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C6 MODIFICATION OF THE PYRIDINONE CORE OF THROMBIN INHIBITOR L-374,087 AS A MEANS OF ENHANCING ITS ORAL ABSORPTION

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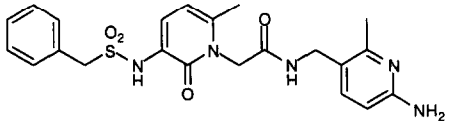
Abstract: **1** (L-374,087) is a potent, selective, efficacious, and orally bioavailable thrombin inhibitor that contains a core 3-amino-2-pyridinone moiety. Replacement of the C6 pyridinone methyl group of **1** by a propyl group gave **5** (L-375,052), which retained all the excellent properties of **1**, and also yielded higher plasma levels after oral dosing in dogs and rats.

Tripeptide templates typified by D-Phe-Pro-Arg have been the active focus of thrombin inhibitor research for the past 20 years.[1,2] In the ongoing quest to discover new therapeutic agents for the treatment of thromboembolic disorders, there is a shift away from such templates in favor of low molecular weight peptidomimetics.[2] Recent disclosures from these laboratories have chronicled the use of structure based drug design in the development of a novel series of potent, achiral, noncovalent 3-amino-2-pyridinone acetamide thrombin inhibitors.[3,4] One of the prototypes of this new class of inhibitors is **1** (L-374,087). In addition to excellent potency (K_i = 0.5 nM), selectivity (relative to trypsin and other serine proteases) and in vivo antithrombotic efficacy (rat ferric chloride thrombosis model),[5] this compound displayed good oral bioavailability in dogs and primates. In rats however, **1** had poor oral bioavailability. LCMS analysis of bile and urine samples from rats dosed with **1** indicated that the pyridinone methyl group was a possible site of metabolism. We therefore embarked on a search for a more metabolically robust surrogate for the methyl group.



1 (L-374,087) K_i 0.5 nM

In deciding what targets to pursue, we examined the X-ray crystallographic structure of **1** bound to the α-thrombin-hirugen complex.[3b] The pyridinone methyl group makes crucial hydrophobic interactions with the side chains of Tyr 60A and Trp 60D of the lipophilic proximal pocket (S2) of the enzyme active site. Deletion of this methyl group to yield **2** results in a 50-fold drop in potency (Table 1).[3b] However, even prior to the synthesis of **1** and the determination of its bound X-ray crystal structure, molecular modeling had made us cognizant of the fact that the P2 fit would be tight, with little room for the S2 pocket to accommodate a group much larger than a methyl group. Nevertheless, we reasoned that the enzyme's insertion loop at residues 60A-

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D, which defines S2, might be flexible enough to accommodate a moderate increase in the size of the C6 substituent. We also believed that a modest loss in potency was deemed an acceptable trade off for potentially improved pharmacokinetics. We therefore decided to investigate small replacement groups, specifically trifluoromethyl and cyclopropyl.

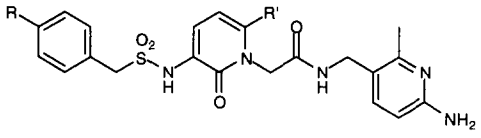
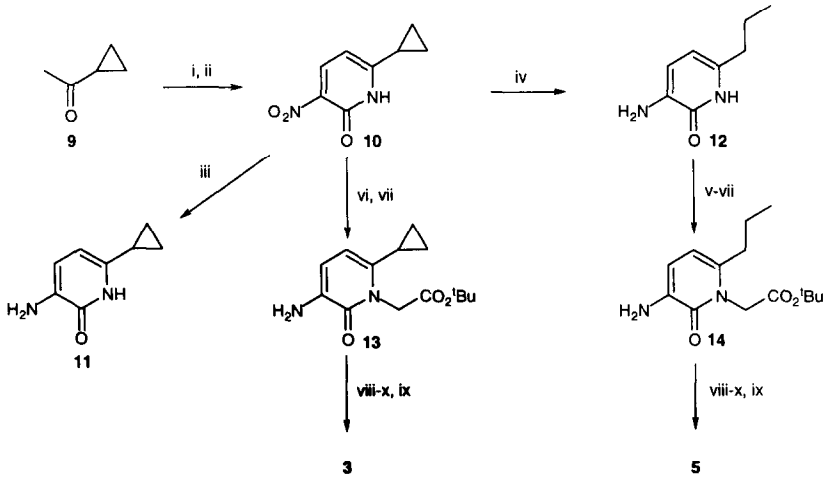


Table 1.

