

Computational Studies of Dipyrindiazepinones as Human Immunodeficiency Virus (HIV) Reverse Transcriptase Inhibitors

Reiner G. Villavicencio and Junie B. Billones*

Department of Physical Sciences and Mathematics, College of Arts and Sciences,
University of the Philippines Manila, Padre Faura St., Ermita, Manila 1000 Philippines

A series of dipyrindiazepinone derivatives that are highly active against wild-type and K103N – Y181C double mutant reverse transcriptase were computationally investigated. Quantitative structure-activity relationship (QSAR) studies, metabolic studies, and docking simulations were performed on these compounds. The logP value and the HOMO-LUMO energies of the compound were found to be critical for activity. The most probable biotransformation reactions include N5 / N11-dealkylation, aliphatic hydroxylation/oxidation at the ethoxy connector, and aromatic hydroxylation in the dipyrindiazepinone nucleus and the aryl group. Docking studies showed that the inhibitor must have at least two aromatic structures to maintain interactions with the Tyr181 – Tyr188 pocket and the Tyr318 region. The nucleus of the molecule must be a hydrophobic structure to interact with Val179 and Gly190 and must be substituted with H-bonding functional groups to interact with Lys101 and Lys/Asn 103.

Key Words: Docking Simulations, E-State, Quantitative -Structure Activity Relationship (QSAR)

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is the disease caused by the human immunodeficiency virus (HIV). HIV contains its genome on RNA strands and for it to insert the genetic material in the genome of the host, a DNA copy of the single-stranded RNA should first be synthesized. This reversal of the transcription process is performed by the enzyme reverse transcriptase. Reverse transcriptase is a multifunctional enzyme with an RNA-directed DNA polymerase, DNA-directed DNA polymerase, and an RNase H activity. The three dimensional conformation of the enzyme consist of a large claw-shaped active site. It is a heterodimer with a 66-kDa (p66) and a 51-kDa (p51) subunit, which are coded by the same gene (Kohlstaedt et al. 1992; Jacobo-Molina et al. 1993; Smerdon et al. 1994; Tantillo et al. 1994; Ding et al. 1995; Ren et al. 1995).

Reverse transcriptase is prone to errors in copying the genome of the virus (about 1/2000). The high level of replication and infidelity of the enzyme give the virus an advantage, especially in this era of extensive drug development. The virus can mutate rapidly, giving rise to drug-resistant variants, and, in some cases, producing drug resistance in a matter of weeks after treatment. The amino acid residues involved in drug resistance of HIV-1 reverse transcriptase are Leu100, Val106, Val108, Val179, Tyr181, Tyr188, Lys103, Gly190, Met230 and Pro236 (Spence et al. 1995). The rapid emergence of resistant strains became the primary reason for the combination of different anti-viral treatments.

Drug design of inhibitors of HIV has focused on three key enzymes required for viral growth, namely protease, integrase, and reverse transcriptase (Smith et al. 2003). Consequently, three classes of anti-HIV drugs are commonly utilized in chemotherapy, the nucleoside reverse transcriptase (NRTIs), non-nucleoside reverse transcriptase

*Corresponding author: jbillones@post.upm.edu.ph

inhibitors (NNRTIs), and the protease inhibitors (PIs). These, however, do not preclude the development of inhibitors of viral envelope proteins HIV-gp120 and HIV-gp41 which are now being considered as potential target of antiviral therapies (Jacobs et al. 2005).

NNRTIs are excellent drug candidates because they do not involve chain terminators and, hence, do not interfere with normal function of polymerases (Kopp et al. 1991; Romero et al. 1991). Some of the NNRTI drugs that have been approved by the U.S. Food and Drug Administration (FDA) are Nevirapine (Viramune, Boehringer Ingelheim), Delavirdine (Rescriptor, Upjohn Pharmacia) and Efavirenz (Sustiva, Du Pont) (Tantillo et al. 1994). Extensive study has been done on this drug class because of their selectivity, antiviral activity, and low cytotoxicity thresholds. These properties allow these drugs to be used routinely without serious side effects (Yarchoan et al. 1989; Richman 1993).

However, there is need for the advancement of second-generation anti-HIV drugs which are also effective against the mutant strains while still maintaining their effect on wild type reverse transcriptase. These drugs should have novel interaction mechanisms with the virus, such as binding with residues which are not prone to mutation (conserved) such as Trp229 (Jacobo-Molina et al. 1993).

