NEW PERSPECTIVES FOR THE TREATMENT OF HIV INFECTIONS

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The HIV replicative cycle reveals several virus-specific events that could function as targets for chemotherapeutic intervention. The compounds that are presently available as anti-HIV drugs are targeted at either the substrate binding site of the reverse transcriptase (zidovudine, didanosine, zalcitabine, stavudine, lamivudine) or a non-substrate binding site of the reverse transcriptase (nevirapine, delavirdine), or the viral protease (saquinavir, ritonavir, indinavir, nelfinavir). Remarkable clinical efficacy has been observed with combinations of different reverse transcriptase inhibitors and protease inhibitors. It may be anticipated that with the advent of newer and more efficient compounds the effectiveness of HIV inhibition could still be improved upon and the prospects for a definitive cure of the disease may be accomplished. An account with 107 references.

Key words: AIDS; HIV; Reverse transcriptase inhibitors; HIV protease inhibitors; Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs); Non-nucleoside reverse transcriptase inhibitors (NNRTIs); Nucleosides; Nucleotides; Peptidomimetics.

1. Introduction

The replicative cycle of human immunodeficiency virus (HIV) can be divided in ten steps: (i) virus adsorption to the cells; (ii) virus-cell fusion; (iii) virus uncoating; (iv) reverse transcription; (v) proviral DNA integration; (vi) proviral DNA replication; (vii) proviral DNA transcription to viral messenger RNA; (viii) viral messenger RNA translation to viral precursor proteins; (ix) viral maturation (proteolysis, myristoylation, glycosylation); (x) budding (release) (Fig. 1).

2. Virus Adsorption Inhibitors

HIV binding to the cells depends on the interaction between the viral envelope glycoprotein gp120 and the cellular CD4 receptor located at the outer cell membrane of human T-lymphocytes[1] (Fig. 2).

The very first compound that was identified as an anti-HIV agent[2] was suramin **1** which was later shown to exert its anti-HIV activity through an interaction with the virus-cell binding process[3]. Suramin is a hexasulfonate and thus its interaction with the virus adsorption process can be attributed to the polyanionic character of the compound. Suramin was also the first anti-HIV agent to be used in the clinic for the treatment of HIV infection[4].

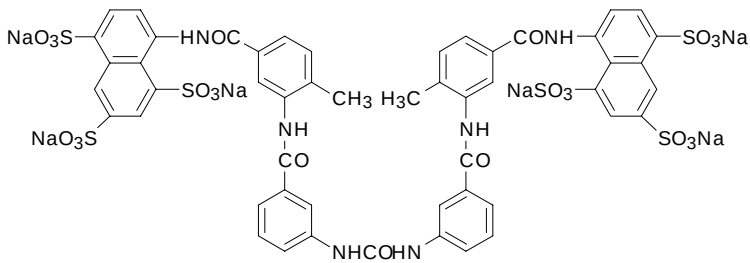

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Figure 3 shows the pharmacokinetics and antiviral effect of suramin in an HIV-infected individual[4] that was treated once a week with 1 gram of suramin given by the intravenous route. As plasma drug levels of suramin built up after each weekly injection, the virus disappeared from the plasma. However, when therapy was stopped, the virus reappeared, suggesting that during suramin therapy the replication of HIV was suppressed but the virus was not eradicated from the organism. More recent studies with newer compounds have essentially revealed the same effect, that is suppression but not eradication of the virus during the treatment period. In this aspect the original results obtained with suramin (which was later discontinued for the treatment of HIV infec-

tions because of toxicity) heralded the results that would be achieved with the more modern and more potent anti-HIV drugs.

In addition to suramin, numerous other polyanionic compounds[5,6] have been shown to block virus adsorption: polysulfates such as dextran sulfate **2** and poly(vinyl alcohol sulfate) (PVAS) **3**, polysulfonates such as poly(vinyl sulfonate) (PVS) **4** and poly(naphthalene sulfonate) **5**. The latter compound[7], also referred to as PRO 2000, is presently in clinical trials for the treatment of HIV infections.

Other polyanionic substances[5,6] that have been found to interfere with the virus adsorption process are the polycarboxylates such as the aurintricarboxylate acid polymer

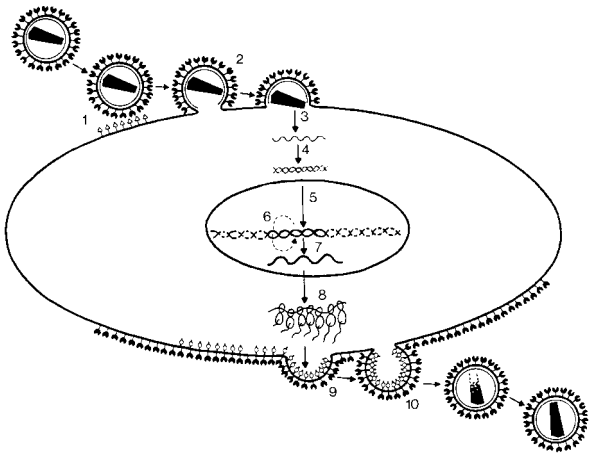


FIG. 1
Essential steps in the HIV replicative cycle 1 adsorption; 2 fusion; 3 uncoating; 4 reverse transcription; 5 integration; 6 DNA replication; 7 transcription; 8 translation; 9 maturation; 10 budding (assembly/release)

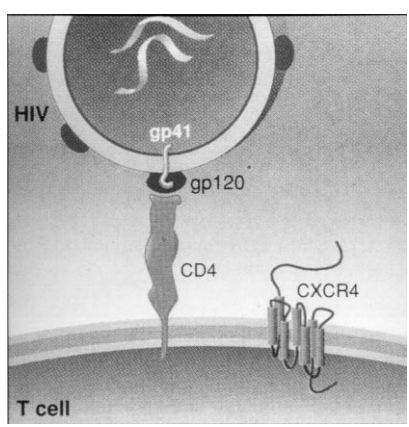
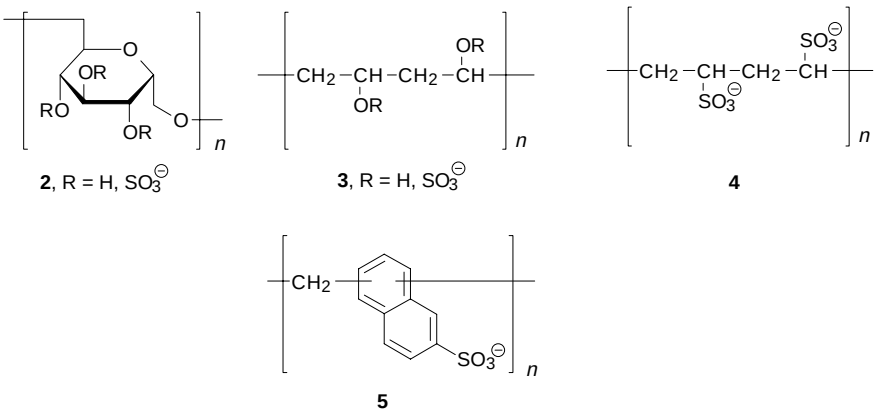


FIG. 2
Adsorption of HIV to the cells: HIV's gp120 binds to the CD4 receptor of the T cell

and various polyoxymetalates (POMs). The polyoxymetalates may assume a globular conformation as represented by the Keggin (Fig. 4a) or Dawson type (Fig. 4b). Yet another example of a polyanionic substance is the polynucleotide zintevir (AR177), a polynucleotide that solely consists of thymine and guanine as the heterocyclic moieties and which forms two stacked G-quartets (Fig. 5) that can bind a potassium ion in the middle. The overall conformation of the molecule is cubic. This molecule has been shown to interfere with the virus adsorption process, although additionally it can also interfere with the viral integrase[8]. Nevertheless, its mechanism of anti-HIV action in cell culture systems can be attributed to an interaction at the virus adsorption level[9].



