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Biochimica et Biophysica Acta 1587 (2002) 258–275

BIOCHIMICA ET BIOPHYSICA ACTA
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Review

New developments in anti-HIV chemotherapy ☆

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Received 24 January 2002; accepted 24 January 2002

Abstract

Virtually all the compounds that are currently used, or are subject of advanced clinical trials, for the treatment of human immunodeficiency virus (HIV) infections, belong to one of the following classes: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs): i.e. zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), emtricitabine [(−)FTC], tenofovir disoproxil fumarate; (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs): i.e. nevirapine, delavirdine, efavirenz, emivirine; and (iii) protease inhibitors (PIs): i.e. saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir. In addition to the reverse transcriptase (RT) and protease reaction, various other events in the HIV replicative cycle can be considered as potential targets for chemotherapeutic intervention: (i) viral adsorption, through binding to the viral envelope glycoprotein gp120 (polysulfates, polysulfonates, polycarboxylates, polyoxometalates, polynucleotides, and negatively charged albumins); (ii) viral entry, through blockade of the viral coreceptors CXCR4 [bicyclam (AMD3100) derivatives] and CCR5 (TAK-779 derivatives); (iii) virus–cell fusion, through binding to the viral envelope glycoprotein gp41 (T-20, T-1249); (iv) viral assembly and disassembly, through NCp7 zinc finger-targeted agents [2,2′-dithiobisbenzamides (DIBAs), azadicarbonamide (ADA)]; (v) proviral DNA integration, through integrase inhibitors such as 4-aryl-2,4-dioxobutanoic acid derivatives; (vi) viral mRNA transcription, through inhibitors of the transcription (transactivation) process (flavopiridol, fluoroquinolones). Also, various new NRTIs, NNRTIs and PIs have been developed that possess, respectively: (i) improved metabolic characteristics (i.e. phosphoramidate and *cyclo*saligenyl pronucleotides by-passing the first phosphorylation step of the NRTIs), (ii) increased activity ["second" or "third" generation NNRTIs (i.e. TMC-125, DPC-083)] against those HIV strains that are resistant to the "first" generation NNRTIs, or (iii) as in the case of PIs, a different, nonpeptidic scaffold [i.e. cyclic urea (mozenavir), 4-hydroxy-2-pyrone (tipranavir)]. Nonpeptidic PIs may be expected to inhibit HIV mutant strains that have become resistant to peptidomimetic PIs. Given the multitude of molecular targets with which anti-HIV agents can interact, one should be cautious in extrapolating the mode of action of these agents from cell-free enzymatic assays to intact cells. Two examples in point are L-chicoric acid and the nonapeptoid CGP64222, which were initially described as an integrase inhibitor or Tat antagonist, respectively, but later shown to primarily act as virus adsorption/entry inhibitors, the latter through blockade of CXCR4.

Keywords: Human immunodeficiency virus (HIV); Reverse transcriptase (HIV); Protease (HIV); CXCR4 (HIV); CCR5 (HIV); Integrase (HIV); Fusion (HIV); Transcription (HIV)

Abbreviations: HIV, human immunodeficiency virus; NRTIs, nucleoside/nucleotide reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; DIBA, 2,2′-dithiobisbenzamide; ADA, azadicarbonamide; AIDS, acquired immune deficiency syndrome; HSV, herpes simplex virus; STD, sexually transmitted disease; MIP-1α and -1β, macrophage inflammatory proteins; SDF-1, stromal-cell derived factor; PBMCs, peripheral blood mononuclear cells; TM4, transmembrane segment; SI, syncytium-inducing; NSI, non-syncytium-inducing; NOBA, 3-nitrosobenzamide; AZT, zidovudine; ddI, didanosine; ddC, zalcitabine; d4T, stavudine; 3TC, lamivudine; ABC, abacavir; bis(POM)-PMEA, bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, adefovir dipivoxyl; bis(POC)-PMPA, bis(isopropyloxycarbonyloxymethyl)-(*R*)-9-(2-phosphonylmethoxypropyl)adenine, tenofovir disoproxil; dOTC, (±)2′-deoxy-3′-oxa-4-thiocytidine; (−)FTC, emtricitabine; DAPD, amdoxovir, (−)-β-D-2,6-diaminopurine dioxolane; bis(SATE)ddAMP, bis(*S*-acetyl-2-thioethyl)phosphotriester of ddA

☆ Proceedings of the 8th International Symposium on Molecular Aspects of Chemotherapy, Gdansk, Poland, 5–9 September 2001.

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0925-4439/02/$ - see front matter
PII: S0925-4439(02)00089-3

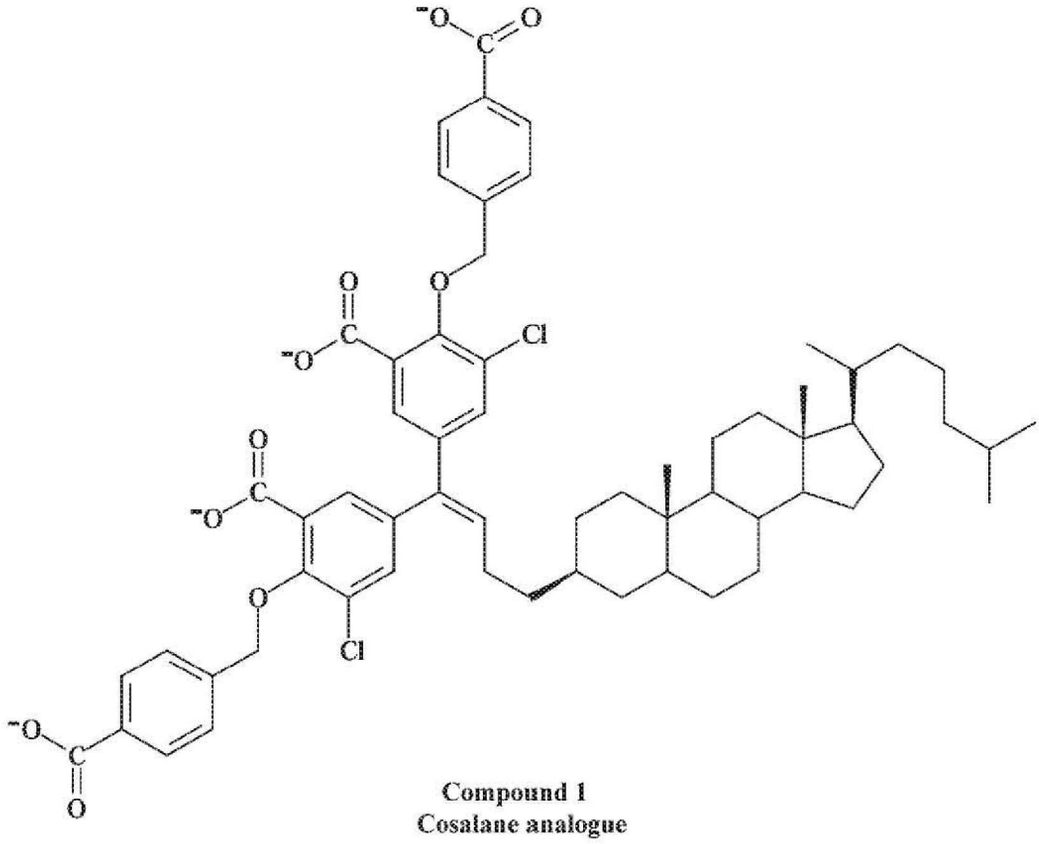
1. Introduction

Combination therapy, comprising at least three anti-human immunodeficiency virus (HIV) drugs, has become the standard treatment of acquired immune deficiency syndrome (AIDS) or HIV-infected patients. Virtually all drugs that have been licensed for clinical use (or made available through expanded access programmes) for the treatment of HIV infections fall into one of the following three categories: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), that, following two phosphorylation steps (tenofovir) or three phosphorylation steps [zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC)], act, as chain terminators, at the substrate binding site of the reverse transcriptase (RT); (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs) that interact with the RT at an allosteric, non-substrate binding site (nevirapine, delavirdine, efavirenz); and (iii) protease inhibitors (PIs) that specifically inhibit, as peptidomimetics, the virus-associated protease (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir). Guidelines to the major clinical trials with these compounds have been recently published [1].

Although the long-term goal of eradicating the virus from latently and chronically infected cells remains forbidding [2], the advent of so many new compounds other than those that have been formally approved, for the treatment of HIV infections, will undoubtedly improve the prognosis of patients with AIDS and AIDS-associated diseases. Here, I will primarily address those new anti-HIV compounds that (i) have emerged as promising anti-HIV drug candidates during the last few years, that (ii) are in preclinical or early-clinical development, and that (iii) are targeted at well-defined steps in the HIV replicative cycle.

