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Studies Towards the Identification of Potent, Selective and Bioavailable Thrombin Inhibitors

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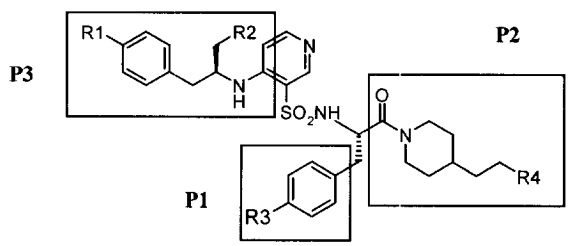
Abstract : The application of selection criteria, based on potency and physicochemical parameters, to a candidate library of thrombin inhibitors is described. The utility of the approach is exemplified by the discovery of a potent, selective and bioavailable thrombin inhibitor **62**.

The serine protease thrombin plays a pivotal role in haemostasis and 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.[1-4]

Existing treatments for thrombotic disease such as oral warfarin[5], subcutaneous injections of heparins[6] and hirudin[7] have a number of limitations and there is a clear need to develop thrombin inhibitors which are safe, effective and can be administered orally. However, despite enormous efforts the discovery of potent, selective and orally bioavailable thrombin inhibitors still remains an elusive goal.[8,9] In previous work[10] we have described the discovery of a series of compounds bearing a novel P3[11] pharmacophore which demonstrate good oral bioavailability. Herein we report the methodology used to exploit a set of 41 key P3, P1 and P2 pharmacophores. Selection of candidate compounds based on an understanding of both the structure activity and structure absorption relationships has lead to the identification of a number of compounds achieving improved pharmacodynamic and pharmacokinetic properties.



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Typically a dynamic relationship exists between adjacent pharmacophores on a molecule and the nature of the ligands will influence drug conformation and electronic properties.[12] These effects are manifest by the

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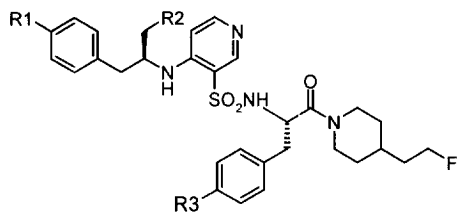
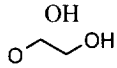
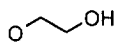
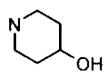
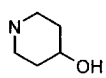
observation that when two independently optimised pharmacophores are combined they do not necessarily contribute in an additive way to potency. However, on inspection of the SAR from compounds based on the general structure **1,** potency contributions from substitutions at P3, P1 or P2 were indeed additive.

Table 1 : Potency Variation by P1 Pharmacophores

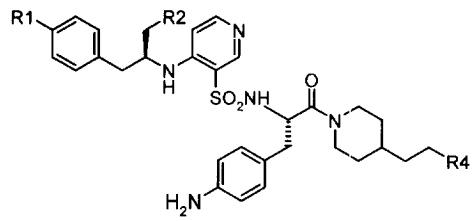


Compound	R1	R2	R3	Ki (nM)
2	H	OH	**H**	100
3	H	OH	**NH_2**	39
4	OMe	OH	**H**	85
5	OMe	OH	**NH_2**	19
6	H		**H**	220
7	H		**NH_2**	49
8	H	$N(CH_3)_2$	**H**	130
9	H	$N(CH_3)_2$	**NH_2**	24
10	H		**H**	61
11	H		**NH_2**	19

This property is exemplified in Table 1 with five pairs of compounds (**2/3**, **4/5**, **6/7**, **8/9** and **10/11**) varying in P1 from phenylalanine to p-anilino-phenylalanine. In each case the increase in potency between the pairs is of the same order within a range of 2.5 to 5.4 fold increase as measured by a K_i determination against human thrombin. In a similar fashion (Table 2) variations at P2 with four pairs of compounds (**12/13**, **14/15**, **16/17** and **18/19**) show a potency trend between the pairs of 2.1 to 3.6 fold increase in activity. In this example comparison is made between the fluoroethyl-piperidine and ethyl-piperidine derivatives at P2.

The consistency with which each of the pharmacophores contributes to potency enables activity predictions to be made. This observation was exploited by the prioritisation and selection of candidate structures for synthesis. This was made necessary since within the given set of P3 (Table 3 and 6), P2 (Table 4) and P1 pharmacophores (Table 5) there are a possible 1176 permutations.[13] Additionally, the *in vivo* pharmacokinetic assay used to determine the absorption of compounds demands 50-100mg quantities of drug and is low throughput in nature.

