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Evaluation of triazolamers as active site inhibitors of HIV-1 protease

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ABSTRACT

Proteases typically recognize their peptide substrates in extended conformations. General approaches for designing protease inhibitors often consist of peptidomimetics that feature this conformation. Herein we discuss a combination of computational and experimental studies to evaluate the potential of triazole-linked β-strand mimetics as inhibitors of HIV-1 protease activity.

The conformational and chemical instabilities as well as poor pharmacological properties of peptides often limit their potential as reagents in molecular biology and drug discovery. Biomimetic oligomers that display protein-like functionality and adopt defined conformations have proven to be invaluable alternatives to peptides.[1–8] We previously reported a new class of nonpeptidic oligomers, termed triazolamers, in which the peptide bond is replaced with 1,2,3-triazole rings but the chiral main-chain and amino acid side chains are preserved (Fig. 1a).[9] We hypothesized that these oligomers may access a restricted set of conformations due to the dipole–dipole interaction between neighboring triazole rings. We have described NMR studies which indicate that short triazolamers adopt zigzag conformations reminiscent of β-strands (Fig. 1b).[9] Optimized solution and solid phase syntheses of triazolamers, which involve iterative diazotransfer and Huisgen 1,3-dipolar cycloaddition steps, have also been reported.[10]

Proteases typically recognize and cleave peptides in extended conformations and, in general, four to five residues provide a majority of binding interactions.[11] To establish the potential of triazolamers as functional β-strand mimetics,[4,5,8,12] we evaluated their ability to target HIV-1 protease (HIVPR) as a model. We began our studies by comparing triazolamers in zigzag configuration with conformations adopted by several known inhibitors of HIVPR. HIVPR is a C_2-symmetrical homodimer and offers four hydrophobic pockets at the interface.[13] Typical inhibitors of HIVPR feature bulky residues to target these pockets. We designed triazolamers that mimic active conformations adopted by prototypical HIVPR inhibitors L-700,417[14] and A-74704[15] (Fig. 2a). Both of these tetrapeptide derivatives target the protease with low nanomolar inhibition constants and have been crystallized in complex with HIV-1, which makes them useful templates for the design of triazolamers as HIVPR inhibitors. Modeling studies suggested that triazolamers **1**–**2** would provide ideal starting points for our studies. Figure 2b–d shows that triazolamer **1** superimposes well onto the conformation of L-700,417 in its protein-bound state. Two key differences between triazolamers and conventional

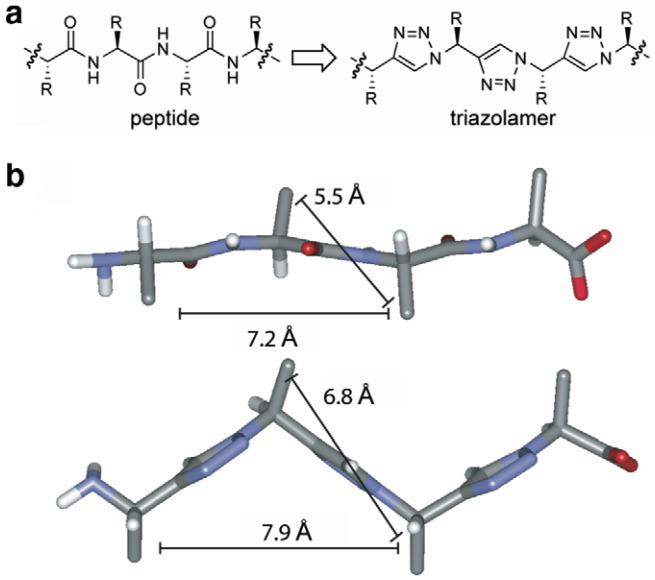

Figure 1. (a) Triazolamers feature a 1,2,3-trizole ring in place of the amide bond. R = amino acid side chain. (b) The triazolamer mimics a peptide β-strand with similar axial distances between the *i* and *i* + 2 side chains. For clarity, side chains are depicted as methyl groups.

