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The Design and Synthesis of Thrombin Inhibitors: The Introduction of In Vivo Efficacy and Oral Bioavailability into Benzthiazolylalanine Inhibitors

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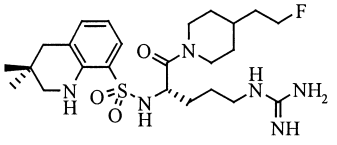
Abstract—The further optimisation of the novel lead compound CGH752 (Fig. 1) is described. By introducing various substituents into the 6-position of the 3,3-dimethyltetrahydroquinoline (DMTHQS) ring we have been able to favourably affect the in vitro and in vivo activity, and the pharmacokinetics of such compounds. One of the inhibitors synthesised (CGH1484) is bioavailable and shows efficacy in animal models of thrombosis. © 2000 Elsevier Science Ltd. All rights reserved.

The development of orally active compounds to specifically inhibit thrombin is a significant therapeutic goal.[1] In the previous letter we have detailed our findings with amino acid replacements for arginine at the P1 position of MD805 derived inhibitors, that culminated in the discovery of CGH752 (Fig. 1) as a novel, potent and selective inhibitor of thrombin.

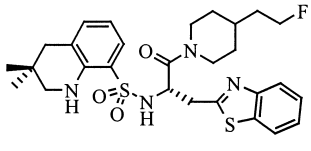
Although having potentially eliminated some of the poor characteristics of a guanidine based inhibitor i.e., removal of the cationic group that prevents satisfactory absorption, there remained a number of properties to improve before realising our goal of an orally effective drug candidate.

The removal of a charged guanidine species and the introduction of a lipophilic Bta group (CGH752 ClogP = 5.38) has serious consequences on the aqueous solubility of these compounds, hence CGH752 is largely insoluble and is not orally bioavailable. The lipophilic profile of CGH752 also alters the effectiveness of the compound to inhibit coagulation in plasma, as measured by the more physiological relevant activated partial thromboplastin time (APTT). This is apparent from a comparison of the APTTs of an arginine compound CGH728 ($K_i = 6$ nM, APTT = 2.1 μM) with CGH752 ($K_i = 26$ nM, APTT = 26.5 μM) (Fig. 1), and can probably be contributed to the increased protein binding of the more lipophilic compound. However, in this new series the possibility of reintroducing some polar groups to tune the physichochemical characteristics, and hence the pharmacokinetics and in vivo activity, was now a possibility. This is somewhat different to the strategies pursued by some other research groups, where the P1 charge interaction has been preserved by introducing a basic amidine group or by modulating the basicity in order to confer favourable properties.[2]

Confident that the presence of Bta in our inhibitors would ensure selectivity for thrombin, we sought to find a site on



CGH728



CGH752 (DMTHQS-Bta-FEP)

Figure 1.

