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Antiviral Drug Design: Computational Analyses of the Effects of the L100I Mutation for HIV-RT on the Binding of NNRTIs

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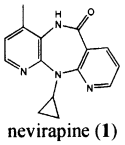
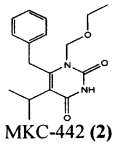
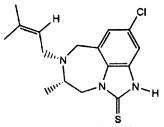
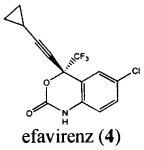
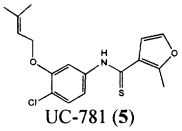
Abstract—Monte Carlo/free energy perturbation (MC/FEP) calculations were used to evaluate the binding free energy change for HIV-RT/inhibitor complexes upon L100I mutation. Inhibitor size and flexibility adjacent to hydrogen-bonding sites are evident as important considerations for antiviral drug design.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) comprise a structurally diverse series of compounds that are highly specific for inhibition of HIV-1 RT.[1] The compounds bind in a pocket that is about 10 Å away from the enzyme's active site.[2] The inhibition mechanism is believed to feature a conformational change in the polymerase site upon binding.[3] Though they represent an important component of anti-HIV chemotherapy, clinical utility of the non-nucleoside inhibitors is adversely affected by the emergence of drug-resistant HIV-1 variants. Thus, it is important to understand the atomic-level origin of the drug resistance and to use that knowledge in the design of improved NNRTIs. At least thirty different classes of NNRTIs have been studied and from them, nevirapine, delavirdine, and efavirenz have been approved by the FDA for clinical use.[4,5] Common RT mutations that confer resistance to NNRTIs include L100I, K103N, V106A/I/L, Y181C, and G190A/T/V. Different mutations have very different effects on different inhibitors. The L100I single point mutation, for example, has little effect on nevirapine, but causes a 100 fold loss of activity for 9-Cl TIBO, as shown in Table 1.

In an attempt to elucidate the variations in drug resistance for NNRTIs, MC/FEP simulations were used to study the L100I mutation for five inhibitors, nevirapine (Viramune), MKC-442 (emivirine), 9-Cl TIBO, efavirenz (Sustiva), and UC-781 (Table 1).

Table 1. Experimental activity of NNRTIs against wild type and L100I mutant HIV-1 RT

NNRTIs	EC_{50}[a]		FR[b]	Ref
	WT	L100I		
 nevirapine (**1**)	0.11	0.07	0.6	6
	0.026	0.053	2.1	7
	0.02	0.10	5.0	12
	0.03	0.38	12.7	8
 MKC-442 (**2**)	0.002	0.02	10.0	9
 9-Cl TIBO (**3**)	0.06	5.60	93.3	8
 efavirenz (**4**)	0.003	0.017	5.9	10
	0.003	0.025	8.6	10
 UC-781 (**5**)	0.009	0.024	2.7	11

[a]EC_{50} in μM; K_i is used for efavirenz.
[b]Fold resistance = L100I/WT.

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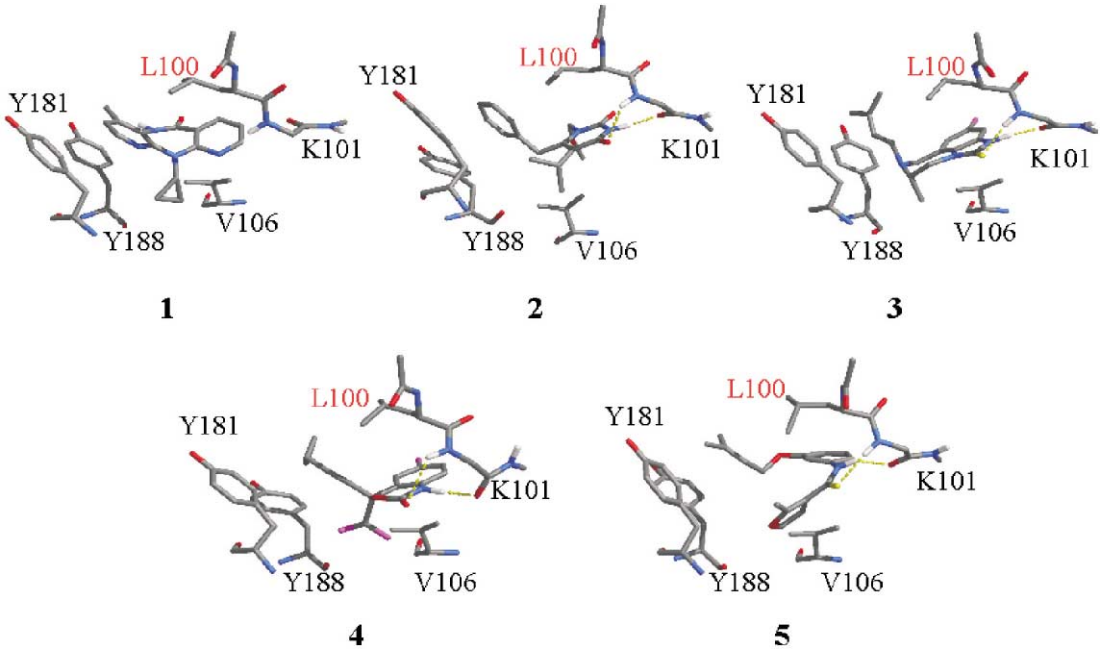