2, R = H, $SO_3^{\ominus}$ **3**, R = H, $SO_3^{\ominus}$ **4**

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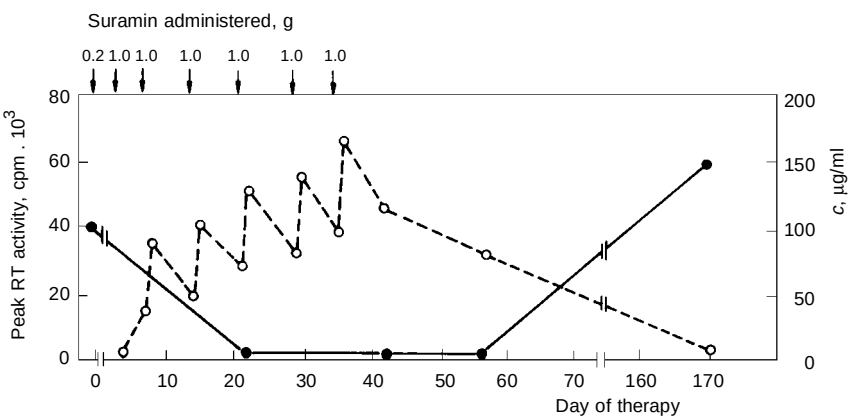


FIG. 3

● Peak reverse transcriptase (RT) activity in cultures of peripheral blood mononuclear cells and ❍ plasma suramin concentrations (*c*) during treatment of a patient who had detectable HIV-1 before the start of suramin therapy[4]

Polyanionic substances may offer great potential in the prophylaxis of HIV infections when applied topically, for instance in gel or cream form to prevent HIV infection by sexual transmission[10,11]. An additional bonus of these compounds is that they are not only effective against HIV but also against some other enveloped viruses such as

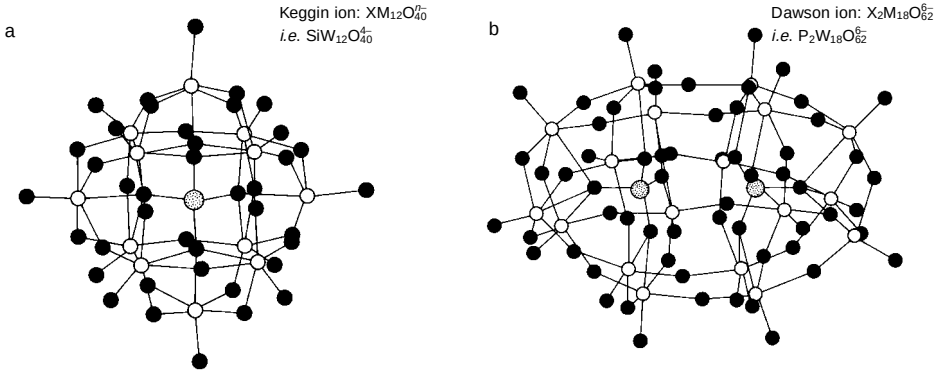


FIG. 4
Keggin (a) and Dawson (b) structures for polyoxometalates

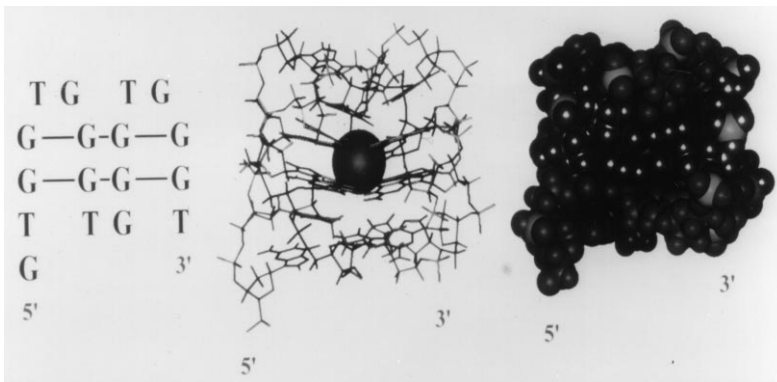
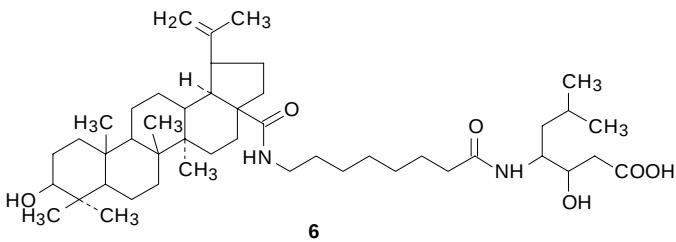


FIG. 5
Predicted three-dimensional structure of AR177 (zintevir) under physiologic conditions. The primary sequence of the oligonucleotides is shown at left. The guanosines participating in tetrad-formation are connected by lines. In the wire-frame rendering (center) and space-filling model (right), the phosphate oxygens are yellow, the tetrad-forming guanosines are gray, and the other bases are blue. The two-stacked G-tetrads (G-octet) are stabilized by potassium cation (red). The structure, obtained using SYBYLL molecular modelling software, was minimized by the conjugate gradient method totaling 4 250 iterations, and the K+ initial constraints were based on the crystal structure for guanine tetraplexes. Electrostatics were calculated using the Gasteiger–Hückel method. The compact, monomeric form is consistent with previously reported NMR data, and energetically favored over multimeric structures

herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), human cytomegalovirus (HCMV), influenza A virus, respiratory syncytial virus and arena- and rhabdoviruses.

3. Virus-Cell Fusion Inhibitors

Virus-cell fusion is initiated by the interaction of the viral envelope gp120 with the coreceptor CXCR4 (fusin), which, like the CD4 receptor, is also embedded in the outer cell membrane[1] (Fig. 6). When gp120 is stripped from the viral envelope, the other envelope glycoprotein gp41 is uncovered so that it can anchor in the cell lipid membrane and ensure the fusion between the lipid bilayers of the virus envelope and the outer cell membrane.



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The virus-cell fusion process can be inhibited by the negatively charged albumins (NCAs, Scheme 1) such as Suc-HSA (succinylated human serum albumin) and Aco-HSA (aconitylated human serum albumin)[12–15]. Other examples of virus-cell fusion inhibitors are the betulinic acid derivatives[16] (*e.g.* RPR 103611, **6**), siamicin I (NP-06) (refs[17,18]) and pentafuside T20. The latter corresponds to a 36 amino acid region in the gp41 glycoprotein[19].

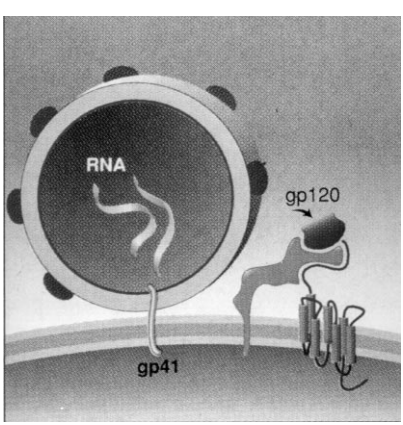
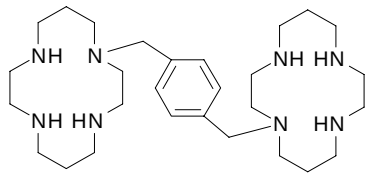


FIG. 6
As the first step in the virus-cell fusion process, HIV's gp120 binds to chemokine receptor (*i.e.* CXCR4) allowing gp41 to breach the cell membrane

Typical fusion inhibitors are the bicyclam derivatives (prototype JM 3100), which contain two cyclam moieties that are tethered through an aliphatic or aromatic bridge[20,21]. The JM 3100 **7** now referred to as AMD3100, inhibits HIV replication at



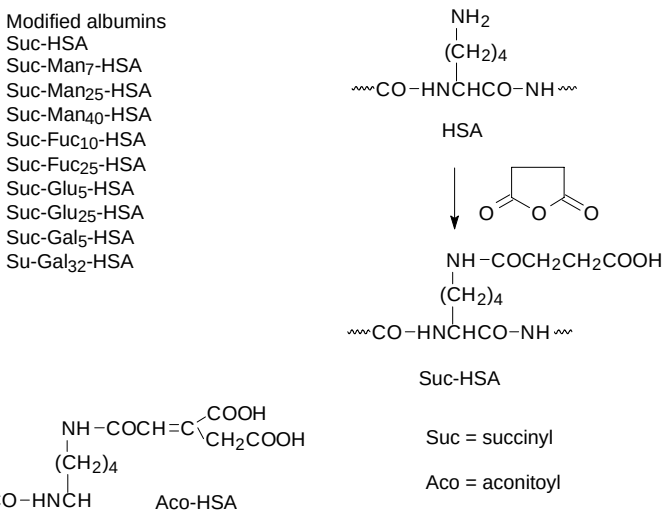
JM 3100

7

nanomolar concentrations, while no toxicity for the host cells is observed at concentrations of 500 μM, which results in selectivity indexes of more than 100 000. Hence, the bicyclam AMD3100 can be considered as one of the most potent and selective anti-HIV agents that have been described so far. We have recently found that the bicyclam AMD3100 binds tightly and specifically with the CXCR4 coreceptor (fusin)[22], and in doing so the bicyclams represent the first low molecular weight compounds shown to inhibit HIV cell fusion through binding to fusin.

