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Validation of a Model for the Complex of HIV-1 Reverse Transcriptase with Nonnucleoside Inhibitor TMC125

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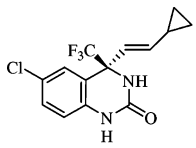
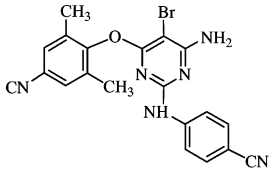
The binding of nonnucleoside inhibitors (NNRTIs) of HIV-1 reverse transcriptase (HIVRT) in a pocket proximal to the polymerase active site disrupts the enyzme's function. Many classes of NNRTIs have been identified, and three inhibitors (nevirapine, delavirdine, and efavirenz) have been approved for clinical treatment of HIV-1 infections.[1] However, the success of NNRTI-inclusive chemotherapy is compromised by the rapid emergence of resistant viral strains carrying mutations at residues that surround the NNRTI binding pocket.[2] Frequently selected single mutations in vivo are L100I, V106A, K103N, Y181C, and Y188C/L. Each effectively disables the approved NNRTIs except Y181C for efavirenz (Sustiva). Second generation NNRTIs including quinoxaline GW420867X, the imidazole derivative S-1153 (capravirine), the quinazolinones DPC961 and DPC083, and members of the ADAM, PETT, QXPT, and PBO series exhibit improved potency against many mutant HIVRT strains.[1] DPC083 and the diarylpyrimidine TMC125 appear particularly promising in phase II clinical trials.[3−5] TMC125 inhibits wild-type (WT) HIVRT, the common NNRTI-resistant single mutants, and the K103N/Y181C double mutant with below 10 nM potency.[4]



DPC083



TMC125

A crystal structure of HIVRT with TMC125 is not available at this time. Therefore, we have (1) computed a structure for the TMC125/HIVRT complex, (2) validated the structure through computation of the effects of key mutations on the binding of TMC125 versus nevirapine and efavirenz, and (3) elucidated the origin of the improved potency of TMC125 against the mutants. This work extends our analyses of binding affinities for structurally diverse inhibitors with different HIVRT forms utilizing free-energy perturbation (FEP) methodology.[6−8]

TMC125 was modeled in the HIVRT binding pocket starting with coordinates from the X-ray structure of the S-1153/HIVRT complex (pdb code 1ep4).[9] The 123 protein residues nearest the inhibitor were included in all calculations for the complexes, as before.[6−8] The pyrimidine core of TMC125 was overlaid on the imidazole of S-1153, and the two cyanophenyl substituents were added using the *GenMol* program.[10] All energy evaluations used the OPLS-AA force field augmented with scaled CM1A charges for the inhibitors.[11] A flexible conformational search inside the binding site resulted in four possible protein−ligand complexes. The two structures with favorable protein−ligand interaction energies were then subjected to 30 million Metropolis Monte Carlo (MC) equilibration steps after explicit inclusion of 851 TIP4P water molecules in a cap with a 22 Å radius.

The final, energetically preferred structure of the TMC125/HIVRT complex reveals familiar interactions observed in other NNRTI/RT complexes and additional features that may contribute to its robustness (Figure 1). A hydrogen bond occurs between TMC125 and the backbone carbonyl of Lys101 and finds precedent in analogous interactions observed for other HIVRT complexes, for example, with efavirenz,[12] 9-Cl TIBO,[13] MKC-442,[14] and UC-781.[15] The backbone NH of Lys101 is positioned between the pyrimidine N1 nitrogen of TMC125 and the C6 amino nitrogen at longer distances. The ether and amino linkages of the two cyanophenyl substituents provide sufficient flexibility to allow favorable aryl−aryl interactions with Tyr181, Tyr188, Trp229, and Tyr318. An additional hydrogen bond is predicted to occur between the C6 amino group and the carboxylate of Glu138. The interactions with Tyr318 and Glu138 are unusual for NNRTI/HIVRT complexes; they presumably promote binding to WT HIVRT and the common mutants. However, these interactions also raise the possibility of emergence of potentially damaging mutations at residues 138 and 318, which were not tested.[4] Finally, a full conformational search was performed for unbound TMC125. There are four key rotatable bonds; eight unique energy minima are found, and the global one is identical to the bound conformation with favorable interaction between the two cyanophenyl rings. Thus, a negligible reorganization penalty is expected for binding TMC125 to WT HIVRT. The next minimum without the aryl−aryl interaction is 1.2 kcal/mol higher in energy.

MC/FEP calculations were then performed to validate the computed TMC125/HIVRT structure by determining the impact of different HIVRT mutations on TMC125 as compared to that on nevirapine and efavirenz. The methodology is based on statistical perturbation theory[16] and features MC simulations for the complexes of the inhibitor with the 123 protein residues and 851 water molecules.[6−8,17] A protein side chain is mutated in the presence of one inhibitor, then another; the resultant difference in free-energy changes, $\Delta\Delta G$, is the difference in the effects of the mutation on the free energies of binding for the two inhibitors. This is expected to parallel differences in the observed effects of the mutation from residue X to Y on the experimental activities for inhibitors A and B:

$$\Delta\Delta G \approx RT(\ln IC_B^Y/IC_B^X - \ln IC_A^Y/IC_A^X)$$

The K103N and Y188L mutations were performed for the efavirenz−TMC125 pair, and the L100I and Y181C mutations were performed for the nevirapine−TMC125 pair.[18] The MC/FEP calculations were carried out with the *MCPRO* program.[19] The computed results in Tables 1 and 2 unequivocally reproduce the observations that TMC125 retains activity better against all four mutants than do the other inhibitors. For example, the IC_{50} for TMC125 remains at 1 nM for WT HIVRT and the irksome K103N mutant, while the IC_{50} for efavirenz goes from 1 to 40 nM giving the "relative fold resistance" of −2.4 kcal/mol in Table 1.[4] Estimated