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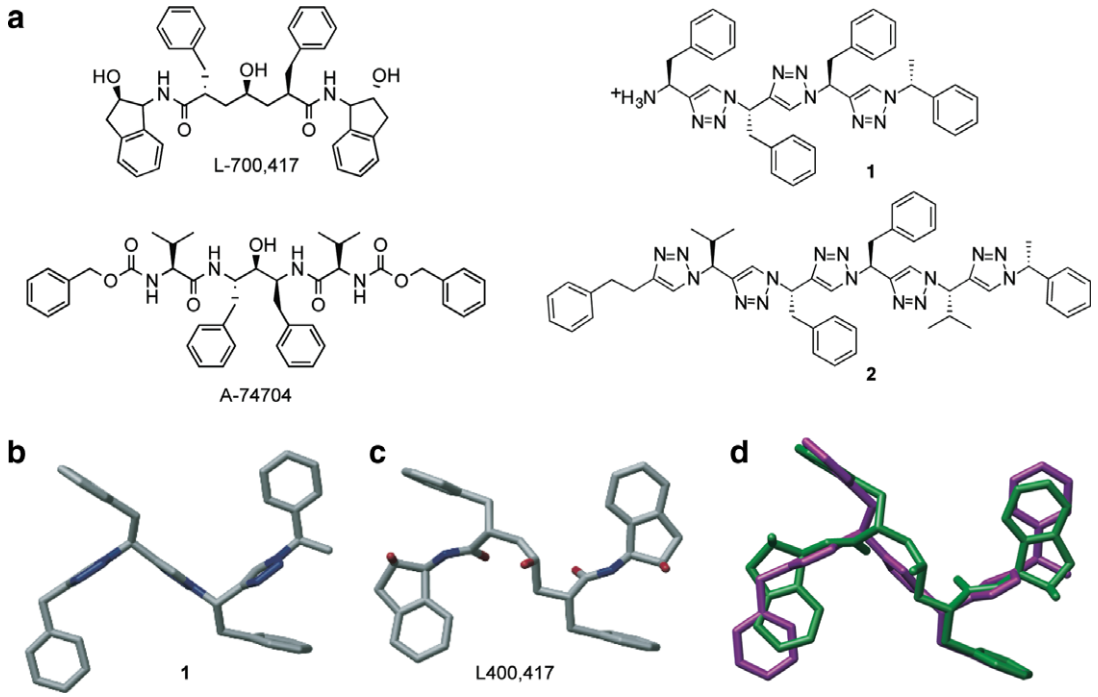


Figure 2. (a) Chemical structures of known HIV-1 inhibitors and their triazolamer mimics. (b and c) Triazolamer **1** and structure of L400,417 in complex with HIV-1 protease (PDB Code: 4phv), and d) overlay of L400,417 (green) and triazolamer **1** (magenta).

peptidomimetic HIVPR inhibitors are (i) the lack of a transition state analog unit, typically a secondary alcohol group, in the backbone of the triazolamers, and (ii) the hydrogen bonding (amide) groups. The triazolamer backbone does not offer a β-strand's hydrogen bond functionality, although the N-2 and N-3 electron pairs may serve as hydrogen bond acceptors.[13] Therefore, the ability of triazolamers to target HIVPR would allow us to gauge the relative importance of the transition state analog units and hydrogen bonding networks versus hydrophobic contacts in a system that provides a preorganized scaffold for binding.

We synthesized and tested twenty triazolamer analogs starting with **1** and **2**; the key results are shown in Table 1. Compounds were synthesized following reported procedures.[10] We utilized previously described FRET-based assays to determine the efficacy of these triazolamers as HIV-1 protease inhibitors.[16] These experiments allow us to develop and test hypotheses about the interaction of these oligomers with biological receptors. The key results from this pilot study reveal suggestive patterns regarding the specificity of triazolamers for the target enzyme. Our best compounds **3** and **8** inhibit the enzyme activity with K_i values of roughly 25 μM. The worst compounds **9**–**10** inhibit with K_i values of over 500 μM. These preliminary results imply a correlation between the number of triazole units in oligomers (**9** vs **6**) and inhibitory activity. Oligomers composed of four triazole linkages (and five side chain groups) are the best leads in this small library. (Uncharged triazolamers are hydrophobic, but incorporation of one amino group allows us to prepare solutions of triazolamers in 10% DMSO in aqueous buffers for the protease assays.) Comparison of **3** with **6** and **7** reveals that replacement of the benzyl group in **3** (as the side chain of second N-terminal residue in these pseudo-symmetric compounds) with isopropyl groups has a detrimental effect on the inhibitory activity of the compounds. To explore the possible reasons for this effect, we utilized AutoDock3 and modeled these compounds in the enzyme cavity (Fig. 3).[17]

