1,2,3-Triazole as a Peptide Surrogate in the Rapid Synthesis of HIV-1 Protease Inhibitors


Given the ubiquitous nature of the peptide linkage in biological molecules, replacement of the amide bond with isosteres in potential drug candidates has been a continual goal of many laboratories. Successful replacements will provide improved stability, lipophilicity, and absorption. Many surrogates have been introduced already,[1] yet the synthesis of many of these isosteres in a combinatorial way is difficult and requires several steps. Thus, the discovery of new peptide surrogates with easier syntheses is an important achievement that could open new opportunities for the study of amide-containing molecules and the development of inhibitors with novel physicochemical properties.

We have used the copper(i)-catalyzed azide–alkyne [3+2] cycloaddition,[2] as a straightforward reaction for the preparation of inhibitor libraries. Over 100 compounds were synthesized in microtiter plates and screened in situ. Two of these compounds (AB2 and AB3) showed the best activity against wild type and mutant HIV-1 proteases, ok? (Table 1).[3] AB2 and AB3, were then computationally docked by using AutoDock3.[4] The docking simulation produced two conformations of approximately equal energy. One conformation placed the triazole in the position normally adopted by the peptide unit—between P2’ and P1’—in peptidomimetic compounds. Furthermore, the central nitrogen of the triazole was perfectly positioned to form a hydrogen bond with the water molecule normally found under the protease flaps. This water molecule also formed a hydrogen bond with the sulfonamide as seen in the crystallographic structure of amprenavir when bound to HIV-1 protease.[5] The other conformation positioned the compounds, ok? in a similar place, but with the triazole rotated by 180°. This allowed for a slightly better fit of the triazole substituent but sacrificed the hydrogen bond with the water molecule. In this work we have solved the ambiguity in binding conformation by solving the crystal structure of two inhibitors derived from a library of triazole compounds with HIV-1 protease, ok? Interestingly, the two structures show that the triazole ring is an effective amide surrogate that retains all hydrogen bonds in the active site (Figure 1).

![Triazole Structure](image)

**Table 1.** Binding constants of 1,2,3-triazole compounds to HIV-1 protease.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Compound AB2</th>
<th>Compound AB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>V82F</td>
<td>6 ± 0.5</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>G48V</td>
<td>19 ± 1</td>
<td>n.d.</td>
</tr>
<tr>
<td>V82A</td>
<td>39 ± 1</td>
<td>n.d.</td>
</tr>
<tr>
<td>V82A</td>
<td>46 ± 1</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

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at the Argonne National Laboratory beamline at a BIO-CARS 14-BM-C station? / XBC station?, please specify, and with a rotating copper anode Rigaku generator on a Mar345 image plate detector. A minimum of 100 frames of 1° oscillation were collected for each data set and processed by using commercial HKL2000 software. The structure was solved by using molecular replacement with protein monomer coordinates from a previous structure. It was then refined by using the SHELXL programs and rebuilt with the molecular graphics program, O, with several rounds of manual model building and automated refinement. The final statistics for each structure are listed in Table 2.

In both structures, the inhibitors are bound in a position identical to that of amprenavir. The large dipole of the triazole (≤ 5 Debye), which bisects the ring plane near atoms N3 and C5, and the capacity of the N2 and N3 electron lone pairs to serve as hydrogen acceptors, taken together, make the triazole an excellent mimic of the peptide group. In the crystallographic structures, N2 takes the position of the carbonyl oxygen, and C5 takes the place of the amide nitrogen (Figure 2). A hydrogen bond is formed from the structural water molecule to this nitrogen, thus locking the inhibitors in place in the active site (Table 3).

In summary, we have demonstrated that the 1,2,3-triazole is an effective replacement for a peptide group in HIV-1 protease inhibitors. This has been illustrated with the combinatorial modification of amprenavir by using azide–alkyne click chemistry followed by inhibition and structural analysis. Work is in progress to modify existing drugs by replacing the amide bond with a 1,2,3-triazol moiety and evaluating the effect of amide replacement on the structure activity of the compound.

**Acknowledgements**

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### Table 2. Statistical data for AB2 and AB3 crystal structures.

<table>
<thead>
<tr>
<th>HIV protease with</th>
<th>AB2</th>
<th>AB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P6(1)22</td>
<td>P6(1)22</td>
</tr>
<tr>
<td>Data resolution [Å]</td>
<td>50–2.02</td>
<td>50–2.02</td>
</tr>
<tr>
<td>Unit cell parameters [Å]</td>
<td>63.1/63.1/82.1</td>
<td>63.1/63.1/82.5</td>
</tr>
<tr>
<td>Data completeness (%)</td>
<td>95.9</td>
<td>98.3</td>
</tr>
<tr>
<td>R-sym (%)</td>
<td>5.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Structure resolution [Å]</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>R-factor (%)</td>
<td>19.5</td>
<td>22.0</td>
</tr>
</tbody>
</table>

### Table 3. Hydrogen bond lengths [Å].

<table>
<thead>
<tr>
<th>HIV-1 protease, AB2, AB3, amprenavir [a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOM 301 to:</td>
</tr>
<tr>
<td>Gly27 O to:</td>
</tr>
</tbody>
</table>

[a] Distances for amprenavir were taken from PDB entry 1hpv.
for molecular replacement and for assistance with X-ray data collection and refinement.

Keywords: click chemistry · inhibitors · peptidomimetics · triazole


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**COMMUNICATIONS**

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1,2,3-Triazole as a Peptide Surrogate in the Rapid Synthesis of HIV-1 Protease Inhibitors

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Substitute for another bond. In this paper we present 1,2,3-triazole as a pepti-de bond surrogate in HIV-1 protease inhibitors. Docking simulations of two potent inhibitors that bear the triazole moiety, in each case produced two conformations of approximately equal energy. Further analysis of the protease by X-ray crystallography solved the ambiguity of the binding mode and revealed that the triazole ring is an effective amide surrogate and retains all the hydrogen bonds in the active site (see figure).