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Review

Perspectives of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection

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Abstract

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have, in addition to the nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), gained a definitive place in the treatment of HIV-1 infections. Starting from the HEPT and TIBO derivatives, more than thirty structurally different classes of compounds have been identified as NNRTIs, that is compounds that are specifically inhibitory to HIV-1 replication and targeted at the HIV-1 reverse transcriptase (RT). Two NNRTIs (nevirapine and delavirdine) have been formally licensed for clinical use and several others are (or have been) in preclinical and/or clinical development [tivirapine (TIBO R-86183), loviride (α-APA R89439), thiocarboxanilide UC-781, HEPT derivative MKC-442, quinoxaline HBY 097, DMP 266 (efavirenz), PETT derivatives (trovirdine, PETT-4, PETT-5) and the dichlorophenylthio(pyridyl)imidazole derivative S-1153]. The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from, the NRTI binding site. NNRTIs are notorious for rapidly eliciting resistance due to mutations of the amino acids surrounding the NNRTI-binding site. However, the emergence of resistant HIV strains can be circumvented if the NNRTIs, preferably in combination with other anti-HIV agents, are used from the start at sufficiently high concentrations. In vitro, this procedure has been shown to 'knock-out' virus replication and to prevent resistance from arising. In vivo, various triple-drug combinations containing NNRTIs, NRTIs and/or PIs may result in an effective viral suppression and ensuing immune recovery. However, this so-called HAART (highly active antiretroviral therapy) may also fail, and this necessitates the design of new and more effective drugs and drug cocktails.

Keywords: NNRTIs (non-nucleoside reverse transcriptase inhibitors); Nevirapine; Delavirdine; Reverse transcriptase; HIV; Triple-drug combinations

1. Introduction

The therapy of HIV (human immunodeficiency virus) infections has, since the advent of azidothymidine, been dominated by the 2′,3′-dideoxynucleoside (ddN) derivatives, five of which are now licensed for clinical use: azidothymidine (AZT, zidovudine, Retrovir®), dideoxyinosine (ddI, didanosine, Videx®), dideoxycytidine (ddC, zalcitabine, Hivid®), didehydrodideoxythymidine (d4T, stavudine, Zerit®) and 3′-thiadideoxycytidine (3TC, lamivudine, Epivir®). Four HIV protease inhibitors, i.e. saquinavir (Invirase®), ritonavir (Norvir®), indinavir (Crixivan®) and nelfinavir (Viracept®) have joined the current anti-HIV drug armamentarium [1,2] and NNRTIs (non-nucleoside reverse transcriptase inhibitors) [3,4] have recently gained an increasingly important role in the therapy of HIV infections. Several NNRTIs have proceeded onto clinical development (i.e. tivirapine, loviride, MKC-442, HBY 097, DMP 266) or are already licensed for clinical use [nevirapine (Viramune®), delavirdine (Rescriptor®)].

The era of the NNRTIs started about a decennium ago with the discovery of 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT) [5,6] and tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) [7,8] as specific HIV-1 inhibitors, targeted at the HIV-1 reverse transcriptase [7–10]. These compounds excelled by their unique specificity for HIV-1: they were highly active against HIV-1, but inactive against HIV-2 or any other retrovirus, and, furthermore, their antiviral action could be attributed to a specific interaction with the viral reverse transcriptase (RT).

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Following the HEPT and TIBO derivatives, nevirapine (BI-RG-587) [11,12], pyridinone derivatives L-696,229 and L-697,661 [13,14], and bis(heteroaryl)-piperazine (BHAP) derivatives U-88204 and U-90152 [15,16] were identified as specific HIV-1 inhibitors. In contrast with HEPT and TIBO, which were first found to inhibit HIV-1 replication in cell culture before their target (HIV-1 RT) was unraveled, nevirapine, pyridinone and BHAP were discovered through an HIV-1 RT screening programme before their antiviral activity in cell culture was established.

Following HEPT, TIBO, nevirapine, pyridinone and BHAP, yet other compounds, i.e. TSAO-T (and TSAO-m^3T) [17–19], loviride [α-APA (R89439)] [20], PETT (LY 300046) [21], new derivatives from HEPT [i.e. I-EBU (MKC-442)] [22] and TIBO [i.e. 8-chloro-TIBO (tivirapine, R86183)] [23], and various other compounds were described as specific HIV-1 RT inhibitors, and all these compounds have been collectively referred to as NNRTIs [3,4] to distinguish them from the ddNs, now also referred to as NRTIs (nucleoside reverse transcriptase inhibitors) [1,2].

2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

To qualify as an NNRTI, the compound should interact specifically with (a non-substrate binding site of) the RT of HIV-1, and inhibit the replication of HIV-1, but not HIV-2 (or any other retrovirus) at a concentration that is significantly lower than the concentration required to affect normal cell viability [3]. The potency and selectivity of the NNRTIs as specific inhibitors of HIV-1 should be evident from the EC_{50} (50% effective concentration, required to inhibit HIV-1 replication in cell culture) and the ratio of their CC_{50} (50% cytotoxic concentration) to the EC_{50}. The latter is termed selectivity index (SI). The interaction of the NNRTIs with HIV-1 RT should be evident from their Ki or IC_{50} (50% inhibitory concentration) for the enzyme.

Based on these premises, more than thirty different classes of NNRTIs could be considered (Figs. 1–7): TIBO derivatives [i.e. 8-chloro-TIBO (R86183, tivirapine)] [23], HEPT derivatives [i.e. I-EBU (MKC-442)] [22,24], dipyridodiazepinones [i.e. nevirapine (BI-RG-587)] [11,12], pyridinones (i.e. L-697,661) [13], BHAP derivatives [i.e. delavirdine (U-90152)] [25], TSAO derivatives (i.e. TSAO-m^3T) [17,18], α-APA derivatives [i.e. loviride (R89439)] [20], PETT derivatives [i.e. trovirdine (LY 300046)] [21,26,27], thiocarboxanilide derivatives (i.e. UC-781) [28,29], quinoxaline derivatives (i.e. HBY 097) [30,31], thiazolobenzimidazole (TBZ, NSC 625487) [32,33], thiazoloisoindolinone (BM + 51.0836) [34,35], indole carboxamide L-737,126

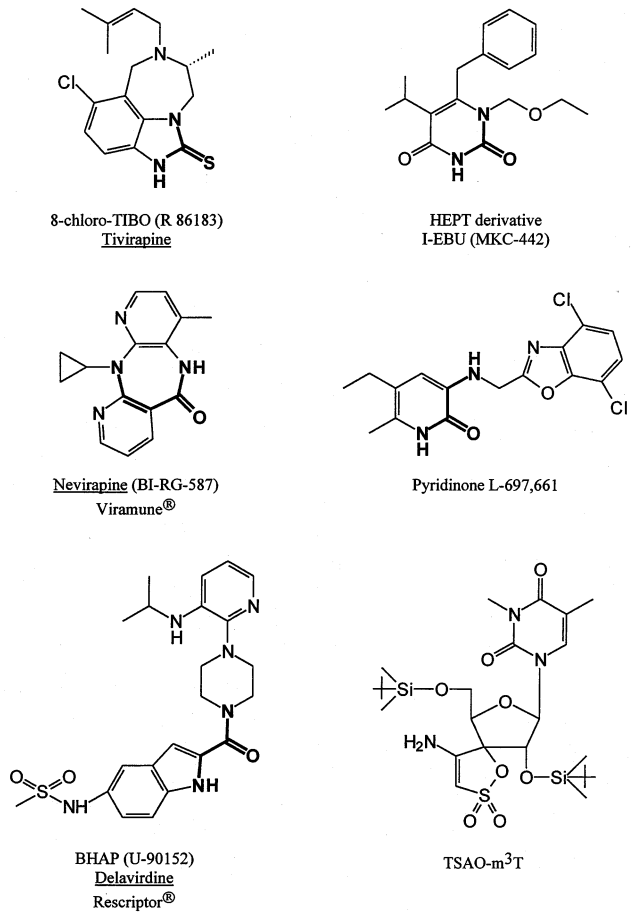


Fig. 1. Structural formulae of the non-nucleoside reverse transcriptase inhibitors (NNRTIs).

