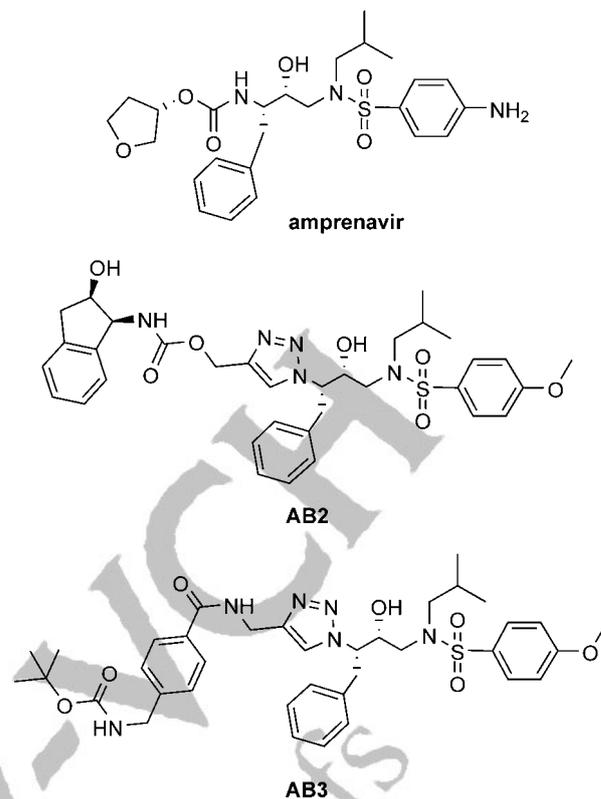


1,2,3-Triazole as a Peptide Surrogate in the Rapid Synthesis of HIV-1 Protease Inhibitors

Ashraf Brik,^[a] Jerry Alexandratos,^[b] Ying-Chuan Lin,^[c] John H. Elder,^[c] Arthur J. Olson,^[c] Alexander Wlodawer,^{*,[b]} David S. Goodsell,^{*,[c]} and Chi-Huey Wong^{*,[a]}

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 Auto-



Dock3.^[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). please provide PDB numbers for structures

HIV-1 protease (3 mg mL⁻¹ in 0.025 M sodium acetate pH 5.4, 10 mM dithiothreitol, 1 mM EDTA) was combined with inhibitor (32 μM in 50% (v/v) dimethylsulfoxide and 2-methylpentane-2,4-diol) at 4°C to give a 2:1 molar ratio of inhibitor to protein, and the mixture was centrifuged to remove the precipitate. The complex was crystallized by the hanging-drop vapor-diffusion method by using protease solution (9.6 μL) over, ok? crystallization buffer (4 μL; 1.34 M ammonium sulfate, 0.1 M sodium acetate, pH 4.8–5.4). Plates were sealed at 20°C for one to two weeks. Data were collected from frozen crystals

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

Enzyme	Compound AB2		Compound AB3	
	IC ₅₀ [nM]	K _i [nM]	IC ₅₀ [nM]	K _i [nM]
wt	6 ± 0.5	1.7 ± 0.1	13 ± 0.5	4 ± 0.5
V82F	19 ± 1	n.d.	n.d.	n.d.
G48V	39 ± 1	n.d.	n.d.	n.d.
V82A	46 ± 1	n.d.	n.d.	n.d.

n.d. = not determined. ok?

[a] Dr. A. Brik, Prof. C.-H. Wong
Department of Chemistry and the Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2409 ok?
E-mail: wong@scripps.edu

[b] Dr. J. Alexandratos, Dr. A. Wlodawer
Macromolecular Structure Laboratory, ABL-Basic Research Program
NCI-Frederick Cancer Research and Development Center
Frederick, MD ZIP? (USA)
Fax: (+1) ok?
E-mail: wlodawer@ncifcrf.gov

[c] Dr. Y.-C. Lin, Prof. J. H. Elder, Prof. A. J. Olson, Prof. D. S. Goodsell
Department of Molecular Biology, The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) ok?
E-mail: goodsell@goliath.scripps.edu

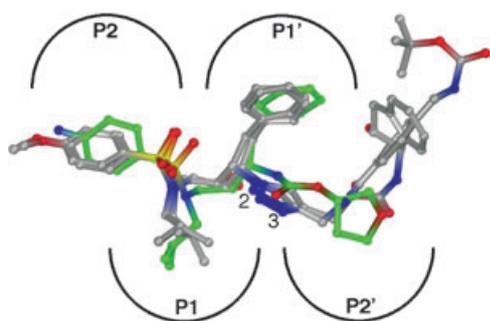


Figure 1. Crystal structure of 1,2,3-triazole compounds. The protein chains of AB2 and AB3 were overlapped with those of the complex with amprenavir, PDB entry 1hvp. The three inhibitor structures are shown. In amprenavir, the carbon atoms are green. Nitrogen atoms 2 and 3 in the triazoles are labeled, and the approximate locations of the protease subsites P2 to P2' are shown.

at the Argonne National Laboratory beamline $\blacksquare\blacksquare$ at a BIO-CARS 14-BM-C station? / X8C station?, please specify $\blacksquare\blacksquare$, 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.^[6] The structure was solved by using molecular replacement with protein monomer coordinates from a previous structure.^[7] It was then refined by using the SHELXL programs^[8] and rebuilt $\blacksquare\blacksquare$ with the molecular graphics program, O,^[9] ok? $\blacksquare\blacksquare$ with several rounds of manual model building and automated refinement. The final statistics for each structure are listed in Table 2.

