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Crosslinked Peptoid-Based Dimerization Inhibitors of HIV-1 Protease

Song-Gil Lee^[a] and Jean Chmielewski^{*,[a]}

^[a] Department of Chemistry Purdue University 560 Oval Drive, West Lafayette, Indiana 47907 (USA)

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HIV-1 protease (PR) is an essential enzyme in the maturation of HIV as it cleaves the gag and gag/pol polypeptides into the functional and structural proteins of the virus.^[1] It has been a major target in the search for useful therapeutics for the treatment of AIDS due to its crucial role in the life cycle of virus. To date, a number of inhibitors have been approved for clinical use that target the active site of HIV-1 PR. Although these agents provide a powerful means to combat viral infection, it is imperative to develop inhibitors with alternate modes of inhibition to overcome problems associated with drug resistance.^[2]

The problems that have been identified with the current group of protease inhibitor drugs serve to underscore the need for novel approaches to inhibitor design. One such approach would be to target the dimerization interface of HIV protease.^[3] The main dimerization region of HIV-1 protease is found in an interdigitating N- and C-terminal four-stranded, antiparallel β -sheet (Figure 1).^[4] Toward this goal, crosslinked peptidic agents have been designed that target the four-stranded, β -sheet dimerization interface of HIV-1 PR,^[3] a region that is relatively free of mutations.^[5] Herein we disclose peptoid-based agents that target this viral protein-protein interaction - an interesting venture considering the β -sheet nature of the dimerization interface of HIV-1 protease.^[4]

Peptidic agents that inhibit the dimerization of HIV PR, such as compound **1**, are composed of Northern and Southern peptide components, derived from interfacial segments of HIV PR, that are crosslinked with an aliphatic tether.^[6] Recent studies demonstrated that the backbone amide hydrogens are not essential for the activity of these agents.^[7] This fact led us to question whether the peptidic backbone of **1** could be modified, since peptides often show undesirable properties, such as proteolytic instability. To this end we investigated peptoids as a peptidomimetic scaffold, since these are known to be proteolytic resistant, while allowing for the convenient synthesis of a diverse library of agents.^[8]

*Fax: (+1) 765-494-0239 chml@purdue.edu.

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In an attempt to systematically convert the peptidic inhibitor into a potent peptoid-based inhibitor, we initially prepared two peptide/peptoid hybrid molecules that contain a peptide backbone in one fragment and a peptoid backbone in the other (**2** and **3**).^[9] The compound containing a complete peptoid backbone based on inhibitor **1** (**4**) was also prepared. All peptoid and peptide fragments were prepared on solid phase with the Rink amide resin for the Northern fragments and the 2-chlorotritylchloride resin for Southern fragments. All peptoid fragments were synthesized based on a two-step monomer addition cycle of acylation with bromoacetic acid and displacement with a primary amine, as reported previously.^[10] Peptides and peptoids were crosslinked with desired tethers in solution with HATU and purified to homogeneity by HPLC.

The inhibitory potency of each agent was determined using a fluorogenic substrate designed by Toth and Marshall.^[11] Each compound was found to be a modest inhibitor of HIV PR with low micromolar IC₅₀ values (Scheme 1). The three peptoid-containing inhibitors were found to be less potent than compound **1**, and as the number of N-alkylglycine residues was increased within the agents the inhibitory efficacy against HIV PR was diminished. The mechanism of inhibition was probed by the Zhang-Poorman kinetic analysis method.^[12] The three peptoid-based inhibitors (**2-4** – concentrations of each compound used in the experiment are specified in Figure 2 caption) were found to act by a dissociative mechanism as indicated by the parallel nature of the lines in relation to uninhibited HIV-1 PR (Figure 2). K_i values were obtained from these data, and the full peptoid-based agent (**4**) was found to be only 9.4-fold less active as compared to its all peptide counterpart (**1**) (Scheme 1). This small decrease in potency is quite remarkable when one considers that five sidechain moieties were relocated in the peptoid structures.

To improve the inhibitory potency of this peptoid-based agent, we set out to make three major modifications to compound **4**: tether length, sidechain length, and sidechain functionality. An expected consequence of the conversion of a peptidic inhibitor into a peptoid-based inhibitor is that each sidechain residue is shifted one atom closer to the tether. Since the tether length has been shown to be a crucial element for inhibition of HIV-1 PR with the peptidic inhibitors,^[6,13] we modified the length of the tether in the peptoid-based agents to determine if this crosslink was optimal. Two compounds were prepared with the tether either decreased or increased by two methylene units (**5** and **6**, respectively). These peptoid-based agents were both found to inhibit the dimerization and activity of HIV-1 PR (Scheme 2 and Table 1). However, no increase in potency was observed in comparison to **4**, indicating that the crosslink in this agent was flexible enough to accommodate the binding of both peptoid arms of the inhibitor to HIV-1 PR.

Next, we attempted to realign the residues of the peptoid-based inhibitor **4** by changing the length of a few select sidechains. In a previous peptide-based study, the aromatic moieties at positions 1 and 5 were found to be most essential for inhibition of HIV PR.^[14] With this in mind, we chose to simultaneously extend the length of each of these sidechains (R1 and R5) in the peptoid inhibitor **4** by one methylene unit (compound **7**, Scheme 2 and Table 1). These modifications resulted in an inhibitor that maintained the dimerization inhibition mechanism and is approximately 2.2-fold more potent than **4** (K_i = 370 nM).

