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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIP OF POTENT BICYCLIC LACTAM THROMBIN INHIBITORS[1]

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Abstract: A simple and versatile method for preparation of (D)-Phe-Pro peptidomimetic bicyclic thiazolidine lactams is presented. These bicyclic lactams have chemical diversity α to the lactam carbonyl and, when linked to electrophilic arginines, provide potent thrombin inhibitors.

Thrombin is a trypsin-like serine protease that plays a critical role in thrombus formation by converting soluble fibrinogen to insoluble fibrin.[2] Thrombus generation can lead to life threatening medical conditions, including pulmonary embolism, myocardial and cerebral infarctions. Inhibition of thrombus formation by using catalytic site directed thrombin inhibitors is an active area of research that may prove to be beneficial in the prevention and treatment of thrombotic disorders.

It is well documented in the literature that the (D)-Phe-Pro dipeptide motif when linked to electrophilic arginines provide potent thrombin inhibitors.[3,4] The mode of interaction of these inhibitors in the catalytic site of thrombin has been well characterized. Three binding subsites S_1, S_2 and S_3 interact with the (D)-Phe-Pro-Arg tripeptide motif in the thrombin catalytic pocket as depicted in Figure 1. The S_3 subsite is lipophilic and interacts with the phenyl ring of (D)-Phe, the S_2 subsite interacts with the proline ring, while the arginine chain goes deeply into the S_1 subsite forming a salt bridge with Asp 189. In addition to these interactions, Ser 195 forms a tetrahedral intermediate with the electrophilic carbonyl.

As a part of our ongoing cardiovascular program, we were interested in the design and synthesis of peptidomimetic based thrombin inhibitors that bind to the catalytic site of thrombin like the (D)-Phe-Pro-Arg tripeptide (Figure 1). These peptidomimetic based inhibitors may have obvious advantages such as enhanced metabolic and proteolytic stability, as well as oral bioavailability. In this paper, we present a convenient and versatile method for the preparation of these thrombin inhibitors with chemical diversity at the P_3 site.

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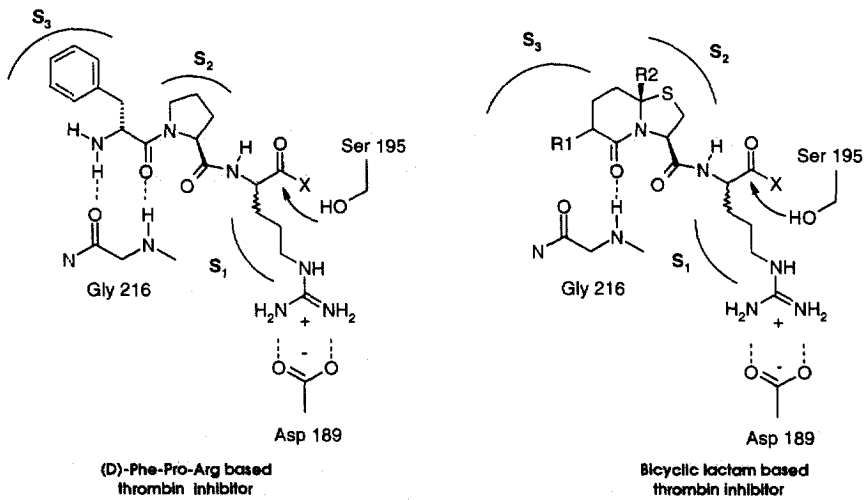
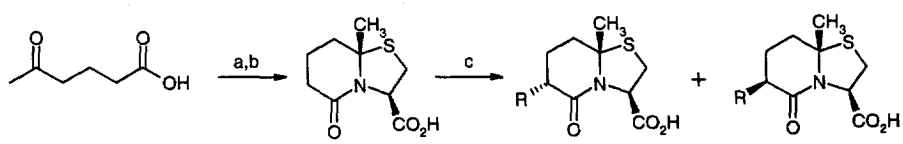


Figure 1. Binding interactions of inhibitors in the catalytic site of thrombin.

Chemistry

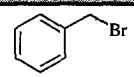
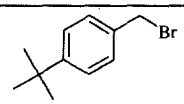
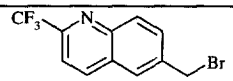
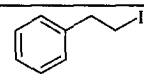
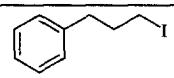
Preparation of α-substituted constrained bicyclic lactams **3–7** (Scheme 1) began with the condensation of 4-acetyl butyric acid **1** and L-cysteine ethyl ester in refluxing toluene to afford, after hydrolysis, bicyclic lactam **2** as a single isomer in good yield. The bicyclic lactam acid **2** was then treated with two equivalents of LiHMDS at -78 °C for 40 min in THF and subsequently quenched with alkylating agents to afford bicyclic lactams **3–7** in modest to good yield.



