Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1103–1108

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Discovery of Orally Available Thrombin Inhibitors : Optimisation of the P1 Pharmacophore

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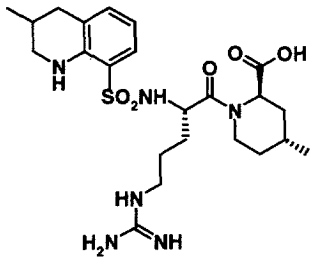
Received 22 December 1998; accepted 9 March 1999

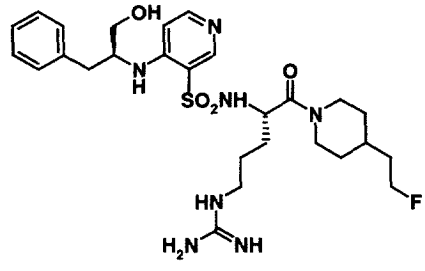
Abstract: Thrombin inhibitors have been designed with the replacement of the strongly basic guanidine P1 pharmocophore with a group that exploits the lipophilicty of the S1 pocket. The approach has lead to the discovery of potent thrombin inhibitors demonstrating good intra-duodenal absorption.

Intravascular clot formation is a causative factor in a number of cardiovascular diseases including myocardial infarction, deep vein thrombosis, and ischemic stroke. Thrombin has become an important target for therapeutic intervention due to its central role in homeostasis and thrombosis.[1] In the clinic, subcutaneous injections of heparins[2] and hirudin[3], or the orally administered coumarins such as warfarin[4], form the mainstay of current therapy, but all suffer limitations particularly for chronic use. The requirement for a potent, selective and orally bioavailable thrombin inhibitor, which can provide predictable anticoagulation across a patient group without the risk of bleeding, has lead to a concentrated effort by several pharmaceutical companies to develop direct thrombin inhibitors[5]. Yet despite this effort, the discovery of suitable drug candidates to progress into the clinic has yet to be accomplished.

Starting from the reversible thrombin inhibitor MD805[6], work done in our laboratory identified the arginine derivative **1** as a potent thrombin inhibitor with a Ki value of 0.016μM. The compound contained the novel '4-amino-pyridyl' P3 pharmacophore[7-8], but displayed no oral bioavailability. We set out to design a new P1 pharmacophore that would not only assist in improving bioavailability but would also be easily accessible synthetically.



MD-805



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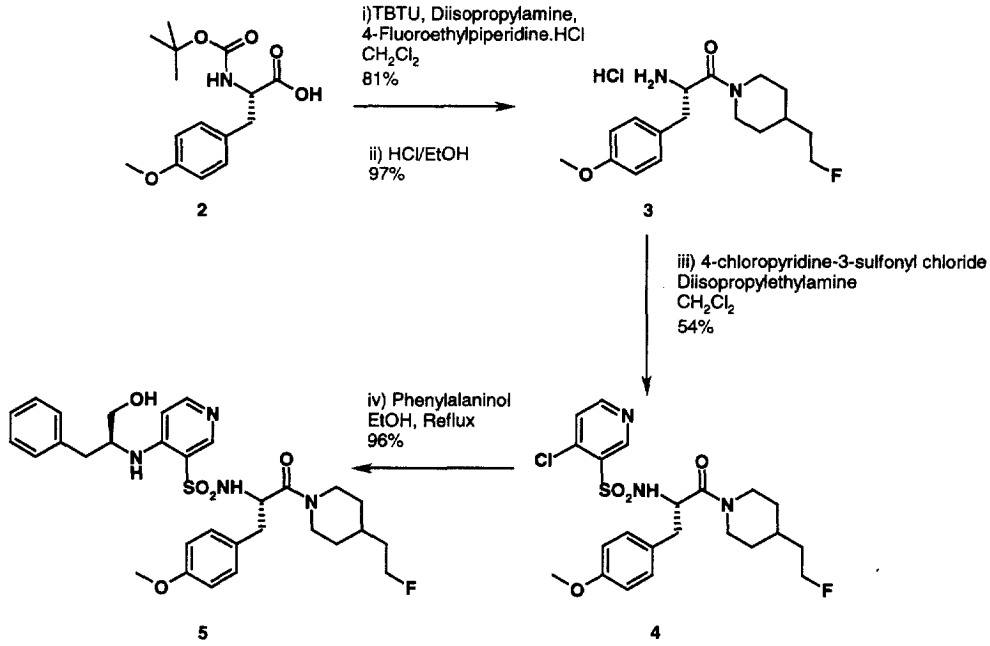
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00138-9

