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The Discovery of Orally Available Thrombin Inhibitors : Studies Towards the Optimisation of CGH1668

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Abstract : The chemical optimisation of CGH1668 **1** is described employing an *in vivo* model of absorption to determine the influence on bioavailability of single point modifications to five key molecular templates. The discovery of an orally bioavailable and selective thrombin inhibitor, **24**, highlights the utility of this approach.

The coagulation cascade is a critical mechanism by which haemostasis is maintained on vascular injury. Thrombin is the final enzyme in the cascade and plays a central role where its principal actions are to convert soluble fibrinogen into insoluble fibrin which forms a mechanical matrix for the developing blood clot and the activation and aggregation of blood platelets. Thrombin also mediates a number of other events such as tissue remodeling and perhaps most importantly its own self amplification leading to an explosive increase in the generation of enzyme locally at the site of injury [1-2]. With the pivotal role played by thrombin in haemostasis the enzyme has become a principal target in the search for new anticoagulants which have potential for the treatment and prevention of cardiovascular disorders such as deep vein thrombosis, myocardial infarction and stroke [3-4].

Existing treatments for thrombotic disease such as oral warfarin [5], subcutaneous injections of heparins [6] and hirudin [7] have a number of limitations associated with patient compliance and adverse side effect profiles. There is a clear need to develop thrombin inhibitors which are safe, effective and can be administered orally to give maximal benefit. Although the field has seen intense interest over a number of years the holy grail of discovering orally active thrombin inhibitors still remains elusive [8,9]. From the archetypal reversible thrombin inhibitor MD805 [10] our efforts have lead to the discovery of a highly potent competitive thrombin inhibitor CGH1668 **1** bearing a weakly basic aminophenylalanine in P1 and a unique "pyridyl" pharmacophore in P3 [11-12]. Further optimisation of the P3, P1 and P2 pharmacophores using five key molecular templates to provide compounds with enhanced pharmacokinetic profiles is described.

Synthetic access to the compounds described in this series is exemplified by the synthesis of **1** (Scheme 1). Standard peptide coupling conditions using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) on a BOC protected p-nitrophenyl alanine **2** followed by acidic deprotection provided **3**. Coupling of **3** with 4-chloro-pyridine-3-sulfonyl chloride **5**, itself generated by $PCl_5/POCl_3$ chlorination of 4-

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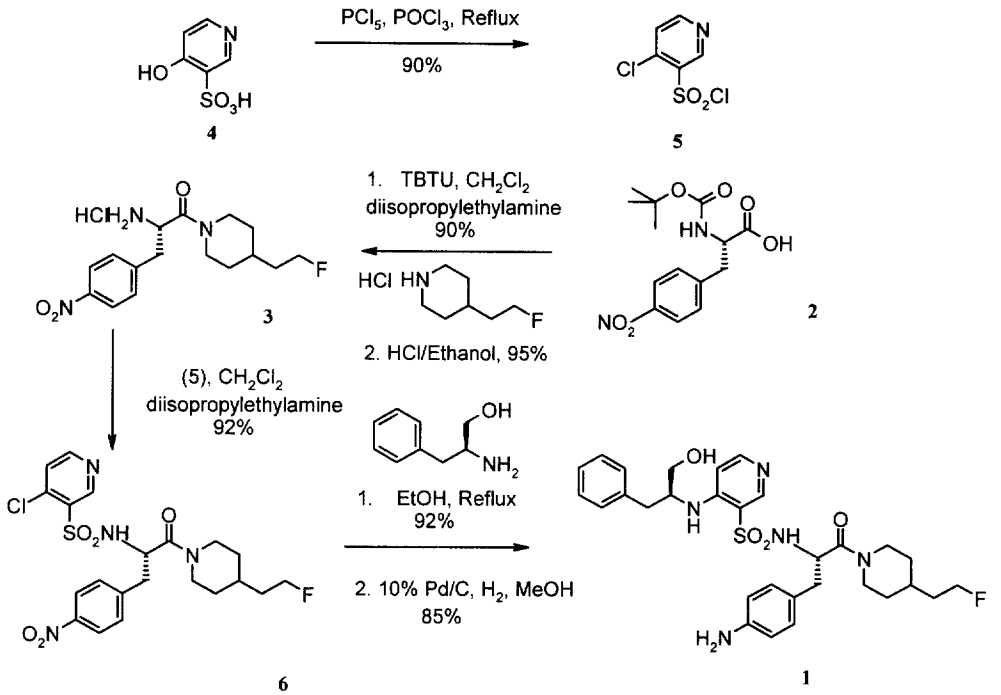
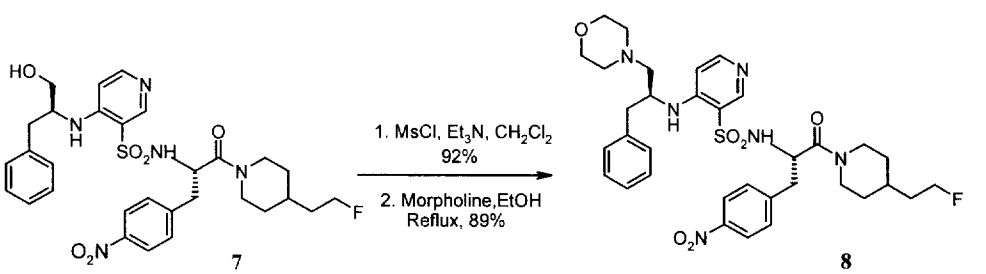
hydroxy-pyridine-sulfonic acid **4**, gave **6** in good overall yield. Finally, coupling of the chloropyridine analog **6** with L-phenylalaninol followed by hydrogenation of the nitro group furnished **1** in 61% overall yield from **2**.



