A Check on Rational Drug Design: Crystal Structure of a Complex of Human Immunodeficiency Virus Type 1 Protease with a Novel γ-Turn Mimetic Inhibitor†

Susan S. Hoog,‡ Baoguang Zhao,‡ Evon Winborne,‡ Seth Fisher,§ David W. Green,§,▽ Renee L. DesJarlais,∥ Kenneth A. Newlander,⊥ James F. Callahan,⊥ Michael L. Moore,⊥ William F. Huffman,⊥ and Sherin S. Abdel-Meguid*,‡

Departments of Macromolecular Sciences, Protein Biochemistry, Physical and Structural Chemistry, and Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406

Received March 23, 1995®

We have previously reported (Newlander et al., *J. Med. Chem.* **1993**, *36*, 2321-2331) the design of human immunodeficiency virus type 1 (HIV-1) protease inhibitors incorporating C_7 mimetics that lock three amino acid residues of a peptide sequence into a γ-turn. The design of one such compound, SB203238, was based on X-ray structures of reduced amide aspartyl protease inhibitors. It incorporates a γ-turn mimetic in the P_2-P_1' position, where the carbonyl of the C_7 ring is replaced with an sp^3 methylene group yielding a constrained reduced amide. It shows competitive inhibition with $K_i = 430$ nM at pH 6.0. The three-dimensional structure of SB203238 bound to the active site of HIV-1 protease has been determined at 2.3 Å resolution by X-ray diffraction and refined to a crystallographic R-factor ($R = \sum|F_o| - |F_c|/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively) of 0.177. The inhibitor lies in an extended conformation in the active site; however, because of the constrained geometry of the C_7 ring, it maintains fewer hydrogen bonds with the protein than in most other HIV-1 protease-inhibitor complexes. More importantly, the inhibitor binds to the enzyme differently than predicted in its design, by binding with the P_2-P_1' α-carbon atoms shifted by approximately one-half a residue toward the N-terminus from their presumed positions. This study illustrates the importance of structural information in an approach to rational drug design.

Introduction

Traditionally, the discovery of drugs has focused on screening for lead compounds in nature and synthetic chemical libraries. Although screening continues to be a powerful tool in drug discovery, knowledge-based or "rational" approaches have gained substantial acceptance, either to complement screening or for use independently in the design of novel therapeutic molecules, particularly enzyme inhibitors.[1] In the design of enzyme inhibitors, the success of the rational approach is greatly accelerated by knowledge of the target enzyme's three-dimensional structure, preferably in the liganded state. This knowledge is not only useful for the design of an initial lead molecule, but can also help in lead optimization through iterative cycles of structure determination and chemical synthesis. Previously, we reported the design and synthesis of novel inhibitors of human immunodeficiency virus type 1 (HIV-1) protease incorporating γ-turn mimetics.[2] As a step toward the design of better such inhibitors, we report herein the determination and analysis of the crystal structure of one of these inhibitors bound to HIV-1 protease.

HIV protease is a 99-amino acid protein whose function is vital for proper assembly and maturation of HIV,[3] the causative factor of acquired immunedeficiency syndrome, AIDS.[4] This protease processes the *gag* and *gag-pol* polyproteins into mature structural proteins and the enzymes needed for viral replication: reverse transcriptase, integrase, and the protease itself. The critical role of the protease makes it an important therapeutic target, where inhibition of the enzyme by synthetic inhibitors during infection *in vitro* has been shown to reduce the amount of infectious viral particles produced.[5] HIV protease is active as a homodimer and is classified as a member of the aspartyl protease family on the basis of its kinetic profile[6] and active-site sequence similarity[7,8] and structural homology[9] to the well-characterized, monomeric enzymes renin and pepsin.

Since the initial characterization of this enzyme, rapid progress has been made toward the development of highly potent peptide analog inhibitors in which the scissile amide bond of the substrate is replaced by a nonhydrolyzable isostere with tetrahedral geometry to mimic the putative transition state of the aspartic protease-catalyzed reaction. These would include statine, hydroxyethylene, difluoroketomethylene, phosphinates, hydroxyethylamine, and reduced amide isosteres and have been reviewed elsewhere.[10,11] Additionally, pseudosymmetric and C_2 symmetric inhibitors have been designed to take advantage of the C_2 symmetric nature of this protease.[12] Recently, C_2 symmetric nonpeptide cyclic ureas have been developed in an attempt to mimic the structural water molecule anchoring the flaps and provide a positive entropic effect with the

† The refined coordinates for the protease-inhibitor complex have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratories, Upton, Long Island, NY 11973, under the file name 1HBV.

‡ Department of Macromolecular Sciences.

§ Department of Protein Biochemistry.

∥ Department of Physical and Structural Chemistry.

⊥ Department of Medicinal Chemistry.

▽ Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000.

® Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3246$09.00/0

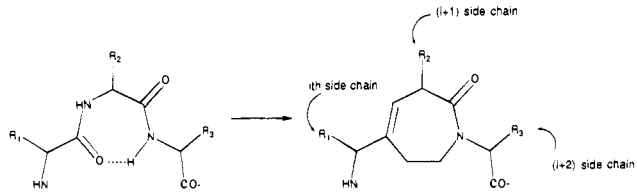
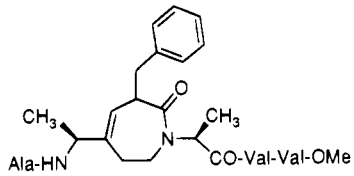


Figure 1. γ-Turn mimetic.



