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LETTERS

IDENTIFICATION AND SAR FOR A SELECTIVE, NONPEPTIDYL THROMBIN INHIBITOR

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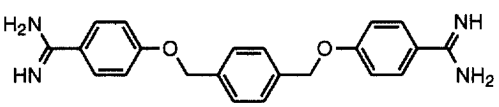
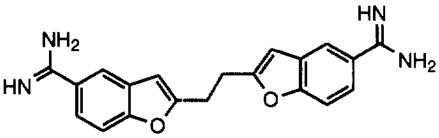
Abstract: A novel, nonpeptidyl thrombin inhibitor, L-636,619 (**1**), was identified via topological similarity searching over the Merck Corporate Sample Database. X-ray crystallographic studies determined the geometry for ligand binding to the enzyme. Chemical modification of the P1 and P3 segments of the ligand resulted in enhanced potency and improvement in the chemical stability of the lead. Analog **9** proved to be the most interesting lead from this structurally novel series. © 1998 Elsevier Science Ltd. All rights reserved.

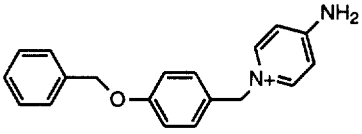
The serine protease thrombin plays a pivotal role in the blood coagulation cascade.[1,2] Thrombin cleaves both fibrinogen to release fibrin and activates blood platelets via the thrombin receptor. Since polymerized fibrin and activated platelets are the chief components of blood clots, the inhibition of thrombin will compromise blood coagulation. Consequently, the identification and development of an anticoagulant agent based upon the direct inhibition of thrombin is a prominent goal for many pharmaceutical laboratories.[3-9]

As a part of our efforts to identify novel, nonpeptidyl inhibitors of thrombin a series of rational database mining studies were performed. Searches based upon bis-amidine ligands that were reported[10] to be nonselective inhibitors of both thrombin and factor Xa (structures below) were used as probe queries.





The TOPOSIM methodology,[11] employing both the atom pairs and topological torsions descriptors, was used to search over the Merck Corporate Sample Database of structures. From such searches, more than 200 compounds were selected and sent for analysis in our standard thrombin kinetic assay (previously described in ref 12). Compound **1**, L-636,619, was identified as a competitive inhibitor lead with a K_i value of 0.7 μM for thrombin versus a K_i value of 112 μM for trypsin.



L-636,619 (**1**)

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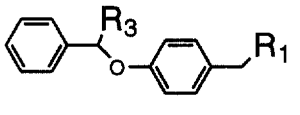
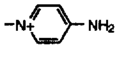
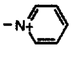
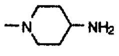
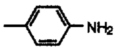
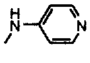
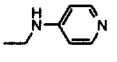
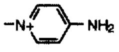
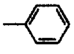
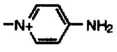
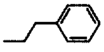
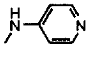
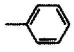
Based upon our previous experience with crystal structures of thrombin ligand complexes it was predicted that the amino pyridinium group of **1** would occupy S1 and the O-benzyl fragment would fill the distal hydrophobic pocket. A crystallographic investigation of the α-thrombin-hirugen complex with **1** was accomplished as previously described.[13,14] The crystal structure was solved at 1.9 Å (R factor of 0.196) and was of sufficient quality to accurately confirm the location and binding of **1** in the enzyme active site. Based on this study, our three-dimensional understanding of the specific interactions of **1** with the enzyme was clarified, as illustrated in Figure 1. As anticipated, the aminopyridinium group filled the specificity pocket. The O-benzyl fragment created a favorable hydrophobic interaction with Ile-174 and Leu-99 as well as made a perpendicular hydrophobic stack with Trp-215. The role of the central aromatic ring was one of scaffold to position the P1 and P3 groups in their respective enzyme sites. The benzylic oxygen atom made no specific hydrogen bond with the enzyme but rather allowed the benzyl fragment to adopt an arrangement off of the central aromatic ring which was co-planar to it.

