REVIEW

RECENT PROGRESS IN THE DEVELOPMENT OF INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INTEGRASE FOR THE MANAGEMENT OF HIV INFECTION

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Summary. – Current therapy of acquired immune deficiency syndrome (AIDS) involves the use of a combination of at least three antiviral drugs to inhibit Human immunodeficiency virus 1 and 2 (HIV-1 and 2, in short HIV) replication via targeting of viral reverse transcriptase and protease. However, all anti-HIV drugs give rise to the new retroviral resistant strains. Therefore, new therapeutic agents against the emerging resistant HIV strains without secondary effects are very much needed. InHIV infection, the integration of viral DNA obtained from RNA genome into the chromosome of the host cell by viral integrase (IN) is essential for an effective viral replication. Moreover, no cellular IN has been found in the cells suggesting that viral IN appears to be one of the best candidates for the development of an antiviral drug. In recent years, promising results have proved that IN inhibitors are useful for treatment of retroviral infections. In this review, we briefly introduce IN and summarize potential inhibitors of IN, which are classified into several groups according to their origin and chemical structure. The resistance to the IN inhibitors is also discussed. Currently, several IN inhibitors are either being used in the clinical treatment or tested in clinical trials. Nevertheless, a great effort must be made to elicit wide knowledge with respect to the design of better viral inhibitors and the synthesis of new chemical derivatives with an anti-HIV activity.

Key words: human immunodeficiency viruses; integrase; antivirals

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Abbreviations: AIDS = acquired immune deficiency syndrome; CAPE = caffeic acid phenylethyl ester; CA-3-OH = 3' hydroxyl ends; HIV = Human immunodeficiency virus 1 and 2; IN = integrase; LTR = long terminal repeat; MAb(s) = monoclonal antibody(ies); PIC = preintegration complex; RNase H = ribonuclease H; ST = strand transfer

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1. Introduction

It is well known that disorders in patients with AIDS are caused by HIV-1 and 2 that have killed more than 25 million people since it was first identified in 1983. Ongoing infection affects more than 40 million people worldwide and infection rate continues to rise around the globe.

Current therapy of AIDS patients involves the use of combined therapy to inhibit replication of HIV via targeting of viral reverse transcriptase and protease. While combination therapy is highly effective at lowering viral loads and extending lives, problems with toxicity and especially drug resistance still pose threats to the effectiveness of these regimens. Indeed, almost 25% of new infections are found to be drug-resistant clearly demonstrating the need for new agents to combat HIV infection. IN is one of the most promising targets for interruption of the viral replication cycle through chemotherapy besides reverse transcriptase and protease.

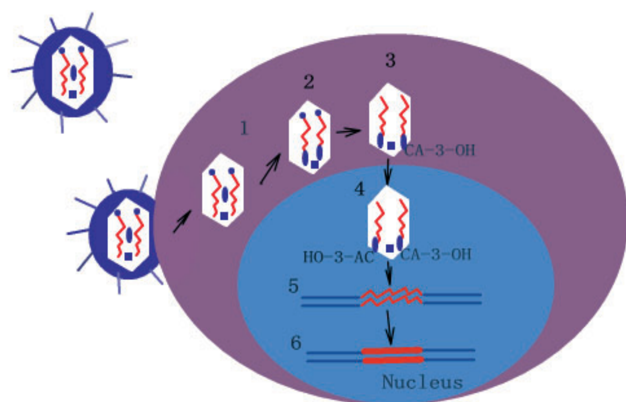

Fig. 1

Schematic representation of the HIV-1 integration process

The blue circle, ellipse and square in the uncoating virus represent reverse transcriptase, integrase, and protease, respectively. 1) Virus is released into cellular plasma. 2) IN binds to the specific sequences in LTR region of viral DNA, which results in a stable viral DNA-IN binding complex. PIC is formed that is responsible for reverse transcription of viral RNA to DNA, transportation to the nucleus, and integration of the viral DNA into the host cell genome. 3) IN removes a pGT dinucleotide at the end of the viral DNA LTRs producing the new CA-3-OH. 4) Viral DNA-IN complex moves into the nucleus. 5) IN binds to the chromosomal DNA and mediates a concerted nucleophilic attack by the 3' hydroxyl residues of the viral DNA on phosphodiester bridges in the target DNA. The processed CA-3-OH viral DNA ends are ligated to the 5'-O-phosphate ends of the target DNA irreversibly binding the viral DNA to the cellular DNA. 6) The viral DNA-cellular DNA combination is a gapped intermediate product. The gap repair is accomplished by the host cell DNA repair enzymes.