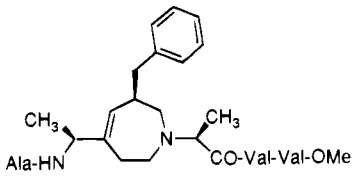


Figure 2. Compounds **III** and **VI**, numbered according to nomenclature of Newlander et al.[2]

conversion of a flexible, linear inhibitor into a rigid cyclic structure with restricted conformations.[13] Conformational restrictions have also been attained through incorporating constrained macrocyclic ring structures into peptide-based inhibitors in an effort to gain an entropic advantage and enhance stabilization toward proteolytic enzymes and increase target enzyme specificity.[14]

Conformational constraints can also be enacted through secondary structure mimetics that force linear peptide sequences into various defined turn conformations.[15] Specifically, a C_7 mimetic has been designed that locks three amino acid residues of a peptide sequence into a γ-turn conformation (Figure 1).[16] In this respect, while the peptide backbone of aspartyl protease inhibitors in general and HIV protease inhibitors in particular is extended, there is a pronounced kink at the scissile bond mimetic that is suggestive of a backbone conformation resembling a γ-turn.[17] This is especially true for reduced amide bond inhibitors, an observation not surprising since reduced amide bonds have been shown to be comparable with γ-turns in both small molecule X-ray crystal studies[18] and theoretical calculations.[19] With this in mind, Newlander et al.[2] prepared compounds **III** and **VI** (SB203238) incorporating a γ-turn mimetic between the P_2 and P_1' positions (Figure 2). Compound **III** was found to be a competitive inhibitor with a K_i of 147 μM. Reduction of the amide bond in the C_7 mimetic resulted in the novel constrained reduced amide mimetic **VI** with a K_i of 430 nM. Additionally, this was also found to be 44-fold better than a similar linear reduced-amide containing inhibitor.[20] These kinetic data suggested that the C_7 mimetic fits the active site and may be acting as a conformational mimetic. In order to explore this possibility, we embarked on determination of the crystal structure of HIV-1 protease complexed with compound **VI** and determined that it does not bind to the active site as previously predicted.

Table 1. Data Collection and Processing Statistics for the HIV-1–**VI** Complex

space group	$P6_1$
cell dimensions	a = 63.4 Å
	b = 63.4 Å
	c = 83.9 Å
	α = 90.0°
	β = 90.0°
	γ =120.0°
molecules/asymmetric unit	1 dimer plus inhibitor
resolution limit	2.3 Å
no. of measured reflections	36 970
no. of unique reflections	8051
data completeness	
∞–2.3 Å	94%
R_{sym}[a]	9.5%

[a] $R_{sym} = \Sigma\Sigma|I_{i(h)} - \langle I_{(h)}\rangle|/\Sigma I_{i(h)}$, where $I_{i(h)}$ is the intensities of multiple measurements of reflection h and $\langle I_{(h)}\rangle$ is their mean.

Results and Discussion

Crystallographic Refinement of the Structure. The details of data collection and reduction are summarized in Table 1. Preliminary phases for the complex were derived from the isomorphous A74704 inhibitor–HIV-1 protease complex.[21] Using the least squares refinement program PROLSQ,[22] a few cycles of rigid-body refinement using data from 10.0 to 3.0 Å resolution for reflections with $|F_o| > 3\sigma|F_o|$ (where F_o is the observed structure factor amplitude) were initially done to obtain the optimal position and orientation of the model. This was followed by several cycles of least squares refinement where data with reflections $|F_o| > 2\sigma|F_o|$ were added in 0.2 Å increments to a final resolution of 2.3 Å, bringing the residual to 25.2%. At this point, Fourier maps with coefficients $|F_o - F_c|$ and $|2F_o - F_c|$ (where F_c is the calculated structure factor amplitude) were calculated and displayed on a Silicon Graphics Onyx workstation. The electron density of the protease dimer was uniformly clear; however, that for the inhibitor was somewhat discontinuous.

A model of **VI** was positioned in the electron density, using the graphics program CHAIN.[23] The starting model was an energy-minimized structure obtained with the program MacroModel (version 4.5) using the AMBER force field,[24] in which the nitrogen atom in the 7-membered ring was protonated. Model adjustment to conventional $|F_o - F_c|$, $|2F_o - F_c|$, and $|3F_o - 2F_c|$ maps failed to resolve ambiguity in the placement of the inhibitor. However, this uncertainty was considerably lessened by the calculation of simulated annealing-refined omit maps,[25] using the program X-PLOR,[26] in which the inhibitor plus a 2 Å radius around it were omitted from the structure factor calculation. The atoms in a 3 Å radius around the omitted region were harmonically restrained in order to prevent them from moving into the omitted region. Subsequent maps calculated using this procedure allowed a clear delineation of the inhibitor conformation. Additional simulated annealing-refined omit maps were calculated. They allowed the identification of residues with large deviations from ideal geometry and permitted straightforward adjustment of these residues. Water molecules were included in the refinement if they were at 3σ above the average density and eliminated if the B-factor rose above 30 Å^2. A summary of the details of refinement is outlined in Table 2.

