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HIV-1 IN Inhibitors: 2010 Update and Perspectives

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Abstract

Integrase (IN) is the newest validated target against AIDS and retroviral infections. The remarkable activity of raltegravir (Isentress®) led to its rapid approval by the FDA in 2007 as the first IN inhibitor. Several other IN strand transfer inhibitors (STIs) are in development with the primary goal to overcome resistance due to the rapid occurrence of IN mutations in raltegravir-treated patients. Thus, many scientists and drug companies are actively pursuing clinically useful IN inhibitors. The objective of this review is to provide an update on the IN inhibitors reported in the last two years, including second generation strand transfer inhibitors (STI), recently developed hydroxylated aromatics, natural products, peptide, antibody and oligonucleotide inhibitors. Additionally, the targeting of IN cofactors such as LEDGF and Vpr will be discussed as novel strategies for the treatment of AIDS.

Keywords

HIV-1 IN; diketo acids; HIV inhibitors; AIDS; IN inhibitors; peptides; oligonucleotides

Introduction

Approximately 30 years of research have produced 25 FDA approved drugs for the treatment of AIDS, 13 of which target viral replication (reverse transcriptase; RT), 9 target maturation (protease), one targets viral fusion, one viral entry (CCR5 antagonist), and one integration (Table 1). The IN inhibitor raltegravir and the CCR5 antagonist maraviroc were both approved by the FDA in 2007. The success of highly active antiretroviral therapy (HAART) combinations with increasingly potent drugs has improved patient long-term survival and tolerability to drug regimens. The current HAART treatments for new patients consist of three drug combinations targeting just two viral enzymes: RT and protease. They consist of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor. However, mutations of the HIV-1 genome confer resistance to those drugs in a large number of patients. Chronic dosing is also commonly associated with side effects that hamper compliance to therapy. Thus, the development of new inhibitors remains essential to reduce side effects and selection of drug-resistant viruses. Simultaneous inhibition of several viral targets remains the best way to circumvent drug resistance because multiple mutations are required for resistance, some of which are bound to be detrimental to viral replication and growth.

With the approval of raltegravir, the antiretroviral drugs can now target all three viral enzymes: RT, protease and IN (IN). The absence of known human homologue to IN

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probably accounts for the low toxicity and high selectivity of raltegravir and of the forthcoming IN inhibitors. That statement, however, should be made with caution since diketo acids can inhibit RAG1/2 recombinase, albeit at drug concentrations 20-fold higher than required to inhibit IN¹. RAG recombinases have mechanistic similarities to HIV-1 IN, further suggesting novel IN inhibitors should be tested against RAG1/2 to assess their selectivity and avoid potential toxicity. Another enzyme with structural similarities to IN is RNase H, which is part of RT activity^{2, 3}. This stresses the value of screening IN inhibitors also against RT-RNase H⁴. The present review describes recent advances in the search for HIV-1 IN inhibitors with a focus on the mechanism of inhibition by diketo acids and small molecules, natural products, peptides, antibody and oligonucleotide inhibitors published in the last two years. It is an update of our prior landmark reviews^{5, 6} and complements other recent reviews^{7–22}.

IN Structural Overview

IN is required for three events leading to viral integration [Fig. (1A)]: 1) site-specific endonucleolytic cleavage of the 3'-ends of the viral DNA (3'-processing, 3'-P), 2) assembly of the preintegration complex (PIC) on the ends of the viral DNA, and 3) insertion of the viral DNA into a host chromosomal DNA (strand transfer, ST). The completion of integration requires an additional step with the processing and attachment of the 5'-ends of the viral DNA to the host genome (5'-processing and gap filling) [Fig. (1A)], which is probably catalyzed by cellular enzymes.

IN is encoded at the 3'-end of the HIV POL gene, which also encodes RT and protease [see scheme in Box 1 p. 240 in ref. 6]. The polyprotein precursor is cleaved by protease during maturation, generating the IN polypeptide, which is packaged within the newly formed HIV virions. HIV-1 IN is a 32,000 Daltons polypeptide of 288 amino acids comprising three functional domains^{3, 23}. The amino-terminal domain (amino acids 1–50) contains a conserved and essential zinc-binding motif HHCC (histidines 12 and 16, cysteines 40 and 43) that coordinates one zinc atom²⁴, though the structure of this region does not resemble a zinc finger²⁵. One known function of the amino-terminal domain region is protein multimerization. The catalytic core domain (amino acids 50–212) contains the catalytic DDE motif, which is conserved among all retroviral INs and consists of the active site residues D64, D116, and E152 in HIV-1 IN (shown in red in Fig. 2). Mutation of any one of these three residues is sufficient to inactivate IN. Crystal structures show that HIV-1 IN binds one magnesium ion between D64 and D116 (pink sphere in Fig. 2A), and that ASV binds an additional Zn²⁺ or Cd²⁺ ion between D64 and E157 (the ortholog of E152)²⁶. Thus, it is likely that the HIV-1 IN active site binds two metal ions (Mg⁺² or Mn⁺²) when complexed with the ends of the viral DNA during the cleavage and joining reactions. Another structural feature of the catalytic core domain is the 10 amino acid flexible loop encompassed between glycine residues G140 and G149. Those two glycines potentially act as hinges for the overall movement of the loop that may serve as a clamp for the binding of the viral DNA ends to the catalytic site of IN. Consistent with this possibility, glutamine 148 (Q148), one of the flexible loop residues has been shown to bind selectively to the penultimate cytosine at the 5'-end of the viral DNA²⁷. Q148 is also a key residue for IN catalytic activity²⁸ and resistance to raltegravir and elvitegravir²⁸. The carboxyl-terminal domain (amino acids 213–288) of HIV-1 IN is important for nonspecific DNA binding of sub-terminal viral DNA and of the host (target) DNA^{29–32}. Its structure contains an SH2-like motif³, which can be considered for rational drug design⁶. While each of the IN domains forms dimers, IN functions as a tetramer^{33–35}.

HIV-1 IN recognizes the specific sequence 5'-GCAGT-3' at the ends of each viral long terminal repeat (LTR) and binds tightly to those LTR ends [Fig. (1A)]. The association of IN

with the host chromosomal (target) DNA is of weaker affinity and specificity ³⁶, which probably explains the integration of viral DNA into many possible genomic regions with relatively little sequence selectivity ³⁷. The integration reaction requires the association of two viral DNA (donor) ends and a host chromosome (acceptor), and an unknown number of IN subunits [at least a tetramer ^{33, 34}]. Unraveling the complexity of the structure of the IN/DNA complex has remained a challenging task.