[36], benzothiadiazine NSC 287474 [37], quinazolinone (**13a**) [38] (L-738,372 [39]), benzoxazinone DMP 266 (efavirenz) [40], calanolide A [41,42], pyrrolobenzodiazepinone (**4**) [43], imidazodipyridodiazepine UK-129,485 [44], imidazopyridazine (**33**) [45], thiadiazolyl dialkylcarbamate (TDA RD-4-2024) [46,47], arylpyridodiazepine and -thiodiazepine derivatives (i.e. MEN 10979) [48], DABO derivatives (i.e. DABO (**12e**)) [49,50], HEPT-pyridinone hybrids (i.e. (**8a**)) [51], benzyloxymethylpyridinone (**18a**) [52], alkoxy(arylthio)-uracil (**18**) [53], indolyldipyridodiazepinone (**7a**) [54], pyrrolobenzoxazepinone (**16e**) [55], highly substituted pyrroles [56], benzylthiopyrimidine U-31355 [57], pyridazinobenzoxazepinones [58], and some imidazobenzodiazepine and imidazoquinazoline derivatives [59]. More recently, benzothiadiazepines and pyrrolobenzothiadiazepines [60], indolobenzothiazepines [61], furopyridinylthiopyrimidinamine PNU-142721 [62], new PETT derivatives (MSC-204, the active metabolite of PETT-4; and PETT-5 or MSH-372) [63], pyrido[1,2a]indole BCH-4989 [64], S-1153 [or 5-(3,5-dichlorophenyl)thio-4-isopropyl-1-(4-pyridyl)methyl-1*H*-imidazol-2-ylmethyl carbamate] [65],

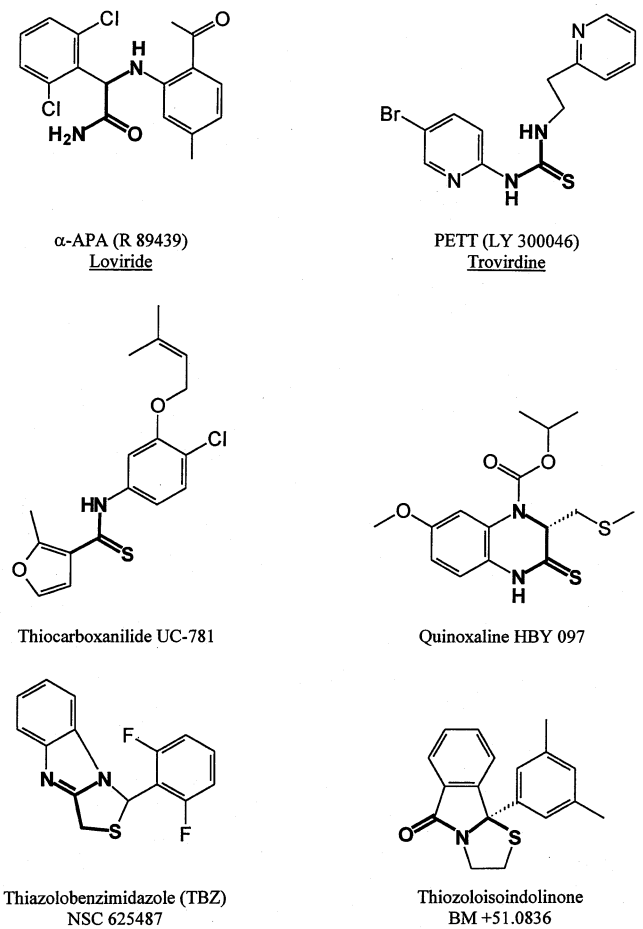


Fig. 2. Structural formulae of the NNRTIs.

trioxothienothiadiazine (TTD) derivatives [66,67], and a variety of new HEPT derivatives (which, however, did not prove as active as I-EBU (MKC-442) [68,69]) have also been described as specific HIV-1 RT inhibitors.

As shown in Table 1, the most potent and most selective congeners among the NNRTIs, i.e. tivirapine, MKC-442, loviride, thiocarboxanilide UC-781, quinoxaline HBY 097, efavirenz and PETT-5, were found to inhibit HIV-1-induced cytopathicity at nanomolar concentrations, with selectivity indexes of 20 000–200 000. All the compounds listed in Table 1 were also found to inhibit HIV-1 RT activity, albeit at widely varying IC_{50} values, which may at least partially be related to the varying assay conditions (i.e. nature of the primer/template) used to monitor RT activity.

2.1. Interaction of NNRTIs with their pocket site at the HIV-1 reverse transcriptase

Whereas the ddN analogues (i.e. AZT, ddI, etc.), following their intracellular phosphorylation to the triphosphate form, interact with the substrate binding site of the HIV reverse transcriptase (RT), the NNRTIs block the HIV-1 RT reaction through interaction with an allosterically located, non-substrate binding site [3,4]. This NNRTI-binding site ('pocket') is located at a close (about 10 Å) distance from the substrate-binding site [70]. It is not only spatially but also functionally [71,72] associated with the substrate-binding site. The cooperative interaction between these two sites [73] provides a means to increase the effectiveness of nucleoside RT inhibitors and non-nucleoside RT inhibitors by using them in combination therapy.

Several studies have revealed a common mode of binding for the chemically diverse NNRTIs with their target site at the HIV-1 RT [74]. The NNRTIs cause a repositioning of the three-stranded β-sheet in the p66 subunit (containing the catalytic aspartic acid residues 110, 185 and 186) [75]. This suggests that the NNRTIs inhibit HIV-1 RT by locking the active catalytic site in an inactive conformation, reminiscent of the conformation observed in the inactive p51 subunit [75]. When bound into their pocket at the HIV-1 RT, the NNRTIs (i.e. α-APA R95846 [76], 8-chloro-TIBO (R86183) [77], 9-chloro-TIBO (R82913) [78] and nevirapine [79]) maintain a very similar conformational 'butterfly-like' shape. They roughly overlay each other in the binding pocket and appear to function as π-electron donors to aromatic side-chain residues surrounding the pocket [79].

The bulky U-90152 (delavirdine) occupies the same pocket as other NNRTIs, but the complex is stabilized quite differently, in particular by hydrogen bonding to the main chain of Lys-103 and extensive hydrophobic contacts with Pro-236. When bound, part of U-90152 protrudes into the solvent creating a channel between Pro-236 and the polypeptide segments 225-226 and 105-106, thus providing evidence for the entry mode of NNRTIs [80]. The greater sensitivity of the P225H recombinant mutant to BHAP U-90152 [81] is in full agreement with the structure of the RT-BHAP complex determined by X-ray crystallography (Fig. 8). To accommodate BHAP, P225 has to swing around by approximately 180°, leaving P225 exposed on the surface of the enzyme. A change from proline to histidine is likely to stabilize the RT-BHAP complex, thereby resulting in an increased sensitivity of the P225H mutant to BHAP U-90152 [81].

The TTD derivative QM96521 [66] can be positioned in the NNRTI binding pocket as shown in Fig. 9. The modeled conformation of QM96521 is similar to the conformation of nevirapine in its complex with HIV-1 RT [74]. Not only do the positions of the fused rings overlap, but the cyclopropyl group of nevirapine (which has a spatial counterpart in all RT-NNRTI structures reported) is mimicked by the N_4-cyanomethyl group of QM96521. Yet, the lack of hydrogen bonding possibilities between the RT and QM96521 makes identification of the correct conformation particularly difficult [66].

The binding of the thiocarboxanilide UC-781 in its HIV-1 RT pocket [82] is shown in Fig. 10. The thiocar-

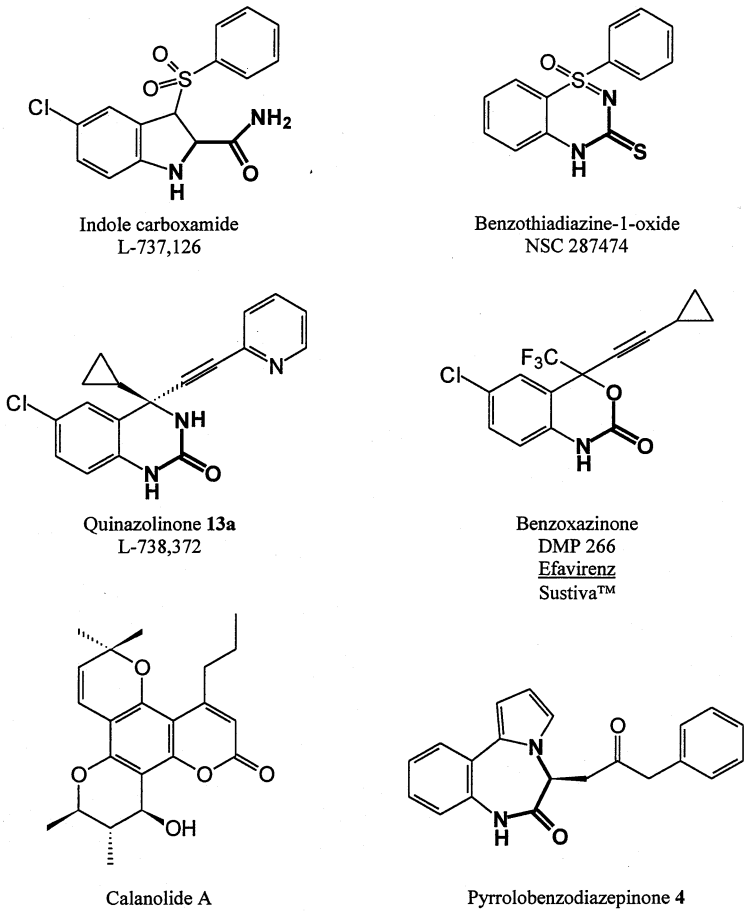


Fig. 3. Structural formulae of the NNRTIs.