In this paper we report the QSAR studies, metabolic prediction, and docking experiments on a set of dipyrrodoiazepinone derivatives.

MATERIALS AND METHODS

QSAR Study

The chemical structures and experimental data of the C8-substituted dipyrrodoiazepinones were taken from literature (O'Meara et al. 2005). A representative structure (Figure 1) (compound **7** in O'Meara et al. 2005) is shown below. The molecular structures of these compounds were modeled using Hyperchem 6.0 (Hypercube, Inc.) using a PC with a 1.59 GHz CPU running on Windows XP.

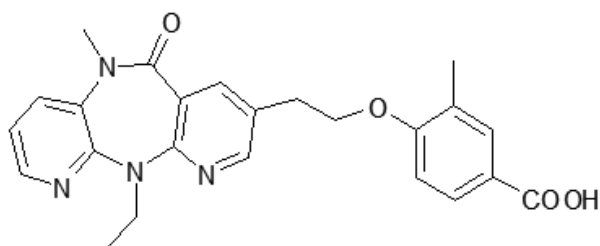


Figure 1. Compound **7** (O'Meara et al. 2005).

Since dipyrrodoiazepinones possess high degree of flexibility, a geometry optimization was performed using a systematic conformational search. An energy minimization was done on a 0.01 kcal/mole convergence criterion. Hyperchem 6.0 package was used in calculating partial atomic charges, solvent accessible area, van der Waals area, volume, hydration energy, logP, refractivity, polarizability, mass, HOMO, and LUMO energies. From the last two data, the absolute hardness (η) and absolute electronegativity (χ) were calculated using the following equations (Meisler & Tarr 1995):

$$\eta = (E_{\text{LUMO}} - E_{\text{HOMO}}) / 2 \quad (\text{Equation 1})$$

$$\chi = -(E_{\text{LUMO}} + E_{\text{HOMO}}) / 2 \quad (\text{Equation 2})$$

The electrotopological indices for each compound were calculated using E-Calc (Kier & Hall 1999). The resulting properties were used as molecular descriptors and were then compiled for multiple linear regression analysis. Multiple linear regression (MLR) was used to investigate the relationship between the biological activity of the dipyrrodoiazepinone derivatives and the calculated molecular properties. The MLR calculations were performed using SPSS v11 package (SPSS Inc.). The number of descriptors in the model was reduced using backward elimination protocol and in accord with the commonly employed criteria for a statistically sound model in QSAR studies: 1 descriptor for every three to five samples (i.e., compounds), high squared correlation coefficient, r^2 and predictive r^2 (or q^2) of at least 0.60 (Wold 1991).

Metabolic Prediction

To assess how these compounds are metabolized in the body, their propensity for biotransformation was also predicted. Metasite 2.7.5 (Zamora et al. 2003; Cruciani et al. 2005) was used to determine which of the hydrogens in the molecule is most susceptible to biotransformation reactions.

The basic concept of MetaSite is to compare the computed patterns of interaction inside the protein with the 3D structure of the ligand. The site of metabolism in substrates of human CYP enzymes is dictated by three factors, namely: enzyme accessibility, E; chemical reactivity, R; and the reaction mechanism, M. The site of metabolism can be described by a probability function PSM (Probability for the Site of Metabolism), which is expressed as product of these three computed components. The probability function, PSM, is considered to be an approximation of the free energy of the overall metabolic process.

The compounds were inputted as single conformers and the site of metabolism was predicted using all predefined cytochromes with reactivity correction. The cytochrome

P450 isoforms included in the study were CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. The three hydrogens with the highest reactivity component were considered for metabolic interpretation.

Docking Experiment

The receptor files of HIV-1 reverse transcriptase were obtained from publicly available X-ray crystal structures in the Protein Data Bank. For the wild type enzyme PDB entry 1VRT was used while for the K103N – Y181C double mutant, the entry 1FKP was used. 1FKP is actually a single mutant enzyme for K103N, the Y181C mutation was introduced later using the mutate tool of Swiss Protein Data Bank Viewer (Guex & Peitsch 1997). To reduce the computational load, each complex was reduced to a smaller size by removing solvent molecules and residues outside the predefined 20-Å nevirapine core.

