ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 56 (2001) 3–12

IL FARMACO

# New developments in anti-HIV chemotherapy

Erik De Clercq *

*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

**Abstract**

Virtually all the compounds that are currently used, or under advanced clinical trial, for the treatment of HIV infections, belong to one of the following classes: (i) nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs): i.e. zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine, tenofovir (PMPA) 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 and amprenavir. In addition, various other events in the HIV replicative cycle are potential targets for chemotherapeutic intervention: (i) viral adsorption, through binding to the viral envelope glycoprotein gp120; (ii) viral entry, through blockade of the viral coreceptors CXCR4 and CCR5; (iii) virus-cell fusion; (iv) viral assembly and disassembly; (v) proviral DNA integration; (vi) viral mRNA transcription. Also, new NRTIs, NNRTIs and PIs have been developed that possess respectively improved metabolic characteristics, or increased activity against NNRTI-resistant HIV strains or, as in the case of PIs, a different, non-peptidic scaffold. Given the multitude of molecular targets with which anti-HIV agents can interact, one should be cautious in extrapolating from cell-free enzymatic assays to the mode of action of these agents in intact cells. 

*Keywords:* HIV; Reverse transcriptase (HIV); Protease (HIV); CXCR4 (HIV); CCR5 (HIV); Integrase (HIV); Fusion (HIV); Transcription (HIV)

## 1. Introduction

Combination therapy, comprising at least three anti-HIV drugs, has become the standard treatment of 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 (adefovir) or three phosphorylation steps (zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir), act, as chain terminators, at the substrate binding site of the reverse transcriptase; (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), that interact with the reverse transcriptase 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). 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 sul-

* Correspondence and reprints:
*E-mail address:* erik.declercq@rega.kuleuven.ac.be (E. De Clercq).

0014-827X/01/$ - see front matter 
PII: S0014-827X(01)01007-2

fated polysaccharides extracted from sea algae [3]. 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) [4] which is necessary for virus attachment to the cell surface heparan sulfate, a primary binding site, before more specific binding occurs to the CD4 receptor of the $CD4^+$ cells. 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 HSV [5], it also explains why polysulfates have a broad-spectrum antiviral activity against HIV, HSV and various other enveloped viruses.

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 also inhibit other sexually transmitted disease (STD) pathogens 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 [6] or CCR5 coreceptor [7]. 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 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 [8,9].

TAK-779, a quaternary ammonium derivative is the first non-peptidic molecule that has been described to block the replication of M-tropic R5 HIV-1 strains at the CCR5 level [10]. 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 [10]. 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. Its therapeutic potential for HIV-1 infections, remains to be further explored.

Almost simultaneously [11–13], three compounds, i.e. the bicyclam AMD3100 [11], [Tyr-5,12,Lys-7]polyphemusin or T22 [12] and the nonapeptide $(D\text{-}Arg)_9$ or ALX40-4C [13] 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 [14,15]. The bicyclams had been known as potent and selective HIV inhibitors for a number of years [16,17], before their target of action was identified as the CXCR4 coreceptor [11,18,19]. The bicyclam AMD3100 inhibits the replication of X4 HIV-1 strains within the nanomolar concentration range [17]. 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 $> 100\,000$.

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 [18,19]. 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 peripheral blood mononuclear cells (PBMC), and, vice versa, various T-tropic and dual-tropic, but not M-tropic, HIV-1 strains have proven sensitive to AMD3100 in PBMC.

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 [20]. 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, that 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 [21]. Following a phase I clinical trial for safety in normal healthy volunteers [22], AMD3100 has recently entered phase II clinical trials in HIV-infected individuals.

## 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), that then anchors through its amino termini (the 'fusion peptides') into the target cell membrane. This initiates the fusion of the two lipid bilayers, that of the viral envelope with that of the cellular plasma membrane [23]. 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 [23].

T-20 (pentafuside) 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 modelled 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 [24]. 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 [25]. These data provide proof-of-concept that HIV fusion inhibitors are able to reduce virus replication *in vivo*.

The betulinic acid derivative RPR 103611 represents the only non-peptidic low-molecular-weight compound known 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 [26], 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. RPR 103611 may be regarded as an interesting lead compound in the pursuit of non-peptidic low-molecular-weight HIV fusion inhibitors targeted at gp41.

## 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 [27] comprise the proposed molecular target for zinc-ejecting compounds such as NOBA (3-nitrosobenzamide), DIBA (2,2′-dithiobisbenzamide), SRR-SB3 (cyclic 2,2′-dithiobisbenzamide) [28], dithiane (1,2-dithiane-4,5-diol,1,1-dioxide,*cis*) [29] and ADA (azodicarbonamide) [30,31]. 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 such 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 [32].

Although NOBA, DIBA, dithiane and ADA have been shown to dock nicely on the NCp7 Zn finger domains [33], 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 [33]. Of the NCp7-targeted compounds, ADA has been the first to proceed to phase I/II clinical trials in advanced AIDS patients. Given the nature of the compound, it will be difficult to pinpoint any in vivo antiviral response to ADA to a specific molecular target. ADA may well interact with a variety of targets, and, certainly its inhibitory effects on T-cell responses in vitro and in vivo [34] can hardly be attributed to an action targeted at the NCp7 Zn fingers.

## 6. Reverse transcriptase (RT) inhibitors targeted at the substrate binding site

The substrate (dNTP) binding site of the HIV-1 is the target for a large variety of NRTI analogues, which have for several years [35] been recognized as efficacious agents for the treatment of HIV infections: i.e. zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC). Other agents in clinical trial include adefovir (PMEA), tenofovir (PMPA) and emtricitabine [(−)FTC]. As a rule, all of these compounds must be phosphorylated to their 5′-triphosphate form, before they can act as competitive inhibitors/substrate analogues/chain terminators at the reverse transcriptase level. In contrast to the nucleoside analogues, the nucleotide analogues PMEA and PMPA are already equipped with a phosphonate group, and thus only need two phosphorylation steps to be converted to the active metabolite [36]. From PMEA and PMPA the oral prodrug forms [bis(POM)-PMEA or adefovir dipivoxyl, and bis(POC)-PMPA or tenofovir disoproxil fumarate, respectively] have been prepared. The former is now in phase III clinical trials for the treatment of hepatitis B virus (HBV) infections, whereas the latter has entered phase III clinical trials for the treatment of HIV infections.

