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X-ray Crystallographic Structure of ABT-378 (Lopinavir) Bound to HIV-1 Protease

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Abstract—The crystal structure of ABT-378 (lopinavir), bound to the active site of HIV-1 protease is described. A comparison with crystal structures of ritonavir, A-78791, and BILA-2450 shows some analogous features with previous reported compounds. A cyclic urea unit in the P_2 position of ABT-378 is novel and makes two bidentate hydrogen bonds with Asp 29 of HIV-1 protease. In addition, a previously unreported shift in the Gly 48 carbonyl position is observed. A discussion of the structural features responsible for its high potency against wild-type HIV protease is given along with an analysis of the effect of active site mutations on potency in in vitro assays.

Introduction

Ritonavir (Fig. 1) was the first HIV protease inhibitor from our antiviral research program to advance to late-stage clinical trials and was licensed for anti-retroviral therapy in 1996.[1] Our search for a second generation inhibitor continued with an extensive list of requirements that included the following: (1) improvement of potency against wild-type HIV in vitro, (2) inclusion of structural features that would offset the effects of specific resistance mutations, and (3) sufficient pharmacokinetic properties to sustain plasma levels well in excess of the in vitro EC_{50}. An intense research effort culminated in the discovery of ABT-378 (Fig. 1, lopinavir), an extremely potent inhibitor ($K_i = 1.3$ pM) of HIV-1 protease possessing the desired in vitro activity in the presence of human serum.[2] Clinical trials with ABT-378/r (a coformulation of lopinavir and ritonavir) demonstrated significant anti-viral activities against both wild-type and drug-resistant HIV infections.[3] In this report, we describe the three-dimensional structure of ABT-378 bound to the active site of HIV-1 protease. While the overall binding mode is similar to previously reported inhibitor-bound structures, several unique features of ABT-378/HIV protease complex are detailed in this report. The potency and resistance profile of this inhibitor is also discussed.

Results and Discussion

There are currently almost 200 co-crystal structures of inhibitor/HIV protease complexes available in the public domain (http://www.ncifcrf.gov/HIVdb/).[12] While several successful crystal growth protocols have been reported which yield crystals of different morphologies and diffraction power,[13] it is our experience that it is difficult to predict the success of any single crystallization and also to control the crystal morphology outcome. This crystallization control is important as some crystals, such as orthorhombic forms (for example, $P2_12_12$), give higher resolution data than other crystals, such as hexagonal forms (for example, $P6_1$). Unfortunately, all of our attempts to grow crystals of ABT-378 in potentially higher resolution orthorhombic form have failed to date. We were successful at obtaining hexagonal crystals, however, and successfully solved that form of the ABT-378/HIV-1 protease complex structure at 2.8 Å resolution.

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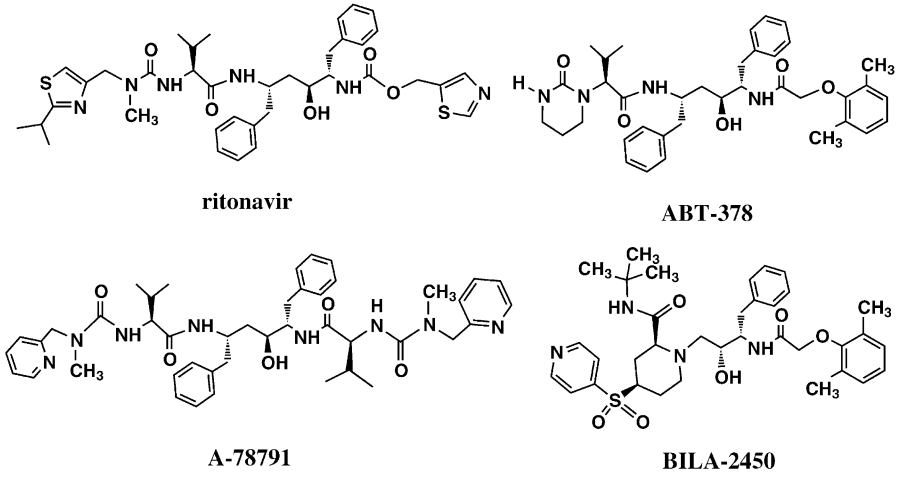

Figure 1. Chemical structures of inhibitors mentioned in this article.