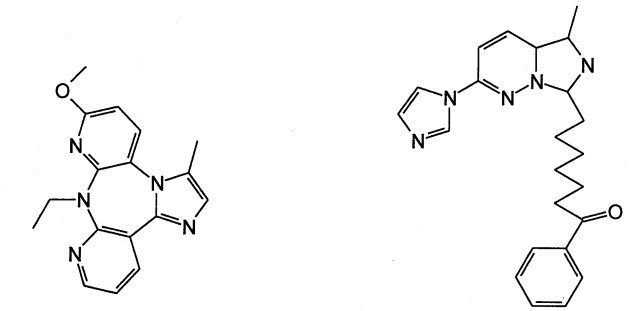
boxanilides bind to their pocket in a similar fashion as the other NNRTIs, i.e. through hydrogen binding with the main chain oxygen of Lys-101 and hydrophobic interactions with Leu-100, Val-106, Val-179, Tyr-188, Phe-227, Leu-234 and His-235. The thiocarboxanilide UC-781 also makes important hydrophobic interactions [82] with Trp-229.

Structural modelling studies have demonstrated that, despite their diverse structures, the positions and conformations adopted by the NNRTIs, when bound to HIV-1 RT, seem to be quite similar, as illustrated in Fig. 11 by the superposition of the thiocarboxanilide UC-781 and the quinoxaline S2720, and in Fig. 12 by the superposition of the TTD QM96521 and nevirapine, relative to the structure of the surrounding protein in the models of the NNRTI-RT complexes [66,82].

2.2. HIV-1 resistance to NNRTIs

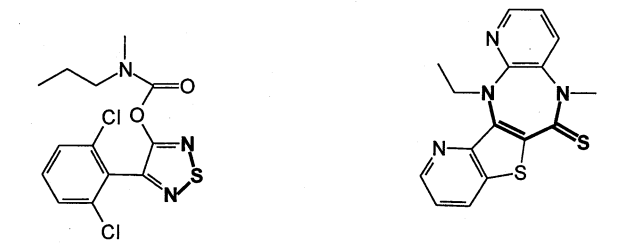
Inevitably, the rapid replication of HIV and its inherent genetic variability must lead to the generation of viral variants that exhibit drug resistance [84]. NNRTIs have been notorious for rapidly triggering the emergence of drug-resistant HIV-1 variants. The first RT mutations shown to be associated with, and to account for, HIV-1 resistance to NNRTIs were the K103N and Y181C mutations, engendering resistance to pyridinone [85], nevirapine [86] and TIBO R82150 [87]. In fact, the mutations K103N and Y181C have been observed with virtually all the NNRTIs (Fig. 13), except for the quinoxalines (i.e. HBY 097). The latter preferentially induce mutations at the RT position 190 (i.e. G190E) [88], particularly under high selective pressure (whereas under low selective pressure, mutations L100I, K103N, V106A/I/L, Y181C and G190A/T/V are induced [89]). Concomitantly with the G190E mutation, HBY 097 induces the mutations L74V and V75I [90,91]. Other 'specific' NNRTI mutations include L100I (TIBO 82150 [92]), E138K (TSAO-T [93,94]), and P236L (BHAPs [95]). Resistance to the HEPT derivatives (i.e. MKC-442) can be associated with mutations K103N, V108I and Y181C [96] or yet others [97], although MKC-442 (I-EBU) still retains sufficient activity against the Y181C mutant (EC_{50}: 0.22 μM) as compared to its activity against the wild-type (EC_{50}: 0.002 μM) [98].

The emergence of NNRTI resistance mutations (which, as a rule, are located at the amino acid residues



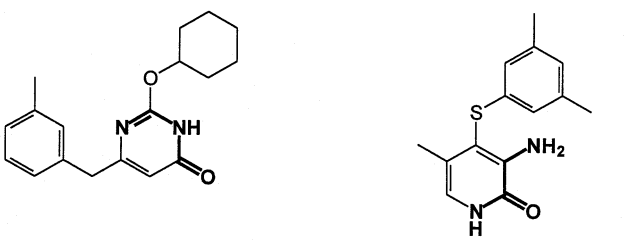
Imidazodipyridodiazepine
UK-129,485

Imidazopyridazine **33**



Thiadiazolyl dialkylcarbamate
(TDA RD 4-2024)

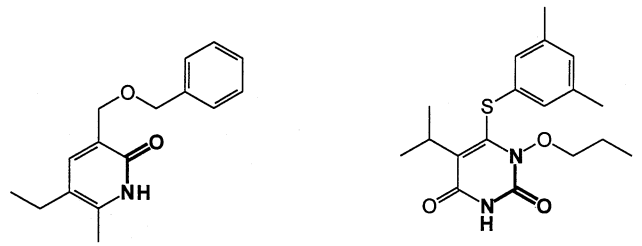
Arylpyridothiodiazepine
MEN 10979



DABO **12e**

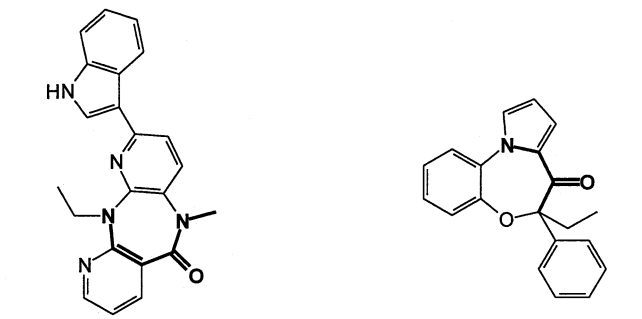
HEPT-pyridinone
hybrid **8a**

Fig. 4. Structural formulae of the NNRTIs.



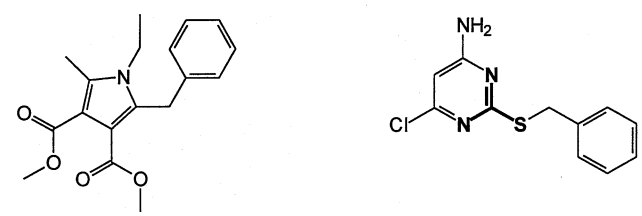
Benzyloxymethylpyridinone **18a**

Alkoxy(arylthio)uracil **18**



Indolyldipyridodiazepinone **7a**

Pyrrolobenzoxazepinone **16e**



Highly substituted pyrrole **3**

Benzylthiopyrimidine
U-31355

Fig. 5. Structural formulae of the NNRTIs.

aligning the NNRTI-binding 'pocket' site) is generally felt as compromising the clinical utility of the NNRTIs. Yet, it should be recognized that several NNRTI classes, viz. quinoxalines (i.e. S-2720, HBY 097) and thiocarboxanilides (i.e. UC-781) still retain pronounced activity against HIV-1 RT mutants containing the L100I, K103N, V106A and Y181C mutation (Table 2) [98–100]. Also DMP 266 (efavirenz) is equally active against the V108I, V179D, Y181C mutant wild-type HIV-1 [40]. The thiocarboxanilides are only 10–20-fold less active against those HIV-1 mutants (i.e. L100I, V106A, E138K and V179D) that they select for in vitro [100]. Also, the imidazole derivative S-1153 does not show more than a 3–10-fold increase in EC_{50} against various HIV-1 mutants (i.e. L100I, K103N, V106A, Y181C, Y188C, G190A, P236L) as compared to its EC_{50} (0.3 ng/ml) for the wild-type virus [65].

