Crystal Structure of HIV-1 Protease in Complex with VX-478, a Potent and Orally Bioavailable Inhibitor of the Enzyme

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HIV protease is an attractive therapeutic target for the treatment of HIV infection and AIDS, due to its obligatory role in the life cycle of the virus.[1] Of the many *in vitro* potent HIV protease inhibitors described so far,[2,3] however, only a few have advanced into clinical trials. Here, we report the structure of HIV-1 protease in complex with VX-478, a potent, low molecular weight, orally bioavailable HIV protease inhibitor currently in advanced preclinical development.[4] VX-478 emerged from a focused program of structure-based drug design[5] that sought to maintain sub-nanomolar *in vitro* potency while reducing inhibitor size, a tactic supported by our analysis of the molecular weight distribution of all marketed drugs (Figure 1). Historically, in the development of angiotensin converting enzyme inhibitors,[6] structural information was similarly used to design smaller, potent inhibitors (like captopril) to specifically resolve issues of *in vivo* efficacy.

In order to enhance inhibitory potency, we sought to optimize binding to the catalytic aspartate residues of the enzyme and to the critical water molecule that mediates inhibitor interactions with the flap.[7] In addition, we tried to minimize unfavorable strain energy by favoring compounds whose conformation would require minimal reorganization on enzyme binding. Other design goals included synthetic accessibility, high antiviral potency, low cellular toxicity, and aqueous solubility without obligate charges. These goals were met by VX-478 (Figure 2), the lead compound in a novel class of N,N-disubstituted (hydroxyethyl)amino sulfonamides. VX-478 has a molecular weight of 506 Da, and inhibits the HIV-1 and HIV-2 proteases competitively, with K_i values of 0.60 and 19 nM, respectively.[4] In addition, the inhibitor has an IC_{90} of 40 nM in CEM cells infected by HIV-IIIB virus as assayed by extracellular p24 levels.[4]

The structure of HIV-1 protease in complex with VX-478, refined at 1.9 Å resolution,[8] shows clear electron density for all atoms of the inhibitor (Figure 3), in a single extended conformation that occupies the S_2 to S_2' binding pockets[9] of the enzyme. Extensive interactions are evident, with a total of 397 Å^2 in solvent accessible surface area[10] excluded on complex formation; nearly 60% (235 Å^2) of the buried surface area is due to nonpolar atoms and can be attributed to hydrophobic interactions. Strong hydrogen bonding to the well-defined flap water molecule (Figures 3 and 4) is observed for the carbonyl oxygen of the inhibitor and one of the two sulfonyl oxygens; the second sulfonyl binds asymmetrically, being partly buried in a hydrophobic environment (Ile50 and Ile84). The central hydroxyl group of VX-478 hydrogen bonds to the carboxylate oxygens of the catalytic Asp25 and Asp25′ residues of the enzyme as shown in Figure 4. In the VX-478 complex, however, the hydroxyl oxygen of the inhibitor is 0.55 Å out of the plane of the catalytic carboxylate groups.

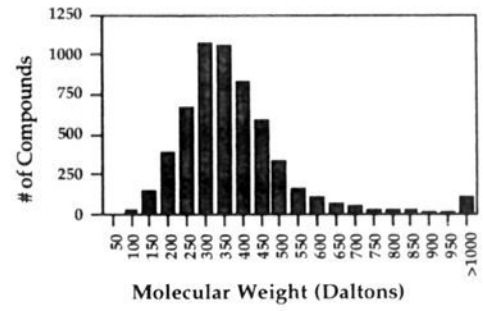

Figure 1. Molecular weight distribution of all marketed drugs administered by oral and systemic routes. The ISIS/Base program[16] was used to select 5687 entries from the CMC-3D data base, an electronic compilation of marketed drugs. The data suggests a rough molecular weight target of 600 Da, below which acceptable pharmacokinetics might be expected of a drug candidate.[11]