Several crystal structures comprising different domains of HIV-1 IN have been solved: core domain ^{38, 39}, core and C-terminal domains ⁴⁰, and core and N-terminal domains ³⁵, but a crystal of the full-length protein with or without DNA remains elusive due to solubility difficulties. Two co-crystal structures with IN inhibitors have been reported: one of HIV-1 IN complexed with 5CITEP ⁴¹, and the other of ASV IN complexed with the inhibitor Y-3 ⁴². Additionally, one study reported the proposed binding site of a mononucleotide inhibitor in the IN active site ⁴³ and a more recent study the proposed binding of pyridoxal 5'-phosphate in the C-terminal domain around lysine 244 ⁴⁴. Another drug binding site has been reported for a photoactivatable coumarin derivative with cysteine 130 and tryptophane 132 ⁴⁵. A crystal structure of the full catalytic complex with inhibitor would be of great benefit to IN inhibitor design. For instance, the development of successful HIV-1 protease and RT inhibitors was achieved through structure-based drug design. Adequate structural information defining the interactions of IN with DNA and inhibitors would certainly speed up similar structure-based drug design efforts ⁴⁶. Solving IN-DNA structures is probably key to the elucidation of the binding site of raltegravir and other strand transfer inhibitors as these drugs are likely to act as interfacial inhibitors ^{47–49} by binding at the interface of the viral DNA LTR end and the DDE catalytic site of IN and thereby preventing the binding of the acceptor (chromosomal) DNA ^{6, 19}.

Mechanism of Integration

The IN reaction occurs biochemically in two steps following reverse transcription and the binding of IN to the LTR ends with several other proteins within the PIC ²². Assembly of the PIC onto the 5'-GCAGT-3' LTR sequence of the viral DNA ends forms a complex competent for integration, although *in vitro*, only IN and DNA are required, Fig. (1A). The first step is an endonucleolytic cleavage, or 3'-P, which results in removal of two nucleotides from the 3'-ends of each viral DNA LTR immediately 3' from the conserved CA dinucleotide (5'-GCAGT-3'). The phosphodiester bond between the deoxyadenosine and deoxyguanosine is hydrolyzed by water as the nucleophile in the presence of Mg²⁺ (the presumably physiological metal) or Mn²⁺ ²⁹. IN can also use glycerol, alcohols, or the 3' viral terminus as the attacking nucleophile in the presence of Mn²⁺ ^{50, 51}. The terminal 3'-end of the viral DNA is released beyond the conserved CA dinucleotide [see Fig. (1B)], and generally a 5'-GT-3' dinucleotide is released (unless the 3'-end has been made longer during reverse transcription ⁵²), resulting in recessed 3'-hydroxyl ends at each terminus of the viral DNA.

Following nuclear translocation of the PIC, integration catalyzes the second transesterification reaction where the 3'-hydroxyl ends of the viral DNA act as nucleophiles to attack the DNA phosphodiester backbone of a host chromosome. HIV-1 IN positions both viral ends to attack both strands of a host chromosome (acceptor DNA) with a five base pair gap on each strand, probably across the major groove of the acceptor DNA [see Fig. 2d in 6]. The result is a staggered insertion that is sealed by host DNA repair enzymes. Raltegravir, elvitegravir (Table 1) and the diketo acid inhibitors reported recently (Table 2) are selective against the ST reaction compared to 3'-P ^{53, 54}. Thus, they are often referred to as STI (strand transfer inhibitors).

Recombinant IN is catalytically active in biochemical assays, which eases the screening of IN inhibitors^{55, 56}. *In vitro*, IN, divalent metal (Mg^{2+} or Mn^{2+}), and short oligonucleotides derived from the DNA sequence of the U5 or U3 LTR are sufficient for integration. The mechanisms of the *in vitro* reactions are shown in Fig. (1B). Most of the inhibitors reported were discovered using oligonucleotide-based assays, with either gel or microtiter plate/fluorescence analysis. In this review, IC_{50} values described as “*in vitro*” were generated using such biochemical assays, unless a cell culture system is specifically described. *In vitro* reactions differ from *in vivo* integration because ST of only one DNA end occurs efficiently in the *in vitro* reactions using short oligonucleotides. Recent reports describe conditions allowing higher efficiency of full-site (concerted) integration^{34, 57}. Concerted joining of both viral DNA ends probably occurs more efficiently *in vivo* due to the presence of long DNA substrates, other PIC components and cellular cofactors.

IN inhibitors in clinical use

IsentresTM (raltegravir, RAL, also known as MK-0518) is the first and still the only IN inhibitor approved by the FDA (in October 2007) for the treatment of patients who are failing HAART (Table 1). Raltegravir is the result of a long-term commitment by Merck and Co. in the development of IN inhibitors²⁰. Raltegravir is well-tolerated, highly potent, and with a good pharmacokinetics (Table 2) 20· 21· 58. Raltegravir is administered orally at the dose of 400 mg twice daily and leads to viral RNA levels below detection before 48 weeks of treatment⁵⁹. It has been difficult to increase raltegravir blood levels by increasing dosing in patients who develop resistance to raltegravir because the raltegravir blood levels remain somewhat limited (see below).

Elvitegravir (EVG = GS-9137 = JTK-303) is the next most advanced IN inhibitor in clinical development (Table 2). It has not yet been approved by the FDA. This quinolone carboxylic acid derivative was originally developed by Japan Tobacco Inc. under the name JTK-303⁶⁰. JTK-303 was subsequently licensed to Gilead Sciences under the name GS-9137 for development in all countries except Japan. Elvitegravir, like raltegravir, is a potent antiviral with single digit nanomolar antiviral potency (Table 2)^{60–62}. Elvitegravir is slightly more potent than raltegravir *in vitro* and *ex vivo* 28· 61 but exhibits a potential higher cytotoxicity in non-infected cells 60 (Marchand et al. unpublished observations). One limitation of elvitegravir is that unlike raltegravir, it is metabolized by cytochrome P450 (CYP3A4/5) and secondarily by glucuronidation (UGT1A1/3) to produce metabolites that are less potent than the parent drug 20· 63. Co-administration of ritonavir with elvitegravir can overcome this metabolic inactivation and can increase the plasma levels of raltegravir[NN1] by 10- to 16-fold 63.

Early diketo acid IN inhibitors and mechanisms of action studies

Both raltegravir and elvitegravir are derived from the diketo acid (DKA) (also referred as ketoenol acid 19) family of IN inhibitors. Diketo acids were the first IN inhibitors reported with selectivity for the strand transfer step of the integration reaction (ST-inhibitor, See 6), high specificity for IN, and antiviral activity that could be related to IN inhibition 54. Historically, the two first reported DKA molecules are 5CITEP from Shionogi & Co. Ltd 41 and the L-731,988 series from Merck and Co. 54 (Table 3). 5CITEP has been the only DKA co-crystallized with the catalytic core domain of IN (Fig. 2A)⁴¹. 5CITEP is stabilized in the active site by hydrogen bonds with one of the catalytic residues (E152) as well as with other amino acids (T66, N155, Q148) and two lysine residues (K156 and K159) previously reported to be involved in DNA substrate binding^{29, 30} and mononucleotide positioning 43 (Fig. 2A). In the 5CITEP-IN co-crystal structure, neither direct contact between the drug and the two other catalytic residues (D64 and D116) nor direct interaction with the magnesium

ion could be detected. Only a van der Waals interaction between the indole ring and one of the four water molecules coordinating the magnesium ion seemed plausible (Fig. 2A).

