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Computational studies of the resistance patterns of mutant HIV-1 aspartic proteases towards ABT-538 (ritonavir) and design of new derivatives

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Abstract

Kinetic characterization and cross resistance pattern studies of HIV-1 aspartic protease (PR) inhibitors have shown that some mutations cause considerable reduction in inhibition efficiency. We have performed a computational study of the binding of ABT-538 (ritonavir) with *wild type* (*wt*) PR and 12 model mutant structures (R8Q, V32I, M46I, V82A, V82F, V82I, I84V, M46I/V82F, M46I/I84V, V32I/I84V, V82F/I84V and V32I/K45I/F53L/A71V/I84V/L89M (6X)) for which inhibition data are available. Our computational studies indicate a significant correlation between computed complexation energies of ABT-538 with the modeled mutant enzyme structures and the corresponding experimental inhibition constants. By evaluating non-bonding interaction energies between the inhibitor and the mutant enzymes, we have carried out a mechanistic analysis to ascertain the reasons underlying the decrease in binding affinities. This analysis indicated that several residues in addition to the mutated residues contribute to the loss of binding. Taking these considerations into account, a number of new derivatives of ABT-538 were designed, so as to increase van der Waal's and hydrogen bonding interactions with selected mutants. A significant improvement in calculated complexation energies towards both mutant and *wt* PR structures was obtained for several of the redesigned analogues.

Keywords: Computational studies; HIV-1 protease; Inhibitors; Model structures; Mutations

1. Introduction

HIV-1 aspartic protease (PR), which is the enzyme responsible for the post-translational processing of gag and gag-pol viral gene products [1], is an important target for anti-AIDS drug design as its inhibition leads to the production of non-infectious viral particles [2–5]. A large number of HIV-1 PR inhibitors, both pseudopeptidic and non-peptidic in nature, have been designed, some of which are extensively used in anti-AIDS chemotherapy [6–13]. The use of PR inhibitors in combination with other anti-HIV drugs has improved the quality of life of AIDS patients tremendously. However, experimental findings indicate the rapid emergence of drug resistance to most of the PR inhibitors in use, due to site specific mutations in the enzyme occurring at one or more residues [14–29]. These mutations are broadly conservative in nature and involve a similar set of amino acids in response to exposure to different inhibitors, and thus often give rise to cross resistance [25]. The current medical strategy for delaying the incidence of resistance is to use a combination of potent antiviral agents acting on different targets. The PR inhibitors used in combination therapy were, however, designed to act against the *wt* enzyme and are less effective against the mutants. Hence, it would clearly be desirable to develop inhibitors that are active also against the mutants responsible for resistance.

Kinetic characterization studies of the cross resistance pattern of the R8Q, V32I, M46I, V82A, V82F, V82I, I84V, M46I/V82F, M46I/I84V, V32I/I84V, V82F/I84V and V32I/K45I/F53L/A71V/I84V/L89M (6X) mutants (Fig. 1) towards a number of PR inhibitors have recently

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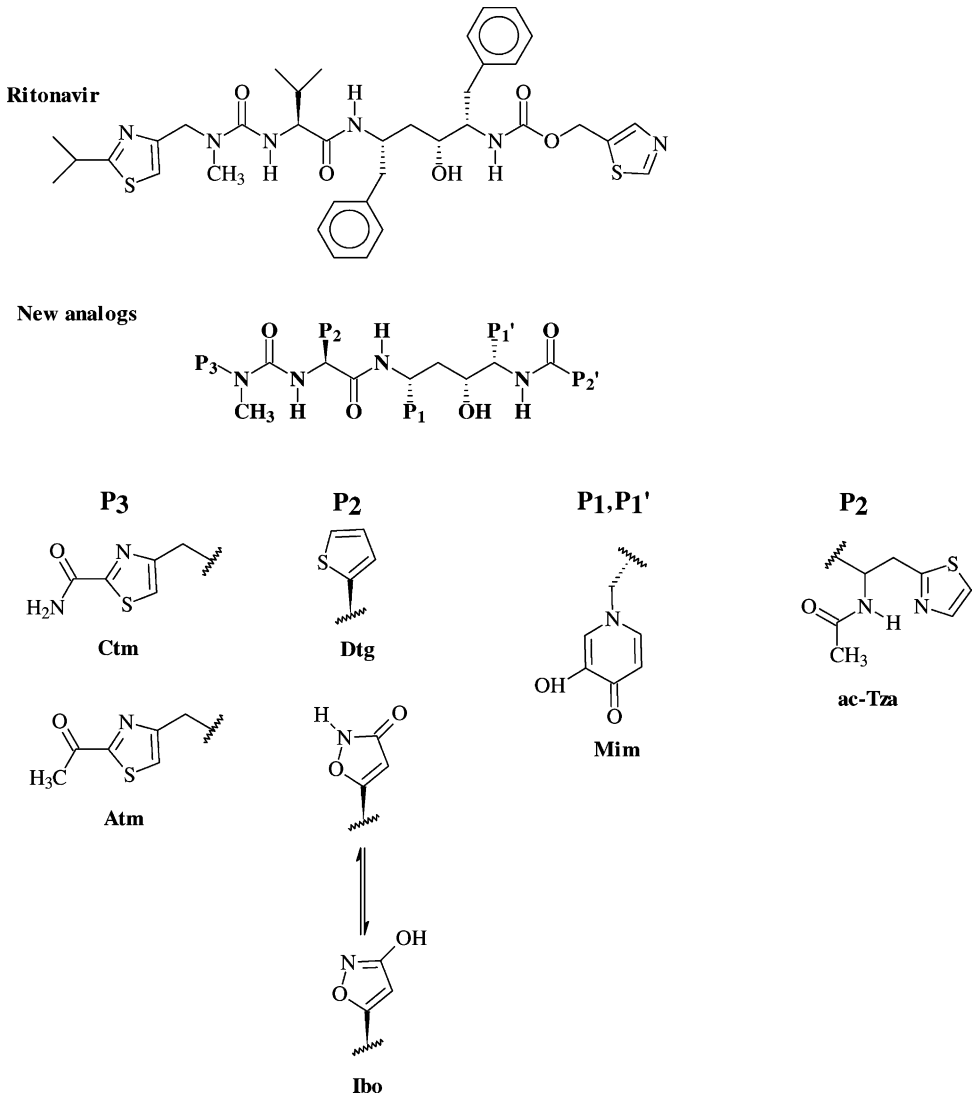