In addition to 3TC and (−)FTC, the structurally related 2′-deoxy-3′-oxa-4′-thiocytidine (BCH-10652,

dOTC) [37], the dioxolane purine nucleoside analogues [38] and the methylenecyclopropane nucleoside analogues (and their phosphoro-L-alaninate diesters) [39,40] have recently been described as new anti-HIV agents. Emtricitabine [(−)FTC] 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. DAPD, (−)-β-D-2,6-diaminopurine dioxolane, which is converted by adenosine deaminase by dioxolane guanine (DXG), has proven active against AZT- and 3TC-resistant HIV-1 strains and has proceeded to phase I/II clinical studies [41]. BCH-10652 (dOTC) has demonstrated activity against HIV-1 in the SCID-hu Thy/Liv model; despite its structural similarity to 3TC it proved also active against 3TC-resistant HIV-1 (M184V), albeit at a relatively high dosage level (400 mg/kg/day) [42].

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] was synthesized and found to be 1000-fold more potent against HIV than the parent compound [43]. 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 [44–46]. 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 [47,48]. *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 [49,50].

## 7. Reverse transcriptase inhibitors targeted at the allosteric non-substrate binding site

More than 30 structurally different classes of compounds have been identified as NNRTIs, i.e. compounds that are specifically inhibitory to HIV-1 replication and targeted at a non-substrate binding site of the reverse transcriptase [51]. 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) 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 [52], which is closely associated with, but distinct from, the substrate binding site. NNRTIs are notorious for rapidly eliciting resistance, 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 [51]. The thiocarboxanilide UC-781 is an exceptionally potent inhibitor of HIV-1 replication [51]. It has been found to restore the antiviral activity of AZT against AZT-resistant HIV-1 [53].

To the new classes of NNRTIs that offer potent anti-HIV-1 activity belong the thieno[3,4-*e*][1,2,4]thiadiazine derivative QM96521 [54], the quinoxaline GW420867X [55], the imidazole derivative S-1153 (AG1549, capravirine) [56], (−) - 6 - chloro - 2 - [(1-furo[2,3 - c]pyridin - 5 - yl - ethyl)thio] - 4 - pyrimidinamine (PNU-142721) [57], *N*-[2-(2,5-dimethoxyphenylethyl]-*N*′-[2-(5-bromopyridyl]-thiourea (HI-236) [58], the pyrido[1,2a]indole derivative BCH-1 [59], the 4-cyclopropylalkynyl - 4 - trifluoromethyl - 3,4 - dihydro - 2(1*H*)-quinazolinones DPC 961 and DPC 963 [60], the thiophene-ethylthiourea (TET) derivative HI-443 [61], the cyclohexenylethylthiourea derivatives HI-346 and HI-445 [62], the *cis*-cyclopropyl urea-PETT derivatives [63], the alkenyldiarylmethane (ADAM) series of compounds [64], the pyrrolobenzoxazepinone (PBO) derivatives [65], and the emivirine (MKC-442) derivative SJ-3366 [66]. As a rule, the 'new' (or second generation) NNRTIs exhibit higher potency than the 'old' (or first generation) NNRTIs against wild-type and NNRTI-resistant HIV-1 [57,60–62,65]. Other remarkable features include the exquisite potency of some of the new NNRTIs such as SJ-3366 [66], which was reported to inhibit HIV-1 replication at concentrations below 1 nM with a therapeutic index greater than 4 000 000, and the fact that the NNRTIs *cis*-cyclopropylurea-PETT [63] and pyrrolobenzoxazepinone (PBO) derivatives [65] are orally bioavailable and penetrate well into the brain. The broad, potent antiviral activity, and favourable pharmacokinetic profile, have led to the selection of PNU-142721 for clinical studies [57]; and DPC 961, DPC 963, DPC 082 and DPC 083 for clinical development [60].

## 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) [67]. 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 review, see Pommier et al. [67]): for example, polyamides, bisdistamycins and lexitropsins [68], polyhydroxylated aromatic type of compounds, including ellagic acid, purpurogallin, 4,8,12-trioxatricornan and hypericin [69], and a series of thiazolothiazepine derivatives, preferably possessing the pentatomic moiety SC(O)CNC(O) with two carbonyl groups [70]. 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 [71–73] is such an example. L-Chicoric acid is structurally reminiscent of curcumin [74], 3,5-dicaffeoylquinic acid [75], rosmarinic acid [76] and dicaffeoyltartaric acids (DCTAs) [77], 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 since a single amino acid substitution (G140S) in the integrase rendered the corresponding HIV-1 mutant resistant to L-chicoric acid [73]. We have recently demonstrated [78], 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 was 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, rather than the proviral DNA integration, stage [78].

Recently, a number of diketo acids (such as L-731,988 and L-708,906) have been described 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 [79]. 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 [79]. 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.

## 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 [80]. Greater specificity, however, can be expected from those compounds that specifically inhibit the transactivation of the HIV LTR promotor by the viral Tat (*trans*-activating) protein [80]. 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 [81]: the basic domain of Tat may be important, not only for translocation but also for nuclear localization and *trans*-activation, and thus targeting of the Tat basic domain may provide great scope for therapeutic intervention in HIV-1 infection [81].

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 [82]. The inhibitory effects of the fluoroquinolines [i.e. 8-difluoromethoxy-1-ethyl-6-fluoro-1,4-dihydro-7-[4-(2-methoxyphe-nyl)-1-piperazinyl]-4-oxoquinoline-3-carboxylic acid (K-12) and 7-(3,4-dehydro-4-phenyl-1-piperidinyl)-1,4-dihydro-6-fluoro-1-methyl-8-trifluoromethyl-4-oxoquinoline-3-carboxylic acid (K-37) on the HIV-1 LTR-driven gene expression may at least in part be attributed to inhibition of Tat [83] but also other RNA-dependent transactivators [84].

Tat peptide analogues, encompassing the Tat core domain (amino acid residues 36–50) [85], or the basic domain (amino acids 48–56: RKKRRQRRR) [86] have been reported to inhibit HIV-1 replication, and, as expected, these peptide analogues were able to effectively block the Tat transactivation process. The 9-mer peptoid CGP64222, 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 [87]. 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 [88], which is, perhaps, not surprising given the structural similarity of CGP64222 to the other, polypeptidic, CXCR4 antagonists such as T22 [12] and nona-arginine (ALX40-4C) [13]. In fact, Tat itself (following its extracellular release) has recently been shown to block CXCR4-dependent HIV-1 infection [89], presumably through blockade of CXCR4 by the above mentioned 48–56 amino acid portion (RKKRRQRRR) of the molecule.