2. Virus adsorption (gp120) inhibitors

A great variety of polyanionic compounds have been described to block HIV replication through interference with virus adsorption (or binding) to the cell surface: i.e. polysulfates, polysulfonates, polycarboxylates, polyphosphates, polyphosphonates, polyoxometalates, etc. This class of compounds also comprises the cosalane analogues (**1**) containing the polycarboxylate pharmacophore [3], as well as the sulfated polysaccharides extracted from sea algae [4]. All these compounds, whether synthetic or of natural origin, are assumed to exert their anti-HIV activity by shielding off the positively charged sites in the V3 loop of the viral envelope glycoprotein (gp120) [5,6], which is necessary for virus attachment to the cell surface heparan sulfate, a primary binding site, before a more specific binding occurs

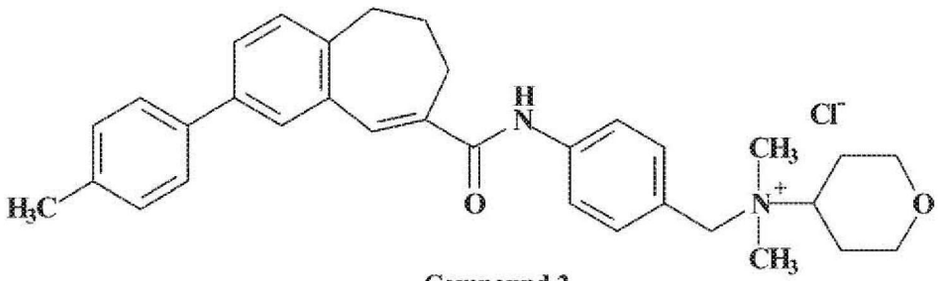


Compound 1
Cosalane analogue

to the CD4 receptor of the $CD4^+$ cells, and to the CXCR4 coreceptor of the $CXCR4^+$ cells (the latter in the case of X4 and dual tropic X4/R5 HIV strains). Heparan sulfate is widely expressed on animal cells and, as it is involved in the virus-cell binding of a broad spectrum of enveloped viruses, including herpes simplex virus (HSV) [7], dengue virus [8] and other flaviviruses (i.e. Japanese encephalitis virus) [9], it also explains why polysulfates have a broad-spectrum antiviral activity against HIV, HSV and various other enveloped viruses [10].

ligand, namely SDF-1 ("stromal-cell derived factor") has been identified. Of these chemokines, the LD78β isoform of MIP-1α has emerged as the most potent chemokine for inhibiting HIV-1 infection in peripheral blood mononuclear cells (PBMCs) [14,15] as well as monocytes/macrophages [16].

TAK-779, a quaternary ammonium derivative (**2**) is the first nonpeptidic molecule that has been described to block the replication of M-tropic R5 HIV-1 strains at the CCR5 level [17].



Compound 2
TAK-779

The major role of polysulfates or polyanionic substances in general in the management of HIV infections may reside in the prevention of sexual transmission of HIV infection, as these compounds, if applied as a vaginal formulation, may successfully block HIV infection through both virus-to-cell and cell-to-cell contact. These compounds therefore merit being pursued as vaginal microbicides. The fact that in addition to their anti-HIV activity, these polyanionic substances, as demonstrated, for example, for poly(sodium(4-styrene)sulfonate), also inhibit other sexually transmitted disease (STD) pathogens, i.e. HSV, *Neisseria gonorrheae* and *Chlamydia trachomatis* [11], further adds to their potential therapeutic and preventive value.

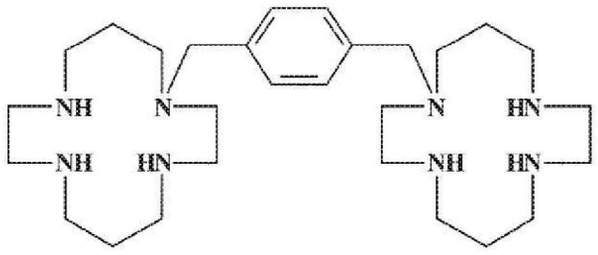
3. Viral coreceptor antagonists

To enter cells, following binding with the CD4 receptor, the HIV-1 particles must interact, again through the viral envelope glycoprotein gp120, with the CXCR4 coreceptor [12] or CCR5 coreceptor [13]. CXCR4 is the coreceptor for HIV-1 strains that infect T-cells (T-tropic or X4 strains), and CCR5 is the coreceptor for HIV-1 strains that infect macrophages (M-tropic or R5 strains). CXCR4 and CCR5 have not evolved simply to act as coreceptors for HIV entry; they normally act as receptors for chemokines (chemoattractant cytokines). The normal ligands for CCR5 are RANTES ("regulated upon activation, normal T-cell expressed and secreted") and MIP-1α and -1β ("macrophage inflammatory proteins"), whereas for CXCR4, only one natural

A binding site for TAK-779 has been identified within the transmembrane helices 1, 2, 3 and 7 of CCR5 [18]. TAK-779 has been found to inhibit R5 HIV-1 strains in the nanomolar concentration range, while not affecting X4 HIV-1 strains at 10,000-fold higher concentrations [17]. TAK-779 is not a "pure" CCR5 antagonist, as it also demonstrates some antagonism towards CCR2b. Unlike RANTES, TAK-779 does not induce internalization of CCR5. The clinical potential of TAK-779 and its congeners [19] in the therapy and/or prophylaxis of HIV-1 infections remains to be further explored. Meanwhile, several new CCR5 antagonists have been reported [20,21], and a lead clinical candidate (SCH C) for further development has been identified.

Almost simultaneously [22–24], three compounds, i.e. the bicyclam AMD3100 [22], [Tyr-5,12,Lys-7]polyphemusin or T22 [23] and the nonapeptide $(D\text{-Arg})_9$ or ALX40-4C [24] were announced as CXCR4 antagonists, blocking the replication of T-tropic X4, but not M-tropic R5, HIV-1 strains through selective antagonism of CXCR4. The bicyclams are the most specific and most potent CXCR4 antagonists that have been described to date [25,26]. The bicyclams had been known as potent and selective HIV inhibitors for a number of years [27,28], before their target of action was identified as the CXCR4 coreceptor [22,29,30]. The bicyclam AMD3100 (**3**) inhibits the replication of X4 HIV-1 strains within the nanomolar concentration range [28]. As it is not toxic to the host cells at concentrations up to 500 μM, its selectivity index, or ratio of 50% cytotoxic concentration (CC_{50}) to 50%

antivirally effective concentration (EC_{50}) can be estimated at > 100,000.



Compound 3
AMD3100

A close correlation has been found, over a concentration range of 0.1–1000 ng/ml, between the AMD3100 concentrations required to inhibit (i) HIV-1 NL4-3 replication, (ii) monoclonal antibody (mAb 12G5) binding to the CXCR4 coreceptor, and (iii) SDF-1-induced signal transduction (Ca^{2+} flux), suggesting an intimate relationship between these three parameters [29,30]. The inhibitory effects of AMD3100 on the T-tropic HIV-1 NL4-3 strain have been demonstrated in a wide variety of cells expressing CXCR4, including PBMCs; and, vice versa, various T-tropic and dual-tropic, but not M-tropic, HIV-1 strains have proven sensitive to AMD3100 in PBMC.