Description of the Overall Structure. The protein portion of the HIV-1 protease–**VI** complex is nearly

Table 2. Refinement Statistics for the HIV-1–**VI** Complex

resolution range	10–2.3 Å
no. of reflections $\|F\| > 2\sigma\|F\|$	6831
no. of protein atoms	1525
no. of inhibitor atoms	42
no. of solvent atoms	33
R-factor[a]	17.7%
rms deviations from ideal geometry	
bond lengths	0.018 Å
bond angles	3.545°

[a] R-Factor = $\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

identical with that of previously reported HIV-1 protease–inhibitor complexes. Superposition of α-carbon atoms from this protease dimer structure with those from other complexes gave root mean square deviations ca. 0.5 Å. The electron density (Figure 3) shows clear delineation of the inhibitor, particularly the benzyl side chain extending off the 7-membered ring. Originally, compound **VI** was a separable mixture of diastereomers where the chirality around the i+1 carbon (Figure 1) was unknown.[2] Examination of the electron density shows this benzyl ring substituent to be in an S conformation at that position. The electron density also reveals some disorder around the γ-turn mimetic, where precise placement of the part of the ring opposite the benzyl substituent was not possible (Figure 3). This disorder is consistent with the results obtained from the Monte Carlo[24] simulation performed on the simplified 7-membered ring of the mimetic. The simulation predicted seven low-energy ring conformations within 5 kcal/mol of the global minimum (Figure 4), of which the one with the lowest energy corresponded to the conformation of the 7-membered ring bound to the enzyme.

Binding of the Inhibitor. Compound **III** was designed on the basis of examination of crystal structures of complexed reduced amide aspartyl protease inhibitors[27] and, in particular, the HIV-1 protease inhibitor MVT-101 (N-acetyl-Thr-Ile-Nle-Ψ[CH_2-NH]-Nle-Gln-Arg·amide),[28] where it was observed that these compounds were binding to the aspartyl proteases in a C_7 conformation, as can be seen in Figure 5. As mentioned above, compound **III** showed competitive inhibition (K_i = 147 μM) and improved binding relative to the linear substrate analog, leading to the hypothesis that it was binding as a conformational mimetic.[2] On this basis, the carbonyl of the C_7 mimetic was replaced with an sp^3 methylene group, affording the constrained reduced amide **VI**. This compound was meant to both stabilize the binding to the protease active site and provide an sp^3 center that might engage the catalytic aspartyl groups, thus improving the affinity of the inhibitor. This was done in analogy to work with linear inhibitors where reducing the amide bond, and therefore incorporating the tetrahedral transition-state geometry found during amide bond hydrolysis, has long been recognized as an efficient method in the development of protease inhibitors.[29] Compound **VI** was subsequently shown to be a better inhibitor of HIV-1 protease with a K_i of 430 nM, providing a 300-fold improvement in inhibitory potency over **III**, thus lending validity to the transition-state hypothesis. However, the conformation of the inhibitor in the crystal structure indicates otherwise.

Figure 6 shows the binding of compound **VI** to the active site of HIV-1 protease. Like other peptide analog inhibitors of HIV-1 protease, it is in an extended conformation in the active site. The similarity ends there, however, in that our inhibitor engages in few hydrogen bonds with the protein and solvent, mainly because of the unusual conformation of the inhibitor backbone caused by the γ-turn mimetic ring. Only four inhibitor heteroatoms engage in hydrogen bonds with the protein either directly or through the centrally located water molecule, found in all HIV-1 protease–inhibitor complexes. Unlike other HIV protease–inhibitor complexes, however, this water molecule is not tetrahedrally coordinated, but instead it maintains only one of the two hydrogen bonds with the inhibitor, that at the carbonyl oxygen of Val_5^{VI}. This result is not surprising since the mimetic replaces the P_2–P_1 amide bond with a trans double bond. In many HIV-1 protease inhibitors, it is the P_2 carbonyl group that makes an important conserved hydrogen bond to the central water molecule, which in turn is hydrogen bonded to Ile50 in the flap, forming part of the hydrogen bond network that holds the flap down over the inhibitor.

The compound **VI**–HIV-1 protease structure also shows that there is only one hydrogen bond with a catalytic aspartate residue, where the carbonyl oxygen of Ala_4^{VI} is engaged in a hydrogen bond (3.3 Å) with the carboxylic acid moiety of Asp25. Notably, this sp^2 carbonyl oxygen is not positioned such that acid hydrolysis could take place. Because of the geometry imposed by the γ-turn mimetic, there is only one hydrogen bond between the inhibitor and the protease along P_3 to P_1. This is in sharp contrast to the numerous hydrogen bond interactions at these sites between HIV-1 protease and the linear reduced amide inhibitor MVT-101, where there are six hydrogen bonds extending from the main chain to the protein. It is, also, noteworthy that the nitrogen atom of the 7-membered ring makes no interactions with the protein. A schematic representation with distances of potential hydrogen bonds between compound **VI** and residues of the active site cleft is shown in Figure 7.

