Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 775–780

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PYRAZOLES, 1,2,4-TRIAZOLES, AND TETRAZOLES AS SURROGATES FOR *cis*-AMIDE BONDS IN BORONATE ESTER THROMBIN INHIBITORS

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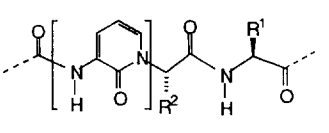
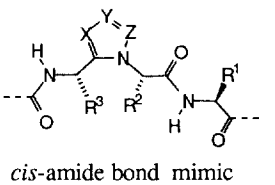
Received 15 December 1997; accepted 11 February 1998

Abstract: Substituted pyrazoles, 1,2,4-triazoles, and tetrazoles are good surrogates for *cis*-amide bonds in a series of boronate ester thrombin inhibitors.

Recently, heterocycles have been used to replace the amide bonds of peptide inhibitors that are in a β-sheet binding arrangement with an enzyme.[1-4] A case in point is the substitution of the 3-amino-2-pyridinon-1-yl group (Figure 1) and its heterocyclic isosteres for dipeptides Ala-Pro, Val-Ala , Phe-Pro, and Asp-Pro, which has led to the discovery of potent semipeptidic human leukocyte elastase (HLE)[2], interleukin-1β converting enzyme (ICE),[3] and thrombin inhibitors.[4] The amide bonds in a β-sheet exist in the *trans*-conformation. The 3-amino-2-pyridinon-1-yl group therefore can be viewed as a *trans*-amide bond mimic. However, there has been only a minimal amount of work devoted to the discovery of *cis*-amide bond surrogates. One such *cis*-bond mimic is the tetrazole, employed by the Marshall group (Figure 1; X, Y, Z = N).[5] The advantage of a tetrazole group is that it can be formed directly from a peptide amide bond. However, the disadvantage of the tetrazole group is that it can only be disubstituted, both positions being used to bond to the peptide backbone. What is needed are heterocycles which would mimic the *cis*-amide bond and which would allow for the incorporation of



trans-amide bond mimic



cis-amide bond mimic

Figure 1. The 3-amino-2-pyridinon-1-yl group as a *trans*-amide bond mimic (R^2 = H for all inhibitors discovered thus far) and the 5-membered ring heterocycle as a *cis*-amide bond mimic.

additional substituents in order to take advantage of other favorable interactions in the active site. We now wish to report on the successful incorporation of not only the tetrazole, but also of pyrazole and 1,2,4-triazole into a series of peptidic boronic ester thrombin inhibitors. These heterocycles act as *cis*-amide bond surrogates while at the same time possess positions at which substituents may be added to increase bonding interactions.

Compound **1** (Figure 2) is a boronic acid thrombin inhibitor with a K_i of 1.7 nM.[6] A close analog of **1**, namely **2** (Figure 2), had its X-ray crystal structure[7] determined bound to the active site of thrombin. We noticed that **2** unexpectedly possesses a *cis*-amide bond between the S_2 and S_3 residues (Figure 3). Compound **1** could also possess a *cis*-conformation about the S_2-S_3 amide bond when bound to thrombin.[6] We envisioned that we could replace this *cis*-amide bond with a five-membered ring heterocycle as shown in Figure 2 in order to decrease the number of amide bonds in the molecule. Our first targets were the tetrazole-containing **3** and 1,2,4-triazole-containing **4** (Table 1).