Autodock 3.0.5 (Goodsell et al. 1996) was used for all the docking calculations. Partial charges of the ligand were retained from the optimization while partial charges and solvation parameters were set up for proteins. AMBER United-Atom charges were loaded for the receptor. The same was done for the ligands but using an All-Atom implementation to monitor hydrogen bonding interactions. A grid box size of 60 x 80 x 60 with a spacing between grid points of 0.375 Å was implemented which covered more than the 8-Å core. The grid was centered upon the mass center of the experimentally-bound nevirapine molecule. A genetic algorithm-local search method was used to generate 100 possible docked structures. Using an initial population of 50 randomly-placed individuals, a maximum number of 2.5×10^5 energy evaluations and a maximum number of 2.7×10^4 generations were utilized. A mutation rate of 0.02 and a crossover rate of 0.8 were used. Results differing by more than 1.0 Å in the positional root-mean-square deviation were clustered together for analysis and comparison.

RESULTS AND DISCUSSION

QSAR Study

Combination of calculated physico-chemical and electrotopological properties were subjected to multi-linear regression analysis with the experimental activity (IC_{50}) and computed properties as dependent variables. For the activity of the ligands against the wild type reverse transcriptase, the following equation was generated:

$$IC_{50} = -104 \log P - 7 \text{ sCH3} + 40 \text{ ssCH2} - 346 \text{ LUMO} + 83 \text{ aaN} - 559 \quad (\text{Equation 3})$$

$$N = 14, q^2 = 0.85$$

The logarithm of the octanol-water partition coefficient ($\log P$) reflects the hydrophobic property of the compound under investigation. The negative value for ($\log P$) suggests that increasing the hydrophobicity of the molecule will decrease the IC_{50} value and thus increases the activity.

The electrotopological descriptors such as sCH3, ssCH2, and aaN are measures of electron richness on methyl, methylene, and aromatic nitrogen, respectively. The negative value for sCH3 indicates that, to increase activity (decrease IC_{50}), there must be nearby functional groups that donate electrons to methyl or no groups that withdraw electrons away from it. On the other hand, the positive value for ssCH2 indicates that methylene groups must be made more electron-deficient. In order to decrease this value, methylene carbons must be in the vicinity of electron withdrawing groups. This can possibly be achieved by shortening the alkyl chains either in the N11-ethyl substituent or the ethoxy linker of the dipyridiazepinone. The latter makes the methylene groups closer to the highly electronegative atoms of oxygen and nitrogen, thus decreasing electron density. However, by doing so, the molecule concomitantly loses some of its hydrophobicity as well as its flexibility so there is a limit by which alkyl chains can be abridged.

The lowest unoccupied molecular orbital (LUMO) energy describes the propensity of an atom or molecule to accept an electron. The negative coefficient for LUMO implies that a higher energy for this orbital correlates with increased activity. The positive coefficient of aaN describes the contribution of the nitrogen heterocycles in the nucleus. To increase activity, electron richness of the aromatic nitrogen should be decreased.

The activity of the dipyridodiazepinone compounds against the mutant enzyme was also modeled. The predictors in equation 3 such as $\log P$, LUMO energy, and aaN were also found to greatly influence the activity of these compounds against the mutant. In addition, HOMO energy and Hmin (i.e., minimum E-state value) were shown to contribute to the observed activity. The negative coefficient of HOMO and Hmin indicates that these should be increased to improve the activity of the compound. This suggests that there should be a limit to the positivity or acidity of hydrogens because extreme values might cause ionization and, therefore, loss of active H-bonding interactions.

Metabolic Prediction

A site of metabolism prediction was conducted to examine the type of reactions the compounds undergo in biological systems. This would facilitate the design of novel drugs

that are more stable in vivo and does not produce reactive or toxic metabolites. Figure 2 shows the site of metabolism in compound **7**.