2. Integrase

IN is a 32 K protein encoded by the 3'-end of the HIV *pol* gene that processes proviral DNA in a step termed 3'-processing, which is followed by the strand transfer (ST) whereby IN inserts the processed proviral DNA into the host genome. Integration of viral DNA into the host cell chromosomal DNA to form a provirus is an essential step in the viral life cycle. IN is composed of one polypeptide chain that contains three distinct functional domains: (i) N-terminal domain (aa 1–50), which is believed to be involved in protein multimerization and contains a HHCC motif that coordinates zinc binding (Burke *et al*., 1992), (ii) dimeric catalytic core domain (aa 50–212), and (iii) C-terminal domain (aa 213–288) that is involved in the non-specific DNA binding (Puras Lutzke and Plasterk, 1998). Since IN is essential for the viral replication and has no counterpart in the host cell, the unique properties of IN make it an ideal target for the drug design. There are four unique processes that can be targeted and potentially inhibited: (1) assembly of a stable complex between IN and specific viral DNA sequences at the end of the HIV long terminal repeat (LTR) regions, (2) 3'-processing, (3) ST, and (4) DNA gap repair and ligation (Hazuda *et al*., 2000). HIV integration process starts from IN binding and ends at integration (Fig. 1). The detailed procedure for HIV integration is described below.

2.1. Integrase binds to viral DNA

After the viral reverse transcriptase enzyme creates a double-stranded DNA product, IN assembles at the ends of the viral DNA and binds to its LTR regions. The viral LTR regions contain specific DNA sequences that are required for recognition of IN resulting in a stable viral DNA-IN binding complex (Wolfe *et al*., 1996). This is a crucial initial step in the integration process.

2.2. First catalytic step – 3'-processing

After binding, IN removes a pGT dinucleotide at each end of the viral DNA LTRs adjacent to a highly conserved CA dinucleotide from the 3' extremity of the linear viral

Table 1. The potential candidates for IN inhibitors

Group	Name	Mode of action	Reference/producer
DKA derivatives	L-708,906	ST reaction	Merck
	L-731,988	3'-processing and ST	Merck
	Raltegravir, L-870,810, L870,812, and L-841,411	IN-DNA binding	Merck
	5-CITEP	IN-DNA binding	Shionogi
	Benzylindolyldiketo acids	ST reaction	Ferro *et al*., 2007
L-CA derivatives	L-CA and L-CA analogs	3'-processing and ST	Robinson *et al*., 1996; Reinke *et al*., 2002
	CAPE and CAPE-like compound	ST reaction	Fesen *et al*., 1993; Ho *et al*., 2005
PLP derivatives	PLP	IN-DNA binding	Williams *et al*., 2005
	Pyrazolopyridine	IN-DNA binding	Ambrilia Biopharma
Small-molecule natural product inhibitors	Catechol and bis-catechol derivatives	3'-processing and ST	Dupont *et al*., 2001
	Styrylquinolines and styrylquinazoline derivatives	3'-processing	Mekouar *et al*., 1998; Lee *et al*., 2002
	Salicylhydrazides and mercaptosalicylhydrazides	IN-DNA binding	Neamati *et al*., 1998; Neamati *et al*., 2002
Dual IN and RT inhibitors	Carbonyl J	IN and RT	Maurer *et al*., 2000
Dual IN and RNase H inhibitors	Madurahydroxylactone derivatives	IN and RNase H	Marchand *et al*., 2008
Novel peptide inhibitors	INH1	ST	Maroun *et al*., 2001
	Isocomplestatin	3'-processing and ST	Singh *et al*., 2001
	Chloropeptin I	3'-processing and ST	Singh *et al*., 2001
Antibody inhibitors	MAb33	IN-DNA binding	Yi *et al*., 2002
Nucleotide inhibitors	L-ddCMP, L-5FddCMP and L-5FddCTP	3'-processing and ST	Mazumder *et al*., 1996
	D-deoxynucleoside with L-isodeoxynucleoside dinucleotide	3'-processing and ST	Taktakishvili *et al*., 2000
	5'-AGAGATTTTC*C* (C* indicates 6-oxocytosine)	IN-DNA binding	Brodin *et al*., 2001
	ODNs 93 and 112	IN and RNase H	de Soultrait *et al*., 2002

