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L-374,087, AN EFFICACIOUS, ORALLY BIOAVAILABLE, PYRIDINONE ACETAMIDE THROMBIN INHIBITOR

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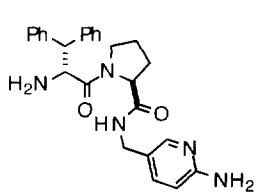
Abstract: Replacement of the amidinopiperidine P1 group of 3-benzylsulfonylamino-6-methyl-2-pyridinone acetamide thrombin inhibitor L-373,890 (**2**) with a mildly basic 5-linked 2-amino-6-methylpyridine results in an equipotent compound L-374,087 (**5**, K_i = 0.5 nM). Compound **5** is highly selective for thrombin over trypsin, is efficacious in the rat ferric chloride model of arterial thrombosis and is orally bioavailable in dogs and cynomolgus monkeys. The structural basis for the critical importance of both methyl groups in **5** was confirmed by X-ray crystallography.

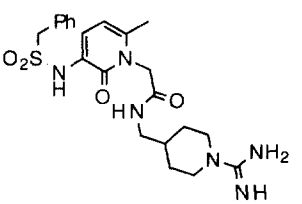
Orally bioavailable thrombin inhibitors that are efficacious in animal models of thrombosis are desirable, but elusive entities.[1] Examples include the arginine aldehyde derivatives CVS-1123[2] and CVS-1801,[3] and the proline derivative L-372,460.[4] Finding the right balance between the lipophilicity and plasma protein binding of such a compound is a design challenge. In the absence of an active transport mechanism, the compound needs to be sufficiently lipophilic to facilitate adequate, passive absorption from the lumen of the gut, without compromising efficacy through excessive binding to plasma proteins.[5] An approach to this delicate problem adopted in these laboratories has been to develop less basic, less polar aminopyridine P1 substituents in a peptide series of ligands.[6] Orally bioavailable compounds such as **1** could then be prepared without having to add large amounts of nonpolar surface area to the P3 region of the molecule.[7]

Recently we reported the use of the pyridinone acetamide peptidomimetic template in the design of the highly selective and efficacious thrombin inhibitor L-373,890 (**2**).[8] Compound **2** displays poor plasma levels following oral administration in cynomolgus monkeys however (at 5 mg/kg, C_{max}< 250 nM, n = 3), presumably as a result of the hydrophilic amidinopiperidine. We then chose to investigate the use of the aminopyridine P1 group as a amidinopiperidine replacement (Figure 1). In this communication we describe the results of this work

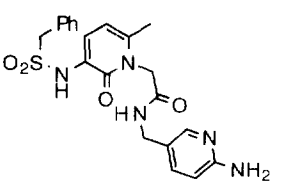
Figure 1



1: K_i = 12 nM[6]



L-373,890 (**2**): K_i = 0.5 nM[8]



3: K_i = 11 nM

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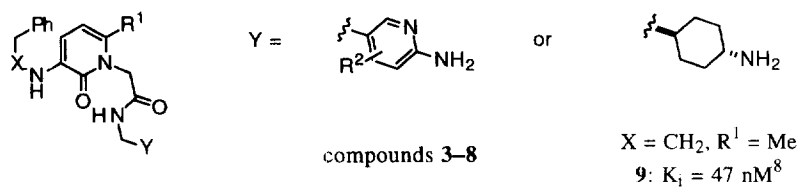
and how we have developed a compound which is orally bioavailable and which retains the exceptional selectivity and efficacy of **2**.