Fig. 1. Ribbon display of HIV-1 protease enzyme complexed with ABT-538 (ritonavir) showing the V32I/K45I/F53L/A71V/I84V/L89M (6X) mutation.

been reported [26–28]. These studies show that mutations such as R8Q, V82A, V82F, I84V, M46I/I84V, V32I/I84V, V82F/I84V and 6X critically affect the inhibition efficiency of ABT-538 (ritonavir), a very potent inhibitor of *wt* PR which is currently used as an AIDS drug. This resistance is presumably mainly due to modifications in the binding cleft of the enzyme. However, as the crystallographic data for mutants complexed with known inhibitors (with a few exceptions, e.g. A77003 [24] and cyclic ureas [30]) are not readily available, both the analysis of the reasons underlying resistance and the structure-based design of new analogs active against mutant PRs remain a difficult task.

As part of a strategy for the rapid and efficient preparation of peptidomimetic HIV-1 PR inhibitors, we have developed a computational approach [31–38] that enables us to estimate the potential inhibitory activity of designed compounds on the basis of calculated complexation energies. This approach involves the structure-based design of new analogs using X-ray crystallographic data of known inhibitors complexed with HIV-1 PR by molecular mechanics calculation of complexation energies. Furthermore, the decomposition of the calculated inhibitor–enzyme non-bonding interaction energy into contributions from individual residues of the enzyme has been used to refine the approach and to quantify the effects of individual or multiple mutations on inhibitor–enzyme binding. Recently, we have carried out computational studies of the binding of inhibitors ABT-538, VX-478 and A77003 towards a selected panel of resistant mutants and a number of derivatives were proposed which could potentially overcome this resistance [35,36].

In this article, we report the analysis of the binding pattern of ABT-538 with *wt* HIV-1 PR and with the 12 previously mentioned mutant PR enzymes. We also present a number of redesigned derivatives of ABT-538 that are predicted to exhibit increased van der Waal's (vdW) and hydrogen bonding interactions towards some representative

mutants. Some of these derivatives in fact show considerably improved calculated complexation energies towards the mutants as well as the *wt* PR when compared to the reference inhibitor ABT-538 and could thus, if synthesized, be potentially useful in overcoming resistance to this drug.

2. Computational methods

The complexation energy E_{compl} for the reversible inhibition of aspartic protease by an inhibitor (PR + I ↔ PR:I) can be calculated from the energies of the free enzyme (E[PR]), of the free inhibitor (E[I]), and of the enzyme–inhibitor complex (E[PR:I]) following the relation:

$$E_{\text{compl}} = E[\text{PR} : \text{I}] - E[\text{PR}] - E[\text{I}]. \quad (1)$$

The crystal structure of ABT-538 bound to *wt* protease (pdb entry: 1HXW [7a]) was used as the reference structure in this work. The energies of the respective species in Eq. (1) were obtained from molecular mechanics calculations using the consistent valence force field (CVFF) of the Discover simulation package of MSI Inc. [39], following the procedure described previously [31–36].

When considering binding of ABT-538 to mutants, the relative complexation energy ($\Delta E^{(\text{M})}_{\text{compl}}$), and its relationship to experimentally determined K_{i} ratios is defined as:

$$\Delta E^{(\text{M})}_{\text{compl}} = E^{\text{mutant}}_{\text{compl}} - E^{wt}_{\text{compl}} \propto -RT \ln \left(\frac{K_{\text{i}}^{\text{mut}}}{K_{\text{i}}^{wt}} \right) \quad (2)$$

where $K_{\text{i}}^{\text{mut}}$ and K_{i}^{wt} are, respectively, the published inhibition constants for mutant and *wt* proteases. An advantage in calculating relative complexation energies ($\Delta E^{(\text{M})}_{\text{compl}}$) is that errors inherent in the force field simulations, and partially in neglecting the entropic contribution, tend to cancel out [36,40].

