STRUCTURE-BASED INHIBITOR DESIGN OF NEW POTENT HIV-1 REVERSE TRANSCRIPTASE, ACTIVE AGAINST MUTANT TYPE

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). The reverse transcriptase (RT) of human immunodeficiency virus type-1 (HIV-1) is the essential enzyme converting the single-stranded viral RNA genome into double-stranded proviral DNA prior to its integration into the host genomic DNA (Jacobo-Molina *et al.*, 1991; De Clercq, 1995a, b). Nowadays, a lot of crystals of a ternary complex that consists of a complex between the HIV-1 RT and the inhibitors, or between the HIV-1 RT and a double-stranded DNA template/primer or free form HIV-1 RT have been deposited at RCSB protein data bank (PDB) (Lawtrakul *et al*., 2004). HIV-1 RT is an asymmetric heterodimer consisting of a 560 residue chains (p66 subunit) and a 440 residue chains (p51 subunit), as shown in Figure 1. Each subunit can be anatomically compared with a right hand and consists of four subdomains, namely the fingers, the palm, the thumb, and the connection domains. The N-terminal 440 amino acids of p66 constitute the polymerase domain, and the C-terminal 120 amino acids comprise the RNase H domain, which is present in the p66 but not in the p51, subunit (Balzarini, 1999). The active (substrate-binding) site of p66 consists of the catalytically essential D110, D185, and D186 triad, which is further surrounded with several highly conserved amino acids.

Nucleoside inhibitors (NRTIs) and non-nucleoside inhibitors (NNRTIs) are two types RT inhibitors that are widely used for the treatment of AIDS. Nucleoside analog inhibitors, such as AZT, ddI and ddC, are competitive inhibitors causing termination of the growing DNA chain (Figure 2a). NRTIs inhibit viral replication because they lack a hydroxyl group of the ribose ring and, when incorporated into viral DNA, act as chain terminators. Mechanism of action of AZT interfering with

HIV-1 RT is shown in Figure 2b (De Clercq, 2004c). AZT needs to be phosphorylated, in three steps, to the triphosphate form before it can interfere with the reverse transcription reaction. Although these nucleoside inhibitors are selective for HIV-1 RT, they are not highly specific and make cellular polymerases to translate into toxic effects (Tantillo *et al.*, 1994; De Clercq, 1995a, b; Sarafianos *et al.*, 2004). Non-nucleoside analog inhibitors, such as nevirapine, TIBO and efavirenz, are non-competitive inhibitors binding in a hydrophobic pocket that is about 10 Å away from the enzyme's active site in the p66 subunit**,** shown in Figure 3. Unlike NRTIs, the NNRTIs are not incorporated in the growing strand of HIV DNA, but directly inhibit the HIV-1 RT by binding in a reversible and non competitive manner to the enzyme. These lead to a significant slowing rate of polymerization catalyzed by the enzyme (Joly and Yeni, 1999). Because of the use at low concentration and the high specificity, non-competitive inhibitors are interesting for developing novel potent inhibitors (De Clercq, 1997, 1998 and 2004a, b). NNRTIs interact with a specific binding site on the enzyme, therefore any slight variation brought about by a single point mutation can have a significant impact on high-level resistance. The efficiency of these inhibitors is limited by drug resistant mutations, such as K103N, Y181C and V106A (Menendez-Arias, 2002). In the clinic, the most important mutations are K103N and Y181C (De Clercq, 2004a, b). From the crystal structures complexed between HIV-1 RT and NNRTIs, it has been suggested that most of the NNRTIs can adopt the similar conformation, as called 'Butterfly-like' shape. Most of them consist of two wing sections. Their wings usually contain significant ¶-electron systems that can interfere efficiently with amino acid functional groups of the binding pocket (Balzarini, 1999). In the pocket of wild-type HIV-1 RT (Figure 4), the necessary interactions with NNRTIs are staking interactions with the aromatic amino acids Y181, Y188, W229 and Y318, electrostatic interactions with K101, K103 and E138, van der waals interactions with L100, V106, Y181, G190, W229, L234 and Y318 and hydrogen bonding with the main-chain peptide bonds (Smerdon *et al.*, 1994; De Clercq, 2002). Therefore, the mutations can affect NNRTIs binding by losing the important contacts between the binding pocket and NNRTIs, changing the size of the binding pocket and interfering the NNRTIs entry into the binding pocket. From the previous work (Hsiou *et al*, 2001) they indicated that the inhibitors active against

K103N mutation would be expected to have favorable interactions with the mutated asparagine side chain. This suggested that design of novel NNRTIs active against protein mutants should make favorable interactions with that mutant residue.