## 10. HIV protease inhibitors

HIV protease inhibitors prevent the cleavage of the gag and gag-pol precursor polyproteins to the functional proteins (p17, p24, p7, p6, p2, p1, protease, reverse transcriptase, integrase), thus arresting maturation and thereby blocking infectivity of the nascent virions [90]. The HIV protease inhibitors have been tailored after the target peptidic linkage in the gag and gag-pol polyproteins that have to be cleaved by the protease, viz. the phenylalanine-proline sequence at positions 167 and 168 of the gag-pol polyprotein. All protease inhibitors that are currently licensed for the treatment of HIV infection, namely saquinavir, ritonavir, indinavir, nelfinavir and amprenavir [90] share the same structural determinant, i.e. an hydroxyethylene (instead of the normal peptidic) bond, that makes them non-scissile substrate analogues for the HIV protease. In addition to the five licensed protease inhibitors, others, such as ABT-378, that are still under clinical development [91], follow the same principle, that is they act as peptidomimetic inhibitors of HIV protease. ABT-378 (in phase III clinical trials) is co-dosed with ritonavir (ABT-3788/r) at 400/100 mg twice daily. The reason for this combination is that ritonavir strongly inhibits the metabolism of ABT-378 and allows ABT-378 to reach much higher plasma drug levels upon oral administration [92].

Resistance mutations have been reported for most, if not all, peptidomimetric inhibitors of HIV protease. This has prompted the search for new, non-peptidic inhibitors of HIV protease, that, in addition to a broader anti-HIV activity spectrum, might also offer increased oral bioavailability and/or pharmacokinetic properties. Examples of non-peptidic protease inhibitors of HIV protease include 4-hydroxycoumarins and 4-hydroxy-2-pyrones [93], sulfonamide-substituted derivatives [94], cyclic ureas (i.e. DMP 323 and DMP 450) [95,96], cyclic cyanoguanidines [97], aza-dipeptide analogues (i.e. CGP 73547) [98], and tipranavir (PNU-140690), a sulfonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone [99–101]. The major advantage of the cyclic urea DMP 450 is its substantial oral bioavailability observed in all species examined, including man [96]. 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 [102]. The new aza-dipeptide analogues (i.e. CGP 73547) 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 [98]. Tipranavir showed low cross-resistance to HIV strains that were resistant to the established (peptidomimetic) inhibitors of HIV protease [101]. Also, tipranavir retained marked activity against HIV-1 isolates derived from patients with multidrug resistance to other protease inhibitors [103].

## 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 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 dicaffeoyltartaric acid 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

Professor 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.

## References

[1] J.A. Tavel, K.D. Miller, H. Masur, Guide to major clinical trials of antiretroviral therapy in human immunodeficiency virus-infected patients: protease inhibitors, non-nucleoside reverse transcriptase inhibitors, and nucleotide reverse transcriptase inhibitors, Clin. Infect. Dis. 28 (1999) 643–676.

[2] Z.-Q. Zhang, T. Schuler, M. Zupancic, S. Wietgrefe, K.A. Staskus, K.A. Reimann, T.A. Reinhart, M. Rogan, W. Cavert, C.J. Miller, R.S. Veazey, D. Notermans, S. Little, S.A. Danner, D.D. Richman, D. Havlir, J. Wong, H.L. Jordan, T.W. Schacker, P. Racz, K. Tener-Racz, N.L. Letvin, S. Wolinsky, A.T. Haase, Sexual transmission and propagation of SIV and HIV in resting and activated $CD4^+$ T cells, Science 286 (1999) 1353–1357.

[3] M. Witvrouw, E. De Clercq, Sulfated polysaccharides extracted form sea algae as potential antiviral drugs, Gen. Pharm. 29 (1997) 497–511.

[4] W.R. Gallaher, J.M. Ball, R.F. Garry, A.M. Martin-Amedee, R.C. Montelaro, A general model for the surface glycoproteins of HIV and other retroviruses, AIDS Res. Hum. Retrovir. 11 (1995) 191–202.