genome producing the new 3' hydroxyl ends (CA-3-OH) (Puras Lutzke *et al*., 1994). This reaction takes place in the cytoplasm of the CD4+ T cell resulting in the formation of a preintegration complex (PIC).

2.3. Second catalytic step – strand transfer

Strand transfer is temporally and spatially separated from 3'-processing and occurs after PIC transport from the cytoplasm through a nuclear pore into the cell nucleus. After the PIC transport, the processed viral DNA-IN complex is ligated to the cellular DNA by IN.

2.4. Gap repair and ligation of viral DNA to cellular DNA

After the ST reaction, the new product (viral DNA-cellular DNA combination) is a gapped intermediate product in which the 5-phosphate ends of the viral DNA are not attached to the 3'-OH ends of the cellular DNA. The integration reaction is completed by the removal of the two unpaired nucleotides at the 5'-end of the viral DNA and by the repair of the single-stranded gaps created between the viral and target DNA (Yoder and Bushman, 2000). Although IN may be involved in these repair reactions, it is not essential, since the host cell has the machinery to carry out such processes.

3. Integrase inhibitors

After a decade of research, several different classes of HIV IN inhibitors have been reported. Merck has recently launched an FDA-approved compound Raltegravir (MK-0518) on the market and Gliead is trying to approve Eltagravir (GS9137) in clinical trials. Given the vast number of compounds, the reported inhibitors have been grouped into several classes, such as nucleotides and analogues, hydroxylated aromatic compounds, DNA-interacting agents, peptides, natural products, and antibodies according to their origin and chemical structure (Dayam *et al*., 2006; Johnson *et al*., 2004; Dayam and Neamati, 2003; Nair, 2003; Puras Lutzke *et al*., 1994). Table

1 shows the potential candidates for IN inhibitors discussed in this study. The following paragraph is meant to be a comprehensive compilation of all candidate compounds that could potentially serve as leads for future consideration.

3.1. Mechanism-based inhibitors

3.1.1. Integrase – DNA binding inhibitors

Pyrano-dipyrimidines have been shown to prevent viral DNA binding to integrase *in vitro* (Pannecouque *et al*., 2002). These compounds that block the replication of various strains of HIV in cell culture were classified as a second class of IN inhibitors. Given the unique mode of action of the pyrano-dipyrimidines, these agents may be potential candidates for further clinical trial (Fikkert *et al*., 2004; Valery *et al*., 2003; Pannecouque *et al*., 2002; Brin *et al*., 2000).

Other studies of several DNA binding agents (e.g., doxorubicin, ellipticine, ethidium bromide) that inhibit both the cleavage and ST steps of the IN mechanism have resulted in some interest in small-molecule, non-nucleotide inhibitors of this enzyme. It has been found that the activity of most of these compounds may be due to a non-specific interaction with the DNA binding domain of IN. These data suggest that IN inhibition is not simply dependent on DNA binding as some compounds with little ability to bind DNA, such as the heteroaromatic systems containing medium chain amines (antimalarial compounds, chloroquin, and primaquine) appear to be the valuable inhibitors of IN (Nair, 2003).

