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# The Design and Synthesis of Thrombin Inhibitors: Analogues of MD805 Containing Non-Polar Surrogates for Arginine at the P1 Position

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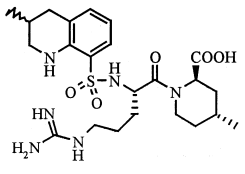
**Abstract**—A series of monocyclic and bicyclic amino acids have been synthesised and incorporated into thrombin inhibitors based on CGH728, an analogue of the Mitsubishi compound MD805. Benzthiazolylalanine (Bta) was found to be a good non-polar substitute for arginine at the P1 position, yielding compounds with low nanomolar potency and good selectivity for thrombin.


The serine protease enzyme thrombin plays a central role in the blood coagulation pathway,[1] directly triggering the production of insoluble fibrin while at the same time providing stimulus for platelet aggregation. The key position of thrombin has made it a popular target for antithrombotic therapy; initial research leading to the discovery of parenterally administered thrombin inhibitors such as hirudin.[2] However, the main effort has been centred on achieving the more difficult goal of an orally active compound.[3]

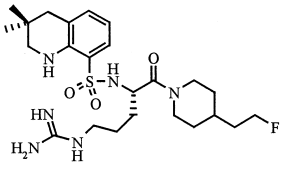
Previous work in our laboratories, based on the Mitsubishi lead MD805,[4] had led to compounds with reduced stereogenicity and increased potency, exemplified by CGH728 that has a $K_i$ of 6 nM for thrombin (Fig. 1).[8]

Although such arginine based inhibitors are potent and selective they tend to have a short duration of action and poor oral bioavailability, properties that are considered largely due to the presence of the very basic guanidine group ($pK_a \sim 14$). Manipulations to reduce the basicity of the P1 amino acid have been demonstrated to increase cell permeability[5] and with this in mind we sought to find a P1 arginine substitute for MD805 type inhibitors that would eventually lead to an efficacious oral drug.

Despite the strong monodentate salt bridge interaction between the arginine guanidine group and Asp-189 of the enzyme, the S1 site of serine proteases tends to be largely lipophilic. This property is accentuated in thrombin over certain other serine proteases by the presence of an alanine residue at position 190 (e.g., serine in trypsin and chymotrypsin). The prospect of making fuller use of this lipophilicity, that is apparently not optimally exploited by arginine, represented a possible opportunity to obtain good binding and selectivity while removing the guanidine. We were therefore interested to explore the P1 position with amino acids totally devoid of amidine or guanidine, but containing a variety of lipophilic side chains. A large number of acyclic,[9] monocyclic and bicylic containing structures were investigated, some of which are shown in Tables 1 and 2.




MD805




CGH728

**Figure 1.**

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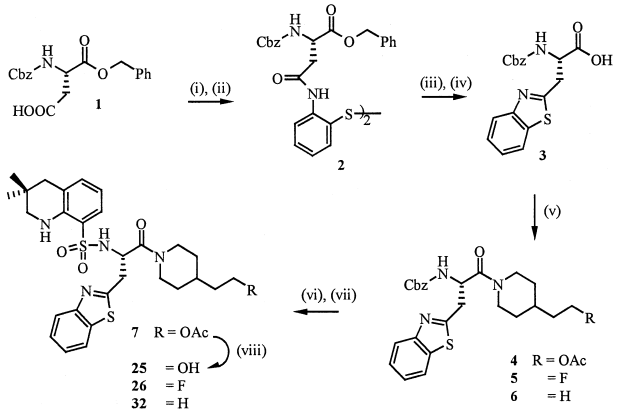
## Chemistry

The majority of compounds were synthesised from a suitably protected aspartic acid precursor. Thus Cbz protected Bta **3** could be prepared in four steps via the acid chloride of Cbz–Asp–OBn and 2-aminophenyl disulphide, using a procedure based on that of Nestor.[6] Sequential coupling with piperidine and sulphonyl chloride was carried out using standard conditions (Scheme 1).

The nitrogen containing analogues of Bta were prepared by the coupling of the hydroxy-amino pyridine **8** or pyrimidine **9** using a reversed mode of thiazolyl ring formation (Scheme 2). The nucleophilicity of benzimidazoles can cause problems during syntheses, a late stage heterocyclic ring formation strategy was therefore favoured for the synthesis of **24**. Cl-DMTHQS–Asp–AEP (**12**) (AEP = acetoxyethylpiperidine) was prepared in 4 steps from Boc-Asp(OBzl)-OH which was then converted to the benzimidazole (**24**).