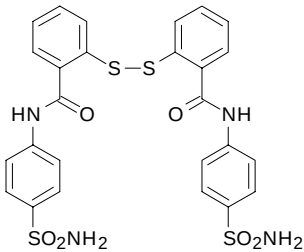
4. Virus Uncoating Inhibitors

Virus uncoating can be envisaged as the dissociation of the viral envelope glycoproteins and viral capsid proteins from the viral genome, which is required for the next



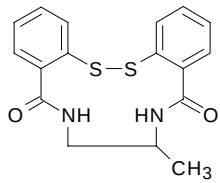
SCHEME 1

step (reverse transcription) to take place. The last protein to be dissociated from the viral genome is the nucleocapsid (NC) protein p7, which contains two zinc fingers (Fig. 7). This protein is believed to be the target of action for the 2,2′-dithiobisbenzamide[23–28] (DIBA, **8a**). We have recently described a macrocylic 2,2′-dithiobisbenzamide SRR-SB3 **8b** that may also achieve its anti-HIV activity through interaction with the NC protein



DIBA

8a



SRR-SB3

8b

p7 (ref.[29]). From time-of-addition experiments[29] whereby the compound was added at different times after HIV infection, we concluded that **8b** may interact with a late stage of virus replication, which may correspond to the assembly of the virus particles. Thus, 2,2′-dithiobisbenzamides may interact with both the uncoating (disassembly) and the maturation (assembly) processes (as the NC protein p7 is involved in both processes).

5. Reverse Transcription Inhibitors

Reverse transcriptase catalyses the conversion of the single-stranded viral RNA genome to the double-stranded proviral DNA which will be subsequently integrated

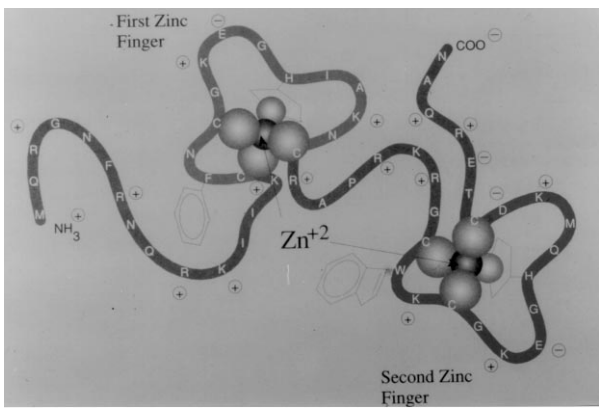
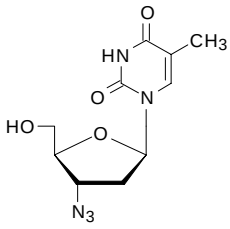


FIG. 7
Nucleocapsid (NC) protein p7, containing two zinc fingers

into the cellular DNA genome. Several domains have been characterized in the reverse transcriptase (RT) heterodimer (p66/p41), of which the palm domain is involved in the catalytic process. There are two targets at this palm domain: the substrate binding site (representing by a dot in Fig. 8) and a non-substrate binding site (represented by an asterisk in Fig. 8), which correspond to the binding sites for the nucleoside/nucleotide RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs), respectively[30].



Zidovudine, AZT

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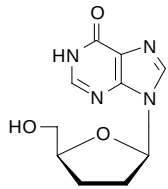
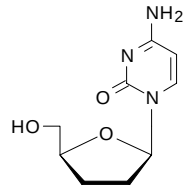


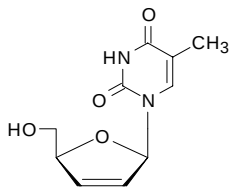
Didanosine, Videx, ddI

10



Zalcitabine, Zerit, ddC

11



Stavudine, d4T

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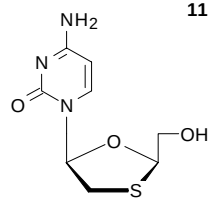


Lamivudine, 3TC

13

Of the NRTIs, five compounds have been approved for the treatment of HIV infections: zidovudine (AZT, **9**), didanosine (ddI, **10**), zalcitabine (ddC, **11**), stavudine (d4T, **12**), and lamivudine (3TC, **13**). All these compounds act in a similar fashion (as demonstrated for AZT in Fig. 9): they need to be phosphorylated intracellularly in three steps to the 5′-triphosphate form, which then interacts at the level of the RT as a competitive inhibitor/alternate substrate. If the inhibitor is incorporated into the DNA chain, further elongation of the DNA chain will become impossible. In this respect, the 5′-triphosphates of AZT, ddI, ddC, d4T and 3TC behave as DNA chain terminators[5,6].

The crucial factor in the anti-HIV activity of the nucleoside analogues is the first phosphorylation step. This phosphorylation from the nucleoside to the nucleotide stage determines why some nucleoside analogues are active as anti-HIV agents whereas others are not and also why nucleoside analogues may be more effective in cells or tissues that have a higher phosphorylating capacity than other cells or tissues. To circumvent this phosphorylation problem we have conceived a class of compounds that are referred to as nucleotide analogues and which correspond to acyclic nucleoside phosphonates **14–16**. The prototypes of this class of compounds are PMEA [9-(2-phosphonomethoxyethyl)adenine] **14a** and PMPA [(*R*)-9-(2-phosphonomethoxypropyl)-

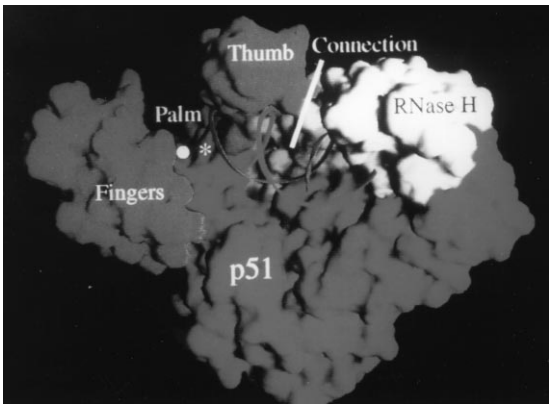


FIG. 8

Locations at the HIV-1 reverse transcriptase of the binding site (dot) for the substrate analogues (ddNTPs, *i.e.* AZT-TP) and the binding site (asterisk) for the non-nucleoside reverse transcriptase inhibitors[30] (NNRTIs). A ribbon representation of the backbone of the template-primer is shown with the template strand in red and the primer strand in purple. The domains of the p66 subunit are colored as follows: blue, fingers; red, palm; green, thumb; yellow, connection; white, RNaseH. The entire p51 subunit is colored magenta