Figure 1. The binding mode of the NNRTIs **1**–**5**.

Computational Methods

The coordinates of the HIV-RT complexes were taken from X-ray crystal structures for nevirapine (PDB entry 1vrt), MKC-442 (1rt1), 9-Cl TIBO (1rev), and UC-781 (1rt4). The initial coordinates for the Sustiva/RT complex were taken from a docked structure produced in this laboratory and verified through MC/FEP calculations of the fold resistances to the Y181C and V106A mutations.[13] A crystal structure of the complex (PDB entry 1fk9) became available subsequently and agrees fully with the docked structure.[14] The coordinates of unliganded RT were taken from PDB entry 1rtj. All residues with an atom within 15 Å of a ligand atom were included and the final system size was 123 residues plus the inhibitor. To relieve initial unfavorable interactions, all complexes were subjected to molecular dynamics (MD) equilibration using the IMPACT program[15] prior to the MC/FEP simulations.

The MC/FEP calculations were performed in the same manner as in our previous study of the Y181C and V106A mutations.[13] All side-chain bond angles and dihedrals on residues within 10 Å of the ligand center were sampled, the protein backbone was fixed after the MD, and each inhibitor was fully flexible. Both the ligand and the complex were solvated in a 22 Å cap of TIP4P water[16] and a 9 Å residue-based cutoff was used. The L100I mutation was accomplished in two steps, L →norvaline→ I. Both FEP steps were divided into 11 windows with double-wide sampling. Each window consisted of 1 million configurations (1 Mcon) of solvent-only equilibration, 10 Mcon of complete equilibration, and 10 Mcon of averaging. All FEP calculations were performed with the MCPRO program[17] utilizing scaled CM1P charges[18] with the OPLS-AA force field,[19] as previously described.[13]

Results and Discussion

Table 2 lists the computed effects of the L100I mutation on the free energy of binding, $\Delta\Delta G$, for inhibitors **1**–**5**. The experimental $\Delta\Delta G$ values are estimated from the fold resistance ratios, which should parallel binding constant ratios.[20] The computed and experimental results concur that nevirapine and UC-781 are less sensitive to this mutation than MKC-442 and Sustiva, while 9-Cl TIBO suffers the largest loss of affinity.

Figure 1 shows the contacts between the inhibitors and RT in each complex. Except for nevirapine, a common feature is the presence of a hydrogen bond between the inhibitor and the backbone oxygen of Lys101 and favorable electrostatic interactions between an adjacent sulfur or oxygen atom and the backbone NH of Lys101. Key additional interactions are mainly hydrophobic contacts, including those with Leu100, Val106, Tyr181, Tyr188, and Trp229.

Table 2. Comparison of calculated and experimental binding free energy (kcal/mol) changes upon L100I mutation

NNRTIs	ΔG	$\Delta\Delta G_{cal}$	$\Delta\Delta G_{exp}$[a]
Nevirapine (**1**)	12.98±0.40	−0.48±0.45	−0.30, 0.46, 0.99, 1.57
MKC-442 (**2**)	14.51±0.26	1.05±0.34	1.42
9-Cl TIBO (**3**)	16.73±0.29	3.27±0.37	2.80
Sustiva (**4**)	14.28±0.21	0.82±0.31	1.09, 1.33
UC-781 (**5**)	13.23±0.26	−0.23±0.34	0.61
Apo enzyme	13.46±0.23	0.00	

[a]$\Delta\Delta G_{exp}$ were estimated as RT ln (FR) at 37 °C from values in Table 1.