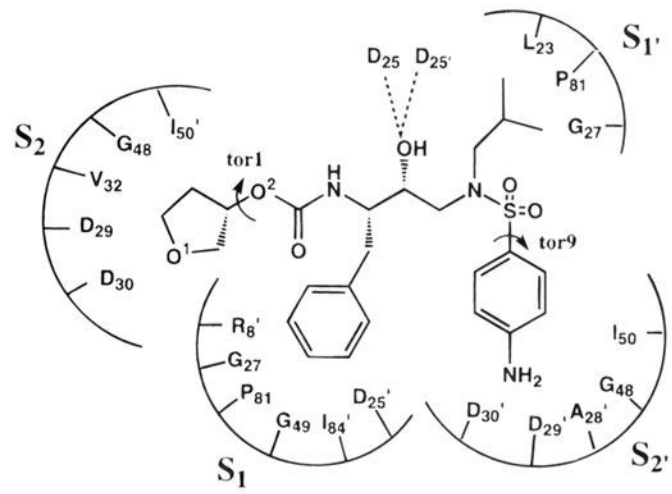

Figure 2. Chemical structure of VX-478, with binding pockets as indicated. All residues within 4.0 Å of the inhibitor are identified. Backbone torsion angles for bound VX-478 are as follows: tor1 =73°; tor2 = −161°; tor3 = −116°; tor4 = −115°; tor5 = 151°; tor6 = 55°; tor7 = 103°; tor8 = 75(75)°; tor9 = 78(91°). The tor8 and tor9 angles in parentheses are the average of the corresponding torsion angles in 10 related structures taken from the Cambridge Crystallographic Database,[12] where none of those structures included ortho substituents.[11] The O1−C−C−O2 torsion angle in the figure is −81°, corresponding to the axial conformation of the 3(*S*)-tetrahydrofuryloxy group.

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(8) (a) Crystals of HIV-1 protease in complex with VX-478 in 15 mM sodium acetate, 5 mM DTT buffer (pH 5.4) grew at room temperature by vapor diffusion against an unbuffered 5% saturated ammonium sulfate reservoir.[8b] Hexagonal rods grew to 0.15 × 0.15 × 1.0 mm^3 in size in about a week. The crystals belong to the space group $P6_1$ with unit cell dimensions $a = b = 63.42$ Å, $c = 83.79$ Å; there is one inhibitor-bound protease dimer per asymmetric unit. Data were collected at room temperature from a single crystal using a Rigaku R-axis IIc image plate area detector (Molecular Structure Corp., Woodlands, TX). A total of 44 734 observations were measured yielding 12 266 unique reflections (of the 16 144 possible) to 1.9 Å resolution, with an overall *R*-merge of 7.14%. All measured reflections between 8.0 and 1.9 Å were included in the structure refinement, which was carried out using X-PLOR.