3.1.2. 3'-processing inhibitors

Styrylquinolines are another class of IN inhibitors described as potent 3'-processing inhibitors directly competing for HIV LTR long terminal repeats *in vitro*. Bonnefant *et al*. (2004) described these compounds as efficient IN inhibitors that acted both on 3'-processing and to a lesser extent on ST activities. The amount of late viral DNA is reduced in cells after the addition of these inhibitors. Furthermore, no accumulation of the circular HIV DNA was detected after styrylquinoline treatment indicating that they are most efficient at inhibiting steps prior to nuclear PIC translocation (Fig. 1) (Bonnenfant *et al*., 2004; Deprez *et al*., 2004; Zouhiri *et al*., 2000). This hypothesis is reinforced by the finding that styrylquinolines are selected for resistance mutations in the IN gene suggesting that they directly target IN (Bonnenfant *et al*., 2004). Mousnier *et al*. (2004) suggested that styrylquinolines probably inhibit the interaction between IN and a cellular factor required for the nuclear import of PIC. This may partially explain the weak effect that these agents retain on the ST process, because preventing nuclear import of PIC would at least indirectly prevent ST. However, styrylquinolines have a higher affinity for the binding to the viral DNA (Deprez *et al*., 2004). Further study offering better corroboration of the specific viral target in cell culture is needed.

3.1.3. Strand transfer inhibitors

ST inhibitors binds the IN portion of the viral IN-DNA complex. Although ST inhibitor is bound to the viral PIC, the 3'-processing step can still occur and the ST inhibitor-PIC complex (ST inhibitor-IN-viral DNA) enters the nucleus of the infected cell via PIC nuclear translocation. Once in the nucleus, ST inhibitor blocks the catalytic site of the IN enzyme and the ST inhibitor-IN-viral DNA complex cannot bind to the cellular DNA (Svarovskaia *et al*., 2004). Then, the viral DNA becomes a substrate for cellular repair enzymes that create the circular byproducts called 2-LTRs (Hazuda *et al*., 2000). Since the 2-LTRs are dead-ends in the HIV infectious process, the virus is irreversibly blocked by the ST inhibitor (Svarovskaia *et al*., 2004). It has been previously shown that 2-LTRs circular forms are increased in the presence of IN inhibitors, especially ST inhibitors (Svarovskaia *et al*., 2004; Fesen *et al*., 1993). Elvitegravir, a dihydroquinoline carboxylic acid that blocks the integration of HIV-1 cDNA through the inhibition of DNA ST, is currently being developed for the treatment of HIV-1 infection (Shimura *et al*., 2008).

3.2. New natural or synthetic inhibitors

3.2.1. 1,3-diketo acid derivatives

1,3-diketo acid derivatives inhibit the integration reaction *in vitro* with a strong specificity for the 3'-processing and ST (Grobler *et al*., 2002; Marchand *et al*., 2002). Two different binding sites for 1,3-diketo acid derivatives may coexist in the active site of the donor and the acceptor sites (Marchand *et al*. 2002). For example, compounds L-708,906 and L-731,988 (Merck) are reportedly able to differentiate between 3'-processing and ST (Hazuda *et al*., 2000). Compound 5-CITEP (Shionogi) binds to the active site of the IN making close contact with the protein (Goldgur *et al*., 1999). Compound L-708,906 is supposed to inhibit preferentially ST reaction and is bound within the acceptor site. On the other hand, 5-CITEP is less selective and seems to bind to the donor and acceptor site at the same time (Deprez *et al*., 2004). ST inhibitors as Raltegravir, compounds L-870,810, L-870,812, and L-841,411 (Merck) bind to IN within the synaptic complex, an early intermediate in the integration pathway, which blocks the docking onto the target DNA and prevents the formation of the ST complex (Pandey *et al*., 2007). A series of rationally designed benzylindolyldiketo acids acting as potent IN strand transfer inhibitors has recently been reported (Ferro *et al*., 2007).