Nevirapine (Viramune®) was the first generation of NNRTI that has been approved by the FDA for the treatment of HIV-1 infection. The binding affinity of nevirapine against wild type and mutant types RT are shown in Table 1 (Dyatkin et al., 1998). Unfortunately, nevirapine showed a lack of affinity upon two important mutations, the K103N and Y181C mutations. From the available the X-ray structures of K103N and Y181C HIV-1 RT mutants in complex with nevirapine, these revealed that the lack of affinity was caused by the lost of important interaction between nevirapine and mutant residues. The orientation of nevirapine in K103N and Y181C HIV-1 RT binding pockets comparing with that in wild-type HIV-1 RT are shown in Figure 5. These structures were taken from pdb code 1vrt (Ren *et al.*, 1995) 1fkp (Ren *et al.*, 2000) and 1jlb (Ren *et al.*, 2001) for wild-type, K103N and Y181C HIV-1 RT structures, respectively. In Figure 5a, the structures supported that the reduced inhibitory efficacy of nevirapine in K103N mutant was caused by the changes in the chemical environment of N103 substituted binding pocket. Between wild-type and K103N mutant complexes, hydrophobic and electrostatic interactions were changed. In Figure 5b, the Y181C binding pocket revealed that ¶-¶ interaction between phenyl groups of nevirapine and tyrosine 181 in wild-type binding pocket was lost when tyrosine was mutated to be cysteine in Y181C.

In the present, several NNRTIs, i.e. PNU-142721, MSC194 and efavirenz, were more potent against the K103N mutation than nevirapine (Lindberg, *et al*., 2002). PNU-142721 is the most active inhibitor of these inhibitors against the K103N mutation. The potent activity of these inhibitors toward the K103N mutation is explained by new interactions with the N103 (Figure 6). From the X-ray structure of PNU-142721 complexed with the K103N mutant, the substituted amino group at the pyrimidine ring revealed the important H-bonds to K101 and N103. Consequently, this structural information of the complexed structure may lead to the discovery of new efficacious NNRTIs against resistant HIV-1 RT.

Once the knowledge of 3D-structure of receptor is known, an approach of drug design searched for a ligand whose orientation and conformation achieves the highest degree of complementarity with respect to all details of the receptor's steric constraints and interaction geometries is called structure-based drug design (Bajorath, 2002; Lengauer *et. al.*, 2002; Oprea, 2002; Seifert *et. al.*, 2003; Shoichet, 2004).

Structure-based drug design is one of the most powerful method in drug discovery process in recent year because the explosion of genomic, proteomic, and structural information has provided hundreds new targets and opportunities for future drug lead discovery (Car and Jhoti, 2002; Lyne, 2002; Schneider and Böhm, 2002; Anderson, 2003). The target's structure reveals the important binding mode of inhibitor, indicates the essential aspects determining its binding affinity and generates new ideas about ways of improving drug efficacy (Klebe, 2000).

In recent year, several successes using structure-based drug design in discovery of new more potent drugs has been published, for example, amprenavir (Agenerase) and nelfinavir (Viracept) (Kaldor *et al.*, 1997). They are two of the first drugs to reach the market by using structure-based drug design method to design them. Structure-based drug design includes several methods, i.e. molecular docking, fragment methods, 3D database techniques, etc (Marrone *et al.*, 1997). Docking compounds from databases to targets of known structure can be utilized to discover or refine new leads. Structures from a database of small-molecule compounds are fit into the target structure using a docking program. The energies of the resulting complexes are evaluated and those that show the most promise can be experimentally tested as possible lead compounds. For 3D database searching, the pharmacophore model was used to search a database of 3D molecular structures and resulted in a molecule having the same pharmacophore of the model.

In this study, virtual screening by means of 3D pharmacophore searching and molecular docking were used to screen the hits from the database. Virtual screening can be defined as the process of reducing a library containing an unmanageable

number of compounds to a limited of potentially promising compounds for the target of interest (Mestres, 2002).