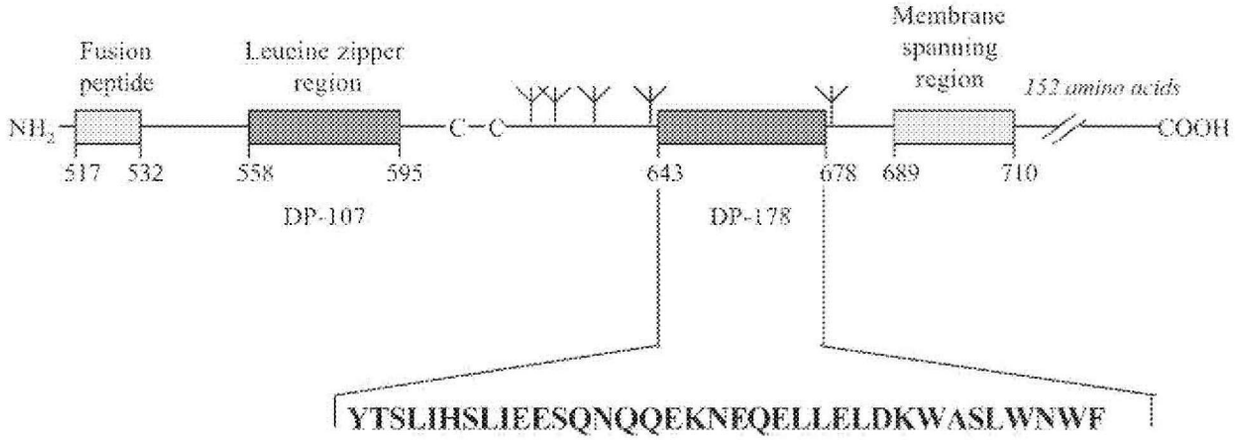
Negatively charged amino acid (i.e. aspartic acid) residues in the extracellular regions of CXCR4 must be involved in its interaction with both AMD3100 and SDF-1, and the V3 loop of X4 HIV gp120, which are all three highly basic. Substitutions of a neutral amino acid residue for aspartic acid in the second extracellular loop generated resistance to AMD3100 [31]. In particular, the aspartate residues at positions 171 and 262, located close to the extracellular sides of the transmembrane segments TM4 and TM6, may represent crucial sites of interaction with the bicyclam AMD3100 [32].

When the bicyclam AMD3100 was added to PBMC infected with clinical HIV isolates displaying the syncytium-inducing (SI) phenotype, these strains reverted to the non-syncytium-inducing (NSI) phenotype, and, concomitantly, these strains switched from CXCR4 to CCR5 coreceptor use [33]. These findings indicate that selective blockade of CXCR4 by AMD3100 may prevent the switch from the less pathogenic M-tropic R5 to the more pathogenic T-tropic X4 strains of HIV, which in vivo heralds the progression to AIDS. AMD3100 has proved efficacious, alone and in combination with other anti-HIV drugs, in achieving a marked reduction in viral load in the SCID-hu Thy/Liv mouse model [34]. Following a phase I clinical trial for safety in normal healthy volunteers [35], AMD3100 recently entered phase II clinical trials in HIV-infected individuals.

Given their high potency and selectivity as CXCR4 antagonists, bicyclams, such as AMD3100, may not only have great potential for the therapy and/or prophylaxis of X4 HIV infections, but also other pathologic processes, such as breast cancer metastasis, which are at least partially dependent of, or mediated by, signaling through CXCR4 [36].

4. Viral fusion (gp41) inhibitors

The interaction of the X4 or R5 HIV-1 envelope glycoprotein gp120 with the coreceptor CXCR4 or CCR5, respectively, is followed by a spring-loaded action of the viral glycoprotein gp41 (normally covered by the bulkier gp120), which then anchors through its amino terminus (the "fusion peptides") into the target cell membrane. This initiates the fusion of the two lipid bilayers, that of the



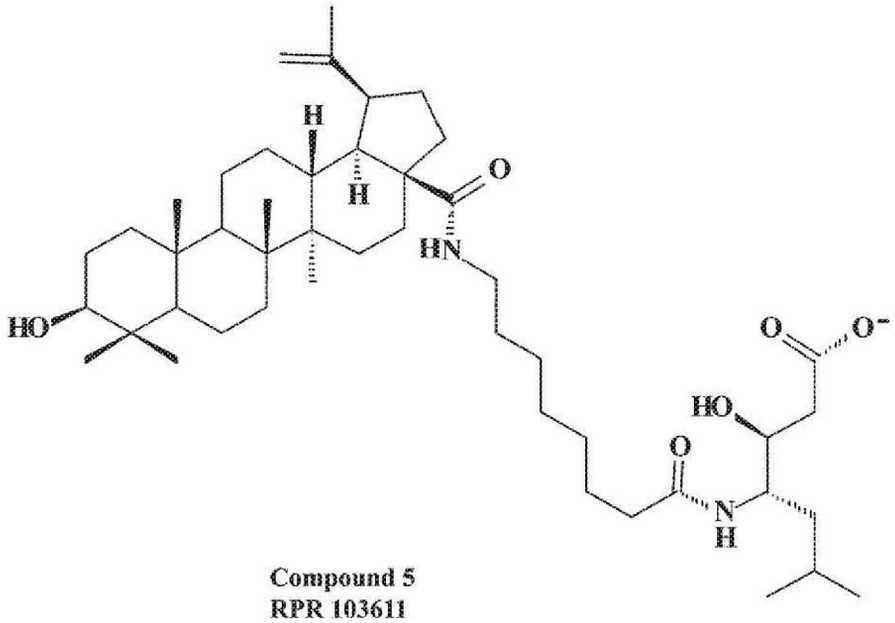
Compound 4
T-20 (pentafuside)

viral envelope with that of the cellular plasma membrane [37]. At the onset of the fusion process, the hydrophobic grooves on the surface of the N36 coiled coil in the gp41 ectodomain become available for binding with extraneous inhibitors, such as DP-178 (T-20), a 36-residue peptide, that binds to the hydrophobic groove of N36 [37].

T-20 (pentafuside) (**4**) is a synthetic, 36-amino acid peptide corresponding to residues 127–162 of the ectodomain of gp41 (or residues 643–678 in the gp160 precursor). T-20, previously called DP-178, was modeled after a specific domain (within gp41) predictive of α-helical secondary structure: DP-178 consistently afforded 100% blockade of virus-mediated cell–cell fusion (syncytium formation) at concentrations ranging from 1 to 10 ng/ml, i.e. 10^4- to 10^5-fold lower than the cytotoxic concentration [38]. An initial clinical trial has been carried out with T-20 at four doses (3, 10, 30 and 100 mg twice daily, intravenously, for 14 days) in 16 HIV-infected adults: at the highest dose (100 mg, twice daily), T-20 achieved by the 15th day a 1.5- to 2.0-fold reduction in plasma HIV RNA [39]. These data provide proof-of-concept that HIV fusion inhibitors are able to reduce virus replication in vivo.

The betulinic acid derivative RPR 103611 (**5**) is the only nonpeptidic low-molecular-weight compound that has been reported to block HIV-1 infection through interaction with gp41: this triterpene derivative has been found to inhibit the infectivity of a number of HIV-1 strains in the 10 nM concentration range [41], apparently through interference with a post-binding, envelope-dependent step involved in the fusion of the virus with the cell plasma membrane.

The exact mode of action of RPR 103611 remains to be elucidated. Sequence analysis of RPR103611-resistant mutants indicated that a single amino acid change, I84S, in HIV-1 gp41 is sufficient to confer drug resistance [42]. However, this I84S mutation did not occur in some of the naturally RPR103611-resistant HIV-1 strains such as NDK. More recently, the action of RPR103611 has been thought to depend on the accessibility of gp41 [43], and for the isomeric betulinic acid derivative IC 9564, HIV-1 gp120, rather than gp41, has been proposed as the prime target (based on the mutations G237R and R252K emerging in gp120 of drug-resistant mutants) [44]. YK-FH312, a betulinic acid derivative unrelated to RPR103611 or IC 9564, was reported to block the assembly and/or budding of HIV particles [45].



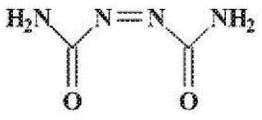
Compound 5
RPR 103611

Meanwhile, T-20 has proceeded to phase II/III clinical trials, and phase I clinical trials have been initiated with T-1249, a 39-amino acid peptide derived from DP-107 (which is a 38-amino acid peptide corresponding to residues 558–595 of gp160); T-1249 would be 10-fold more potent than T-20 when evaluated in vitro against HIV under the same conditions [40].

5. Nucleocapsid protein (NCp7) Zn finger-targeted agents

The two zinc fingers [Cys-X_2-Cys-X_4-His-X_4-Cys (CCHC), whereby X = any amino acid] in the nucleocapsid (NCp7) protein [46] comprise the proposed molecular target for zinc-ejecting compounds such as 3-nitrosobenzamide

(NOBA), 2,2′-dithiobisbenzamide (DIBA), SRR-SB3 (cyclic DIBA) [47], 1,2-dithiane-4,5-diol,1,1-dioxide (dithiane) [48] and azadicarbonamide (ADA) [49,50]. These compounds should be able to interfere with both early (uncoating, disassembly) and late phases (packaging, assembly) of retrovirus replication. Their effect at the early phase (disassembly) may also be ascribed to cross-linkage among adjacent zinc fingers. The DIBAs are able to enter intact virions and the cross-linkage of NCp7 in virions correlates with loss of infectivity and decreased proviral DNA synthesis during acute infection [51]. Electron microscopically, the effect bestowed by DIBAs on virus morphology could be described as "core-freezing" [52].