[5] D. Shukla, J. Liu, P. Blaiklock, N.W. Shworak, X. Bai, J.D. Esko, G.H. Cohen, R.J. Eisenberg, R.D. Rosenberg, P.G. Spear, A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry, Cell 99 (1999) 13–22.
[6] J.F. Berson, D. Long, B.J. Doranz, J. Rucker, F.R. Jirik, R.W. Doms, A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains, J. Virol. 70 (1996) 6288–6295.
[7] B.J. Doranz, Z.H. Lu, J. Rucker, T.-Y. Zhang, M. Sharron, Y.-H. Cen, Z.-X. Wang, H.-H. Guo, J.-G. Du, M.A. Accavitti, R.W. Doms, S.C. Peiper, Two distinct CCR5 domains can mediate coreceptor usage by human immunodeficiency virus type 1, J. Virol. 71 (1997) 6305–6314.
[8] P. Menten, S. Struyf, E. Schutyser, A. Wuyts, E. De Clercq, D. Schols, P. Proost, J. Van Damme, The LD78β isoform of MIP-1α is the most potent CCR5 agonist and HIV-1-inhibiting chemokine, J. Clin. Invest. 104 (1999) R1–R5.
[9] R.J.B. Nibbs, J. Yang, N.R. Landau, J.-H. Mao, G.J. Graham, LD78β, a non-allelic variant of human MIP-1α (LD78α), has enhanced receptor interactions and potent HIV suppressive activity, J. Biol. Chem. 274 (1999) 17478–17483.
[10] M. Baba, O. Nishimura, N. Kanzaki, M. Okamoto, H. Sawada, Y. Iizawa, M. Shiraishi, Y. Aramaki, K. Okonogi, Y. Ogawa, K. Meguro, M. Fujino, A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity, Proc. Natl. Acad. Sci. USA 96 (1999) 5698–5703.
[11] D. Schols, S. Struyf, J. Van Damme, J.A. Esté, G. Henson, E. De Clercq, Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4, J. Exp. Med. 186 (1997) 1383–1388.
[12] T. Murakami, T. Nakajima, Y. Koyanagi, K. Tachibana, N. Fujii, H. Tamamura, N. Yoshida, M. Waki, A. Matsumoto, O. Yoshie, T. Kishimoto, N. Yamamoto, T. Nagasawa, A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection, J. Exp. Med. 186 (1997) 1389–1393.
[13] B.J. Doranz, K. Grovit-Ferbasi, M.P. Sharron, S.-H. Mao, M.B. Goetz, E.S. Daar, R.W. Doms, W.A. O'Brien, A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor, J. Exp. Med. 186 (1997) 1395–1400.
[14] E. De Clercq, Inhibition of HIV infection by bicyclams, highly potent and specific CXCR4 antagonists, Mol. Pharmacol. 57 (2000) 833–839.
[15] G.J. Bridger, R.T. Skerlj, S. Padmanabhan, S.A. Martellucci, G.W. Henson, S. Struyf, M. Witvrouw, D. Schols, E. De Clercq, Synthesis and structure–activity relationships of phenylenebis(methylene)-linked bis-azamacrocycles that inhibit HIV-1 and HIV-2 replication by antagonism of the chemokine receptor CXCR4, J. Med. Chem. 42 (1999) 3971–3981.
[16] E. De Clercq, N. Yamamoto, R. Pauwels, M. Baba, D. Schols, H. Nakashima, J. Balzarini, Z. Debyser, B.A. Murrer, D. Schwartz, D. Thornton, G. Bridger, S. Fricker, G. Henson, M. Abrams, D. Picker, Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event, Proc. Natl. Acad. Sci. USA 89 (1992) 5286–5290.
[17] E. De Clercq, N. Yamamoto, R. Pauwels, J. Balzarini, M. Witvrouw, K. De Vreese, Z. Debyser, B. Rosenwirth, P. Peichl, R. Datema, D. Thornton, R. Skerlj, F. Gaul, S. Padmanabhan, G. Bridger, G. Henson, M. Abrams, Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100, Antimicrob. Agents Chemother. 38 (1994) 668–674.
[18] D. Schols, J. Esté, G. Henson, E. De Clercq, Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4, Antiviral Res. 35 (1997) 147–156.
[19] G.A. Donzella, D. Schols, S.W. Lin, J.A. Este, K.A. Nagashima, P.J. Maddon, G.P. Allaway, T.P. Sakmar, G. Henson, E. De Clercq, J.P. Moore, AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor, Nature Med. 4 (1998) 72–77.
[20] J.A. Esté, C. Cabrera, J. Blanco, A. Gutierrez, G. Bridger, G. Henson, B. Clotet, D. Schols, E. De Clercq, Shift of clinical human immunodeficiency virus type 1 isolates from X4 to R5 and prevention of emergence of the syncytium-inducing phenotype by blockade of CXCR4, J. Virol. 73 (1999) 5577–5585.
[21] R. Datema, L. Rabin, M. Hincenbergs, M.B. Moreno, S. Warren, V. Linquist, B. Rosenwirth, J. Seifert, J.M. McCune, Antiviral efficacy in vivo of the anti-human immunodeficiency virus bicyclam SDZ SID 791 (JM 3100), an inhibitor of infectious cell entry, Antimicrob. Agents Chemother. 40 (1996) 750–754.
[22] G.W. Hendrix, C. Flexner, R.T. MacFarland, C. Giandomenico, E.J. Fuchs, E. Redpath, G. Bridger, G.W. Henson, Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers, Antimicrob. Agents Chemother. 44 (2000) 1667–1673.
[23] J.G. Sodroski, HIV-1 entry inhibitors in the side pocket, Cell 99 (1999) 243–246.
[24] C.T. Wild, D.C. Shugars, T.K. Greenwell, C.B. McDanal, T.J. Matthews, Peptides corresponding to a predictive α-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection, Proc. Natl. Acad. Sci. USA 91 (1994) 9770–9774.
[25] J.M. Kilby, S. Hopkins, T.M. Venetta, B. DiMassimo, G.A. Cloud, J.Y. Lee, L. Alldredge, E. Hunter, D. Lambert, D. Bolognesi, T. Matthews, M.R. Johnson, M.A. Nowak, G.M. Shaw, M.S. Saag, Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry, Nature Med. 4 (1998) 1302–1307.
[26] J.-F. Mayaux, A. Bousseau, R. Pauwels, T. Huet, Y. Hénin, N. Dereu, M. Evers, F. Soler, C. Poujade, E. De Clercq, J.B. Le Pecq, Triterpene derivatives that block entry of human immunodeficiency virus type 1 into cells, Proc. Natl. Acad. Sci. USA 91 (1994) 3564–3568.
[27] W.G. Rice, J.A. Turpin, L.O. Arthur, L.E. Henderson, Highly conserved retroviral zinc fingers as targets for HIV-1 therapeutic management, Int. Antiviral News 3 (1995) 87–89.
[28] M. Witvrouw, J. Balzarini, C. Pannecouque, S. Jhaumeer-Laulloo, J.A. Esté, D. Schols, P. Cherepanov, J.-C. Schmit, Z. Debyser, A.-M. Vandamme, J. Desmyter, S.R. Ramadas, E. De Clercq, SRR-SB3, a disulfide-containing macrolide that inhibits a late stage of the replicative cycle of human immunodeficiency virus, Antimicrob. Agents Chemother. 41 (1997) 262–268.
[29] W.G. Rice, D.C. Baker, C.A. Schaeffer, L. Graham, M. Bu, S. Terpening, D. Clanton, R. Schultz, J.P. Bader, R.W. Buckheit, Jr., L. Field, P.K. Singh, J.A. Turpin, Inhibition of multiple phases of human immunodeficiency virus type 1 replication by a dithiane compound that attacks the conserved zinc fingers of retroviral nucleocapsid proteins, Antimicrob. Agents Chemother. 41 (1997) 419–426.
[30] M. Vandevelde, M. Witvrouw, J.C. Schmit, S. Sprecher, E. De Clercq, J.-P. Tassignon, ADA, a potential anti-HIV drug, AIDS Res. Hum. Retrovir. 12 (1996) 567–568.
[31] W.G. Rice, J.A. Turpin, M. Huang, D. Clanton, R.W. Buckheit, Jr., D.G. Covell, A. Wallqvist, N.B. McDonnell, R.N. DeGuzman, M.F. Summers, L. Zalkow, J.P. Bader, R.D. Haugwitz, E.A. Sausville, Azodicarbonamide inhibits HIV-1 replication by targeting the nucleocapsid protein, Nature Med. 3 (1997) 341–345.
[32] J.A. Turpin, C.A. Schaeffer, S.J. Terpening, L. Graham, M. Bu, W.G. Rice, Reverse transcription of human immunodeficiency virus type 1 is blocked by retroviral zinc finger inhibitors, Antiviral Chem. Chemother. 8 (1997) 60–69.

[33] M. Huang, A. Maynard, J.A. Turpin, L. Graham, G.M. Janini, D.G. Covell, W.G. Rice, Anti-HIV agents that selectively target retroviral nucleocapsid protein zinc fingers without affecting cellular zinc finger proteins, J. Med. Chem. 31 (1998) 1371–1381.

[34] J. Tassignon, J. Ismaili, A. Le Moine, F. Van Laethem, O. Leo, M. Vandevelde, M. Goldman, Azodicarbonamide inhibits T-cell responses in vitro and in vivo, Nature Med. 5 (1999) 947–950.

[35] E. De Clercq, Toward improved anti-HIV chemotherapy: therapeutic strategies for intervention with HIV infections, J. Med. Chem. 38 (1995) 2491–2517.

[36] L. Naesens, R. Snoeck, G. Andrei, J. Balzarini, J. Neyts, E. De Clercq, HPMPC (cidofovir), PMEA (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections, Antiviral Chem. Chemother. 8 (1997) 1–23.

[37] J.-M. De Muys, H. Gourdeau, N. Nguyen-Ba, D.L. Taylor, P.S. Ahmed, T. Mansour, C. Locas, N. Richard, M.A. Wainberg, R.F. Rando, Anti-human immunodeficiency virus type 1 activity, intracellular metabolism and pharmacokinetic evaluation of 2′-deoxy-3′-oxa-4′-thiocytidine, Antimicrob. Agents Chemother. 43 (1999) 1835–1844.