The DKA derivatives selectively inhibit ST at nanomolar IC_{50} values and inhibit integration without interfering with viral DNA synthesis in cells⁵⁴. Validation of HIV-1 IN as a molecular target was obtained by generation of drug-resistant mutant viruses, which were found to bear single or multiple mutations in their IN coding sequence. The resulting mutations (T66I, L74M, E92Q, G140G/A, Y143R/C, Q148R/H/K, S153Y, M154I, N155H) all cluster around the DDE catalytic triad of amino acids (D64, D116, E152) (Fig. 2C&2D)^{54, 64, 65}. Consistent with the drug binding within the IN catalytic site, inhibition was found to be metal-dependent⁶⁶ and to interfere with binding of the flexible loop to the viral LTR DNA end⁶⁷. Selective IN inhibition in HIV infected cells was also suggested by the increased formation of 2-LTR viral circles, which are abortive viral replication intermediates⁵⁴. Binding studies revealed that DKA compounds only bind to the catalytic site of HIV-1 IN specifically in the presence of viral LTR DNA⁶⁸. Other studies suggested that these compounds also interact with the 5'-end of the viral DNA^{53, 69}. Mutating one of the HIV IN core catalytic residues is sufficient to abrogate drug binding in the presence of a viral DNA substrate⁶⁸. Prior binding of the target DNA substrate to the IN-viral DNA complex prohibits binding and inhibition of integration by DKAs in a concentration-dependent manner, suggesting a competition between diketo acids and DNA in the ST complex^{17, 53, 68}.

DKA derivatives mediate their antiviral activity through metal chelation^{69–71}. Although the type and number of metals required for IN activity have still not been determined, it has been postulated that the integration mechanism involves two divalent metals (magnesium) in the catalytic site of the enzyme^{69–71}. The first magnesium atom, as observed in the different crystal structures, is coordinated by the two catalytic residues D64 and D116 (Fig. 2). The second would be coordinated either by D116 and E152^{69–71}.

Shionogi & Co. Ltd. has developed IN inhibitors in partnership with GlaxoSmithKline and both went into phase I/II clinical trials with S-1360, a derivative of 5CITEP⁷². The compound was active and well tolerated but the trial was terminated for undisclosed reasons⁸. Merck & Co. after demonstrating the efficacy of DKA derivatives to suppress SIV viral replication in rhesus macaques⁶⁵, developed the naphthyridine carboxamides (L-870,810, Table 3), a class of IN inhibitors derived from the DKA family⁷³. L-870,810 is a potent ST inhibitor with improved bioavailability compared to previously reported DKA. L-870,810 inhibits the IN ST activity *in vitro* with an IC_{50} value of 8 nM and is antiviral with an EC_{95} value of 15 nM in a cell culture assay (Table 3)⁷³. During clinical trials, L-870,810 was terminated in Phase II due to toxicity in dogs⁸.

Resistance to raltegravir and elvitegravir and second generation ST inhibitors

Despite its recent success in the clinic, emergence of resistance leading to treatment failure has already been reported for raltegravir and elvitegravir^{20, 28, 61, 62}. Consistent with the selective targeting of IN by raltegravir and elvitegravir, resistance pathways have been described in relationship with point mutations of IN residues surrounding the IN catalytic core site. Three main pathways appear to drive resistance. They involve “primary mutations” Q148R/H/K, N155H or Y143R/C^{28, 61, 62, 74–77}. Each tends to be associated with “secondary mutations” that appear to restore viral fitness in those primary mutants: for instance G140S with Q148H or G140A with Q148R^{75, 77} (Marchand and Pommier, unpublished). In a recent study, we showed that the resistance profile of elvitegravir closely

resembles that of raltegravir, suggesting that elvitegravir is unlikely to overcome resistance to raltegravir²⁸.

Current research efforts are now devoted to the discovery and the development of novel compounds and model systems to circumvent raltegravir and elvitegravir resistance. Merck has already developed second generation ST-inhibitors such as the tricyclic MK-2048 (Table 3), which exhibits an EC95 value of 41 nM and excellent pharmacokinetics in preclinical species²⁰. Gilead Sciences is developing its own next generation tricyclic inhibitors. GS-9160 has been designed on the naphthyridine carboxamide scaffold (Table 3) and exhibits a single digit nanomolar antiviral activity with over 3 logs of selectivity index⁷⁸. Bristol-Myers Squibb is also committed to develop ST inhibitors with the use of compound ST1 (Table 3) to study integration mechanisms⁷⁹. Academic groups are also actively involved in the development of novel IN inhibitors. One example is the quinolinonyl DKA, which shares with elvitegravir the same quinolone structural motif (Table 3)⁸⁰ and novel fused DKA compounds (see Table 5)^{81–83}. The rest of this chapter will be devoted to other compounds, many of which inhibit both 3'-processing and strand transfer.

L-Chicoric Acid Derivatives

Caffeic acid phenylethyl ester (CAPE) was one of the first reported natural product (from bee hives) inhibitors of IN (Table 4)^{84, 85}. Furthermore, CAPE was the first compound found to inhibit ST with selectively over 3'-P with IC₅₀ values of 5 μ M^{84, 85}. L-Chicoric acid (L-CA) is a related bidentate catechol that has also been identified as an inhibitor of IN (Table 4). Inhibition has been shown at sub-micromolar concentrations through a noncompetitive and reversible mechanism both *in vitro* and *ex vivo*^{86, 87}. However, L-CA enters cells poorly and its antiviral activity has been proposed to result primarily from inhibition of viral entry by interaction with gp120⁸⁸. These two molecules, CAPE and L-CA, have served as parent structures for the identification of new molecules with improved properties. For example, an L-CA derivative (Table 4) has shown improved selectivity against the integration step in cell culture according to quantitative real-time PCR^{86, 87}. In addition, several L-CA derivatives have shown clinical potential based on their ability to readily enter cells and increased activity against IN. Notably, the clinically relevant IN mutations G140S and F185K confer significant resistance to L-CA and its derivatives⁸⁷.

The caffeic acid derivative in which two caffeoyl moieties are bridged by a tartaric acid (Table 4) is active against HIV-1 IN at submicromolar concentrations and against HIV-1 replication at micromolar concentrations⁸⁶. Unfortunately, toxicity of this molecule is also in the same concentration range leading to poor selectivity indexes⁸⁹. A chicoric acid derivative substituted with maleic anhydride (Table 4) represents a potent inhibitor of IN with an IC₅₀ as low as 3.8 μ M⁹⁰. However, this compound is unable to inhibit HIV-1 replication under nontoxic concentrations. A series of caffeoylamino acid derivatives have also been generated (Table 4). For example, catechol ring substitutions have shown to reduce toxicity. In addition, substitution of the carboxylic acid with an amide moiety results in a decrease potency *in vitro*, but has demonstrated improved cell entry⁹¹.

A second set of compounds has been designed based on L-CA and dicaffeoyl derivatives^{89, 90, 92}. These molecules contain a dicaffeoyl group connected by a furan ring linker containing hydrogen bond accepting or donating groups (Table 4). They exhibit IC₅₀ values in the range of 0.5–12 μ M. Unfortunately, these compounds do not inhibit HIV-1 replication in MT-4 cells at non-toxic concentrations.