3.2.2. L-chicoric acid derivatives

The flavone, caffeic acid phenylethyl ester (CAPE), was the first compound developed from L-chicoric acid and inhibits IN with some selectivity for ST. CAPE inhibits both the 3'-processing and ST (Fesen *et al*., 1993). Although L-chicoric acid inhibits integrase *in vitro,* its antiviral activity results primarily from inhibition of viral entry by interaction with gp120 (Pluymers *et al*., 2000). In contrast to the ST specificity of the natural product, CAPE, L-chicoric acid, and its analogs are equally inhibitory against 3'- processing and ST (Robinson *et al*., 1996; King *et al*., 1999). A series of derivatives was synthesized to improve cell entry by substituting an amide for the carboxylic acid group and reducing drug toxicity by catechol ring substitutions (Reinke *et al*., 2002). Four compounds exhibit increased specificity for IN compared with other L-chicoric acid analogs dicaffeoyl-L-lysine, dicaffeoyl-D, L-isoserine and digalloyltartaric acid. In contrast to L-chicoric acid, dicaffeoyl-D, L-isoserine inhibits viral replication. Additionally, quantitative real-time PCR employed to amplify 2-LTRs of HIV circle DNA has shown that these compounds have improved anti-IN activity in cell culture. L-chicoric acid analogs that can enter cells and show an increased activity against IN, may also have some clinical potential.

Different CAPE-like compounds have been identified for inhibition of HIV. Fesen *et al*. (1993) found that CAPE was a type of an IN inhibitor. The CAPE isothiocyanates were synthesized by appending an isothiocyanate group to several sites of the phenylethyl aryl ring of the CAPE (Goldgur *et al*., 1999). These isothiocyanates are reactive toward nucleophiles such as cysteine and lysine and the CAPE isothiocyanates are thought to react irreversibly with these amino acids on the active site of IN. Some of these compounds (Grobler *et al*., 2002; Pais *et al*., 2002; Rice and Baker, 2001; Yoder and Bushman, 2000) inhibit both the 3'-processing and ST reaction (Zhang *et al*., 2001). Although these compounds show better potency than CAPE, they are not able to inhibit irreversibly IN. This fact may be due to the structural incompatibility of the ligands with the active site of IN. These compounds cannot form a covalent bond with any of the active nucleophilic functional groups of IN. Our laboratory has also reported and patented the effect of CAPE-like compounds including CAPE, methyl caffeate, ethyl 3-(3,4-dihydroxyphenyl) acrylate, phenethyl dimethyl caffeate, and phenethyl 3-(4-bromophenyl) acrylic on mechanisms of HIV replication, cytokine modulation, and selective anti-cancer activity (Ho *et al*., 2005; Lee *et al*., 2000). Our results suggested that these compounds affected antiviral and cytokine-modulating responses via different mechanisms. Both the antiviral and cytokine-modulating mechanisms to these treatments need to be elaborated in further studies in order to derive the structural features of more effective compounds.

3.2.3. Pyridoxal-5'-phosphate derivatives

Pyridoxal-5'-phosphate, a ubiquitous enzymatic cofactor in many biochemical processes was reported as an inhibitor of IN with a unique mechanism of action (Williams *et al*., 2005). It was suggested that pyridoxal-5'-phosphate inhibits IN by binding to its C-terminal domain instead of its catalytic core domain. Pyrazolopyridine compounds binding to a site on IN similar to that for pyridoxal-5'-phosphate have recently been developed by Ambrilia Biopharma, Canada (Wu *et al*., 2007).

3.2.4. Small-molecule natural product inhibitors

Numerous IN inhibitors have been isolated from a variety of natural products. Marine, plant, and fungal extracts that exhibit an ample structural diversity have been reported for their anti-IN activity (Dayam and Neamati, 2003). Catechol derivatives were synthesized by modifying the groups attached to the aromatic ring with the hope that these compounds would be more potent and less toxic. It was found that the catechol moiety was not required for IN inhibition (Molteni *et al*., 2000). These drugs exhibit differential inhibition in the presence of manganese or magnesium, but the specific interactions with these metals are unknown. A recent study about the new rigid and semi-rigid catechol and bis-catechol derivatives has reported three principal inhibitory compounds (Dupont *et al*., 2001). This group of drugs inhibits both 3'-processing and ST with comparable potency (Maurer *et al*., 2000).