Scheme 1. *Conditions and reactions*: (a) L-cysteine ethyl ester hydrochloride, toluene, reflux (88%); (b) $LiOH{\bullet}H_2O$, THF/H_2O (80%); (c) 2 equiv LiHMDS, THF, -78 °C, 40 min then R-X, -78 °C to room temperature.

Diastereoselectivity of the alkylation reaction (Table 1) was not high and slightly favored the *endo* isomer that, for this type of reaction, is well precedented in the literature.[5] The stereochemistry of the major isomer was unambiguously confirmed by an X-ray crystallographic study[6] of the quinolinyl derivative **5a** (Figure 2).

Table 1. Alkylation of bicyclic lactam **2** with various electrophiles

Entry	R-X	a (endo)	b (exo)
3		46%	6%
4		46%	23%
5		54%	25%
6		15%	10%
7		26%	11%

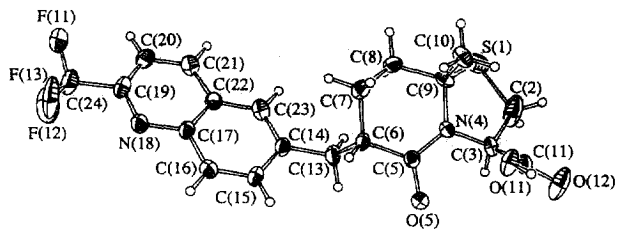
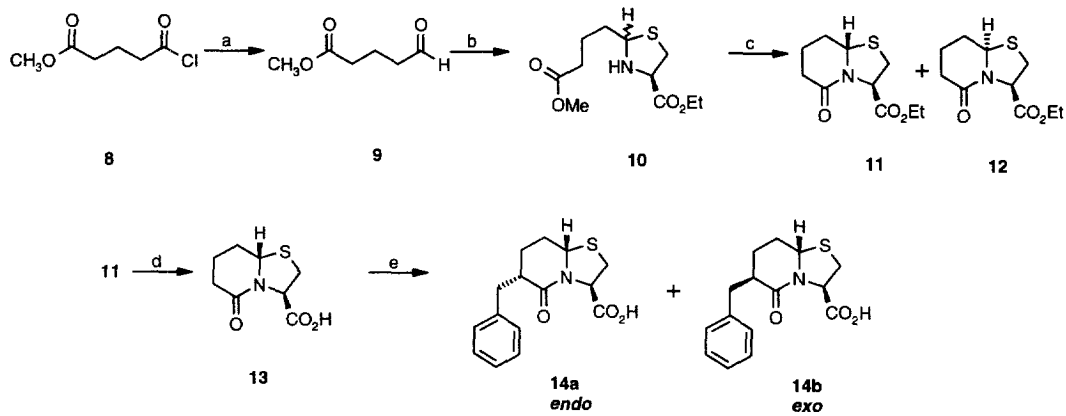


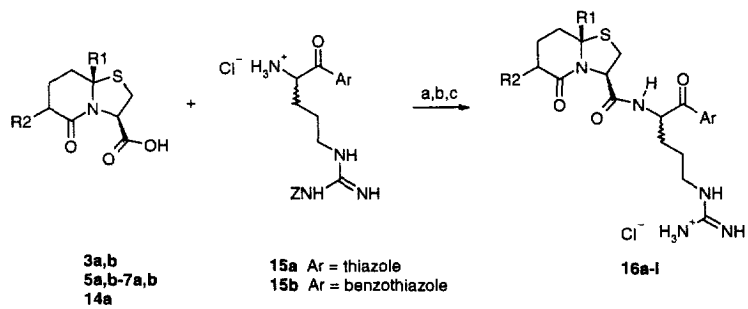
Figure 2. ORTEP view of quinolinyl derivative **5a**

