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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1032–1036

Phenolic P_2/P_3 core motif as thrombin inhibitors—Design, synthesis, and X-ray co-crystal structure

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Received 21 September 2005; revised 14 October 2005; accepted 24 October 2005
Available online 15 November 2005

Abstract—Prototypical thrombin inhibitors were synthesized based on a trisubstituted phenol as a core motif. A naphthylsulfonamide analogue showed excellent antithrombin activity. An X-ray co-crystal structure showed the expected interactions.

The proteolytic activity of the enzyme thrombin on endogenous proteins such as fibrinogen, and the involvement of other serine proteases such as Factor VIIa and Factor Xa in a series of complex biochemical events leads to blood clots, with serious thrombotic health consequences when procoagulant activities are unnaturally high.[1] Selective inhibitors of key coagulation factors such as thrombin could offer a unique method for the management of cardiovascular thrombotic states in the heart and elsewhere. The availability of X-ray crystallographic data on thrombin with a host of small molecules offers attractive options for the design and synthesis of structure-based potential inhibitors.[2] Indeed, several peptidomimetic type drug candidates known to interact with thrombin at specific sites are in advanced stages of clinical studies.[3] More recent efforts have focused on non-peptidic inhibitors based on heteroaromatic or heterocyclic core structures.[4] Such comparatively ''simpler'' molecules are equally effective in inhibiting thrombin, since they are deployed with requisite functionality for optimal binding in the active site.

We have reported on the design, synthesis, and X-ray co-crystal structure of indolizidinones with thrombin.[5] Low to moderate nanomolar inhibition was observed, thus validating the initial design concept which focused on constrained and conformationally rigidified analogues of the natural substrate D-Phe-Pro-Arg. We have also reported on related indolizidinones designed to target Factor VIIa.[6] The marine natural products Dysinosin A[7] and Oscillarin[8] recently synthesized in our laboratories exhibit inhibitory activity against thrombin and Factor VIIa.

We now report on the synthesis and antithrombin activity of a series of achiral sulfonamides appended to a trisubstituted phenolic core structure represented by the generic expression **1** (Fig. 1). The requisite P_1, P_2, P_3 interactions with their respective subsites in the enzyme as suggested by molecular modeling augured well for anticipating in vitro activity as well. During the

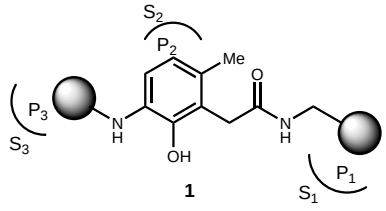

Figure 1. Prototype of phenolic core with variations in the P_1 and P_3 subunits.

Keywords: Thrombin; Inhibitor; Trypsin; Phenol; Sulfonamide.
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doi:10.1016/j.bmcl.2005.10.082

biological evaluation of our set of compounds, a patent appeared from the 3-D Pharmaceuticals Inc. group, in which related compounds were reported but limited data were provided on their antithrombotic activity.[9]

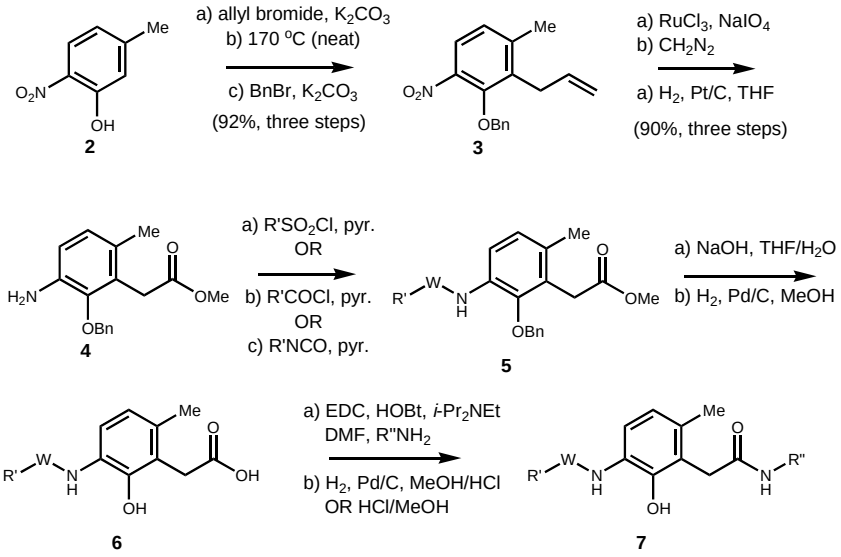
The phenolic core containing a diverse set of P_3 appendages was synthesized as shown in Scheme 1, by a somewhat obvious protocol. Thus, the commercially available 2-nitro-5-methyl phenol **2** was O-allylated and the product subjected to an *ortho*-Claisen rearrangement followed by O-benzylation to give **3** in excellent overall yield. Oxidative cleavage of the double bond and subsequent treatment of the resulting carboxylic acid with diazomethane yielded the corresponding methyl ester. Chemoselective reduction of the nitro group with Pt/C led to the amine **4**. Sulfonylation or acylation of **4** with the desired P_3 moiety afforded **5**. Hydrolysis of the ester followed by debenzylation yielded **6**, which was submitted to amide formation and subsequent deprotection to give the desired analogue **7**. In order to provide sufficient diversity in the library, different aromatic P_1 substituents were also prepared using the general protocol (Fig. 2).