Despite the constrained geometry, the inhibitor does engage in favorable van der Waals contacts with hydrophobic residues in the binding site of the enzyme. Geometry of the C_7 ring allows the side chain of Ala_4^{VI} to engage in favorable interactions with Ile84′, and the inhibitor is positioned such that Val_5^{VI} and Val_6^{VI} engage in favorable hydrophobic contacts at the S_1' and S_2' enzyme-binding sites. However, comparison with MVT-101 (Figure 8) shows that although compound **VI** has a similar extended backbone conformation, the geometry imposed by the C_7 ring permits fewer favorable hydrophobic interactions in the S_3–S_1 pockets. Specifically, positions of the β-carbon atoms of Ala_2^{VI} and Phe_3^{VI} are different by ca. 1 and 1.5 Å from those of the Ile and Nle residues in MVT-101, respectively. Also, the benzyl ring of Phe_3^{VI} is extending toward the solvent by 2.5 Å as compared to Nle in the P_1 position and thus avoids additional van der Waals contacts with Ile84 and Ile50′.

Most important, however, Figure 8 shows the reduced amide within the C_7 ring to be far from the reduced amide in MVT-101. It is not, as had been predicted,[2] positioned between the catalytic aspartate residues as if mimicking a transition state intermediate and is instead 1.6 Å from the reduced amide of MVT-101.

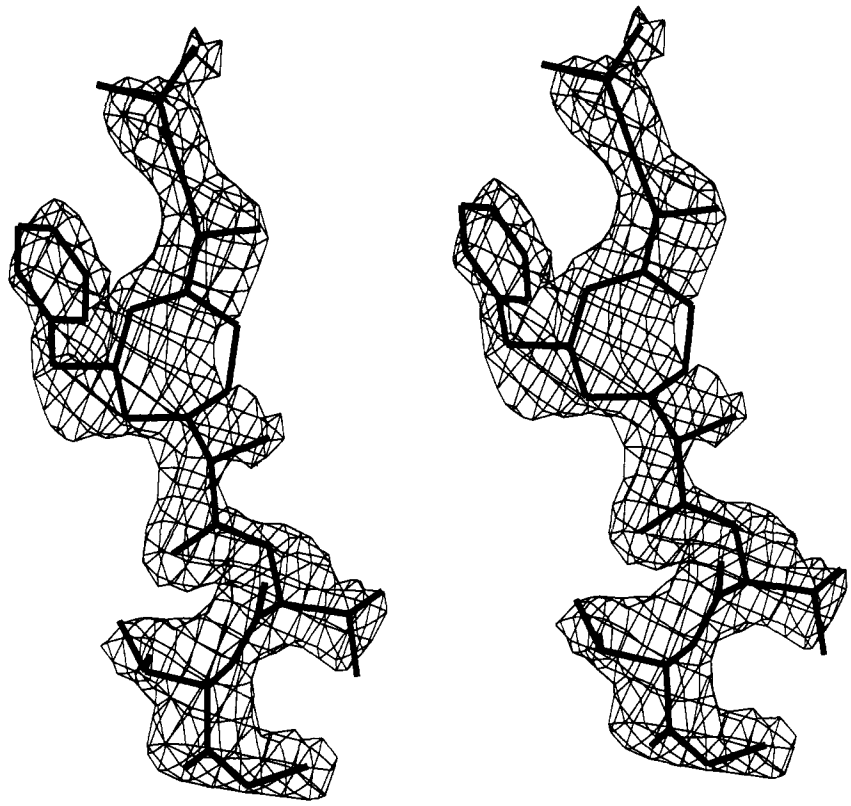


Figure 3. Stereoview showing the fit of **VI** to the final $2|F_o| - |F_c|$ electron density map, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

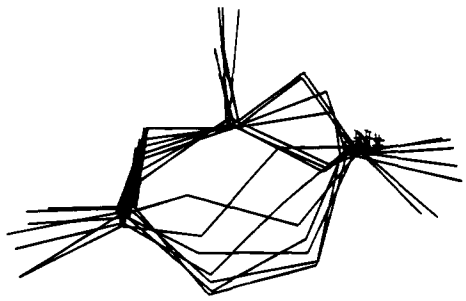


Figure 4. Overlay of the seven low-energy conformations of the simplified mimetic.

Structural Implications of Inhibitory Potencies of γ-Turn Mimetics. In spite of the constrained geometry imposed by the C_7 ring, compound **VI** does maintain enough electrostatic and hydrophobic contacts to provide inhibitory activity that compares quite favorably with the micromolar K_i's for most published reduced amide inhibitors.[17] Favorable enzyme–inhibitor contacts in the protein binding subsites are maximized mainly at the C-terminal end of the inhibitor at Val_5^{VI} and Val_6^{VI} further from the C_7 ring, where three of the four hydrogen bonds to the enzyme are found.

In view of the unexpected binding mode of compound **VI** in the HIV-1 protease active site, it would be unwise to speculate on the structural implications of the 300-fold increase in inhibitory potency of **VI** over compound **III**, since they may not bind similarly in the active site. However, if we assume that the two compounds bind to the active site in a similar manner, then the C_7 amide carbonyl will be within 1 Å of both the Asp25 δ_1 oxygen and the Gly27′ carbonyl oxygen. Consequently, the repulsion between these atoms would cause a conformational change within the ring, thereby preventing most of the favorable contacts seen between compound **VI** and the protease in the present structure. Clearly, only a crystal structure of compound **III** bound to HIV-1 protease would allow determination of the exact nature of the interaction.