In the D-diphenylalanine-proline peptide series, switching from a cyclohexylamine[9] to an aminopyridine P1 group (**1**) resulted in a 120-fold loss in potency.[6] However, the 3-benzylsulfonylamino-6-methyl-2-pyridinone acetamide can well complement an aminopyridine P1 group, relative to a cyclohexylamine group. Thus, **3** (K_i = 11 nM) is only half as potent as the corresponding cyclohexylamine derivative.[8]

We then chose to investigate the effects of substitution of methyl groups at the 4- and 6-positions of the pyridine ring (Table 1). In the peptide series, such a strategy has been explored as a means to increase the selectivity of the inhibitors for thrombin over trypsin,[6] and more importantly, addition of a single methyl group to the pyridine 6-position can improve the potency for thrombin.[10] In the pyridinone series the results are dramatic. A 6-methyl substituent sharply boosts the affinity for thrombin, without significantly affecting the affinity for trypsin. Compound **5** (L-374,087, K_i = 0.5 nM) is 22-fold more potent than **3**, making it equipotent with **2**. The net increase in selectivity seen with compound **5** is consistent with the proposal that the specificity pocket (S1) of thrombin is larger and more lipophilic than that of trypsin.[11] On the other hand, contrary to this proposal, substitution of a methyl group at the 4-position (compound **4**) has a negligible effect on the K_i for thrombin and increases the affinity for trypsin, although the effect is slight.



compounds **3–8**

X = CH_2, R^1 = Me
9: K_i = 47 nM[8]

Table 1

compound[12]	X	R^1	R^2	K_i(thrombin)/nM[13]	K_i(trypsin)/nM[13]
3	SO_2	Me	H	11	3,000
4	SO_2	Me	4-Me	12	1,500
5	SO_2	Me	6-Me	0.5	3,200
6	CH_2	Me	6-Me	2.3	4,300
7	SO_2	Me	4,6-diMe	0.33	2,100
8	SO_2	H	6-Me	26	20,000

The striking potency of **5** prompted us to determine its precise binding conformation by X-ray crystallography (Figure 2). The crystals of **5** bound to the α-thrombin-hirugen complex were prepared and the diffraction data collected as described previously.[13b,14] The crystal structure was solved at 2.0 Å with an R factor of 0.19. A comparison of the bound conformations of **5** and the previously reported cyclohexylamine derivative **9**[8] illustrates their shared binding features, most of which were anticipated by modeling. As expected, the benzyl group fills the distal pocket,[15,16] the pyridinone-6-methyl group occupies the proximal pocket (S2) and the aminopyridine sits in S1. The pseudo-antiparallel β-sheet array of hydrogen bonds to the enzyme are shorter for this ligand than for **9**, with the sulfonamide nitrogen 2.9 Å from the carbonyl oxygen of Gly-216, the pyridinone

oxygen 3.2 Å from the amide nitrogen of Gly-216 and the acetamide nitrogen 3.2 Å from the carbonyl oxygen of Ser-214. The corresponding atomic separations in the crystal structure of **3** are 3.3 Å, 3.5 Å and 3.3 Å, respectively. The absence of any direct or indirect hydrogen bonding between the enzyme and the sulfonamide oxygens of **5** implies that, structurally, the sulfonyl group acts simply as a conformational mimic of a methylene group in **9**. This is supported by the fact that there is only a modest difference between the K_i's of **5** and its methylene analog **6**.

Figure 2. X-ray crystal structure of **5** bound in the thrombin active site.

The position and hydrogen bonding of the aminopyridine of **5** in S1 is similar to the bound structure of a related compound in the peptide series.[6b] It sits in such a way that the amino group interacts with both the carboxylate group of Asp-189 at the bottom of S1 and the carbonyl group of Gly-219. In this case the N-O separation are both 2.9 Å. This is similar to the P1 amino group of **9**, which makes the same interactions with separation distances of 3.1 and 2.8 Å, respectively. An ordered water molecule forms a bridge between the pyridine nitrogen and the Asp-189 carboxylate with N-O and O-O separations of 2.5 and 2.6 Å, respectively. This additional hydrogen bond between the P1 group and the ordered water is lacking in the crystal structure of cyclohexylamine derivative **9** and presumably contributes to the increased potency of **5**.