Synthesis of bicyclic lactam with an hydrogen at the angular position was accomplished as depicted in Scheme 2. A modified Rosenmund reduction of commercially available methyl glutaryl chloride **8** with palladium using 2,6-lutidine as base afforded aldehyde **9** with 72% yield. Condensation of aldehyde **9** with L-cysteine ethyl ester in dichloromethane afforded thioaminals **10** as a mixture of diastereisomers in quantitative yield. Subsequent cyclization of crude thioaminals **10** in refluxing toluene yielded lactams **11** and **12** in 32% and 19% yield, respectively. Bicyclic lactam **11** was then hydrolyzed to corresponding acid **13** in 73% yield. An X-ray crystallographic[6] study of **13** established the *cis* orientation of the angular hydrogen with respect to the carboxylic acid. Hydrolysis of the other isomer **12** proved to be difficult leading only to a small amount of the desired product. Alkylation of the acid **13** using the method described earlier for the methyl analog provided the *endo* and the *exo* isomers **14a**[7] and **14b** in 18% and 8% yield, respectively (Scheme 2).



Scheme 2. *Conditions and reactions*: (a) 2,6-lutidine, H_2, Pd/C 5%, THF (72%); (b) L-cysteine ethyl ester hydrochloride, dichloromethane, K_2CO_3, $MgSO_4$, room temperature (100%); (c) toluene, pTSA (cat.) (**11**, 32%), (**12**, 19%); (d) LiOH•H_2O, THF/H_2O (73%); (e) 2 equiv LiHMDS, THF, -78 °C, 40 min then benzyl bromide, -78 °C to room temperature (**14a**, 18%), (**14b**, 8%).

Preparation of thrombin inhibitors **16a–l** is described in Scheme 3. Peptide coupling of the arginines **15a** or **15b**[8] with bicyclic lactam acids prepared in Schemes 1 and 2 provided, after deprotection, HPLC purification and chloride exchange, compounds **16a–16l** (Table 2).



Scheme 3. *Conditions and reactions*: (a) BOP, NMM, DMF; (b) BBr_3, dichloromethane, -78 °C, followed HPLC purification; (c) Amberlite IRA-400 (Cl) (overall yields 12–42%).

Results and Discussion

Compounds **16a–16l** were evaluated in vitro against human α-thrombin and showed a broad range of binding affinities from 2 to 550 nM. One of the most interesting features affecting potency is related to the stereochemistry of P_3 hydrophobic group α to the amide. It is clear from Table 2 that the preferred stereochemistry for P_3 hydrophobic groups, which interact with the S_3 lipophilic subsite, is *S* (*endo*) (e.g., **16l** vs **16k**, **16i** vs **16h**, **16f,g** vs **16e**, **16d** vs **16c**). Differences in binding affinities between the *endo* (*S*) and the *exo*

(*R*) isomers can be as much as 50-fold especially for compounds **16e** and **16g**. This difference in potency is more pronounced for compounds in the low nM range (e.g., **16e** vs **16g**) compared to compound in the high nM (e.g., **16c** vs **16d**).

Table 2. In vitro activity of inhibitors **16a–l** against human α-thrombin.[9]

Entry	R_1	R_2	Ar	K_i (nM)
16a	CH_3	benzyl, (*S*)[a]	benzothiazole	18
16b	CH_3	benzyl, (*S*)	thiazole	16
16c	CH_3	phenethyl, (*R*)	benzothiazole	550
16d	CH_3	phenethyl, (*S*)	benzothiazole	235
16e	CH_3	phenylpropyl, (*R*)	thiazole	100
16f	CH_3	phenylpropyl, (*S*)[b]	thiazole	6
16g	CH_3	phenylpropyl, (*S*)[c]	thiazole	2
16h	CH_3	2-(trifluoromethyl)-6-quinolinyl, (*R*)	thiazole	150
16i	CH_3	2-(trifluoromethyl)-6-quinolinyl, (*S*)	thiazole	10
16j	CH_3	2-(trifluoromethyl)-6-quinolinyl, (*S*)	benzothiazole	18
16k	H	benzyl, (*R*)	benzothiazole	1112[d]
16l	H	benzyl, (*S*)	benzothiazole	8

[a]All entries represent compounds that are a mixture of epimers at the arginine moiety unless indicated otherwise. [b]One single isomer, slow moving component on HPLC. [c]One single isomer, fast moving component on HPLC.[10] [d]IC_{50} value (In general, for these kinds of compounds, K_i values are about ten times less than IC_{50} values)

Binding in the S_3 subsite was shown to be dependent on the nature of P_3 lipophilic group. For example, high activity in the low nanomolar range were displayed by the benzyl (**16b**, **16l**) and phenylpropyl group (**16f**, **16g**). Moreover, the quinolinyl analog was found to be well tolerated in the S_3 subsite (**16i** and **16j**). The best binding of 2 nM was obtained by the phenylpropyl derivative **16g**, which presumably goes deeper in the S_3 subsite than the benzyl analog **16b** that could account for a K_i slightly higher (16 nM). It remains unclear why the phenethyl analog **16c,d** that has an intermediate length did not show an halfway value of binding affinity.