Conclusions

The rationale in the design of the reduced amide C_7 mimetic **VI** was based upon crystal structures of complexed linear reduced amide inhibitors and supported by the kinetic inhibition data, leading to the hypothesis that the inhibitor was binding to the protease active site as a conformational mimetic. However, the structure determination of the **VI**–HIV-1 complex illustrated that the C_7 γ-turn mimetic inhibitor is not bound in the predicted transition-state conformation and instead appears to be binding in a distorted conformation in which the P_2 and P_1 α-carbon atoms are shifted by approximately one-half a residue toward the N-terminus relative to the equivalent residues in the MVT-101 structure. As a consequence, the inhibitor was unable to take full advantage of many possible backbone interactions with the protein.

In summary, this study illustrates the importance of structure determination of enzyme–inhibitor complexes in order to understand the structure–activity relationship within a series of compounds as part of the rational design approach.

Experimental Section

Enzyme Preparation. Recombinant HIV-1 protease was expressed in *Escherichia coli* and subsequently purified to apparent homogeneity as previously described.[30,31] The synthesis of the inhibitor **VI** has also been previously described.[2] The protease–**VI** complex was prepared by adding a 3-fold

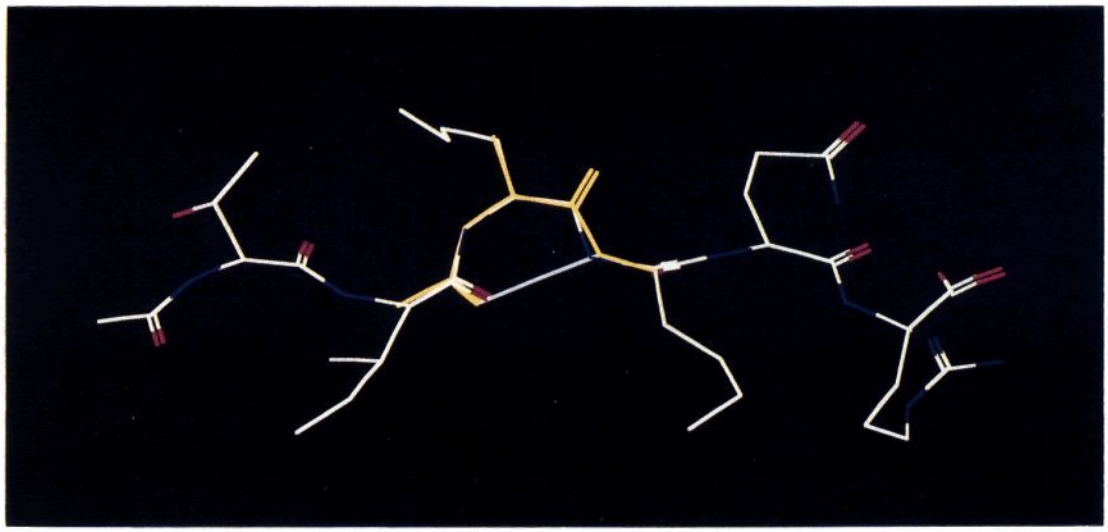


Figure 5. Overlay of the X-ray structure of the reduced amide inhibitor MVT-101 with an idealized C_7 turn, shown in yellow, with hydrogens removed for clarity. The location of the hydrogen bond in the C_7 structure is shown in light blue. [Reprinted with permission from: Moore, M. L.; Dreyer, G. B. *Prespect. Drug Dis. Des.* **1993**, *1*, 85. Copyright 1993 by ESCOM Science Publishers B.V., Leiden, The Netherlands.]

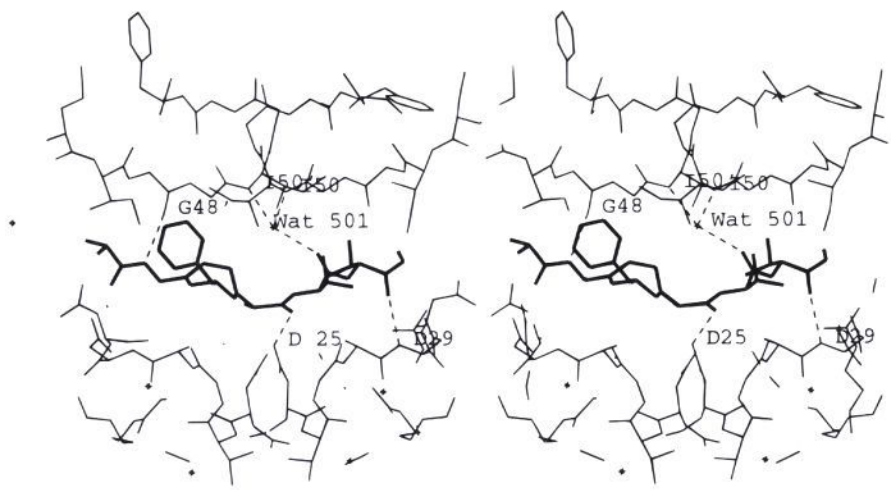


Figure 6. Binding of **VI** to the active site of HIV-1 protease. The inhibitor is shown in thick lines, and hydrogen bonds are dashed. The centrally located water, Wat501, is engaged in a single hydrogen bond with the inhibitor. Additionally, the carbonyl of the P_1' Ala residue is hydrogen bonded to a catalytic aspartate residue; however, the skewed geometry does not favor hydrolysis.