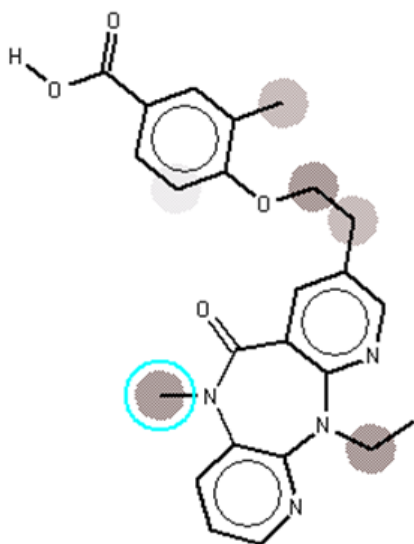


Figure 2. Sites of metabolism for compound **7** on Cyp1A2

The hydrogens with the high reactivity component were highlighted and should be considered for the design of novel reverse transcriptase inhibitors. In general, three biotransformation reactions are most likely to ensue, namely: N-dealkylation, aliphatic hydroxylation/oxidation, and aromatic hydroxylation. The most possible sites of these reactions were predicted based on the complementarity of the hydrophobic molecular interaction field (MIF) distances, charges, and H-bond donor and acceptor distances for the cytochrome and the inhibitor. The N-dealkylation reactions would most probably occur at the N5 with the methyl group (in blue circle) and N11 with the ethyl group. These reactions have the highest reactivity component in almost all cases. Thus, in the design of novel compounds, the effects of other carbon groups at these positions were evaluated. Cyclopropane and cyclopentane were tested but with little success. To preserve the hydrophobic nature of the molecule without interference in binding, the prototype compound was substituted with an ethyl group at the N5 position. Although still susceptible to N-dealkylation, we found that it is not as reactive as the methyl component. In addition adding a slightly longer carbon chain increases the hydrophobic nature of the compound which according to the QSAR study (equation 3) is crucial for activity. The aliphatic hydroxylations occur at the methylene carbons connecting C8 to the methylbenzoic acid moiety, the N11-ethyl substituent and, occasionally, on the methyl substituent of the methylbenzoic acid. Adding halogens

could prevent the oxidation of these methylene groups but their activity would be sacrificed because of severe loss of conformational flexibility. These groups should be left unchanged during the design of a prototype compound. Aromatic hydroxylation, on the other hand, occurs at C2, C3, and C9. The metabolic reactions for the other dipyrrodo-diazepinone derivatives to the different cytochrome P450 isoforms were generally similar to the model compound **7**.

Docking Experiment

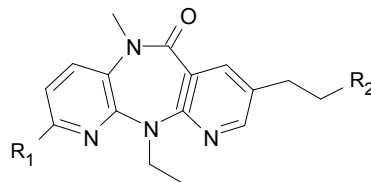
Table 1 shows that the dipyrrodo-diazepinone derivatives have a much lower docking energy than the drug nevirapine in both the wild type and mutant enzyme. The calculated energies are almost two-fold lower than the reference compound. The RMSD values can be considered acceptable if their values are about 1.0 Å (Mai et al. 2005). Although, there were values as high as 3 Å, the similarity of structure to the overall conformation was also considered upon screening. The reason for their high cluster RMSD is that we have previously randomized the input of the initial conformations to prevent the introduction of any bias in the experiment. Occasionally there will be structures that are very far from the conformations taken by most of the training set. Nevertheless, upon docking, these random conformations would find their energy minimum in the allosteric site but would sacrifice their RMSD values in the process.

In determining the binding modes, the Chimera program (Pettersen et al. 2004) and Ligplot (Wallace et al. 1995) were used. The binding of nevirapine to the wild type reverse transcriptase only showed hydrophobic and aromatic interactions (Figure 3). The residues involved are Trp229, Tyr181, Leu234, Val179, Gly190, Val106, Pro236, and Tyr318.

The tryptophan residue is critical in the interaction of the ligand with the aromatic region of the allosteric site. This residue is not susceptible to mutation and is therefore a reliable basis for the design of the novel inhibitors of reverse transcriptase. Another residue important for inhibition is Tyr181 which improves the binding through π - π interaction with the aromatic heterocycles of the compound. The outline in the ligand determines the solvent accessibility of the molecule with a lighter shade, denoting more accessibility to water molecules.

Figure 4 shows that nevirapine does not form any fixed or efficient interactions with any region because it cannot approach all these binding pockets at the same time. This is the reason for the conjugation of the nevirapine nucleus with an ethoxy chain connected to an aromatic ring to allow the molecule to bind effectively with these




Table 1. Docking energies and root-mean-square deviation (RMSD) values for the different RT inhibitors.