Compound	R	R'	K_i (thrombin)/nM	K_i (trypsin)/nM
1 (L-374,087)	H	Me	0.5[3b]	3,200
2	H	H	26[3b]	20,000
3	H	cyclopropyl	16	1,700
4	H	trifluoromethyl	12	2,500
5 (L-375,052)	H	n-propyl	0.85	1,400
6	Cl	Me	0.12	2,600
7	Cl	cyclopropyl	4	1,900
8	Cl	trifluoromethyl	2.6	3,400

Table 1 shows the K_i values for the two analogs **3** and **4** in which the C6 methyl group of **1** has been replaced by cyclopropyl and trifluoromethyl groups, respectively.[6] These results were consistent with the molecular modeling prediction. Both C6 methyl replacements resulted in significant losses in potency. Even though their respective inhibitory potencies did not fall within an acceptable range, **3** and **4** were evaluated orally in dogs. Neither displayed a better pharmacokinetic profile than **1**. The potency could be partially restored by placing substituents on the phenyl ring of the benzylsulfonamide moiety.[7] For example, the addition of a chlorine to the *para* position gives a four fold potency boost (**7** versus **3** and **8** versus **4**). Although more potent, neither **7** nor **8** was evaluated further for reasons directly related to their highly lipophilic nature. In the case of **8**, the increased lipophilicity resulted in poor solubility. This caused formulation difficulties which prevented evaluation of its in vivo antithrombotic efficacy. As shown in Table 2, in the case of **7**, the issue was poor anticoagulant and antithrombotic efficacy as judged by performance in the 2x APTT assay (1.43 μM) and rat ferric chloride model of arterial thrombosis.[5] At an iv infusion rate of 10 μg/kg/min, 4/6 vessels occluded. At the end of the experiment, the average plasma concentration of **7** was 347 nM. By comparison, when the lead compound **1** was dosed in rats at 10 μg/kg/min, full efficacy (0/6 occlusions) was observed (average final plasma concentration 586 nM). **1** has a Log P of 0.69. It is 78% protein bound in human plasma where it has a 2x APTT value of 0.22 μM. It is 47% protein bound in rat plasma where it has a 2x APTT value of 0.25 μM. Although all of these parameters for **7** are not available for a complete comparison, it was determined that **7** is more lipophilic (Log P 1.98) and highly protein bound (94% in human plasma) than **1**. In human plasma, **7** also has a much higher 2x APTT value (1.43 μM) than **1**. It seems reasonable to speculate that **7** is also more highly protein bound than **1** in rat plasma as well which would translate into a higher rat 2x APTT value. When taken

in conjunction with the lower observed plasma levels of **7**, the observed decrease in antithrombotic efficacy can be rationalized. These results are consistent with observations made by Tucker and coworkers in a peptide series of thrombin inhibitors.[8] It was demonstrated that for an effective in vivo thrombin inhibitor, there is a delicate balance of potency, lipophilicity and protein binding which must be exhibited by a single molecule.[8] In the series at hand, one solution to this quandary came unexpectedly as a result of synthetic considerations which arose during the preparation of **3** (Scheme 1).



(i) $Me_2NCH(OMe)_2$, cat pTsOH, (ii) $NO_2CH_2CONH_2$, aq piperidinium acetate (iii) H_2, PtO_2, EtOH, 1 atm, 0 °C (iv) H_2, 10% Pd/C, EtOH, 60 psi (v) CbzCl, Na_2CO_3, CH_2Cl_2 (vi) NaH, $BrCH_2CO_2{}^tBu$, THF (vii) H_2, 10% Pd/C, EtOAc, 1 atm (viii) $BnSO_2Cl$, pyridine (ix) HCl, EtOAc, 0 °C, (x) Boc amine, EDC, HOBt, DMF.