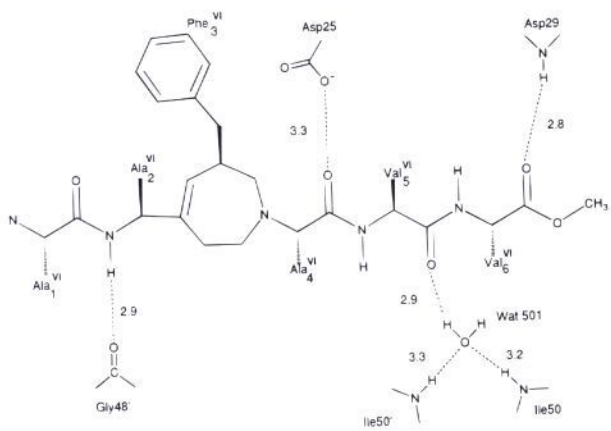


Figure 7. Schematic representation of potential hydrogen bond interactions between **VI**, HIV-1 protease, and solvent. Distances are given in Ångstroms.

molar excess of inhibitor per protease dimer and concentrated to 2.0 mg/mL for crystallography in a buffer containing 50 mM sodium acetate, pH 5.0, 2 mM EDTA, 5 mM DTT, 5% DMSO, and 150 mM NaCl. The complex was stored for no longer than 1 week before crystallization experiments were set up.

Crystallization. Crystals were grown by vapor diffusion in hanging drops.[32] Briefly, 1 mL of precipitant solution containing 16–28% ammonium sulfate and 200 mM sodium acetate, pH 5.4–5.6, was equilibrated with 3 μL of protein solution and 3 μL of precipitant at room temperature. Crystals appeared in 2 days and grew to full size over 1 week. The crystals were mounted into glass capillaries and used for data measurement.

Data Collection. Diffraction data were collected on a Siemens four-circle area detector mounted on a Siemens X-ray generator operating at 50 kV and 96 mA and reduced using the XENGEN software.[33] The diffraction pattern revealed symmetry consistent with the hexagonal space group $P6_122$ or $P6_1$ with strong noncrystallographic 22 symmetry reflecting the 2-fold symmetry of the protein. Space group $P6_1$ was chosen to be consistent with previously published reports of HIV-1 protease complexes.[34,21] The unit cell dimensions are $a = b = 63.4$ Å and $c = 83.9$ Å, with the asymmetric unit containing one complete copy of the complex (a protein dimer plus an inhibitor). Approximately 37 000 reflections were

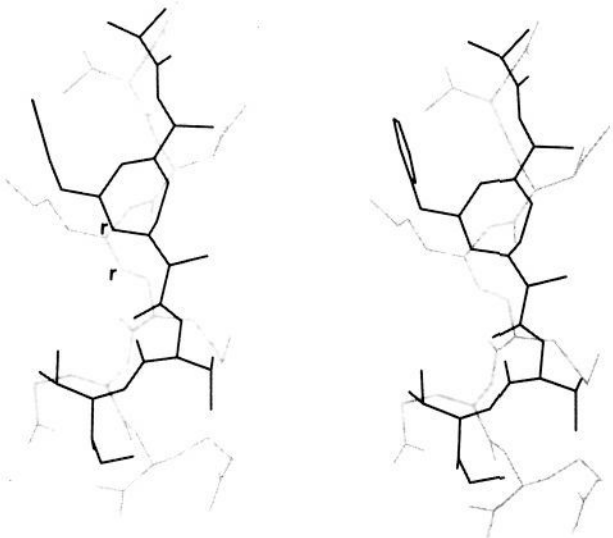


Figure 8. Stereoviews of superposition of compound **VI** (dark lines) with MVT-101, where the inhibitor chains run down vertically from the N- to C- terminus. An r marks the location of the reduced amide in both structures. Superposition was achieved by aligning α-carbon atoms of the protease residues of both complexes.

collected from a single crystal over 2 days and reduced to 8051 unique reflections representing 94% of the data to 2.3 Å resolution.

Energy Minimization. The conformational search of the simplified 7-membered ring was performed using MacroModel (Version 4.5).[24] Monte Carlo simulation generated 2500 ring conformations that were minimized using the Amber force field within a water matrix. Seven conformations were obtained that were within 5 kcal/mol of the global minimum.

Acknowledgment. This work was supported by NIH Grant 1 RO1 GM50579-01.

References

(1) Reddy, M. R.; Varney, M. D.; Kalish, V.; Viswanadhan, V. N.; Appelt, K. Calculation of Relative Differences in the Binding Energies of HIV-1 Protease Inhibitors-A Thermodynamic Perturbation Approach. *J. Med. Chem.* **1994**, *37*, 1145−1152.

(2) Newlander, K. A.; Callahan, J. F.; Moore, M. L.; Tomaszek, T. A.; Huffman, W. F. A Novel Constrained Reduced-Amide Inhibitor of HIV-1 Protease Derived from the Sequential Incorporation of γ-Turn Mimetics into a Model Substrate. *J. Med. Chem.* **1993**, *36*, 2321−2331.

(3) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. Active Human Immunodeficiency Virus Protease is Required for Viral Infectivity. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686−4690.

(4) Gallo, R. C.; Montagnier, L. AIDS in 1988. *Sci. Am.* **1988**, *259*, 40−48.

(5) McQuade, T. J.; Tomasselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, F. K.; Heinrikson, R. L.; Tarpley, W. G. A Synthetic HIV-1 Protease Inhibitor with Antiviral Activity Arrests HIV-Like Particle Maturation. *Science* **1990**, *247*, 454−456.