The styrylquinolines are a type of 3'-processing inhibitors reported several years ago as IN inhibitors with antiviral activity (Zouhiri *et al*., 2000; Mekouar *et al*., 1998). More potent styrylquinazoline derivatives were discovered recently (Lee *et al*., 2002) and their inhibition effect was six-fold higher than the inhibition of the initial styrylquindin (Mekouar *et al*., 1998).

Salicylhydrazides that inhibit integrase in a recombinant assay only in the presence of manganese are also IN inhibitors (Neamati *et al*., 1998). In contrast, mercapto-salicylhydrazides inhibit integrase in the presence of either manganese or magnesium. Experiments performed with an IN mutant and molecular modeling studies suggested that mercaptosalicylhydrazide forms a disulfide bond in integrase active site (Neamati *et al*., 2002). This interaction facilitates positioning of mercaptosalicylhydrazide to allow divalent metal chelation. Additionally, the mercaptosalicylhydrazides are 300 times less toxic than other known salicylhydrazides and exhibit antiviral activity (Puras Lutzke *et al*., 1995).

3.2.5. Dual integrase and reverse transcriptase inhibitors

A combination of structure-based computer modeling and combinatorial chemistry was used to make derivatives of a front reverse transcriptase inhibitor, carbonyl J (Maurer *et al*., 2000).

The structure and inhibition data for this parent compound, as well as for calcomine orange as the best derivative, show the potential to inhibit viral replication by blocking both reverse transcriptase and IN activities simultaneously.

3.2.6. Dual integrase and RNase H inhibitors

Madurahydroxylactone is a secondary metabolite produced by the soil bacterium *Nonomuria rubra* and belongs to the family of benzo[a]naphthacenequinones. A series of madurahydroxylactone derivatives exhibiting dual inhibitory effects for both IN and RNase H of HIV has been recently reported suggesting that distinct pharmaceuticals could be generated (Marchand *et al*., 2008).

3.2.7. Novel peptide inhibitors

Peptide-based inhibitors derived from the dimeric interface of the IN enzyme have been obtained through a combinatorial screening. The first IN peptide inhibitor reported was the hexapeptide HCKFWW (Puras Lutzke *et al*., 1995). Sourgen *et al*. (1996) have described two synthetic peptides containing aa 147–175 of the IN. The first one contained the same sequence and the second one a sequence with mutation L159 replaced by P. The inhibitory effect was tested on ST, 3'-processing, and autointegration activities *in vitro,* but was clearly positive only for ST.

Several peptide inhibitors of IN have been derived from polypeptide helices found within the IN structure. These helices show a crystal structure of the core domain dimer. Maroun *et al*. (2001) described two peptide inhibitors INH1 and INH5, both derived from the sequences of the α1 and α5 helices of the catalytic core that inhibited the ST activity of the enzyme. The INH5 peptide disturbed the association-dissociation equilibrium of the whole enzyme and catalytic core. Zhao *et al*. (2003) synthesized five interfacial peptides derived from the dimeric interface of IN. Three of these are inhibitors of enzyme 3'-endonuclease activity and are able to disrupt crosslinking of the IN dimer.

Indolicidin, a natural antimicrobial peptide, was found to have IN inhibitory activity. Further, improved activity has also been demonstrated for analogues of indolicidin with short hexapeptide sequences (Robinson *et al*., 1998). Krajewski *et al*. (2003) prepared dimers and tetramers by connecting the C-terminal ends of monomers using different linkers. The tetramers proved to be more potent than the dimeric peptides in terms of both 3'-processing and ST assay. These peptides seem to act as multimeric inhibitors that bind simultaneously to two or four neighboring catalytic sites within the IN oligomeric complex. The continued development of peptide inhibitors of IN has provided biochemical data helping to define further the structure and dimerization properties of the enzyme.

