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Design, synthesis and SAR of a series of 1,3,5-trisubstituted benzenes as thrombin inhibitors

Richard C. A. Isaacs [a,*], Christina L. Newton [a], Kellie J. Cutrona [a], Swati P. Mercer [a], Linda S. Payne [a], Kenneth J. Stauffer [a], Peter D. Williams [a], Jacquelynn J. Cook [c], Julie A. Krueger [b], S. Dale Lewis [b], Bobby J. Lucas [b], Elizabeth A. Lyle [c], Joseph J. Lynch [c], Daniel R. McMasters [d], Adel M. Naylor-Olsen [d], Maria T. Michener [c], Audrey A. Wallace [c]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA
[b] Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA
[c] Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA
[d] Department of Molecular Systems, Merck Research Laboratories, West Point, PA 19486, USA

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ABSTRACT

A novel 1,3,5-trisubstituted benzamide thrombin inhibitor template was designed via hybridization of a known aminopyridinoneacetamide and a known 1,3,5-trisubstituted phenyl ether. Optimization of this lead afforded a novel potent series of biaryl 1,3,5-trisubstituted benzenes with excellent functional anticoagulant potency.

As part of our efforts to design and develop novel small molecule thrombin inhibitors for the treatment and prevention of coagulation disorders we routinely seek to exploit new structure-activity relationships which evolve from our own work as well as others in the field. We have established a research interest in 3-aminopyridinone and related thrombin inhibitors exemplified by **1** (Fig. 1) which is a potent inhibitor (K_i = 0.5 nM).[1] The aminopyridine moiety binds to the specificity pocket S1 of the thrombin active site. The methyl group on the pyridinone ring binds to the S2 pocket and the benzyl group of the sulfonamide chain binds to the S3 pocket of the enzyme.[1] The related analog **2** was found to be similarly potent (K_i = 2.2 nM). While the binding mode of **1** in the thrombin active site was established by X-ray crystallography and has been disclosed,[1a] an X-ray crystal structure of **2** bound to thrombin was never obtained. Compound **2** was originally targeted based on molecular modeling, with one of the isobutyl groups having been designed to interact with the S3 pocket of thrombin, mimicking the role of the phenyl group in **1**. While this work was in progress, a series of patent applications detailing 1,3,5-trisubstituted benzenes exemplified by **3** was published.[2] Although at the time, no binding data for **3** was disclosed,[3] we were struck by the obvious structural similarity to **2**, namely the similarity of the 1,3 relative disposition of the methyl and sulfonate groups in **3** when compared to the 1,3 relative disposition of the methyl and carboxamide groups in **2**. This suggested to us that these two compounds likely had similar binding modes across the S3S2 span of the enzyme. The ether linked guanylpiperidine moiety of **3** was presumed to be capable of binding directly to Asp189 in the S1 pocket in a manner similar to the aminopyridine in compounds **1** and **2**.

We decided to prepare and evaluate hybrids of these two types of compounds. In this paper we will describe our work to prepare, evaluate and optimize the inhibitory potency and anticoagulant properties of said hybrids.

Although we did not have any actual experience handling compounds such as **3**, we did intuitively have reservations about the hydrolytic susceptibility of the sulfonate linkage and reasoned that replacement by an amide would likely confer improved stability. In evaluating our options for surrogates for the guanylpiperidine moiety at P1 we decided to focus primarily on weakly basic heterocycles and the noncharged 2,5-disubstituted phenyls previously pioneered in these labs.[4] Based on our experience, these P1 ligands demonstrated an increased propensity to confer physicochemical properties conducive to good oral bioavailability, while minimizing the undesirable side effects which have been observed with more basic groups.[5] Molecular

* Corresponding author.
E-mail address: richard_isaacs@verizon.net (Richard C. A. Isaacs).

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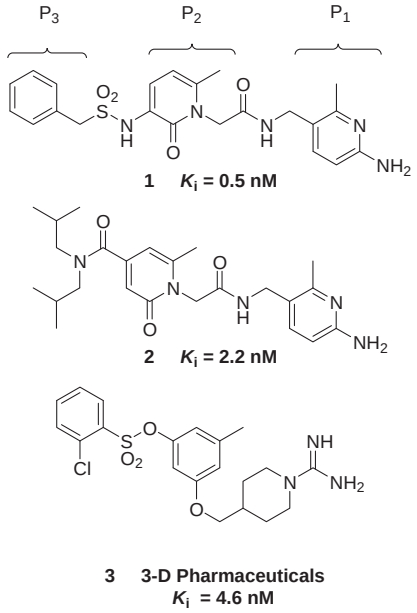


Figure 1.

modeling suggested that the optimum tether length for connecting these P1 groups to the phenolic oxygen of the hybrid template was two carbons (Fig. 2).