More remarkably, the P236L mutation that confers resistance to BHAP U-90152 (delavirdine) causes hypersensitivity to other NNRTIs such as nevirapine, TIBO and pyridinone [95]. Recently, a novel mutation (P225H) was identified that consistently appeared in a V106A mutant background and conferred additional resistance to all NNRTIs, except for delavirdine, which actually showed hypersensitivity towards the P225H mutant [81,101]. Another novel mutation (F227L) that arose in a V106A mutant background was found to confer high-level resistance to virtually all NNRTIs that were examined [102]. It has become increasingly clear that combinations of different RT mutations (i.e. L100I + K103N, or K101D + K103N, or K103N + Y181C, or V106A + F227L) are required for engendering high-level resistance to NNRTIs. It is not clear, however, whether such double-mutants readily arise in vivo, in patients under NNRTI treatment.

With nevirapine, the most common resistance mutation observed in vivo is Y181C, and this mutation is prevented from emerging by coadministration of AZT [103]. Vice versa, the Y181C mutation [104] and L100I mutation [105] in an AZT resistance background (T215Y) significantly suppress (phenotypic) resistance to AZT. Concomitant combination of 3TC with HBY

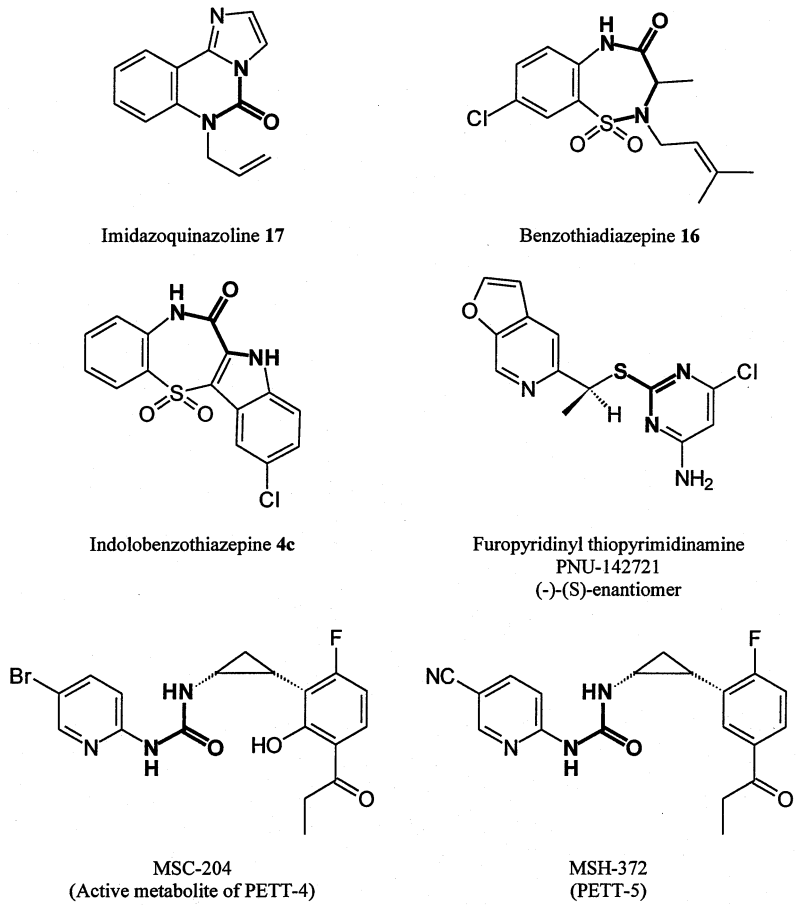


Fig. 6. Structural formulae of the NNRTIs.

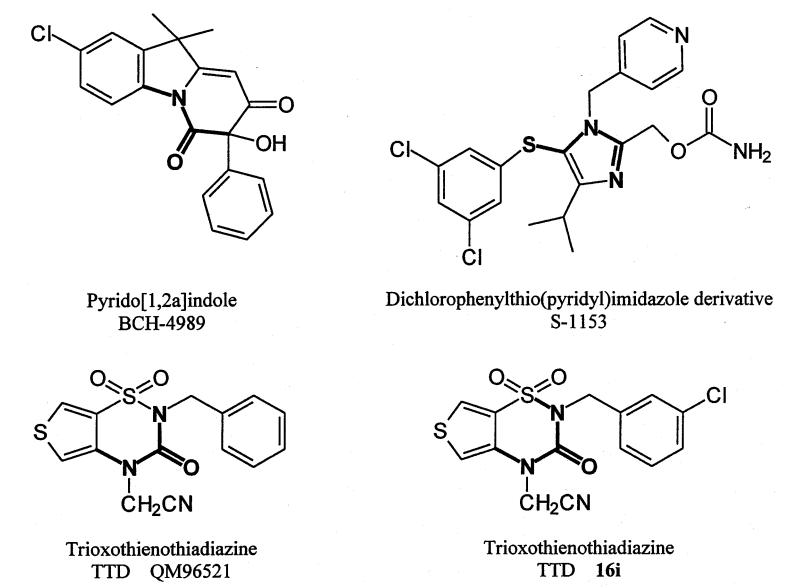


Fig. 7. Structural formulae of the NNRTIs.

097 prevents the emergence of virus resistance to HBY 097 [106]. In fact, an AZT-resistant HIV-1 strain was found to retain marked sensitivity to HBY 097 when-subcultured in the combined presence of HBY 097 and 3TC [107].

2.3. Optimization of NNRTI treatment regimens

The mutually antagonistic effects of different resistance mutations (i.e. Y181C or L100I versus T215Y), and the hypersensitivity that is seen under some condi-

Table 1
Inhibitory effects of NNRTIs on HIV-1 RT activity and HIV-1 cytopathicity[a]

Compound	HIV-1 RT activity	HIV-1 cytopathicity			Refs.
	IC_{50} (μM)[b]	EC_{50} (μM)[c]	CC_{50} (μM)[d]	SI[e]	
Tivirapine [8-chloro-TIBO (R86183)]	0.05	0.0046	138	30 000	[23]
HEPT [I-EBU (MKC-442)]	0.012	0.014	>100	>7000	[22]
		0.005	141	28 000	[24]
Nevirapine (BI-RG-587), Viramune®	0.084	0.048	>50	>1,000	[4,11,12]
Pyridinone L-697,661	0.019	0.012	>60	>4800	[13]
Delavirdine (BHAP U-90152), Rescriptor®	0.26	0.01	>100	>10 000	[25]
TSAO-m^3T	4.7	0.034	139	4088	[17,18]
Loviride (α-APA R89439)	0.2	0.013	710	54 615	[20]
Trovirdine (PETT LY 300046)	0.007	0.016	87	5438	[21,26,27]
Thiocarboxanilide UC-781	0.02[f]	0.002[f]	>100[f]	>50 000	[28,29]
Quinoxaline HBY 097	0.08	0.001	200	200 000	[31]
Thiazolobenzimidazole NSC 625487 (TBZ)	0.5	0.21	60	292	[32,33]
Thiazoloisoindolinone BM +51.0836	0.016	0.01	>50	>5000	[34,35]
Indole carboxamide L-737,126	0.003	<0.003			[36]
Benzothiadiazine NSC 287474	1.2	4	>130	>32	[37]
Quinazolinone **13a**, L-738,372	0.012	<0.025			[38,39]
Efavirenz (Benzoxazinone DMP 266)	0.003	0.001	80	80 000	[40]
Calanolide A	0.07	0.1	20	200	[41,42]
Pyrrolobenzodiazepinone **4**	0.04[f]	<0.3[f]	10[f]	>33	[43]
Imidazodipyridodiazepine UK-129,485	0.156	<0.002	>10	>5000	[44]
Imidazopyridazine **33**	0.0006	0.01	26	2600	[45]
TDA RD 4-2024	0.5	0.012	28	2280	[46,47]
MEN 10979	0.18	0.0025	38	15 000	[48]
DABO **12e**	1.8	0.8	>335	>418	[50]
HEPT-pyridinone hybrid **8a**	0.1	0.01	>10	>1000	[51]
Benzyloxymethylpyridinone **18a**		12	100	8	[52]
Alkoxy(arylthio)uracil **18**	12.3	0.064	33	516	[53]
Indolyldipyridodiazepinone **7a**		0.028			[54]
Pyrrolobenzoxazepinone **16e**	0.25	0.47	4.9	10	[55]
Highly substituted pyrrole **3**	0.64	2.3	175	76	[56]
Benzylthiopyrimidine U-31355	50–80	0.3	>16	>50	[57]
Benzothiadiazepine **16**	3.3	0.8	>200	>250	[60]
Indolobenzothiazepine **4c**	3.1	0.6	>200	>333	[61]
Furopyridinylthiopyrimidinamine PNU-142721	0.02	0.001[g]			[62]
MSC-204 (active metabolite of PETT-4)		0.01	58	5800	[63]
MSH-372 (PETT-5)		0.002	48	24 000	[63]
Pyrido[1,2a]indole BCH-4989	4	0.7	23	33	[64]
Dichlorophenylthio(pyridyl)imidazole S-1153	0.45	0.0014[f]	11[f]	7860	[65]
Trioxothienothiadiazine					
TTD QM96521	26	0.9	504	560	[66]
TTD **16i**		0.09	>340	3780	[67]

