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L-373,890, AN ACHIRAL, NONCOVALENT, SUBNANOMOLAR THROMBIN INHIBITOR

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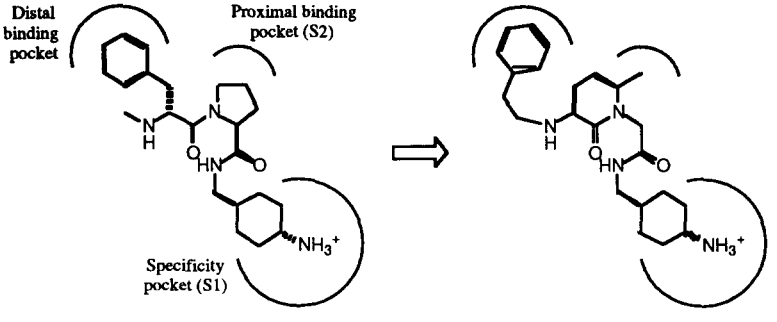
Abstract: L-373,890, a highly selective and efficacious pyridinone acetamide thrombin inhibitor was designed using a combination of X-ray crystallography, molecular modeling and empirical structure optimization.

Since the first report that the glycylproline amide backbone of peptide inhibitors of human leukocyte elastase (HLE) may be effectively replaced by a 3-amino-2-pyridinone or 5-amino-6-pyrimidinone acetamide template,[1] a wealth of data has emerged from the Zeneca group concerning the use of these templates in the design of covalent HLE inhibitors.[2] Very recently others have disclosed the use of these templates in the design of covalent interleukin-1β converting enzyme inhibitors[3,4] and thrombin inhibitors.[5] We too were inspired by the simplicity of the pyridinone template. Given the recent report from these laboratories that *trans*-substituted cyclohexylamine can be incorporated at P_1 to give highly selective low nanomolar peptide thrombin inhibitors which do not require a serine trap,[6,7] we elected to couple this P_1 group with a suitably functionalized pyridinone P_2/P_3 scaffold. In this communication, we describe our initial work in this area of peptidomimetic chemistry and how it has opened up the possibility of building achiral, noncovalent thrombin inhibitors.