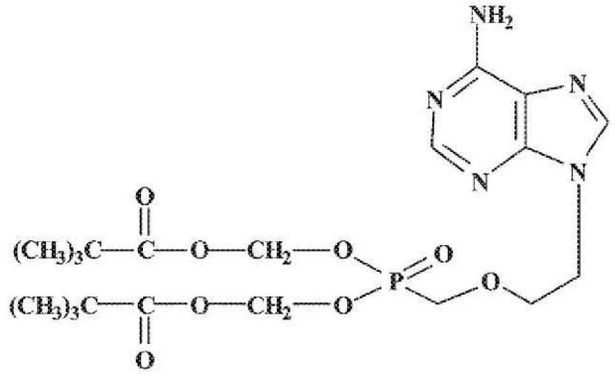


Compound 6
ADA (azodicarbonamide)

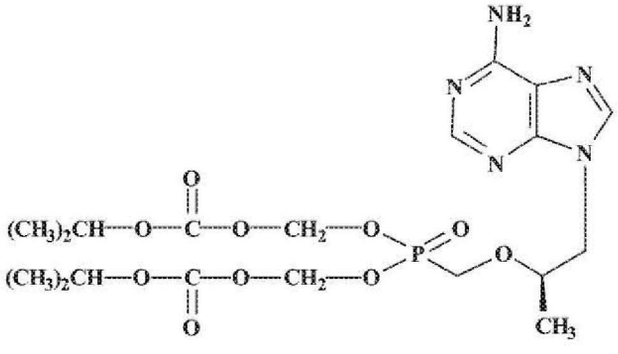
Although NOBA, DIBA, dithiane and ADA have been shown to dock nicely on the NCp7 Zn finger domains [53] and are believed to selectively target these Zn fingers without affecting the cellular Zn finger proteins, their selectivity indexes [ratio of CC_{50} (50% cytotoxic concentration) over EC_{50} (50% effective concentration)] are not that impressive [53]. Of the NCp7-targeted compounds, ADA (**6**) has been the first to proceed to phase I/II clinical trials in advanced AIDS patients. Some preliminary evidence of efficacy was witnessed with add-on ADA in patients failing current antiretroviral therapy [54]; these studies should be further extended. Although ADA is an HIV NCp7 Zn-finger inhibitor, its action in vivo is likely to be multipronged. ADA may well interact with a variety of targets and, certainly, its inhibitory effects on T-cell responses in vitro and in vivo [55] can hardly be attributed to an action targeted at the HIV NCp7 Zn fingers.

6. RT inhibitors targeted at the substrate binding site

The substrate (dNTP) binding site of the HIV-1 RT is the target for a large variety of NRTI analogues, which have for several years [56] been recognized as efficacious drugs for the treatment of HIV infections: i.e. AZT, ddI, ddC, d4T, 3TC, ABC, and the yet experimental drug emtricitabine [(−)FTC]. Fozivudine tidoxil is a thioether lipid AZT conjugate that has recently passed phase II clinical trials [57] and should be as effective as, and potentially better tolerated than, AZT. As a rule, all these compounds must be phosphorylated to their 5′-triphosphate form, before they can act as competitive inhibitors/substrate analogues/chain terminators at the RT level. In contrast to the nucleoside analogues, the nucleotide analogues PMEA and PMPA are already equipped with a phosphonate group, and therefore only need two phosphorylation steps to be converted to the active metabolite [58]. From PMEA and PMPA, the oral prodrug forms [bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine (bis(POM)-PMEA) or adefovir dipivoxyl (**7**), and bis(isopropyloxycarbonyloxymethyl)-(***R***)-9-(2-phosphonylmethoxypropyl)adenine (bis(POC)-PMPA) or tenofovir disoproxil (**8**) fumarate, respectively] have been prepared. The former is in advanced phase III clinical trials for the treatment of hepatitis B virus (HBV) infections, whereas the latter has completed phase III clinical trials for the treatment of HIV infections. A new drug application (NDA) and market authorization application (MAA) has been recently filed for tenofovir disoproxil fumarate with the FDA (US) and EMEA (EU), respectively. In rhesus macaques infected with the highly pathogenic chimeric virus SHIV, tenofovir treatment initiated 1 week post-infection, at a time when disseminated infection and extensive viral replication had already been established and $CD4^+$ T-cell loss had begun, led to prompt, virtually complete suppression of viral replication and long-term stabilization of $CD4^+$ T-cell levels, which were sustained, even after withdrawal of tenofovir (after 12 weeks of treatment) [59].

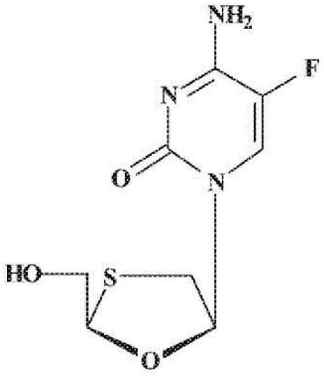


Compound 7
bis(POM)-PMEA
Adefovir dipivoxyl

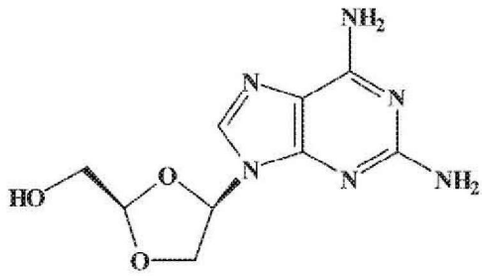


Compound 8
bis(POC)-PMPA
Tenofovir disoproxil

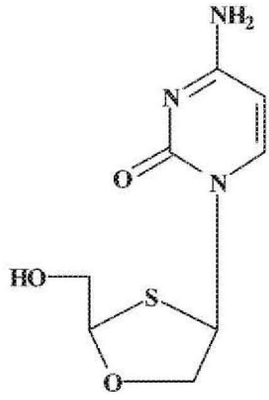
In addition to 3TC and (−)FTC, the structurally related (±)2′-deoxy-3′-oxa-4′-thiocytidine (BCH-10652,dOTC) [60], the dioxolane purine nucleoside analogues [61], the methylenecyclopropane nucleoside analogues (and their phosphoro-L-alaninate diesters) [62,63] and the 4′-ethynyl nucleoside analogues [64] have recently been described as new anti-HIV agents. [(−)FTC] (**9**) is in phase III trials for HIV and phase I/II trials for HBV; it is considered for use in the multidrug combination therapy of HIV-1 and HBV infections. Amdoxovir [DAPD, (−)-β-D-2,6-diaminopurine dioxolane] (**10**), which is converted by adenosine deaminase to dioxolane guanine (DXG), has proven active against AZT- and 3TC-resistant HIV-1 strains and has proceeded to phase I/II clinical studies [65,66]. BCH-10652 (dOTC) (**11**) has demonstrated activity against HIV-1 in the SCID-hu Thy/Liv model. Despite its structural similarity to 3TC, dOTC proved also active against 3TC-resistant HIV-1 (M184V), albeit at a relatively high dosage level (400 mg/kg/day) [67]. Also in vitro, dOTC and its (+) and (−) enantiomers still retained, albeit reduced, activity against 3TC-resistant M184V and M184I HIV-1 mutants [68].



