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Beta-lactam compounds as apparently uncompetitive inhibitors of HIV-1 protease

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Abstract—Compounds of a combinatorial monocyclic beta-lactam library were found to be apparently uncompetitive inhibitors of HIV-1 protease, providing lead compounds for a new class of HIV protease inhibitors.

The pivotal role of the protease of human retroviruses in virus replication has served as a rationale to design protease inhibitors as chemotherapeutic agents to block acquired immunodeficiency syndrome (AIDS).[1] The HIV-1 PR is a homodimeric aspartic protease. In the absence of ligand it is almost perfectly symmetrical with two highly flexible flap regions, which are closed down on the bound inhibitors as well as substrates (Fig. 1). The HIV protease inhibitors currently used in therapy are peptidomimetic compounds, which bind to the substrate-binding site of the enzyme,[2] as indicated in Figure 1. Unfortunately, viral variants that are cross-resistant to the existing PR inhibitors are continuously emerging, indicating that there is a need for new, more potent peptidomimetic inhibitors[3] or for designing drugs targeting alternative sites in the protease.

Beta-lactam compounds have been found to be promising inhibitors of various proteases. Monocyclic beta-lactams were reported to be inhibitors of human tryptase,[4,5] and HCMV protease.[6] Previously we have found that cephalosporin oligopeptides are able to inhibit HIV-1 PR.[7] To search for other beta-lactam lead compounds as inhibitors of HIV-1 PR, we have applied a colorimetric microtiter plate method[8] to screen a 126-member combinatorial monocyclic beta-lactam library[9] (Fig. 2) for inhibition of the enzyme. Using this high-throughput screening method several of the inhibitors provided greater than 60% inhibition (Table 1). Inhibition with three compounds (shown in bold italics in Table 1) was also verified by an HPLC method under similar assay conditions.[10] Using this assay, the K_i values for the compounds were determined as shown in Table 2. Surprisingly, compound **I/13f** appeared to be substantially less potent when assayed with the

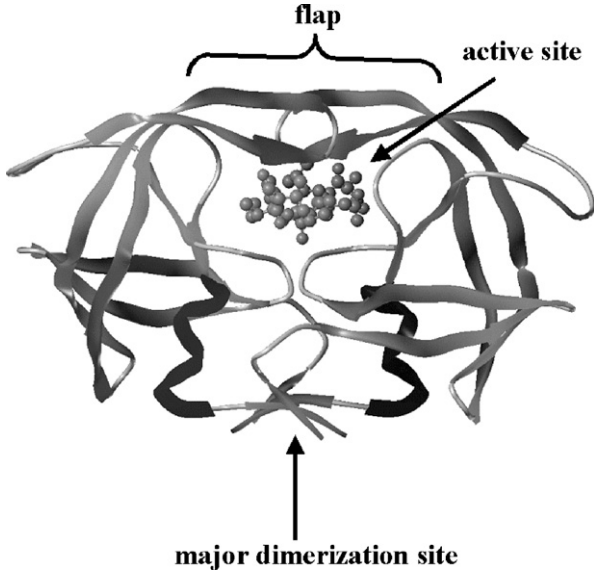

Figure 1. Schematic diagram of the HIV-1 protease with the features that could be targeted in drug design.

Keywords: HIV protease; Beta-lactam library; Enzyme kinetics; Inhibition.
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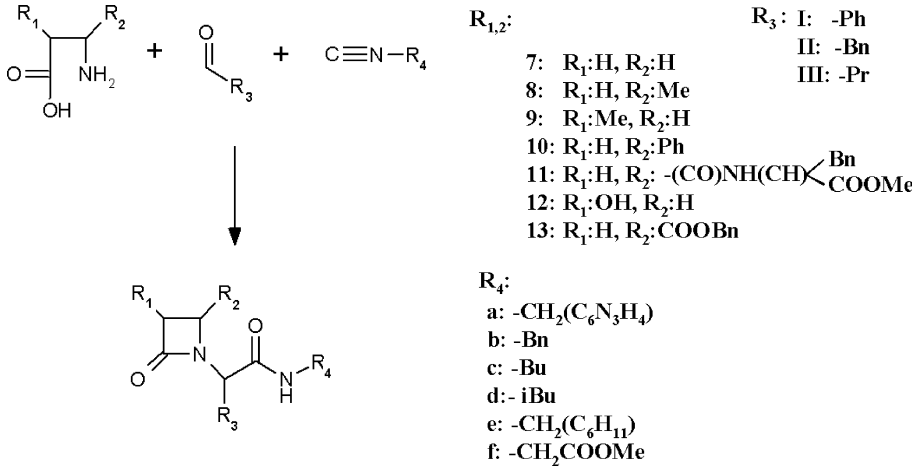

