M. J.; Masryanoff, B. E.; Hecker, L. R.; Schott, M. R.; Yabut, S. C.; Zhang, H-C.; Andrade-Gordon, P; Kauffman, J. A.; Lewis, J. M.; Krishnan, R.; Tulinsky, A. *J.Med. Chem.*, **1996**, *39*, 3039.
9. Tamura, S. Y.; Shamblin, B. M.; Brunck, T. K.; Ripka, W. C. *Bioorg. Med Chem. Lett.* **1997**, *7*, 1359.
10. Finkle, C. D.; St-Pierre, A.; Leblond, L.; Deschenes, I.; DiMaio, J.; Winocour, P. D. *Thrombosis and Haemostasis* **1998**, *79*, 431.