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PRELIMINARY STRUCTURAL ANALYSIS OF THE MUTATIONS SELECTED BY NON-NUCLEOSIDE INHIBITORS OF HIV-1 REVERSE TRANSCRIPTASE

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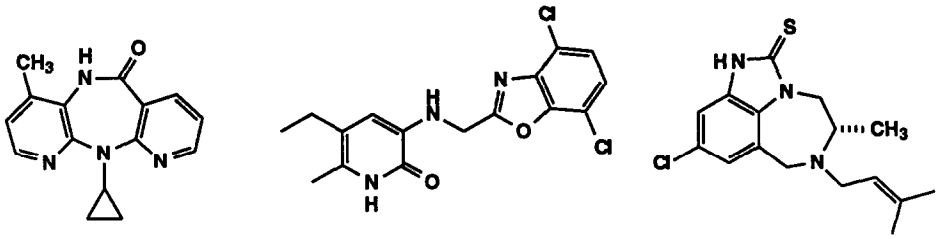
Abstract The crystal structure of HIV reverse transcriptase complexed with nevirapine showed that most of the mutations that confer resistance to non-nucleoside inhibitors of RT are clustered around the nevirapine binding pocket. Establishment of favorable interactions with conserved residues in this pocket is essential for the design of second generation RT inhibitors.

The inhibition of HIV-1 reverse transcriptase (RT), the enzyme responsible for converting viral RNA to double-stranded viral DNA, has been demonstrated to be a clinically useful approach for the treatment of AIDS.[1] To date, only nucleoside-based inhibitors (AZT, ddI and ddC) have been approved, though these drugs are sub-optimal in their antiviral and toxicological profiles.[2] More recently, several non-nucleoside inhibitors (nevirapine, Merck L697,661, TIBO R82913; Fig.1) have been described as very specific and potent inhibtors of HIV-1 RT and several compounds have progressed to clinical evaluation.[3]



Nevirapine **Merck L697,661** **TIBO R82913**

Figure 1. Three of the RT Non-nucleoside Inhibitors.

One of the severe limitations of all the RT inhibitors results from the high mutation rate of the target enzyme, which is ascribed to the poor replication fidelity of retroviral polymerases.[4] The presence of drugs which inhibit RT (and thus viral replication) selects escape mutants of HIV.[5]

This observation has been made in cell culture experiments and in AIDS patients as well (Table 1).[5] The resultant mutations which render RT less sensitive to inhibitors are likely the reason that only short term clinical effectiveness is achieved with compounds such as AZT.

With respect to the non-nucleoside inhibitors, the structural basis for the resistance mutations can begin to be rationalized based on the recent crystal structure at 3.5Å resolution of HIV-1 RT complexed with nevirapine (Fig. 1).[6] Nevirapine binds RT in a pocket adjacent to the polymerase active site and presumably causes enzyme inhibition through an allosteric effect. The pocket is hydrophobic in nature and is formed by residues from β-strands 5, 6, 9, 10, 12, 13, and 14, corresponding roughly to amino acid residues 95-110 (strands 5 and 6), 178-191 (strands 9 and 10), and 220-240 (strands 12, 13 and 14) (Fig. 2). The pocket is V-shaped with strands 6, 9 and 10 forming one side and strands 12, 13, and 14 forming the other side. Strand 5 and the loop connecting strands 5 and 6 cover the top of the 'V', enclosing nevirapine in the hydrophobic pocket.

Table 1. Some of the Resistance Mutations of Non-nucleoside RT Inhibitors[5]

Inhibitor	Mutations observed from	
	Cell Culture	Patients
Nevirapine	Y181C	Y181C, V106A, Y188C, Y188L
Merck L697,661	Y181C, K103N	Y181C, K103N
TIBO R82913	Y181C, L100I	Y181C

The crystal structure reveals that the A ring of nevirapine lies in close proximity to the side chain of Tyr181, consistent with previous photo-affinity labeling[7] and site-directed mutagenesis studies.[8] The cyclopropyl ring of nevirapine is situated close to Gly190. The side chains of Val106, Val108, Val179 and Tyr188 in strands 6, 9 and 10 also contribute to the hydrophobic environment of the pocket (Fig. 3). The resistance mutations so far identified from cell culture studies and clinical trials with nevirapine are all located in this pocket (Table 1). It is expected that the first mutations that are selected upon treatment with a given drug should be located in a region of strong inhibitor-protein interaction. Resistance mutations identified with other non-nucleoside inhibitors (Fig. 1) also cluster near this binding site (Table 1), suggesting that these non-nucleoside inhibitors share this binding pocket. The differences in the pattern of resistance mutations imply that the non-nucleoside RT inhibitors interact with different, though overlapping, regions of this pocket, as resistance mutations that are selected by one compound generally confer resistance to other non-nucleoside inhibitors as well.[9] The mutations in RT selected with nevirapine (Table 1) usually lead to an 100 to 1000-fold reduction in the potency of nevirapine against RT.[5]