Compound 9
Emtricitabine [(-)FTC]

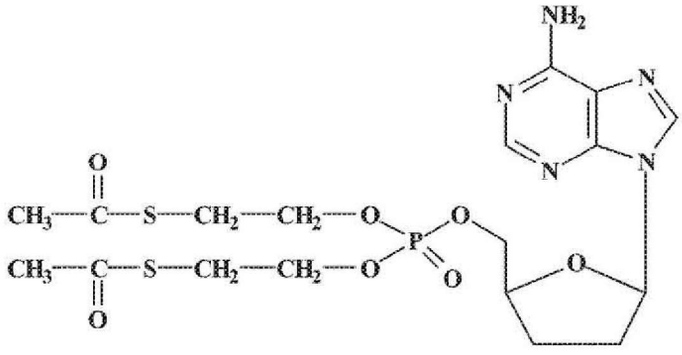


Compound 10
Amdoxovir
[DAPD, (-)-β-D-2,6-diaminopurine dioxolane]

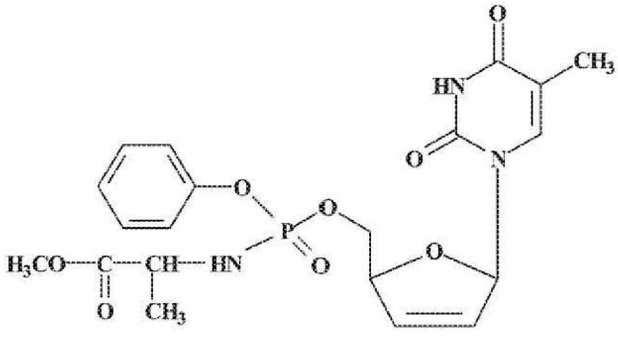


Compound 11
(±)2'-deoxy-3'-oxa-4'-thiocytidine (dOTC)

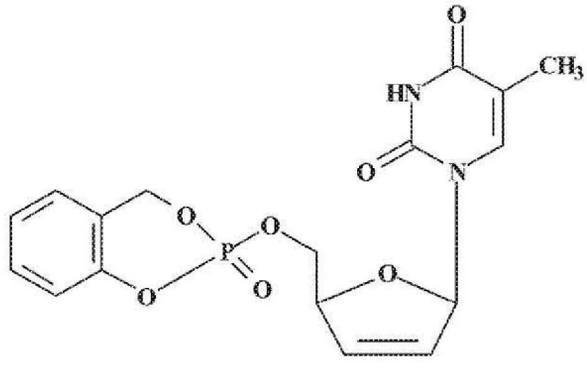
The bottleneck in the metabolic pathway leading from AZT and the other 2′,3′-dideoxynucleoside (ddN) analogues to their active 5′-triphosphate form is the first phosphorylation step. Therefore, attempts have been made at constructing 2′,3′-dideoxynucleotide (ddNMP) prodrugs that, once they have been taken up by the cells, deliver the nucleotide (ddNMP) form. This approach has proven particularly successful for a number of NRTIs such as 2′,3′-dideoxyadenosine (ddA) and d4T. Thus, the bis(*S*-acetyl-2-thioethyl)phosphotriester of ddA [bis(SATE]ddAMP] (**12**) was synthesized and found to be 1000-fold more potent against HIV than the parent compound ddA [69]. Similarly, aryloxyphosphoramidate derivatives of d4T [i.e. So324, a d4T-MP prodrug containing at the phosphate moiety a phenyl group and the methylester of alanine linked to the phosphate group through a phosphoramidate linkage] have been constructed [70–72]. After the d4T aryloxyphosphoramidate (**13**) has been taken up by the cells, d4TMP is released intracellularly and then processed onto its active metabolite d4TTP [73]. This "thymidine kinase bypass" explains the high anti-HIV activity of d4T aryloxyphosphoramidate derivatives in thymidine kinase deficient cells and resting monocytes/macrophages [74]. The thymidine kinase (in the case of d4T) and the adenosine deaminase (in the case of ddA) can also be bypassed by using the cyclic saligenyl approach [75,76]. ***Cyclo***saligenyl pronucleotides of d4T and ddA deliver exclusively the nucleotides d4TMP and ddAMP, not only under chemical-simulated hydrolysis conditions but also under intracellular conditions [77,78]. This has been convincingly shown for the *cyclo*-saligenyl derivative of d4TMP (**14**) in a number of cell lines [79].



Compound 12
bis(S-acetyl-2-thioethyl)phosphotriester of ddA [bis(SATE)ddAMP]



Compound 13
d4T aryloxyphosphoramidate



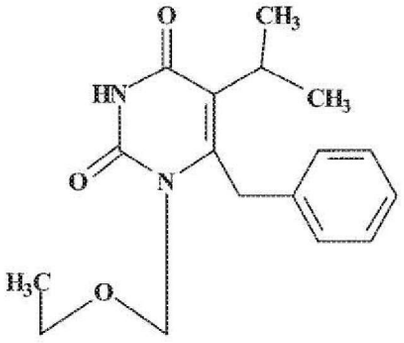
Compound 14
***cyclo*saligenyl d4TMP**

7. RT inhibitors targeted at the allosteric, nonsubstrate binding site

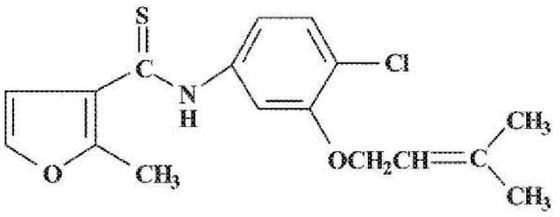
More than 30 structurally different classes of compounds have been identified as NNRTIs, viz. compounds that are specifically inhibitory to HIV-1 replication and targeted at a nonsubstrate binding site of the RT [80]. Three NNRTIs (nevirapine, delavirdine and efavirenz) have so far been formally licensed for clinical use in the treatment of HIV-1 infections, emivirine (MKC-442) (**15**) is in advanced (phase III) clinical trials, and others are in preclinical or early clinical development. The NNRTIs interact with a specific "pocket" site of the HIV-1 RT [81], which is closely associated with, but distinct from, the substrate binding site. NNRTIs are notorious for rapidly eliciting resistance [82], resulting from mutations at the amino acid residues that surround the NNRTI-binding site of HIV-1 RT. However, emergence of NNRTI-

resistant HIV strains can be prevented if the NNRTIs are combined with NRTIs and used from the beginning at sufficiently high concentrations [80].

The thiocarboxanilide UC-781 (**16**) is an exceptionally potent inhibitor of HIV-1 replication [80]. It has been found to restore the antiviral activity of AZT against AZT-resistant HIV-1 [83]. UC-781 has been recognized as a (retro)virucidal agent, capable of reducing the infectivity of HIV-1 virions, and, therefore, yielding considerable promise for the use in (retro)virucidal formulations to prevent the transmission of HIV from infected to noninfected individuals [84]. UC-781 would seem an ideal candidate for application as a vaginal microbicide (virucide), i.e. when formulated in replens gel [85].

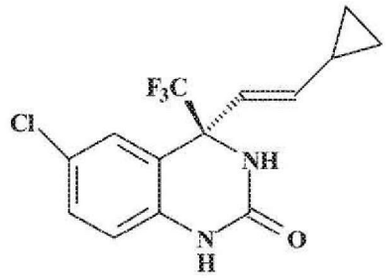


Compound 15
Emivirine (MKC-442)

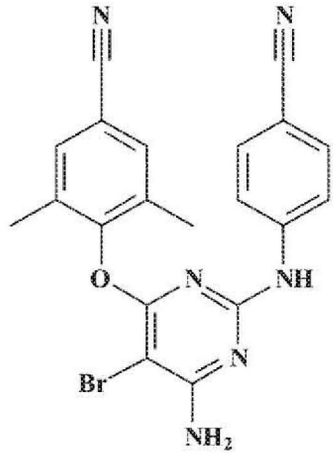


Compound 16
Thiocarboxanilide UC-781

To the new classes of NNRTIs that offer potent anti-HIV-1 activity belong the thieno[3,4][1,2,4]thiadiazine derivative QM96521 [86], the quinoxaline GW420867X [87], the imidazole derivative S-1153 (AG1549, capravirine) [88–90], (−)-6-chloro-2-[(1-furo[2,3-c]pyridin-5-yl-ethyl)thio]-4-pyrimidinamine (PNU-142721) [91], *N*-[2-(2,5-dimethoxyphenylethyl]-*N*′-[2-(5-bromopyridyl]-thiourea (HI-236) [92], the pyrido[1,2*a*]indole derivative BCH-1 [93], the 4-cyclopropylalkynyl-4-trifluoromethyl-3,4-dihydro-2(1*H*) quinazolinones DPC 961 and DPC 963, the 4-cyclopropylalkenyl-4-trifluoromethyl-3,4-dihydro-2(1*H*)quinazolinones DPC 082 and DPC 083 [94], the thiophene-ethylthiourea (TET) derivative HI-443 [95], the cyclohexenylethylthiourea derivatives HI-346 and HI-445 [96], the *cis*-cyclopropyl urea-PETT derivatives [97], the alkenyldiarylmethane (ADAM) series of compounds [98], the pyrrolobenzoxazepinone (PBO) derivatives [99], the quinoxalinylethylpyridyl thioureas (QXPTs) [100], the emivirine (MKC-442) derivative SJ-3366 [101] and R165335(TMC125) [102]. As a rule, the "new" ("second" or "third" generation) NNRTIs exhibit higher potency than the "old" ("first" generation) NNRTIs against wild-type and NNRTI-resistant HIV-1 [91,94–96,99,102]. This is particularly prominent for DPC 083 (**17**) and R165335 (TMC125) (**18**) that showed activity against L100I, K103N, Y181C, Y188L, K103N+L100I and K103N+Y181C RT mutant strains in the nanomolar concentration range [102]. This makes R165335 (TMC125) an excellent candidate for further clinical development.