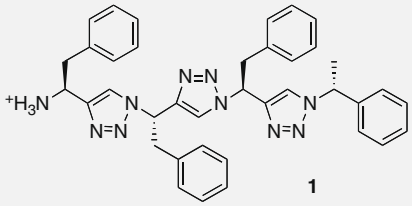
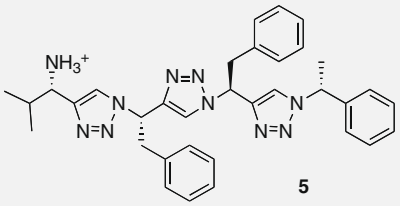
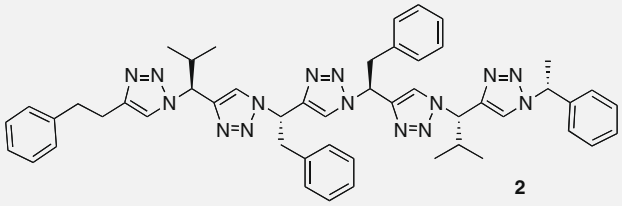
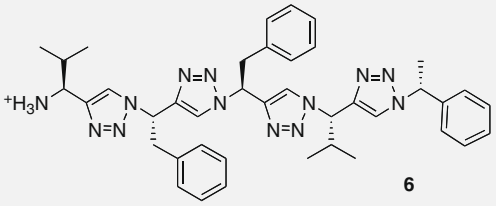
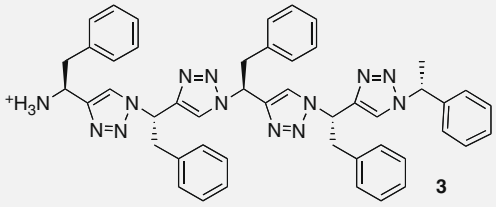
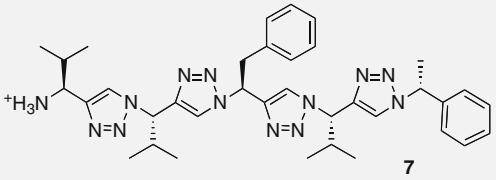
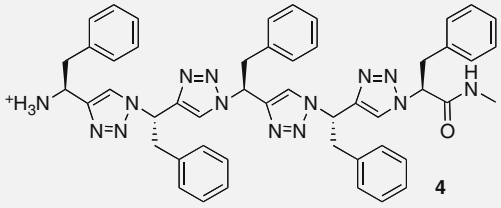
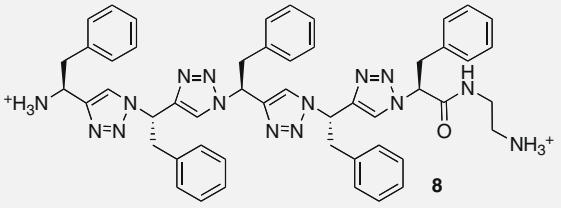
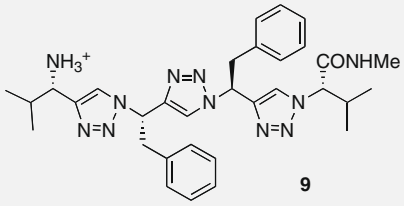
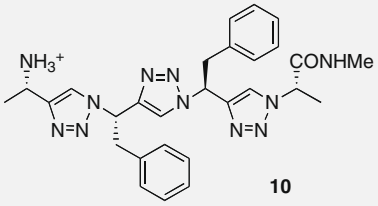
The A-74704/HIV-1 structure[15] (PDB code 9HVP) was used as a starting point for the AutoDock calculations: the protein and ligands were treated using the united-atom approximation, and prepared using the molecular modeling program InsightII.[18] Only polar hydrogens were added to the protein, and Kollman united-atom partial charges were assigned using AutoDock Tools.[19] Atomic solvation parameters were assigned to the protein atoms using the AddSol utility in AutoDock3. The grid maps were calculated using AutoGrid 3.0. In all cases grid maps contained $61 \times 61 \times 61$ points with spacing of 0.375 Å. The partial charges of the ligands were calculated using the Gasteiger–Marsili method.[20] Rotatable bonds in the ligand were defined by the AutoTors utility. Ten dockings with the Lamarkian Genetic Algorithm search method were performed for triazolamer compounds **3**, **6**, and **7**.