Nowadays, because of advances in quantum chemical methods and together with impressive improvements in computer speed, quantum chemical calculations are widely used to calculate the electronic structure, structural property and energetic properties (such as geometries, energies, NMR properties, etc.) (Bell, 2004). The details of quantum are described in Appendix A. In quantum chemical calculations, semi-empirical, *ab initio*, and density functional methods can be performed to determine the interaction energy between ligand and surrounded amino acid residues in the binding pocket (Kuno *et al.*, 2003; Nunrium *et al.*, 2005; Mei *et al.*, 2005; Saen-oon *et al.*, 2005). The results can provide the clearer elucidation in protein-ligand binding.

For improving the efficiency of inhibitors insensitive to the K103N and Y181C mutations, structure-based drug design approaches by using 3D-pharmacophore searching and molecular docking were used to find novel inhibitors. In addition, quantum chemical calculations were used to calculate the interaction energy between the designed compound and mutated residue. The works have been separated into two parts.

In chapter I, virtual screening procedures based on the pharmacophores of nevirapine, PNU-142721 and some NNRTIs are used to find novel inhibitors insensitive to the K103N and Y181C mutations from an ‘in-house’ or ‘Bioinfo’ database of a set of 500k commercially available drug-like compounds. The details of virtual screening procedures are explained as following. Firstly, some docking methods, i.e. FlexX, GOLD and Surflex, have been performed with the nevirapine-RT complex to validate and select the best possible strategy. Secondly, before performing the molecular docking with the database, the compounds in the database were filtered by using pharmacophore searching. The 3D pharmacophore models were constructed based on the known important interactions between the amino acid in the binding pocket and NNRTIs. Then, after filtering the database, the selected compounds were

applied to docking. The hits from docking were selected and classified by scaffold diversity using ClassPharmer program. Finally, the selected compounds from the classification were tested for HIV-1 RT inhibition.

In chapter II, for finding novel nevirapine analogue insensitive to the K103N and Y181C mutations, Nevirapine derivatives were designed using a combinatorial library design approach (Krier *et al.*, 2005). In this study, the GOLD program was used to dock nevirapine derivatives the binding pocket of K103N and Y181C HIV-1 RT. The hits from docking were further submitted to a post-docking filtering by topologically analyzing predicted interactions with the SILVER program. The hits presented a significant percentage of their surface buried upon binding (>80%) and exhibiting H-bonds to either N103 or C181 residues of the HIV-RT were finally selected. Furthermore, quantum chemical calculations were performed to calculate the interaction energy between nevirapine derivatives and mutant residues. The results will give more information on the interaction between nevirapine derivatives and N103 or C181.

Therefore, structure-based drug design approaches by using 3D-pharmacophore searching, molecular docking and quantum chemical calculations were applied to screen and design new inhibitors insensitive to the K103N and Y181C mutations with the aims of:

1. To find the novel inhibitors active against the K103N and Y181C mutations by performing the virtual screening procedures with ‘Bioinfo’ database.

2. To design the new nevirapine analogues active against the K103N and Y181C mutations by using combinatorial library design approach, molecular docking and quantum chemical calculations.