[a] The data, as listed, were obtained under different assay conditions (i.e. with different templates, for determination of HIV-1 RT activity; and with different virus strains and cell types, for determination of HIV-1-induced cytopathicity). This makes a direct comparison of the individual compounds rather difficult.
[b] 50% inhibitory concentration, or concentration required to inhibit HIV-1 RT activity by 50%.
[c] 50% effective concentration, or concentration required to inhibit HIV-1-induced cytopathicity by 50%.
[d] 50% cytotoxic concentration, or concentration required to reduce viability of the host cells by 50%.
[e] Selectivity index, or ratio of CC_{50} to EC_{50}.
[f] These values are expressed in μg/ml (instead of μM).
[g] 90% Effective concentration, or concentration required to inhibit p24 production by 90%.

tions (i.e. with the P236L mutation towards some NNRTIs), argue in favour of the combined use of NNRTIs with NRTIs (nucleoside/nucleotide reverse transcriptase inhibitors), and different NNRTIs with each other. While achieving synergism in their anti-HIV action, different drugs combined may also reduce the risk of HIV drug resistance development and diminish toxic side effects (through reduction of the individual doses). The compounds should not be given in sequential order [108], as such procedure may enable the virus to acquire resistance mutations to all the compounds [109]. Instead, concomitant combination therapy, as

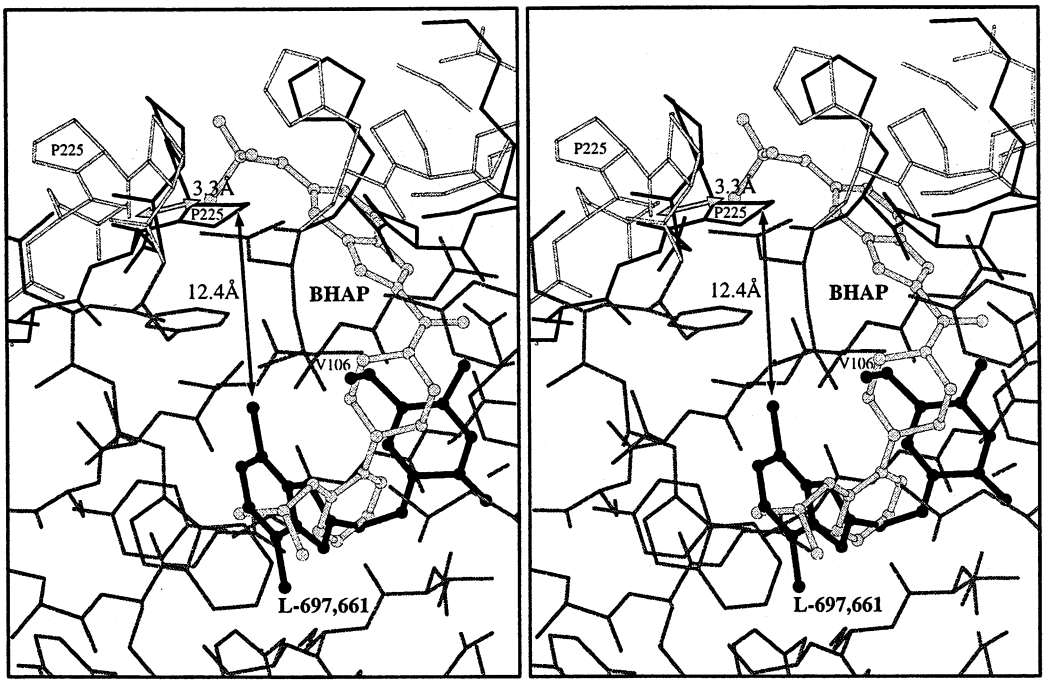


Fig. 8. Positioning of BHAP U-90152 and pyridinone L-697,661 in the NNRTI-binding pocket of HIV-1 RT [81]. Stereodiagram showing the position of residues P225 and V106 in HIV-1 RT relative to the bound NNRTI in the complexes with BHAP U-90152 (X-ray structure; grey ball-and-stick model) and pyridinone L-697,661 (model structure; black ball-and-stick model).

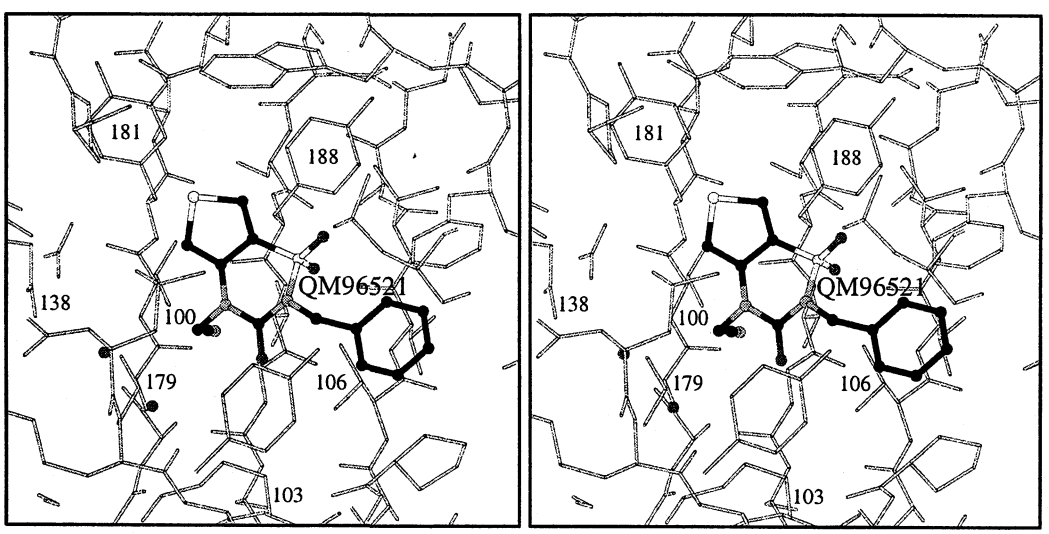


Fig. 9. Positioning of the TTD QM9651 in the NNRTI-binding pocket of HIV-1 RT [66]. Stereodiagram showing QM96521 in ball-and-stick representation (C, black; O, dark gray; N, mid-gray; S, light-gray).

demonstrated particularly with 3TC and NNRTIs [109], should be recommended.

Synergistic anti-HIV activity may be expected if NNRTIs are combined with NRTIs. For example, synergistic anti-HIV activity has been described for combinations of AZT with any of the NNRTIs, i.e. 9-chloro-TIBO R82913 or 8-chloro-TIBO R86183 (tivirapine) [110], nevirapine [111], delavirdine [112], MKC-442 [113,114], or S-1153 [65]. Synergistic anti-HIV activity has also been reported for the combination of delavirdine with protease inhibitors (i.e. U-75875) or interferon-α [115], for the combination of HEPT with interferon-α [116], and for the combination of MKC-442 with virtually all other anti-HIV agents (NRTIs, NNRTIs and PIs) [117]. Thiocarboxanilides (i.e. UC-781) show an additive inhibitory effect on HIV-1 replication when combined with other antiretroviral drugs (reverse transcriptase inhibitors or HIV protease inhibitors) [118]. The different drug combinations that have proved to confer an additive/synergistic anti-HIV activity in vitro and/or in vivo (the latter, in patients) are illustrated in Fig. 14.