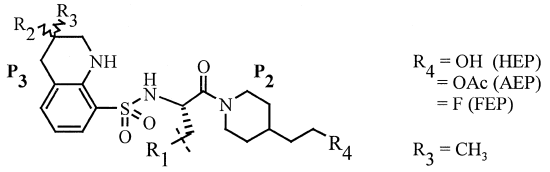
The amino acid precursors to the 4-substituted thiazolyl compounds **27** to **29** were easily obtained from asparagine, first by conversion to the thioamide with Lawsson's reagent,[7] followed by cyclisation onto the appropriate chloromethyl ketone. The fully assembled 4-chloromethylthiazolyl compound was converted to the azide and then smoothly reduced to the amine **29** with triphenylphospine in the presence of water. Other amino acids were obtained from commercial sources (Scheme 3).

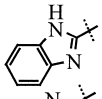
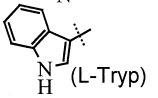
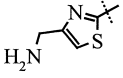
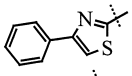
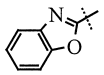
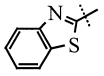
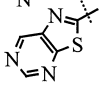



**Scheme 1.** Synthesis of benzthiazolylalanine (Bta) containing inhibitors. Reaction conditions: (i) $SOCl_2$ (99%); (ii) *i*-$Pr_2EtN$, (2-$H_2N$-$C_6H_4)_2S_2$ (88%); (iii) Zn, AcOH, then TEA, dioxan (81%); (iv) 1M NaOH (EtOH/$H_2O$) (98%); (v) 4-(*R*-ethyl)piperidine, DCC, HOBt (>90%); (vi) HBr, AcOH (97%); (vii) $RSO_2Cl$, NMM, DCM (50–70%); (viii) NaOMe, MeOH (90%).

Compounds containing the monomethyl tetrahydroquinolyl (MTHQS) P3 were prepared by coupling the respective amino precursor to the known 3-methylquinoline-8-sulfonyl chloride with subsequent catalytic

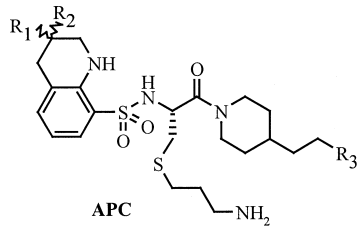
**Table 1.** Monocyclic and bicyclic amino acids at the $P_1$ position

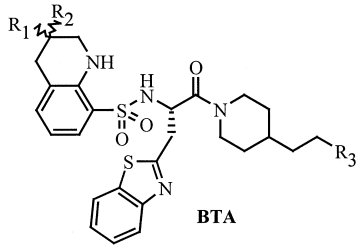



| Compd | $R_2$ | $R_4$ | $R_1$ | $K_i$ (μM) | Comp | $R_2$ | $R_4$ | $R_1$ | $K_i$ (μM) |
|---|---|---|---|---|---|---|---|---|---|
| **16** | H | HEP |  | 6.7 | **24**[a] | $CH_3$ | AEP |  | 0.452 |
| **17** | H | HEP |  | 47.3 | **25** | $CH_3$ | HEP |  | 0.0798 |
| **18** | $CH_3$ | HEP |  | 132.7 | **26** (CGH752) | $CH_3$ | FEP |  | 0.026 |
| **19** | $CH_3$ | HEP |  | 19.58 | **27** | $CH_3$ | HEP |  | 2.19 |
| **20** | H | HEP |  (L-Tryp) | 1.57 | **28** | $CH_3$ | HEP |  | 0.76 |
| **21** | H | HEP | D-Tryp | 59.9 | **29** | $CH_3$ | HEP |  | 3.28 |
| **22** | H | HEP |  | 3.1 | **30** | $CH_3$ | FEP |  | 0.81 |
| **23** | H | HEP |  | 0.118 | **31** | $CH_3$ | FEP |  | 4.56 |

[a]Contains 6-chlorodimethyltetrahydroquinoline at the P3 position.