General structural features of the ABT-378 active site orientation

The ABT-378 inhibitor occupies the active site much as anticipated by our earlier modeling studies[2] with important deviations from this model noted below. The inhibitor provides hydrophobic sidechains that fill four pockets of the active site that are closest to the catalytic Asp residues, the S_2, S_1, $S_{1'}$, and $S_{2'}$ subsites. We observe two clear binding modes of the inhibitor which represent a 2-fold 180° disorder in the inhibitor orientation. There is approximately an equal mixture of both the nonprime-to-prime and prime-to-nonprime orientations. This 2-fold occupancy has been observed in many inhibited complexes of HIV protease and has been described in detail.[8] The overlay of one of the ABT-378 orientations is shown with ritonavir in Figure 2. As anticipated from our drug design strategy,[2] there is greatly diminished van der Waals interaction with Val 82, relative to that exhibited by ritonavir. Specifically, the terminal isopropyl group of ritonavir that makes several good VdW contacts with the sidechain of Val82 has been eliminated in ABT-378. The isopropylthiazolylmethyl urea unit of ritonavir has been replaced by the significantly shortened cyclic urea unit of ABT-378. This lack of interaction with Val 82 is in good accord with the observation of retention of wild-type inhibitory potency by ABT-378 toward single-site mutated HIV protease in assays of enzymatic activity.[2] For example, the fold-loss in potency for ritonavir against V82A, T, and F mutated enzymes, relative to wild-type is 12-, 30- and 52-fold, respectively. The corresponding losses for ABT-378 are all less than 3.8-fold.

The pseudo-C_2-symmetrical diphenylhexane mono-ol P_1/$P_{1'}$ core unit of ABT-378

The pseudo-symmetric core unit of ABT-378 has its structural roots in our early reports of C_2-symmetric HIV protease inhibitors.[14] In Figure 3, the core unit of ABT-378 is overlaid on the structure of an identical unit of an earlier analogue, A-78791 (Fig. 1).[15] The overlay indicates that the core pieces of the two inhibitors are quite close, particularly in the central diaminoalcohol backbone, with slightly increased variation in the position of the P1 and P1′ benzyl units.

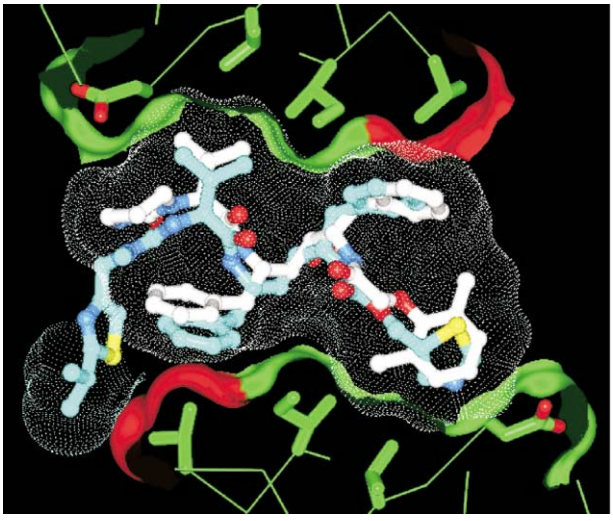

Figure 2. Active-site overlay of ABT-378 (white carbon atoms with white dotted surface) and ritonavir (blue carbon atoms with blue dotted surface covering the terminal isopropyl group in contact with Val 82) in HIV-1 protease. Side chains of protein residues Asp30, Val30, Val82, and Ile84—both C_2-symmetrical partner residues—are thick-bonded in green with a green solid surface indicating the outline of the active site. The protein surfaces of Val 82 and its C_2-symmetrical partner residue are colored red to indicate the region of resistance in V82 mutants.