The sets of sulfonamides and amides harboring the phenolic core motif exhibited excellent purities by LC/MS. The IC_{50} values against thrombin and trypsin of the phenolic sulfonamides and amides in the 4-amidinobenzyl P_1 series (**A**) are listed in Table 1.

Several mono- and disubstituted sulfonamides exhibited IC_{50} values in the range of 16–82 nM with selectivity over trypsin (Table 1, entries 1–6). Unfortunately, no clear-cut SAR could be observed by varying the position of aromatic substituents (Table 1, entries 2–10). The benzyl sulfonamide analogue displayed similar activity as the analogous phenylsulfonamide (Table 1, compare entries 7 and 11). Amides and ureas were also included and proved to be detrimental to antithrombin activity (Table 1, entries 12–14). Although the activities could not be directly correlated with the nature or position of aromatic substitution on the arylsulfonamide part, we were intrigued that the 1-naphthylsulfonamide showed excellent antithrombin activity with a 67-fold selectivity over trypsin (Table 1, entry 1).

In Tables 2–4 are listed the IC_{50} values of another set of substituted phenolic sulfonamides with simultaneous variations of the aromatic substituents and the P_1 subunits (series **B**, Table 2). Sulfonamides in the 2-amino-5-aminomethyl-6-methyl pyridines were almost equipotent with IC_{50}'s in the 190–590 nM range against thrombin and with excellent selectivity. The same trend was observed with the 2-amino-4-aminomethyl pyridine P_1 subunit (series **C**, Table 3), as well as with the 2-aminomethyl-3-fluoro-6-methyl pyridine P_1 subunit (series **D**, Table 4), although the activity against thrombin was greatly reduced compared to the 4-amidinobenzyl series **A** (Table 1).

Determination of the X-ray crystal structure of compound **8** in complex with thrombin revealed the same general binding mode as observed for CVS1695[4c,10] (Figs. 3 and 4). The 4-amidinobenzyl group occupies



Scheme 1. General protocol for the synthesis of the phenolic analogues.

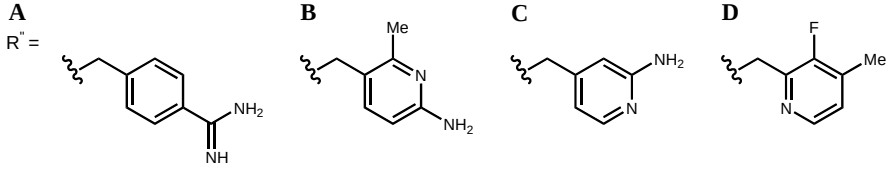
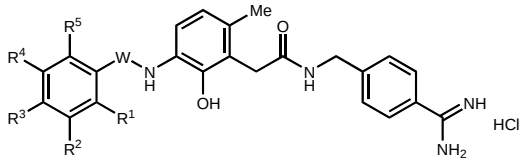


Figure 2. Different P_1 substituents.