When considering new derivatives (D) of ABT-538 (A), $\Delta E^{(\text{D})}_{\text{compl}}$ is instead defined as:

$$\Delta E^{(\text{D})}_{\text{compl}} = E^{(\text{D})^*}_{\text{compl}} - E^{(\text{A})wt}_{\text{compl}} \quad (3)$$

where $E^{(\text{D})}_{\text{compl}}$ and $E^{(\text{A})}_{\text{compl}}$ are the complexation energies for the designed analogue and ABT-538, respectively, and the asterisk indicates either *wt* or mutant protease structures.

Using the Insight II molecular modeling software [39], mutant PR enzymes were modeled from the reference pdb structure of the ritonavir:*wt* enzyme complex (pdb code: 1HXW) by manually altering relevant residues. Subsequently, structural analogs were designed by modifying the bound ABT-538, within either the *wt* or the modeled mutant structures. A detailed conformational search was carried out around the side-chains of mutated residues and on the altered ABT-538 fragments so as to identify the lowest energy structure. For some cases, a short (20 ps) molecular dynamics calculation at 350 K combined with annealing was also carried out to avoid lingering in local minima.

Molecular mechanics calculations were performed to evaluate total energies (E[PR:I], E[PR] and E[I]) using the CVFF force field without a cut-off for the non-bonding interactions. A gradual relaxation of the model structures was carried out during the minimization process, employing steepest descent and then conjugate-gradient methods until the average gradient fell below 0.01 kcal mol^{-1} Å^{-1}. The effective dielectric constant, $\varepsilon = 4$, was employed to account for the dielectric shielding in proteins.

In order to understand in detail the loss of affinity of the ABT-538 inhibitor with mutant PRs, non-bonding interaction energies (E_{int}) between individual ABT-538 moieties and the mutated residues of the enzyme were calculated using a custom written computer program within the CVFF framework [31–36].

To evaluate the role of enzyme residues other than the mutated ones in altered binding, the structures of *wt* and selected model mutants were superimposed residue by residue using the Insight II software after which the RMS deviation was determined. A deviation significantly above the background level was taken to indicate a local modification, which could potentially affect binding to the inhibitor. This was subsequently probed by calculating the difference in non-bonding interaction energy between the inhibitor and that residue in the two structures, as described earlier.

3. Results and discussion

Experimental characterization of cross resistance patterns for HIV-1 PR mutants selected under drug pressure from several different inhibitors [26–28] have shown that mutations in the binding cleft of the protease enzyme result in reduced binding affinity for most inhibitors [15,16,29]. The effect of 12 mutations, namely R8Q, V32I, M46I, V82A, V82F, V82I, I84V, M46I/V82F, M46I/I84V, V32I/I84V, V82F/I84V and V32I/K45I/F53L/A71V/I84V/L89M (6X) on the binding constant of ABT-538 has been experimentally determined [26–28] (Table 1). The most critical mutations in lowering the inhibition efficiency appear to be V82F/I84V, V32I/I84V and 6X. In particular, V82F and V32I introduce relatively large hydrophobic moieties at one set of positions, and I84V creates a void at another set of positions in the active site of these double mutants. Although, the 6X mutant and the V32I/I84V mutant share only two mutations in common (involving four residues), both of them exhibit similar loss in inhibitor binding (Table 1) since the remaining four mutations of the 6X mutant reside far from the active site. A decrease in binding affinity is also observed for single mutations which involve residues in the binding site, such as R8Q, V82A and I84V, but not for the M46I mutation which is in the flap region [26,27]. There is, however, some discrepancy in the published binding constants, particularly concerning the V82F and V82I mutations [26–28].

Table 1
Calculated relative complexation energies ($\Delta E^{(M)}_{compl}$) and reported inhibition constants (K_i^{mut}/K_i^{wt}) for *wild type* and mutant HIV-1 proteases towards ABT-538

Enzyme	$\Delta E^{(M)}_{compl}$ (kcal mol^{-1})	K_i^{mut}/K_i^{wt} [a]	$\ln(K_i^{mut}/K_i^{wt})$
wt	0	1	–
V82I	0.6	1.5 (14.7)[b]	0.4 (2.7)
M46I	0.9	1.9	0.6
V32I	1.5	3.4	1.2
I84V	4.8	6.7 (11.2) [14][c]	1.9 (2.4) [2.6]
R8Q	5.7	23.1	3.1
V82F	6.3	23.5 (0.8) [90]	3.2 (−0.2) [4.5]
V82A	7.2	10.4 (11.2)	2.3 (2.4)
M46I/84V	3.9	9.4	2.2
V32I/I84V	6.1	63.5	4.1
6X[d]	6.8	43.7	3.8
M46I/V82F	7.2	2.6	0.9
V82F/I84V	9.0	(700)	(6.5)

$\Delta E^{(M)}_{compl}$ is the calculated complexation energy relative to *wt* protease. K_i^{wt} and K_i^{mut} are the published inhibition constants for the inhibitor with the *wt* and indicated mutant proteases.
[a] Data from Gulnick et al. [26].
[b] Data in parentheses from Klabe et al. [27].
[c] Data in square brackets from Wilson et al. [28].
[d] 6X indicates the V32I/K45I/A71V/I84V/L89M six point mutation.