Figure 2. Schematic description of the beta-lactam peptide library used in this study. Seven β-amino acids (**7**–**13**), six isocyanides (**a**–**f**), and three aldehydes (**I**–**III**) were combined as described in Ref. 9. Final products are characterized by the individual set of R_3, $R_{1,2}$, and R_4 as indicated in the assignment of the compounds (e.g., **I/11a**, see Table 1).

Table 1. Inhibition of HIV-1 protease by members of a combinatorial β-lactam library observed by using a microtiter plate method

Compound	Inh. %	Compound	Inh. %	Compound	Inh. %	Compound	Inh. %
II/8a[a]	62	**III/10f**	68	**II/10c**	74	**I/9b**	86
I/10c	63	**I/7a**	68	**III/7a**	74	**I/11c**	86
I/9f	63	**II/11f**	69	**I/10b**	75	**III/13f**	87
II/7b	65	**I/7f**	70	**II/9e**	76	**I/13e**	92
II/7d	65	**I/13c**	71	**I/9a**	76	**III/10c**	93
II/12e	66	**II/8e**	71	**I/10a**	78	***I/13f***	***97***
II/13a	66	**I/11d**	71	**II/9c**	78	***I/13a***	***97***
II/13f	67	**III/11a**	71	**I/7e**	79	***I/11a***	***97***
I/8b	67	**II/9a**	71	**III/13a**	83	**III/11f**	(103)
II/11d	68	**II/8d**	72	**I/8e**	83	**I/13b**	(119)

[a] Structure of the compounds has been described in Figure 2 and in Ref. 9. Measurements were performed as described in Ref. 8 using a photometric determination of cleavage product of substrate Acetyl-RKIL↓FLDG. Inhibitors shown in bold italics were further studied. Last two compounds gave artificial values (higher than 100% inhibition) therefore these were not studied further.

Table 2. Inhibition of HIV-1 protease by members of a combinatorial β-lactam library

Compound	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}s^{-1}$)	[I] (μM)	K_I (μM)	Type of inhibition
None (DMSO only)	1.678	0.250	0.149	—	—	
I/13a	0.832	0.126	0.151	60	60.3	Uncompetitive
I/11a	0.645	0.100	0.155	80	63.2	Uncompetitive
I/13f	0.920	0.144	0.157	1000	1140	Uncompetitive

Measurements were performed as described in Ref. 10 using HPLC detection of cleavage products of substrate IRKIL↓FLDG. The K_I values were determined from the IC_{50} values measured at the indicated inhibitor concentration by using the following equation: $K_I = (IC_{50} \times [S])/(K_m + [S])$.

HPLC method as compared to compounds **I/13a** and **I/11a**. Since the HPLC method is a very reliable system, this discrepancy suggests a limitation for the colorimetric screening method used. Interestingly, the type of inhibition was found to be apparently uncompetitive as illustrated in Figure 3. These assays were performed under low ionic strength conditions. At high ionic strength optimal for the HIV protease[11] a similar level of inhibition was observed at much greater protease activities, but the type of inhibition appeared to be mixed (data not shown). Since in uncompetitive inhibition the compound reacts only with the ligand-bound enzyme, taking into account the mechanism of HIV-1 protease, which involves the closing of the flap region of the substrates, we envisioned that the inhibitor might interact with the closed flap region of the enzyme–substrate complex, as schematically represented in Figure 4A. A molecular model of this interaction[12] is provided in Figure 4B. The molecular model of the compound–flap interaction was tested by a 100 ps molecular dynamics simulation. Analysis of the last 80 ps (where the system seemed stable) showed that two H-bonds were formed and remained stable between the flaps of the enzyme and the β-lactam compound. One H-bond was formed to the carbonyl oxygen of Gly51 (averaged distance 3.18 ± 0.25 Å), while the other was directed to the nitrogen atom of Phe53′ (averaged distance 2.81 ± 0.20 Å). Both monomers were involved in these