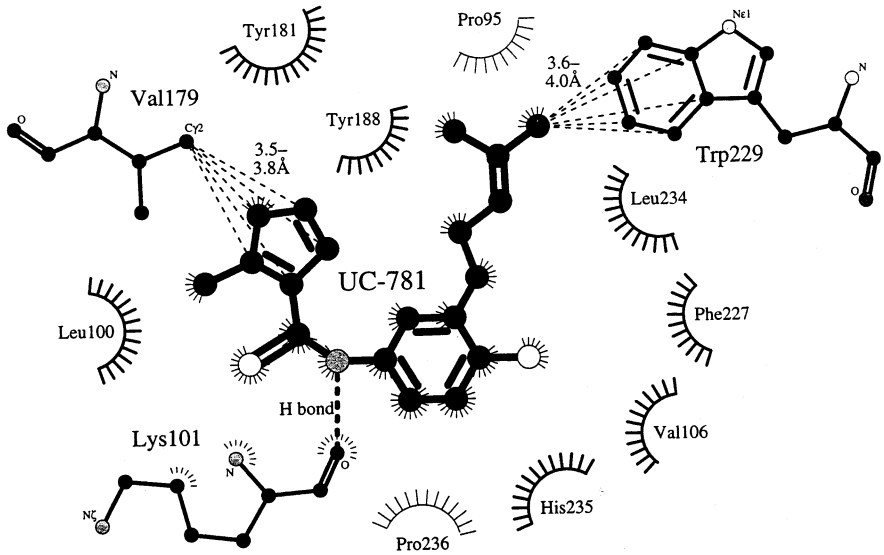


Fig. 10. Schematic diagram of the features stabilizing the HIV-1 RT complex with thiocarboxanilide UC-781 [82]. The hydrogen bond between UC-781 and the main chain of Lys-101 and the two methyl group–aromatic ring interactions are shown explicitly. Figure modified from Esnouf et al. [82].

In contrast with the ddN analogues (AZT, ddI, …), the NNRTIs TIBO, BHAP and nevirapine were found to completely suppress HIV-1 infection, if added to the cells at a sufficiently high concentration (1-10 μM, or 100 times their EC_{50}) [129]. The infected cells could apparently be cleared from virus by the NNRTIs when used at these 'knocking out' concentrations, and the resultant healthy cell culture could be subsequently maintained without drug with no evidence of latent proviral DNA [129,130]. The more potent quinoxaline [131] and thiocarboxanilide [100] derivatives were found to achieve this 'knocking out' effect at even lower concentrations (0.1–1 μM) (Fig. 15); i.e. concentrations that should be readily attainable in the plasma following systemic administration to patients. Also, DMP 266 (efavirenz) was found to completely suppress virus replication in peripheral blood mononuclear cells (PBMC) at a concentration of 0.96 μM, and no regrowth of virus occurred in the presence of compound after 10 weeks or in the absence of compound for 3 additional weeks [132].

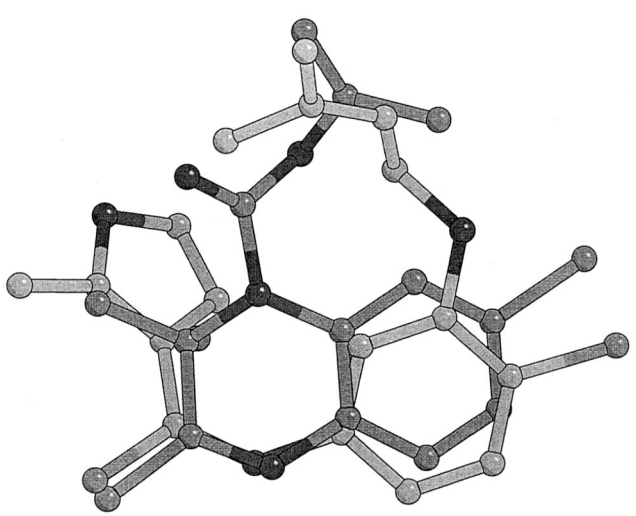


Fig. 11. Superposition of thiocarboxanilide UC-781 and quinoxaline S2720 within the NNRTI-binding pocket of HIV-1 RT [82]. Ball-and-stick models are shown for the thiocarboxanilide UC-781 (pale grey carbon atoms) and for the quinoxaline S2720 (dark grey carbon atoms). The UC-781 ether group and the S2720 ester group adopt conformations that allow terminal methyl groups to interact strongly with the aromatic ring of Trp-229. The figure was drawn using Bobscript [83].

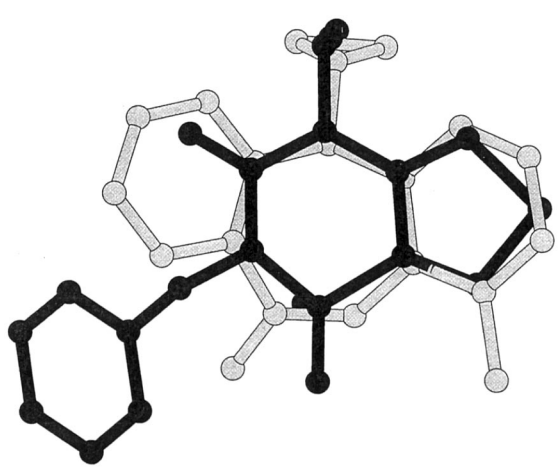


Fig. 12. Superposition of the TTD QM96521 and nevirapine within the NNRTI-binding pocket of HIV-1 RT [66]. Positions and conformations for QM96521 (model structure; black ball-and-stick) and nevirapine (X-ray structure; grey ball-and-stick) in their respective complexes with RT. The structures were superimposed based on the surrounding protein residues, as described by Ren et al. [74].

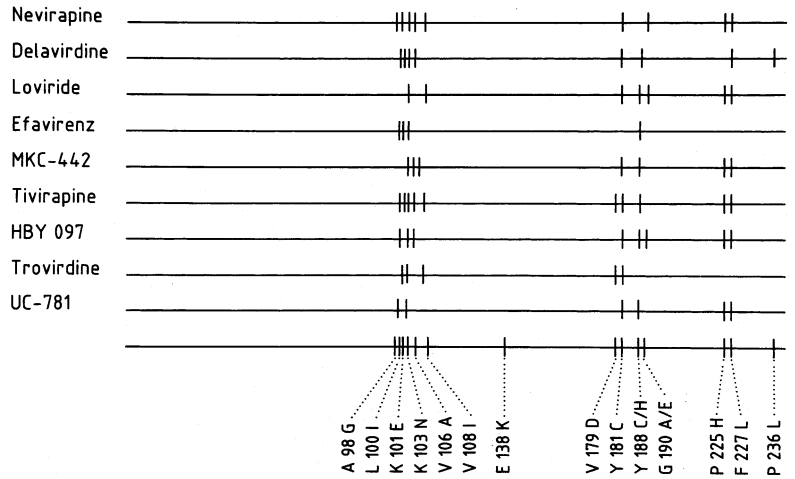


Fig. 13. Mutations in the HIV-1 reverse transcriptase that confer resistance to the NNRTIs (see also Schinazi et al. [84]).

Table 2
Antiviral activity spectrum of NNRTIs[a]

NNRTI	Active against[b]						
	Wild type (HIV-1 III_B)	Mutant					
		HIV-1 L100I	HIV-1 K103N	HIV-1 V106A	HIV-1 E138K	HIV-1 Y181C	HIV-1 Y188H
TIBO R82913	■	□	□	□	■	□	□
I-EBU (MKC-442)	■	■	□	■	■	■	□
Nevirapine	■	■	□	□	■	□	□
Pyridinone (L-697,661)	■	■	□	■	■	□	□
BHAP U-88204	■	□	□	■	■	■	■
BHAP U-90152 (Delavirdine)	■	□	■	■	■	■	■
TSAO-m^3T	■	■	■	□	□	□	□
TTD QM96521	■	□	□	□	□	□	□
Quinoxaline S-2720	■	■	■	■	■	■	■
Thiocarboxanilide UC-10	■	■	□	■	■	■	■
Quinoxaline HBY 097	■	■	■	■	■	■	■
Thiocarboxanilide UC-781	■	■	■	■	■	■	■
DMP 266 (Efavirenz)	■	■	■	■	■	■	■

[a] Data from Balzarini et al. [98–100] and unpublished, and Witvrouw et al. [66].
[b] ■, EC_{50} ≤1 μM; □, EC_{50}+1 μM.

Furthermore, if the NNRTIs are used in combination with the ddN analogues, i.e. delavirdine combined with AZT [25], or MKC-442 combined with AZT [119], or delavirdine combined with 3TC [120], or MKC-442 combined with 3TC [120], or thiocarboxanilides combined with other NNRTIs [99], the drugs are able to suppress virus breakthrough for a much longer time, and at much lower concentrations than when the compounds are used individually (Fig. 16).

Thus, when optimizing the NNRTI-containing drug treatment regimens, the drugs, in combination, should be administered from the start at the highest possible doses so as to achieve complete virus suppression and prevent virus drug resistance from arising.