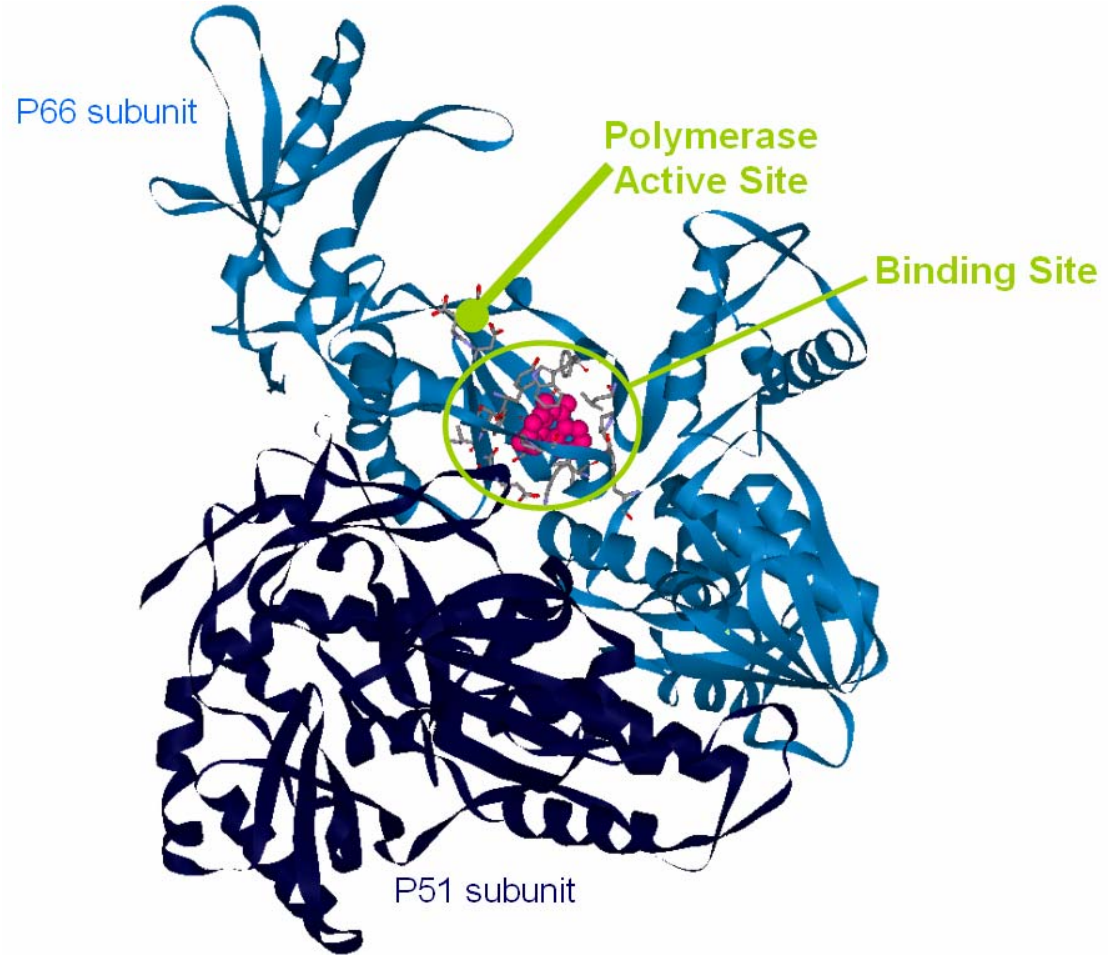
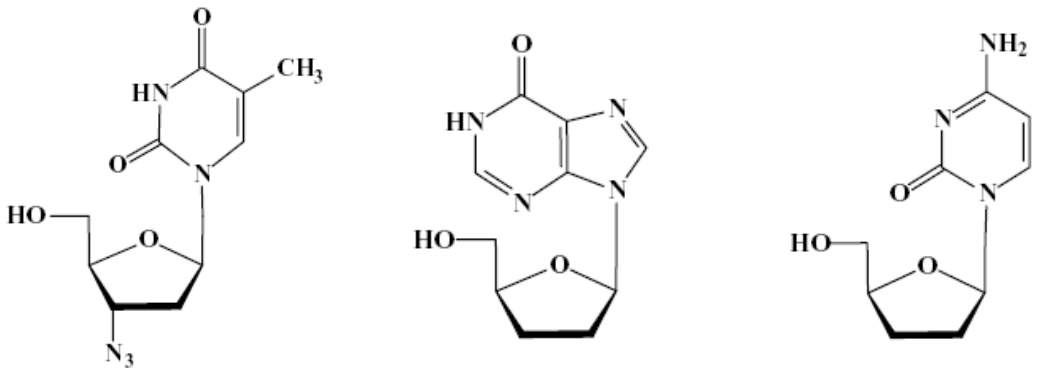
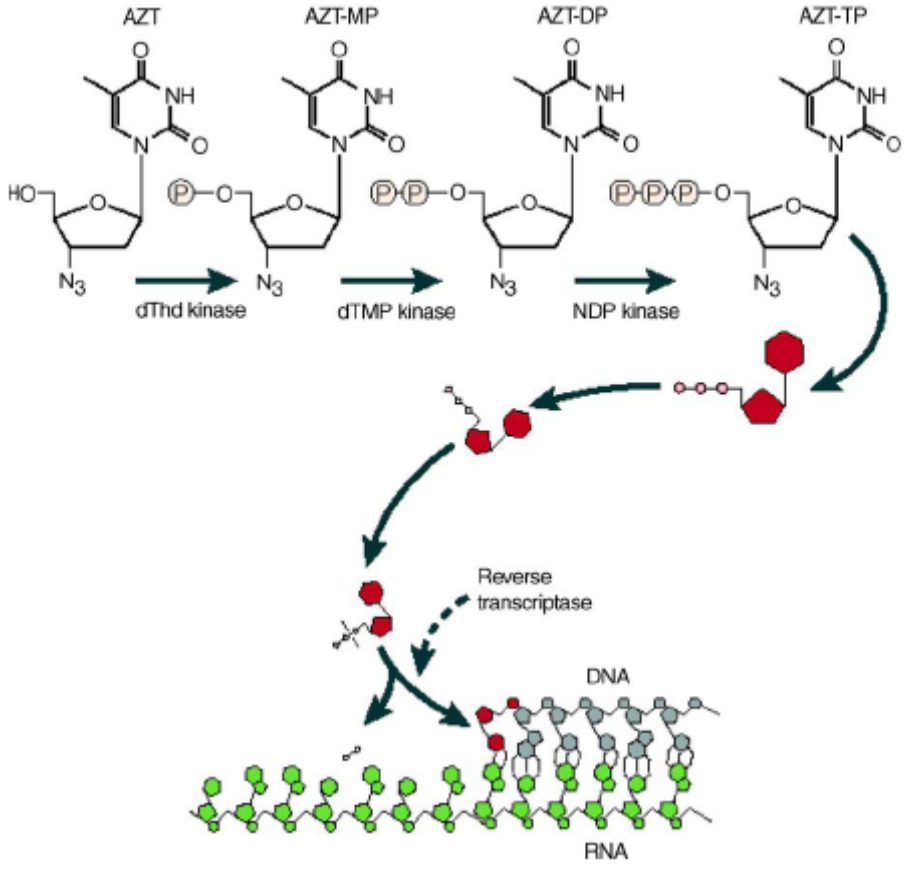