The L100I mutation is fundamentally different from Y181C and V106A. L100I is an isosteric replacement, though without adjustment, the branching at C^{β} rather than C^{γ} would vector the Ile γ-methyl group directly towards a ring of each ligand and adjacent to any group that is hydrogen bonding with Lys101. Thus, the L100I mutation is likely to inflict either a steric penalty or to destabilize the hydrogen bond to Lys101. If a hydrogen-bonding inhibitor is too large or inflexible, it will be vulnerable to this mutation. In contrast, the Y181C and V106A mutations represent significant size reductions, which diminish favorable hydrophobic contacts for the ligands. The structural effects of the L100I mutation can be seen in Figure 2, where average structures of the ligands, Leu/Ile 100, and Lys101 are overlaid.

In the nevirapine-RT complexes, the position of the inhibitor is essentially unchanged. However, the C^{α} of Ile100 is shifted ca. 1.2 Å towards the cleft in the ligand to relieve steric interactions with the pyridine ring. Since nevirapine does not have the hydrogen bond with Lys101, the shift is not disruptive and nevirapine tolerates the L100I change. Negligible movement of Ile100 was observed for the other four complexes. Significant movement could adversely affect the hydrogen bond between the inhibitors and Lys101. The average NH–O hydrogen bond distances in the four complexes are computed to vary by less than 0.1 Å.

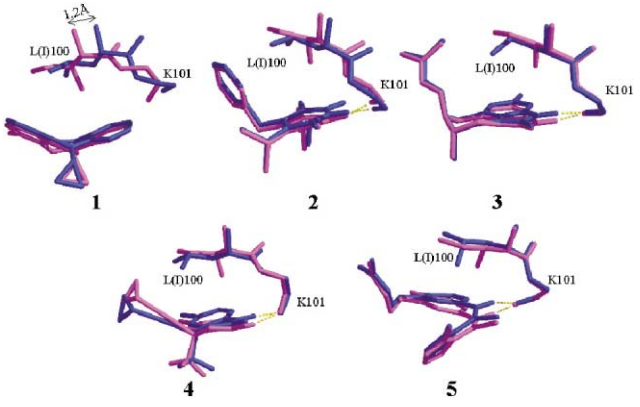

Figure 2. Average structures obtained from 100 snapshots during the MC simulations (blue: wild type; magenta: L100I mutant).

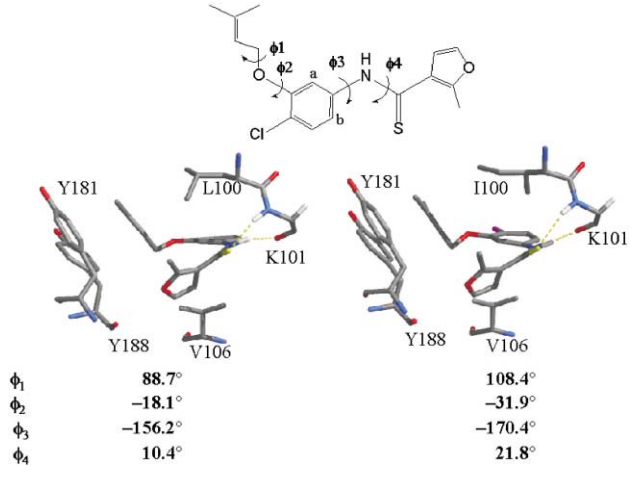

Figure 3. Average dihedral angles for **5** with WT and L100I mutant RT.

9-Cl TIBO represents the worst case, it is the largest inhibitor and its ring system is rigid in the vicinity of the thiocarboxamide fragment that hydrogen bonds with Lys101. The clash with the γ-methyl group of Ile100 cannot be avoided. Prior work has elucidated a similar fate for the 8-Cl isomer.[21] MKC-442 fares better because it is smaller and is pushed down in the pocket to help accommodate the γ-methyl group.