(6) Meek, T. D.; Dayton, B. D.; Metcalf, B. W.; Dreyer, G. B.; Strickler, J. E.; Gorniak, J. G.; Rosenberg, M.; Moore, M. L.; Magaard, V. W.; Debouck, C. Human immunodeficiency virus 1 protease expressed in *Escherichia coli* behaves as a dimeric aspartic protease. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1841−1845.

(7) Toh, H.; Ono, M.; Saigo, K.; Miyata, T. Retroviral Protease-Like Sequence in the Yeast Transposon Ty1. *Nature* **1985**, *315*, 691−692.

(8) Power, M. C.; Marx, P. A.; Bryant, M. L.; Gardner, M. B.; Barr, P. J.; Luciew, P. A. Nucleotide Sequence of SRV-1; a Type-D Simian Acquired Immune Deficiency Syndrome Retrovirus. *Science* **1986**, *231*, 1567−1572.

(9) Pearl, L. H.; Taylor, W. R. A Structural Model for the Retroviral Proteases. *Nature* **1987**, *329*, 351−354.

(10) Meek, T. D. Inhibitors of HIV-1 Protease. *J. Enzyme Inhib.* **1992**, *6*, 65−98.

(11) Abdel-Meguid, S. S. Inhibitors of Aspartyl Proteinases. *Med. Res. Rev.* **1993**, *13*, 731−778.

(12) Greer, J.; Erickson, J. W.; Baldwin, J. J.; Varney, M. D. Application of the Three-Dimensional Structures of Protein Target Molecules in Structure Based Design. *J. Med. Chem.* **1994**, *37*, 1035−1054. Also: Hosur, M. V.; Bhat, T. N.; Kempf, D. J.; Baldwin, E. T.; Liu, B.; Gulnik, S.; Wideburg, N. E.; Norbeck, D. W.; Appelt, K.; Erickson, J. W. Influence of stereochemistry on activity and binding modes for C_2 symmetry-based inhibitors of HIV-1 protease. *J. Am. Chem. Soc.* **1994**, *116*, 847−855.

(13) Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. Rational Design of Potent, Bioavailable, Nonpeptide Cyclic Ureas as HIV Protease Inhibitors. *Science* **1994**, *263*, 380−384.

(14) Podlogar, B. L.; Farr, R. A.; Friedrich, D.; Tarnus, C.; Huber, E. W.; Cregge, R. F.; Schirlin, D. Design, Synthesis, and Conformational Analysis of a Novel Macrocyclic HIV-Protease Inhibitor. *J. Med. Chem.* **1994**, *37*, 3684−3692.

(15) Morgan, B. A.; Gainor, J. A. Approaches to the Discovery of Non-Peptide Ligands for Peptide Receptors and Peptidases. *Annu. Rep. Med. Chem.* **1989**, *24*, 243−252.

(16) Huffman, W. F.; Callahan, J. F.; Codd, E. E.; Eggleston, D. S.; Lemieux, C.; Newlander, K. A.; Schiller, P. W.; Takata, D. T.; Walker, R. F. Mimics of Secondary Structural Elements of Peptides and Proteins. In *Synthetic Peptides: Approaches to Biological Problems;* Tam, J., Kaiser, E. T., Eds.; A. R. Liss: New York, 1989; pp 257−266.

(17) Moore, M. L.; Dreyer, G. B. Substrate-based inhibitors of HIV-1 protease. *Perspect. Drug Dis. Des.* **1993**, *1*, 85−108.

(18) Toniolo, C.; Valle, G.; Crisma, M.; Kaltenbronn, J. S.; Repine, J. T.; vanBinst, G.; Elseviers, M.; Tourwe, D. Ψ[CH_2NH] Backbone-Modified Peptides: First Unequivocal Observation of a C_7 Structure in a Linear Peptide. *Pept. Res.* **1989**, *2*, 332−337.

(19) Dauber-Osguthorpe, P.; Jones, D. K.; Campbell, M. M.; Semple, G.; Osguthorpe, D. J. Reduced and Retro-Reduced Peptide Analogues-Conformations and Energies. *Tetrahedron Lett.* **1990**, *31*, 917−920.

(20) Dreyer, G. B.; Metcalf, B. W.; Tomaszek, T. A.; Carr, T. J.; Chandler, A. C.; Hyland, L.; Fakhoury, S. A.; Magaard, V. W.; Moore, M. L.; Strickler, J. E.; Debouck, C.; Meek, T. D. Inhibition of human immunodeficiency virus 1 protease in vitro: Rational design of substrate analogue inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9752−9756.

(21) Erickson, J.; Neidhard, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Widenurg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. Design, Activity, and 2.8 Å Crystal Structure of a C_2 Symmetric Inhibitor Complexed to HIV-1 Protease. *Science* **1990**, *249*, 527−533.

(22) Hendrickson, W. A. Stereochemically Restrained Refinement of Macromolecular Structures. *Methods Enzymol.* **1985**, *115*, 252−270.

(23) Sack, J. S. CHAIN: A Crystallographic Modeling Program. *J. Mol. Graph.* **1988**, *6*, 224−225.

(24) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel--An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−476.

(25) Hodel, A.; Kim, S.-H.; Brünger, A. T. Model Bias in Macromolecular Crystal Structures. *Acta Crystallogr.* **1992**, *A48*, 851−858.