Figure 1. A stereoview of the active site region of the α-thrombin-hirugen-**1** crystal structure. The catalytic triad is colored green, the specificity pocket (S1) aspartic acid is colored red, the residues of the proximal hydrophobic pocket (S2) are colored yellow, and the residues of the distal hydrophobic pocket are colored magenta. The ligand is colored by atom type such that carbon atoms are light green, the oxygen atom is red and the nitrogens are blue.

As a lead structure, **1** offered the advantage of being small, nonpeptidyl, and selective relative to trypsin. Analogs of **1** were synthesized and assayed in an effort to improve the potency. The results are tabularized below.[15]

Initial attention was focused upon the P1 region of the molecule. A concern associated with this group was the potential for the quaternary pyridinium moiety to covalently react and hence contribute to possible toxicity. The question at hand was whether the heterocycle could be modified or replaced and potency maintained or enhanced. Either the removal of the amino group, as in compound **2** (K_i = 581 μM), or the reduction of the pyridinium ring to the piperidine, as in compound **3** (K_i >500 μM), resulted in analogs with more than an 800-fold drop in potency. Substitution of an aniline group for P1, as in compound **4** (K_i >10 μM), indicated that our potency loss was at least 14-fold. If the point of attachment was changed to the amino group at the 4-position of the pyridine, as in analog **5** (K_i = 4.7 μM), potency diminished by less than sevenfold. The introduction of an additional methylene unit in this reversed linkage between P1 and the central aromatic, as in analog **6** (K_i = 7.9 μM), produced a 1.5-fold drop in potency relative to the shorter compound, **5**. Other heterocyclic replacements for P1 failed to maintain or enhance potency. These results drew our attention back to the 4-aminopyridine P1, recently reported by Boehringer Mannheim,[16] as worthy of further structural modification.



Analog	R1	R3	K_i* (μM)	Analog	R1	R3	K_i* (μM)
1		H	0.700 (77)	**2**		H	>500
3		H	581	**4**		H	>10 (>10)
5		H	4.7 (233)	**6**		H	7.9 (568)
7			0.058 (517)	**8**			0.16 (16)
9			1.3 (77)				

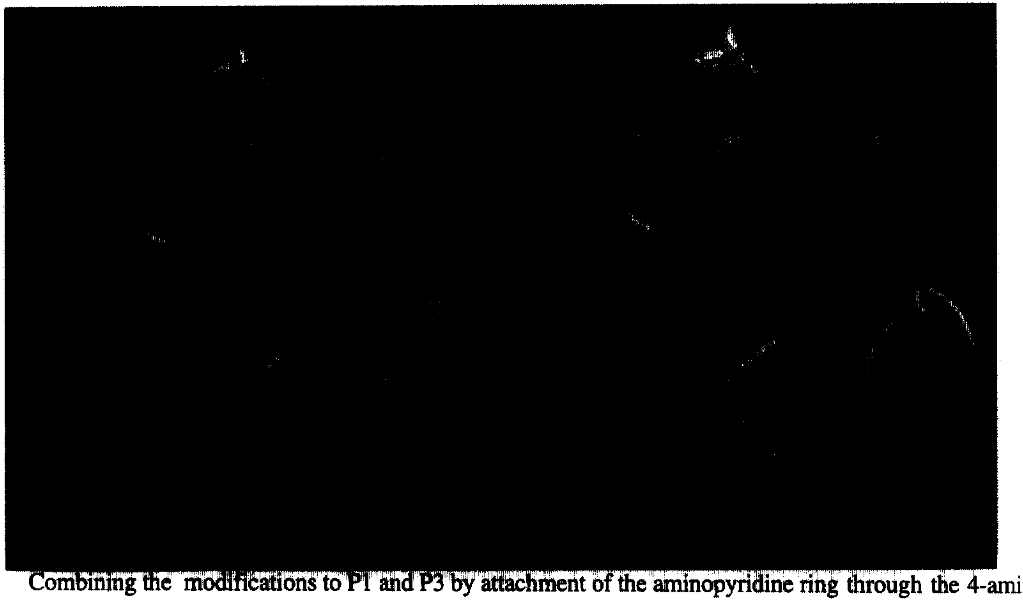
* Initial values given correspond to thrombin inhibition while those presented in parentheses correspond to trypsin.