3.1. Regression analysis between complexation energies and inhibition constants

We have evaluated the complexation energies ($\Delta E^{(M)}_{compl}$) for ABT-538 with the 12 mutant proteases relative to *wt* protease, and compared these values with the $\ln(K_i^{mut}/K_i^{wt})$ (Table 1). The correlation between the $\ln(K_i^{mut}/K_i^{wt})$ values taken from the more extensive data set of Gulnick et al. [26] and the calculated complexation energies is plotted in Fig. 2. This correlation may at first sight seem to be only qualitative ($R = 0.70$, $s = 0.99$, $F = 7.61$, where R, s and F are the correlation coefficient, standard error of estimate and significance of regression, respectively). The disagreement comes mainly from the M46I/V82F double mutation (without which $R = 0.88$, $s = 0.64$, $F = 27.62$), and to a lesser extent from the V82A mutation (without both $R = 0.95$, $s = 0.43$, $F = 72.84$). The correlation between calculated $\Delta E^{(M)}_{compl}$ and experimental K_i^{mut}/K_i^{wt} values for the remaining nine mutants is thus quite good (see Fig. 2), confirming that resistance is principally due to a decrease in complexation energy between the mutant protease and the inhibitor. Regarding the two cases of discrepancy, it is somewhat difficult to explain the experimentally determined K_i for the M46I/V82F double mutant. Consistent with our prediction that the M46I single mutation has only limited influence on binding ($\Delta E^{(M)}_{compl} = 0.9$ kcal mol^{-1}), the observed K_i is affected only marginally in this single mutant, and would thus be expected to behave similarly in the double mutant. This mutation is located in the flap region and, hence, should not affect the binding pattern of the inhibitor significantly [15,16,26], although, it does appear to influence the catalytic efficiency [15]. Regarding the V82A mutation, the problem appears to be in the calculated complexation energy, which predicts a considerable destabilization in binding not borne out by the measured K_i value.

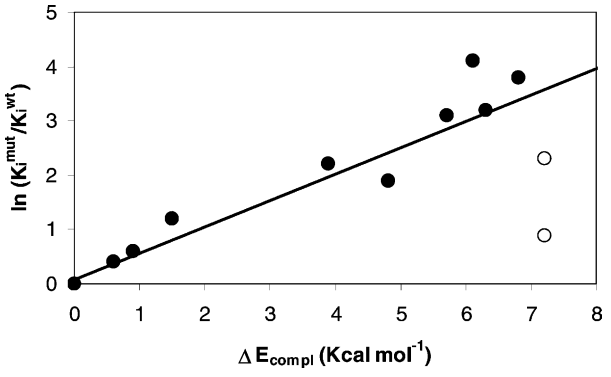

Fig. 2. Correlation between calculated complexation energies ($\Delta E^{(M)}_{compl}$) and reported (Gulnick et al. [26]) inhibition constants ($\ln(K_i^{mut}/K_i^{wt})$).

Considering another source of experimental data from Klabe et al. [27], the K_i values correlate with the calculated $\Delta E^{(M)}_{compl}$ values for the I84V mutant (Table 1), while the reported K_i values for V82F and V82I mutants are curiously inverted with respect to those of Gulnick et al. [26] and thus, do not fit well. The K_i value for V82A confirms that measured by Gulnick et al. [26], and the very high K_i^{mut}/K_i^{wt} observed for V82F/I84V corresponds to a highly positive (i.e. disfavored) calculated $\Delta E^{(M)}_{compl}$ value (9.0 kcal mol^{-1}). A reasonable correspondence is also observed for the data of Wilson et al. [28] (Table 1). These comparisons give evidence that our method for calculating relative complexation energies of mutant enzyme–inhibitor complexes, modeled from the available X-ray structure of the *wt* enzyme–inhibitor complex, represents a valid approach for predicting the effect of mutations in HIV-1 PR on inhibition.

3.2. Analysis of inhibitor–enzyme binding patterns in mutant PRs

The nature of the decrease in binding efficiency displayed by ABT-538 towards some mutants was further analyzed by evaluating the non-bonding interaction energy between the inhibitor as a whole and each of the altered residues of the mutant PR model structures. Table 2 shows the summed interaction energy (E_{int}) for symmetric pairs of residues involved in mutations with the inhibitor, and the effect of these mutations on the relative interaction energy ($\Delta E_{int} = E_{int}^{mutant} - E_{int}^{wt}$).