Compound	R1	R2	Wild Type RT		K103N-Y181C RT	
			Docking Energy (kcal/mol)	Cluster RMSD	Docking Energy (kcal/mol)	Cluster RMSD
nevirapine			-8.41	0.514	-8.42	1.141
7	H		-14.1	0.264	-13.7	0.255
22	F		-14.3	0.316	-14.2	0.248
23	OMe		-15.1	0.063	-13.9	1.056
24	H		-12.2	2.932	-11.6	0.973
25	H		-11.0	2.809	-12.1	0.766
26	H		-14.0	0.380	-13.1	1.005
27	H		-12.7	0.188	-11.7	0.664
28	H		-13.4	0.056	-12.9	0.772
29	H		-14.3	0.404	-14.3	0.180
30	H		-14.4	0.163	-6.3	0.974
31	H		-14.7	1.634	-15.4	3.275

see Table 1 continuation next page

Table 1 continuation

32	H		-15.6	1.55	-14.9	0.240
33	Cl		-15.2	0.240	-14.2	0.924
34	Me		-14.6	0.207	-14.7	2.800
Average RMSD			0.781	Average RMSD	1.018	

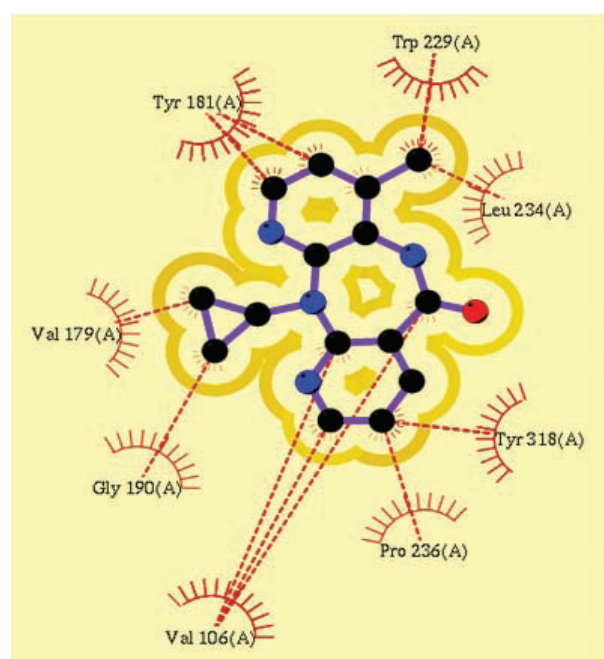


Figure 3. Hydrophobic interactions of nevirapine with aromatic and aliphatic amino acid residues of the wild type reverse transcriptase.

interaction centers. The cyclopropane group has an affinity to the depression because of the cooperative hydrophobic contributions of the aliphatic chains of Val179 and Gly190. The methyl pyridine ring interacts with the aromatic region consisting of Tyr188, Tyr181, and Trp229 while the other pyridine ring interacts with Tyr318 which is at the right corner of the diagram.

The conformation of nevirapine docked to the mutant enzyme is more or less the same, but because the Tyr181 residue is mutated to cysteine, the aromatic interaction is not as effective. The cysteine residue (Figure 5, yellow) forms a cavity in the aromatic region and disperses the aromatic residues.

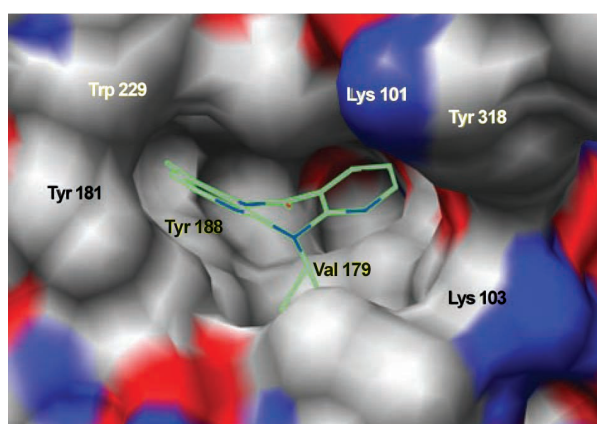


Figure 4. Surface model of wild-type reverse transcriptase complex with nevirapine.