Several highly modified cyclic and noncyclic peptide-based natural products such as complestatins and integramides isolated from microbial extracts were shown to be as potent as 1,3-diketo acid inhibitors (Singh *et al*., 2002, 2001). Isocomplestatin and chloropeptin I (bicyclic hexapepetide) inhibit the 3'-processing and ST activities of IN (Singh *et al*., 2001). Moreover, an increase of approximately 10 folds has been observed for isocomplestatin and its derivatives in terms of selectivity towards IN 3'-processing activity over the ST activity (Singh *et al*., 2001). These findings suggest that these compounds may preferentially bind to the non-complexed enzyme.

3.2.8. Antibody inhibitors

A library of monoclonal antibodies (MAbs) have been prepared against IN. Characterization of these antibodies has provided information with respect to the mechanism of IN inhibition through interaction with protein domains, as well as an insight into the subunit arrangement of the enzyme. Several anti-IN MAbs have been characterized: MAb17 that binds to the N-terminal domain, MAb4 that binds to the catalytic domain and MAb32, MAb33 that bind to the C-terminal domain of IN. Most of these antibodies inhibit IN activity *in vitro* (Asante-Appiah and Skalka, 1997). MAb17 and the corresponding Fab17 fragment inhibit 3'-processing (Yi *et al*., 2000). This antibody binds the N-terminal domain and inhibits the enzyme possibly by interfering with protein-protein interaction or conformation without disturbing dimerization or DNA binding. The most potent antibody MAb33 and the corresponding Fab33 fragment inhibit binding of the C-terminal domain of IN to DNA (Yi *et al*., 2002).

3.2.9. Nucleotide inhibitors

Mononucleotide inhibitors - IN is inhibited at both the 3'-processing and DNA-integration stages by 3'-azido-3'-deoxythymidine mono-, di-, and tri-phosphates (Mazumder *et al*., 1994). The inhibition of an IN mutant containing only aa 50–212 sequence of the catalytic domain implies that these nucleotides bind to IN somewhere in this region. More active nucleotides for inhibition of integrase in the 3'-processing and ST activities are β-D-2',3'-dideoxycytidine monophosphate (L-ddCMP), β-L-5-fluoro-2',3'-dideoxycytidine monophosphate (L-5FddCMP), and β-L-5-fluoro-2',3'-dideoxycytidine triphosphate (L-5FddCTP) (Mazumder *et al*., 1996). The L-related isomeric dideoxynucleoside, (S, S)-isodideoxyadenosine monophosphate, (S, S-isoddA) is a weak inhibitor of IN (Nair *et al*., 1995). Isodideoxynucleotides with ring-extended bases are somewhat more active (Zhang *et al*., 1998).

Dinucleotide inhibitors – HIV DNA that undergoes tailoring or 3'-processing leaves recessed ends in the DNA that terminates in CA-3-OH. Residues immediately upstream

of the dinucleotide cleavage site may provide critical recognition/binding sites for IN. According to this theory, several researchers have investigated, designed, and studied natural dinucleotides and/or non-natural dinucleotides as potential IN inhibitors (Nair *et al*., 2000; Taktakishvili *et al*., 2000; Mazumder *et al*., 1997). Recently, it has been reported that linear dinucleotides with adenine and cytosine bases are IN inhibitors (Taktakishvili *et al*., 2000; Mazumder *et al*., 1997). A non-natural dinucleotide with a conformationally unusual internucleotide phosphodiester bond that joins a D-deoxynucleoside and an L-related isodeoxynucleoside also inhibits both the 3'-processing and ST steps (Taktakishvili *et al*., 2000). Moreover, it was suggested that changing the position of the surrogate nucleoside component of the dinucleotide can dramatically change the mode of inhibitory activity (Chi and Nair, 2005).