Based on this analysis we targeted hybrid compounds **4** generally and **5** specifically for synthesis to validate our structure-driven design hypothesis (Fig. 3).

Compound **5** was synthesized as outlined in Scheme 1. The methyl ester of the known carboxylic acid **6**[6] was protected as a benzyl ether. Compound **7** was hydrolyzed and the resulting acid converted to the diisobutylamide **8**. Hydrogenolysis of the benzyl ether gave phenol **9.** Commercially available imidazole aldehyde **10** was protected as the trityl derivative (a mixture of regioisomers) and the aldehyde group homologated to give nitrile **11** using potassium *t*-butoxide and tosylmethylisocyanide (Tosmic). During hydrolysis of nitrile **11** with 6 M HCl, the trityl protecting group was compromised and was therefore reinstalled. The resulting acid **12** was esterified and then reduced with LAH to give alcohol **13**. Alcohol **13** was converted to mesylate **14** which was then used to alkylate phenol **9**. Removal of the trityl group with TFA and triethylsilane furnished compound **5**.

Compound **5** was found to be a potent (K_i = 3.5 nM) low molecular weight (372) thrombin inhibitor which displayed

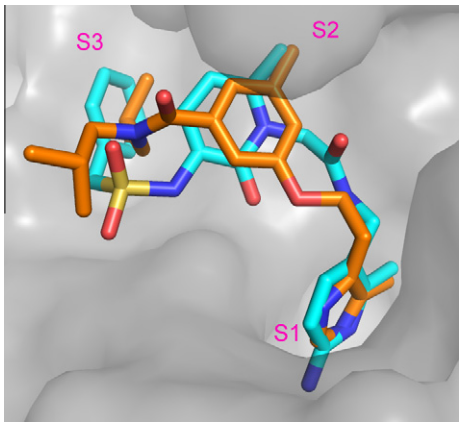


Figure 2. X-ray crystal structure of inhibitor **1** (in blue) bound to the active site of human α-thrombin overlayed by a modeled hybrid **5** (in orange).

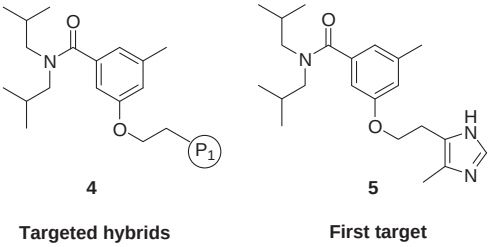
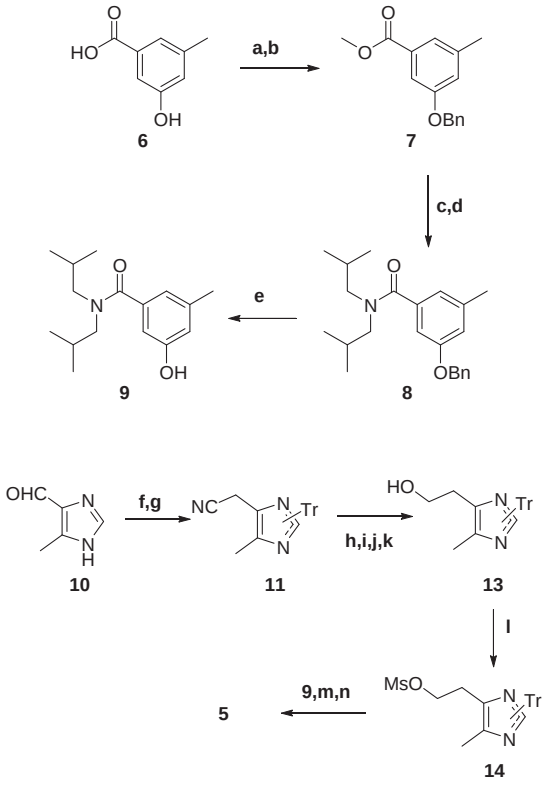


Figure 3.



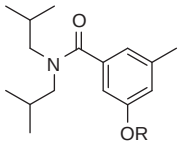
Scheme 1. Synthesis of hybrid **5**. Reagents and conditions: (a) $TMSCHN_2$, CH_2Cl_2/MeOH, 0 °C; (b) NaH, BnBr, THF, 0 °C; (c) LiOH, THF, MeOH; (d) iBu_2NH, EDC, HOBt, Et_3N, DMF; (e) H_2, Pd/C, ethanol; (f) TrCl, TEA, CH_2Cl_2; (g) tBuOK, Tosmic; (h) 6 M HCl, dioxane, 80 °C; (i) $TMSCHN_2$, CH_2Cl_2/MeOH, 0 °C; (j) TrCl, TEA; (k) $LiAlH_4$, THF; (l) Ms_2O, TEA, CH_2Cl_2; (m) Cs_2CO_3, DMF, 80 °C, (n) Et_3SiH, TFA, CH_2Cl_2.