Scheme 1

Elaboration of the P3 pharmacophore in **1**, as exemplified by the synthesis of **8** (Scheme 2), was readily achieved by mesylation of the alcohol **7** followed by displacement under refluxing conditions by the requisite amine. Subsequent hydrogenation of **8** furnished the desired p-anilino-phenylalanine derivatives.

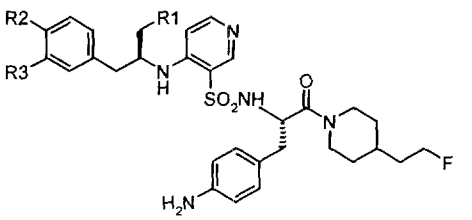


Scheme 2

A study of the structure activity relationships of derivatives at P3, P1 and P2 was conducted and in tandem the influence of structure on the absorption of compounds was measured. The *in vitro* potency of compounds was evaluated as the inhibition constant (K_i) determined against human thrombin. The influence of compounds on the doubling of a human clotting time assay (activated partial thromboplastin time, APTT) was

also determined. Additionally, compounds were dosed intra duodenally (3mg/kg) in PEG400 to anesthetised Sprague-Dawley rats and samples of blood taken from the hepatic portal vein and carotid artery over a 2hr period. The compound concentration in each of the blood samples was determined by HPLC, and the area under curve (portal vein), AUCp and the area under curve (carotid artery), AUCc calculated as indicators of bioavailability.

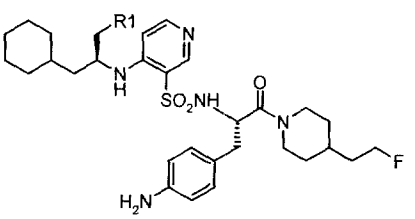
Table 1



Compound	R1	R2	R3	Ki (nM)	APTT (μM)	AUCp	AUCc
1	**OH**	**H**	**H**	**39**	**2.5**	**113**	**18**
9	OH	OMe	H	19	2.9	24	4
10	H	OMe	H	15	9.0	102	7
11	H	H	H	18	7.1	45	7
12	NHMe	H	H	35	2.4	0	0
13	OH	OMe	OMe	49	2.5	45	7

The subtle influence of substitutions at R1, R2 and R3 in P3 is illustrated by the derivatives **1**, **9** and **10** (Table 1). Placing a methoxy substitution at R2 in **1** to give **9** maintains *in vitro* potency however absorption is dramatically reduced. Removal of the hydroxyl in R1 leads to derivative **10** where absorption, at least into the hepatic portal vein, is increased however with a reduction in APTT. Substitution of a basic residue in R1 to give **12** still provides a compound with good potency in both K_i and APTT terms however all potential for absorption is abolished.

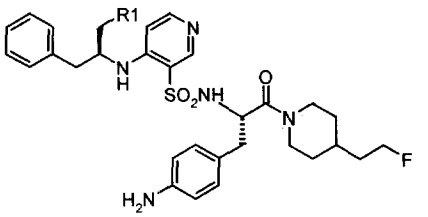
Table 2

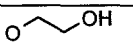
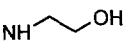
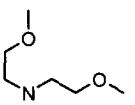
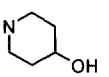
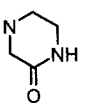
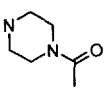


Compound	R1	Ki (nM)	APTT (μM)	AUCp	AUCc
14	OH	18	2.6	91	4
15	NHMe	33	2.8	5	0

Increasing the lipophilicty of the aryl ring in P3 to the cyclohexyl derivative **14** (Table 2) results again in compounds with high *in vitro* potency. Whilst absorption in to the hepatic portal vein is modest (AUCp, 91) systemic values as determined by AUCc are low indicating extensive first pass clearance for both **14** and **15**. As with derivative **12** incorporation of a methylamino group in R1 to give **15** reduces absorption.