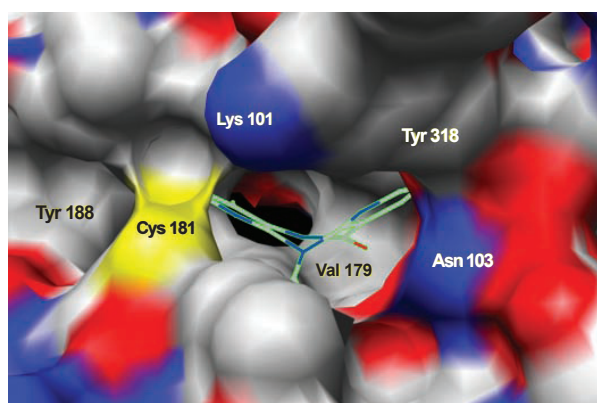


Figure 5. Interaction of nevirapine with the K103N – Y181C mutant reverse transcriptase.

As the representative compound of the dipyridodiazeponone derivatives, compound **7** was modeled into the allosteric site of the enzyme. The residues involved in the interaction were Trp229, Tyr181, Leu234, Val179, Gly190, Val106, Pro236, Lys101, and Lys103.

Figure 6 shows that compound **7** is flipped in such a way that N11 atom faces the tryptophan residue instead of N5. This is to allow the methylbenzoic acid moiety to scaffold so that it can reach Tyr318 in the other side of the hydrophobic pocket. The dipyridodiazepinone nucleus is imbedded in the aromatic pocket of the allosteric site. In addition, the hydrophobic interaction with the aliphatic chain of Val179 is diminished because the sterically hindered methylene connector cannot fold to the depression of the hydrophobic pocket. The distance of the ethoxy connector to Lys101 may also suggest H-bonding interactions. The interactions of compound **7** with the mutant enzyme (Figure 7) are basically the same but with loss of contact points. The nucleus is still in the same conformation as it was when docked to the wild type enzyme but it is more entrenched in the aromatic pocket. Interestingly, the methylbenzoic acid moiety sacrificed the interactions to the Tyr318 residue in order for the carboxylate to form H-bonding interaction with the mutated Asn103. This interaction can be a basis for the activity of compound **7** and other C8-substituted

dipyridodiazepinones against the double mutant enzyme. This mount point is also assisted by the interaction of the oxygen in the chain with an H-bond to Lys101. This proves that H-bonding is a more favored interaction by this class of compounds.

The dipyridiazepinone class of compounds form less interactions with the Lys103 residue than other non-nucleoside reverse transcriptase inhibitors. This unique binding mode enables these drug molecules to effectively interact with the hydrophobic site of the enzyme even in the presence of the double mutation K103N – Y181C.

Compound **7** is a representative of the dipyridiazepinone derivatives discussed in literature because of its pharmacokinetic profile and remarkable stability in metabolic studies (Wallace et al. 1995). The remaining members of this drug class bind in much the same way in the wild type and mutant enzyme. The compounds bind in a folded manner in the wild-type reverse transcriptase while they bind in an extended conformation upon substitution with the double mutant enzyme. There are occasionally novel binding mechanisms that can be attributed to these other compounds but preference was made to compound **7** since its drug properties surpass the other compounds making it a good basis for the design of more active and stable non-nucleoside reverse transcriptase inhibitors.

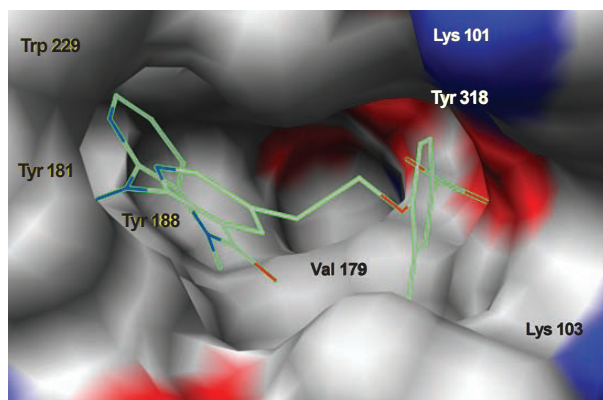


Figure 6. Surface model of wild-type reverse transcriptase in complex with compound.