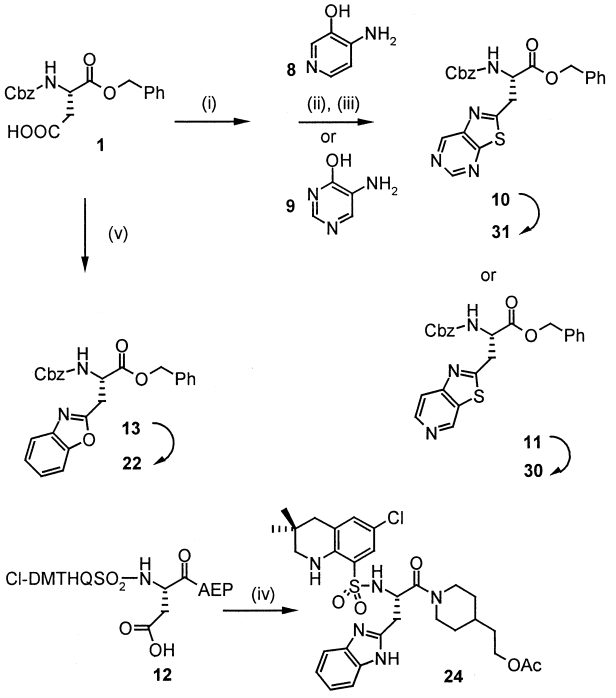
**Table 2.** Serine protease selectivity profile of thrombin inhibitors containing Benzthiazolylalanine (Bta) and Aminopropylcysteine (APC) at P1



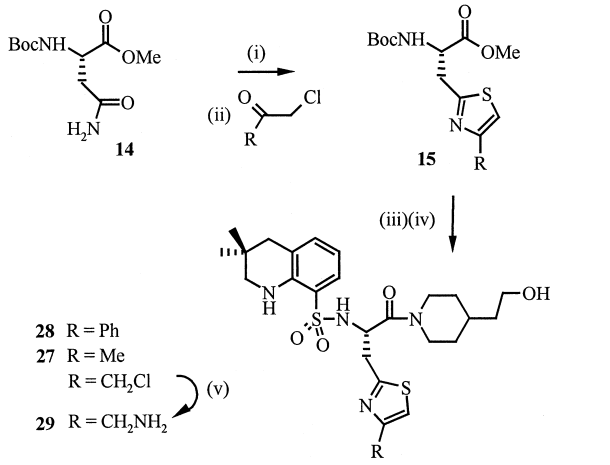




| | | | | $K_i$ (μM) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | P1 | $R_1$, $R_2$ | $R_3$ | Thrombin | Trypsin | Chymotrypsin | Plasmin | Kallikrein | Xa |
| **34** | APC | $R_1=R_2=CH_3$ | F | 0.126 | 1.32 | 11.95 | 4.0 | Φ [a] 87 | Φ[a]87 |
| **35** | APC | $R_1=R_2=CH_3$ | OH | 0.28 | 1.78 | 384 | 6.2 | Φ87 | Φ87 |
| **32** | BTA | $R_1=R_2=CH_3$ | H | 0.248 | 260 | 4.91 | 240 | Φ92 | Φ92 |
| **26** | BTA | $R_1=R_2=CH_3$ | F | 0.026 | ND[b] | 8.4 | ND | ND | ND |
| **33** | BTA | $R_1=H, R_2=CH_3$ | OH | 0.118 | 126 | 7.55 | 109 | Φ45 | Φ45 |

[a]Φ = Inactive at stated concentration.
[b]ND = not determined.




**Scheme 2.** Synthesis of analogues of Bta. Reaction conditions: (i) $SOCl_2$ (99%); (ii) pyridine, DMAP, **8** or **9** (93% and 21% respectively); (iii) Lawesson's reagent, toluene, 80 °C (68% & 48%); (iv) (a) iBuOCOCl, phenylenediamine, TEA, THF; (b) AcOH; (v) (a) iBuOCOCl, *o*-aminophenol (55%); (b) AcOH, reflux (42%).