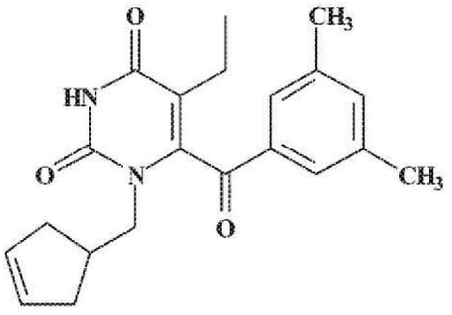


Compound 17
DPC 083



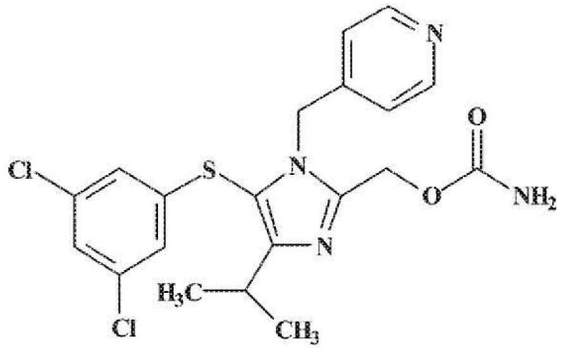
Compound 18
R165335 (TMC125)

Some of the new NNRTIs, such as SJ-3366 (**19**), possess remarkable features. This compound was reported to inhibit HIV-1 replication at a concentration below 1 nM with a therapeutic index greater than 4,000,000, and to inhibit HIV-2 replication (albeit at higher concentrations than those required for inhibition of HIV-1) at the viral entry stage [101].



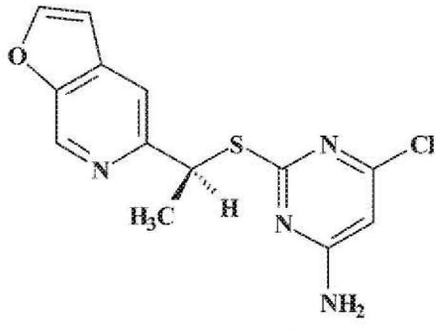
Compound 19
SJ-3366

Capravirine (AG1549) (**20**) has a favorable profile of resilience to many drug resistance mutations, which has been attributed to extensive main chain hydrogen bonding involving the main chain of residues 101, 103, and 236 of the p66 RT subunit [89]. Capravirine has proceeded to phase II/III clinical trials [90].



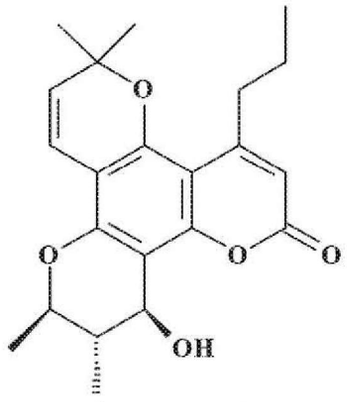
Compound 20
Capravirine (AG1549)

The NNRTIs *cis*-cyclopropylurea-PETT [97] and PBO derivatives [99] are orally bioavailable and penetrate well into the brain. The broad, potent antiviral activity, and favorable pharmacokinetic profile, have led to the selection of PNU-142721 (**21**) for clinical studies [91]; and DPC 961, DPC 963, DPC 082 and DPC 083 for clinical development [94].



Compound 21
PNU-142721

(+)-Calanolide A (**22**) is the only naturally occurring NNRTI: it was first isolated from a tropical tree (*Calophyllum lanigerum*) and has already been the subject of a phase I clinical study in healthy, HIV-negative individuals [103].



Compound 22
(+)-Calanolide A

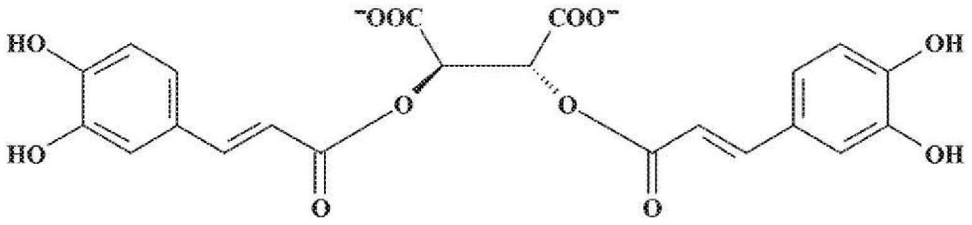
Recently, an unexpected effect of NNRTIs on HIV-1 RT dimerization was documented [104]: several NNRTIs, including efavirenz, were found to enhance the association between the RT subunits p66 and p51, apparently due to a conformational change in the p66 subunit that resulted in enhanced binding to the p51 subunit. It remains to be established if this enhanced dimerization has any bearing on the anti-HIV-1 potency of the NNRTIs.

8. HIV integrase inhibitors

Retrovirus integration requires at least two viral components, the retroviral enzyme integrase, and *cis*-acting sequences at the retroviral DNA termini U3 and U5 ends of the long terminal repeats (LTRs). Since HIV, like other retroviruses, cannot replicate without integration into a host chromosome, integrase has been considered as an attractive therapeutic target. Numerous compounds have been described as inhibitors of HIV-1 integrase (for a recent review, see Ref. [105]): for example, polyamides, bisdistamycins and lexitropsins [106], polyhydroxylated aromatic type of compounds, including ellagic acid, purpurogallin, 4,8,12-trioxatricornan and hypericin [107] and a series of thiazolothiazepine derivatives, preferably possessing the pentatomic moiety SC(O)CNC(O) with two carbonyl groups [108]. The problem with integrase inhibitors is that while they might be effective in an enzyme-based assay, their anti-HIV activity in cell culture may be masked by cytotoxicity, and if they do exhibit anti-HIV activity, this could, at least in some cases, be attributed to antiviral actions targeted at other steps in the HIV replicative cycle.

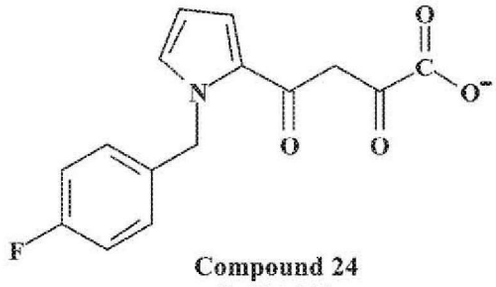
L-chicoric acid [109–111] is such a case. L-chicoric acid is structurally reminiscent of curcumin [112], 3,5-dicaf-

feoylquinic acid [113], rosmarinic acid [114] and dicaffeoyltartaric acids (DCTAs) [115], and all these compounds have been reported to inhibit HIV-1 integrase. Integrase was identified as the molecular target for the action of L-chicoric acid (**23**) since a single amino acid substitution (G140S) in the integrase rendered the corresponding HIV-1 mutant resistant to L-chicoric acid [111]. We have recently demonstrated [116], however, that L-chicoric acid owes its anti-HIV activity in cell-culture to an interaction with the viral envelope gp120. Upon repeated passages of the virus in the presence of the compound, mutations were found in the V2, V3 and V4 loop of gp120, while no mutations were seen in the integrase. We did confirm that in an enzymatic assay L-chicoric acid inhibited HIV integrase activity, but integrase carrying the G140S mutation appeared to be as sensitive to the inhibitory effect of L-chicoric acid as the wild-type integrase. Furthermore, L-chicoric acid proved inactive against HIV strains that were resistant to polyanionic compounds known to interact at the virus adsorption level, and time-of-addition experiments further corroborated an interaction of L-chicoric acid at the virus adsorption stage [116].