2.4. In vivo efficacy of NNRTIs

Given their high specificity as inhibitors of HIV-1 infection (for which there is no adequate animal model), NNRTIs have only rarely been studied in animal retrovirus models. When evaluated in HIV-1-infected hu-PBL-SCID mice, the NNRTIs delavirdine (BHAP U-90152) and thiocarboxanilide UC-781 protected the mice against HIV-1 infection [100]. Similarly, nevirapine proved effective in preventing HIV-1 infection in chimpanzees [133].

In humans, several NNRTIs have been the subject of short-term clinical studies: 9-chloro-TIBO R82913 [134,135], pyridinone L-697,661 [136,137], nevirapine

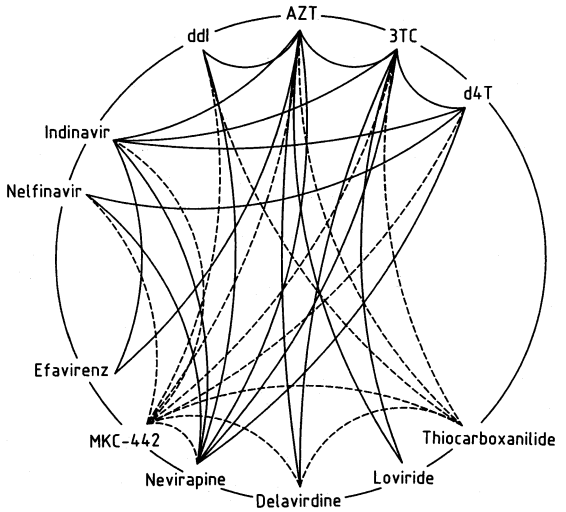


Fig. 14. Additive/synergistic anti-drug interactions shown in vitro [99,111–114,118–128], and personal communications from G. Tarpley, M. Myers and D. Mayers (1998).

[138], α-APA R89439 (loviride) [139], MKC-442 [140] and HBY 097 [141]. As a rule, the NNRTIs were very well tolerated (although rash developed in about half of the patients treated with nevirapine [138]); they efficiently suppressed plasma viral load (up to 1.38 $\log_{10}$) [141], but could not prevent the emergence of drug-resistant virus strains [136,138]. As shown for pyridinone L-697,661, suppression of virus replication was only transient: this suppressive effect disappeared coincidentally with the emergence of resistant virus (within 6 to 12 weeks) [136,137]. Even following high-dose nevirapine (400 mg/day) therapy, nevirapine-resistant virus was isolated from all subjects tested at 12 weeks [138], and by that time plasma HIV-1 RNA load had returned to baseline values [142]. Although some patients treated with high-dose nevirapine (i.e. 400 mg daily) may experience sustained reduction in plasma HIV RNA despite the presence of resistant virus [143], this does not seem to hold true in previously untreated HIV-1-infected persons [142].

Although monotherapy with NNRTIs may rapidly lead to the emergence of drug-resistant HIV strains [136,137], the rate of emergence of NNRTI-resistant virus can be markedly reduced in subjects receiving the NNRTI (i.e. pyridinone L-697,661) concomitantly with AZT [121,123]. As could be predicted from the in vitro studies (see supra), NNRTIs such as nevirapine [124] and delavirdine [125] achieve higher efficacy in HIV-1-infected patients when combined with ddN analogues such as AZT than when used alone. If these combinations (AZT with nevirapine, or AZT with delavirdine) are extended by yet another drug (i.e. ddI), the efficacy (in both immunological and virological terms) is further increased [125,126]. Combined treatment of nevirapine with AZT and ddI has also proved efficacious in providing a sustained reduction of the plasma HIV-1 RNA load in infants [127]. The latter authors also stated that "therapy with potent combinations of antiretroviral drugs should be started as early as possible in infants with maternally acquired infection (probably within the first 2–4 weeks), to minimize the likelihood that antiretroviral resistance will emerge and to maximize the opportunity for long-term control of HIV-1 replication" [127].

From a comprehensive study of 1330 HIV-1-infected patients enrolled in several antiretroviral treatment trials [144], it appeared that having either a reduction in HIV-1 RNA level or an increase in CD4 lymphocyte count, or both, are associated with a delay in clinical disease progression. This implies that patient prognosis can be assessed using both HIV-1 RNA and CD4 cell responses to therapy [144]. In fact, the characteristic responses of CD4 cell counts (increase) and HIV RNA plasma levels (decrease) following therapy could be depicted as a mirror image [145]: the magnitude of the CD4 cell response tends to mirror the magnitude of the viral RNA response (although the peak response in CD4 cell counts lags behind the maximum reduction in plasma HIV RNA levels) (Fig. 17). Pictured here are the responses of CD4 cell counts and plasma HIV RNA levels towards monotherapy (AZT), bitherapy (AZT + 3TC) and tritherapy (AZT + 3TC + protease inhibitor), but it is obvious that similar patterns may also be seen in response to NNRTI-containing drug regimens. It has, in fact, been demonstrated that extension of the dual-drug (AZT + ddI) therapy to the triple-drug (AZT + ddI + nevirapine) regimen resulted in higher CD4 cell counts paralleled by lower plasma HIV-1 RNA levels, thus attesting to the immunologic and virologic benefit afforded by the addition of nevirapine to the combination of AZT and ddI [126].

As already mentioned above, the triple-drug combination AZT + ddI + delavirdine proved more potent than either two-drug combinations or monotherapy [125]. Similarly, the triple-drug combination AZT + 3TC + delavirdine proved superior to the double-drug combination AZT + 3TC, which in turn, proved more efficacious than the double-drug combination AZT + delavirdine, when monitored either virologically (decrease of HIV RNA plasma levels) or immunologically (increase of CD4 cell counts) [G. Tarpley, personal communication (1998)].

In the CAESAR trial (a large randomized trial, involving more than 3000 patients) the addition of 3TC, or 3TC + loviride, to AZT-containing treatment regimens (AZT monotherapy, or AZT + ddC, or AZT + ddI combination therapy), was found to slow the progression of HIV disease and improve survival [128]; and again the incremental benefit resulting from the addition of 3TC and loviride was reflected by the increase in CD4 cell counts and decrease of plasma HIV RNA levels.

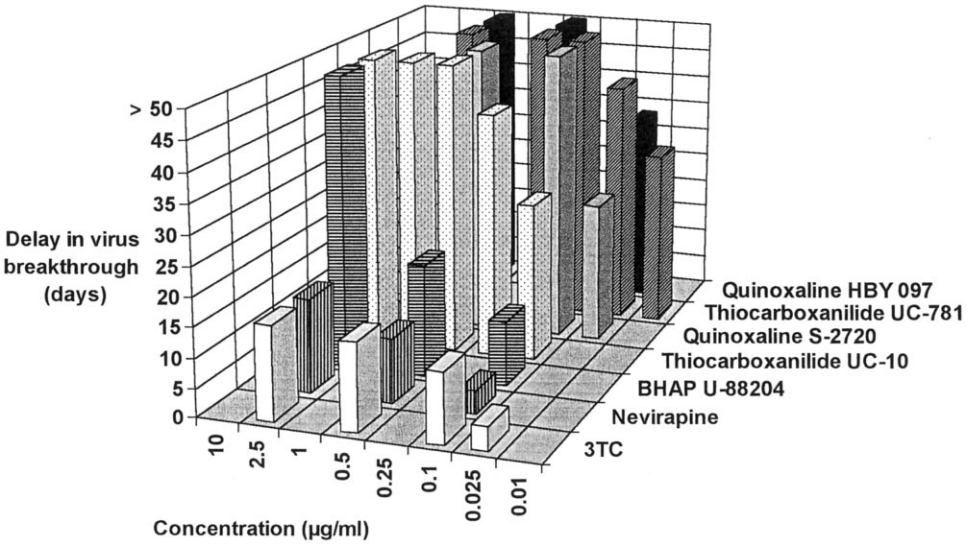


Fig. 15. Suppression of virus breakthrough in CEM cells infected with HIV-1(III_B) and treated with individual drugs at different concentrations. The delay in virus breakthrough corresponds to the number of days required for 50% viral cytopathicity to develop. Data taken from Balzarini et al. [99,100,120,131].