Figure 1 Structure of HIV-1 RT (taken from PDB entry 1vrt).



AZT ddI ddC

a)

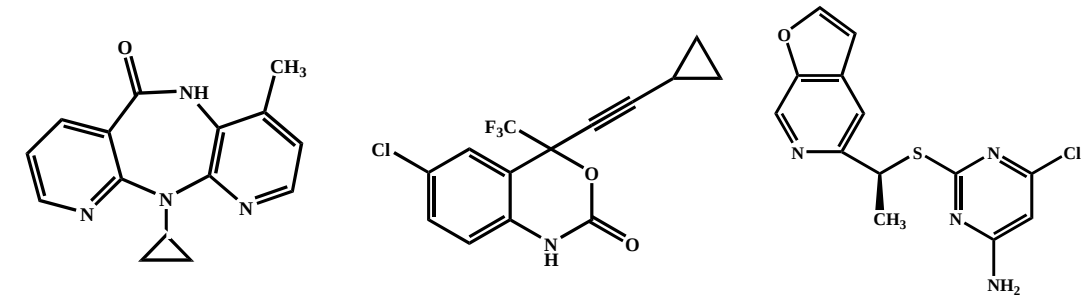


b)

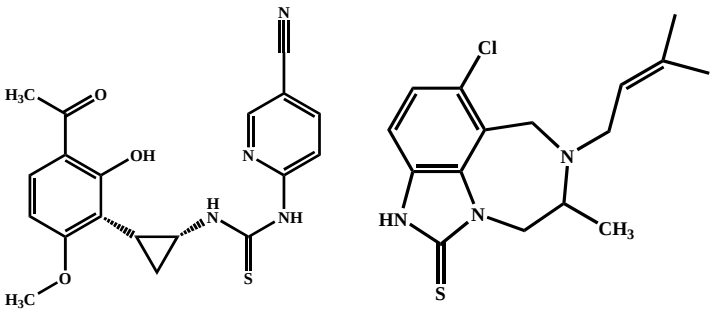
Figure 2 a) Structures of nucleoside reverse transcriptase inhibitors (NRTIs).

b) Mechanism of action of AZT interfering with the HIV-1 RT reaction.