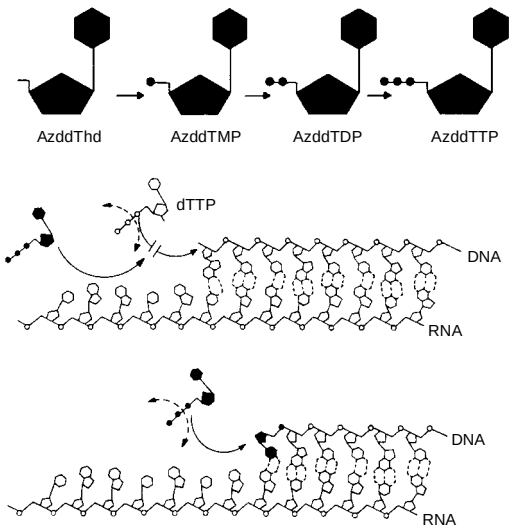
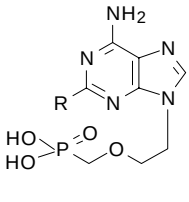


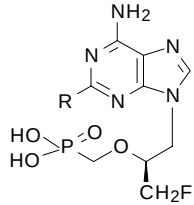
FIG. 9

Mechanism of anti-HIV action of 2′,3′-dideoxynucleoside analogues, as exemplified for AZT (AzddThd). Following intracellular phosphorylation of AzddThd to its 5′-triphosphate (AzddTTP), the latter interacts with the reverse transcriptase reaction as either competitive inhibitor or alternate substrate with respect to the natural substrate (dTTP); the incorporation of AzddTMP in the DNA product leads to chain termination

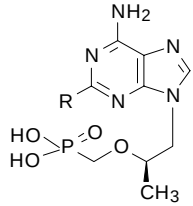
adenine] **16a**. These compounds need only two phosphorylations to be converted to the active (diphosphate) form, *i.e.* PMEApp, PMPApp, which then interact as chain terminators at the RT level (Fig. 10).



14a, R = H : PMEA
14b, R = NH_2 : PMEDAP

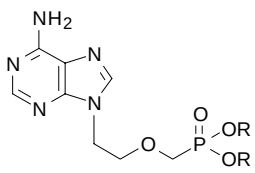


15a, R = H : (*S*)-FPMPA
15b, R = NH_2 : (*S*)-FPMPDAP

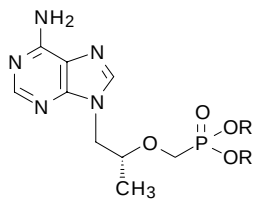


16a, R = H : (*R*)-PMPA
16b, R = NH_2 : (*R*)-PMPDAP

The acyclic nucleoside phosphonates PMEA and PMPA have proved to be potent and selective inhibitors of retrovirus (*i.e.* HIV) replication, in cell culture, animal models and humans[31,32]. Since these compounds are not sufficiently bioavailable by the oral route, they have been formulated in an oral prodrug form, *i.e.* bis(POM)PMEA, **17** and bis(POC)PMPA, **18**. Bis(POM)PMEA is now in advanced clinical trials and



bis(POM)PMEA
17, R = $CH_2OC(O)C(CH_3)_3$



bis(POC)PMPA
18, R = $CH_2OC(O)CH(CH_3)_2$

bis(POC)PMPA has just entered clinical trials in HIV-infected individuals. Both compounds offer great promise for the treatment of HIV infections. They may be administered in a convenient way, that is one oral dose per day; they are not only active against HIV but also against hepatitis B virus (HBV). PMEA is also active against the replication of HCMV and may be expected to be effective in the prophylaxis of HCMV infections. On the other hand, PMPA has proved to completely prevent simian immunodeficiency virus infection (SIV) in Rhesus macaques monkeys[33].

Of the NNRTIs, two compounds have presently been approved, namely nevirapine **19** and delavirdine **20**, and several others are in either preclinical or clinical development: loviride **21**, MKC-442 **22**, Tivirapine R-86183 **23**, DMP-266, HBY-097 **24**, and the thiocarboxanilides **25** (ref.[34]). All these compounds, although belonging to different

chemical classes, assume a butterfly-like structure (Fig. 11). They dock into a pocket ("niche") located in the vicinity of the substrate binding site, as shown for loviride in Fig. 12. Consequently, the NNRTIs interfere allosterically with RT activity and processivity.

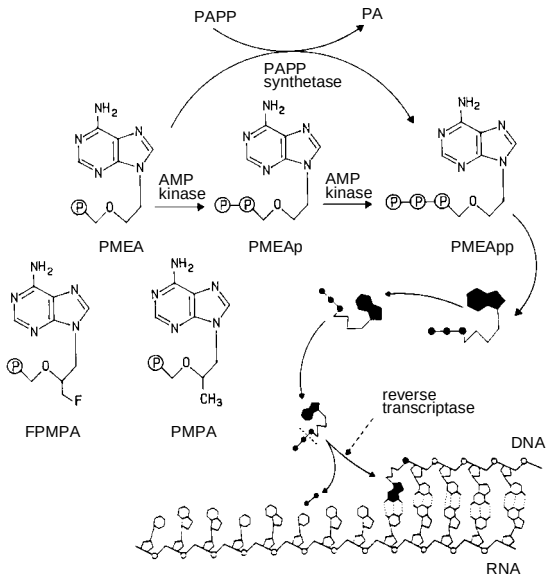


FIG. 10
Mechanism of anti-HIV action of acyclic nucleoside phosphonates (PMEA, FPMPA, PMPA). After intracellular phosphorylation of these compounds to their diphosphate form (which can occur in one or two steps catalyzed by the 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase or the (d)AMP kinase, respectively), the latter (*i.e.* PMEApp, FPMPApp or PMPApp) interact as competitive inhibitors/alternate substrates with respect to the natural substrate (dATP). The incorporation of PMEA, FPMPA or PMPA in the DNA product leads to chain termination

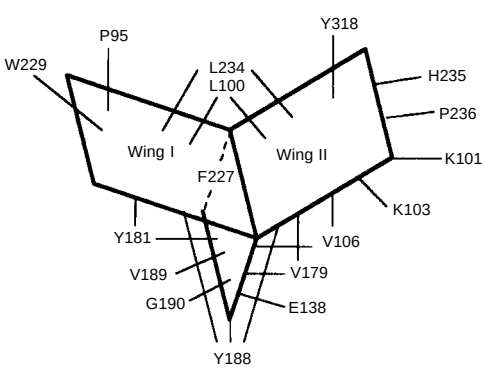
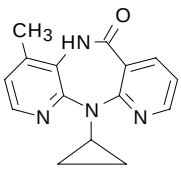


FIG. 11
Schematic representation of the NNRTIs showing the butterfly shape (with the two wings) and the interactions between different positions of the butterfly and the surrounding amino acid residues[34]. All amino acid residues making contact with the NNRTIs are from the p66 subunit, except for E138, which is from the p51 subunit



Nevirapine

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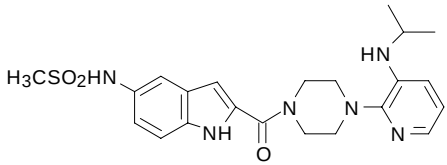

Delavirdine, U-90152

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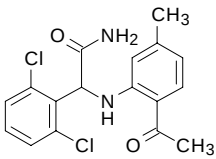
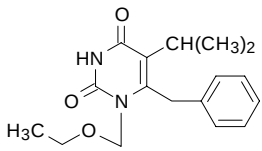


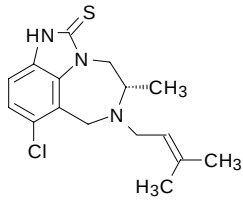
Loviride, R-89439

21



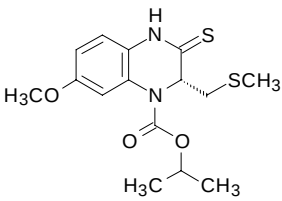
I-EBU, MKC-442

22



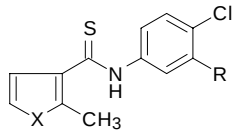
Tivirapine, R-86183, 8-Cl-TIBO

23



HBY-097

24



25	X	R	Code
a	O	CH=N-O-C(CH$_3$)$_2$	UC-10
b	O	OCH$_2$CH=C(CH$_3$)$_2$	UC-781
c	S	OCH$_2$CH=C(CH$_3$)$_2$	UC-82

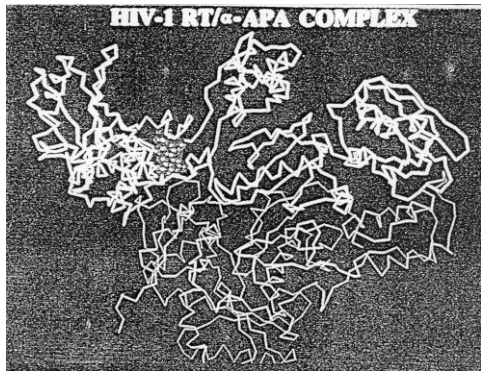
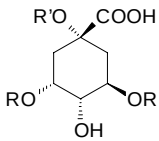