We next undertook a proof of principle study to determine if enhanced potency could be obtained through modification of the peptoid sidechain moieties. Since 4-aminophenylalanine (4-Paf) and 1-naphthylalanine (1-Nal) had been shown to be important elements for the potency of peptide-based inhibitors at positions 4 and 5, respectively,^[3b] a 4-aminobenzyl group at position R4 (compound **8**) and a 1-naphthalenemethyl group at position R5 (compound **9**) were individually inserted into these positions of the peptoids (Scheme 2 and Table 1). Both agents showed improved efficacy as compared to inhibitor **4**, and inhibited the activity and dimerization of HIV-1 PR. Compound **9** was only slightly more active as compared to **4**, whereas **8** was found to be 3.7-fold more potent than **4**. Interestingly, this same trend was observed with peptidic agents; compounds containing 4-Paf found to be more potent than those containing 1-Nal. Finally, an agent that contained three modifications within the northern fragment was prepared (compound **10**) and the most potent peptoid-based, dimerization inhibitor of HIV-1 PR was obtained (Scheme 2 and Table 1), with an efficacy that is only 1.6-fold less than the starting peptidic inhibitor **1**.

In conclusion, we have successfully developed the first potent dimerization inhibitors against HIV-1 PR that employ a crosslinked peptoid scaffold. The potency obtained is quite remarkable when one considers that five sidechain moieties were relocated in the peptoid structures, and that the hydrogen-bonding network with the dimerization interface was compromised. These data serve to underscore the importance of inhibitor sidechain interactions with HIV-1 PR, and support a diminished role of hydrogen bonding in blocking this protein-protein interaction. The results herein may be useful in guiding the further development of potent peptoid-based inhibitors of protein-protein interactions involving beta-sheet structures, since peptoids are known to be proteolytic resistant, but allow for ease of diversification.^[8]

Experimental Section

Peptoid synthesis and purification

All peptoid syntheses and purification details can be found in the supporting information.

Enzymatic assay

For the determination of the IC₅₀ values, HIV-1 protease solution (180 µl of a 50 nM) in assay buffer (20 mM phosphate, 1 mM DTT, 10% glycerol, and 0.1% CHAPS at pH 5.5) was incubated with inhibitor solution (36 µl) at the desired concentrations in DMSO for one hour at room temperature. This solution (60 µl × 3) was added to three different aliquots (40 µl) of a substrate solution (150 µM, 10% DMSO and 90% assay buffer) in a 96-well plate. The final concentration of DMSO was maintained at 14%. The change in fluorescence intensity at 430 nm (ex: 360) was immediately measured upon the addition of the protease to the substrate solution over a period of 14 minutes. For the Zhang-Poorman kinetic assay, varying concentrations of HIV-1 protease solution (180 µl, 5 – 40 nM) in assay buffer (20 mM phosphate, 1 mM DTT, 10% glycerol, and 0.1% CHAPS at pH 5.5) was incubated with inhibitor solution (36 µl) at the desired concentrations in DMSO for one hour at room temperature. This solution (60 µl × 3) was added to three different aliquots (40 µl) of a substrate solution (62.5 µM, 10% DMSO and 90% assay buffer) in a 96-well plate. The final

concentration of DMSO was maintained at 14%. The change in fluorescence intensity at 430 nm (ex: 360nm) was immediately measured upon the addition of the protease to the substrate solution over a period of 14 minutes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) Structure of dimeric HIV-1 protease and b) expansion of the region of the β -sheet dimerization interface (box) of HIV-1 protease.

Figure 2. Zhang-Poorman analysis of agents 2-4; ■ uninhibited HIV-1 PR, ▲ 2 (9 μM), ◆ 3 (2.6 μM), ● 4 (5.2 μM). E_0 is the total enzyme concentration, and V is the initial velocity.

Scheme 1.
Results of peptoid-based modifications to inhibitor **1**.

Scheme 2.
Structures of the amines used in the library.

Table 1

Results of mutation study based on Inhibitor 4.

Compound	n	Northern Component			Southern Component		IC ₅₀ (μM) ^[a]	K _i (nM) ^[a]
		<i>R1</i>	<i>R2</i>	<i>R3</i>	<i>R4</i>	<i>R5</i>		
4	8	A4	A7	A5	A6	A1	8.8 ± 0.4	801 ± 11
5	6	A4	A7	A5	A6	A1	5.2 ± 0.1	960 ± 10
6	10	A4	A7	A5	A6	A1	18.7 ± 0.3	3700 ± 50
7	8	A8	A7	A5	A6	A9	3.6 ± 0.1	370 ± 2
8	8	A4	A7	A5	A4	A1	2.0 ± 0.1	215 ± 4
9	8	A4	A7	A5	A6	A2	7.4 ± 0.3	720 ± 20
10	8	A4	A3	A4	A4	A1	0.87 ± 0.01	135 ± 5

^[a] IC₅₀ and K_i values against HIV-1 PR from triplicate measurements.