The two methyl groups of **5** are critical for its potency. The pyridinone-6-methyl group, serves both as a nonpolar group to occupy S2 and as a pre-organizational element,[8] and compound **8**, which is missing this methyl group, is 50-fold less potent than **5**. This result contrasts with the finding that structurally related arginine aldehyde derivatives are essentially equipotent with or without the pyridinone methyl group.[3] The crystal structure provides an explanation for the importance of the pyridine-6-methyl group. It points back into S1, making contact with the aliphatic side chain of Val-213. In principal, the 4-methyl group of compound **4** could make the same contact if the pyridine ring was turned about 180°. However, the intricate hydrogen bonding

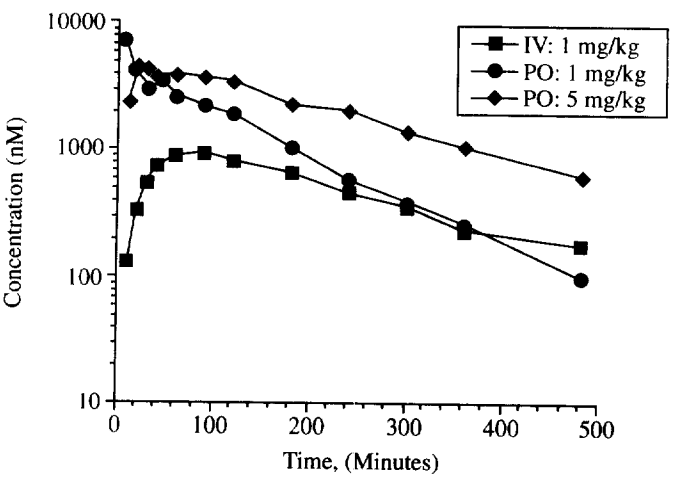
network between the aminopyridine and the Asp-189 carboxylate would be disrupted. A model of the 4,6-dimethyl compound **7** (K_i = 0.33 nM) shows that it should be able to bind in the same conformation as **5** and without making any additional contacts with the enzyme.

Compound **5** is 6,400-fold selective for thrombin over trypsin and is inactive (K_i >80 μM) against the serine proteases plasmin, tPA, activated protein C, plasma kallikrein, and chymotrypsin. In free solution, **5** would exist predominantly in the uncharged state since the pK_a in water for the aminopyridine and the sulfonamide were determined to be 6.6 and 8.8, respectively, by spectrophotometric titration. The protein binding in citrated human plasma is 79% (1 μM spike, n = 6) and the log P is 0.69 (octanol/water, pH 7.4). The on-rate for binding to thrombin is 90 $\mu M^{-1}s^{-1}$, near the diffusion controlled limit. The final assay concentrations required to double the APTT in human and rat plasma are 210 and 250 nM, respectively. In the rat ferric chloride model of arterial thrombosis,[17] the efficacy is dose proportional (Table 2) with 0/9 occlusions at an infusion rate of 10 mg/kg/min (final plasma concentration 586 ± 59 nM).

Table 2. Efficacy of **5** in the rat ferric chloride arterial thrombosis model.[17]

Dose (μg/kg/min iv)	Incidence of occlusion
1	5/6
3	2/6
5	1/6
10	0/9

Figure 3. Plasma levels of **5** in dogs, mean values (n = 2 each)



The pharmacokinetic data for **5** (administered as the HCl salt) in beagle dogs is shown in Figure 3. At an iv dose of 1 mg/kg, the iv half life dose was 90 min and after oral administration at the same dose, the C_{max} of 0.99 μM was reached at 75 min, giving an oral bioavailability of 44% (n = 2). In cynomolgus monkeys, under comparable conditions the iv half life was 145 min (n = 2) and the oral bioavailability was 19% (5 mg/kg, n = 2

and 5.8 mg/kg, n = 2). The plasma levels attained in rats were severely limited after oral administration (F < 5%). This latter result is most probably a result of high first pass hepatic clearance in tandem with an absorption rate which is moderated by the low solubility of **5** at neutral to slightly alkaline pH (16 μg/mL in citrate buffer, pH 7.2).

In conclusion, replacement of the amidinopiperidine P1 group of pyridinone acetamide thrombin inhibitor L-373,890 (**2**) with a mildly basic 5-linked 2-amino-6-methylpyridine results in an equipotent compound L-374,087 (**5**) which is highly selective, efficacious and orally bioavailable. This rare combination of properties in a simple, achiral structure makes it an attractive lead in the search for a clinically useful antithrombotic drug.

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