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P3 optimization of functional potency, in vivo efficacy and oral bioavailability in 3-aminopyrazinone thrombin inhibitors bearing non-charged groups at the P1 position

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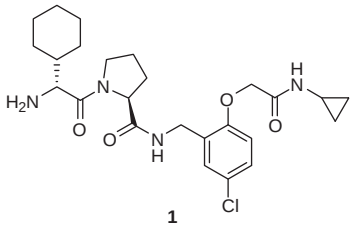
ABSTRACT

Although the S3 pocket of the thrombin active site is lined with lipophilic amino acid residues, the accommodation of polarity within the lipophilic P3 moiety of small molecule inhibitors is possible provided that the polar functionality is capable of pointing away from the binding pocket outwards toward solvent while simultaneously allowing the lipophilic portion of the P3 ligand to interact with the S3 amino acid residues. Manipulation of this motif provided the means to effect optimization of functional potency, in vivo antithrombotic efficacy and oral bioavailability in a series of 3-aminopyrazinone thrombin inhibitors which contained non-charged groups at the P1 position.

The serine protease thrombin is a key enzyme in the blood coagulation cascade and a prime therapeutic target for the development of small molecule drugs to combat coagulation disorders.[1] Disclosures from these laboratories have chronicled the development of potent, noncovalent active site thrombin inhibitors in which the typical electrostatic interaction between a basic group at the P1 position of the inhibitor and the carboxylate of Asp189 in the specificity pocket of the thrombin active site is absent.[2] Compensatory lipophilic binding interactions are instead made between the enzyme and a 2,5-disubstituted phenyl group also at the P1 position of the inhibitor. An early example of such an inhibitor is the dipeptide **1**.[2b]



1

K_i 0.61 nM

Compound **1** has excellent intrinsic thrombin binding potency (K_i = 0.61 nM) and excellent functional in vitro anticoagulant potency as measured by the concentration required to double the activated partial thromboplastin time in human plasma[3] (2 × APTT = 0.42 μM). It also has very good in vivo efficacy when evaluated in a ferric chloride induced thrombosis model in rats[4] (1/6 occlusions at an iv infusion rate of 10 μg/kg/min). Good oral bioavailability was observed in rats (20%) and cynomologous monkeys (34%).

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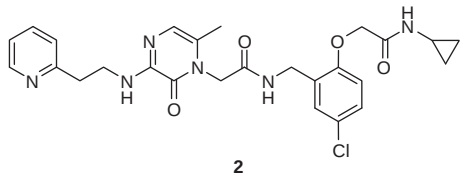
Our work in the field of thrombin inhibitors gradually evolved away from the early dipeptide inhibitors like **1** towards nonpeptide inhibitors, particularly those based on a 3-aminopyrazinone P2 template.[5] This shift was initially primarily driven by a desire to develop new P3P2 inhibitor scaffolds offering the potential for improved physicochemical and drug-like properties when used in conjunction with more traditionally basic or moderately basic P1 ligands which directly bind to Asp189. Incorporation of the neutral P1 ligand of **1** into the 3-aminopyrazinone template[5] provided picomolar thrombin inhibitor **2**.

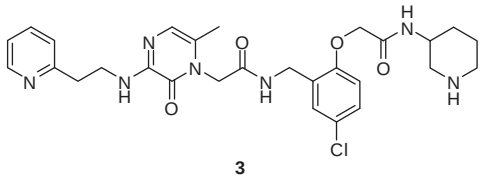


2

K_i 15 pM

While compound **2** has exquisite intrinsic thrombin potency (K_i = 15 pM), that is, 40-fold greater than its dipeptide analog **1**, its functional anticoagulant potency (2 × APTT = 0.45 μM) and its efficacy in the rat ferric chloride thrombosis model (2/6 occlusions) are essentially identical. This disconnect between enzyme and functional activity was found to be a general trend in 3-aminopyrazinone analogs bearing this type of non-charged oxyacetamide P1 ligand.[6] Prior work on the dipeptide series of thrombin inhibitors bearing more conventional basic P1 groups demonstrated that high protein binding and low solubility could significantly impact functional potency and in vivo efficacy, respectively.[7] The fact that the free fraction of **2** in human plasma is only 1% as compared to 12% for **1**, suggested that functional activity of **2** was likely being compromised in part by its greater lipophilicity. Based on this premise, we evaluated the effect of polar modifications to compound **2** as a means of improving functional potency.