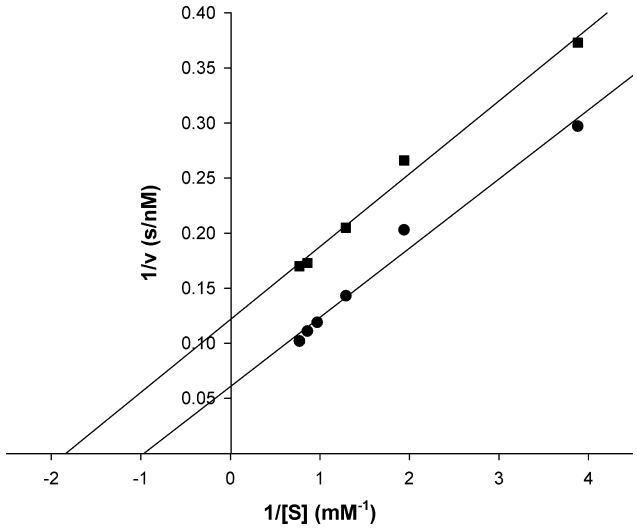

Figure 3. Uncompetitive inhibition observed with compound **I/11a**. Measurements were performed with DMSO alone (–●–) or in the presence of 80 μM inhibitor (–■–) as described in Ref. 10, using HPLC detection of cleavage products of substrate IRKIL↓FLDG.

anchoring interactions and they could only form when the flaps were closed down on the substrate. The model agreed well with the result of the enzyme kinetic measurements that suggested an uncompetitive nature of the inhibition, where the enzyme–substrate–inhibitor ternary complex was typically formed. The interaction may be increased by Van der Waals interactions between aromatic rings of the β-lactam compound and Phe53′, Pro79, and Pro81 at one side of the enzyme, and with Phe53, Pro79′, and Pro81′ at the other half of the enzyme.

Change in the type of inhibition at higher ionic strength may be due to the more favorable binding of these compounds to the active site of the enzyme through hydrophobic contact with the appropriate side chains, as also demonstrated previously for peptidomimetic inhibitors, which bind much more strongly toward the active site of the enzyme in high ionic strength.[13]

In further support that the targeted region is the closed-down flap, we studied the inhibition of compound **I/13a**