Table 3

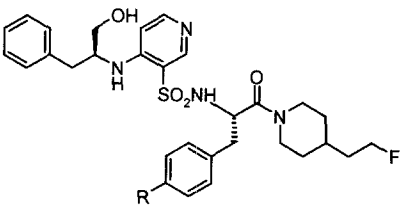


Compound	R1	Ki (nM)	APTT(μM)	AUCp	AUCc
16		49	2.5	45	7
12	NHMe	35	2.4	0	0
17	$N(CH_3)_2$	24	3.4	80	9
18	NHEt	99	3.2	6	6
19	NHnPr	47	3.3	43	2
20	NHnBu	43	5.1	-	-
21	$N(CH_2CH_3)_2$	45	3.56	93	11
22		48	3.4	91	12
23		25	6.1	139	11
24		**22**	**2.9**	**107**	**21**
25		72	2.0	5	2
26		19	3.7	124	8
27		19	1.73	21	1
28		90	2.43	6	1
29		24	2.4	83	10

The influence of changes at P3 was further explored in a series of compounds with restricted modifications at R1, entries (**16-29**) (Table 3). Despite the poor availability of **12** the amine series was pursued because of favorable solubility characteristics. Formation of the tertiary dimethyl amine **17** encouragingly gave an increase in relative absorption *vis a vis* **12**. This trend is also observed with **21**, **22** and **23** although as lipophilicity increases so too does the relative APTT value. This trend can also be seen in the homologous series of secondary amines **12**, **18**, **19**, and **20** where APTT values rise from 2.4μM to 5.1μM. Incorporation of a polar functionality at the terminus of the side chain in R1 appears to compensate for the loss of APTT as witnessed by

a comparison of ethyl amine **18** (APTT 3.2μM) and hydroxyethyl amine **25** (APTT 2.0μM). A similar observation can be noted with entries **24**, **26**, **27-29** with **24** giving good absorption characteristics in respect of both the AUCp and AUCp values.

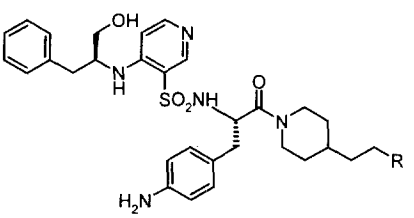
Table 4



Compound	R	Ki (nM)	APTT(μM)	AUCp	AUCc
29	H	100	11.5	-	-
1	NH_2	39	2.5	113	18
30	OH	41	1.91	28	3
31	OMe	36	8.1	-	-
32	F	65	11.7	126	9
33	NHMe	12	4.1	119	2

A limited study based on P1 modifications at the para position of the phenylalanine ring highlighted a number of potent derivatives (Table 4). Remarkably even the phenylalanine derivative **29** itself demonstrated good potency (K_i 100nM), an observation which is atypical of many thrombin inhibitors which require a basic residue in S1 to maintain potency [9]. Other neutral or weakly basic P1 substituents were also tolerated illustrating that in this series a basic residue in S1 is not esential. The trend in activity follows a simple Craig analysis where optimum potency is observed with substituents having -σ and -π values [13]. In parallel with our previous observations the best APTT values were found with the more polar substituents exposed at the surface of the molecule for example in **1**, **30** and **33**.

Table 5



Compound	R1	Ki (nM)	APTT (μM)	AUCp	AUCc
1	F	39	2.5	113	18
34	OH	634	7.4	0	0
35	Cl	27	4.4	62	0
36	H	110	5.5	55	1

Improvement of the pharmacokinetic profile of **1** by variations at P2 were unsuccessful with only the chloropiperidine **35** and ethylpiperidine **36** showing moderate absorption (Table 5). With reference to the AUCc values, none of the compounds with the exception of **1** showed any systemic availability.

In conclusion, based on a potent and well absorbed lead compound CGH1668 **1** we have used a template strategy to explore derivatives at the P3, P1 and P2 positions on the molecule. From this approach we have discovered a number of structure absorption and structure activity relationships which have been applied in the design of more potent and orally bioavailable thrombin inhibitors. One such compound, **24,** demonstrates improved pharmacokinetics in the rat over **1** with an absolute bioavailability of 61% after p.o. administration (10 mg/kg). In i.v. dosing studies with **24**, at 3 mg/kg, plasma clearance is approximately 110 ml/min/kg with an elimination half-life of 59 min. and a volume of distribution of 5092 ml.

References and Notes

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11. For convenience pharmacophores residing in the S9 pocket, also known as the distal or aryl pocket, of thrombin are referred to as P3.

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