For the *wt* protease, the most negative (i.e. favorable) calculated E_{int} value is between the inhibitor and the following symmetric pairs of enzyme residues (in descending order of favorability): A8/B8, A82/B82, A84/B84, A32/B32 and A45/B45. The interaction with other pairs is only marginal. Considering the single mutant proteases, the ΔE_{int} values

Table 2
Summed non-bonding interaction energies (E_{int}) for ABT-538 with symmetric pairs of residues in both *wild type* and mutant proteases

Positions	*wt* residue	E_{int}^{wt} [a] (kcal mol^{-1})	Mutant residue	E_{int}^{mut} [b] (kcal mol^{-1})	ΔE_{int} [c] (kcal mol^{-1})
A84, B84	I	−6.2	V	−4.8	1.4
	(6X)			−5.6	0.6
A82, B82	V	−7.0	A	−5.0	2.0
			F	−11.1	−4.1
			I	−8.4	−1.4
A8, B8	R	−8.3	Q	−4.9	3.4
A46, B46	M	−0.3	I	−0.3	0
A32, B32	V	−2.9	I	−3.2	−0.3
A45, B45	K	−2.0	I	−0.3	1.7
A53, B53	F	−0.8	L	−0.3	0.5
A71, B71	A	0.0	V	0.0	0
A89, B89	L	−0.1	M	−0.1	0

The positions are given as residue numbers in either chain A or B of the protease homodimer.
[a] E_{int}^{wt} is the sum of interaction energies for the symmetric pair of *wild type* protease residues in that position with the inhibitor.
[b] E_{int}^{mut} is the sum of interaction energies for the symmetric pair of mutated residue with the inhibitor.
[c] $\Delta E_{int} = E_{int}^{mut} - E_{int}^{wt}$.

also decrease in the order R8Q, V82A, and I84V indicating that in these single mutants, the reported resistance towards the inhibitor may be attributed in part to a reduced interaction of the inhibitor with the mutated residues in particular. On the other hand, the V82F, V82I and V32I single mutants are predicted by our calculations to exhibit an enhanced interaction with the inhibitor, so that factors other than direct interaction between the inhibitor and the mutated residues must be influencing the enzyme–inhibitor complex formation. The ΔE_{int} values in the double mutants reflect those in the single mutations, and hence the same considerations may apply. For the 6X mutant, the modification at position 45 (A45 and B45) appears to result in a particularly marked reduction in binding.

One can conclude from the earlier analysis that the resistance shown by mutant enzymes towards ABT-538, as evidenced by diminished binding affinity (K_i), cannot simply be ascribed to changes at the level of the mutated residues and the consequent local reduction in interaction with the inhibitor. More extensive enzyme rearrangements appear in some cases to be playing a major role in determining resistance. To analyze this, the structure of the *wt* PR inhibitor complex (pdb code: 1HXW) was superimposed on that of each model mutant PR and the all-atom RMS deviation was calculated for the enzyme backbone (using the Insight II *superimpose* option). It was found that those mutants showing either little change in $\Delta E_{compl}^{(M)}$ for ABT-538 (M46I) or a change in ΔE_{int} that correlated with that in $\Delta E_{compl}^{(M)}$ (e.g. R8Q, V82A) had a relatively low RMS deviation value (0.097 and 0.104, respectively), indicating that the structure was not greatly affected. For those mutants where the change in ΔE_{int} did not correlate well with the calculated $\Delta E_{compl}^{(M)}$ value, (e.g. V82F, V82I, and V32I) the RMS was instead considerably higher (0.16–0.30), suggesting a significant rearrangement of the enzyme structure.

Choosing the V82F mutant as an example, the structure of the *wt* protease–inhibitor complex was superimposed on that of the mutant:inhibitor model structure, residue by residue, to localize the residues most affected by rearrangement. The RMS deviation calculated at that level (results not shown) was significantly above background, particularly for those residues that were spatially close to the mutated residue, but in an asymmetric manner, and specifically for A10, A48–A50, A81, A83, B8, B23, B25, B34, B50, B77, B80–B84. The non-bonding interactions with the inhibitor were found to be particularly reduced (0.5–1.0 kcal mol^{-1}) for A49, B23, B8, A27, B80, A48, B25 in that order. It is interesting that the aromatic rings at positions A82 and B82 in the mutant structure orient differently, so that the effect of mutation appears to be greater in the B chain. These results confirm the importance of structural rearrangements beyond the mutated residue in determining complexation energies.

3.3. Design of new analogues of ABT-538

From the earlier analysis, the reported resistance of mutants towards ABT-538 can be explained by the overall trend toward the less favorable calculated complexation energies. However, it is not necessarily due principally to the changes in specific interactions with the mutated residues only. Modifications to ABT-538 that would increase the complexation energy with mutant PRs and overcome resistance should thus not aim merely at compensating the loss or gain of groups on the mutated residues with complementary groups on the appropriate inhibitor fragments, but more generally at increasing vdW and hydrogen bonding interactions with the mutants.