FIG. 12
Schematic presentation of the HIV-1 reverse transcriptase (RT) with loviride (R-89439, α-APA) docked into the NNRTI-binding site ("pocket") of the enzyme[5]

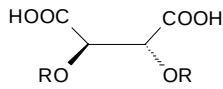
6. Proviral DNA Integration Inhibitors

Proviral DNA integration into the cell DNA genome encompasses several steps (*i.e.* endonuclease, strand transfer, disintegration, ligation) (Fig. 13) which makes it *a priori* a hard task to conceive specific inhibitors for any of these steps.

There is a number of compounds that have been reported to interfere with the integration process: among them, zintevir (Fig. 5), which is also known to block the virus adsorption process[8,9]. Although zintevir clearly inhibits the HIV integrase through blocking the formation of the initial stable complex[35], it is not clear that this particular action may play a role in the overall anti-HIV activity of the compound.

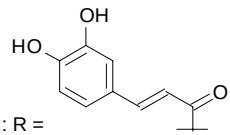


26a, R' = H
26b, R' =COCOOCH$_3$



27

In formulae **26**, **27** : R =



Other compounds that have been shown to interfere with HIV proviral DNA integration are the dicaffeoylquinic acid derivatives. These compounds inhibit the HIV integrase on the one hand and HIV replication in cell culture on the other hand[36,37] Time-of-addition experiments should be carried out to ascertain that the inhibitory effect of the dicaffeoylquinic acid derivatives **26**, **27** on HIV replication is really due to inhibition of the HIV integrase.

7. Proviral DNA Replication Inhibitors

No inhibitors are known that specifically interfere with the replication of the proviral DNA.

8. Proviral DNA Transcription Inhibitors

The proviral DNA transcription to viral messenger RNA is a multi-facetted process involving several host cell factors (including the RNA polymerase) as well as the viral regulatory protein Tat (*trans*-acting transcription transactivator)[38] (Fig. 14).

A few compounds have been described to interfere with the Tat transactivation process: the benzodiazepine derivative Ro 5-3335 **28** (ref.[39]), and the non-benzodiazepine derivative GCPK **29** (ref.[40]). The so-called Tat antagonists do not interfere directly with the viral Tat protein as such but with other (cellular) factor(s) that are involved in the Tat transactivation process[41].

Another class of compounds that interact with the Tat transactivation process are the carbocyclic adenosine analogues (for instance neplanocin A **30a**, 3-deazaneplanocin A **30b**, 5′-noraristeromycin **31**, and others **32–35**). These compounds are known inhibitors of the *S*-adenosylhomocysteine (SAH) hydrolase[42]. They lead to an accumulation of

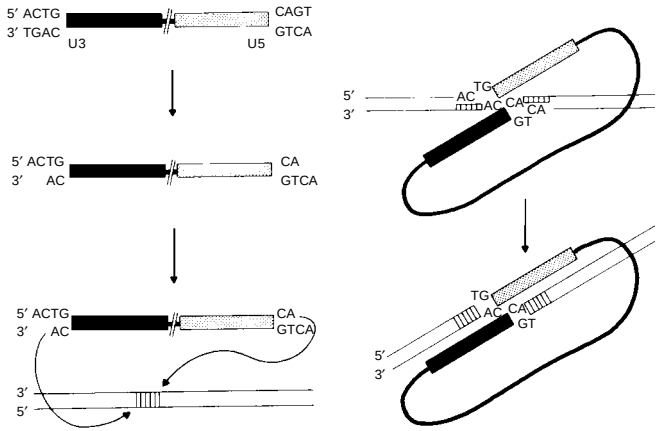


FIG. 13
Proviral DNA integration into the host cell DNA genome is a multistaged process involving DNA-integrase complex formation, endonucleolytic cleavage, DNA strand transfer, disintegration and ligation

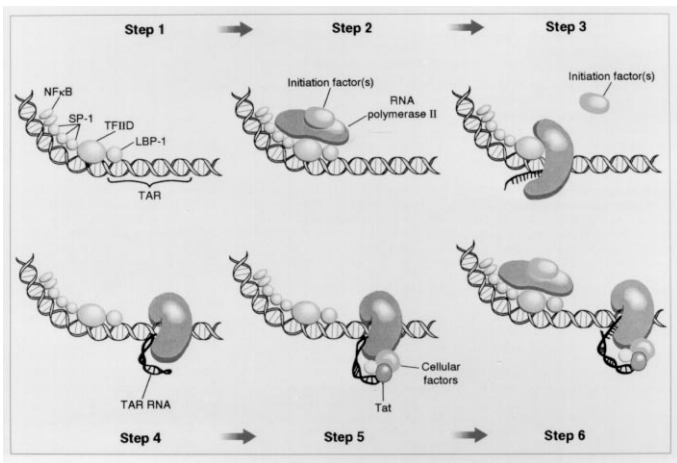
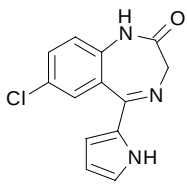


FIG. 14
Successive steps in the transcription of proviral DNA to mRNA (ref.[38]). Transactivation of the transcription process by Tat (step 5) following formation of TAR RNA (step 4). Tat is the *trans*-acting transcription transactivator, whereas TAR is the Tat-responsive element

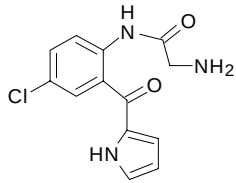
SAH which is a product/inhibitor of transmethylation reactions that depend on *S*-adenosylmethionine (SAM) as the methyl donor. These transmethylation reactions are assumed to play a role in the maturation of viral mRNAs and they may also be involved in the formation of mature HIV mRNA from the proviral DNA. This could then explain the inhibitory effect of the carbocyclic adenosine analogues on the HIV mRNA transcription process[43].

9. Viral Messenger RNA Translation Inhibitors

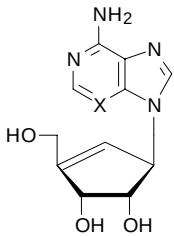
The viral mRNA can serve as the target for oligonucleotides (*i.e.*, ribozymes, antisense oligonucleotides) that specifically hybridize with well-defined nucleotide sequences in the viral mRNA: *i.e.* the GEM91, 25-mer oligodeoxynucleotide phosphorothioate that



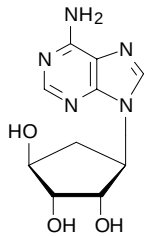
Ro 5-3335
28



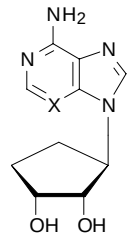
GCPK
29



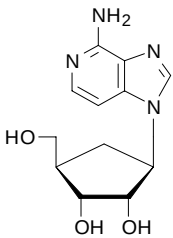
30a, X = N : Neplanocin A
30b, X = CH : 3-Deazaneplanocin A



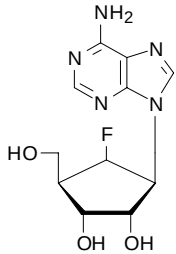
31, 5'-Noraristeromycin



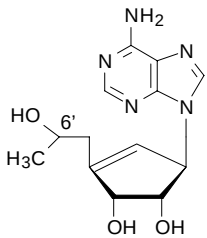
32a, X = N : DHCaA
32b, X = CH : c^{3}DHCaA



33, 3-Deazaaristeromycin, C-c^{3}Ado



34, 6'-Fluoroaristeromycin, F-C-Ado



35a, 6'-(*R*)-Methylneplanocin A
35b, 6'-(*S*)-Methylneplanocin A

is complementary to the translation-initiation site of the viral mRNA (refs[44,45], Fig. 15). Antisense oligonucleotides may be expected to cause viral mRNA translation arrest through their hybridization with the mRNA. *A conditio sine qua non* for their anti-HIV activity is that they must be able to penetrate into the cells to reach their target sequence at the viral mRNA level. This condition is hard to fulfill and requires judicious chemical manipulations of the oligonucleotide structure.