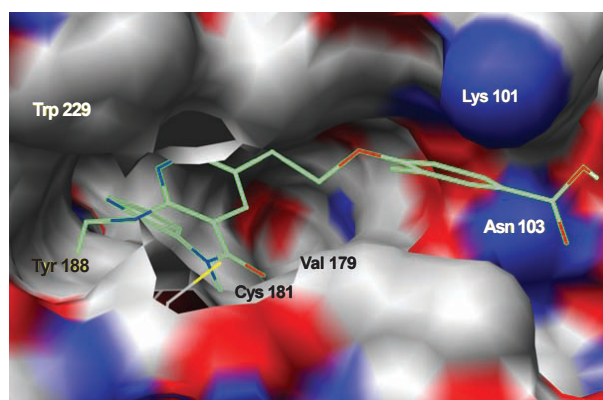


Figure 7. Surface model of K103N – Y181C mutant reverse transcriptase in complex with compound **7**

CONCLUSION

Quantitative structure-activity relationship study, metabolic prediction, and docking experiments were undertaken on nevirapine and the dipyridodiazepinone class of compounds. In the QSAR study, it was demonstrated that the hydrophobic nature of the compound and the destabilization of its frontier orbitals, among others, promote activity. The metabolic study revealed that the functional groups susceptible to biotransformation should be made more electron-dense to prevent nucleophilic attack. It was predicted that the most probable site of biotransformation is the N5-methyl group via demethylation. The docking experiments showed the significance of aromatic groups for efficient interaction by direct binding and acting as mount points for more stable interactions such as H-bonding. These results are expected to facilitate the design and development of next-generation HIV reverse transcriptase inhibitors. In fact, the subsequent molecular design of novel HIV RT inhibitors is now the subject of intense efforts in our group.

ACKNOWLEDGMENT

We thank the Scripps Research Institute for the Autodock software.

REFERENCES

- CRUCIANI G, ARISTEI Y, VIANELLO R, BARONI M. 2005. GRID-Derived Molecular Interaction Fields for Predicting the Site of Metabolism in Human Cytochromes. In: *Molecular Interaction Fields: Applications in Drug Discovery and ADME Prediction*. Weinheim: WILEY-VCH Verlag GmbH and Co. KGaA. 310 p.
- DING J, DAS K, MOEREELS H, KOYMANS L, ANDRIES K, JANSSEN PA, HUGHES SH, ARNOLD E. 1995. Structure of HIV-1 RT/TIBO R 86183 reveals similarity in the binding of diverse nonnucleoside inhibitors. *Nature Struct Biol* 407-415.
- GOODSELL DS, MORRIS GM, OLSON AJ. 1996. Automated docking of flexible ligands: applications of AutoDock. *J Mol Recognit* 9: 1-5.
- GUEX N, PEITSCH MC. 1997. Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* 1997. 18:2714-2723.
- JACOBO-MOLINA A, DING J, NANNI RG, CLARK AD JR, LU X, TANTILLO C, WILLIAMS RL, KAMER G, FERRIS AL, CLARK P, ARNOLD E. 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double stranded DNA at 3.0Å resolution shows bent DNA. *Proc Natl Acad Sci* 90: 6320-6324.
- JACOBS A, SEN J, RONG L, CAFFREY M. 2005. Alanine scanning mutants of the HIV gp41 loop. *The J Biol Chem* 280: 27284-27288.
- KIER LB, HALL LH. 1999. *Molecular Structure Description: The Electrotopological State*; Lemont B. Ed. New York: Academic Press. 286 p.
- KOHLSTAEDT LA, WANG J, FRIEDMAN JM, RICE PA, STEITZ TA. 1992. Crystal structure at 3.5Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 256: 1783 – 1790.
- KOPPEB, MIGLIETLA JJ, SHRUTKOWSKI AG, SHIH CK, GROB PM, SKOOG MT. 1991. Steady state kinetics and inhibition of HIV-1 reverse transcriptase by a non-nucleoside dipyridodiazepinone, BI-RG-587, using a heteropolymeric template. *Nucleic Acids Res* 19: 3035-3039.
- MAI A, ARTICO M, RAGNO R, SBARDELLA G, MASSA S, MUSIU C, MURA M, MARTURANA F, CADEDDU A, MAGA G, LA COLLA P. 2005. 5-alkyl-2-alkylamino-6-(2,6-difluorophenylalkyl)-3,4-dihydropyrimidin-4(3H)-ones, a new series of potent, broad-spectrum non nucleoside reverse transcriptase inhibitors belonging to the DABO family. *Bioorg Med Chem* 13: 2065-2067.
- MEISLER G, TARR D. 1995. *Inorganic Chemistry* 2nd Ed. New Jersey: Prentice Hall, Inc. 480 p.
- O' MEARA J, YOAKIM C, BONNEAU P, BOS M, CORDINGLEY M, DEZIEL R, DOYON L, DUAN J, GARNEAU M, GUSE I, LANDRY S, MALENFANT E, NAUD U, OGILVIE W, THAVONEKHAM B, SIMONEAU B. 2005. Novel 8-Substituted Dipyridodiazepinone Inhibitors with a Broad-spectrum of Activity against HIV-1 Strains Resistant to Non-nucleoside Reverse Transcriptase Inhibitors. *J Med Chem* 48: 5580 – 5588.
- PETTERSEN EF, GODDARD TD, HUANG CC, COUCH GS, GREENBLATT DM, MEN EC, FERRIN TE. 2004. UCSF Chimera - A Visualization System for Exploratory Research and Analysis. *J Comput Chem* 25(13):1605-1612.
- REN J, ESNOUF R, GARMAN E, SOMERS D, ROSS C, KIRBY I, KEELING J, DARBY G, JONES Y, STUART D, AND OTHERS. 1995. High Resolution Structures of HIV -1 RT from four RT-inhibitor complexes. *Nature Struct Biol* 2: 293.
- RICHMAN DD. 1993. Resistance of clinical isolates of human immunodeficiency virus to antiretroviral agents. *Antimicrob. Agents. Chemother* 37: 1207-1213.
- ROMERO DL, BUSSA, M, TAN CK, REUSSER F, PALMER JR, POPPE SM, ARISTOFF PA, DOWNERY KM, SO AG, RESNICK L, TARPLEY WG. 1991. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. *Proc Natl Acad Sci U.S.A.* 88: 8806-8810.
- SMERDON SJ, JAGER REN J, ESNOUF R, GARMAN E, SOMERS D, ROSS C, KIRBY I, KEELING J, DARBY G, JONES Y, STUART D. 1994. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. *Nature Struct Biol* 2: 293.
- SMITH MB, HOSE BM, HAWKINS A, LIPCHOCK J, FARNSWORTH DW, RIZZO RC, TIRADO-RIVES J, ARNOLD E, ZHANG W, HUGHES SH,