A recent study on cyclic urea derivatives by Jadhav et. al. [41] suggests in fact that the greater the ability of an inhibitor to engage in hydrogen bonding and vdW interactions

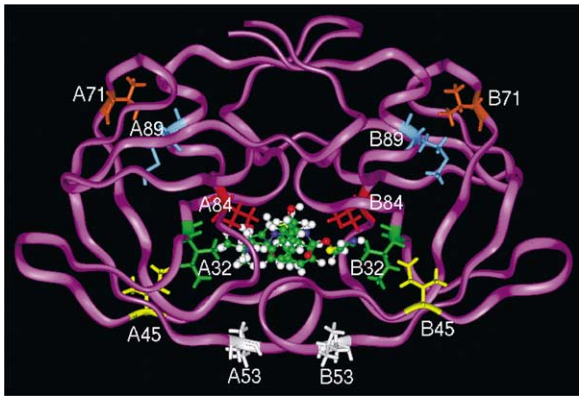

Fig. 3. Structure of ABT-538 and proposed side-chain modifications. Ctm: 4-(2-carbamoyl thiazolyl)methyl; Atm: 4-(2-acetyl thiazolyl) methyl; Leu: leucine; Dtg: D-thienylglycine; Ibo: ibotenic acid; Mim: mimosine; ac-Tza: *N*-acetyl-(2-thiazolyl)-L-alanine.

with the enzyme, the greater the potency and the better the resistance profile against mutant PRs. To be useful, however, theoretical strategies for modifying an inhibitor in this direction must also take into account the synthetic feasibility and bioavailability of the proposed derivatives. Thus, we focused our modifications to those compatible with the commercial availability of components.

We selected the V82F and R8Q single mutants, as well as the 6X multiple mutant that contains the V32I and I84V active site modifications, as targets for the redesigned ABT-538 derivatives. New ABT-538 derivatives were designed by modifying one or more of the P_3, P_2, P_1, P'_1 or P'_2 positions. The selected fragments for modification are given in Fig. 3. The calculated relative complexation energies ($\Delta E^{(D)}_{compl}$) against either the *wt* protease or mutant proteases are listed in Table 3.

In analogs **I1** and **I2**, the valine at position P_2 is replaced by D-thienylglycine (Dtg) and ibotenic acid (Ibo), respectively, and both yielded improved $\Delta E^{(D)}_{compl}$ values against all three structures and especially against the *wt* protease structure and the modeled V82F mutant structure. This finding is in agreement with previous studies [36] that demonstrated the beneficial effect of introducing five-membered heterocyclic rings at P_2. Substitution of this position with polar residues such as threonine (**I3**), (R)-amino-3-sulfamoylpropionic acid (Ams), or 2-amino-3-hydroxypentanoic acid (Ahp) was also tested (data not shown), but results were less encouraging. Extending the valine in P_2 by a methylene group to leucine (**I4**) results in a net improvement in complexation for the modeled mutant structures but not for the *wt* enzyme, confirming the importance of increased vdW interactions over complementary side-chain modifications.

The combined substitution of an acetylated (2-thiazolyl)-L-alanine (Tza) moiety at P'_2 and Dtg or Ibo at P_2 (Fig. 3) leads to compounds (**I5** and **I6**, respectively) which show a further improvement in calculated $\Delta E^{(D)}_{compl}$ for both mutants. Regarding the Ibo group, in particular, the enolic tautomer leads to more negative $\Delta E^{(D)}_{compl}$. Substitution of polar residues (Thr, Ahp, or Ams) or the hydrophobic Leu at the P_2 position in place of a five-membered heterocycle, while maintaining acetylated Tza at P'_2, does not lead to a substantial improvement (results not shown).

Single substitution of the unnatural amino acid mimosine (Mim) side-chain into the P_1 position of ABT-538 (**I7**) is predicted to have a moderately stabilizing effect on bind-

Table 3
Relative complexation energies ($\Delta E^{(D)}_{compl}$) for ABT-538 analogues with *wt* and selected mutant enzymes

	Modifications[a]					$\Delta E^{(D)}_{compl}$[b] (kcal mol^{-1})			
						wt	6X	V82F	V82F/I84V
ABT-538	R_3	R_2	R_1	R'_1	R'_2	0	6.8	6.3	9.0
I1	–	Dtg	–	–	–	−4.2	5.5	−0.4	
I2	–	Ibo[c]	–	–	–	−4.6 (−4.9)	4.7 (4.8)	0.9 (−0.6)	
I3	–	Thr	–	–	–	−0.8	5.8	0.5	
I4	–	Leu	–	–	–	3.9	3.4	−0.2	
I5	–	Dtg	–	–	ac-Tza	4.4	−0.4	−2.2	
I6	–	Ibo	–	–	ac-Tza	3.4 (4.1)	−1.9 (−3.5)	−1.6 (−3.3)	
I7	–	–	Mim	–	–	−1.7	−1.0	−1.3	
I8	–	–	Mim	–	ac-Tza	−0.2	0.4	−4.2	
I9	–	Dtg	Mim	–	ac-Tza	0.6	−3.4	−4.9	5.9
I10	–	Ibo	Mim	–	ac-Tza	0.4 (−0.7)	−4.3 (−6.3)	−4.4 (−6.1)	3.0
I11	–	–	Mim	Mim	–	−4.1	−3.4	−3.1	
I12	–	Ibo	Mim	Mim	–	−4.9 (−5.9)	−6.2 (−7.4)	−4.5 (−6.3)	1.7
I13	Ctm	–	–	Mim		−8.2	−4.6[d]		
I14	Ctm	Dtg	–	Mim		−7.8	−6.6[d]		
I15	Ctm	Ibo	–	Mim		−7.2 (−8.9)	−7.4 (−7.8)[d]		