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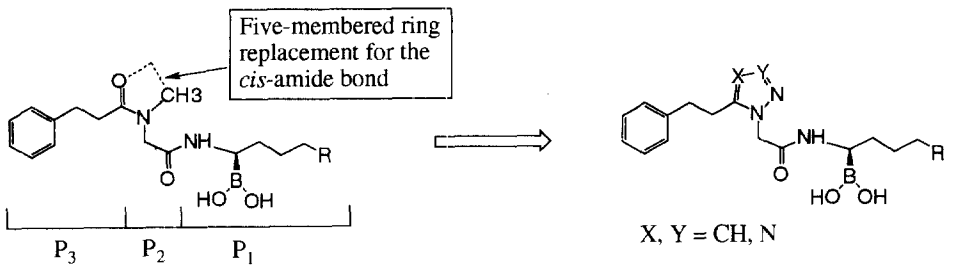
PII: S0960-894X(98)00104-8

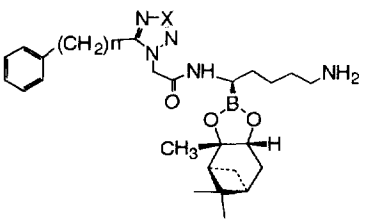


1: R = $-CH_2NH_2$ $K_i = 1.71 \pm 0.38$ nM
2: R = $-S(C{=}NH)NH_2$ $K_i = 0.30 \pm 0.026$ nM

Figure 2. Replacement of the *cis*-amide bond with 5-membered ring heterocycles.

As can be seen from the data, both tetrazole **3** and triazole **4** bind equally well to thrombin, although the affinity of both has decreased about 20-fold from that of **1** (the pinanediol ester hydrolyzes under assay conditions to the boronic acid). Shortening the phenethyl side-chain to a benzyl (**5**) does not have a dramatic effect on the K_i. However, extending to a phenylpropyl side-chain (**6**) reduces binding affinity. An X-ray crystal structure determination was performed on **5** bound in the active site of thrombin and is shown in Figure 3 together with that of **2**. The close overlap of the compounds except for the phenyl groups shows that the triazole is indeed a good surrogate for the *cis*-amide bond of **2**. We decided to pursue the 1,2,4-triazoles since

Table 1. Tetrazole and 1,2,4-triazole analogs of 1.



3	X = N	n = 2	$K_i = 33$ nM
4	X = CH	n = 2	$K_i = 36$ nM
5	X = CH	n = 1	$K_i = 31$ nM
6	X = CH	n = 3	$K_i > 42$ nM

we believed we could improve on their potency by attaching additional functionality onto the triazole ring. We chose to make analogs of **5** since the shorter benzyl side-chain might allow us to substitute more functionality onto the phenyl ring.

We noticed that the positively charged ammonium group of Lys^{60F} resides near the triazole 3-position. We postulated that a negatively charged group at this position should increase the binding affinity dramatically. However, **7** (Table 2), containing a negatively charged $-CH_2$-tetrazole group, exhibited a loss in affinity (K_i = 350 nM). The synthetic precursor to the tetrazole[8] was cyano-containing compound **8** (Table 2). Suprisingly, this compound showed better binding affinity with a K_i = 3.5 nM. X-ray crystal structures were determined for both compounds (Figure 4). We see that both the negatively charged tetrazole and the cyano groups bind to Lys^{60F}, one being an ionic interaction, the other being an H-bond. Both the cyano and tetrazole nitrogens are 3.6 Å away from the positively charged ammonium group of Lys^{60F}. One explanation why tetrazole **7** showed such poor affinity is that it might form an intramolecular salt-bridge between the tetrazole and the borolysine

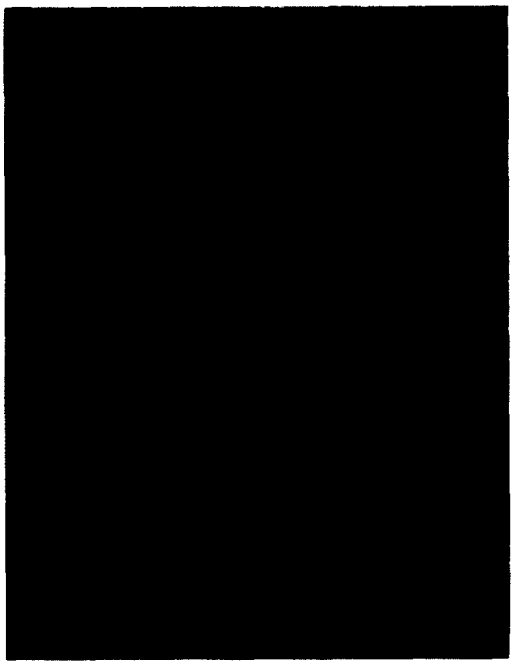


Figure 3. The X-ray crystal structures of compound **2** (red) and **5** (yellow) in the active site of thrombin.