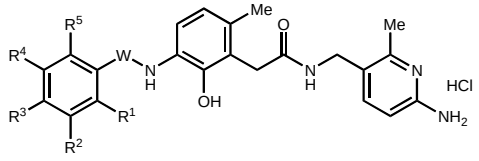
Table 1. IC_{50} values for the 4-amidinobenzyl P_1 subunit (series **A**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**8**	SO_2	CH=CH–CH=CH_2		H	H	H	0.017	1.1	67
2	**9**	SO_2	Me	H	H	Me	H	0.025	0.11	4.5
3	**10**	SO_2	F	H	Cl	H	H	0.082	0.68	8.4
4	**11**	SO_2	H	OMe	H	H	H	0.016	0.28	17
5	**12**	SO_2	H	OMe	OMe	H	H	0.039	0.34	8.7
6	**13**	SO_2	H	H	OMe	H	H	0.050	0.91	18
7	**14**	SO_2	H	H	H	H	H	0.097	0.60	6.1
8	**15**	SO_2	H	CF_3	H	H	H	0.12	2.8	23
9	**16**	SO_2	H	H	Me	H	H	0.25	1.1	4.4
10	**17**	SO_2	H	H	Cl	H	H	0.13	0.94	7.2
11	**18**	SO_2CH_2	H	H	H	H	H	0.19	3.1	17
12	**19**	$COCH_2$	H	H	H	H	H	4.7	4.0	0.86
13	**20**	CO	H	H	H	H	H	1.9	—	—
14	**21**	$CONHCH_2$	H	H	H	H	H	2.4	2.7	1.0

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

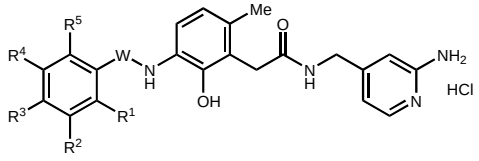
Table 2. IC_{50} values for the 2-amino-5-aminomethyl-6-methyl pyridine P_1 subunit (series **B**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**23**	SO_2	Me	Cl	H	H	H	0.19	>44	222
2	**24**	SO_2	Me	H	H	H	H	0.26	>44	212
3	**25**	SO_2	H	F	Me	H	H	0.59	>44	75
4	**26**	SO_2CH_2	H	H	H	H	H	0.57	>44	78

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

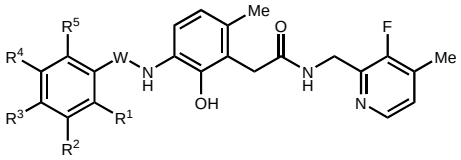
Table 3. IC_{50} values for the 2-amino-4-aminomethyl pyridine P_1 subunit (series **C**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**28**	SO_2	Cl	H	H	Cl	H	1.0	>44	44
2	**29**	SO_2	Cl	H	H	H	H	3.0	>44	15

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

Table 4. IC_{50} values for the 2-aminomethyl-3-fluoro-4-methyl pyridine P_1 subunit (series **D**)



Entry	Compound	W	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)		Ratio[a]
								Thrombin	Trypsin	
1	**30**	SO_2	H	Cl	Me	H	H	20	>44	2.2
2	**31**	SO_2	H	Cl	H	H	H	14	>44	3.2
3	**32**	SO_2CH_2	H	Cl	H	H	H	15	>44	3.0

[a] Ratio of (IC_{50} trypsin)/(IC_{50} thrombin).

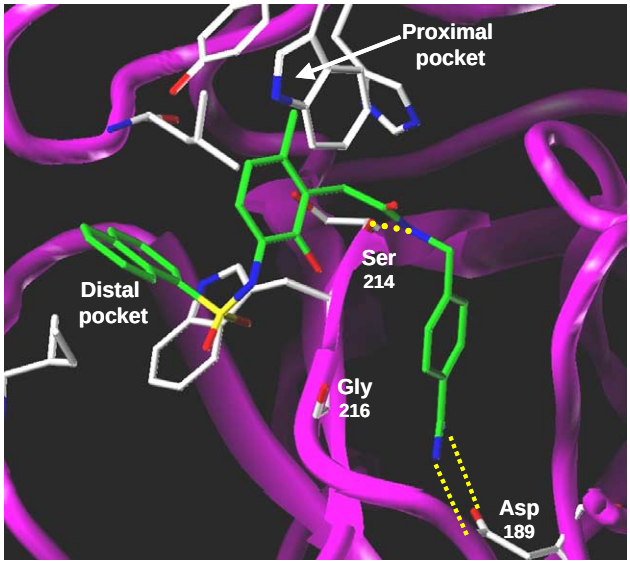


Figure 3. X-ray crystal structure of **8** bound in thrombin active site. Essential H-bonds to Asp 189 and Ser 214, and to sidechains of the proximal and distal pockets involved in lipophilic interactions are shown (PDB ID code 2BDY).