Compound 23
L-chicoric acid

Recently, the structure of the HIV-1 integrase core domain complexed with an inhibitor [1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)-propenone] has been described as a platform for structure-based design of novel HIV-1 integrase inhibitors [117]. This was followed by the description of a number of diketo acids (such as L-731,988 and L-708,906) as inhibitors of the integrase-mediated strand transfer reaction that leads to the covalent linkage of the viral DNA 3′ ends to the cellular (target) DNA [107]. These compounds were also found to inhibit HIV-1 replication in cell culture. Furthermore, mutations in the HIV-1 integrase conferred resistance to the inhibitory effects of the compounds on both strand transfer and HIV-1 infectivity [118]. Thus, it was surmised that these diketo acids owe their antiviral activity exclusively to inhibition of one of the two catalytic functions of integrase, namely strand transfer [the other catalytic function being endonucleolytic processing of the (pro)viral DNA to remove the terminal dinucleotide (GT) from the 3′ end]. Starting from L-731,988 (**24**), additional 4-aryl-2,4-dioxobutanoic acid derivatives have been reported as inhibitors of HIV-1 integrase [119].



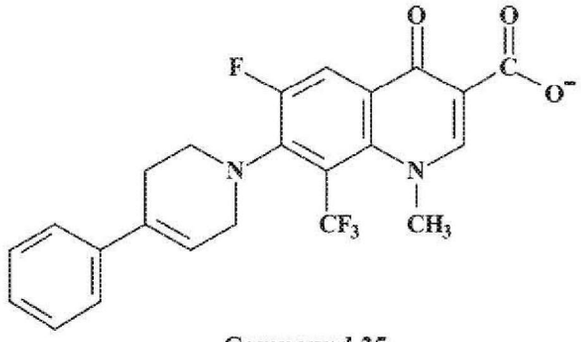
Compound 24
L-731,988

9. Transcription (transactivation) inhibitors

At the transcription level, HIV gene expression may be inhibited by compounds that interact with cellular factors that bind to the LTR promoter and that are needed for basal level transcription, such as the NF-κB inhibitors [120]. Greater specificity, however, can be expected from those compounds that specifically inhibit the transactivation of the HIV LTR promoter by the viral Tat (*trans*-activating) protein [120]. Tat has pleiotropic effects: it not only activates the transcription of HIV-1 RNA, but also binds to a number of receptors, i.e. on smooth muscle and skeletal muscle cells [121]: the basic domain of Tat may be important, not only for translocation but also for nuclear localisation and *trans*-activation, and thus targeting of the Tat basic domain may provide great scope for therapeutic intervention in HIV-1 infection [121].

A number of compounds have been reported to inhibit HIV-1 replication in both acutely and chronically infected cells through interference with the transcription process: i.e. fluoroquinoline derivatives [122]. The inhibitory effects of the fluoroquinolines (K-12) [8-difluoromethoxy-1-ethyl-6-fluoro-1,4-dihydro-7-[4-(2-methoxyphe-nyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic acid] and (K-37) [7-(3,4-dehydro-4-phenyl-1-piperidinyl)-1,4-dihydro-6-fluoro-1-methyl-8-trifluoromethyl-4-oxoquinoline-3-carboxylic acid] (**25**) on the HIV-1 LTR-driven gene expression may at least in part

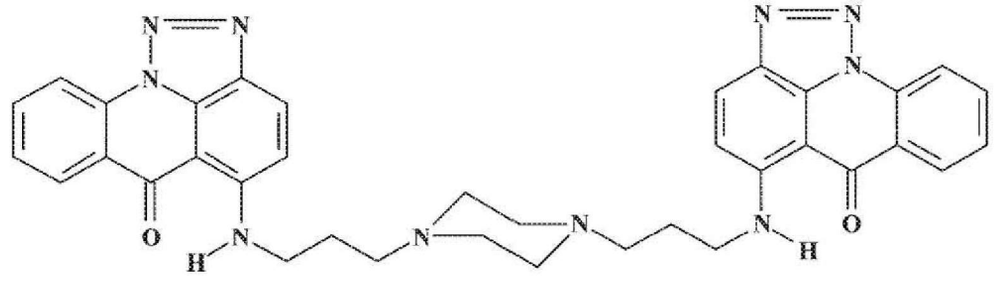
be attributed to inhibition of Tat [123] or other RNA-dependent transactivators [124].



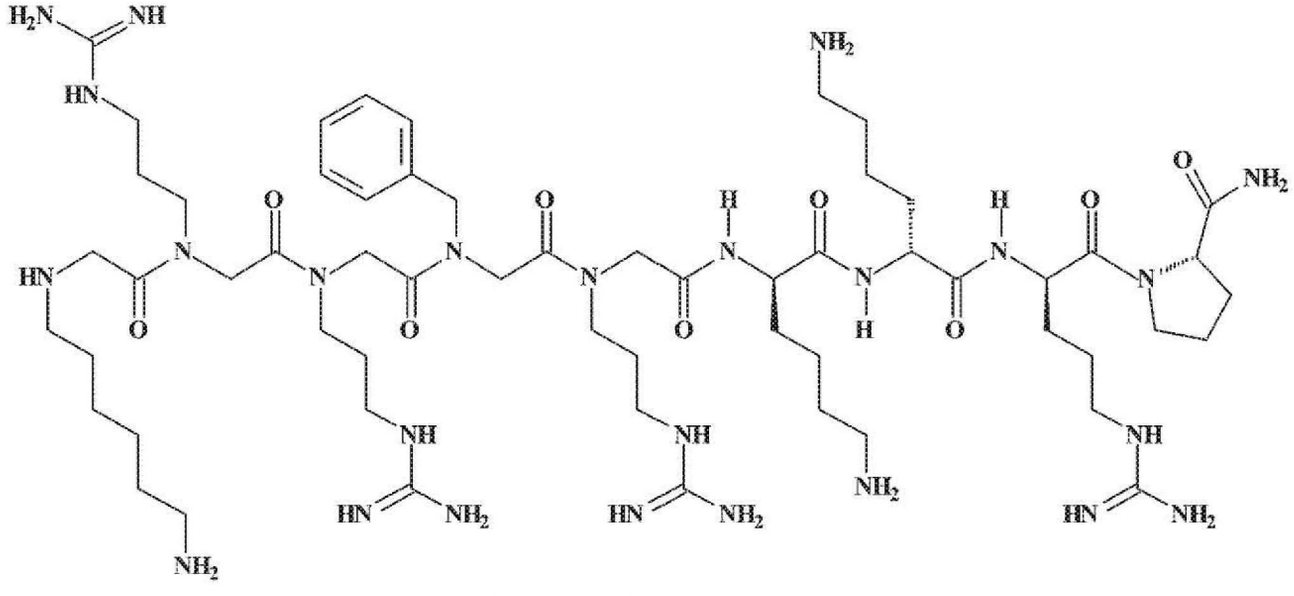
Compound 25
Fluoroquinoline K-37

The bistriazoloacridone temacrazine [1,4-bis(3-(6-oxo-6H-v-triazolo[4,5,1]acridin-5-yl-aminopropyl)piperazine] (**26**) was found to block HIV-1 RNA transcription from the HIV proviral DNA without interfering with the transcription of any cellular genes [125]: the compound inhibited HIV-1 replication in both acutely and chronically infected cells. Resistance was generated upon serial passage of the virus in the presence of temacrazine and was associated with several unique nucleotide changes in HIV-1 LTR at positions −1, −2 and +111 relative to the start of transcription [126].

Tat peptide analogs, encompassing the Tat core domain (amino acid residues 36–50) [127], or the basic domain (amino acids 48–56: RKKRRQRRR) [128] have been reported to inhibit HIV-1 replication, and, as expected, these peptide analogs were able to effectively block the Tat transactivation process. The 9-mer peptoid CGP64222 (**27**), which is structurally reminiscent of the amino acid 48–56 sequence RKKRRQRRR of Tat, was also reported, on the one hand, to block the Tat/TAR interaction, and, on the other hand, to suppress HIV-1 replication [129]. We have demonstrated, however, that the peptoid CGP64222 owes its anti-HIV activity in cell culture primarily to an interaction with CXCR4, the coreceptor for X4 HIV strains [130], which is, perhaps, not surprising given the structural similarity of CGP64222 to the other, polypeptidic, CXCR4



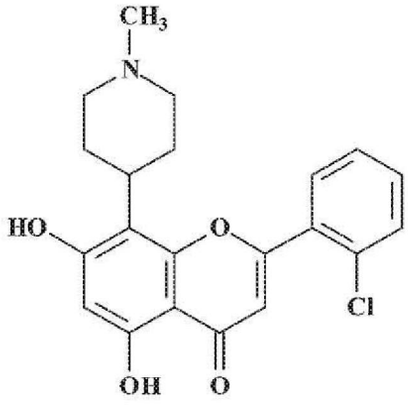
Compound 26
Temacrazine



Compound 27
CGP64222

antagonists such as T22 [23] and nona-arginine (ALX40-4C) [24]. In fact, Tat itself (following its extracellular release) has recently been shown to block CXCR4-dependent HIV-1 infection [131], presumably through blockade of CXCR4 by the above-mentioned 48–56 amino acid portion (RKKRRQRRR) of the molecule.

