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Small, Low Nanomolar, Noncovalent Thrombin Inhibitors Lacking a Group to Fill the 'Distal Binding Pocket'

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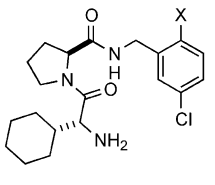
Abstract—Use of a chlorophenoxyacetamide P1 group with a pyridinone acetamide P2/P3 gave an exceptionally potent thrombin inhibitor ($K_i = 40$ pM). Truncation of the molecule at the N-terminus gave unique, low nanomolar, non-covalent thrombin inhibitors which do not have a group to fill thrombin's 'distal binding pocket'. A co-crystal structure indicates the importance of an intramolecular hydrogen bond between the P1 side chain and P1/P2 amide link in this series.

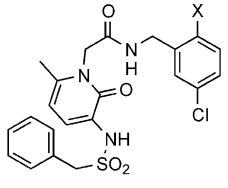
Regular monitoring of a patient's coagulation parameters is a necessary burden when using warfarin, the existing oral anticoagulant. This fact is driving the search for alternative antithrombotics. Both intravenous and orally active direct thrombin inhibitors have been shown to be clinically effective antithrombotics. Consequently, the development of a selective, orally active thrombin inhibitor which has predictable pharmacokinetics and which is suitable for once or twice a day dosing is a prominent medicinal chemistry goal.[1] A key assumption underlying the design of such a compound is that it should have a group, typically, although not necessarily, basic to occupy S1 (the 'specificity pocket') linked in some fashion to a second, lipophilic group which binds in the 'distal binding pocket'. Both of these features are believed to be necessary in order for the compound to achieve useful levels of inhibition. To our knowledge there is no reported example of a potent thrombin inhibitor which contradicts this assumption. In this communication we describe a unique series of small, potent, non-covalent thrombin inhibitors which lack a group to fill the 'distal binding pocket'.

Previous reports from these laboratories describes the development of uncharged 2,5-disubstituted phenyl P1 groups in a D-cyclohexylglycylproline based series of inhibitors (**1** and **2**).[2,3] In this series, a compound containing the 2,5-dichlorophenyl P1 group was found to be significantly more potent than the corresponding monosubstituted 3-chlorophenyl derivative. When an oxyacetamide group was incorporated at the 2-position in place of a chlorine (**2**), an increase in potency was seen and this was ascribed to the hydrogen bond between the oxyacetamide carbonyl group and the NH of Gly 219 seen in the crystal structure of **2** bound in thrombin's active site.[3]

We applied this class of P1 group to our pyridinone acetamide template in a search for viable alternatives to the previously described aminopyridines.[4–6] First we found that the 2,5-dichlorophenyl derivative **3** was less potent than the 3-chlorophenyl derivative **4**, in contrast to the proline based series. Secondly and more significantly, there was a 600-fold increase in potency on switching from a chloro group to the oxyacetamide group at the 2-position (**5**, $K_i = 40$ pM), an improvement *two orders of magnitude greater than that seen in the proline based series* (Table 1).



1: X = Cl[3]
2: X = $OCH_2CONHEt$[3]



3: X = Cl
4: X = H
5: X = $OCH_2CONHEt$

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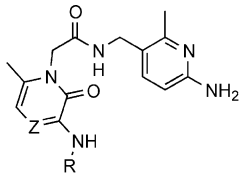
Table 1. In vitro assays for compounds **1**–**15**

Compd	Thrombin inhibition K_i, nM[a]	Trypsin inhibition K_i, nM[a]	2×APTT μM[b]	Protein binding %free[c]
1[3]	5	29,000	0.95	4
2[3]	0.74	23,000	0.41	15
3	24	>100,000	2.7	—
4	7	20,000	2.8	—
5	0.04	2000	0.79	—
6[5]	0.8	1800	0.41	6
7	900	23,000	—	—
8	340	20,000	—	—
9	0.07	1000	1.2	1
10	12	7200	1.6	20
11	8.5	10,000	2.1	—
12	18	14,000	1.3	—
13	7.4	11,000	0.7	18
14	2100	>100,000	—	—
15	15	7100	2.5	—

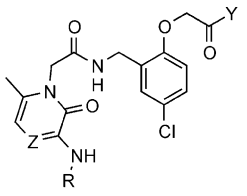
[a]See ref 10.
[b]Concentration of compound required to double the human activated partial thromboplastin time.
[c]Human plasma.