New Catechol Derivatives

The dihydroxybenzoic acid hydrazides family of IN inhibitors is also derived from a catechol structure (Table 5). These compounds are potent IN inhibitors, but do not display selectivity for 3'-P or ST. Inhibition is observed with both Mg^{2+} and Mn^{2+} cofactors. The hydrazide containing inhibitors exhibit high inhibitory potency only when Mn^{2+} is used⁸².

Dihydroxyisoindolone derivatives are more potent and selective for ST⁸² (Table 5). To improve potency and reduce toxicity, halo-substitutions of the aromatic ring were performed. The 3-chloro-4-fluoro-derivative is the most potent inhibitor with an IC_{50} for ST of 0.16 μM . It also exerts an 80-fold ST selectivity compared to 3'-P inhibition⁸¹. Antiviral activities have been found at submicromolar concentrations for these inhibitors, but with relatively high cytotoxicity suggesting that they may have relatively unfavorable therapeutic indexes. The dihydroxynaphthyl derivatives have variable selectivity for ST. Specifically, the dihydroxynaphthyl derivative with catechol-DKA hybrids (Table 5) is active against IN at low micromolar concentrations with a two- to three-fold decrease in selectivity for ST compared to DKA⁹³.

The styroquinolines (SQL) (Table 5) are IN inhibitors with specificity for 3'-P⁹⁴. SQL block viral replication at nontoxic concentrations⁹⁵ and inhibit 3'-P activity *in vitro* by competing with binding of the viral DNA substrate⁹⁶. Recently more potent SQL derivatives have been produced replacing the C-8 methoxy by a hydroxy group (Table 5)⁹⁷. Inhibition of 3'-P by styrylquinazoline was 6-fold increased compared to the initial SQL. The cellular target of SQLs was confirmed by the appearance of mutations in the IN gene when viruses were grown in the presence of increasing concentrations of SQLs⁹⁸. Finally, these mutations led to SQL-resistant viruses when introduced into the wild-type sequence.

Another catechol derivative, the dihydroxyindole dimeric with piperazine (Table 5) inhibits IN *in vitro* with an IC_{50} value at 7 μM and exhibits less toxicity than other catechols with a CC_{50} value above 20 μM ^{45, 92}.

Dinitrorosmarinic acid (Table 5) is a very potent IN inhibitor *in vitro* with a high selectivity for ST⁹⁹. The IC_{50} for 3'-P and ST are 52 μM and 70 nM, respectively. The mechanism most likely involves the chelation of the divalent cation. This molecule also inhibits viral replication in MT-4 cells with EC_{50} at 12 μM but its therapeutic index is only 3.8.

The coumarin caffeoyl derivative (Table 5) exhibits potent inhibitory activity at micromolar concentration *in vitro*¹⁰⁰. In cells, this molecule is less effective with an EC_{50} value of 31 μM . Interestingly, some coumarin derivatives can interact directly to IN *in vitro* in the catalytic core region between residues ¹²⁸AACWWAGIK¹³⁶⁴⁵. This peptide segment is located close to the dimer interface of IN. Thus, coumarin-containing molecules represent a new type of inhibitors that inhibit IN *in vitro* by shifting its oligomerization state.

Salicylhydrazide Derivatives

Salicylhydrazides (Table 6) were previously reported as an IN inhibitor. However, inhibition occurs *in vitro* only in the presence of manganese¹⁰¹, which, unlike magnesium is believed not to be the relevant divalent catalytic metal. Due to the high toxicity of the molecule (CC_{50} = 0.1 μM), the antiviral properties could not be determined. Two new derivatives (Table 6) inhibit IN in the presence of either manganese or magnesium¹⁰². These compounds displayed low micromolar activity against IN *in vitro* (range 3–13 μM) and are several hundred-fold less cytotoxic than the parent molecules (CC_{50} around 20–30 μM). Experiments performed with the integrase mutant C65S and molecular modeling studies suggested that mercaptosalicylhydrazide (MSH) forms a disulfide bond with C65 in the IN

active site¹⁰². This interaction has been proposed to facilitate positioning of MSH to allow divalent metal chelation.

Dual inhibitors of IN and RT

Some IN inhibitors such as V-165 (Table 7) have been described to have some activity against HIV-1 reverse transcriptase (RT)^{98, 103, 104}. This RT inhibition can be due in some cases to a specific inhibition of the RNase H domain of RT that shares structural similarities with IN¹⁰⁵. Even if it is commonly admitted that a good inhibitor must be highly specific and selective for a single target, it may be valuable to develop IN-RT dual inhibitors as an antiretroviral therapy for the treatment of HIV and AIDS.

Since 2004, several other dual inhibitors have been reported. Beta-thujaplicinol, a natural tropolone derivative, inhibit IN and RNase H probably by metal chelation^{106–108}. More recently, madurahydroxylactone and hydroxyisoquinoline dione derivatives (Table 7) have also been reported as dual inhibitors of IN and RNase H^{4, 109}. An interesting approach in the development of such dual inhibitors consists of rationally designing a chimeric drug based on the fusion of an RT-specific and an IN specific scaffold. The resulting “portmanteau compounds” (Table 7) exhibits nanomolar antiviral activity^{110–112}.

Peptide Inhibitors from screening

The first IN peptide inhibitor reported was the hexapeptide HCKFWW¹¹³. This peptide (Table 8), discovered by systematic screening of peptide libraries inhibits HIV IN 3'-P and ST with an IC₅₀ value of 2 µM. Dimerization of this peptide via a thioether or a dithiomethylene linker lowers the IC₅₀ values of both 3'-P and ST as much as 10–20-fold^{114, 115}.

Using the yeast 2-hybrid system, a 33 amino acids long peptide was isolated with an IC₅₀ of 9 µM for 3'-P and for ST¹¹⁶. The amino-terminal portion of the peptide, EBR28 (Table 8), shows increase activity with an IC₅₀ of 5 µM against 3'-P. This peptide forms an amphipathic alpha helix in solution and interacts with the catalytic domain to block DNA binding. EBR28 also inhibits HIV-1 infectivity of CD4⁺ HeLa cells with an IC₅₀ of 40 µM and apparently low toxicity.

Integramides A and B (Table 8) are non-ribosomal peptides isolated from *Dendrodochium* sp. with IC₅₀ values of 17 and 10 µM, respectively¹¹⁷. Antiviral activity could not be tested because these compound exhibited toxicity at IC₅₀ levels. Recently, a total chemical synthesis was performed and the synthetic product was comparable to the natural product¹¹⁸.

Indolicidin (Table 8) is an antimicrobial peptide isolated from the large granules of bovine neutrophils with virucidal properties against HIV-1¹¹⁹. The complete effect was obtained at 174 µM and the 50% inhibitory dose was between 39 and 58µM. *In vitro* activities are in the same range of concentration with IC₅₀ value of around 60 µM for both 3'-P and ST¹²⁰ and result from interference in the DNA-IN complex formation by direct binding of indolicidin to DNA¹²¹. Peptide toxicity is important with a CC₅₀ value around 20 µM; but by connecting monomers with a lysine linker, *in vitro* activities can be increased. The most potent derivative is a tetramer with an IC₅₀ value of 0.6 µM for both 3'-P and ST¹²⁰.