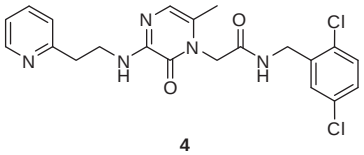
The first polar modification made was the replacement of the cyclopropyl group in **2** with amino-substituted alkyl groups. Compound **3** serves to illustrate the general SAR trends observed. Although the 3-piperidine compound **3** (K_i = 125 pM) is a five-fold more potent thrombin inhibitor than **1** (K_i = 0.61 nM) and an eight-fold less potent thrombin inhibitor than **2** (K_i = 15 pM), it displayed two-fold increased functional potency (2 × APTT = 0.24 μM) over both **1** and **2**. Compound **3** also displayed improved (i.e., full) efficacy in the rat ferric chloride assay (0/6 occlusions). This improvement in efficacy may be a result of increased free fraction of **3** in human plasma (12% for **3** compared to 1% for **2**).



3

K_i 125 pM

Having improved the functional potency and in vivo efficacy, we turned our attention to bioavailability. While the 3-aminopyrazinone series of thrombin inhibitors with basic P1 groups tend to exhibit good oral bioavailability in rats and dogs,[5] inhibitors such as **2** and **3** which bear the neutral oxyacetamide P1 group generally suffer from low absorption. For example, when compound **3** was administered to dogs (at 1 mpk p.o.) and rats (at 10 mpk p.o.), negligible absorption was observed in each species. Since the high molecular weight (568) of compound **3** could be a contributing factor to its poor bioavailability we considered ways to reduce the size while maintaining the attractive biochemical properties. Based on these observations we refocused optimization efforts on the smaller analog **4** (MW 446) which does not contain the oxyacetamide moiety.[2b] One deficit of this lead was the modest efficacy in the rat ferric chloride thrombosis assay (3/6 occlusions).



4

K_i 0.75 nM

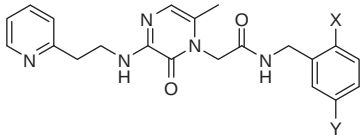
With reduced molecular weight as a potential advantage of this approach, we set out to improve the functional potency of **4** through structural modifications designed to increase polarity while simultaneously minimizing molecular weight increases. We renewed our efforts by focusing on the 2,5-disubstituted phenyl P1 portion of inhibitor **4**. Removal of the chlorine substituent at the C2 position was predicted to increase polarity. The resulting compound **5** had a surprisingly attractive activity profile: thrombin K_i = 3 nM, trypsin K_i = 37 μM, molecular weight 411, free fraction in human plasma 6%, 2 × APTT = 1 μM, near complete efficacy in the rat ferric chloride assay (1/6 occlusions). Unlike **3**, compound **5** exhibited good oral absorption in dogs (C_{max} 2.82 μM, half-life 54 min at 1 mpk p.o.).

Encouraged by these results we expanded our evaluation of substituents at both the C2 and C5 positions of the P1 phenyl group with a view towards increasing polarity and simultaneously limiting molecular weight increases. Table 1 shows potency and efficacy data for some key compounds developed during this effort. These analogs of **4** generally exhibit good thrombin inhibitory potency and excellent selectivity for thrombin versus trypsin. Functional potency across the series was marginal with 2 × APTT values varying from 0.5 to 3 μM. Efficacy in the rat ferric chloride assay was also generally marginal. When dosed orally to dogs, these compounds exhibited very good absorption but consistently short half-life (Table 2). Pharmacokinetic profile in rats was markedly better than in dogs. These results represent significant overall improvements over the chlorophenoxyacetamide-containing analogs.

The following analysis outlines our thought process for attempting to further improve functional potency in the 2 × APTT assay. Although the thrombin S3 site is comprised of lipophilic amino acid residues, in considering the tolerability of a P3 pyridine in compounds like **9**, X-ray structural data for earlier lead analogs bound in the thrombin active site revealed that the C3C4C5 edge of the pyridine ring makes the required lipophilic contacts with the thrombin S3 pocket while the C2N1C6 edge remains solvent exposed. This suggested that replacement of the pyridine ring by a piperidine ring should also be tolerated by the enzyme. Such a modification would be expected to increase polarity and improve performance in the 2 × APTT assay which in turn could provide improved efficacy in the rat ferric chloride thrombosis assay. Table 3 summarizes data for the racemic piperidine analog of **9** and similar compounds containing small 2,5-substituents on the P1 phenyl ring. In all cases, functional potency improved by two-fold or greater when compared to the corresponding pyridine analog, and excellent to full efficacy was observed in the rat ferric chloride assay.