[38] Z. Gu, M.A. Wainberg, N. Nguyen-Ba, L. L'Heureux, J.-M. de Muys, T.L. Bowlin, R.F. Rando, Mechanism of action and in vitro activity of 1′,3′-dioxolanylpurine nucleoside analogues against sensitive and drug-resistant human immunodeficiency virus type 1 variants, Antimicrob. Agents Chemother. 43 (1999) 2376–2383.

[39] H. Uchida, E.N. Kodama, K. Yoshimura, Y. Maeda, P. Kosalaraksa, V. Maroun, Y.-L. Qiu, J. Zemlicka, H. Mitsuya, In vitro anti-human immunodeficiency virus activities of *Z*- and *E*-methylenecyclopropane nucleoside analogues and their phosphoro-L-alaninate diesters, Antimicrob. Agents Chemother. 43 (1999) 1487–1490.

[40] K. Yoshimura, R. Feldman, E. Kodama, M.F. Kavlick, Y.-L. Qiu, J. Zemlicka, H. Mitsuya, In vitro induction of human immunodeficiency virus type 1 variants resistant to phosphoralaninate prodrugs of *Z*-methylenecyclopropane nucleoside analogues, Antimicrob. Agents Chemother. 43 (1999) 2479–2483.

[41] P.A. Furman, R. Chin, K. Borroto-Esoda, B. Dent, A. Bartholomeusz, S. Locarnini, DAPD: a novel inhibitor of HIV and HBV replication is active against drug resistant viruses, 4th International Workshop on Drug Resistance & Treatment Strategies, 12–16 June 2000, Sitges, Spain, Antiviral Ther. 5 (Suppl. 3) (2000) 5 (abstract 5).

[42] C.A. Stoddart, M.E. Moreno, V.D. Linquist-Stepps, C. Bare, M.R. Bogan, A. Gobbi, R.W. Buckheit, Jr., J. Bedard, R.F. Rando, J.M. McCune, Antiviral activity of 2′-deoxy-3′-oxa-4′-thiocytidine (BCH-10652) against lamivudine-resistant human immunodeficiency virus type 1 in SCID-hu Thy/Liv mice, Antimicrob. Agents Chemother. 44 (2000) 783–786.

[43] C. Perigaud, A.-M. Aubertin, S. Benzaria, H. Pelicano, J.-L. Girardet, G. Maury, G. Gosselin, A. Kirn, J.-L. Imbach, Equal inhibition of the replication of human immunodeficiency virus in human T-cell culture by ddA bis(SATE)phosphotriester and 3′-azido-2′,3′-dideoxythymidine, Biochem. Pharmacol. 48 (1994) 11–14.

[44] C. McGuigan, H.-W. Tsang, D. Cahard, K. Turner, S. Velazquez, A. Salgado, L. Bidois, L. Naesens, E. De Clercq, J. Balzarini, Phosphoramidate derivatives of d4T as inhibitors of HIV: the effect of amino acid variation, Antiviral Res. 35 (1997) 195–204.

[45] A.Q. Siddiqui, C. Ballatore, C. McGuigan, E. De Clercq, J. Balzarini, The presence of substituents on the aryl moiety of the aryl phosphoramidate derivative of d4T enhances anti-HIV efficacy in cell culture: a structure–activity relationship, J. Med. Chem. 42 (1999) 393–399.

[46] A.Q. Siddiqui, C. McGuigan, C. Ballatore, F. Zuccotto, I.H. Gilbert, E. De Clercq, J. Balzarini, Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for in vitro activity and QSAR, J. Med. Chem. 42 (1999) 4122–4128.

[47] C. Meier, M. Lorey, E. De Clercq, J. Balzarini, Cyclic saligenyl phosphotriesters of 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) – a new pro-nucleotide approach, Bioorg. Med. Chem. Lett. 7 (1997) 99–104.

[48] C. Meier, T. Knispel, E. De Clercq, J. Balzarini, ADA-bypass by lipophilic *cyclo*Sal-ddAMP pro-nucleotides. A second example of the efficiency of the *cyclo*Sal-concept, Bioorg. Med. Chem. Lett. 7 (1997) 1577–1582.

[49] C. Meier, M. Lorey, E. De Clercq, J. Balzarini, *cyclo*Sal-2′,3′-dideoxy-2′,3′-didehydrothymidine monophosphate (*cyclo*Sal-d4TMP): synthesis and antiviral evaluation of a new d4TMP delivery system, J. Med. Chem. 41 (1998) 1417–1427.

[50] C. Meier, T. Knispel, E. De Clercq, J. Balzarini, *cyclo*Sal-pronucleotides of 2′,3′-dideoxyadenosine and 2′,3′-dideoxy-2′,3′-didehydroadenosine: synthesis and antiviral evaluation of a highly efficient nucleotide delivery system, J. Med. Chem. 42 (1999) 1604–1614.

[51] E. De Clercq, The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection, Antiviral Res. 38 (1998) 153–179.

[52] J. Ren, R.M. Esnouf, A.L. Hopkins, J. Warren, J. Balzarini, D.I. Stuart, D.K. Stammers, Crystal structures of HIV-1 reverse transcriptase in complex with carboxanilide derivatives, Biochemistry 37 (1998) 14394–14403.

[53] G. Borkow, D. Arion, M.A. Wainberg, M.A. Parniak, The thiocarboxanilide nonnucleoside inhibitor UC781 restores antiviral activity of 3′-azido-3′-deoxythymidine (AZT) against AZT-resistant human immunodeficiency virus type 1, Antimicrob. Agents Chemother. 43 (1999) 259–263.

[54] M. Witvrouw, M.E. Arranz, C. Pannecouque, R. Declerq, H. Jonckheere, J.-C. Schmit, A.-M. Vandamme, J.A. Diaz, S.T. Ingate, J. Desmyter, R. Esnouf, L. Van Meervelt, S. Vega, J. Balzarini, E. De Clercq, 1,1,3-Trioxo-2H,4H-thieno[3,4-e][1,2,4]thiadiazine (TTD) derivatives: a new class of nonnucleoside human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitors with anti-HIV-1 activity, Antimicrob. Agents Chemother. 42 (1998) 618–623.

[55] W. Prince, K. Moore, L. Cass, N. Dallow, A. Jones, J.-P. Kleim, P. Mutch, M. St. Clair, GW420867X, a new non-nucleoside reverse transcriptase inhibitor (NNRTI) – initial phase I evaluation. Abstracts of the 12th International Conference on Antiviral Research, Jerusalem, Israel, March 21–25, 1999, Antiviral Res. 41 (1999) A48.