Using phage display, several small peptides were isolated including Pep1 (Table 8). This peptide is able to bind specifically to the C-terminus of IN and inhibits only the ST reaction with an IC₅₀ value of 70 µM¹²². The postulated mechanism was an inhibition of the pre-cleaved substrate binding.

Peptide nucleic acid (PNA) are DNA analog in which sugar-phosphate skeleton are replaced by peptidic group as [N-(2-amino-ethyl)-glycine]. The resulting molecules (Table 8) are not sensitive to nucleases but can still interact tightly with DNA in a Watson-Crick manner. Recently, a N-terminal lipid functionalized PNA, PNA4 (Table 8) was reported to be antiviral with inhibition of both RT and IN ¹²³. *In vitro*, this molecule inhibits both 3'-P and ST with an IC₅₀ of 22 μ M.

Peptide Inhibitors derived from IN

The continued development of peptide inhibitors of HIV-1 IN has provided biochemical data helping to further define the structure and dimerization properties of the enzyme. Several peptide inhibitors of HIV-1 IN have been derived from polypeptide helices found within the IN structure. These helices are highlighted in Fig. (3B), which shows a crystal structure of the core domain dimer of IN ³⁸. The inhibitory data for the polypeptide inhibitors are summarized in Table 9.

Alpha helices 1 and 5 form the dimerization interface between the core domains from different IN subunits. Synthetic peptides INH1 and INH5 derived from α 1 helix (amino acids 93–107) and α 5 helix/loop (amino acids 167–187), respectively, inhibit HIV-1 IN in the nanomolar range ¹²⁴. INH5 is the most active of these peptides with IC₅₀ values of 0.085 μ M against 3'-P and 0.060 μ M against ST *in vitro*. Fluorescence spectroscopy and size exclusion chromatography showed that INH5 binds the core domain in the full-length protein and induces the dissociation of IN oligomers.

Helix 4 of IN [amino acids 151–172, shown in green in Fig. (2B)] is an amphipathic alpha helix containing several amino acids shown to interact with the HIV-1 LTR 29–31 and nucleotides ⁴³ including G152, K156, and K159. A peptide inhibitor (K159) derived from the α 4 helix of IN was reported to inhibit HIV-1 IN *in vitro* with a relatively low potency (IC₅₀ of 600 μ M and complete inhibition at 1.2 mM) ¹²⁵. Circular dichroism and chemical cross-linking showed auto-association of K159. It has been suggested that the inhibitory activity of the peptide results from a coiled-coil interaction stabilized through a hydrophobic interface with the peptide and IN helix 4, blocking the catalytic residue E152 ¹²⁶. Mutagenesis of K159 leads to a new peptide, EAA26, with increased helical properties and enhanced potency against IN compared to K159 peptide ¹²⁷.

Subsequently, a monoclonal antibody against K159 (anti-K159) was developed and characterized ¹²⁸. The functional epitope was identified as amino acids 163–175, in a helical form important for DNA binding. Filter binding studies showed that interaction between anti-K159 and IN decreases DNA binding. The reported IC₅₀ for inhibition of DNA binding is approximately 0.6 μ M for anti-K159 and the IC₅₀ values for ST and 3'-P are respectively 25 nM and 16 nM.

Peptide Inhibitors derived from viral or cellular cofactors

Throughout the replication process in cells, viral DNA is part of a large nucleoprotein complex, which moves from the reverse transcription complex to the pre-integration complex (PIC) by gain and loss of viral and cellular factors. PICs have been difficult to fully resolve due to their potentially dynamic composition and to the fact that cofactors can vary depending on the purification protocol.

Using the yeast 2-hybrid system, IN was found to interact with Ini1 (IN interactor 1), also known as SNF5, and part of the SWI/SNF complex ^{129,130}. This interaction could be implicated in the targeting of the integration and so as a possible therapeutic target. Fragment S6 (Table 10) of Ini1 (aa 183–294) is the minimal interacting sequence with IN.

S6 when expressed in cells exhibits a trans-dominant effect and inhibits viral production and replication. S6 does not exhibit apparent toxicity and should not be immunogenic due to its host origin¹³¹. It appears that In1 is important for early steps of replication^{132, 133} and S6 inhibition is dependent on its oligomerization state¹³⁴.

Searching into cells with an IN-flag allowed the isolation of a large complex composed by a tetramer of IN bound to a 76 kDa nuclear protein previously known as Lens-Epithelium Derived Growth Factor (LEDGF/p75)³³. The IN binding domain (IBD) corresponds to amino acids 347–429 of LEDGF (Table 10) and is composed of 4 or 5 alpha helix¹³⁵. A deletion of LEDGF in cells by siRNA or knock down induces a three to five fold decrease of viral replication due to a lack of integration¹³⁶. On the other hand, when IBD is over-expressed in cells, HIV-1 infection is strongly inhibited after DNA translocation into the nucleus but before integration¹³⁷. The LEDGF-IN interaction is attractive as therapeutic target because disruption of the LEDGF-IN interaction induces a failure in viral replication. But LEDGF is a cellular factor, so side effects can be important when modifying its expression or activity. A number of small molecules have been reported for their capacity to specifically interfere with the LEDGF-IN interaction. CHIBA-3003 (2-hydroxy-4-(4-hydroxy-1H-indol-3-yl)-4-oxobut-2-enoic acid) inhibits the LEDGF-IN complex formation *in vitro* with an IC₅₀ of 35 µM¹³⁸.

Another way to disrupt an important interaction with IN without risk of side effect is to target a viral co-factor. To date, several proteins have been described in the PICs including MA, Vpr, or RT¹³⁹. By screening a complete library of RT-, Rev- and Vpr-derived peptides, several peptides were isolated with IN inhibitory activity (Table 10). Peptides 4286 and 4321 are derived from reverse transcriptase palm (166–185) and RNase H domain (514–528), respectively¹⁴⁰. Both 3'-P and ST are inhibited with IC₅₀ between 4.5 and 6.9 µM. Rev13–23 and Rev 53–67 inhibit both 3'-P and ST *in vitro* with an IC₅₀ of 25 µM and inhibit IN *ex vivo* at low micromolar concentrations¹⁴¹. In fact, Rev-derived peptides act as shiftide *in vitro* resulting in the tetramerization and *de facto* inhibition of 3'-P¹⁴². This mechanism of inhibition can also be reproduced with unrelated peptides like IN1 (WQCLTLTHRGFVLLTITVLR), which was isolated by yeast 2-hybrid and found to inhibit IN with IC₅₀ at 38 (3'-P) and 12 µM (ST)¹⁴³. C-terminal domain of Vpr is also able to bind and stimulate ST *in vitro*¹⁴⁴. Derived peptides can bind and/or inhibit both RT (polymerase and RNase H activities) and IN (3'-P and ST)¹⁴⁵. The most potent peptide, Vpr 61–75, exhibits IC₅₀ at single digit micromolar concentration for all these activities.