This pyridine to piperidine modification was also applied to a number of inhibitors with a variety of different P1 ligands (aminopyridines, indoles, azaindoles and neutral oxyacetamides)

Table 1
Thrombin and trypsin inhibition constants, in vitro anticoagulant potency, and in vivo antithrombotic efficacy for compounds **4**–**12**



Compound	X	Y	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)	$FeCl_3$
4	Cl	Cl	0.75	52	0.98	3/6
5	H	Cl	3	37	1.01	1/6
6	OMe	Cl	2	161	1.05	5/6
7	OMe	Me	8	902	2.9	ND
8	Me	Cl	1.1	>50	0.87	4/6
9	CN	OMe	1.6	>1000	0.72	2/6
10	CN	Me	1.3	102	0.56	ND
11	OCH_2CF_3	Cl	0.5	32	0.79	3/6
12	OCH_2CF_3	Me	1.5	91	0.89	3/5

Table 2
Dog[a] and rat[b] pharmacokinetic data for selected compounds **5**–**6**, **8**–**11**

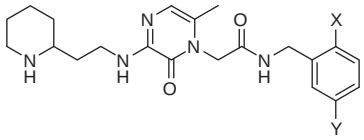
Compound	Dog C_{max} (μM)	Dog $T_{1/2}$ (min)	Rat C_{max} (μM)	Rat $T_{1/2}$ (min)
5	2.82	52	2.98	60
6	1.48	60	0.57	224
8	2.52	71	1.29	220
9	2.26	88	2.28	125
10	2.3	51	ND	ND
11	3.24	78	1.06	117

[a] Dogs dosed at 1 mpk po.
[b] Rats dosed at 10 mpk po.

and similar improvements in functional potency and in vivo efficacy were observed.[6] These P3 piperidine compounds did not suffer from any of the undesired off-target activities typically observed in analogs with basic groups at the P1 position.[8] However, unlike their P3 pyridine counterparts, these compounds were not orally bioavailable in rats or dogs.

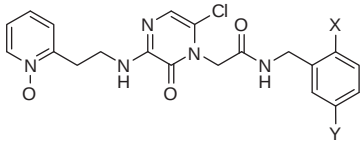
Another approach to increasing polarity through modification of the P3 pyridine involved conversion to the N-oxide. In addition, optimization of the central pyrazinone core had revealed that substitution of 6-methyl with 6-chloro provided compounds with improved metabolic stability and only modest increases in lipophilicity.[9] Therefore, we next targeted the N-oxide of C6-chloro analog **16**.

Table 3
Thrombin and trypsin inhibition constants, in vitro anticoagulant potency, and in vivo antithrombotic efficacy for compounds **13**–**15**

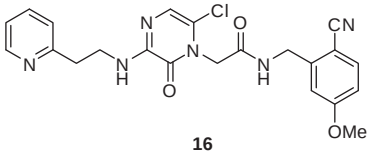


Compound	X	Y	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)	$FeCl_3$
13	OMe	Cl	4.8	58.9	0.65	0/6
14	Me	Cl	2.3	38	0.44	1/6
15	CN	OMe	1.8	136.5	0.27	1/5

Table 4
Thrombin and trypsin inhibition constants and in vitro anticoagulant potency for compounds **17**–**21**



Compound	X	Y	Thrombin K_i (nM)	Trypsin K_i (μM)	2 × APTT (μM)
17	CN	OMe	1.1	278	0.41
18	Me	Cl	1.1	94	0.69
19	Cl	Cl	0.76	138	0.65
20	OMe	Cl	1.8	64	0.67
21	OCH_2CF_3	Cl	0.31	17	0.6



16

When **16** (thrombin K_i = 1.5 nM, trypsin K_i >100 μM, 2 × APTT = 0.83 μM) was converted to its N-oxide **17**, thrombin potency (K_i = 1.1 nM) and trypsin selectivity (K_i = 278 μM) were preserved. Again as predicted, the resulting increase in polarity was sufficient to increase functional potency as measured by the 2 × APTT assay by a factor of two (Table 4). Several other N-oxides were prepared and evaluated (Table 4). It soon became clear that the N-oxidation solution to balancing activity and physical properties was generally applicable, and indeed, the application of these findings to a related series of pyrazinone thrombin inhibitors bearing a substituted pyridine at P1, resulted in the identification of a clinical development candidate with optimized thrombin potency, trypsin selectivity, functional potency, in vivo efficacy and oral bioavailability.[9]

Summary: A number of strategies for increasing polarity in a series of aminopyrazinone thrombin inhibitors bearing a non-charged 2,5-disubstituted benzylamide at the P1 position were undertaken with the goal of maximizing functional potency, in vivo efficacy and oral bioavailability. The incorporation of a pyridine N-oxide group at the P3 position proved to be the most versatile modification and was crucial to the identification of a preclinical development candidate for the treatment of thromboembolic disorders.

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