L-371,912[6] (**1**): $K_i = 5.0 \pm 0.5$ nM

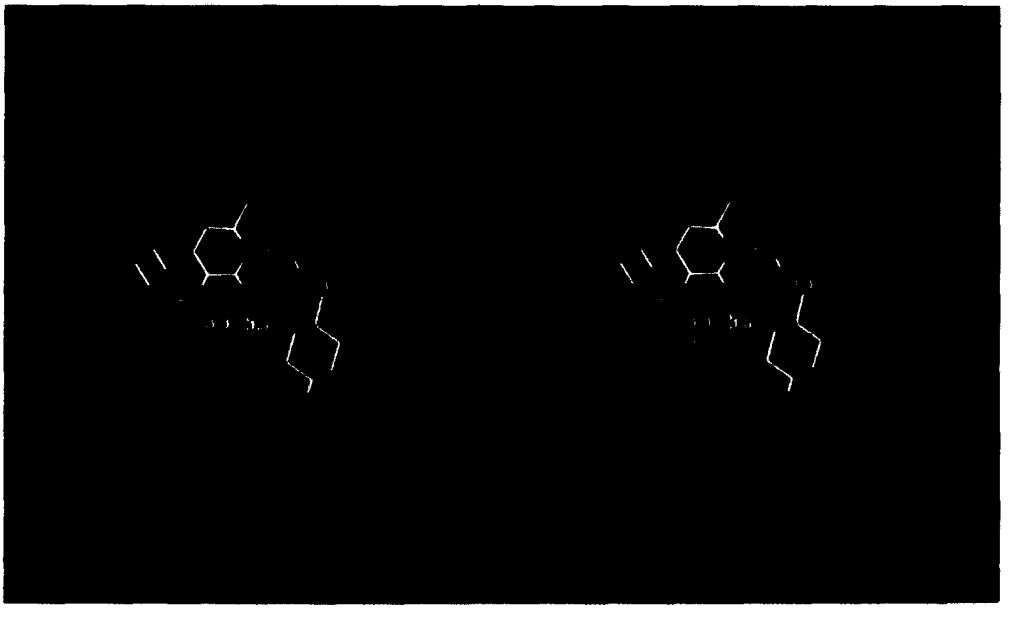
2: $K_i = 47$ nM

The design process began with the X-ray crystal structure of L-371,912 (**1**) bound to the α-thrombin-hirugen complex[6] and subsequently used OPTIMOL with the MMFF force field[8] for energy minimization of the proposed ligands in the enzyme active site. Initially, we confirmed that a 3-amino-2-pyridinone acetamide template could be accommodated by the enzyme in a position that allowed it to form hydrogen bonds to the Ser-214, Trp-215, Gly-216 β-sheet element of the protein, mimicking the hydrogen bonds from the peptide backbone of **1** to the enzyme. To fill the hydrophobic distal binding pocket,[9] a phenyl group could be tethered to the amino group of the new scaffold via a carbon chain containing two methylene units. Modeling also suggested that a methyl substituent at the 6-position of the pyridinone would fill the hydrophobic proximal binding pocket

(S2). This substituent would also confer a measure of preorganization to the acetamide, forcing the CO-CH_2 bond out of the plane of the ring in a conformation which mimics the nascent turn of the bound peptide.

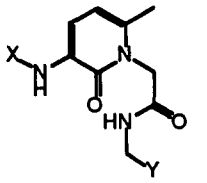
On the basis of this modeling we chose first to make the 3-(2-phenethylamino)-6-methylpyridinone **2**.[10] Encouraged by the affinity of **2** for thrombin (K_i = 47 nM)[11] we confirmed its binding mode by X-ray crystallography (figure below). The crystals of **2** bound to the α-thrombin-hirugen complex were prepared and the diffraction data collected as described previously.[6,12] The crystal structure was solved at 2.1 Å (R factor of 0.14) and was of sufficient quality to accurately confirm the location and binding mode of **2** in the enzyme active site.

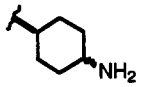
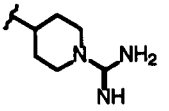
X-ray crystal structure of 2 in the thrombin active site.



As suggested by the modeling, the phenethyl substituent fills the distal pocket and the 6-methyl group occupies S2. The aminocyclohexyl P_1 group of **2** sits in the specificity pocket (S1) in a manner similar to that portion of **1** when bound to the enzyme. While the pyridinone ligand makes the predicted series of hydrogen bonds with the enzyme, the hydrogen bond lengths are longer than optimal. The 3-amino nitrogen atom of **2** is 3.3 Å from the carbonyl oxygen of Gly-216, the pyridinone oxygen atom is 3.5 Å removed from the amide nitrogen of Gly-216 and the acetamide nitrogen of **2** is 3.3 Å from the carbonyl oxygen of Ser-214. The corresponding atomic separations in the crystal structure of **1** are 3.1 Å, 2.7 Å, and 2.9 Å, respectively. The longer hydrogen bonding distances observed with **2** could be due to non-optimal P_1 and P_3 appendages attached to the scaffold.

Compound **2** is also chemically unstable, slowly forming highly colored impurities on standing in solution or in the solid state, presumably through the oxidation of the electron rich pyridinone ring. To chemically and structurally optimize **2** we chose to prepare a series of compounds in which the aminopyridinone is deactivated by derivatizing the amine as an amide, carbamate or sulfonamide. In addition, we chose to investigate the use of the amidinopiperidine P_1 group.[9,13] The results are shown in the table.



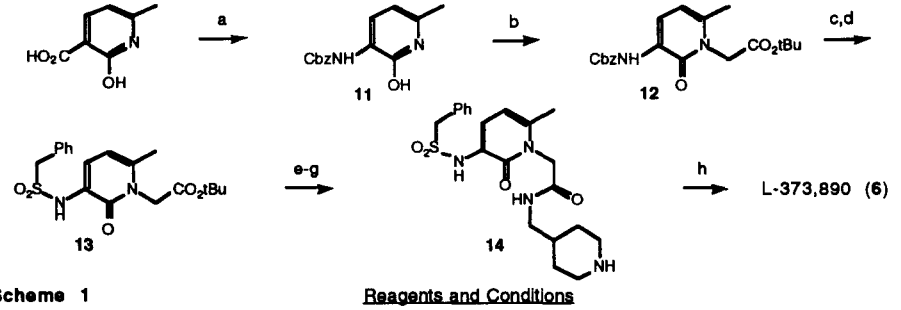
Y:  c-HexNH$_2$ or  amidinopip.