Moreover, antisense oligonucleotides may also interfere with processes other than viral mRNA translation, such as virus adsorption to the cells (because of their polyanionic character), the reverse transcription (by competition with the RNA template), proviral DNA integration (by interference with any of the steps of the integration process), and proviral DNA transcription (by triplex formation with proviral DNA).

10. Viral Maturation (Proteolysis) Inhibitors

When the viral particles are assembled to mature virions, the precursor capsid proteins (Pr 55 gag, Pr 160 gag-pol) need to be cleaved by the HIV protease into smaller, mature capsid proteins (p17, p24, p9, p7). The peptide linkage that is involved in the proteolytic cleavage between p17 and p24 is the Tyr-Pro linkage (Fig. 16).

The protease inhibitors have been tailored after the peptide that is normally cleaved by the HIV protease. Thus, in the protease inhibitors the hydrolysable peptide linkage has been replaced by a non-hydrolysable hydroxyethylene bond, and this motif is present in the different protease inhibitors that have so far been approved for the treatment of HIV infections[46]: saquinavir **36**, ritonavir **37**, indinavir **38**, and nelfinavir **39**.

Protease inhibitors, as exemplified for indinavir (Fig. 17), can cause a dramatic decrease in plasma HIV RNA load, paralleled by a concomitant increase in the $CD4^+$

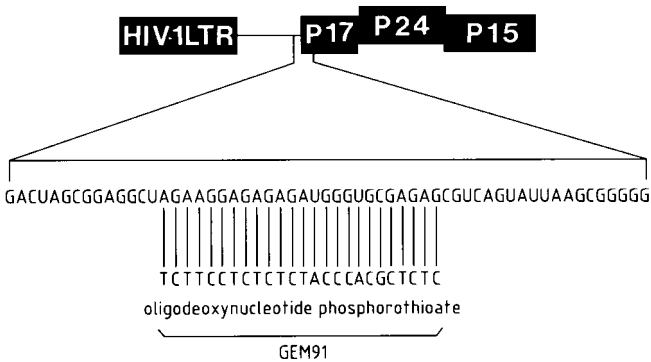
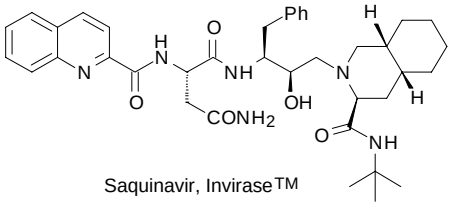
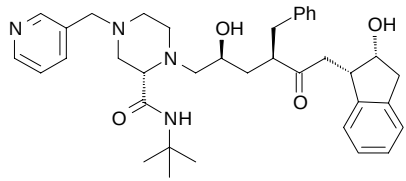


FIG. 15
GEM91, a 25-mer oligodeoxynucleotide phosphorothioate, is complementary, and thus hybridizes with the *gag* mRNA of HIV-1 at the initiator (AUG) codon region[45]



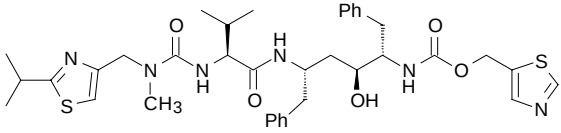
Saquinavir, Invirase™

36



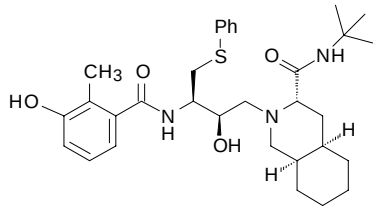
Indinavir, Crixivan™

38



Ritonavir, Norvir™

37



Nelfinavir, Viracept™

39

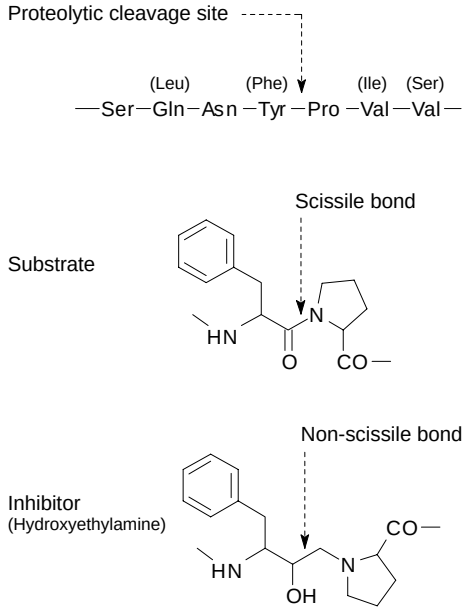


FIG. 16

Substrate-based (peptidomimetic) inhibitors of HIV-1 protease. Example of substrate sequence cleaved by the HIV-1 protease: Tyr(Phe)-Pro. In the protease inhibitor the scissile bond (CONH) is replaced by a non-scissile bond [$CH(OH)CH_2NH$]

counts[47]. Their effects on both viral load and $CD4^+$ counts seem to be more pronounced than that of the nucleoside analogues AZT and 3TC combined.

11. Virus Budding (Release) Inhibitors

No inhibitors are known that specifically interfere with the virus budding (release) process.

12. Outlook for Future anti-HIV Therapy

All the HIV inhibitors may be expected to select for mutations that engender resistance to the compounds. This resistance development may obviously compromise the clinical usefulness of the different anti-HIV agents. Therefore, strategies should be envisaged to circumvent or prevent the development of resistance. One such strategy is based on the use of the compounds from the beginning at sufficiently high concentrations so as to completely suppress the virus so that is has no chance to develop resistance.

As shown in Fig. 18, loviride **21** (R-89439) is able to completely prevent virus breakthrough when applied to the cells at a concentration of 2.5 μg/ml, whereas at lower concentrations (0.1, 0.3 and 0.5 μg/ml) it only temporarily prevents virus breakthrough, that is for 3, 7 or 10 days, respectively[48]. Similarly, the quinoxaline **40** (S-2720) completely prevents virus breakthrough is used at a concentration as low as 0.1 μg/ml (ref.[49]). Furthermore, when the virus-infected cells that had been exposed to the quinoxaline **40** at 0.1 μg/ml were analyzed by multiple cycles of PCR for the detection of proviral DNA, no such proviral DNA was detectable (Fig. 19). This suggests that virus-

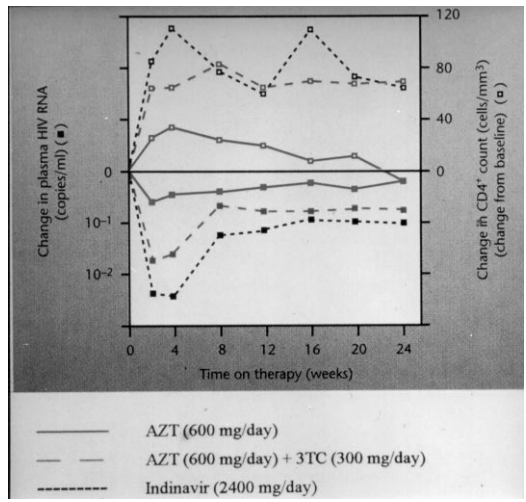
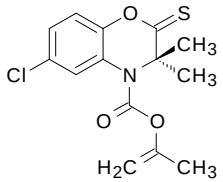
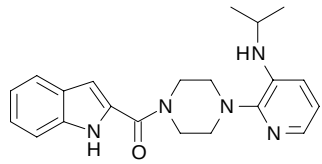


FIG. 17
Composite of plasma HIV RNA and $CD4^+$ cell count responses among a group of patients receiving three different anti-retroviral regimens: zidovudine monotherapy (AZT); zidovudine plus lamivudine (AZT + 3TC), and the protease inhibitor indinavir (MK-639). All patients were naive to their respective treatment regimens. For each treatment group, the relative HIV RNA and $CD4^+$ count treatment responses appear inversely proportional, although individual exceptions to this association exist[47]

infected cells that had been passaged in the presence of this anti-HIV agent had completely lost the proviral DNA, which in turn suggests that the cells had been cleared ("cured") from the virus. Further experiments[50] have indicated that the "newer" NNRTIs such as quinoxaline HBY-097 **24** and thiocarboxanilide UC-781 **25b** are able to prevent virus breakthrough for a longer time and at a lower concentration than the "older" compounds such as nevirapine **19** and BHAP (U-88204) **41** (Fig. 20).