Antibody Inhibitors

A library of monoclonal antibodies against HIV-1 IN was developed¹⁴⁶. Characterization of these antibodies has provided information on their mechanism of inhibition of IN through interaction with protein domains, as well as insight into subunit arrangement of the enzyme. Four monoclonal antibodies (Table 11) have been characterized: mAb17 binds to the N-terminal domain, mAb4 binds to the catalytic domain, and mAb32 and mAb33 bind to the C-terminal domain of IN. Three of these antibodies inhibit IN *in vitro* (Table 11)¹⁴⁷. MAb17 and Fab17 inhibit 3'-P with IC₅₀ values of 0.4 and 4.5 µM, respectively¹⁴⁸. These antibodies bind the N-terminal domain and inhibits the enzyme possibly by interfering with protein-protein interactions or conformation, without disturbing dimerization or DNA binding.

Mab32 has a weak inhibition against IN 3'-P (IC₅₀ of 2 µM) and Fab32 lacks inhibitory activity. The most potent antibody, mAb33 and the corresponding isolated Fab33 fragment, bind to IN C-terminal domain to DNA (220–270) inhibiting DNA binding^{149, 150}. The IC₅₀ values for 3'-P are 0.18 and 1.8 µM for mAb33 and Fab33, respectively. An IN

conformational change that occurs upon metal binding is prevented by the addition of mAb33, and if the conformational change already occurred, antibody binding is blocked¹⁴⁷. Therefore, mAb33 may act by locking the enzyme in the catalytically inactive conformation prior to metal binding.

Recently, it was shown that IgG isolated from HIV-infected patients can act as abzyme and hydrolyse specifically IN¹⁵¹. The mechanism involved is similar to autoimmune proteolytic abzyme but the potential role of these immunoglobulins during infection is not known. Nevertheless, hydrolyzing antibodies can be useful for diagnosis purposes.

The potential for antibody-based therapeutics may be realized using a gene therapy approach. Intracellular expression of a recombinant antibody gene has been reported to inhibit effectively many HIV-1 proteins including reverse transcriptase, REV, IN, and CXCR4^{152–156}. Transfection of human peripheral blood monocytic cells (PBMCs) with retroviral vectors expressing the single chain variable (SFv) region of mAb33 (IN#33) dramatically reduces HIV-1 replication¹⁵⁴. Further studies showed reduced HIV-1 infection and reduced virion infectivity in T cells stably transfected with a Vpr-IN-SFv fusion construct¹⁵³. Greater than 95% of IN#33 transduced CD34⁺ progenitor T cells from rhesus macaque and human fetal bone marrow cells expressed the IN antibody, and high expression continued after transplantation into SCID mice¹⁵⁷. These or similar stably transduced genes may be useful in treating diseases residing in CD34⁺ cells. Recently, a reduced number of HIV-1 infected thymocytes in human thymic grafts was demonstrated in a mouse model, suggesting SV40-derived vectors may provide efficient *in vivo* therapeutic delivery of genes encoding IN antibodies¹⁵⁸.

Update on Oligonucleotide Inhibitors

Zintevir™ (AR177), a guanosine quartet (G4)-forming oligonucleotide^{159–161} was the first anti-IN drug tested in clinical trials by Aronex Pharmaceuticals. Mono- and di-nucleotides IN inhibitors were the focus of prior reviews^{5, 9, 18} and are not discussed further here. An oligonucleotide containing 6-oxocytosine has been reported to inhibit IN in the sub-micromolar range¹⁶². The best oligonucleotide inhibitor described contains the sequence 5'-AGAGATTTTC*C*, where C* indicates 6-oxocytosine substitution for cytosine (Table 12). This oligonucleotide inhibits IN with IC₅₀ values of 0.3 μM for both 3'-P and ST. The sequence is identical to the sequence beginning seven bases from the 5' end of the non-cleaved strand of the U5 LTR. Substitution of two cytosines with 6-oxocytosine residues results in a potent IN inhibitor even though the unmodified oligonucleotide has similar affinity for IN but fails to inhibit IN activity¹⁶³. Inhibition was dependent on sequence, presence of 6-oxocytosine, and positioning the 6-oxocytosine at the terminus of the oligonucleotide^{162, 163}. The 6-oxocytosine-containing oligonucleotide inhibits binding of IN to substrate DNA, when added either before or after binding of the substrate DNA¹⁶³.

Based on this same principle, small single stranded DNA harboring a 3'-acridine group are able to inhibit IN activities *in vitro* in the low micromolar concentrations¹⁶⁴. This GTGT-Acr dissociates the IN-DNA complex and the acridine modification seems to be the dissociating factor while the DNA sequence only acts a targeting factor.

Oligonucleotides as potential therapeutics have several limitations. Nucleic acids in a cellular context can be subject to degradation and chemical modifications unless they are modified as phosphorothioates or with other special structural modifications to confer resistance to nucleases. Guanosine quadruplexes (G-quartets) are especially stable and efficient inhibitors of HIV-1 IN *in vitro*¹⁵⁹. The G-quartet T30177 (Table 12) inhibits IN 3'-P and ST with IC₅₀ values of 0.079 and 0.049 μM, respectively¹⁵⁹. HIV-1 replication is also inhibited with an IC₅₀ of 0.075 μM¹⁶¹ and, as mentioned at the beginning of this

section, a derivative of T30177 was tested as the first IN inhibitor in clinical trials in 1996 as Zintevir™ by Aronex Pharmaceuticals. However, T30177 was also shown to inhibit gp120¹⁶⁵ and resistant viruses obtained during T30177 treatment exhibited mutations in the gp120 gene¹⁶⁶. Increasing the length and sequence of the G-quartet did not decrease inhibition efficiency and modeling suggested the G-quartet structure interacted with IN in a “face-to-face” orientation occurring between the G-quartet loops and the IN active site¹⁶⁰.

Oligonucleotides selected against and inhibitors of RNase H are also able to inhibit IN¹⁶⁷. 93del (Table 12) forms specific dimeric G-quartets¹⁶⁸ and inhibits recombinant HIV-1 IN with IC₅₀ values in the nanomolar range. Consistent with the formation of G4s, IN inhibition is significantly improved in the presence of potassium ion. This aptamer blocks HIV-1 replication in human P4 cells with EC₅₀ values of 20 nM. However this oligonucleotide does not inhibit cell fusion, suggesting that IN may be the primary target of this second generation G4 inhibitors. Selecting against the isolated RNase H domain, other G-rich oligonucleotides, including ODN 60del (Table 12), have been selected. They are not inhibitors of RNase H in the RT context but inhibit IN *in vitro*¹⁶⁹. The IC₅₀ of 60del against the 3'-P and ST is quite higher than those of 93del and other G-quartet but still remain around 100 nM for both reactions. According to the high specificity of aptamers and the stability of G-quadruplexes, it is reasonable to think that oligonucleotides can be a good alternative to treat HIV.