[56] T. Fujiwara, A. Sato, M. El-Farrash, S. Miki, K. Abe, Y. Isaka, M. Kodama, Y. Wu, L.B. Chen, H. Harada, H. Sugimoto, M. Hatanaka, Y. Hinuma, S-1153 inhibits replication of known drug-resistant strains of human immunodeficiency virus type 1, Antimicrob. Agents Chemother. 42 (1998) 1340–1345.

[57] D.G. Wishka, D.R. Graber, L.A. Kopta, R.A. Olmsted, J.M. Friis, J.D. Hosley, W.J. Adams, E.P. Seest, T.M. Castle, L.A. Dolak, B.J. Keiser, Y. Yagi, A. Jeganathan, S.T. Schlachter, M.J. Murphy, G.J. Cleek, R.A. Nugent, S.M. Poppe, S.M. Swaney, F. Han, W. Watt, W.L. White, T.-J. Poel, R.C. Thomas, R.L. Voorman, K.J. Stefanski, R.G. Stehle, W.G. Tarpley, J. Morris, (−)-6-Chloro-2-[(1-furo[2,3-c]pyridin-5-ylethyl)thio]-4-pyrimidinamine, PNU-142721, a new broad spectrum HIV-1 non-nucleoside reverse transcriptase inhibitor, J. Med. Chem. 41 (1998) 1357–1360.

[58] C. Mao, E.A. Sudbeck, T.K. Venkatachalam, F.M. Uckun, Rational design of *N*-[2-(2,5-dimethoxyphenylethyl)]-*N′*-[2-(5-bromopyridyl)]-thiourea (HI-236) as a potent non-nucleoside inhibitor of drug-resistant human immunodeficiency virus, Bioorg. Med. Chem. Lett. 9 (1999) 1593–1598.

[59] D.L. Taylor, P.S. Ahmed, P. Chambers, A.S. Tyms, J. Bedard, J. Duchaine, G. Falardeau, J.F. Lavallée, W. Brown, R.F. Rando, T. Bowlin, Pyrido[1,2-a]indole derivatives identified as novel non-nucleoside reverse transcriptase inhibitors of human immunodeficiency virus type 1, Antiviral Chem. Chemother. 10 (1999) 79–86.

[60] J.W. Corbett, S.S. Ko, J.D. Rodgers, S. Jeffrey, L.T. Bacheler, R.M. Klabe, S. Diamond, C.-M. Lai, S.R. Rabel, J.A. Saye, S.P. Adams, G.L. Trainor, P.S. Anderson, S.K. Erickson-Viitanen, Expanded-spectrum nonnucleoside reverse transcriptase inhibitors inhibit clinically relevant mutant variants of human immunodeficiency virus type 1, Antimicrob. Agents Chemother. 43 (1999) 2893–2897.

[61] F.M. Uckun, S. Pendergrass, D. Maher, D. Zhu, L. Tuel-Ahlgren, C. Mao, T.K. Venkatachalam, *N′*-[2-(2-thiophene)ethyl]-*N′*-[2-(5-bromopyridyl)]thio-urea as a potent inhibitor of NNI-resistant and multidrug-resistant human immunodeficiency virus-1, Bioorg. Med. Chem. Lett. 9 (1999) 3411–3416.

[62] F.M. Uckun, C. Mao, S. Pendergrass, D. Maher, D. Zhu, L. Tuel-Ahlgren, T.K. Venkatachalam, *N*-[2-(1-cyclohexenyl)ethyl]-*N′*-[2-(5-bromopyridyl)]-thiourea and *N′*-[2-(1-cyclohexenyl)ethyl]-*N′*-[2-(5-chloropyridyl)]-thiourea as potent inhibitors of multidrug-resistant human immunodeficiency virus-1, Bioorg. Med. Chem. Lett. 9 (1999) 2721–2726.

[63] M. Högberg, C. Sahlberg, P. Engelhardt, R. Noréen, J. Kangasmetsä, N.G. Johansson, B. Öberg, L. Vrang, H. Zhang, B.-L. Sahlberg, T. Unge, S. Lövgren, K. Fridborg, K. Bäckbro, Urea–PETT compounds as a new class of HIV-1 reverse transcriptase inhibitors. 3. Synthesis and further structure–activity relationship studies of PETT analogues, J. Med. Chem. 42 (1999) 4150–4160.

[64] A. Casimiro-Garcia, M. Micklatcher, J.A. Turpin, T.L. Stup, K. Watson, R.W. Buckheit, M. Cushman, Novel modifications in the alkenyldiarylmethane (ADAM) series of non-nucleoside reverse transcriptase inhibitors, J. Med. Chem. 42 (1999) 4861–4874.

[65] G. Campiani, E. Morelli, M. Fabbrini, V. Nacci, G. Greco, E. Novellino, A. Ramunno, G. Maga, G. Spadari, S. Spadari, G. Caliendo, A. Bergamini, E. Faggioli, I. Uccella, F. Bolacchi, S. Marini, M. Coletta, A. Nacca, S. Caccia, Pyrrolobenzoxazepinone derivatives as non-nucleoside HIV-1 RT inhibitors: further structure–activity relationship studies and identification of more potent broad-spectrum HIV-1 RT inhibitors with antiviral activity, J. Med. Chem. 42 (1999) 4462–4470.

[66] S. Palmer, J.D. Russell, R.W. Buckheit, Jr., J.-W. Lee, S.-H. Lee, J.W. Oh, H.-S. Kwon, S.G. Chung, E.-H. Cho, Activity of SJ-3366, a novel non-nucleoside reverse transcriptase inhibitor of HIV-1, in combination with other anti-HIV agents against wild-type and SJ-3366-resistant viral isolates, 4th International Workshop on Drug Resistance & Treatment Strategies, 12–16 June 2000, Sitges, Spain, Antiviral Ther. 5 (Suppl. 3) (2000) 9 (abstract 12).

[67] Y. Pommier, A.A. Pilon, K. Bajaj, A. Mazumder, N. Neamati, HIV-1 integrase as a target for antiviral drugs, Antiviral Chem. Chemother. 8 (1997) 463–483.

[68] N. Neamati, A. Mazumder, S. Sunder, J.M. Owen, M. Tandon, J.W. Lown, Y. Pommier, Highly potent synthetic polyamides, bisdistamycins, and lexitropsins as inhibitors of human immunodeficiency virus type 1 integrase, Mol. Pharmacol. 54 (1998) 280–290.

[69] C.M. Farnet, B. Wang, M. Hansen, J.R. Lipford, L. Zalkow, W.E. Robinson, Jr., J. Siegel, F. Bushman, Human immunodeficiency virus type 1 cDNA integration: new aromatic hydroxylated inhibitors and studies of the inhibition mechanism, Antimicrob. Agents Chemother. 42 (1998) 2245–2253.