Scheme 1

The synthesis of **3** began with the β-*N,N*-dimethylaminoenone derived from condensation of DMF dimethyl acetal with cyclopropyl methyl ketone **9**. Subsequent condensation with nitroacetamide gave 3-nitro-6-cyclopropyl-2-pyridinone **10**.[9,10] Hydrogenation to the corresponding 3-amino-6-cyclopropyl-2-pyridinone **11** was complicated by competitive reduction of the cyclopropyl group. Under most conditions, mixtures of partially and completely reduced material were obtained. Complete reduction of both groups (to give **12**) was also achievable (H_2, 10% Pd/C, EtOH, 60 psi).[11] Conditions were eventually found that allowed selective reduction of the nitro group with retention of the cyclopropyl group (H_2, 1 atm, PtO_2, EtOH, 0 °C). Even though the amino group of **11** could be protected and then the pyridinone ring alkylated, the route to **3** was simplified when it was found that following N-alkylation of 3-nitro-6-cyclopropyl-2-pyridinone **10**, the cyclopropyl group was then inert towards hydrogenation. This rendered the completion of the synthesis of **3** straightforward.[10] Sulfonylation of the amine **13**, deprotection of the *tert*-butyl ester, coupling of the resulting carboxylic acid with 3-aminomethyl-6-*tert*-butoxycarbonylamino-2-methylpyridine and a final removal of the Boc group gave **3**. Similarly, 3-amino-6-propyl-2-pyridinone **12** was first protected as its Cbz derivative and then taken through the same sequence to give **5** (L-375,052).

Contrary to our initial thoughts, **5** is essentially equipotent with **1** (Table 1). A priori, we had no reason to expect that an *n*-propyl group would be accommodated by the S2 pocket of thrombin whereas a cyclopropyl would not. Although the crystal structure of **5** was not determined, the *n*-propyl group and the 60A-D insertion loop presumably have the flexibility to interact with each other without causing gross distortions of other key interactions with the enzyme backbone. The linear alkyl chain can probably flex out of a modified or adjusted S2 pocket without coming in contact with the adjacent acetamido linkage.[12] The more rigid cyclopropyl group of **3** when accommodated by the S2 insertion loop may prevent other sites on the inhibitor from achieving optimal binding with the enzyme.

As shown in Table 2, in going from **1** to **5**, although the methyl to *n*-propyl substitution significantly increased the lipophilicity (Log P 1.52 for **5** versus 0.69 for **1**) and protein binding (88% for **5** versus 78% for **1** in human plasma and 75% for **5** vs. 47% for **1** in rat plasma), there was not a commensurate loss in antithrombotic efficacy as was the case for the cyclopropyl substituted compound **3**. So whereas in the rat ferric chloride assay, **1** at 10 μg/kg/min had an incidence of occlusion of 0/6 rats, the efficacy of **5** was 1/6. The average final plasma concentration of **5** during the experiment was 389 nM which is essentially identical to its 2x APTT value (0.4 μM) in rat plasma.

Table 2. Comparative clotting and lipophilicity data for **1**, **5** and **7**.

	2x APTT (μM) rat plasma	2x APTT (μM) human plasma	% protein binding rat plasma	% protein binding human plasma	log P	occlusions at 10 μg/kg/min$^{\psi}$	final plasma conc. (μM)$^{\psi}$
1	0.25	0.22	47	78	0.69	0/6	0.586
5	0.40	0.32	75	88	1.52	1/6	0.389
7	ND	1.43	ND	94	1.98	4/6	0.347

ψ Rat ferric chloride thrombosis model (ref. 5)

The plasma concentration profile of **5** after oral dosing in dogs was found to be superior to that of **1**. As shown in Table 3 and Figure 1, at 5 mpk po, the peak plasma concentration of **5** was 9.46 μM whereas for **1** it was 5.31 μM. Duration of action was also improved. The level of **5** in dog plasma was 1.5 μM 8 h post dosing compared to 0.6 μM for **1**. This improvement in oral absorption was not only confined to dogs. While **1** was not detectable in rat plasma following po dosing at 20 mpk, **5** reached peak concentrations of 250 nM following po dosing in rats at 10 mpk.

Table 3. Comparative pharmacokinetic parameters for **1** and **5** in dogs.

Dog (5 mpk po)	C_{max} (μM)	$t_{1/2}$ (min)	AUC (μM*hr)
1	5.31	151	18.8
5	9.46	189	40.6
Dog (1 mpk iv)	**Vd_{ss} (l/kg)**	**Cl (ml/min/kg)**	**AUC (μM*hr)**
1	0.46	3.80	9.92
5	0.54	5.82	5.93

In conclusion, although the S2 pocket of thrombin provides for a very tight fit for 6-methyl pyridinone based thrombin inhibitors, a propyl group is still very well tolerated. Such a substitution provides **5**, which retains the excellent potency, selectivity and antithrombotic efficacy of **1** but imparts improved oral absorption

in rats and dogs. These findings are considered important in the continuing optimization of the pharmacokinetic properties of this class of compounds.