**Scheme 3.** Synthesis of thiazolyl containing inhibitors. Reaction conditions: (i) Lawesson reagent, toluene, 80 °C; (ii) $RCOCH_2Cl$, molecular sieve, THF; (iii) (a) 1M NaOH, $MeOH/H_2O$; (b) 4-acetoxyethylpiperidine, DCC, HOBt; (iv) (a) TFA, DCM; (b) $DMTHQSO_2Cl$, NMM, DCM; (c) 1M NaOH, $MeOH/H_2O$; (v) (a) $NaN_3$, NaI, DMF; (b) $Ph_3P$, $H_2O$.

reduction of the quinoline ring. Dimethyl tetrahydroquinolyl sulphonyl chloride ($DMTHQSO_2Cl$) was prepared from *p*-chloroaniline and dimethyl malonic acid in 7 steps,[8] 4- fluoroethylpiperidine (FEP) was prepared from *N*-Boc-4-hydroxyethylpiperidine (Boc-HEP) by treatment with DAST.[8]

## Results and Discussion

From Table 1 it can be seen that benzthiazolylalanine (Bta) compounds (**23**, **25** and **26**) are the most potent, indeed Bta in combination with DMTHQS and FEP at P3 and P2 respectively[9,10] (**26**, **CGH752**) gave a $K_i$ of 26nM, a potency comparable to arginine based inhibitors.

Alterations to this ring system always led to a reduction in potency. The enhanced activity of Bta can mainly be rationalised in terms of good lipophilic interaction with the P1 pocket, an explanation born out by modelling[11] (Fig. 2) and later confirmed by the crystal structure of an inhibitor/thrombin complex. While the sulphur atom

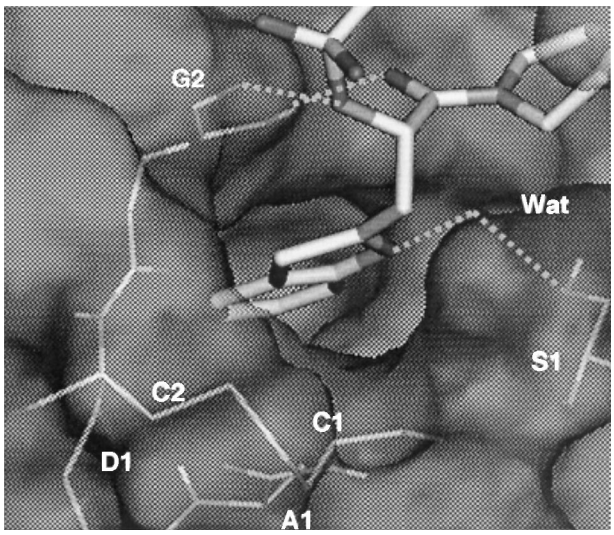



**Figure 2.**

and benzene ring of the inhibitor make good contact in a low energy conformation with the S1 pocket of thrombin, the thiazolyl ring nitrogen hydrogen bonds with a water molecule associated with serine. This same water interacts with the piperidine carboxyl group of MD805 in thrombin. The difference in activity observed between **22** and **23** probably reflects a combination of the superior lipophilic influence of sulphur on the whole system and the increased bond length facilitating better fit. It is more difficult to interpret the intermediate activity of **24**, but it is feasible that this compound may adopt a different binding conformation.

In Table 2 a selectivity comparison is made between Bta compounds and inhibitors from an acyclic series (**34**, **35**).[12] From this it can be seen that Bta is a P1 amino acid which confers good selectivity for thrombin over a number of other serine proteases, especially trypsin, a potentially important pre-requisite for oral activity. Although the Bta compounds prepared had low aqueous solubility and no appreciable oral bioavailable, they represented a unique starting point to totally circumvent the problems inherent to guanidine and amidine based thrombin inhibitors. A detailed description of our work to introduce the desired properties into Bta based inhibitors is covered in the following paper.

## Conclusion

In summary a series of novel, reversible thrombin inhibitors have been synthesised in which the cationic arginine moiety has been substituted by neutral, low polarity groups. Bta derived inhibitors exhibit excellent potency and selectivity for thrombin, and represent a major step forward in the search for orally active compounds.

## References and Notes

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9. MD805 and Bta containing inhibitors bind to thrombin with a different backbone orientation to that of fibrinogen, with the MTHQS ring occupying S8 and S9, termed P3* by Banner,[10] and the piperidine moiety occupying S2. For convenience we term MTHQS and DMTHQS P3 and the piperidide moiety P2.
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12. Details concerning acyclic P1 inhibitors of this type will be published elsewhere.