Source: De Clercq (2004c).



Nevirapine Efavirenz PNU-142721



MSC-194 8-Cl TIBO

Figure 3 Structures of non-nucleoside reverse transcriptase inhibitors (NNRTIs).

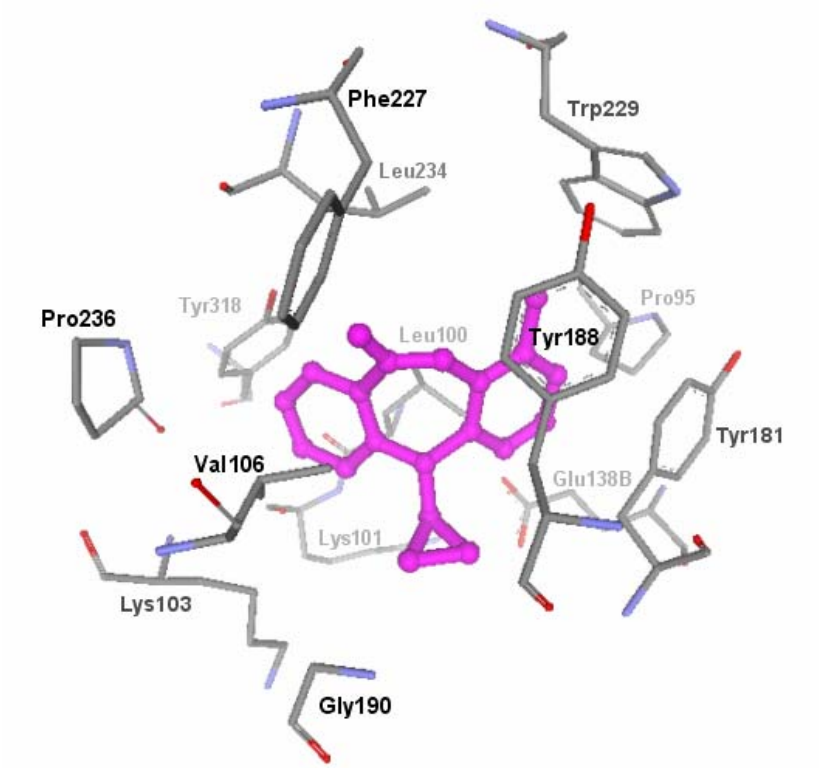
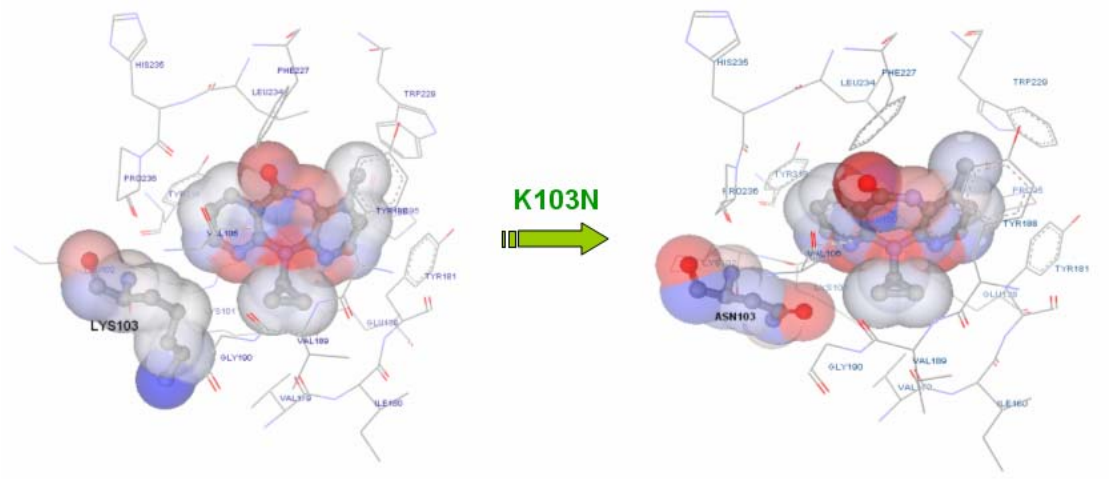