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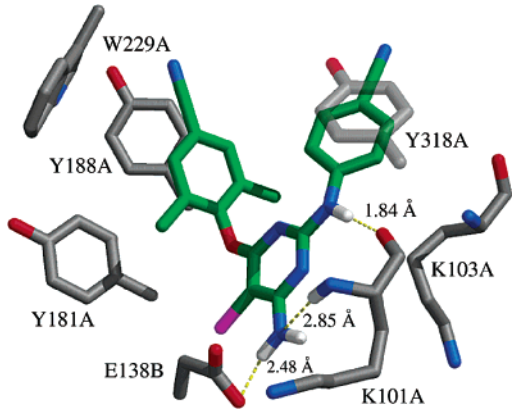

Figure 1. Proposed binding mode for the TMC125/HIVRT complex.

Table 1. Effects of Mutations, $\Delta\Delta G$ (kcal/mol), on TMC125 Relative to Efavirenz

	ΔΔG (K103N)		ΔΔG (Y188L)	
	exp[a]	calc	exp[a]	calc
efavirenz	0.0	0.00	0.0	0.00
TMC125	−2.4	−2.00 ± 0.40	−2.4	−2.07 ± 0.23

[a] From experimental anti-HIV activities in ref 4.

Table 2. Effects of Mutations, $\Delta\Delta G$ (kcal/mol), on TMC125 Relative to Nevirapine

	ΔΔG (L100I)		ΔΔG (Y181C)	
	exp[a]	calc	exp[a]	calc
nevirapine	0.0	0.00	0.0	0.00
TMC125	−0.9	−2.36 ± 0.42	−2.6	−1.36 ± 0.28

[a] From experimental anti-HIV activities in ref 4.

uncertainties in the experimental results are 0.5−1.0 kcal/mol based on multiple determinations.[6−8] Thus, the good quantitative agreement between the computed and experimental results supports the validity of the computed structure for the TMC125/HIVRT complex (Figure 1). The accompanying structural results can then be analyzed for insights on the origin of the improved performance of TMC125.

The L100I mutation generally affects binding of NNRTIs either by destabilizing the hydrogen bond to the backbone of Lys101 or by introducing unfavorable protein−ligand interactions.[6,8] TMC125 remains effective against this mutant with the hydrogen bonds to Lys101 and Glu138 computed to have similar lengths in both WT and L100I HIVRT. Enough torsional flexibility exists for the aryl rings attached to the C2 NH group to accommodate the Ile γ-methyl group; TMC125 is pressed a little deeper into the binding pocket, while retaining all key stabilizing interactions.

Reduced activity against the Y181C mutant is mostly attributed to loss of $\pi-\pi$ interactions between the tyrosine and aryl or allyl groups of many NNRTIs (e.g., nevirapine, MKC-442, and 9-Cl TIBO).[7] TMC125 makes less significant contact with Tyr181 through a methyl group of the dimethylcyanophenyl substituent (Figure 1), while π stacking occurs with Tyr188. Thus, replacement of the diminished interaction with Tyr181 has little impact on the activity of TMC125. A similar explanation was provided for the limited effect of Y181C on the activity of efavirenz, for which the interaction between the ethynylcyclopropyl group and Y181 is also not optimal.[7]

The K103N mutation confers resistance to almost all NNRTIs including efavirenz. The origin of the diminished potency of efavirenz has been attributed to unfavorable interactions between Asn amide hydrogen Hδ2 and the hydrogen on nitrogen in the benzoxazin-2-one ring of efavirenz, which also weakens the hydrogen bond between the ring hydrogen and the backbone carbonyl oxygen of Lys101. The corresponding donor hydrogen in TMC125 is farther removed from the Asn, and its hydrogen bond with Lys101 is not affected by this mutation with an average computed H···O length of 1.76 Å as compared to 2.13 Å for efavirenz. Favorable interactions with other residues in the binding pocket are also retained.

The Y188L mutation affects the binding of NNRTIs in a manner similar to Y181C, by reducing favorable protein−ligand $\pi-\pi$ interactions. TMC125 should be susceptible to this effect, so the reported factor of 3 loss in activity seems modest.[4] However, the flexibility of the ether linkage appears to allow TMC125 to better adjust to the Y188L replacement and to yield more favorable hydrophobic contact with L188. Overall, balanced flexibility, which permits an inhibitor to accommodate changes in the binding pocket by small conformational adjustments, while retaining favorable specific interactions, is key to the design of antiviral therapeutics that remain active against microbial mutants. The more rigid fused polycyclic cores of earlier generation NNRTIs, although they provide good potency against WT HIVRT, lead to poorer resistance profiles.

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