- JORGENSEN WL, MICHEJDA CJ, SMITH RH JR. 2003. Molecular modeling calculations of HIV-1 reverse transcriptase nonnucleoside inhibitors: Correlation of binding energy with biological activity for novel 2-aryl-substituted benzimidazole analogues. *J Med Chem* 46: 1940 – 1947.
- SPENCE RA, KATI WM, ANDERSON KS, JOHNSON KS. 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. *Science* 267: 988 – 993.
- SPSS INC. 2001. SPSS for Windows Release 11.0.1 Chicago; Illinois: SPSS Inc.
- TANTILLO C, DING J, JACOBO-MOLINA A, NANNI RG, BOYER PL, HUGHES SH, PAUWELS R, ANDRIES K, JANSENN PA, ARNOLD E. 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase, Implications for mechanisms of drug inhibition and resistance. *J Mol Biol* 243: 369-387.
- WALLACE AC, LASKOWSKI RA, THORNTON JM. 1995. LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Protein Eng* 8:127-134.
- WANG J, KOHLSTAEDTLA, CHIRINO AJ, FRIEDMAN JM, RICE PA, STEITZ TA. 1994. Structure of the binding site for nonnucleoside inhibitors of reverse transcriptase of human immunodeficiency virus type 1. *Proc Natl Acad Sci* 91: 3911-3915.
- WOLD S. 1991. Validation of QSAR's. *Quantitative Structure – Activity Relationship* 10: 191-193.
- YARCHOAN R, MITSUYA H, THOMAS RV, PLUDA JM, HARTMAN NR, PERNO CF, MAREZYK KS, ALLAIN JP, JOHNNS DG, BRODER S. 1989. In vitro activity against HIV and favorable toxicity profile of 2',3'-dideoxyinosine. *Science* 215: 412-415.
- ZAMORA I, AFZELIUS L, CRUCIANI G. 2003. Predicting Drug Metabolism: A Site of Metabolism Tool Applied to the Cytochrome P450 CYP2C9. *J Med Chem* 46: 2313 – 2324