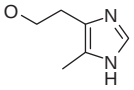
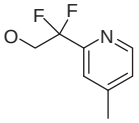
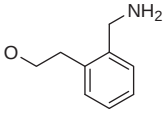
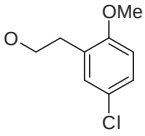
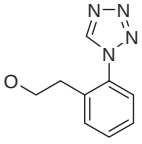
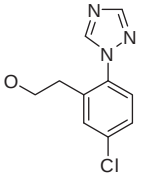
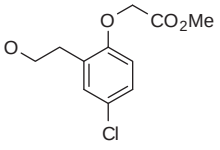
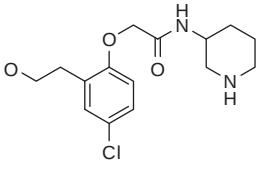
modest selectivity for thrombin versus trypsin (K_i = 2.6 μM). The in vitro anticoagulant potency of **5** as measured by the concentration required to double the activated partial thromboplastin time in human plasma (2 × APTT = 4.11 μM) was poor.[7]

Based on these results, compound **5** was deemed to be a reasonable new lead structure for further optimization. We started by screening a variety of different weakly basic and neutral groups at P1. Representative results are shown in Table 1.

These data demonstrate that with respect to thrombin binding affinity, the diisobutylamide template is compatible with the same variety of weakly basic and neutral P1 groups as the pyridinone and pyrazinone template from which it was derived in part. The level of binding potency however did not translate into functional anticoagulant potency in human plasma (generally, 2 × APTT >3 μM). This is not surprising given the lipophilic nature of these analogs. Compound **21** is a particular standout in that it contains the most basic group (i.e., piperidine) of all the analogs shown but is among the least active analogs in the 2 × APTT assay.[8]

Table 1
Thrombin and trypsin inhibition constants and in vitro anticoagulant potency for compounds **5** and **15**–**21**



Compound	OR	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)
5		3.5	2.6	4.1
15		37	>100	ND
16		2.9	29	3.6
17		34	>100	ND
18		1.7	37	4.4
19		0.93	28	7.1
20		0.73	>100	>8.2
21		1.6	78	>8.2

After working with these compounds for some time we developed concerns about the chemical and metabolic viability of the ether linkage. All of the analogs shown in Table 1 were synthesized using an etherification procedure as previously described for compound **5** using different means of activating the requisite P1 containing alcohol (e.g., trifilate, bromide, Mitsunobu). These reactions typically gave only modest yields of the ether. Competitive elimination to give styrene-like P1 containing by-products was a recurring issue. In vitro metabolic profiling of selected analogs in dog, rat and human liver microsomes served to also highlight the metabolic vulnerability of the ether linkage in these molecules. For these reasons we sought ether

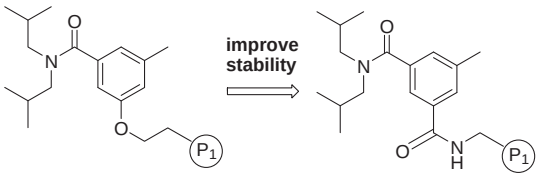
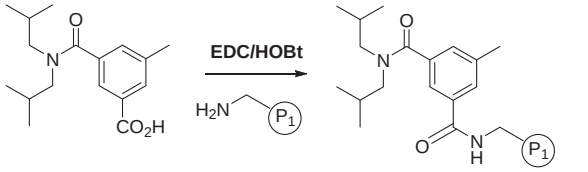


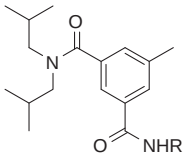
Figure 4.



Scheme 2.

replacements with potential for improved chemical and metabolic stability. Molecular modeling indicated that the ether could be replaced by an amide group (second generation hybrid **22**, Fig. 4) and still allow for productive binding to all three pockets (S3, S2,

Table 2
Thrombin and trypsin inhibition constants and in vitro anticoagulant potency for compounds **24**–**27**



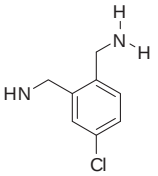
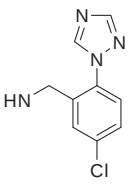
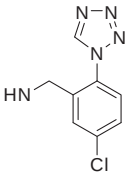
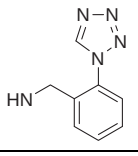
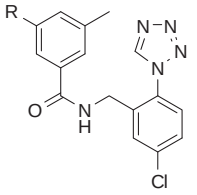
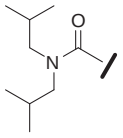
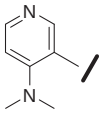
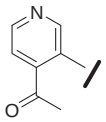
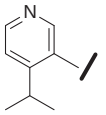
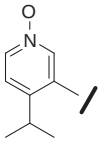
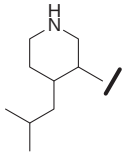
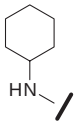
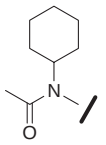
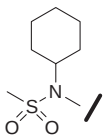
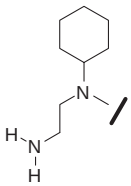
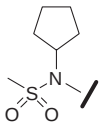
Compound	NHR	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)
24		0.68	111	1.6
25		7	>100	5.3
26		0.27	29	1.2
27		50	>100	ND