The potency of compound **5** prompted us to explore the possibility of truncating the molecule by deleting the benzylsulfonyl group. This would compensate for the mass of the P1 group (**5**: M_r 561). In addition it could potentially improve the physical characteristics of the molecule. However, simply deleting the benzylsulfonyl group would leave an electron rich 3-aminopyridinone which we have found to be chemically unstable.[7] 3-Aminopyrazinones are chemically stable 3-aminopyridinone isosteres,[5] so we first tried this truncation strategy on a pyrazinone analogue of **5**.[8]

We anticipated a steep drop in potency upon deletion of the P3 group since in the P1 aminopyridine series,[5] with the same transformation, there is an 1100-fold K_i difference (**6** and **7**). However, with a phenoxyacetamide P1 group we saw a relatively modest 180-fold K_i difference between the phenethyl substituted compound **9** and its truncated form, compound **10** ($K_i = 12$ nM). The advantage to deleting the large, lipophilic P3 side chain becomes apparent when the protein binding is taken into consideration. Whereas **9** is 99% bound in human serum, **10** is 80% bound. So despite the gap in potency, the concentration of **10** required to double the activated partial thromboplastin time (2×APTT) is only slightly higher than that for compound **9** (1.6 μM, vs 1.2 μM respectively). Presumably the increased free fraction compensates for the drop in enzyme affinity.



6: Z = N R = $Ph(CH_2)_2$[5]
7: Z = N R = H
8: Z = CCF_3 R = H



	Z	R	Y
9	N	$Ph(CH_2)_2$	NHEt
10	N	H	NHEt
11	CCF_3	H	NHEt
12	CCF_3	H	$NH(CH_2)_2NMe_2$
13	CCF_3	H	$NH(CH_2)_3NMe_2$
14	CCF_3	H	NMe_2
15	CCF_3	H	NHMe

Another way to stabilize a 3-aminopyridinone is to incorporate an electron withdrawing group such as trifluoromethyl at the 4-position. Examination of the crystal structures of pyridinone and pyrazinone acetamide thrombin inhibitors bound in the enzyme active site showed that the enzyme can accommodate such a group at the 4-position as this position is open to solvent.[4,5,7,9] Consequently we prepared a series of 4-trifluoromethyl substituted pyridinones.[6] For comparison, the P1 aminopyridine derivative **8** was 3-fold more potent than the corresponding pyrazinone. Incorporation of the phenoxyacetamide P1 group gave compound **11** ($K_i = 8.5$ nM). To improve the physical characteristics of compound **11** we introduced a dimethylamine substituent

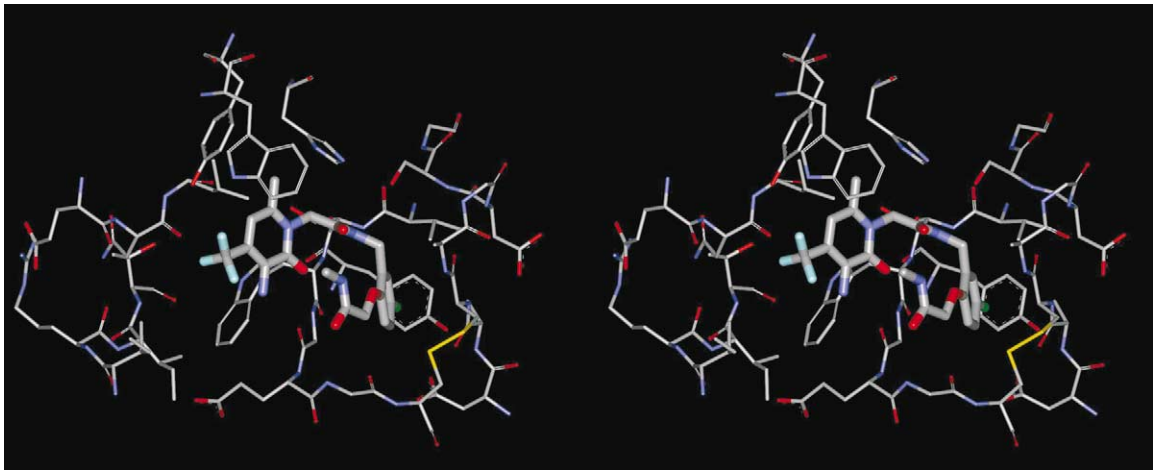


Figure 1. The crystal structures **13** in the thrombin active site. The density map of the dimethylaminopropyl chain of **13** is not well defined and the side chain has been omitted.