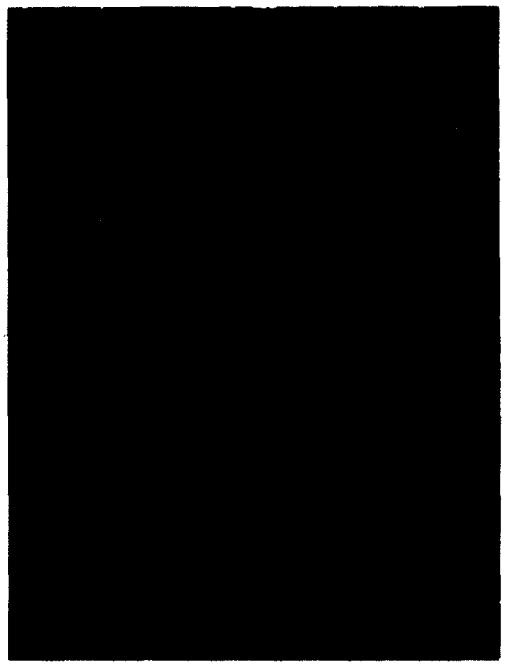
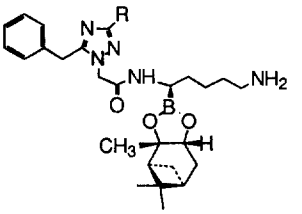


Figure 4. X-ray crystal structure of **7** (red) and **8** (yellow) in the active site of thrombin.

ammonium group. The cyano group of **8**, on the other hand, can only H-bond to Lys^{60F} resulting in an increase in the affinity by a factor of 9 over that of compound **5**. Having discovered this H-bonding interaction, we subsequently made analogs at the 3-position of the 1,2,4-triazole to see if we could duplicate this interaction with other functional groups. These 3-position analogs are summarized in Table 2.

We were surprised by compound **28** which possesses a 3-methyl group and a K_i of 8.6 nM. Thus it appears that part of the cyanomethyl group's increase in affinity might be due to a lipophilic interaction. Other H-bonding substituents did not fare so well probably due to steric constraints which do not permit their proper orientation for H-bonding to Lys^{60F}. Negatively charged acetic acid derivative **9** is worse than tetrazole **7**, since the COOH group is smaller than a tetrazole and thus its oxygen atoms are located further away from Lys^{60F}. Two compounds, **11** and **16**, contain elongated and lipophilic 3-substituents and were practically as potent as **8**. For compound **16**, the reason for the good affinity is that a different type of interaction occurs with the active site. In its X-ray crystal structure (not shown), the triazole in compound **16** has moved closer to Trp^{215} and has rotated around the N1-CH_2 bond so that the 3-substituent and the benzyl group exchange places in the active site. Compound **11** most likely assumes a similar orientation. In Table 2, selectivity for all compounds against other serine proteases was generally good except for trypsin.