UC-781 tolerates the L100I mutation well because it is relatively flexible. The mutation causes the phenyl ring to move down in the binding site, presumably to lessen the contact with the γ-methyl. However, the ligand's head (dimethylallyl group) and tail (2-methylfuranyl group) largely maintain their original positions as with Leu100. There are six rotatable bonds in the molecule and four of them rotate by 10–20° to adapt to the mutation, as shown in Figure 3.

9-Cl TIBO and UC-781 are very different in chemical structure. However, when their bound conformations for L100 RT are superimposed (Fig. 4), the chlorine atoms (magenta), thio-carboxamide groups (yellow S and blue N), and dimethylallyl groups lie in almost identical positions. Although the thio-carboxamide group in UC-781 adopts the *E* configuration to mimic the three fused rings of 9-Cl TIBO, there is nevertheless a large difference in free energy change upon L100I mutation, −0.2 kcal/mol versus 3.3 kcal/mol (Table 2). The importance of molecular flexibility as a determinant in drug resistance is clear.

Finally, Sustiva is relatively compact, but its benzoxazinone ring system is rigid, and the mutation results in a rotation of the whole molecule. As shown in Figure 2, the γ-methyl group of Ile100 (magenta) points towards the chlorophenyl ring. The ligand appears to tilt to minimize the contact, which displaces the cyclopropyl ethynyl group towards the void left by the C^{δ} methyl of Leu100. This is achieved by a rotation of ca. 15° around the NH–O hydrogen bond axis.

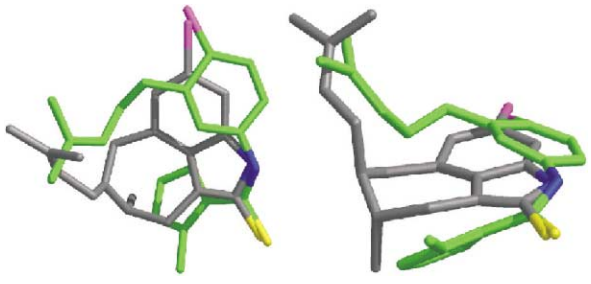

Figure 4. Top view (left) and side view (right) of overlaid structures for 9-Cl TIBO (grey) and UC-781 (green).

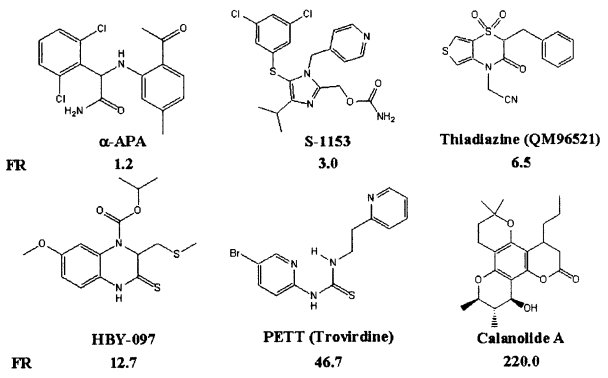

Figure 5. Other NNRTIs and their fold resistances for L100I mutation.

Summary

MC/FEP calculations for five NNRTIs complexed with HIV-RT have elucidated the inhibitors' response to the L100I mutation. The hydrogen bond between the backbone oxygen of Lys101 and most NNRTIs is important for binding, but makes them vulnerable to L100I mutation. Rigid, especially fused, ring systems which contain the hydrogen-bond donor hinder L100I resilience, while proximal rotatable bonds are desirable.

Given this, the observed effects of the L100I mutation on the activity of other NNRTIs can be rationalized (Fig. 5). α-APA[22] and thiadiazine[23] do not hydrogen bond with Lys101, while S-1153[7] forms a water-bridged hydrogen bond, but it is flexible. Thus, their fold resistances are not large. However, HBY-097[22] and PETT[24] are too inflexible near the hydrogen-bonding thioamide owing to the fused ring system for HBY-097 and internal NH–N hydrogen bond for PETT.[24] For Calanolide A,[25] the great loss of activity is due to its size and rigid polycyclic ring system, as for 9-Cl TIBO.

Fundamentally, anti-viral drug resistance can arise from mutations that (I) reduce favorable protein–drug interactions, (II) introduce or enhance unfavorable protein–drug interactions, or (III) rigidify the complex. For HIV-RT, the Y181C and V106A mutations are type I, and L100I is largely type II. Careful tuning of the drug including size and flexibility is the antidote.

Acknowledgements

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