HIV protease with	AB2	AB3
space group	<i>P</i> 6(1)22	<i>P</i> 6(1)22
data resolution [Å]	50–2.02	50–2.02
unit cell parameters [Å]	63.1/63.1/82.1	63.1/63.1/82.5
data completeness [%]	95.9	98.3
<i>R</i> -sym [%]	5.4	6.8
structure resolution [Å]	2.0	2.0
<i>R</i> -factor [%]	19.6	22.0

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 $\blacksquare\blacksquare$ that is positioned under the flaps, ok? $\blacksquare\blacksquare$ to this nitrogen, thus locking the inhibitors in place in the active site (Table 3). Similar hydrogen bonds to the N2 of 1,2,3-triazole were observed in the structures of the acetylcholinesterase inhibitor,^[10] and in the recent structure of a triazole-modified α -helical coiled coil.^[11] This latter structure also showed a CH...O hydrogen bond from the triazole hydrogen at the 5 position to a

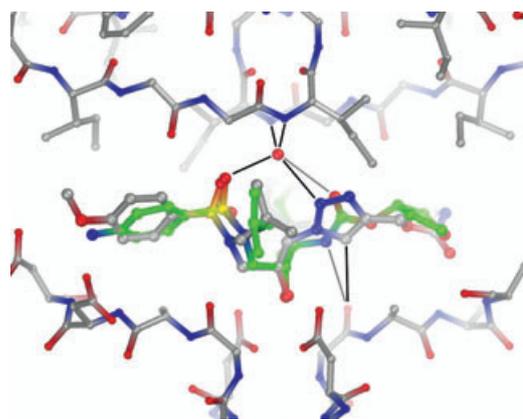


Figure 2. Detail of hydrogen-bonding interactions in AB2. A cross section through the active site is shown, with the protease flaps at the top and the two active site aspartates at the bottom. The inhibitor runs horizontally through the center. The position of amprenavir is also shown, with carbon atoms in green. Key hydrogen bonds to the structural water molecule and to the main chain of Gly27 are shown with black lines.

$\blacksquare\blacksquare$ HIV-1 protease, ok? $\blacksquare\blacksquare$	AB2	AB3	amprenavir ^[a]
HOH 301 to:	triazole N2 = 2.892	triazole N2 = 2.395	peptide O = 3.021
Gly27 O to:	triazole C5 = 3.816	triazole C5 = 3.810	peptide N = 3.580

[a] Distances for amprenavir were taken from PDB entry 1hvp.

neighboring carbonyl in the modified α -helix. In the HIV-1 protease structures, the hydrogen at the C5 position is pointed directly at the peptide oxygen of Gly27, at a distance of 3.8 Å in both structures, and therefore forms a similar CH...O hydrogen bond.

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

We thank the National Institutes of Health for financial support. Part of diffraction data used in this study were collected at the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID, located at the Advanced Photon Source, Argonne National Laboratory. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38. We are grateful to Mi Li for providing HIV protease coordinates

for molecular replacement and for assistance with X-ray data collection and refinement.

Keywords: click chemistry · inhibitors · peptidomimetics · triazole

- [1] For reviews on the subject, see: a) A. F. Spatola, *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, Marcel Dekker, New York, **1983**, 267–357; b) J.-M. Ahn, N. A. Boyle, M. T. MacDonald, K. D. Janda, *Mini-Rev. Med. Chem.* **2002**, *2*, 463–473.
- [2] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.
- [3] A. Brik, J. Muldoon, Y.-C. Lin, J. H. Elder, D. S. Goodsell, A. J. Olson, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *ChemBioChem* **2003**, *4*, 1246.
- [4] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998**, *19*, 1639–1662.
- [5] E. E. Kim, C. T. Baker, M. D. Dwyer, M. A. Murcko, B. G. Rao, R. D. Tung, M. A. Navia, *J. Am. Chem. Soc.* **1995**, *117*, 1181.
- [6] Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307–326.
- [7] M. Li, G. M. Morris, T. Lee, G. S. Laco, C. H. Wong, A. J. Olson, J. H. Elder, A. Wlodawer, A. Gustchina, *Proteins* **2000**, *38*, 29–40.
- [8] G. M. Sheldrick, SHELXS97 and SHELXL97. **1997**, University of Göttingen, Germany. <http://shelx.uni-ac.gwdg.de/SHELX/index.html>
- [9] T. A. Jones, M. Kjeldgaard, *Methods Enzymol.* **1997**, *277*, 173–208.
- [10] Y. Bourne, H. C. Kolb, Z. Radić, K. B. Sharpless, P. Taylor, P. Marchot, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 1449–1454.
- [11] W. S. Horne, M. K. Yadav, C. D. Stout, M. R. Ghadiri, *J. Am. Chem. Soc.* **2004**, *126*, 15366–15367.

Received: March 14, 2005

Published online on ■ ■ ■ ■, 2005



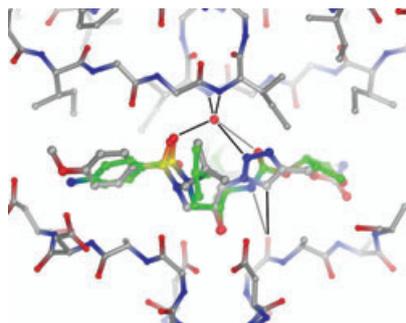
WILEY-VCH
Galley Proofs

COMMUNICATIONS

A. Brik, J. Alexandratos, Y.-C. Lin,
J. H. Elder, A. J. Olson, A. Wlodawer,*
D. S. Goodsell,* C.-H. Wong*



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



Substitute for another bond. In this paper we present 1,2,3-triazole as a peptide 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).



WILEY-VCH
Galley Proofs