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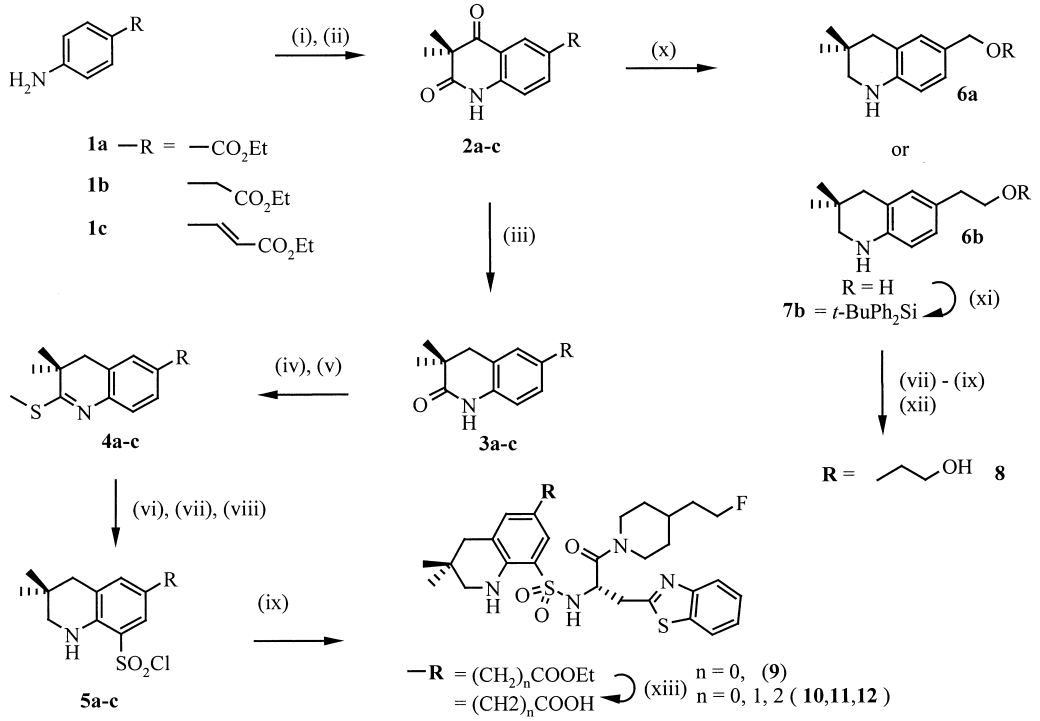
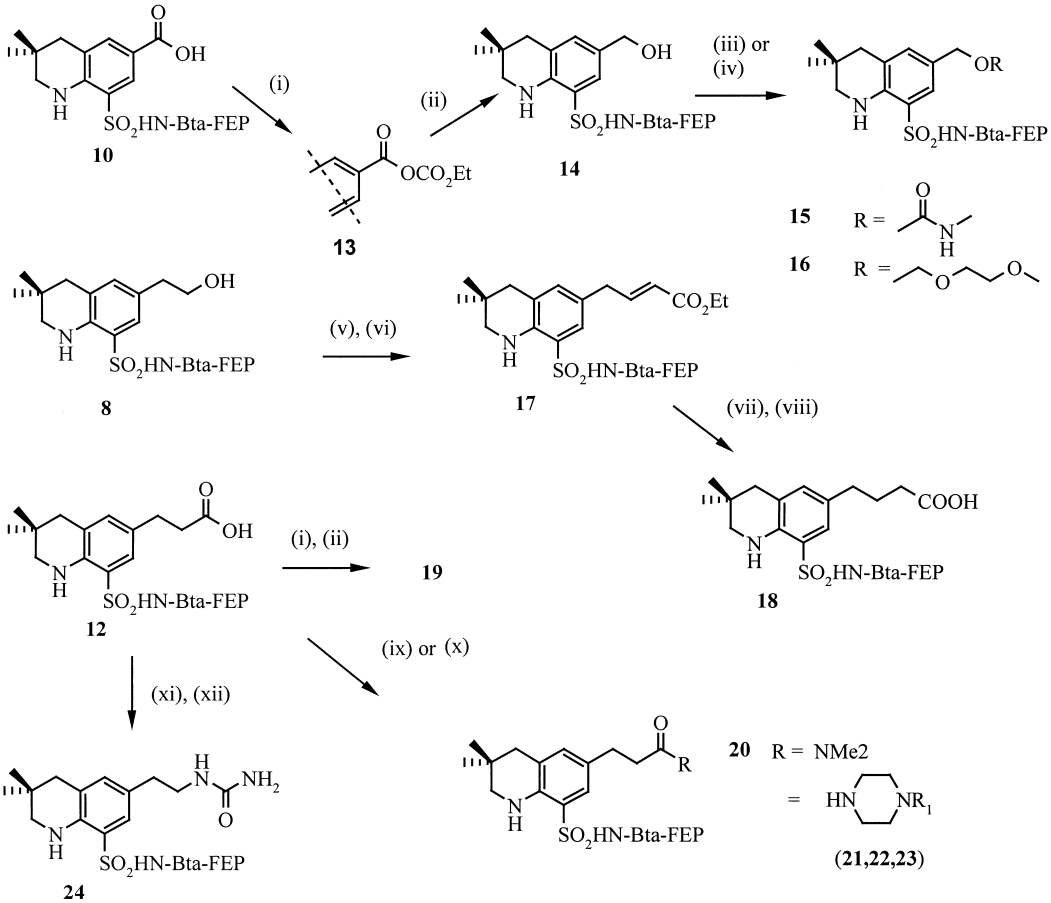


Scheme 1. Reaction conditions: (i) (a) dimethylmalonic acid, $SOCl_2$, , THF, reflux; (b) 4-sub-aniline (2 equiv), 20 °C; (ii) P_2O_5, CH_3SO_3H, 70 °C; (iii) Pd/C, AcOH, H_2; (iv) (a) P_2S_5 or Lawesson's reagent, dioxan; (v) MeI, KOtBu, THF; (vi) Na $CNBH_3$; (vii) Pyr.SO_3 Pyr, reflux; (viii) Pyr, $POCl_3$, CH_3CN, ultrasound 20 °C; (ix) H-Bta-FEP, DIPEA, DCM; (x) $AlCl_3$, LAH, THF/Et_2O, 20 °C to reflux; (xi) *t*-$BuPh_2SiCl$, imidazole; (xii) TBAF, THF; (xiii) 1M NaOH, H_2O/MeOH.