Table 2. 5-Benzyl substituted 1,2,4-Triazole Thrombin Inhibitors

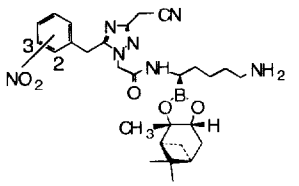


		K_i (nM)[a]					
No.	**R**	**thrombin**	**TT[b]**	**trypsin**	**fVIIa**	**fXa**	**TF[c]**
5	H	31	2500				
7	CH_2CN_4H	350					
8	CH_2CN	3.5	500	5.6	4200	>6000	
9*	CH_2CO_2H	1700			>14000	>6000	
10*	CH_2CO_2Me	85			14000	>6000	
11	$(CH_2)_2CO_2$-t-Bu	5.5		1.4	>3000	760	670
13	CH_2CH_2COOH	230		7.1	>3000	>6000	>10000
14	*trans*-CH_2=CHCOOtBu	150		36	2700	>6000	2800
15	CH_2OH	84			7700	>6000	
16	CH_2OCH_2Ph	8.7	1300		1500	1600	
17	CH_2SCH_3	65	4500	1.9	2500	>6000	
18	CH_2SOCH_3	47		4.8	2100	6000	3600
19	$CH_2SO_2CH_3$	11		2.1	1900	>6000	2700
20	$CH_2NHSO_2CF_3$	210		>1200	>3000	>6000	3700
21	CH_2NHCHO	33		6.6	2200	>6000	2700
22	$CH_2NHCOCH(CH_3)_2$	160		9.6	>3000	>6000	
23	$CH_2NHCOCH_2$-t-Bu	33		2.7	>3000	4300	1490
24	3-NO_2-Ph	85			2600	4200	
25	3-NH_2-Ph	150		4.2	1400	>6000	
26	Ph	173					
27	CH_2-Ph	41			3940	>6000	
28	CH_3	8.6	6000		7160	>6000	
29	$CH_2CH_2CH_3$	73		2.3	2400	>6000	

*Denotes a 1:1 mixture of N-1 and N-2 triazoleacetyl analogs. (a) The inhibitory constant (K_i) assays were performed as described in Kettner, C.; Mersinger, L.; Knabb, R. *J. Biol. Chem.* **1990**, *265*, 18289. (b) The thrombin time is defined as the concentration of inhibitor necessary to double the time required for clot formation induced by the addition of 4 NIH u/mL (final concentration) bovine thrombin; see Knabb, R. M.; Kettner, C. A.; Timmermans, P. B. M. W. M.; Reilly, T. M. *Thromb. Haemostasis* **1992**, *67*, 56. ; (c) Tissue factor.

We could further increase the affinity of **8** by placing H-bonding substituents on the phenyl ring. For example, substitution of a nitro group in the 2-position led to **30** (K_i = 0.8 nM) and in the 3-position to **31** (K_i = 1.8 nM) (Table 3).

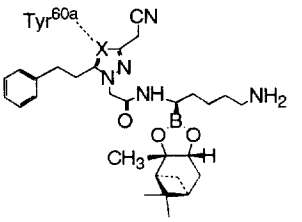
Table 3. Compounds 30 and 31.



K_i (nM)

		Thrombin	Trypsin	fVIIa	fXa	factor I
30	2-NO_2	0.8	8.4	3000	4900	2000
31	3-NO_2	1.8	23	>3000	>6000	1900

Table 4. 1,2,4-Triazole 32 and pyrazole 33.



K_i (nM)

		Thrombin	Trypsin	fVIIa	fXa	factor I
32	X=N	2.6	12	>3000	>6000	6800
33	X=CH	4.6	35		>6000	13000

We also examined the effect on substituting a 3-cyanomethyl group onto 5-phenethyl-1,2,4-triazole **4**. The result was compound **32** with a K_i = 2.6 nM (Table 4) which is about the same as benzyl analog **8** (3.5 nM). Replacement of the 1,2,4-triazole ring of **32** with a pyrazole yielded compound **33** which had a K_i = 4.6 nM (Table 4). The triazole's N-4 might form a weak H-bond with the OH of Tyr^{60a} in the active site (Table 4). Therefore, the pyrazole, which contains a CH at that position, binds with a slightly weaker affinity to thrombin.

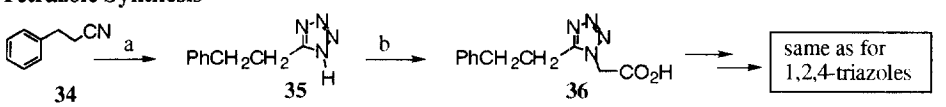
Conclusion.