[70] N. Neamati, J.A. Turpin, H.E. Winslow, J.L. Christensen, K. Williamson, A. Orr, W.G. Rice, Y. Pommier, A. Garofalo, A. Brizzi, G. Campiani, I. Fiorini, V. Nacci, Thiazolothiazepine inhibitors of HIV-1 integrase, J. Med. Chem. 42 (1999) 3334–3341.

[71] W.E. Robinson, Jr., M.G. Reinecke, S. Abdel-Malek, Q Jia, S.A. Chow, Inhibitors of HIV-1 replication that inhibit HIV integrase, Proc. Natl. Acad. Sci. USA 93 (1996) 6326–6331.

[72] W.E. Robinson, Jr., L-Chicoric acid, an inhibitor of human immunodeficiency virus type 1 (HIV-1) integrase, improves on the in vitro anti-HIV-1 effect of zidovudine plus a protease inhibitor (AG1350), Antiviral Res. 39 (1998) 101–111.

[73] P.J. King, W.E. Robinson, Jr., Resistance to the anti-human immunodeficiency virus type 1 compound L-chicoric acid results from a single mutation at amino acid 140 of integrase, J. Virol. 72 (1998) 8420–8424.

[74] A. Mazumder, K. Raghavan, J. Weinstein, K.W. Kohn, Y. Pommier, Inhibition of human immunodeficiency virus type-1 integrase by curcumin, Biochem. Pharmacol. 49 (1995) 1165–1170.

[75] W.E. Robinson, Jr., M. Cordeiro, S. Abdel-Malek, Q. Jia, S.A. Chow, M.G. Reinecke, W.M. Mitchell, Dicaffeoylquinic acid inhibitors of human immunodeficiency virus integrase: inhibition of the core catalytic domain of human immunodeficiency virus integrase, Mol. Pharmacol. 50 (1996) 846–855.

[76] A. Mazumder, N. Neamati, S. Sunder, J. Schulz, H. Pertz, E. Eich, Y. Pommier, Curcumin analogs with altered potencies against HIV-1 integrase as probes for biochemical mechanisms of drug action, J. Med. Chem. 40 (1997) 3057–3063.

[77] B. McDougall, P.J. King, B.W. Wu, Z. Hostomsky, M.G. Reinecke, W.E. Robinson, Jr., Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase, Antimicrob Agents Chemother 42 (1998) 140–146.

[78] W. Pluymers, N. Neamati, C. Pannecouque, V. Fikkert, C. Marchand, T.R. Burke, Y. Pommier Jr, D. Schols, E. De Clercq, Z. Debyser, M. Witvrouw, Viral entry as the primary target for the anti-HIV activity of chicoric acid and its tetraacetyl esters, Mol. Pharmacol. 58 (2000) 641–648.

[79] D.J. Hazuda, P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J.A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau, M.D. Miller, Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells, Science 287 (2000) 646–650.

[80] D. Daelemans, A.-M. Vandamme, E. De Clercq, Human immunodeficiency virus gene regulation as a target for antiviral chemotherapy, Antiviral Chem. Chemother. 10 (1999) 1–14.

[81] K. Watson, R.J. Edwards, HIV-1 *trans*-activating (Tat) protein. Both a target and a tool in therapeutic approaches, Biochem. Pharmacol. 58 (1999) 1521–1528.

[82] M. Baba, M. Okamoto, M. Makino, Y. Kimura, T. Ikeuchi, T. Sakaguchi, T. Okamoto, Potent and selective inhibition of human immunodeficiency virus type 1 transcription by piperazinyloxoquinoline derivatives, Antimicrob. Agents Chemother. 41 (1997) 1250–1255.

[83] M. Baba, M. Okamoto, M. Kawamura, M. Makino, T. Higashida, T. Takashi, Y. Kimura, T. Ikeuchi, T. Tetsuka, T. Okamoto, Inhibition of human immunodeficiency virus type 1 replication and cytokine production by fluoroquinoline derivatives, Mol. Pharmacol. 53 (1998) 1097–1103.

[84] H. Okamoto, T.P. Cujec, M. Okamoto, B.M. Peterlin, M. Baba, T. Okamoto, Inhibition of the RNA-dependent transacti-

vation and replication of human immunodeficiency virus type 1 by a fluoroquinoline derivative K-37, Virology 272 (2000) 402–408.

[85] F. Kashanchi, M.R. Sadaie, J.N. Brady, Inhibition of HIV-1 transcription and virus replication using soluble Tat peptide analogs, Virology 227 (1997) 431–438.

[86] I. Choudhury, J. Wang, A.B. Rabson, S. Stein, S. Pooyan, S. Stein, M.J. Leibowitz, Inhibition of HIV-1 replication by a Tat RNA-binding domain peptide analog, J. Acquir. Immun. Defic. Syndrom. Hum. Retrovirol. 17 (1998) 104–111.

[87] F. Hamy, E.R. Felder, G. Heizmann, J. Lazdins, F. Aboul-ela, G. Varani, J. Karn, T. Klimkait, An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication, Proc. Natl. Acad. Sci. USA 94 (1997) 3548–3553.

[88] D. Daelemans, D. Schols, M. Witvrouw, C. Pannecouque, S. Hatse, S. Van Dooren, F. Hamy, T. Klimkait, E. De Clercq, A.-M. Vandamme, A second target for the peptoid Tat/transactivation response element inhibitor CGP64222: inhibition of human immunodeficiency virus replication by blocking CXC-chemokine receptor 4-mediated virus entry, Mol. Pharmacol. 57 (2000) 116–124.

[89] S. Ghezzi, D.M. Noonan, M.G. Aluigi, G. Vallanti, M. Cota, R. Benelli, M. Morini, J.D. Reeves, E. Vicenzi, G. Poli, A. Albini, Inhibition of CXCR4-dependent HIV-1 infection by extracellular HIV-1 Tat, Biochem. Biophys. Res. Commun. 270 (2000) 992–996.

[90] C. Flexner, HIV protease inhibitors, New Engl. J. Med. 338 (1998) 1281–1292.

[91] A. Molla, G.R. Granneman, E. Sun, D.J. Kempf, Recent developments in HIV protease inhibitor therapy, Antiviral Res. 39 (1998) 1–23.

[92] H.L. Sham, D.J. Kempf, A. Molla, K.C. Marsh, G.N. Kumar, C.-M. Chen, W. Kati, K. Stewart, R. Lal, A. Hsu, D. Betebenner, M. Korneyeva, S. Vasavanonda, E. McDonald, A. Saldivar, N. Wideburg, X. Chen, P. Niu, C. Park, V. Jayanti, B. Grabowski, G.R. Granneman, E. Sun, A.J. Japour, J.M. Leonard, J.J. Plattner, D.W. Norbeck, ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease, Antimicrob. Agents Chemother. 42 (1998) 3218–3224.