S-2720
40



BHAP, U-88204
41

When different compounds are combined, such as 3TC **13** with MKC-442 **22**, or 3TC with delavirdine **20**, or 3TC with thiocarboxanilide **25b**, virus breakthrough may be delayed for much longer time than when the compounds are used individually[51]. For example (Fig. 21), 3TC at a concentration of 0.1 μg/ml delays virus breakthrough for about 10 days and so does MKC-442 at a concentration of 0.04 μg/ml, but if both 3TC at 0.1 μg/ml and MKC-442 at 0.04 μg/ml are combined, they can delay virus breakthrough for more than 50 days.

It has become increasingly evident that future anti-HIV therapy will consist of combinations of two, three, four or even more drugs. If only limited to the reverse transcrip-

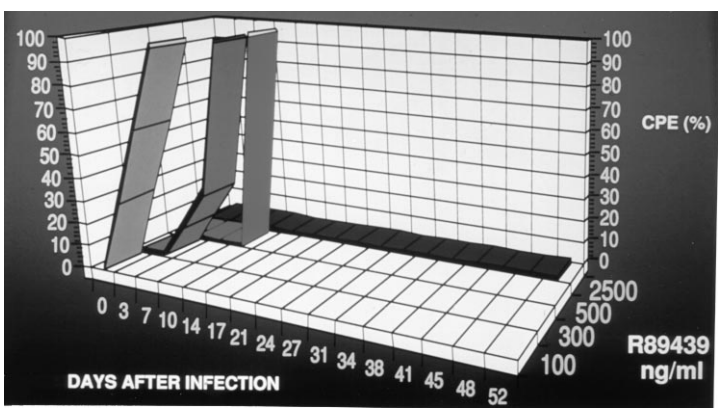


FIG. 18
Virus suppression [in HIV-1(III_B)-infected MT-4 cells] by different concentrations (100, 300, 500 and 2 500 ng/ml) of the HIV-1-specific RT inhibitor loviride (R-89439)[48]

tase inhibitors and protease inhibitors, there are more than 30 compounds (Table I) that we could choose from to concoct drug combination regimens[46]. The choice for the ideal drug combination will have to be guided by several criteria, such as availability from the supplier, convenience of administration, compliance by the patient, and possible drug interactions in the organism. There must exist a wealth of ideal drug combinations, and these will have to be defined in the future.

The aim of the future anti-HIV therapy will be based on the installment of the multiple (*i.e.*, triple, quadruple,…) drug combinations as early as possible during the course of the HIV infection whenever there is evidence of ongoing HIV replication. The compounds should be used at sufficiently high doses so as to completely suppress

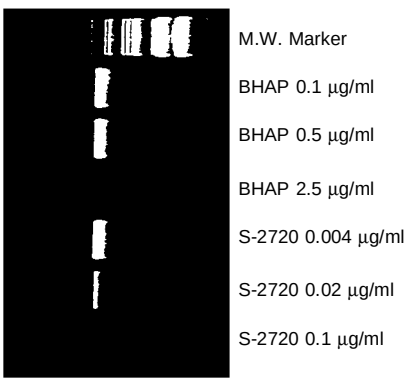


FIG. 19
Lack of proviral DNA detection in HIV-1-infected CEM cell cultures after the 10th subcultivation in the continuous presence of BHAP (U-88204), **41** (at 2.5 μg/ml) or quinoxaline S-2720, **40** (at 0.1 μg/ml) (ref.[49])

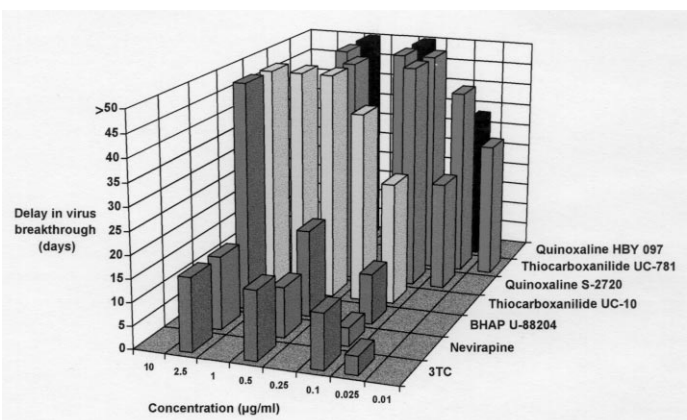


FIG. 20
Suppression of virus breakthrough in CEM cells infected with HIV-1(III_B) and treated with individual drugs at different concentrations. The delay in virus breakthrough corresponds to the number of days required for virus-induced cytopathicity to develop

virus replication. If different compounds are combined, the doses could be lowered so as to still generate a total antiviral response in the absence of untoward side-effects. Mellors *et al.*[52] have pointed out that the risk for the development of AIDS is directly

TABLE I
Candidate compounds among nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors[46] (PIs), that could be considered for multiple drug combinations in attempts to knock-out HIV-1

NRTIs	NNRTIs	PIs
Zidovudine (AZT) **9**	Nevirapine **19**	Saquinavir **36**
Didanosine (ddI) **10**	Delavirdine (BHAP, U-90152) **20**	Ritonavir **37**
Zalcitabine (ddC) **11**	I-EBU (MKC-442) **22**	Indinavir **38**
Stavudine (d4T) **12**	Loviride (α-APA, R-89439) **21**	Nelfinavir **39**
Lamivudine (3TC) **13**	Benzoxazinone DMP-266 **49**	VX-478 (141W94) **48**
Adefovir (PMEA) **14a**	Pyridinone L-697,661 **47**	DMP-323 **51**
Adefovir dipivoxil [bis(POM)PMEA] **17**	Tivirapine (8-Cl-TIBO) **23**	KNI-272 **50**
PMPA **16a**	Trovirdine (LY 300046) **46**	Lasinavir (CGP-61755) **52**
Bis(POC)PMPA **18**	Quinoxaline HBY-097 **24**	RPI 312 **53**
1592U89 **42**	Thiocarboxanilide UC-781 **25b**	SC-52151 **54**
F-ddA **43**		SDZ PRI 053 **55**
(–)-FTC **45**		PNU-140690 **56**

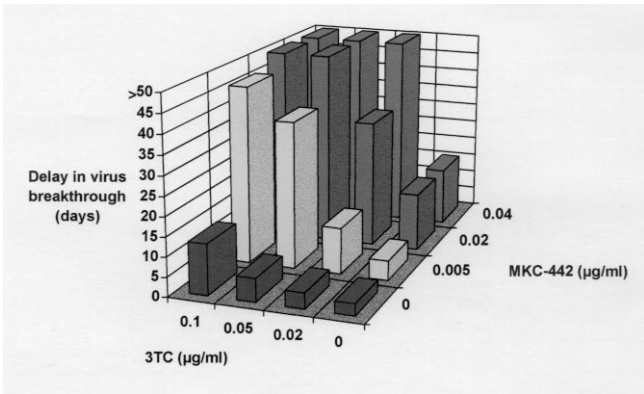
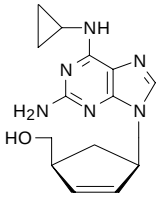
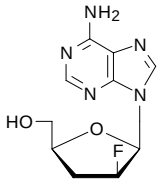