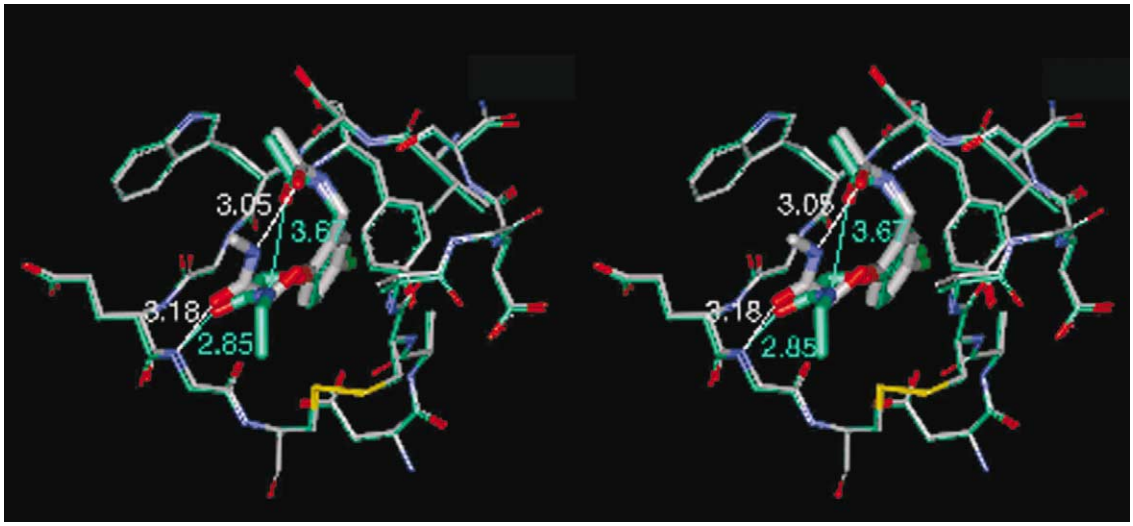


Figure 2. An overlay of the crystal structures of the P1 regions of **2** (carbons in green) and **13** (carbons in grey) in their respective active sites.

into the side chain to give **12**. Compound **12** was less potent in the enzyme assay than **11** but it had an improved 2×APTT. Extending the chain by one methylene gave dimethylaminopropyl compound **13** which was equipotent to **11** in the enzyme assay and was submicromolar in the APTT assay (2×APTT = 0.7 μM).

The crystal structure of **13** bound in the active site of thrombin is shown in Figure 1.[11] The density map for the dimethylaminopropyl side chain is not well defined so the group has been omitted. The pyridinone is orientated in the expected fashion and the 'distal binding pocket' bounded by the side chains of Leu-99, Ile-174 and Trp-215 is vacant. Figure 2 is an overlap of the P1 portions of **2** and **13** in their respective active sites.[3] The two structures are subtly distinct. Both inhibitors form a hydrogen bond between the carbonyl of the oxyacetamide side chain and the NH of Gly 219 although O–N distance is shorter for **2** (2.85 Å vs 3.18 Å). Compound **13** also forms an intramolecular hydrogen bond (3.05 Å) between the same amide and the carbonyl group of the amide link to the pyridinone. The corresponding distance for **2** is 3.67 Å, outside hydrogen bonding range. Interestingly, dimethylamide **14** (K_i = 2,100 nM) is 140-fold less potent than the methylamide **15** (K_i = 15 nM). The difference in potency between these two compounds would suggest that this intramolecular hydrogen bond (and possible ligand preorganization) lies at the heart of the potency of this class of P1 group in the pyridinone and pyrazinone series.

In conclusion, we have exploited the inherent potency of the 4-chlorophenoxyacetamide P1 group in the 3-aminopyridinone and pyrazinone acetamide series to make compound **10**, a low nanomolar, low molecular weight (408), non-covalent thrombin inhibitor which does not have a group to fill thrombin's 'distal binding pocket'. We also prepared a series of chemically stable 3-amino-4-trifluoromethylpyridinones using the same design strategy. This series was optimized to give compound **13** which is as potent as 3-benzylsulfonylaminopyridinone **5** in the 2×APTT assay. The compounds which have resulted from this design strategy constitute a unique new class of thrombin inhibitors. This work provides further illustration of the utility of the 3-aminopyridinone and pyrazinone acetamide templates in the design of potent thrombin inhibitors.

Acknowledgements

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