Scheme 2. Reaction conditions: (i) $ClCO_2Et$, DMF, TEA; (ii) $NaBH_4$, EtOH; (iii) meNCO, DCM, AcOH, 50 °C; (iv) DIPEA, DCM, RCl; (v) $COCl_2$, DMSO, TEA; (vi) $Ph_3P{=}CHCO_2Et$, DCM, rt; (vii) $NaBH_4$, CuCl, MeOH; (viii) NaOH, H_2O, meOH, then d.HCl; (ix) PyBOP, DIPEA, DMF, rt; (x) TBTU, DIPEA, DCM, piperazine; (xi) $(PhO)_2P(O)N_2$, TEA, Toluene, 100 °C; (xii) NH_3, DCM.

the CGH752 structure where property-modifying groups could be introduced without impairing the good binding affinity. Modelling first suggested the P2 piperidine moiety had potential for such a campaign; there appears to be considerable space within the enzyme to accommodate structural changes to the substituent group on piperidine. However, from previous studies in the arginine series we knew that it was not easy to make changes at this position and maintain good K_i and APTT values.[3] This was confirmed by the synthesis of a number of P2 variants in the Bta series, findings that largely paralleled those of the equivalent arginine compounds. The other possible site was the aromatic ring of the dimethyltetrahydroquinoline (DMTHQS) P3, where a small amount of direct evidence already existed that this could be substituted in the 6-position without detriment to potency.[3] The selection of the DMTHQS P3 6-position was also born out by a systematic modelling study, and the synthesis and evaluation of compounds with various substitutions at this position was therefore undertaken (Scheme 1).

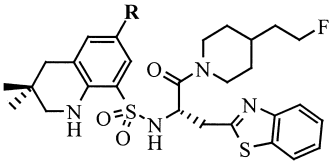
Chemistry

Key 6-substituted DMTHQS intermediates were prepared by a route adapted from that originally used for the unsubstituted version.[3] The respective *para*-substituted aniline was coupled to in situ prepared dimethylmalonyl chloride and the resultant amide carboxylic acid efficiently cyclised to the ketoamides **2a–c** in Eaton's reagent.[4] These were then converted in a stepwise fashion, first by reduction of the benzylic ketone (and double bond for **3c**) by catalytic hydrogenation, and then by reduction of the amide via the intermediary *S*-methyl thioimidate compounds **4a–c**, thus preserving the ester functionality in the 6-positive. Alternatively a total reduction was carried out with LAH in the presence of aluminium chloride leading to the 6-hydroxymethyl and ethyl compounds **6a** and **6b** respectively. Regioselective sulphonation, chlorination and coupling with the free base of Bta-FEP completed the synthesis. The preparation of Bta-FEP is detailed in the preceding letter. The fully coupled compounds could be conveniently transformed to more complex structures. Thus the carboxylic acid **10** was reduced via the mixed anhydride (Scheme 2) and the alcohol (**14**) reacted with methyl isocyanate to form **15** or alkylated to **16**. Swern oxidation of **8** followed by Wittig reaction led to alkene **17**, which could then be reduced with copper (I) chloride/sodium borohydride,[5] with no interference from Bta, and hydrolysed to the acid **18**. The carboxylic acid **12** was coupled to a variety of amines typically using PyBOP or TBTU (Scheme 2). A Curtius rearrangement was accomplished with diphenyl-phosphoryl azide and the isocyanate trapped with ammonia to give the urea **24**, a number of substituted ureas and urethanes could be prepared in this way.

Results and Discussion

As new compounds bearing substituents at the 6-position were synthesised and assayed, it became apparent that a range of chain length and functionality could be incorporated while retaining reasonable activity, a finding that was in contrast to our experience in the P2 area. The various compounds shown in Table 1 all have submicromolar activity and the majority have improved APTTs when compared to the original lead. As was previously stated there appears to be a relationship between logP (and logD) and APTT at a gross level,[6] but the effectiveness of an inhibitor in plasma must be governed by a multiplicity of factors making rationalisation and prediction difficult at the present time. Using **12** (bioavailability = 50% id) as a template a variety of amides were prepared, with piperazine amides in particular displaying good properties. The compound **23** (**CGH1484**) (Table 2), which contains the solubilising elements of a basic nitrogen (piperazine ring) and morpholine, had good K_i and APTT; moreover this compound showed reasonable bioavailablity (28%) when dosed to rats by *per oral* administration. The effects of CGH1484A (HCl salt) on arterial thrombosis in the rat was determined using an acute model of injury-induced thrombus formation in the rate dorsal aorta.[7] CGH 1484A of a 10 or 20 mg/kg (po)

Table 1.