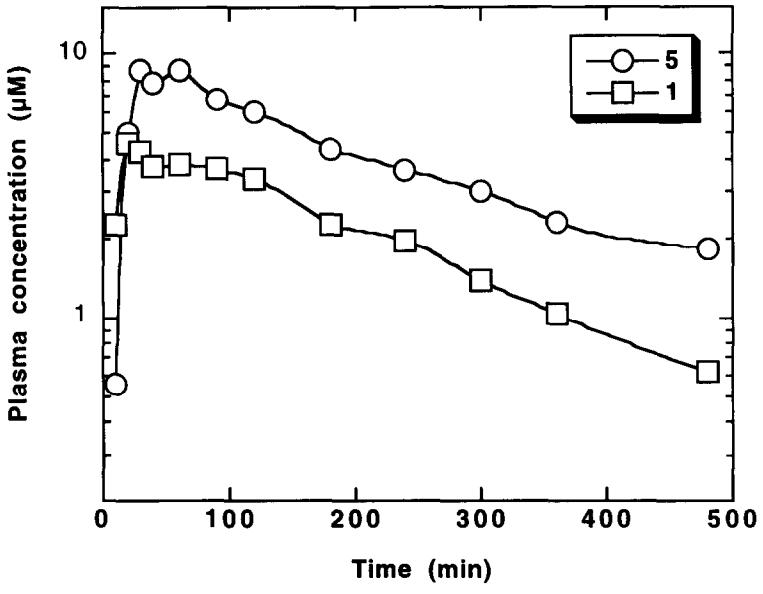


Figure 1. Comparative plasma concentration profile of **1** and **5** following 5 mpk po dosing in dogs (n = 2).

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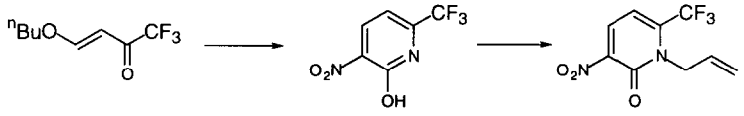
6. 

The synthesis of **3** will be described later on in the text. The synthesis of **4** began with (*E*)-4-butoxy-1,1,1-trifluoro-3-buten-2-one.[13] Reaction with nitroacetamide in refluxing ethanolic sodium ethoxide gave 2-hydroxy-3-nitro-6-trifluoromethylpyridine. All attempts to alkylate the sodium or cesium salt of this compound with haloacetic acid esters resulted in exclusive *O*-alkylation. Attempted alkylation of the Cbz-protected amino derivative was similarly fruitless. However, treatment with π-allyl palladium in refluxing THF to gave the *N*-allylated compound exclusively. The remainder of the synthesis was relatively straightforward. The nitro group was reduced with tin (II) chloride and the resulting amine then sulfonylated with α-toluenesulfonyl chloride. Oxidative cleavage of the olefin was accomplished using the Katsuki/Sharpless procedure[14] to give a mixture of the aldehyde and the corresponding acid. Jones oxidation completed the transformation. Finally, coupling and deprotection as described for **3** gave **4**.

7. The full results of SAR studies of the P3 ligand in our pyridinone derived thrombin inhibitors will be disclosed at a later date.

8. For a thorough discussion of the interactive effects of potency, lipophilicity and protein binding on oral bioavailability and antithrombotic efficacy see Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, R. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I. -W.; Dancheck, K. B.; Vacca, J. P. *J. Med. Chem.* **1997**, *40*, 1565.

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11. The reduction of the cyclopropyl group yielded only the *n*-propyl group. None of the *iso*-propyl isomer was ever observed.

12. This flexibility of the S2 pocket has been observed in the crystal structure of a non-peptide series of thrombin inhibitors. See Naylor-Olsen, A. M.; Ponticello, G. S.; Lewis, S. D.; Mulichak, A. M.; Chen, Z.; Habecker, C. N.; Sanders, W. M.; Tucker, T. J.; Shafer, J. A.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, xxx.

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