Table 3
Thrombin and trypsin inhibition constants and in vitro anticoagulant potency for compounds **26** –**37**



Compound	R	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)
26		0.27	29	1.2
28		67	302	ND
29		85	>100	ND
30		0.27	>100	1.5
31		0.77	483	0.38
32		1.8	278	1.42
33		9.6	>100	>8.2
34		4.1	>100	1.67
35		0.93	>100	0.87
36		1.6	75	1.48
37		1.25	>100	0.75

S1) of the enzyme. We reasoned that the amide could potentially be a stable replacement for the ether linkage.

The second generation bis-amide hybrids **22**, were prepared in a straightforward manner (Scheme 2). EDC mediated coupling of 5-methylisophthalic acid with diisobutylamine gave carboxylic acid **23** which was then coupled with the appropriate aminomethyl P1 fragments.

We were pleased to find that this modification did in fact deliver a potent series of thrombin inhibitors. Table 2 summarizes thrombin and trypsin binding data as well as functional potency (2 × APTT) data for four bisamide analogs (**24**–**27**). The 2,5-disubstituted benzylamine P1 groups used in these analogs delivered the best combination of intrinsic thrombin binding potency and functional anticoagulant potency (2 × APTT).

In the context of the 2,5-disubstituted benzylamine P1 ligands, the bisamide template was capable of yielding sub-nanomolar thrombin inhibitors (compounds **24** and **26,** K_i = 0.68 nM and 0.27 nM respectively) with excellent selectivity versus trypsin (>100,000 fold). In spite of this level of intrinsic binding potency, functional potency as measured in the 2 × APTT assay was marginal (1.6 and 1.2 μM respectively). We reasoned based on our previous experience, that functional potency in this series was being compromised by high plasma protein binding.[8] Of the two amide groups present in the current template, we reasoned that the di-isobutyl amide was the greater contributor to lipophilicity and sought polar replacements to address the issue. Given the lipophilic nature of the amino acid residues which line the S3 pocket of thrombin, we designed amide replacements which contained both a hydrophobic terminus (for interacting with the S3 pocket) and a polar terminus to be compatible with solvent exposure. We also evaluated replacing the central phenyl ring with a pyridine ring; however, that modification led to a significant loss in thrombin potency. Likewise, attempts to reduce polarity by replacing the P2 methyl group by polar surrogates (cyano, aminomethyl), led to loss of thrombin activity. Table 3 summarizes data for a number of polar di-isobutylamide replacements which were tolerated by the enzyme.

Replacement of the di-isobutylamide in compound **26** with a 4-isopropylpyridine gave compound **30** which was equipotent with respect to both thrombin activity and potency in the 2 × APTT assay. Activity in the 2 × APTT assay could be significantly improved by increasing polarity via oxidation to the pyridine N-oxide (compound **31**, 2 × APTT = 0.38 μM). Aminocycloalkanes were also found to be potent replacements for the diisobutylamide (compounds **33**–**37**) with conversion of the amine to the methanesulfonamide providing sufficient increase in polarity to improve activity in the 2 × APTT assay (compounds **35** and **37**).

Summary: Structure based design techniques were used to exploit the putative similarity in binding mode of an aminopyridinone thrombin inhibitor **2** and a trisubstituted benzene inhibitor **3** to generate a new lead inhibitor **5**. SAR work at both the amide and ether carbons of the central benzene ring in conjunction with optimization of the P1 ligand led to the development of a new series of potent inhibitors exemplified by compound **31**. Several of these inhibitors displayed good in vitro functional potency as judged by the concentration of compound required to double the activated partial thromboplastin time (2 × APTT) in human plasma. None displayed animal pharmacokinetics comparable to compound **1**.

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References and notes

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7. We consider human plasma 2 × APTT values of 1 μM or less to be generally predictive of good in vivo efficacy with lower concentrations being predictive of greater efficacy. We use this 1 μM mark routinely as a gate for determining whether to evaluate a compound further in vivo in the rat ferric chloride efficacy assay (see Ref. 4). In practice, in optimizing compounds, we strive to achieve 2 × APTT values of <0.5 μM.
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