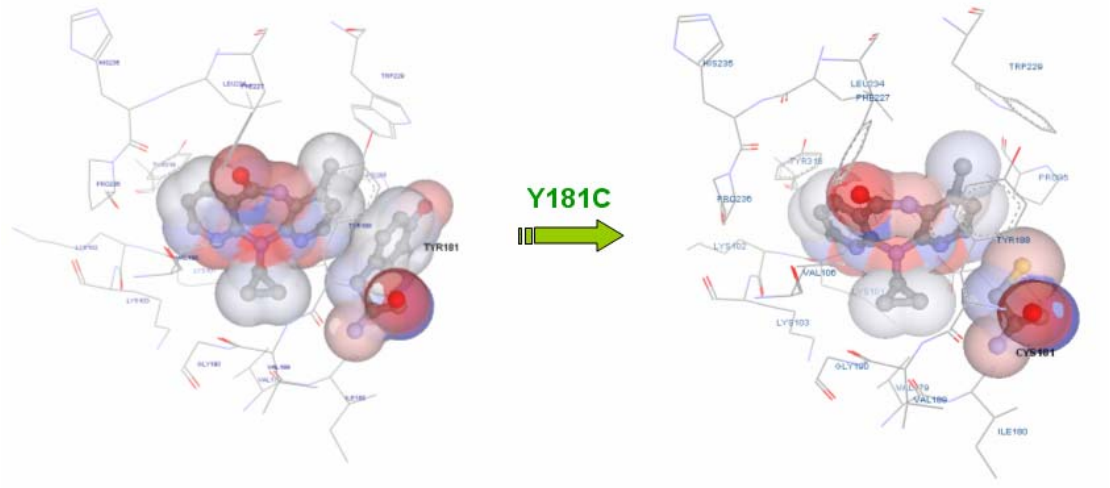
Figure 4 Binding pocket of wild-type HIV-1 RT complex with nevirapine (taken from PDB entry 1vrt).

Table 1 Binding affinities of nevirapine against wild type and mutant types RT.

Compound	IC_{50} (μM)				
	Wild Type	Y181C	P236L	K103N	L100I
Nevirapine	0.06	3.2	0.18	1.3	0.17



a)



b)

Figure 5 Binding pocket of wild-type, K103N (a) and Y181C (b) HIV-1 RT complex with nevirapine. The electrostatic potential of nevirapine and the mutant residues are shown on the van der Waals surface as red for negative and blue for positive values.

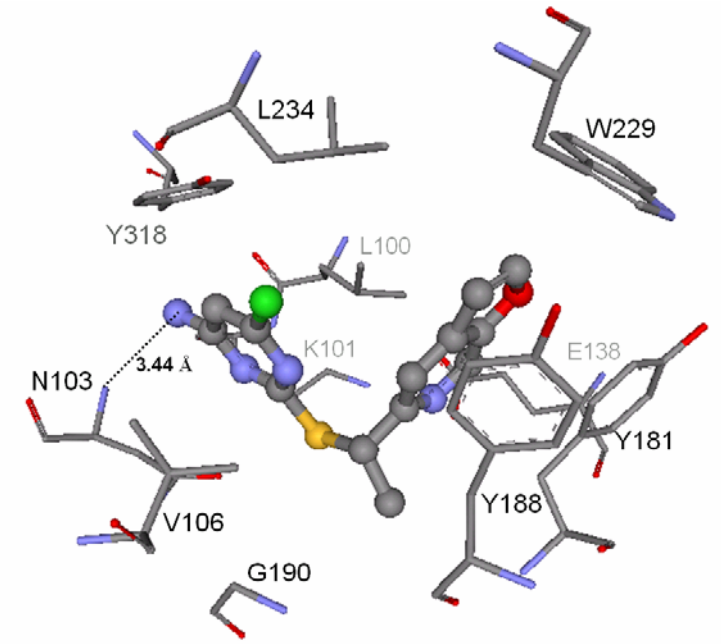


Figure 6 Orientation of PNU-142721 (displayed in ball and stick model) in the K103N binding pocket (taken from PDB code 1ikx (Lindberg, *et al.*, 2002)).