[8c] The complex structure was refined using the slow-cool algorithm[8d] with starting HIV-1 protease coordinates taken from an isomorphous complex structure determined by Erickson *et al.*[8b] (with inhibitor A-700,417; Brookhaven Protein Data Bank[8e] accession code 9hvp). The program QUANTA (Version 4.0b; Molecular Simulations Inc.; Burlington, MA) was used for model building. The final model of the complex, including 92 solvent molecules, led to an *R*-value of 0.196 with root mean square deviations from ideality of 0.016 Å and 3.5° in bond distances and bond angles, respectively. (b) Erickson, J. W.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenfouse, J. W.; Turon, M. ; Wideburg, N.; Kohbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Dnigge, M. *Science* **1990**, *249*, 527−533. (c) Brunger, A. T. *X-PLOR. A system for X-ray Crystallography and NMR*, Version 3.1; Yale University Press: New Haven, 1992. (d) Brunger, A. T.; Krukowski, A.; Erickson, J. W. *Acta Crystallogr.* **1990** *A46*, 585−593. (e) Bernstein, F. C.; Koetzel, T. F.; Williams, G. J. G.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, T. *J. Mol. Biol.* **1977**, *112*, 535−542.
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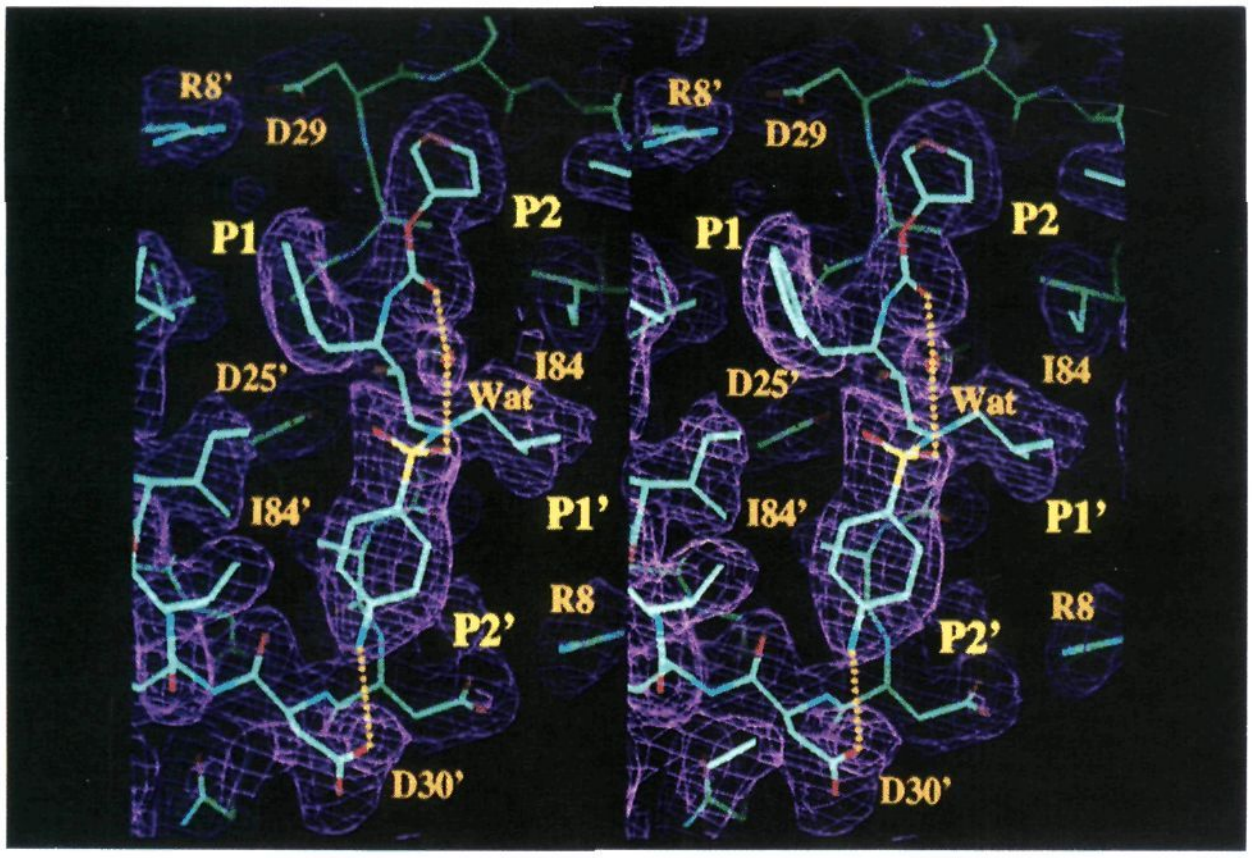