The nitrogen atom of the phenylalanine in the (D)-Phe-Pro-Arg tripeptide is involved in hydrogen bonding with Gly 216 (Figure 1) and initially, it was not clear how the absence of this nitrogen atom α to amide (in the bicyclic lactams) would affect the potency. From the work presented herein, it is clear that the lack of a nitrogen atom in the compounds prepared above did not have any significant effect on potency when proper P_3 hydrophobic groups were chosen. By taking full advantage of the lipophilicity of the S_3 subsite, it is possible to compensate and overcome the absence of one major interaction in catalytic site of thrombin.

Since the objective of the present study was mainly to investigate the P_3 lipophilic group, one can not say much about the P_2 and the P_1' site. Nevertheless, the presence of a hydrogen at the angular position (P_2 site) (entry **16l**) compared to a methyl (entry **16a**) does not make a significant change by improving slightly the

potency by only twofold. Finally, replacement of the benzothiazole unit by a thiazole ring at the P_1' site does not have any significant effect on potency (**16a** vs **16b**, **16i** vs **16j**).

Conclusion

In summary, we have developed a simple and versatile method for obtaining peptidomimetic based thrombin inhibitors having chemical diversity at P_3. The method allowed us to investigate systematically the effect of lipophilic groups at the P_3 site essential for enhanced affinity against thrombin. Compounds **16f,g** having a phenylpropyl group in P_3 site showed the highest affinities of 6 and 2 nM, respectively.

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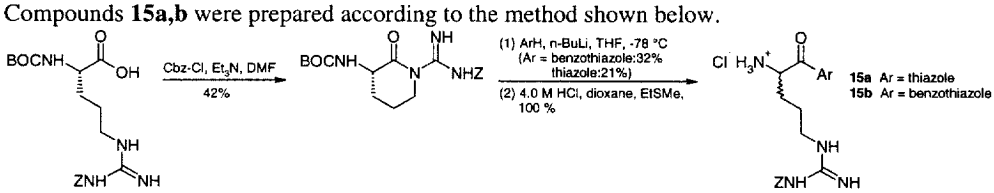
References and Notes

1. Presented at the 214th American Chemical Society Meeting (Las Vegas, NV, September 7-11, 1997)
2. Hirsh, J.; Marder, V. J.; Saizman, E. W. *Hemostasis and Thrombosis: Basic Principles and Clinical Practice*, 3rd Ed., Collman, R. W., Ed.; J. B. Lippincott: Philadelphia, 1994; p 1638.
3. Edmunds, J. J.; Rapundalo, S. T.; Siddiqui, M. A. *Ann. Rep. Med. Chem.* **1996**, *31*, 51.
4. Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N. S. *TiPS* **1993**, *14*, 366.
5. Meyers, Z. I.; Romo, D. *Tetrahedron* **1991**, *46*, 9503.
6. The authors have deposited X-ray crystallographic data with the Cambridge Crystallographic Data Centre.
7. The NMR spectrum of **14a** is identical to a sample prepared using a different route, see: Siddiqui, M. A.; Préville, P.; Tarazi, M.; Warder S. E.; Eby P.; Gorseth, E.; Puumala, K.; DiMaio J. *Tetrahedron Lett.* **1997**, *38*, 8807.
8. Compounds **15a,b** were prepared according to the method shown below.



9. For determination of binding affinities, see: Finkle, C. D.; St-Pierre, A.; Leblond, L.; Deschênes, I.; DiMaio, J.; Winocour, P. D. *Thromb. Haemostasis.* **1998**, *79*, 431.
10. Since these inhibitors (e.g. **16f–16g**) are derived from coupling of peptidomimetic acid with racemic thiazoloketoarginine (**15a**), corresponding hemiaminal isomers are expected to have four diastereoisomers. Moreover, nature of hemiaminal moiety is prone to undergo solvent dependant equilibration, thus the ratio of four diastereisomers is likely to vary from solvent to solvent. The fact that the two isomers (**16f–16g**), are stable entities and the ratio of isomers is solvent independant suggests that there are epimers at the arginine. However, each epimer undergoing equilibration to form hemiaminal under assay conditions can not be ruled out.