[a] See Fig. 2 for side-chain structures, dash indicates no modification.
[b] $\Delta E^{(D)}_{compl}$ is the calculated complexation energy for the proposed analog relative to *wt* protease.
[c] Values in parentheses correspond to the enolic tautomer of Ibo (see Fig. 3).
[d] Values are under R8Q mutant.

ing for both the *wt* and mutant proteases. The combination of this modification with that placing the acetylated Tza into P_2' (**I8**) is calculated to have a strong effect, particularly against the V82F mutant. Furthermore, introduction of a five-membered heterocyclic ring into P_2 leads to the triply modified compounds (**I9** and **I10**) with quite negative (favorable) calculated $\Delta E^{(D)}_{compl}$ values towards all three enzymes, especially in the case of the Ibo tautomer.

The doubly modified ABT-538 derivative with Mim at both P_1 and P_1' positions (**I11**) was also modeled with the enzymes, and showed somewhat better $\Delta E^{(D)}_{compl}$ values against all three enzymes than the singly modified derivative. Further replacement of valine at P_2 with Ibo (**I12**) leads to a triply modified derivative with a very large decrease in $\Delta E^{(D)}_{compl}$ in all cases.

Among the proposed analogues, **I9**, **I10**, **I11** and **I12** can be considered, on the basis of the calculated complexation energies, to be potential inhibitors of HIV-1 protease that could overcome resistance to the 6X and V82F mutants. The heterocyclic five-membered ring at P_2 in these derivatives (Dtg or Ibo) has significantly improved the vdW interaction with the mutated residues as well as the non-mutated residues, particularly with the A28, A32, A76 and A84 residues of the 6X mutant (distance 3.5–4.0 Å). This can be illustrated by comparing the binding mode of ABT-538 in the pdb structure, 1HWX, with that of the **I12** enolic tautomer in the binding site of our modeled 6X mutant enzyme (Fig. 4). As can be seen from Fig. 4(a) and (b), favorable vdW and hydrogen bonding patterns exist for the new derivative, **I12**. The acetyl group of the Tza moiety at position P_2' (**I9**, **I10**) also improves vdW interactions with the mutated B32 residue (Val to Ile) in the 6X mutant (distance < 3.8 Å), as well as with other neighboring residues. Inhibitor candidates **I7**–**I12**, each possessing the Mim residue at P_1 or both P_1 and P_1' positions, were found to have strong hydrogen bond interactions through the Mim hydroxyl group interacting with the hydroxyl groups in the side-chains of the A80 and B80 Thr residues (distances = 2.0–2.3 Å), and through the carbonyl group with the backbone amino hydrogen of A82 and B82 Val residues (distance = 2.1–2.3 Å). There also exists the possibility of improved vdW interactions with residues at positions 32 and 84 (see Fig. 4(b)).

Derivative **I9** and the enolic tautomers of derivatives **I10** and **I12** were calculated to have particularly negative complexation energies with the 6X and V82F mutant structures. These derivatives were thus also computationally tested against the modeled V82F/I84V mutant structure, as the corresponding mutant has been found to be highly resistant to ABT-538. All three derivatives showed an improvement in $\Delta E^{(D)}_{compl}$ with respect to ABT-538, in particular **I12** for which it dropped from 9 to 1.7 kcal mol^{-1}. On the basis of our correlation (Fig. 2), this result would predict a reduction in K_i^{mut} to a value comparable to that for the *wt* enzyme.

Design of ABT-538 analogs against the R8Q mutant were carried out by modifications at the P_3, P_2 and P_1' fragments