Figure 3. Stereo diagram of the $2|F_o| - |F_c|$ electron density map in the active site region of the VX-478 complex with HIV-1 protease, contoured at 1.0σ above background,[8] where VX-478 was omitted in the map calculation. Key elements in the complex structure are shown, along with the tetrahedrally coordinated flap water molecule,[7] labeled "Wat". VX-478 was fitted unambiguously to difference electron density[11] in a single extended conformation, with all inhibitor atoms well defined in the map.

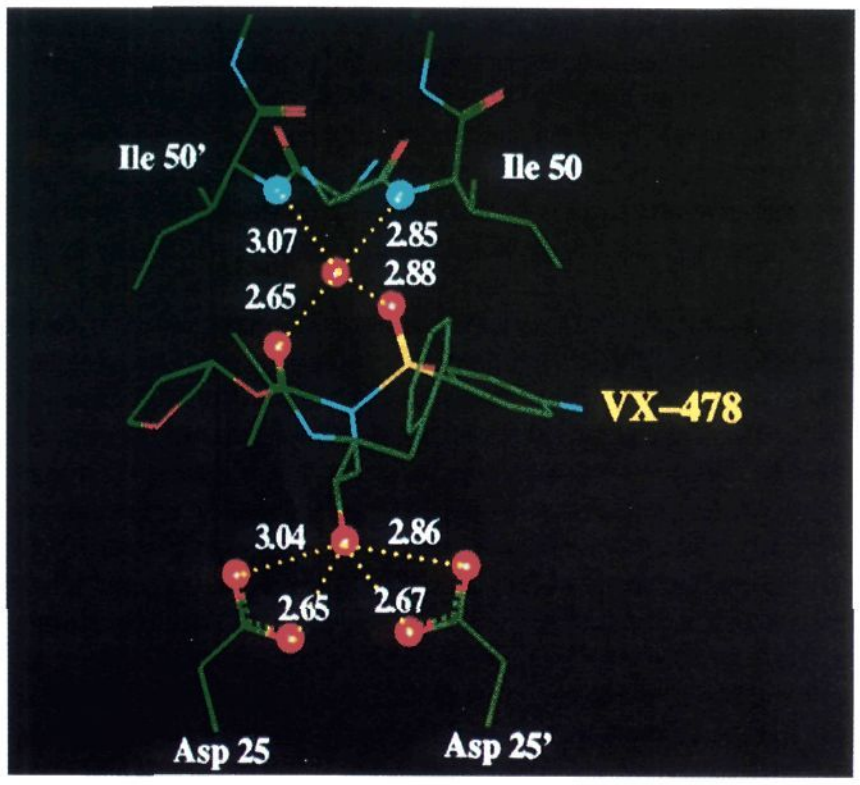

Figure 4. Hydrogen-bonding interaction between VX-478 and HIV-1 protease. Hydrogen bonding distances are shown in angstroms. The tetrahedrally coordinated water is labeled "Wat".

The conformational preference around the *N,N*-dialkylbenzenesulfonamide segment of VX-478 in the protease complex matches the average of the corresponding torsion angles (tor8 and tor9) in 10 related structures[11] from the Cambridge Crystallographic Database[12] (Figure 2). *Ab initio* calculations carried out on *N,N*-diethylbenzenesulfonamide,[13] for example, show the global minimum structure to be within 2 kcal/mol of the bound conformation of the corresponding portion of VX-478 in the complex, suggesting that the strain energy in the bound conformation is low.[14] The conformation of the 3(*S*)-tetrahydrofuryloxy group in P_2 is axial, with the oxygen of the ring (O1 in Figure 2) weakly interacting with the Asp29 and Asp30 backbone amides (at 3.4 and 3.5 Å, respectively). The ester oxygen of the carbamate makes no hydrogen bonds with the enzyme, though it seems to play a crucial role in positioning the THF moiety in the S_2 pocket, as does the energetically preferred axial orientation of the 3-oxy-THF moiety. As noted, the carbamate carbonyl forms a strong hydrogen bond with the flap water. The backbone amide of VX-478, however, does not hydrogen bond to the carbonyl oxygen of Gly27 as in other complexes.[3,7]

Aqueous solubility emerged as a design goal when its correlation with compound bioavailability was noted in early inhibitors in our program. At 190 μg/mL (375 μM),[15] VX-478 is significantly higher in aqueous solubility than other members of the series,[4] without recourse to obligate charge. This enhancement can be attributed to the 4-amino substituent on the aryl sulfonamide of the inhibitor, which also forms a strong hydrogen bond (2.8 Å) to the side chain oxygen of Asp30′ (Figure 3), contributing to enzyme binding affinity thereby.

We have presented the refined crystal structure of HIV-1 protease in complex with VX-478, emphasizing those interactions that promote high enzyme inhibitory potency in a small molecular weight compound. These properties, in conjunction with a favorable aqueous solubility profile, enhance the possibility that VX-478 will emerge from clinical development as an orally available therapeutic agent against AIDS.

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Supplementary Material Available: Analysis of the molecular weight distribution of marketed drugs, sulfonamide torsion angles of 10 related structures from the Cambridge Crystallographic Database, and a difference electron density map of the inhibitor (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information. Atomic coordinates for the structures of HIV-1 protease in complex with VX-478 have been deposited with the Brookhaven Protein Data Bank.[8e] The accession code is 1HPV.

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(11) Experimental details are provided in the supplementary material.
(12) Cambridge Crystallographic Database, version 5.7.
(13) Geometries were optimized at the HF/6-31G* and MP2/6-31G* levels of theory using Gaussian 92, Revision E.2, 1992, Gaussian, Inc., Pittsburgh, PA.
(14) Murcko, M. A.; Kim, E. E.; Rao, B. G. Unpublished results.
(15) Solubility determined in saturated phosphate-buffered saline solution at pH 6.8.
(16) ISIS/Base, Version 1.0; Molecular Design Ltd.; San Leandro, CA.