FIG. 21
Suppression of virus breakthrough in CEM cells infected with HIV-1(III_B) and treated with the combination of 3TC + MKC-442 at different concentrations. The delay in virus breakthrough corresponds to the number of days required for virus-induced cytopathicity to develop



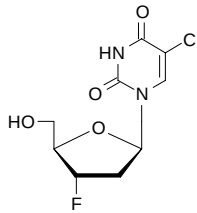
1592U89

42



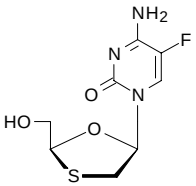
F-ddA

43



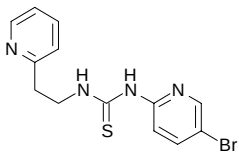
F-ddClUrd, 935U83

44



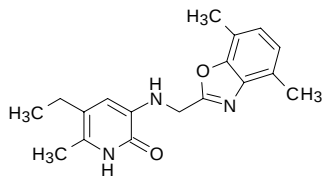
(–)-FTC

45



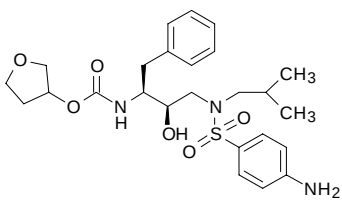
Trovirdine, PETT, LY-300046

46



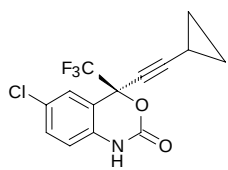
Pyridinone, L-697639

47



VX-478, 141W94

48



L-743,726, DMP-266

49

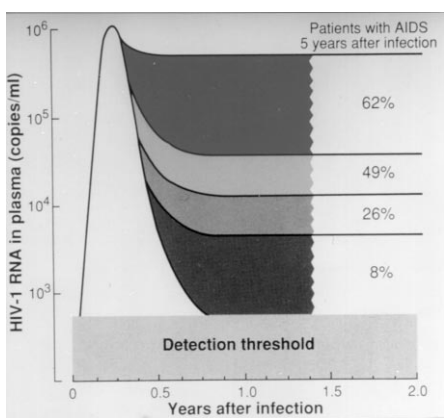
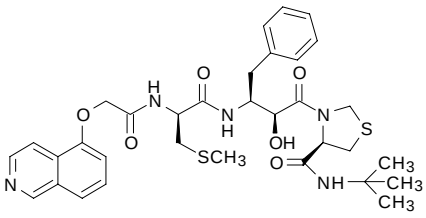


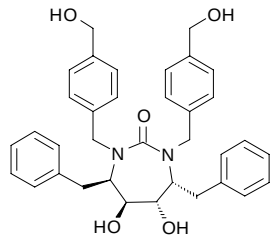
FIG. 22
Variable virologic setpoints after acute HIV-1 infection and their prognostic values in terms of progression to AIDS (ref.[53])

proportional to the initial plasma viral load. If this viral load can be brought down to below the detection threshold (Fig. 22), the risk for the development of AIDS should be minimal[53]. We thus envision that treatment with different drugs in combination and at sufficiently high doses, started as early as possible during the course of the HIV infection, may completely prevent progression to the disease.



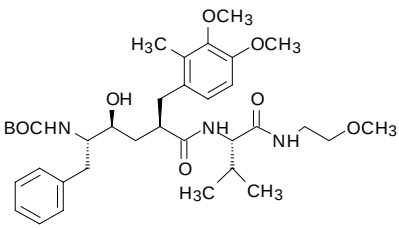
KNI-272

50



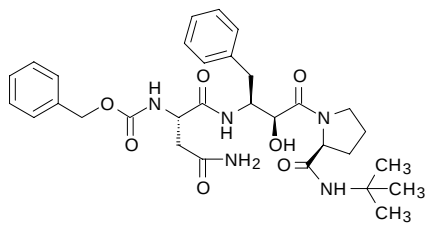
DMP-323

51



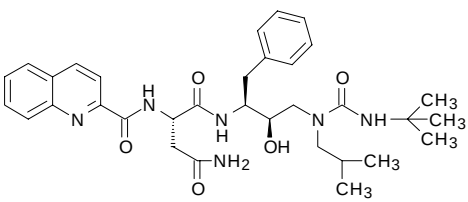
Lasinavir, CGP-61755

52



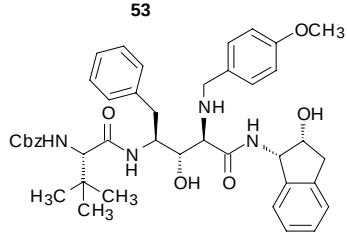
RPI 312

53



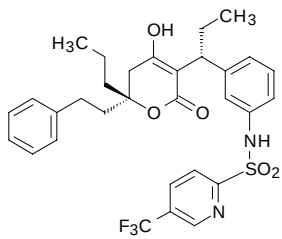
SC-52151

54



SDZ PRI 053

55



PNU-140690

56

ADDENDA

List of drug names, their synonyms and code specification

Formula	Name	Synonym	Code	Ref.
1	Suramin	Germanin	Bayer 205	2
2	Dextran sulfate			5, 6
3	Poly(vinyl alcohol sulfate)		PVAS	5, 6
4	Poly(vinyl sulfonate)			7
5	Poly(naphthalene-2-sulfonate)		PRO 2000	7
6			RPR 103611	56
7			JM 3100	57
8a			DIBA	58
8b			SRR-SB3	59
9	Zidovudine	Retrovir, azidothymidine	AZT	60
10	Didanosine	Videx™	ddI	61
11	Zalcitabine	Zerit™	ddC	62
12	Stavudine		d4T	63
13	Lamivudine	Epivir™	BCH-189	64
14a	Adefovir		PMEA	65
14b			PMEDAP	65
15a			(*S*)-FPMPA	66
15b			(*S*)-FPMPDAP	66
16a			(*R*)-PMPA, GS-1278	67
16b			(*R*)-PMPDAP	67
17	Adefovir dipivoxil		bis(POM)PMEA	68
18			GS-4331, bis(POC)PMPA	69
19	Nevirapine	Viramun™	BIRG-587	70
20	Delavirdine	Rescriptor™	U-90152, BHAP	71
21	Loviride		R-89439, α-APA	34
22			MKC-442, I-EBU	72, 73
23	Tivirapine		R-86183, 8-Cl-TIBO	74
24			HBY-097	75
25a			UC-10	76
25b			UC-781	76
25c			UC-82	76

List of drug names (*Continued*)

Formula	Name	Synonym	Code	Ref.
26a				36, 37
26b				36, 37
27				36, 37
28			Ro 5-3335	77
29			GCPK	78
30a	Neplanocin A			79
30b	3-Deazaneplanocin A			80
31	5′-Noraristeromycin			81
32a			DHCaA	82
32b			c^3DHCaA	82
33			C-c^3Ado	83
34	6′-Fluoroaristeromycin		F-C-Ado	84
35a	6′-(*R*)-Methylneplanocin A			85
35b	6′-(*S*)-Methylneplanocin A			85
36	Saquinavir	Invirase™	Ro-31-8959	86
37	Ritonavir	Norvir™	ABT-538	87
38	Indinavir	Crixivan™	MK-639, L-735	88
39	Nelfinavir	Viracept™	AG-1343	89
40			S-2720	90
41			U-88204, BHAP	91
42			1592U89	92
43			F-ddA	93
44			FddClUrd, 935U83	106
45			(–)-FTC	94
46	Trovirdine		LY 300046, PETT	95
47	Pyridinone		L-697,639	96
48			141W94, VX-478	97
49			DMP-266, L-743,726	98
50			KNI-272	99
51			DMP-323	100
52	Lasinavir		CGP-61755	101
53			RPI312	102
54			SC-52151	103
55			SDZ PRI 053	104
56			PNU-140690	105

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