compound	X	Y	K_i (thrombin)/nM[11]	K_i (trypsin)/nM[11]
2	$Ph(CH_2)_2$	*c*-HexNH$_2$	47	2,200
3	4-MePhSO$_2$	*c*-HexNH$_2$	47	2,100
4	$PhCH_2SO_2$	*c*-HexNH$_2$	4.6	3,000
5	4-MePhSO$_2$	Amidinopip.	29	140
6	$PhCH_2SO_2$	Amidinopip.	0.5	570
7	PhCO	Amidinopip.	690	1,500
8	$Ph(CH_2)_2CO$	Amidinopip.	95	940
9	Ph_2CHCH_2CO	Amidinopip.	166	890
10	$PhCH_2OCO$	Amidinopip.	77	350

The bound structure of **2** shows that in order for an aromatic ring to fill the distal binding pocket, while maintaining the hydrogen bonding array between the ligand and the enzyme, the link between the aromatic ring and the amine must be flexible enough to allow the side chain to make a sharp turn out of the plane of the pyridinone. This is born out by the results given in the table. Thus, the benzamide **7** binds relatively poorly since it has a strong preference to remain coplanar with the pyridinone. The more flexible dihydrocinnamoyl and Cbz derivatives **8** and **10** are approximately tenfold more potent and the *p*-toluenesulfonamides **3** and **5** are somewhat better still. With the amidinopiperidine in P_1, *p*-toluenesulfomide **5** is only modestly better than its cyclohexylamine counterpart **3**. However the breakthrough compound is benzylsulfonamide **6** (L-373,890, K_i 0.5 nM) which is nearly tenfold more potent than its cyclohexylamine counterpart **4**, presumably because the full benefit of the intrinsically more potent amidinopiperidine group is made possible by the more flexible benzyl group.

Compound **6** retains good selectivity against trypsin (K_i = 570 nM) and is inactive ($K_i \geq 20$ μM) against the serine proteases plasmin, tPA, activated protein C, plasma kallikrein and chymotrypsin. The concentration required to double the APTT is 170 nM (human plasma). Occlusion was prevented in 6 out of 6 animals in the rat ferric chloride model of arterial thrombosis[14] at an IV infusion rate of 10 μg/kg/min.

The synthesis of **6** starts from commercially available 2-hydroxy-6-methylpyridine-3-carboxylic acid (Scheme 1). Curtius rearrangement of the acyl azide and trapping of the isocyanate with benzyl alcohol



Scheme 1 Reagents and Conditions

(a) DPPA, Et_3N, Dioxane, reflux 16 h, then BnOH, reflux 24 h (59% recrystallized from MeOH). (b) NaH, $BrCH_2CO_2tBu$, THF, 2 h (97%). (c) H_2 (50 psi), $Pd(OH)_2$, 4:1 EtOH:H_2O, 2 h (99%). (d) $BnSO_2Cl$, pyridine, 1 h (97%). (e) HCl, EtOAc, 1.5 h (82%). (f) DCC, 4-aminomethyl-1-BOC-piperidine, CH_2Cl_2, 3 h. (g) HCl, EtOAc, 1 h. (h) pyrazole-1-carboxamidine.HCl, DMF, 60 h [17% over 3 steps after C_{18} prep. HPLC (CH_3CN/H_2O/0.1% TFA)].

generates the Cbz protected amine **11**. The sodium salt of **11** cleanly reacts on the ring nitrogen with *t*-butylbromo acetate in THF to give **12**, and hydrogenolysis of the Cbz group followed by sulfonylation of the free amine in pyridine gives the benzyl sulfonamide **13**. Deprotection, followed by coupling to 4-aminomethyl-1-BOC-piperidine[15] and a second acidic deprotection gives the piperidine **14**, which is reacted with pyrazole-1-carboxamidine[16] to give the final product.

In conclusion, we have extended the use of the pyridinone acetamide peptidomimetic template in its first successful application for the synthesis of potent, noncovalent inhibitors of either a cysteine or a serine protease. The power of the template is illustrated by the fact that **6**, L-373,890, is the first achiral, subnanomolar thrombin inhibitor. Further work in this promising area will be reported in due course.

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