P2′ phenoxyacetyl unit of ABT-378

The phenoxyacetyl structural unit has been the subject of a recent detailed report.[16] The crystal structure of one of the compounds from that study, BILA-2450 (Fig. 1), is available for comparison, and the overlay with ABT-378 is shown in Figure 4. The overlap of the phenoxyacetyl unit from the two inhibitors is quite close. Subtle differences in the two inhibitors might be attributed to the chirality difference at the central hydroxy substituent for BILA-2450 and ABT-378, leading to slight

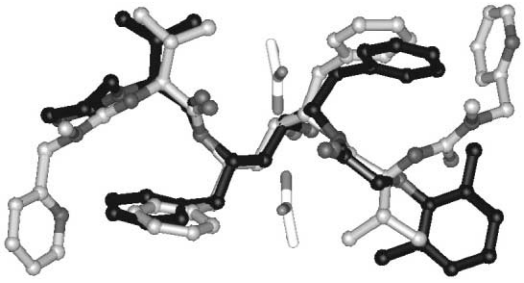

Figure 3. Overlay of ABT-378 (black carbons) with A-78791 (grey carbons) showing the close overlap of the central diaminoalcohol backbone core units of each inhibitor. The two catalytic Asp residues of the protein are shown in white.

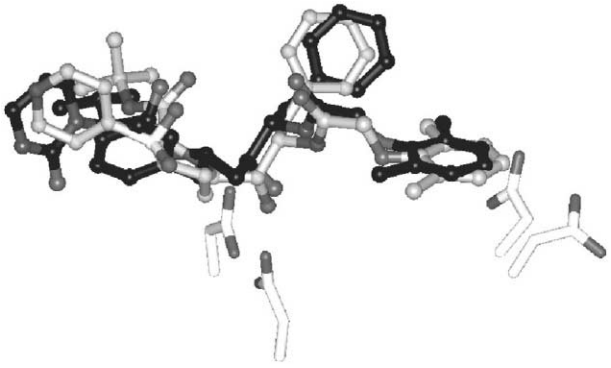

Figure 4. Overlay of ABT-378 (black carbons) with BILA-2450 (grey carbons) showing the close overlap of the $P_{2'}$ phenoxyacetyl units of each inhibitor. The catalytic Asp residues and other protein residues of the S_2 subsite are shown in white.

changes in projection vectors for the respective P2′ units.

Cyclic urea P2 unit of ABT-378

ABT-378 differs from all previously disclosed HIV protease inhibitors in the use of a cyclic urea unit as a P2–P3 peptide amide bond replacement. Initial molecular modeling experiments led to two models for the orientation of a cyclic urea in this region of the active site.[2] A static protein model indicated that a hydrogen bond interaction with Asp 30 would be possible, but a protein model which allowed slight backbone movement near Gly 48 indicated that a hydrogen bond interaction with Asp29 would be possible. Our previous experience[17] with designing inhibitors that interact via hydrogen bonds with Asp 29 and/or Asp 30 suggested that potency gain could be substantial with this design strategy, regardless of which Asp was selected. Analogues possessing this cyclic urea unit were quickly identified as potent enzyme inhibitors; however, the binding ambiguity remained until the crystallographic analysis reported here established that the observed mode of binding involves Asp 29. A picture of this interaction is shown in Figure 5. The urea carbonyl of ABT-378 accepts a hydrogen bond from the backbone N–H of Asp 29, and the terminal urea N–H of ABT-378 donates a hydrogen bond to the sidechain of Asp 29. As anticipated, slight protein movement in the backbone of the protein near residue 48 occurred to accommodate the

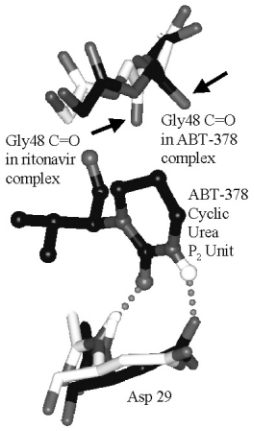

Figure 5. Close-up view of the bidentate H-bond interactions between the terminal P_2 cyclic urea unit of ABT-378 (black carbons) and Asp 29, and the VdW contact of the trimethylene portion of this unit with Gly48. Two proteins are shown: the protein corresponding to ABT-378 is shown with black carbons, the protein corresponding to ritonavir is shown with white carbons.

trimethylene portion of the cyclic urea of ABT-378 (also illustrated in Fig. 5). Our observations suggest that the energetic cost of inducing this backbone rotation is low as ABT-378 is a very potent HIV protease inhibitor with $K_i = 1.3$ pM. We see no evidence for the alternate binding interaction with Asp 30 crystallographically. The identical bidentate interaction between Asp 29 and the terminal cyclic urea unit of other inhibitors has been observed crystallographically at high resolution (< 1.5 Å) in many crystal structures from our medicinal chemistry effort leading to ABT-378 (results to be published elsewhere).