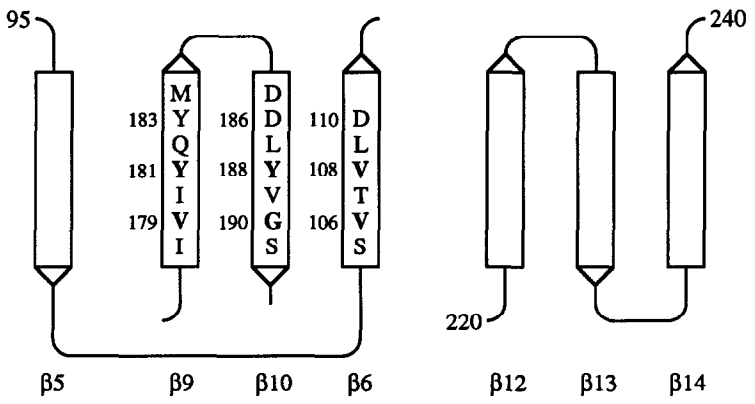


Figure 2. Topological Drawing of the Nevirapine Binding Pocket.
Residues in strands β6, β9 and β10 that face nevirapine are shown in bold.

Drug resistance arises as a result of changes in the binding pocket, through possibly 3 mechanisms: 1) loss of favorable interaction between the inhibitor and the protein (*e.g.* Tyr181 to Cys mutation may result in a loss of π stacking with nevirapine, and presumably TIBO and the Merck compounds as well (Table 1)); 2) creation of steric hindrances between the inhibitor and the protein, (*e.g.* Mutations at Gly190 may alter the preferred binding orientation of nevirapine due to the repulsive steric interactions between the amino acid side chain and the cyclopropyl ring of nevirapine); and 3) induction of main chain conformation changes in the binding pocket as a result of the mutations. It can be expected that mutations at residues 106, 108, 179, 181, 188 and 190 may confer resistance to non-nucleoside RT inhibitors. Mutations at those residues in the other β-strands and the connecting loops that face this binding pocket may also lead to resistance (Table 1).

Comparisons of reverse transcriptase amino acid sequences from different immunodeficiency viruses showed that many of the residues in or close to the nevirapine binding pocket are highly conserved (Table 2). Mutations at these residues may not be tolerated by the virus. For example, mutations of the catalytic aspartic acid residues (Asp110, Asp185 and Asp186) and Tyr183 resulted in inactive RT enzymes.[10] Preliminary results showed that mutation of Trp229 into Ala led to a substantial decrease in RT activity.[11]

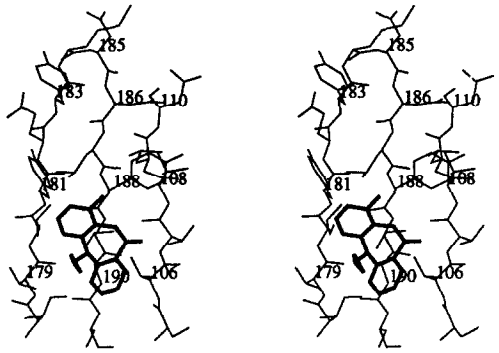


Fig. 3. Stereo drawing showing nevirapine (thick lines) and residues in strands β6, β9 and β10 (thin lines). The model was derived based on the C_α positions, which was determined from the crystallographic study[6]. The main chain atoms were positioned with hydrogen-bonding restraints and the side chains were assigned extended conformations using the program FRODO[12].

Table 2. Alignment of RT Sequences Around the Nevirapine Binding Pocket*

	95 100 105 110	180 185 190	220 225 230 235 240
(1)	PHPAGLKKKKSVTVLD	IVIYQYMDDLYVGSD	KHQKEPPFLWMGYELHPDKWT
(2)	----------	-T----------	----------
(3)	------R-MRQI----	LT-V------W---Q	-M-----YE----K-W-H--E
(4)	------QI--Q-----	LD-------I-I--N	-L-E---YT--------LT--
(5)	-Y-P-I-ECEHL-AI-	VML-------LI--N	-V-E-.RVK-I-F--T-K--R
(6)	------A---RI----	VILI-----ILIA--	-F--D---Q------W-T--K
(7)	------A-R-RI----	VTLV-----ILIA--	-F--D---Q------W-T--K
(8)	------A---RI----	VTLI-----ILIA--	-F--D---Q------W-T--K
	β5 and β6	β9 and β10	β12, β13 and β14

* The sequences used in the alignment -- (1) HIV-1, (2) Chimpanzee, (3) Simian (African green monkey), (4) Feline, (5) Bovine, (6) HIV-2, (7) Simian (Macaque), and (9) Simian (Mangaby).

The principal goal in the design of second generation RT inhibitors based on nevirapine is to construct compounds which have a broad spectrum of activity against the various mutated, yet enzymatically competent, RT enzymes. An important component of this process is the identification of conserved residues in and near the nevirapine binding pocket and the establishment

of favorable interactions between the inhibitors and these residues. The success of this strategy will be demonstrated by the synthesis of new compounds which not only are potent inhibitors of wild type RT as well as existing drug resistant mutants, but also cause a significant delay in the emergence of new resistant strains in cell culture and eventually in patients.

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