From the X-ray structure of crystallised thrombin[9], it is clear that the S1 site is a deep pocket with Asp 189 at its end, capable of forming a salt-bridge with positively charged residues such as arginine or lysine. However, the remainder of the pocket is hydrophobic and therefore permits the design of inhibitors with lipophilic P1 side chains[10]. There are many examples of potent thrombin inhibitors in the literature that use in the P1 side chain either a guanidine or benzamidine group[5]. However we have found from an extensive study of arginine based derivatives[11] similar to compound **1**, that inhibitors containing strongly basic groups are unlikely to be passively absorbed across the gastrointestinal tract. Herein we describe our efforts in designing a P1 pharmacophore to exploit a lipophilic interaction in the S1 pocket. By avoiding the use of very basic side chains that have a detrimental effect on absorption, we have obtained potent inhibitors displaying good bioavailability.

Scheme 1

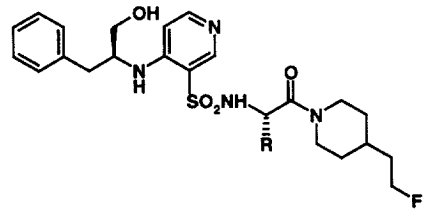


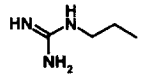
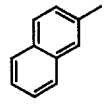
Potency for each compound was measured by a Ki determination[12] against Human thrombin and by their influence on the doubling of a human clotting time assay (the activated partial thromboplastin time APTT)[13]. For candidates with APTT values below an arbitrary 10μM, an indication of the bioavailabilty was obtained by intra-duodenal administration (3mg/kg) of the compound in PEG400 to anaesthetised Sprague-Daley rats, followed by sampling of blood to the portal vein and carotid artery over a 2-hour period. HPLC analysis of the samples provided AUC(portal) and AUC(carotid) values that were interpreted as a measure of intestinal absorption and an indicator of potential bioavailability respectively.

The methods used to synthesise the derivatives described are exemplified by the synthesis of compound **5** shown in Scheme 1. 4-fluoroethyl piperidine was condensed with a BOC protected amino acid using 2-(1H-

benzatriazole-1-yl)-1,1,3,3 tetramethylammonium tetrafluoroborate (TBTU) as the peptide coupling agent, followed by removal of the BOC protecting group by treatment with 6M HCl/ethanol to afford the amine **3** as its HCl salt. The amine was coupled to chloropyridine-3-sulfonyl chloride[14] to give the chloropyridyl derivative **4**. Substitution of the 4-chloro group with excess phenyl alaninol was performed in a sealed vessel using ethanol as solvent at 90°C to furnish the product **5** in good yield.

Table 1



Compound	R group	Ki (μM)	APTT (μM)
6	Me	49.89	--
7	iPr	6.51	--
8		11.93	--
9	$(CH_2)_4NH_2$	1.8	--
1		0.016	0.75
10		0.1	11
11		11.81	--
12		0.069	26

The importance of the P1 pharmacophore in providing tight binding inhibitors is clearly demonstrated by the weak binding affinity of the alanine derivative **6** (Table 1). Extending a lipophilic chain into the pocket, as in examples **7** and **8**, has little effect, whilst introducing a terminal basic amino group, as in lysine derivative **9**, results in a modest increase in binding for this series of inhibitors. However, introduction of a phenyl ring, as in the phenylalanine derivative **10**, resulted in a significant increase in binding affinity, demonstrating the effect of an enhanced lipophilic interaction and optimum topology in the S1 pocket. The analogous cyclohexyl derivative **11** is two orders of magnitude less potent than **10**, suggesting a requirement for planar groups. The need for planarity is further supported by the equally good activity of the napthyl derivative **12**.