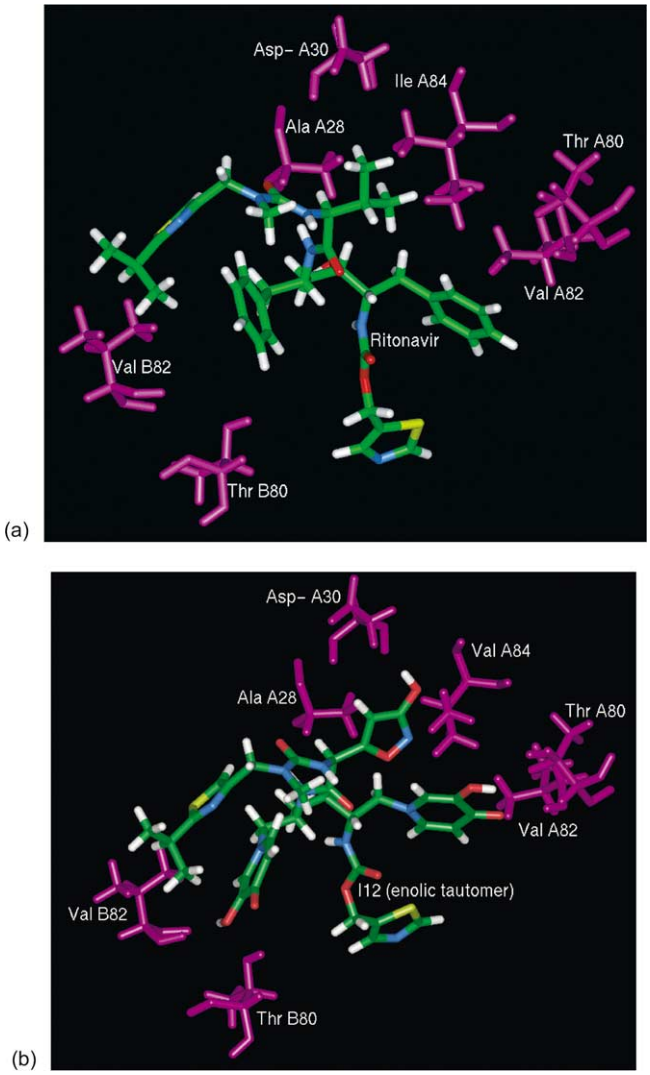

Fig. 4. HIV-1 PR binding site containing ABT-538 (ritonavir) and proposed novel derivative **I12** (enolic tautomer). Enzyme residues Thr A80 and B80, Val A82 and B82, Ala A28, Asp^- A30 and Ile A84, with which the ligands come into close vdW and hydrogen bonding contact, are also shown. (a) ABT-538 in the binding site of *wt* HIV-1 PR; (b) new derivative **I12** (enolic tautomer) in the binding site of the 6X model mutant structure.

of the reference inhibitor (Table 3). An amide (–CO–NH_2) group replaces the isopropyl substituent on the thiazole ring at P_3 with the aim to improve hydrogen bonding interactions with the side-chain of the B8 glutamine residue in the mutant enzyme. Five-membered heterocyclic residues (Dtg or Ibo) to replace Val in P_2 and/or Mim to replace Phe in P_1' were used with the aim to improve the hydrogen bonding and vdW interactions with enzyme residues. Modifications at P_3 (amide) and P_1' (Mim) only (**I13**) result in markedly improved binding characteristics toward the *wt* PR, and to a lesser extent also toward the mutant PR. The additional modification at P_2 (**I14** and **I15**) improves binding to the R8Q mutant, due to the earlier mentioned increased vdW and hydrogen bonding interactions, without greatly affecting

binding to the *wt* enzyme. Again the Ibo tautomer was found to be very effective towards both mutant and *wt* enzymes. Derivatives analogous to **I13**, **I14** and **I15** but with the acetyl (–CO–CH_3) substitution at the P_3 position led to comparable $\Delta E^{(D)}_{compl}$ values to the amidated derivatives. Introduction of the polar Ams residue into P_2, instead of a heterocyclic ring, is predicted not to improve binding towards the R8Q mutant.

Analysis of neighboring interactions for the **I13**, **I14** and **I15** derivatives with residues in the *wt* and model mutant structures revealed an improved dipole interaction between the –CO–NH_2 group in the inhibitor and the B8 residues of either *wt* or R8Q mutant structures, as well as interactions such as those previously discussed for Dtg and Ibo. The amide carbonyl can interact with either the amino hydrogen of the B8 glutamine in the mutant structure (distance = 3.7 Å), or the guanidyl hydrogen of the arginine side-chain in the *wt* structure. The amide amino group displays favorable vdW interactions with the B82 residue in either protease. Another beneficial interaction may occur between the hydroxyl oxygen of the P_1' Mim moiety and the amino hydrogen of the A8 glutamine residue in the mutant structure or the A8 arginine amino group in the *wt* structure. Of course, it should be noted that seemingly small structural modifications such as those suggested above might possibly lead to dramatic alterations in the solubility and/or bioavailability of the proposed inhibitor [42,43].

4. Concluding remarks

A molecular modeling study of the resistance pattern for the PR inhibitor ABT-538 (also known as ritonavir or A-84538) was carried out on homology model mutant structures derived from the published X-ray structure of the inhibitor bound to *wt* enzyme. These studies have revealed good agreement between computed complexation energies and experimentally observed $\ln(K_i^{mut}/K_i^{wt})$ values for most of the subject mutants. Variations in complexation energies appear to involve binding interactions between the inhibitor and the enzyme globally, rather than just to changes in local interactions with the mutated residues.

Based on this analysis, new analogues of ABT-538 were designed to compensate for the effect of the 6X, V82F, R8Q and V82F/I84V mutants by increasing vdW contacts and H-bonding interactions. From these computational studies, we have identified some very promising analogues which appear feasible from a synthetic point of view and which could help to deal with the problem of drug resistance in AIDS chemotherapy.

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