Conclusions

The structure of the complex of HIV-1 protease with ABT-378 has been determined. The binding orientations of the core and phenoxyacetyl units of ABT-378 are in good accord with previous observations of these units in other inhibitors. The cyclic urea unit of ABT-378 provides a novel hydrogen bonding arrangement with Asp 29 not previously observed. To accommodate this interaction with Asp 29, a slight shift in the protein backbone near Gly 48 has occurred. In addition to the structural aspects described here, other pharmacological properties such as serum-protein binding, half-life, and metabolism were simultaneously optimized in an extensive medicinal chemistry effort.[2] On the basis of demonstrated efficacy in clinical trials, the ABT-378/r coformulated regimen was licensed (as Kaletra™) for anti-retroviral therapy in 2000.

Experimental

ABT-378 was synthesized at Abbott Laboratories.[4] An *Escherichia coli*-codon optimized synthetic gene representing bases 1419–1907 of the BH-10 isolate (GenBank M15654) of HIV-1 protease was cloned into a pET11b expression vector in BL21/pLysS. The expressed 19 kDa protein autoproteolyzed to yield the mature 11 kDa BH-10

protease. The soluble protein was purified by sequential chromatography using SP-Sepharose Fast Flow (Pharmacia) and Superose 12 prep grade (Pharmacia). Purified protein was then concentrated to 4–5 mg/mL and stored at −80 °C before use. Co-crystals of ABT-378 bound to HIV protease were prepared by incubating a 1:1.1 molar ratio of protein to inhibitor for 1 h and then crystallizing by the hanging drop vapor diffusion method over a grid of acetate buffer (pH 4.0–5.0) and 0.4–0.8 M NaCl.[5] Data were collected at the Argonne National Laboratory synchrotron on the IMCA ID17 beamline using a Mar 165 CCD detector. The co-crystals of HIV protease/ABT-378 were soaked for approximately 10 min in a cryoprotectant containing 0.1 M Na acetate, 0.6 M NaCl, and 25% glycerol at pH 5.2 and data were collected at 100 K using an Oxford Cryo System. Data were processed using HKL2000.[6] The crystals belong to the hexagonal space group $P6_1$, with unit cell dimensions $a = b = 63.3$ Å, $c = 83.6$ Å. and $\gamma = 120°$. The initial fit of ABT-378 was to a difference Fourier map calculated after rigid body refinement, using the protein coordinates from the Brookhaven protein data bank, entry 9HVP[7] which clearly indicated the presence of ABT-378 (at 3–4 σ). The observed electron density was symmetrical about the central core (including the P2/P3 substituents); however, ABT-378 is not a symmetrical molecule, indicating that ABT-378 was bound in two orientations, analogous to the two orientations described by Miller et al. for MVT-101.[8] The observed electron density was best fit when ABT-378 was bound in an approximately 1:1 mixture of the two orientations. The final structure of the HIV protease/ABT-378 inhibited complex was refined using CNX,[9,10] an R_{free} set of 7.0%, and two orientations (refined as alternate conformations), to an $R = 26\%$, $R_{free} = 32\%$, at 2.8 Å resolution. Waters were not modeled into the refined structure because of the modest resolution (2.8 Å) of the data. Coordinates for ritonavir (1HXW), A-78791 (1HVJ), and BILA-2450 (1IDB) were taken from the Protein Data Bank.[11] Overlays of the ABT-378 structure with other inhibitor/protease complexes were generated by least squares fit of the 198 alpha carbons of the corresponding protein structures. Coordinates of the ABT-378 complex will be available from the Protein Data Bank upon publication.

Acknowledgements

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