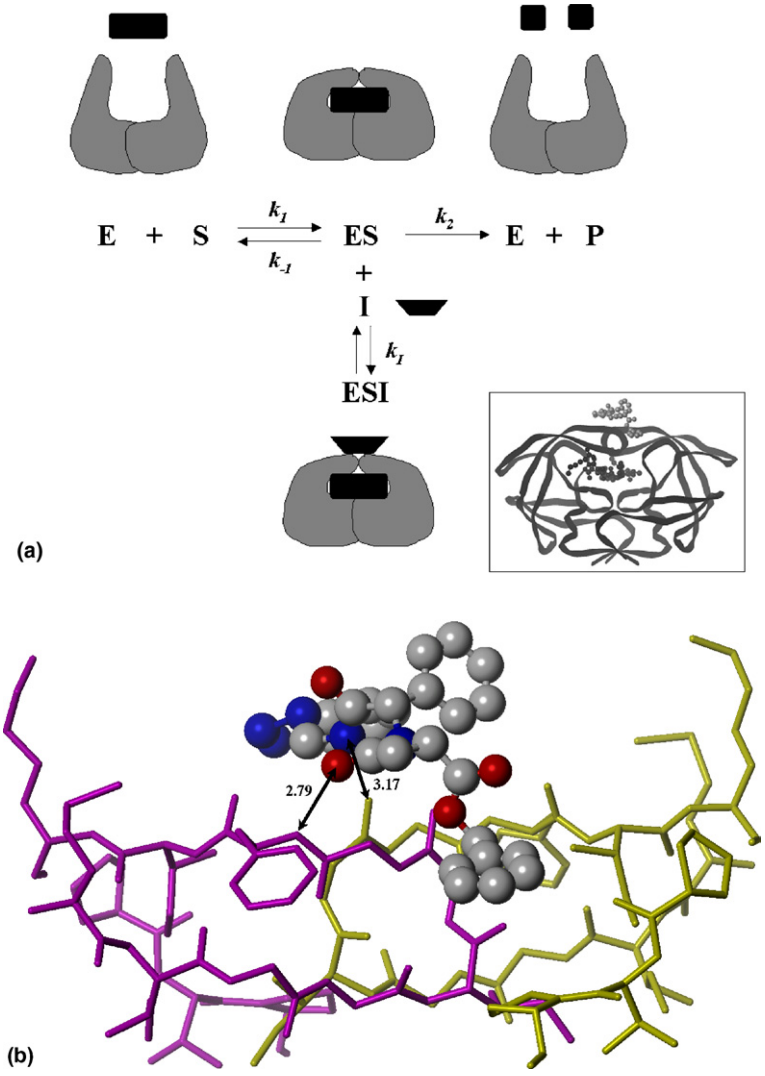

Figure 4. A. Proposed mechanism of action of the beta-lactam compounds. Insert shows the ribbon model of the ESI complex. B: molecular model of ligand–flap interaction after a short molecular dynamics simulation. Flap of the two subunits are shown in yellow and magenta, while the inhibitor is shown in a ball-and-stick representation.

on single-chain HIV-1 protease, which forms a significantly more stable dimer as compared to the wild type enzyme,[14] as well as its catalytically substantially weaker mutant, (Des-50-52)-[Asn48], which contained a truncated, modified flap in one subunit.[15] While the single-chain HIV-1 protease was substantially inhibited by the compound, the flap mutant was not sensitive (data not shown). Nevertheless, verification of the flap region as the actual binding site of these compounds requires structural studies, such as the determination of the crystal structure of the enzyme–inhibitor complexes.

To test whether these compounds could serve as lead compounds for broad-spectrum retroviral PR inhibitors, we have tested **I/13a** as an inhibitor of murine leukemia virus protease,[16] an enzyme only distantly related to HIV-1, which is not sensitive towards most of the peptidomimetic inhibitors currently used in AIDS therapy.[17] The protease was inhibited by the compound, with a fourfold higher IC_{50} value as found for HIV-1 PR, although due to the high K_m values, the type of inhibition could not be determined (data not shown).

In this study we have utilized a multicomponent chemistry strategy to identify lead compounds as inhibitors of HIV-1 protease. A similar approach was recently applied to find nonpeptidic inhibitors of this enzyme.[17] A major problem in anti-AIDS therapy is the development of resistance, which continuously requires the development of more potent peptidomimetic inhibitors or designing drugs targeting alternative sites in the protease. Besides the active-site directed inhibitors, attempts were made to target the dimerization of the protease (Fig. 1) by either using chemically synthesized compounds,[18,19] or by expressing trans-dominant negative protease subunits.[20] The flap region provides a third, so far unexplored region of the enzyme. The only inhibitory compound that has so far been shown to bind to the flap region is a tight-binding monoclonal antibody capable of binding to the exposed loop structures at the N-terminal end of the flap.[21]

To our knowledge, these are the first cases of apparently uncompetitive inhibitors of HIV-1 protease, providing lead compounds for a new class of protease inhibitors, which may complement in combination therapy the currently used active site-targeted drugs.

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Sybyl using Tripos force field and partial charges of Gasteiger–Marsili method implemented in Sybyl. Five hundreds Powell iterations were applied and only the β-lactam compound was allowed to move. Next, the complex was gradually heated up to 300 K by short molecular dynamics runs and finally a 100 ps molecular dynamics was applied, where only the β-lactam compound was allowed to move.

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