(26) Brünger, A. T. *X-PLOR Version 3.1 Manual*; Yale University Press: New Haven, CT, 1992.

(27) Suguna, K.; Padlan, E. A.; Smith, C. W.; Carlson, W. D.; Davies, D. R. Binding of a reduced peptide inhibitor to the aspartic proteinase from *Rhizopus chinesis:* Implications for a mechanism of action. *Proc Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7009−7013.

(28) Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Kent, S. B. H.; Wlodawer, A. Structure of Complex of Synthetic HIV-1 Protease with a Substrate-Based Inhibitor at 2.3 Å Resolution. *Science* **1989**, *246*, 1149−1152.

(29) Examples of reduced amide inhibitors in HIV-1 protease and renin, respectively, include: (a) Urban, J.; Konvalinka, J.; Stehlikova, J.; Gregorova, E.; Majer, P.; Soucek, M.; Andreansky, M.; Fabry, M.; Strop, P. Reduced-bond tight-binding inhibitors of HIV-1 protease. *FEBS Lett.* **1992**, *298*, 9−13. (b) Szelke, M.; Leckie, B.; Hallet, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. Potent new inhibitors of human renin. *Nature* **1982**, *299*, 555−557.

(30) Debouck, C.; Gorniak, J. G.; Strickler, J. E.; Meek, T. D.; Metcalf, B. W.; Rosenberg, M. Human Immunodeficiency Virus Protease Expressed in Escherichia coli Exhibits Autoprocessing and Specific Maturation of the gag Precursor. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8903−8906.

(31) Grant, S. K.; Deckman, I. C.; Minnich, M. D.; Culp, J.; Franklin, S.; Dreyer, G. B.; Tomaszek, T. A., Jr.; Debouck, C.; Meek, T. D. Purification and Biochemical Characterization of Recombinant Simian Immunodeficiency Virus Protease and Comparison to Human Immunodeficiency Virus Type 1 Protease. *Biochemistry* **1991**, *30*, 8424–8434.

(32) McPherson, A. *Preparation and Analysis of Protein Crystals*, 2nd ed.; Robert E. Krieger Publishing Co., Inc.: Malabar, FL, 1989; pp 96–97.

(33) Howard, A. J.; Gilliland, G. L.; Finzel, B. C.; Puolos, T. L.; Ohlendorf, D.; H.; Salemme, F. R. The Use of an Imaging Proportional Counter in Macromolecular Crystallography. *J. Appl. Crystallogr.* **1987**, *20*, 383–387.

(34) Ambiguity in the identification of hexagonal space group in HIV-1 protease–inhibitor complexes has been previously noted, including: (a) Dreyer, G. B.; Lambert, D. M.; Meek, T. D.; Carr, T. J.; Tomaszek, T. A.; Fernandez, A. V.; Bartus, H.; Cacciavillani, E.; Hassell, A. M.; Minnich, M.; Petteway, S. R.; Metcalf, B. W.; Lewis, M. Hydroxyethylene Isostere Inhibitors of Human Immunodeficiency Virus-1 Protease: Structure-Activity Analysis Using Enzyme Kinetics, X-ray Crystallography, and Infected T-Cell Assays. *Biochemistry* **1992**, *31*, 6646–6659. (b) Dreyer, G. B.; Boehm, J. C.; Chenera, B.; DesJarlais, R. L.; Hassell, A. M.; Meek, T. D.; Tomaszek, T. A.; Lewis, M. A Symmetric Inhibitor Binds HIV-1 Protease Asymmetrically. *Biochemistry* **1993**, *32*, 937–947. (c) Abdel-Meguid, S. S.; Zhao, B.; Murthy, K. H. M.; Winborne, E.; Choi, J.-K.; DesJarlais, R. L.; Minnich, M. D.; Culp, J. S.; Debouck, C.; Tomaszek, T. A.; Meek, T. D.; Dreyer, G. B. Inhibition of Human Immunodeficiency Virus-1 Protease by a C_2 Symmetric Phosphinate. Synthesis and Crystallographic Analysis. *Biochemistry* **1993**, *32*, 7972–7980. (d) Abdel-Meguid, S. S.; Metcalf, B. W.; Carr, T. C.; Demarsh, P.; DesJarlais, R. L.; Fisher, S.; Green, D. W.; Ivanoff, L.; Lambert, D. M.; Murthy, K. H. M.; Petteway, S. R.; Pitts, W. J .; Tomaszek, T. A.; Winborne, E.; Zhao, B.; Dreyer, G. B.; Meek, T. D. An Orally Bioavailable HIV-1 Protease Inhibitor Containing an Imidazole-Derived Peptide Bond Replacement: Crystallographic and Pharmacokinetic Analysis. *Biochemistry* **1994**, *33*, 11671–11677. (e) Thompson, S. K.; Murthy, K. H. M.; Zhao, B.; Winborne, E.; Green, D. W.; Fisher, S. M.; DesJarlais, R. L.; Tomaszek, T. A.; Meek, T. D.; Gleason, J. G.; Abdel-Meguid, S. S. Rational Design, Synthesis, and Crystallographic Analysis of a Hydroxyethylene-Based HIV-1 Protease Inhibitor Containing a Heterocyclic P_1'-P_2' Amide Bond Isostere. *J. Med. Chem.* **1994**, *37*, 3100–3107.

JM950215X