Subsequent efforts at improving the potency of the lead compound focused on the P3 region. The introduction of an additional aromatic ring, a technique pioneered by researchers at the Thrombosis Research Institute[17] which also proved quite useful in a peptidyl series of thrombin inhibitors,[18] was investigated. A phenyl group directly attached to the benzylic site of P3, as in compound **7** (K_i = 58 nM), proved to be 12-fold more potent than the original lead compound. The addition of a phenethyl substituent to the benzylic site, as in analog **8** (K_i = 160 nM), also produced an analog that was more potent than the lead compound but threefold less potent than **7**. Efforts at producing an analog with a benzyl group attached to the benzylic site were thwarted because the compound eliminated to yield a stilbene structure.

The introduction of additional aromatic rings at the P3 site also revealed important structural insights about ligand binding to the enzyme. We expected the additional aromatic ring of **7** to interact with the Ile-174 residue of the distal hydrophobic pocket, as observed previously in the crystal structure of a diphenylalanine inhibitor complexed with thrombin.[18] The crystal structure of **7** bound to the α-thrombin-hirugen complex was characterized in a manner similar to those previously reported from this laboratory.[13,14] The structure was solved at 1.9 Å (R factor of 0.178) and revealed that the bulkier P3 group actually placed the new phenyl ring in the *proximal* hydrophobic site defined by the 60-A to 60-D insertion loop, instead of the distal hydrophobic pocket.

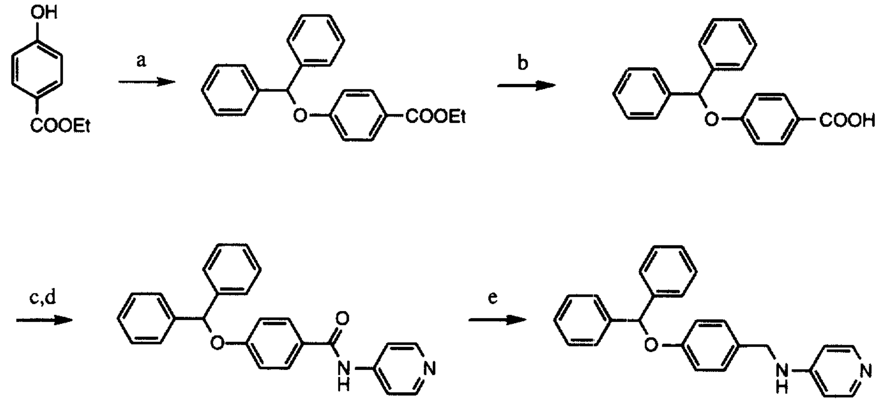
This was accomplished via a torsional difference in the phenyl-O linkage relative to the structure of **1** bound to thrombin. The additional aromatic ring of compound **7** formed a parallel π-stack with Tyr-60A and displaced Trp-60D by ~1.5Å, as illustrated in Figure 2. Obviously, the S2 pocket defined by this insertion loop is somewhat more accommodating than previously observed.

Figure 2. A stereoview of the active site region of the α-thrombin-**7** crystal structure colored as described in the legend to Figure 1.



Combining the modifications to P1 and P3 by attachment of the aminopyridine ring through the 4-amino group and the addition of another benzene ring yielded the most promising compound in the series. Analog **9**, L-373,363, has a K_i value of 1.3 μM. The synthetic route to **9** is given in Scheme 1.