Table 2 : Potency Variation by P2 Pharmacophores



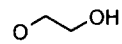
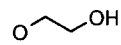
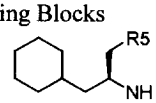
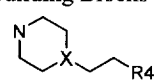
Compound	R1	R2	R4	Ki (nM)
12	H	OH	**H**	110
13	H	OH	**F**	39
14	OMe	OH	**H**	40
15	OMe	OH	**F**	19
16	H		**H**	147
17	H		**F**	49
18	H		**H**	62
19	H		**F**	22

Table 3 : P3 Building Blocks



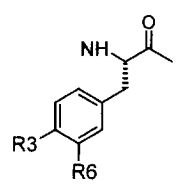
Compound	R5
20	OH
21	NHMe
22	OCH_2CH_2OH

Table 4 : P2 Building Blocks



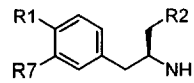
Compound	X	R4
23	CH	F
24	CH	OH
25	CH	Cl
26	CH	H
27	CH	NHCONHMe
35	N	F

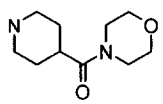
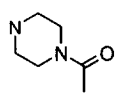
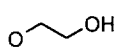
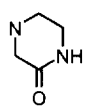
Table 5 : P1 Building Blocks

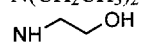
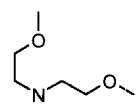
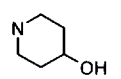
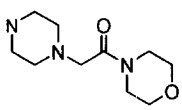


Compound	R3	R6
28	H	H
29	NH_2	H
30	OH	H
31	OMe	H
32	F	H
33	NHMe	H
34	OCH_2O	

Table 6 : P3 Building Blocks



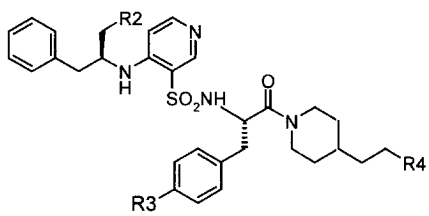
Compound	R7	R1	R2
36	H	H	OH
37	OMe	OMe	OH
38	H	OMe	OH
39	H	OMe	H
40	H	H	H
41	OMe	OMe	H
42	H	H	
43	H	OMe	NHMe
44	OMe	OMe	NHMe
45	H	H	
46	H	H	
47	H	H	
48	H	H	

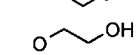
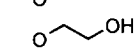
Compound	R7	R1	R2
49	H	H	NHMe
50	H	H	$N(CH_3)_2$
51	H	H	NHEt
52	H	H	NHnPr
53	H	H	NHnBu
54	H	H	
55	H	H	
56	H	H	$N(CH_2CH_3)_2$
57	H	H	
58	H	H	
59	H	H	
60	H	H	

By a consideration of the impact that each of the building blocks has on potency the activity of all of the 1176 putative structures was estimated. In this way the initial set of 1176 compounds was dramatically reduced to a sub set of 392 candidate structures possessing the highest calculated potency values. In a retrospective analysis of the compounds finally synthesised the lowest potency measured was 220nM, demonstrating the high degree of certainty with which activity could be estimated. Indeed 96% of compounds synthesised fell below a K_i of 100nM. To further limit the candidate compounds requiring synthesis the log D of each of the 392 compounds were calculated.[14] From our previous studies and in agreement with a number of examples in the literature[15] the absorption of compounds across the gastrointestinal tract is best observed with compounds in the log D range of 2-4. The log D values of each of the 392 candidates was calculated at the pH of the duodenum (pH 6.2), which is the site of administration in the *in vivo* pharmacokinetic model. Excluding compounds outside the clog D window of 2-4 resulted in a new set of 198 compounds requiring synthesis and evaluation.

The *in vitro* potency of compounds was evaluated as the inhibition constant (Ki) determined against human thrombin.[16] The influence of compounds on the doubling of a human plasma clotting time assay (activated partial thromboplastin time, APTT) was also determined.[17] Additionally, compounds were dosed (3mg/kg) intra duodenally 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 AUCp (the area under curve portal vein), and AUCc (the area under curve carotid artery) were calculated as indicators of bioavailability.

Table 7



Compound	R2	R3	R4	Ki (nM)	APTT (μM)	AUCp	AUCc
61		NH_2	F	22	2.9	107	21
62		NH_2	H	147	5.0	286	91
63		OH	Cl	28	4.4	46	15
64	$N(CH_3)_2$	H	F	130	9.6	141	18

From this exercise a number of compounds **61**, **62**, **63**, and **64** (Table 7) demonstrated good absorption from the duodenum as witnessed by the high AUCp values and more importantly systemic availability in the carotid artery (AUCc). The most promising compound from the series was **62** which has a bioavailability in the rat of 55 % after p.o. administration (30 mg/kg), a C_{max} of 3.36 $\mu g.ml^{-1}$ and an elimination half-life of 120-180 min. In an *in vivo* rat model of venous thrombosis, **62** dose-dependently inhibits thrombus formation when administered orally one hour before induction of stasis.[17] Nearly complete inhibition of thrombus formation is achieved with an oral dose of 30 $mg.kg^{-1}$ of **62** where the duration of the antithrombotic effect is approximately 4 hours.

In conclusion we have employed selection criteria based on a knowledge of the structure activity and structure absorption relationships of compounds under investigation. In this way we have derived a sub set of compounds for synthesis which are biased towards providing candidates with both good pharmacodynamic and pharmacokinetic characteristics. From our studies we have identified a novel thrombin inhibitor **62** possessing a

weakly basic P1 ligand. Compound **62** is highly selective for thrombin when compared to a panel of other serine proteases (trypsin, chymotrypsin, plasmin, kallikrein and factor Xa) and has insignificant effects on the complement cascade *in vitro*. Thrombin inhibitor **62** represents a compound with antithrombotic efficacy *in vitro*, and *in vivo* on oral administration.

References and Notes

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