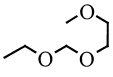
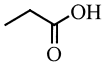
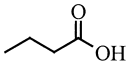
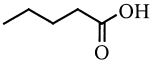
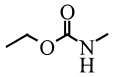
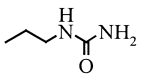
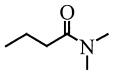
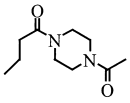
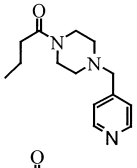
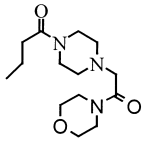
	-R	K_i (nM)	APTT (μM)	ClogP
752		26	26.5	5.38
14		64	16.8	4.57
8		39	13.7	4.58
19		119	19	5.1
16		125	18	
9		723	105.4	6.45
10		359	13	5.46
11		500	14.9	4.25
12		82	10.7	5.1
18		94	16.6	5.63
15		77	20.7	4.85
24		47	10.2	4.03
20		34	9.2	4.54

Table 2.

	-R	K_i (nM)	APTT (μM)	Bio Avl.	Aqu sol. (mg/ml)
12		82	10.7	50% (id) 0% (po)	0.00515 @pH7
21		34	7.5	N.D.[b]	N.D.
22		27	9.7	N.D.	N.D.
23[a] **CGH1484***		57	7	38% (id) 28% (po)	0.13 @pH7 15 @ pH2

[a]K_i trypsin = 153 μM, chymotrypsin = 12.2 μM, plasmin = 212 μM, Xa = >200 μM, TF/VIIa = 11600 μM.
[b]N.D. = not determined.

dose, administered 1 h prior to injury to the dorsal aorta, significantly inhibited platelet deposition by approximately 60%. The effect was maintained with the higher dose for up to 3 h. The effect on fibrin(ogen) deposition was less marked with a 48% and 43% inhibition being noted at 1 and 2 h following the oral administration of 20 mg/kg CGH1484A.

Conclusion

The presence of Bta in inhibitor compounds ensures good potency and selectivity for thrombin over other serine proteases. The introduction of a variety of groups into the 6-position of the DMTHQS aromatic ring is possible without adversely affecting the good binding of Bta-FEP based inhibitors and we believe these substituents extend onto the surface of the enzyme. These findings have allowed us to introduce groups that markedly influence the water solubility and APTT of inhibitors, and have led to a compound (CGH 1484) with oral efficacy in animal models of thrombosis.

References and Notes

1. (a) Brundish, D. E. *Current Opinion in Therapeutic Patents (Current Drugs)* **1992**, pp 1457–1466; (b) Ripka, W. C. *Curr. Opin. Chem. Biol.* **1997**, *1*, 242; Wiley, M. R.; Fisher, M. J. *Expert Opin. Ther. Pat.* **1997**, *7*, 1265. (d) Hauptmann, J.; Sturzebecher, J.; *Thromb. Res.* **1999**, *93*, 203.
2. (a) Misra, R. N.; Kelly, Y. F.; Brown, R. B.; Roberts, D. G. M.; Chong, S.; Seiler, S. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2165. (b) von der Saal, W.; Kucznierz, R.; Leinert, H.; Engh, R. A.; *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1283. (c) Sanderson, P. E. J.; Naylor-Olsen, A. *Curr. Med. Chem.* **1998**, *5,* 289.
3. Brundish, D.; Bull, A.; Donovan, D.; Fullerton, J. D.; Garman, S.; Hayler, J.; Janus, D.; Kane, P. D.; McDonnell, M.; Smith, G. P.; Wakeford, R.; Walker, C. V.; Howarth, G.; Hoyle, W.; Allen, M. C.; Ambler, J.; Butler, K.; Talbot, D. *J. Med. Chem.* **1999**, *42*, 4584.
4. McGarry, L. W.; Detty, M. R. *J. Org. Chem.* **1990**, *55*, 4349.
5. Narisada, M.; Horibe, I.; Watanabe, F.; Takeda, K. *J. Org. Chem.* **1989**, *54*, 5308.
6. Unpublished results.
7. Butler, K. D.; Ambler, J.; Dolan, S.; Giddings, J.; Talbot, M. D.; Wallis, R. B. *Blood Coag. Fibrinol.* **1992**, *3*, 155.