Scheme 1



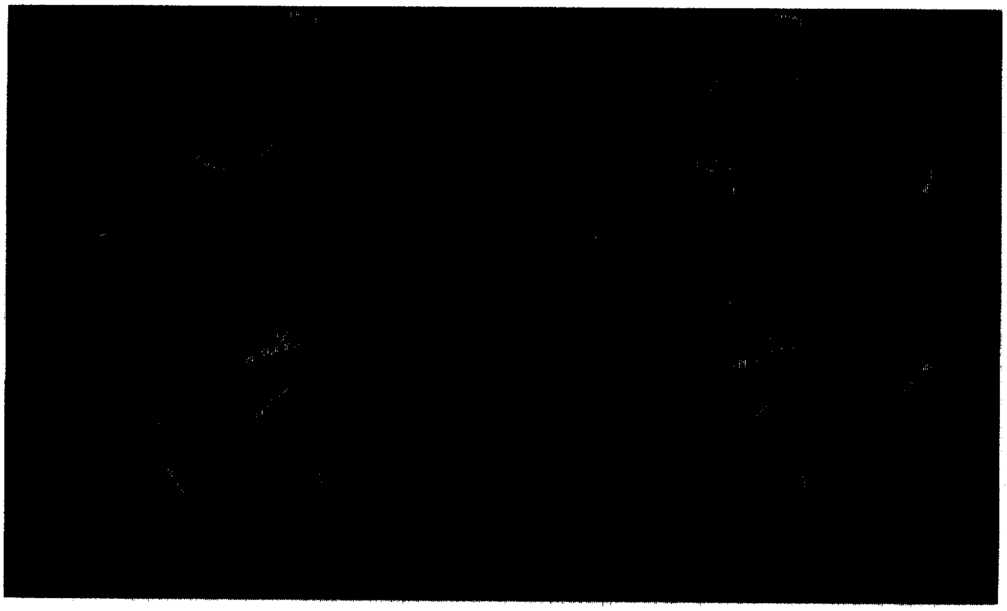
<u>Reagents and Conditions:</u>

(a) 1 eq Cs_2CO_3, DMF, 1.1 eq $(C_6H_5)_2CHBr$, 60 °C, 18 h (100%) (b) 10% aq NaOH-EtOH, rt, 18 h, 80 °C, 0.5 h (100%) (c) 1.5 eq EDC, 1.5 eq HOBT (d) 1.5 eq Et_3N, DMF, 1.05 eq 4-aminopyridine, rt, 18 h (80%) (e) 1.5 eq $LiAlH_4$, THF, 60 °C, 18 h (95%).

While analog **9** is more than 20-fold less potent than the corresponding quaternary compound **7**, it retains a good biological and specificity profile yet lacks the potentially reactive P1 moiety.

The crystal structure of **9**, at 1.9 Å (R factor Of 0.172), indicated that the 4- aminopyridine group fills S1. This structure also revealed an alternate binding mode for the bulkier P3 group, as illustrated in Figure 3. In the case of the thrombin-**9** complex, the phenyl-O linker torsion is comparable to that observed in the thrombin-**1** complex and one of the aromatics of the P3 group occupies the distal hydrophobic pocket. The additional aromatic ring of **9** sits in a new region on the solvent exposed edge of Trp-60-D of the enzyme. This is in marked contrast to the binding observed for analog **7**, described above, which contains the same P3 group. By simply changing the connectivity of the P1 fragment, we have discovered yet another mode for ligand binding in the region of the distal hydrophobic pocket.

Figure 3. A stereoview of the active site region of the α-thrombin-**9** crystal structure colored as described in the legend to Figure 1.



In conclusion, these studies identified a novel, nonpeptidyl thrombin inhibitor which by modifications of the P1 and P3 segments of the ligand led to a superior class of non-quaternary compounds which retained good in vitro potency. Further investigations on this series of analogs are in progress and will be reported in due course.

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