Flavopiridol (L86-8275, HMR1275) is a cyclin-dependent kinase (Cdk) and P-TEFb inhibitor, which is in clinical trials for the treatment of cancer because of its antiproliferative properties. P-TEFb is a protein kinase composed of Cdk9 and cyclin T1 and secures the elongation phase of transcription by RNA polymerase II (through phosphorylation of the carboxyl-terminal domain). Tat forms a triple complex with P-TEFb (composed of Cdk9 and cyclin T1) and the nascent transcript from the HIV-1 LTR promoter. Consistent with its ability to block P-TEFb, flavopiridol (**28**) was found to block Tat transactivation, and, concomitantly, also inhibited HIV replication [132].

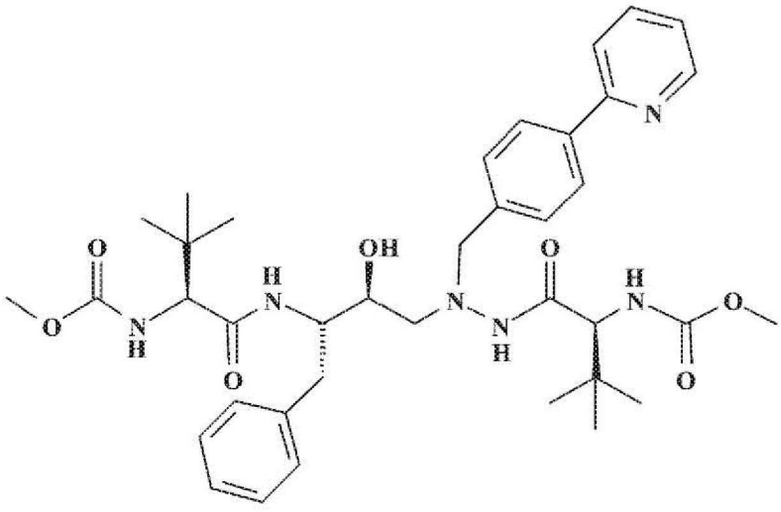


Compound 28
Flavopiridol

10. HIV PIs

HIV PIs prevent the cleavage of the gag and gag-pol precursor polyproteins to the structural proteins (p17, p24, p7, p6, p2, p1) and functional proteins (protease, RT/RNase H, integrase), thus arresting maturation and thereby blocking infectivity of the nascent virions [133]. The HIV PIs have been tailored after the target peptidic linkage in the gag and gag-pol polyproteins that are cleaved by the protease, viz. the phenylalanine–proline sequence at positions 167 and 168 of the gag-pol polyprotein. All PIs that are currently licensed for the treatment of HIV infection, namely saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir, share the same structural determinant, i.e. an hydroxyethylene (instead of the normal peptidic) bond, which makes them nonscissile substrate analogues for the HIV protease. All six licensed PIs follow the same principle; that is, they act as peptidomimetic inhibitors of HIV protease [134]. Lopinavir is co-dosed with ritonavir at 400/100 mg twice daily. The reason for this combination is that ritonavir strongly inhibits the metabolism of lopinavir and allows lopinavir to reach much higher plasma drug levels upon oral administration [135]. In phase III clinical trials is atazanavir (BMS-232632) (**29**), which has been accredited with a favorable resistance profile that does not parallel any of the other PIs currently in clinical use, as well as a favorable pharmacokinetic profile that would allow once-daily dosing [136].

Resistance mutations have been reported for most, if not all, peptidomimetric inhibitors of HIV protease. This has prompted the search for new, nonpeptidic inhibitors of HIV protease, which, in addition to a broader anti-HIV activity spectrum, might also offer increased oral bioavailability and/or pharmacokinetic properties. Examples of nonpeptidic PIs of HIV protease include 4-hydroxycoumarins and 4-



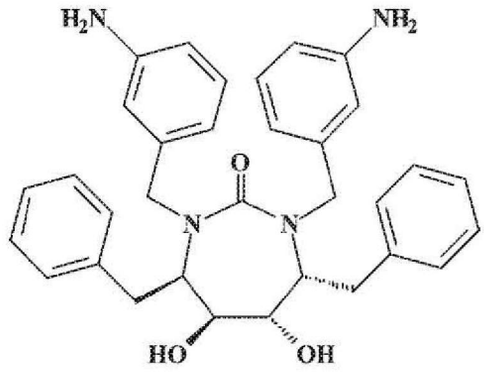
Compound 29
Atazanavir (BMS-232632)

hydroxy-2-pyrones [137], sulfonamide-substituted derivatives [138], cyclic ureas (i.e. DMP-323 and DMP-450) [139,140], cyclic cyanoguanidines [141], aza-dipeptide analogues [142], and tipranavir (PNU-140690), a sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone [143–145]. The major advantage of the cyclic urea mozenavir (DMP-450) (**30**) is its substantial oral bioavailability observed in all species examined, including man [140]. DMP-450 has been the subject of phase I/II dose-escalating clinical studies and appears to have good antiviral activity and tolerability at all doses tested [146]. The new aza-dipeptide analogues combine excellent anti-HIV potency with high blood drug levels after oral administration, and, furthermore, they show no cross-resistance with saquinavir-resistant HIV strains [142]. Tipranavir (**31**) showed low cross-resistance to HIV strains that were resistant to the established (peptidomimetic) inhibitors of HIV protease [145]. Also, tipranavir retained marked activity against HIV-1 isolates derived from patients with multidrug resistance to other PIs [147].

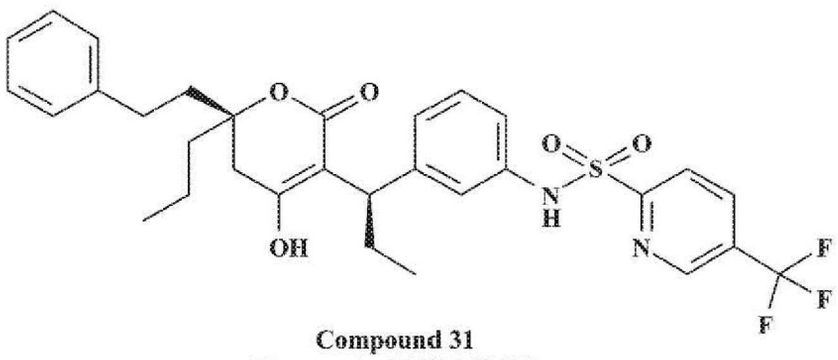
shown to inhibit gag polyprotein processing as well as HIV maturation and release [148]. While a potentially interesting approach, it remains to be seen whether inhibitors of the proteasome/ubiquitin system display sufficient specificity in their anti-HIV action so as to suppress virus replication without (overt) cytotoxicity.

11. Conclusions

In recent years, an ever increasing number of compounds have been uncovered as anti-HIV agents targeted at virtually any step of the virus replicative cycle: adsorption, entry, fusion, uncoating, reverse transcription, integration, transcription (transactivation) and maturation. In addition to the "newer" NRTIs, NNRTIs and PIs, various other compounds, i.e. those that are targeted at viral entry (i.e. CXCR4 and CCR5 antagonists) and virus-cell adsorption/fusion (i.e. compounds interacting with either gp120 or gp41), offer



Compound 30
Mozenavir (DMP-450)



Compound 31
Tipranavir (PNU-140690)

Independently of the HIV protease itself, proteasomes play a role in the processing of the gag polyprotein, and proteasome inhibitors, such as epoxomicin, have been great potential for the treatment of HIV infections. Quite a number of compounds are capable of interacting with more than one target. Two examples in point are the DCTA L-

chicoric acid, and the nonapeptoid CGP 64222. L-chicoric acid was originally identified as an integrase inhibitor, and the nonapeptoid as a transactivation (Tat) antagonist, and their anti-HIV activity in acutely infected cells was ascribed to interference with the integration and transactivation process, respectively. As it now appears, L-chicoric acid primarily interacts as a virus adsorption inhibitor, and the nonapeptoid as a CXCR4 antagonist, and thus these compounds owe their anti-HIV activity mainly to interference with an early event (adsorption, entry) of the HIV replicative cycle.

Acknowledgements

Prof. Erik De Clercq holds the Professor P. De Somer Chair of Microbiology at the Katholieke Universiteit Leuven School of Medicine and thanks Christiane Callebaut for her invaluable editorial assistance.

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