Intracellular penetration of the G-quartets such as T30177 or 93del has been considered a potential limitation. Nevertheless early studies with ³²P-radiolabeled T30177 demonstrated this not to be case¹⁶¹. Delivery of T40214 was achieved using a liposomal delivery system¹⁷⁰. T40214 enters the nuclei of 3T3, CEMSS and MT4 cells and decreases HIV-1 replication with an EC₅₀ of 0.2 μM, suggesting that IN can be targeted intracellularly by G-quartets. Inhibition of IN in cells has not yet been demonstrated. Recently, T30923 and 93del were used to study the cellular uptake¹⁷¹. It appears that both 93del and T30923 are able to enter different cell lines including lymphocyte cells at nanomolar concentrations. This entrance is enhanced using transfection agent and can be directed into a cell compartment depending on the cationic lipid used. In presence of HIV-1 particles, these G-rich forming quartet oligonucleotides exhibit also a very high entrance, comparable to a transfection experiment, which highlights a probable favoring role of the virus in the oligonucleotide uptake. Further experiments have to be done to discriminate the exact role of the virus; the mechanism is apparently gp120-independent as VSV-G pseudo-typed viruses induce the same pattern of oligonucleotide entry. Further studies are therefore warranted to elucidate whether other targets besides IN exist in HIV-infected cells.

Conclusions

The approval and remarkable activity and tolerance of raltegravir, and the clinical activity of elvitegravir have demonstrated that targeting IN can lead to effective therapies against AIDS and HIV infections. Judging from the other HIV targets, RT and protease, it is expected that many more drugs targeting IN are on their way to the clinic. The availability of the raltegravir-resistant IN mutants and viruses should enable the selection and development of drugs that will overcome such resistance. Thus, IN inhibitors are likely to have a major impact on AIDS and HIV infections. It is also possible that IN could be used as a target for other retroviral infections.

Abbreviations

PIC pre-integration complex

LTR	long terminal repeat
L-CA	L-chicoric acid
3'-P	3'-processing
ST	strand transfer

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Figure 1.

The mechanism of HIV integration. A: The 3'-P and ST reactions result in integration of the viral DNA ends into a host chromosome. B: *In vitro* assay to analyze IN catalysis and drug inhibition. Dinucleotide cleavage shortens the DNA substrate by two nucleotides (21mer to 19mer) during the 3'-P reaction. The subsequent ST reaction generates bands that migrate slower than the 19mer on a denaturing sequencing gel.

Figure 2.

Panels A, C and D are derived from the crystal structure of the IN core domain complexed with 5CITEP 41. The catalytic amino acids are shown in red, the magnesium ion is colored in magenta and the four coordinating water molecules are yellow. A: 5CITEP interactions within the HIV-1 IN active site. Amino acids with direct interactions with 5CITEP 41 are highlighted in green. The view angle is the same as in panel C. B: Crystal structure of the IN core domain dimer³⁸. Alpha helices targeted by peptide inhibitors are colored and labeled. Helices 1 (cyan) and 5 (magenta) form the dimerization interface between two IN monomers. Helix 4 (green) is proximal to the active site and includes the catalytic amino acid E152. The three catalytic residues, D64, D116, and E152 are shown in red. C and D: Illustration of the amino acids that are mutated in diketo acid resistant viruses. The side chains of the amino acids conferring resistance to DKA are highlighted in gold. Panel C is a view in the same orientation as in panel A. In panel D, the IN is rotated horizontally 90°.

Table 1

Approved anti-AIDS therapies

FDA Approval	Brand Name	Generic Name	Manufacturer
<i>Nucleosides Reverse Transcriptase Inhibitors (NRTIs)</i>			
1987	Retrovir	zidovudine, azidothymidine, AZT, ZDV	GlaxoSmithKline
1991	Videx	didanosine, dideoxyinosine, ddI	Bristol-Myers Squibb
1992	Hivid	zalcitabine, dideoxycytidine, ddC	Roche Pharmaceuticals
1994	Zerit	stavudine, d4T	Bristol-Myers Squibb
1995	Epivir	lamivudine, 3TC	GlaxoSmithKline
1997	Combivir	lamivudine + zidovudine	GlaxoSmithKline
1998	Ziagen	abacavir sulfate, ABC	GlaxoSmithKline
2000	Trizivir	abacavir+ lamivudine+ zidovudine	GlaxoSmithKline
2000	Videx EC	enteric coated didanosine, ddI EC	Bristol-Myers Squibb
2001	Viread	tenofovir disoproxil fumarate, TDF	Gilead Sciences
2003	Emtriva	emtricitabine, FTC	Gilead Sciences
2004	Epzicom	abacavir+ lamivudine	GlaxoSmithKline
2004	Truvada	emtricitabine + tenofovir disoproxil fumarate	Gilead Sciences
<i>Non-Nucleosides Reverse Transcriptase Inhibitors (NNRTIs)</i>			
1996	Viramune	nevirapine, NVP	Boehringer Ingelheim
1997	Rescriptor	delavirdine, DLV	Pfizer
1998	Sustiva	efavirenz, EFV	Bristol-Myers Squibb
2008	Intelence	etravirine	Tibotec Therapeutics
<i>Protease Inhibitors (PIs)</i>			
1995	Invirase	saquinavir mesylate, SQV	Roche Pharmaceuticals
1996	Norvir	ritonavir, RTV	Abbott Laboratories
1996	Crixivan	indinavir, IDV	Merck
1997	Viracept	nelfinavir mesylate, NFV	Pfizer
1997	Fortovase	saquinavir (no longer marketed)	Roche Pharmaceuticals
1999	Agenerase	amprenavir, APV	GlaxoSmithKline
2000	Kaletra	lopinavir+ ritonavir, LPV/RTV	Abbott Laboratories
2003	Reyataz	atazanavir sulfate, ATV	Bristol-Myers Squibb
2003	Lexiva	fosamprenavir calcium, FOS-APV	GlaxoSmithKline
2005	Aptivus	triplanavir, TPV	Boehringer Ingelheim
2006	Prezista	darunavir	Tibotec Therapeutics
<i>Fusion Inhibitors</i>			
2003	Fuzeon	Enfuvirtide, T-20	Roche Pharmaceuticals & Trimeris
<i>Entry Inhibitors</i>			
2007	Selzentry	maraviroc	Pfizer
<i>IN Inhibitors</i>			
2007	Isentress	raltegravir	Merck

From <http://www.fda.gov>

Table 2

The first approved and the most advanced IN inhibitors.

Name	Structure	IC ₅₀	EC ₅₀	EC ₉₅	CC ₅₀	Ref.
Raltegravir		87 10	8.8	33 31	ND	20, 28, 58, 61

Name	Structure	IC ₅₀	EC ₅₀	EC ₉₅	CC ₅₀	Ref.
Elvitegravir		7.2, 28	1.1 0.7 0.9 1.8	ND	4000	28, 60–62

Curr Top Med Chem. Author manuscript; available in PMC 2010 April 28.

ND means not determined or not disclosed. Values are expressed in nM.