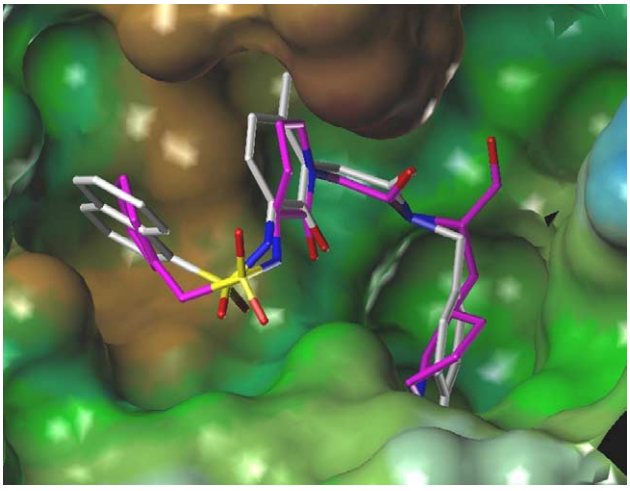


Figure 4. Superposition of the X-ray crystal structures of **8** (white) and CVS1695 (magenta) in complexes with thrombin.

the S_1-pocket with a salt bridge between the amidino moiety and Asp 189 (d = 2.62 and 2.97 Å). Similarly, the H-bond from the P_1–P_2 amide linkage to the C=O of Ser 214 is conserved (d = 3.07 Å). The P_2-group of **8** aligns well with that of CVS1695 creating hydrophobic interaction with the proximal pocket (S_2-pocket). The A-ring of the P_3-naphthyl group of **8** occupies almost the same space as the phenyl ring of CVS1695 with interactions in the lipophilic distal (D) pocket (S_3-pocket). The major difference is the absence of hydrogen bonds between the 2-aminophenol group and Gly 216 (d = 4.33 Å phenol-O to NH of Gly 216 and 3.90 Å NH to C=O of Gly 216). This may partially explain the slightly lower potency of this series of compounds compared to similar P_2-motifs recently disclosed, which in general exhibit an antiparallel β-sheet hydrogen bond network to Gly 216.[11] This difference is in line with the stronger H-bond donating capability of the amide oxygen in CVS1695 and similar P_2-motifs compared to that of the phenolic oxygen in our series.[12] This also provides an explanation why removal of the phenolic hydroxyl group (as in the P_2 phenyl analogue of the benzylsulfonamide **18**) only reduced the potency two times (IC_{50} 0.41 μM thrombin, 3.3 μM trypsin).

The B-ring of the naphthyl group is evidently too big to be accommodated in the D pocket and merely interacts with bulk water. Notably, compound **8** is four times as active as phenyl analogue **14** (IC_{50} 0.017 and 0.097 μM). Most likely, this is due to intramolecular stabilization of the torsion around the N–S–C–C bond of the flanking B-ring. In agreement with this, improved potency is also observed for compound **9** having an *ortho*-methyl group (IC_{50} 0.025 μM).

In conclusion, we have shown that a trisubstituted phenol can be used as central P_2 scaffold and that substituted phenylsulfonamides can be most effective P_3 subunits. Depending on the nature of the P_1 subunit, activity and selectivity over trypsin can be nicely modulated. The X-ray crystal structure of the 1-naphthylsulfonamide analogue **8** corroborates the anticipated interactions in the binding site of thrombin (Figs. 3 and 4, PDB ID code 2BDY).

Clearly, the 4-amidinobenzyl P_1 subunit (**A**) is favored for thrombin with some selectivity against trypsin. Selectivity is dramatically increased with less basic P_1 subunits at the expense of a 3- to 20-fold loss of antithrombin activity compared to the amidine series.

The simplicity of these structures and their relative ease of synthesis should pave the way to a better understanding of the relative roles of the P_2/P_3 subunits, and the subtle influences of aromatic substitutions on the bioactivity.[13]

Acknowledgments

We thank the NSERC of Canada and AstraZeneca (Mölndal, Sweden) for financial assistance through the Medicinal Chemistry Chair program. W.A.L.v.O thanks the NRF (South Africa) for partial post-doctoral support. We also thank Sofi Nielsen at AstraZeneca for IC_{50} determinations.

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