The pyrazole, 1,2,4-triazole and tetrazole effectively mimic a *cis*-amide bond. Their incorporation into a peptidic boronic acid thrombin inhibitor caused a slight loss in affinity for thrombin. However, the affinity was regained and even increased by the substitution of appropriate functionality onto the triazole and pyrazole rings.

Chemistry.

The synthetic routes to the triazoles, tetrazoles, and pyrazoles are shown below. Tetrazole **35** was synthesized by the tin azide procedure.[8] This tetrazole was subsequently elaborated as discussed for the 1,2,4-triazoles: nitrile **37** was converted to its ethyl imidate and then to triazole **39** via known methods.[9,10] Alkylation, separation of isomers, identification of the isomers by nOe, saponification and coupling with **42**[6,7] yielded bromide **43**. Conversion to product **44** was done via known methods.[7] Finally, pyrazole **47** was synthesized[11] and elaborated as discussed for the 1,2,4-triazoles.

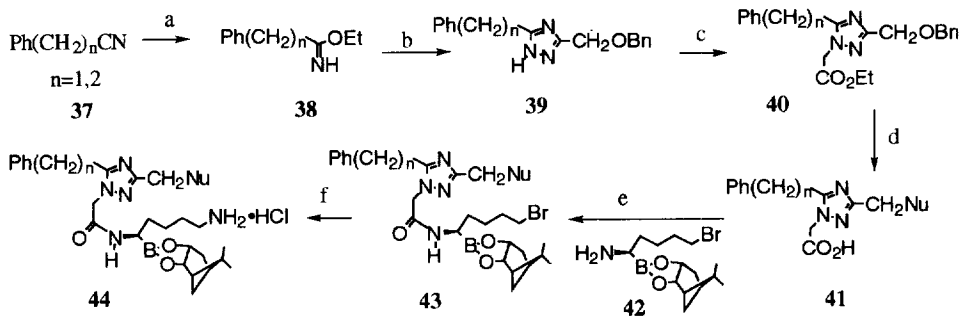
Tetrazole Synthesis



34 **35** **36**

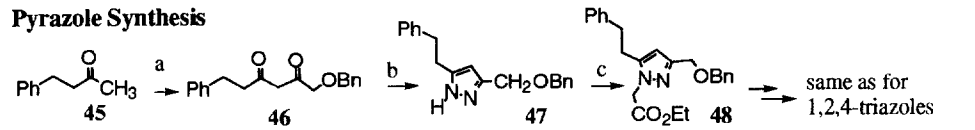
(a) 1. Bu_3SnCl, NaN_3, xy, reflux, 16 h. 2. Ph_3CCl, Et_3N 3. 6 N HCl, THF, H_2O. (b) 1. K_2CO_3, DMF. 2. $EtOCOCH_2Br$. 3. Chromatography to separate regioisomers 4. ^-OH.

1,2,4-Triazole Synthesis



(a) 1. EtOH, 0 °C, HCl. 2. NH_3, -20 °C. (b) 1. $BnOCH_2CONHNH_2$, EtOH, 0 °C. 2. 130 °C, 10 min. neat. (c) 1. NaH, DMF or K_2CO_3, DMF. 2. $EtOCOCH_2Br$ 3. Chromatography to separate regioisomers. (d) 1. $H_2/Pd(OH)_2$, MeOH 2. MsCl, Et_3N, -78 °C. 3. Nu^- (nucleophile). 4. LiOH, THF, H_2O. (e) N-methylmorpholine, isobutylchloroformate, THF, -20 °C. 2. **42**. (f) 1. NaN_3, DMSO 2. H_2 Pd/C $CHCl_3$ (3 eq))/MeOH.

Pyrazole Synthesis



(a) NaOEt, Tol, $CH_3O(C{=}O)CH_2OBn$, reflux. (b) N_2H_4, EtOH, reflux. (c) 1. K_2CO_3, DMF 2. $EtOCOCH_2Br$. 3. Chromatography to separate regioisomers

References and Notes.

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