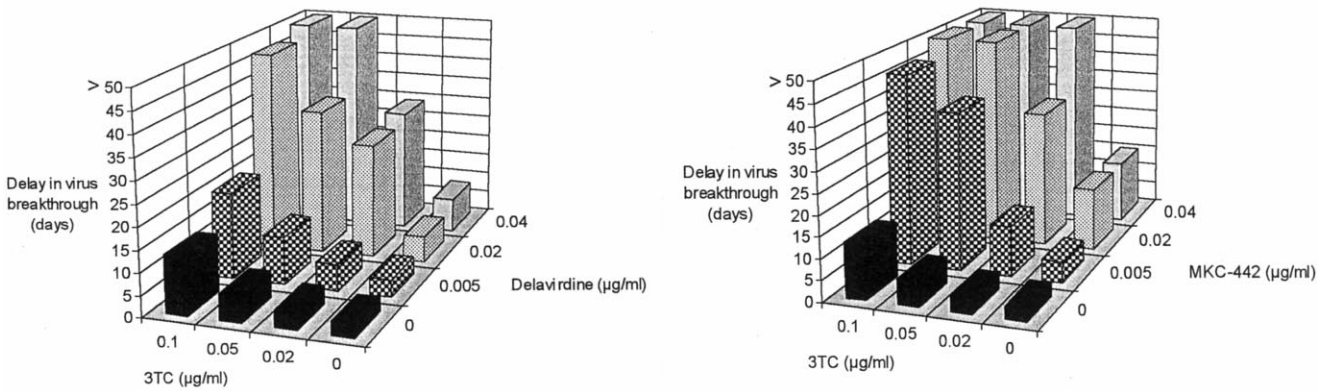


Fig. 16. Suppression of virus breakthrough in CEM cells infected with HIV-1(III_B) and treated with dual drug combinations at different concentrations: 3TC plus delavirdine or 3TC plus MKC-442. For some dual drug combinations, i.e. 3TC (at 0.1 μg/ml) with MKC-442 (at 0.02 or 0.04 μg/ml) or delavirdine (at 0.04 μg/ml), the cell cultures remained p24 negative when the drugs were removed on day 52 and the cells were further passaged in the absence of the compounds. The delay in virus breakthrough corresponds to the number of days required for 50% viral cytopathicity to develop. Data taken from Balzarini et al. [120].

However, triple-drug combinations (typically composed of AZT + 3TC + protease inhibitor), or HAART (highly active antiretroviral therapy), afford an optimal response in only a fraction of the patients [146]. There are individuals who experience an optimal response to treatment, as reflected by effective viral suppression and ensuing immune recovery (Fig. 18, panel A). There are others with increasing CD4 cell counts in the presence of ongoing viral replication (Fig. 18, panel B), or lack of immune recovery despite viral load reduction (Fig. 18, panel C), or, finally, complete failures (no reduction in viral load and no immune recovery) (Fig. 18, panel D) [146]. Therapy failure may be attributed to poor adherence to HAART, prior exposure to antiretroviral drugs in mono- or bitherapy, the sequential addition of drugs to a failing treatment regimen, the counteractive interactions among the drugs used, and inevitably emanating from all this, virus drug resistance development.

3. Conclusion

HIV-1 viremia, the hallmark of HIV infection, is sustained by a highly dynamic process involving continuous rounds of de novo virus replication and cell turnover [147,148]. Productively infected cells have, on average, a life-span of 2.2 days (half-life + 1.6 days) and plasma virions have a mean life-span of 0.3 days (half-life + 0.24 days). The average total HIV-1 production is ca. 10^{10} virions per day, and the minimum duration of the HIV-1 life cycle in vivo is 1.2 days on average [149].

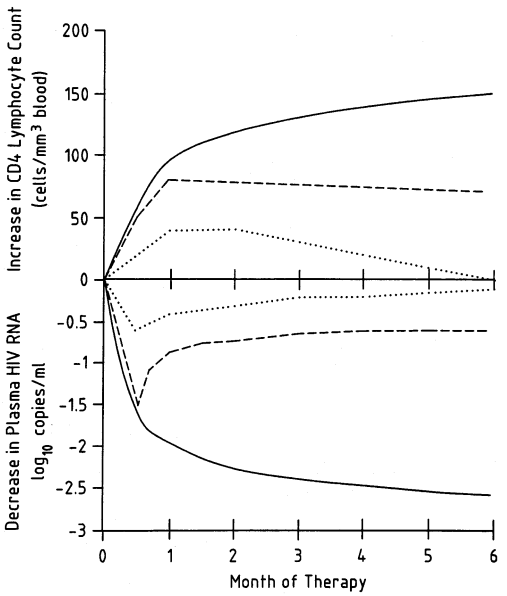


Fig. 17. Idealized response to tri-, di- and monotherapy, as exemplified for AZT (…), AZT plus 3TC (- - -) and AZT plus 3TC plus protease inhibitor (—). According to Havlir and Richman [145], modified.

Virus replication is the driving force in the progression to AIDS. Low viral load is associated with long-term non-progression to AIDS [150,151], and, while both plasma HIV RNA levels and CD4 cell counts are valid predictors of the clinical progression of HIV disease [152,153], plasma viral load is a more accurate predictor of progression to AIDS than the number of CD4 cells [154]. Furthermore, the clinical course of HIV-1 infection may already be determined at the earliest phase of the disease, and this necessitates initiation of definitive treatment very early in HIV-1 infection [155].

Table 3
Recommendations for clinical use of anti-HIV drugs, including NNRTIs

1. Use different compounds in multiple (double, triple, quadruple, …) drug combinations.
2. At sufficiently high (but subtoxic) doses.
3. Starting as soon as possible after the HIV infection.
4. With the aim to achieve complete suppression of virus replication (plasma viral load below detection limit).
5. And to prevent the development of virus drug resistance.
6 Ensuring full compliance (patient taking his/her medicine).
7. While improving on the convenience of drug dosing (preferably once daily).
8. Minimizing adverse side effects of the drug.
9. Continuing drug treatment as long as required for a sustained suppression (and, ideally, eradication of the virus from the organs and from the organism).
10. Making the anti-HIV drugs widely available (at affordable costs).

In view of all these considerations, and the remarks made above on the optimization of drug treatment regimens, a few recommendations could be formulated so as to ensure a successful treatment of HIV infections. These recommendations (Table 3) concern NNRTIs as well as any other anti-HIV drugs. It would be of paramount importance that the NNRTI-containing drug regimens should be started as soon as possible after HIV infection, and that the individual compounds should be used at the highest possible doses so as to completely suppress virus replication (and prevent drug-resistant virus strains from emerging) for as long a time as necessary. How long the treatment should be continued, and whether it could be discontinued or adjusted at certain time points, remain unsettled issues. The fact is that, despite prolonged suppression of plasma

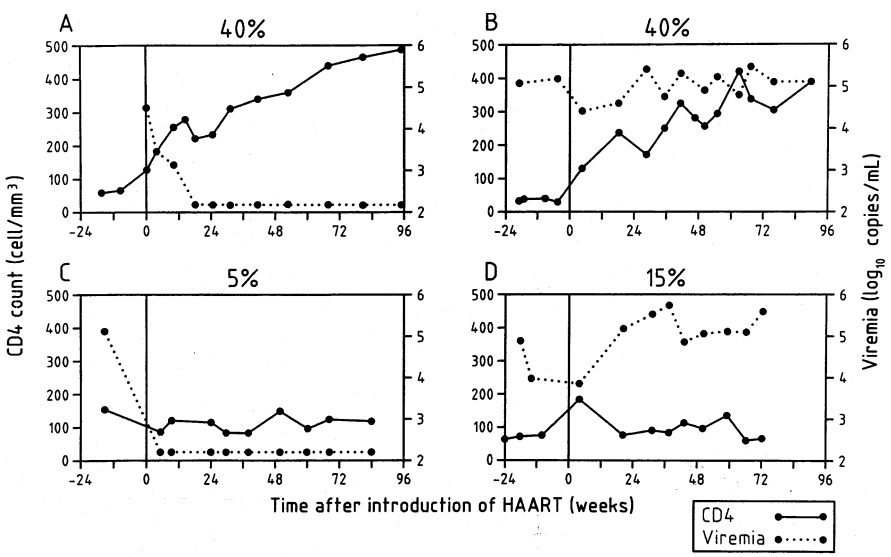


Fig. 18. Different types of patients' responses to HAART, pertaining to the complexity of what is definition of failure or success of HIV treatment. According to Perrin and Telenti [146].

viremia, replication-competent virus could still be recovered from resting CD4 T lymphocytes [156,157], even after 30 months of HAART. It thus appears that with the current drug treatment regimes, virus replication may be suppressed, resistance development prevented, and progression to AIDS arrested, albeit without eradication of the virus from its reservoir(s).

Acknowledgements

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