Although planar lipophilic pharmacophores in P1 can clearly provide a significant contribution to binding affinity, such groups increase the lipophilicity of the compounds[15] resulting in both poor APTT values and low intra-duodenal absorption. From previous studies on compounds within this series, we have found an increased probability of absorption with compounds within a clogD[16] window of 2 to 4. Consequently compounds with lower clogD values were synthesised.

Introducing a heteroaromatic ring in P1 provided compounds with lower clogD values (Table 2). The 2-pyridyl derivative **13** showed good absorption and, although of similar Ki potency to compound **10**, the APTT value is lower by a factor of more than two. The position of the pyridyl nitrogen is clearly important, with the 3 & 4-pyridyl derivatives **14 & 15** seeing a significant reduction in binding affinity. The thiazole derivative **16**, with a clogD of 2.24 also demonstrates good absorption.

Table2

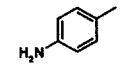
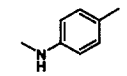
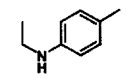
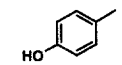
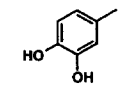
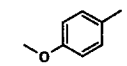
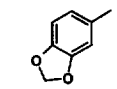
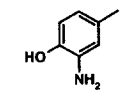
Compound	R group	Ki (μM)	APTT (μM)	AUCp	AUCc	cLogD @pH 6.2
10		0.1	11	22(±6)	13(±1.5)	3.83
13		0.081	3.87	126(±42)	19(±3)	2.49
14		1.61	20.33	--	--	2.45
15		1.54	--	--	--	2.45
16		0.28	9.17	101(±17)	4(±3)	2.24

Reduction in clogD was also achieved by addition of polar groups onto the phenyl ring, as shown in Table 3. Derivatives **17, 18** and **19** containing para amino substituents show good absorption, although only the primary amino compound **17** has systemic exposure. The phenol containing compounds **20** and **21** were poorly absorbed, but the tyrosine derivative **20** had a marked effect on lowering the APTT value as well as providing a modest reduction in potency over the parent phenylalanine derivative **10**. Compound **20** clearly demonstrates that in this series of thrombin inhibitors, a basic amino group is not required in the P1 side chain for tight binding. Masking the phenol groups in **20** gave the methylene dioxy derivative **22,** which was well absorbed.

In conclusion, the P1 pharmacophore has been optimised by replacing the strongly basic guanidine side chain of compound **1** with a more lipophilic pharmacophore, affording compounds with good intra-duodenal

absorption, whilst maintaining strong binding affinity and anti-coagulant activity. One such compound, CGH1668 **17**, is a selective thrombin inhibitor (Ki Thrombin 0.013μM, Trypsin(hum.) 25.18μM, Chymotrypsin(hum.) 115.25μM and Plasmin >86μM), with a low APTT (2.51μM) and after oral dosing (10mg/kg p.o.) gave an oral bioavailability of 12(±2)%. Further optimisation of pharmacokinetic parameters in this compound series is the subject of a future communication.

Table 3

Compound	R group	Ki (μM)	APTT (μM)	AUCp	AUCc	cLogD @pH 6.2
10		0.1	11	22(±6)	13(±1.5)	3.83
17		0.013	2.51	112(±21)	16(±3)	1.80
18		0.012	4.08	119(±29)	2(±2)	3.41
19		0.575	17.4	102(±24)	2(±1)	3.91
20		0.041	1.91	28(±8)	3(±1.5)	2.27
21		0.082	6.17	5(±1)	0	1.70
5		0.036	8.013	--	--	3.90
22		0.043	6.39	151(±7)	12(±5)	2.55
23		0.026	1.53	6	0	1.22

References and Notes

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7. For convenience pharmacophores residing in the S9 pocket of thrombin are referred to as P3

8. Studies relating to the discovery of the '4-amino-pyridyl' P3 pharmacophore in press

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11. Studies relating to development of novel thrombin inhibitors based on Argatroban in press. (J.Med.Chem.)

12. n>4 for all Ki values measured

13. n>3 for all APTT values measured

14. The chloropyridine-3-sulfonyl chloride was made by $PCl_5/POCl_3$ chlorination of available 4-hydroxypyridine-3-sulphonic acid.

15. cLogD @pH6.2 for compound **12** is 5.10

16. cLog D values calculated using PrologD software version 2.0, Compudrug, Budapest, Hungary.