[93] S. Thaisrivongs, P.K. Tomich, K.D. Watenpaugh, K.-T. Chong, W.J. Howe, C.-P. Yang, J.W. Strohbach, S.R. Turner, J.P. McGrath, M.J. Bohanon, J.C. Lynn, A.M. Mulichak, P.A. Spinelli, R.R. Hinshaw, P.J. Pagano, J.B. Moon, M.J. Ruwart, K.F. Wilkinson, B.D. Rush, G.L. Zipp, R.J. Dalga, F.J. Schwende, G.M. Howard, G.E. Padbury, L.N. Toth, Z. Zhao, K.A. Koeplinger, T.J. Kakuk, S.L. Cole, R.M. Zaya, R.C. Piper, P. Jeffrey, Structure-based design of HIV protease inhibitors: 4-hydroxycoumarins and 4-hydroxy-2-pyrones as non-peptidic inhibitors, J. Med. Chem. 37 (1994) 3200–3204.

[94] H.I. Skulnick, P.D. Johnson, W.J. Howe, P.K. Tomich, K.-T. Chong, K.D. Watenpaugh, M.N. Janakiraman, L.A. Dolak, J.P. McGrath, J.C. Lynn, M.-M. Horng, R.R. Hinshaw, G.L. Zipp, M.J. Ruwart, F.J. Schwende, W.-Z. Zhong, G.E. Padbury, R.J. Dalga, L. Shiou, P.L. Possert, B.D. Rush, K.F. Wilkinson, G.M. Howard, L.N. Toth, M.G. Williams, T.J. Kakuk, S.L. Cole, R.M. Zaya, K.D. Lovasz, J.K. Morris, K.R. Romines, S. Thaisrivongs, P.A. Aristoff, Structure-based design of sulfonamide-substituted non-peptidic HIV protease inhibitors, J. Med. Chem. 38 (1995) 4968–4971.

[95] P.Y.S. Lam, Y. Ru, P.K. Jadhav, P.E. Aldrich, G.V. DeLucca, C.J. Eyermann, C.-H. Chang, G. Emmett, E.R. Holler, W.F. Daneker, L. Li, P.N. Confalone, R.J. McHugh, Q. Han, R. Li, J.A. Markwalder, S.P. Seitz, T.R. Sharpe, L.T. Bacheler, M.M. Rayner, R.M. Klabe, L. Shum, D.L. Winslow, D.M. Kornhauser, D.A. Jackson, S. Erickson-Viitanen, C.N. Hodge, Cyclic HIV protease inhibitors: synthesis, conformational analysis, P2/P2′ structure–activity relationship, and molecular recognition of cyclic ureas, J. Med. Chem. 39 (1996) 3514–3525.

[96] C.N. Hodge, P.E. Aldrich, L.T. Bacheler, C.-H. Chang, C.J. Eyermann, S. Garber, M. Grubb, D.A. Jackson, P.K. Jadhav, B. Korant, P.Y.S. Lam, M.B. Maurin, J.L. Meek, M.J. Otto, M.M. Rayner, C. Reid, T.R. Sharpe, L. Shum, D.L. Winslow, S. Erickson-Viitanen, Improved cyclic urea inhibitors of the HIV-1 protease: synthesis, potency, resistance profile, human pharmacokinetics and X-ray crystal structure of DMP 450, Chem. Biol. 3 (1996) 301–314.

[97] P.K. Jadhav, F.J. Woerner, P.Y.S. Lam, C.N. Hodge, C.J. Eyermann, H.-W. Man, W.F. Daneker, L.T. Bacheler, M.M. Rayner, J.L. Meek, S. Erickson-Viitanen, D.A. Jackson, J.C. Calabrese, M. Schadt, C.-H. Chang, Nonpeptide cyclic cyanoguanidines as HIV-1 protease inhibitors: synthesis, structure–activity relationships, and X-ray crystal structure studies, J. Med. Chem. 41 (1998) 1446–1455.

[98] G. Bold, A. Fässler, H.-G. Capraro, R. Cozens, T. Klimkait, J. Lazdins, J. Mestan, B. Poncioni, J. Rösel, D. Stover, M. Tintelnot-Blomley, F. Acemoglu, W. Beck, E. Boss, M. Eschbach, T. Hürlimann, E. Masso, S. Roussel, K. Ucci-Stoll, D. Wyss, M. Lang, New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development, J. Med. Chem. 41 (1998) 3387–3401.

[99] K.S. Fors, J.R. Gage, R.F. Heier, R.C. Kelly, W.R. Perrault, N. Wicnienski, A convergent, scalable synthesis of HIV protease inhibitor PNU-140690, J. Org. Chem. 63 (1998) 7348–7356.

[100] S.R. Turner, J.W. Strohbach, R.A. Tommasi, P.A. Aristoff, P.D. Johnson, H.I. Skulnick, L.A. Dolak, E.P. Seest, P.K. Tomich, M.J. Bohanon, M.-M. Horng, J.C. Lynn, K.-T. Chong, R.R. Hinshaw, K.D. Watenpaugh, M.N. Janakiraman, S. Thaisrivongs, Tipranavir (PNU-140690): a potent, orally bioavailable nonpeptidic HIV protease inhibitor of the 5,6-dihydro-4-hydroxy-2-pyrone sulfonamide class, J. Med. Chem. 41 (1998) 3467–3476.

[101] S.M. Poppe, D.E. Slade, K.-T. Chong, R.R. Hinshaw, P.J. Pagano, M. Markowitz, D.D. Ho, H. Mo, R.R. Gorman, III, T.J. Dueweke, S. Thaisrivongs, W.G. Tarpley, Antiviral activity of the dihydropyrone PNU-140690, a new nonpeptidic human immunodeficiency virus protease inhibitor, Antimicrob. Agents Chemother. 41 (1997) 1058–1063.

[102] J. Sierra, S. Nino, P. Volkow, D. Sereni, P. Yeni, S. Staszewski, J. Gatell, L. Wang, N. McMillan, F. Rousseau, G.D. Miralles, Preliminary profile of the antiviral activity, metabolic effects and safety of DMP-450, a novel cyclic urea protease inhibitor, 4th International Workshop on Drug Resistance & Treatment Strategies, 12–16 June 2000, Sitges, Spain, Antiviral Ther. 5 (Suppl. 3) (2000) 6 (abstract 6).

[103] S. Rusconi, S.L.S. Catamancio, P. Citterio, S. Kurtagic, M. Violin, C. Balotta, M. Moroni, M. Galli, A. D'Arminio-Monforte, Susceptibility to PNU-140690 (tipranavir) of human immunodeficiency virus type 1 isolates derived from patients with multidrug resistance to other protease inhibitors, Antimicrob. Agents Chemother. 44 (2000) 1328–1332.