The HIV-1 dimer has a C-2 symmetry; according to our AutoDock model, the R^2 and R^3 residues of the triazolamer compound make contacts with the symmetrical hydrophobic pockets on the individual units of HIV-1 protease (Fig. 3). The computational studies reveal that the benzyl group resides close to a hydrophobic pocket composed of Leu23, Ile50, Val82, and Ile-84, corresponding to the S_1 and S_1' binding pockets of HIV-1 protease.[15] The docking results are consistent with previous studies that show bulky hydrophobic groups of the ligand occupying the S_1 or S_1' pockets.[21] Table 2 lists the correlation between the AutoDock results and experimental IC_{50} values for triazolamers **3**, **6**, and **7**. We find that the potency of triazolamers can be predicted by the distance between the R_2 or R_3 side chain and Ile-84 of the protease.

The docking studies do not predict interactions between the nitrogens on the triazole rings and side chain or backbone atoms in the protease. Given the importance of hydrogen bonding networks in protease targeting, we expect that addition of hydrogen bond donor groups to the triazole rings will enhance the inhibitory activity of these compounds. Studies that explore such modified triazolamers are currently underway.

In conclusion, these studies suggest that nonpeptidic β-strand mimetics, termed triazolamers, offer attractive starting points for the rational design of protease inhibitors. Docking studies were used to gain insights into binding mode of the basic scaffold and

Table 1
Structures of selected triazolamers and their 50% inhibitory concentrations (IC_{50}) against HIV-1 protease[a]

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[b] (μM)
 **1**	78 ± 7	 **5**	129 ± 15
 **2**	101 ± 12	 **6**	60 ± 7
 **3**	29 ± 9	 **7**	131 ± 19
 **4**	72 ± 18	 **8**	25 ± 7
 **9**	>500	 **10**	>500

[a] Values obtained from a FRET-based assay. Please see the Supplementary data for details.
[b] Values are means of three experiments.

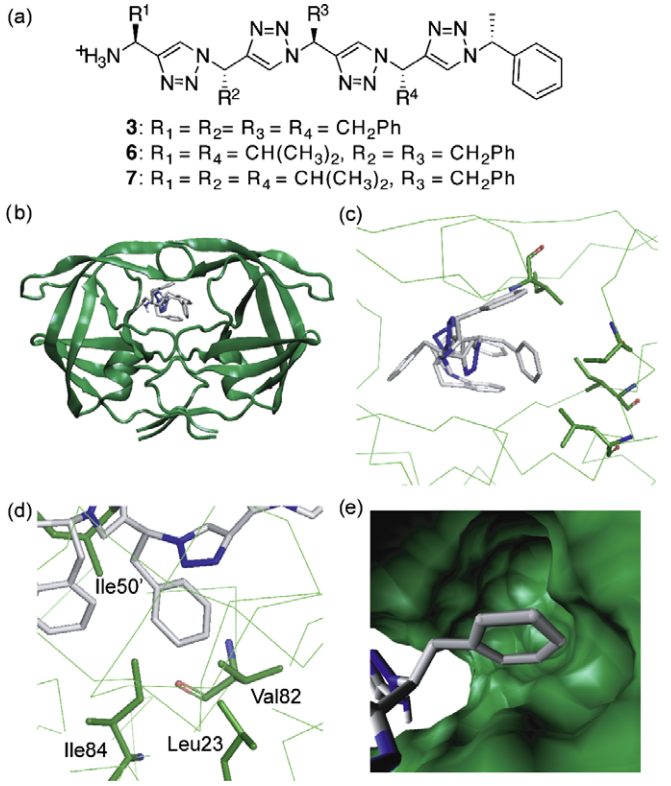


Figure 3. (a) Chemical structure of triazolamers **3**, **6** and **7** with the residue positions numbered, (b) full view of compound **3** docked into active site (PDB code 9HVP), (c) front view of the residues of HIV-1 protease composing the hydrophobic cavity around R^2 and R^3 of compound **3**, (d) side view of residues of HIV-1 protease composing the hydrophobic cavity around R^2 and R^3 of compound **3**, and (e) view of the hydrophobic cavity.

Table 2
Correlation of AutoDock calculations and experimental results

Triazolamer	Closest enzyme residue	Distance[a] (Å)	IC_{50} (μM)
7	Ile-84	5.50	131
6	Ile-84	3.49	60
3	Ile-84	3.19	29

[a] Distance between side chains of residues R_2 and R_3 on the triazolamers and Ile-84 of the enzyme.

derivatives. This analysis provides a persuasive explanation for the differential activities of triazolamers and offers a platform for continuing studies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.049.

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