Table 3

Diketo acid derivatives

Name	Structure	IC ₅₀	EC ₅₀	EC ₉₅	
5CTEP		2000	NA	NA	41
L-731,818		50	1000	ND	54
L-870,810		8	ND	15	73

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Name	Structure	IC ₅₀	EC ₅₀	EC ₉₅	
GS-9160		28	2.1 1.7 8.5	4000	78, 172, 173
STC-175		15	ND	ND	79

Name	Structure	IC ₅₀	EC ₅₀	EC ₉₅	
RDS1625		40 60	1170	ND	67, 80

NA means not antiviral, and ND means not determined or not disclosed. Values are expressed in nM.

Table 4

Caffeic acid derivatives and related catechols

Name	Structure
CAPE	<chem>Oc1cc(O)ccc1/C=C/C(=O)OCCc2ccc(O)cc2</chem>

Name	Structure
L-chicoric Acid (L-CA)	<chem>O=C(O)[C@H](OC(=O)/C=C/c1cc(O)cc(O)c1)OC(=O)/C=C/c1cc(O)cc(O)c1C(=O)O</chem>

Name	Structure
Caffeic acid derivative
Chicoric acid derivative

Name	Structure
Caffeoylamino acid derivative	<chem>Oc1ccc(cc1C/C=C/C(=O)N[C@@H](Cc2cc(O)c(O)cc2)C(=O)O)O</chem>

ND means not determined or not disclosed. Values are expressed in μM .

Table 5

New catechol derivatives

Name	Structure	IC ₅₀		EC ₅₀	Ref.
		3 ^o -P	ST		
Dihydroxybenzoic acid hydrazides <i>Curr Top Med Chem.</i> Author manuscript; available in PMC 2010 April 28.		6,9	1,6	1,7	82
Dihydroxyisoindolones		13	0,16	0,48	83

Name	Structure	IC ₅₀		EC ₅₀	Ref.
		3'-P	ST		
Dihydroxy naphthyl derivatives		1.9	0.9	46	93
Styrylquinazoline		21	ND	ND	97
Catechol Derivative		7	ND	ND	45

Name	Structure	IC ₅₀		EC ₅₀	Ref.
		3'-P	ST		
Dinitrosma trinic acid		52	0.07	12	99
Coumarin Caffeoyl derivative		1	ND	31	100

Curr Top Med Chem. Author manuscript; available in PMC 2010 April 28.

ND means not determined or not disclosed. Values are expressed in μM .

Table 6

Salicylhydrazide derivatives

Name	Structure
Salicylhydrazide
Salicylhydrazide

Name	Structure
Mercapto-salicylhydrazide	<p>The chemical structure shows two salicylhydrazide units linked by a disulfide bond. Each unit consists of a benzene ring with a hydrazide group (-CONHNH-) at the 1-position and a thiol group (-SH) at the 2-position. The two thiol groups have reacted to form a disulfide bridge (-S-S-). The hydrazide groups are oriented towards each other, with the text 'HN-NH' appearing above the top unit and below the bottom unit.</p>

ND means not determined or not disclosed. Values are expressed in μM .

Table 7

Dual inhibitors of HIV-1 RT and IN.

Name	Structure	IC ₅₀		EC	Ref.
		3'-P	ST		
V-165		0.9	16.1	8.9	103, 178

Name	Structure	IC ₅₀		EC	Ref.
		3'-P	ST		
β-thujaplicinol		117	22	ND	106-108

Name	Structure	IC ₅₀		EC	Ref.
		3'-P	ST		
MHL 2e		ND	4.06	ND	4
Compound 1		ND	1.24	22	109

Name	Portmanteau ⁸	Structure	IC ₅₀		EC	Ref.
			3'-P	ST
		ND	2.4	0.0033	110–112

Table 8

Peptide inhibitors from screening.

Peptide Name	Sequence	IC ₅₀	Ref.
	HCKFWW-NH ₂ ^b	2, 3'-P/ST	113
EBR28	YQLLRMIYKNI	5, 3'-P	116
Integramide A: R=H B: R=CH ₃		A=17 B= 10	117
Indolicidin	ILPWKWPWWPWRR	ND	119, 121, 179
pep1	FHNHGKQGGGS	70, ST	122
PNA4 B=thymine-1-yl R ¹ =CH ₃ (CH ₂) ₁₆ -		22, 3'-P/ST	180

PNA = Peptide Nucleic Acid. ND means not determined or not disclosed. Values are expressed in μM .

Table 9

Peptide inhibitors derived from IN.

Peptide Name	Sequence	IC ₅₀	Ref.
INH1	ATGQETAYFLKLAGKA-CONH ₂	250, 3'-P 150, ST	124
INH5	DQAEHLKTAVQMAVFIHNYKA-CONH ₂	0.085, 3'-P 0.060, ST	124
K159	SQGVVESMNKELKKIIGQVRDQAEHLKTAY	600, ST	125

Values are expressed in μM .

Table 10

Peptide inhibitors derived from other proteins.

Peptide Name	Sequence	IC ₅₀	Ref.
S6	Ini1 183–294	60, ST	181
IBD	LEDGF 355–377	ND	135
4286 (RT palm)	KILEPFRKQNPDIVIYQYMD	4.8, 3'-P 4.5, ST	140
4321 (RNaseH)	ELVNQIEQLIKKEKVYLAW	6.9, 3'-P 5, ST	140
Rev13–23	LKTVRLIKFLY	25	182
Rev53–67	RSISGWILSTYLGRP	25	182
Vpr 61–75	IRILQQLFIHFRIG	1.3, 3'-P 1, ST	145

Ini1 = IN interactor 1 ; IBD = IN Binding Domain ; LEDGF = Lens Epithelium-Derived Growth Factor. ND means not determined or not disclosed. Values are expressed in μM .

Table 11

Antibody inhibitors of HIV-1 IN.

Antibody	Target Domain	IC ₅₀ 3'-P	Ref.
mAb4	Catalytic core	ND	146
mab17	N-terminal	0.4	148
mAb32	C-terminal	2	149
mAb33	C-terminal	0.18	149, 150
IgG	ND	ND	151

ND means not determined or not disclosed. Values are expressed in μM .

Table 12

Oligonucleotide inhibitors.

Name	Sequence	IC ₅₀	EC ₅₀	Ref.
6-oxycytosine (*)	5'-AGAGATTTTC*C*-3'	500, ST	ND	163
GTGT-Acr (* acridine)	5'-GGTTTTTGTGT*-3'	2500, 3'-P 2000, ST	ND	164
6-oxycytosine (*)	5'-AGAGATTTTC*C*-3'	500, ST	ND	163
T30177 (*phosphorothioate)	5'-G*TGGTGGGTGGGTGGG*T-3'	79, 3'-P 49, ST	75	159
T30923	5'-GGGTGGGTGGGTGGGT-3'	75, 3'-P 80, ST	65	183
93del	5'-GGGTGGGAGGAGGGT-3'	42, 3'-P 166, ST	20	167
60del	5'-GGGGGGGCCAGGCCATGG-3'	≈100, 3'-P ≈100, ST	ND	169

ND means not determined or not disclosed. Values are expressed in nM.