Oligonucleotide inhibitors – a number of oligonucleotides are known to inhibit IN. For example, an oligonucleotide with 17 nts in length that has only deoxyguanosine and thymidine components and contains single phosphorothioate internucleoside linkages at its 5'- and 3'-ends for stability, was found to be a potent inhibitor of IN (Ojwang *et al*., 1995). This oligonucleotide does not possess significant sequence homology and no complementary anti-sense sequence motifs with the HIV genome. Anti-HIV activity of this inhibitor has been reported in several infected cell lines (Ojwang *et al*., 1995).

The best oligonucleotide inhibitor described contains the sequence 5'-AGAGATTTTC*C*, where C* indicates 6-oxocytosine substitution for cytosine (Brodin *et al*., 2001). Its sequence is identical to initial seven bases from the 5'-end of the non-cleaved strand of the 5'-LTR. Substitution of two cytokines with 6-oxocytosine residues results in a potent IN inhibitor, even though the unmodified oligonucleotide has similar affinity for IN, it fails to inhibit enzyme activity (Brodin *et al*., 2002). This inhibition was dependent on sequence and presence of 6-oxocytosine positioned at the oligonucleotide terminus (Brodin *et al*., 2002, 2001). The 6-oxycytosine containing oligonucleotide inhibits binding of IN to substrate DNA, when added either before or after binding of the substrate DNA.

Two of the reported oligonucleotide inhibitors, DNA aptamers (ODN 93 and ODN 112), are also Rnase H inhibitors associated with HIV reverse transcriptase (de Soultrait *et al*., 2002). Shorter DNA aptamers derived from ODNs 93 and 112 (ODNs 93del and 112del) were able to inhibit integrase in the nanomolar range.

4. Integrase inhibitor resistance

All anti-HIV drugs give rise to the new retroviral resistant strains, because HIV displays wide genetic variability. The nucleic acid sequence of isolates from HIV can differ by as much as 20%. This variability arises because the HIV reverse transcriptase is highly error-prone and lacks the proofreading activities of other DNA polymerases. Mutations in the HIV genome that confer resistance to antiretroviral agents have been reported for all compounds used to treat patients infected with the virus. Thus, the same individual patient may carry a mixture of genetically distinct but related viral strains. Resistance to the IN inhibitors arises from the selection of viral variants with genetic mutations that change the target enzyme by creating conformational changes in the IN catalytic site or by affecting a metal ion coordination (Barreca *et al*., 2003; Zouhiri *et al*., 2000). The balance between the different strains may evolve over time in the same individual according to a defined "pressure". This leads to following consequences: i) emergence of drug-resistant viral strains leading to failure of the treatment used to halt AIDS progression in treated individuals; ii) one or more mutants may develop the capability for evading the immune response leading to the disease progression and death in nearly every infected individual.

Similar to protease inhibitor resistance, the number of IN mutations also confers an additive effect on IN inhibitor resistance (Fikkert *et al*., 2004; Valery *et al*., 2003). Amino acid substitutions T66I and E92Q in the integrase molecule contributing mainly to elvitegravir resistance have recently been reported (Shimura *et al*., 2008). The replication capacity of elvitegravir-resistant variants was significantly reduced relative to both wild-type virus and other IN inhibitor-resistant variants selected by compound L-870,810. Zolopa *et al*. (2007) documented that E92Q mutation was principally associated with resistance to elvitegravir in concert with other IN mutations. However, both Q148 and N155 mutational pathways can also confer resistance to this drug. Grinsztejn *et al*. (2007) reported that single mutations at positions N155 and Q148 confer only low-level resistance to raltegravir and do not seem to coexist within the same virus. However, the addition of other mutations in each of these pathways can act upon simultaneous restoration of the viral replicative capacity and drug resistance. The possibility that raltegravir and elvitegravir may share overlapping mutational pathways and potential cross-resistance between these two agents cannot be excluded.

Several approaches offer a possibility to overcome the emergence of virus-drug resistance: (i) switching from one class of the inhibitors to another, since different inhibitors do not necessarily lead to a cross-resistance; (ii) combining of the various compounds showing differences with respect to their mutation-resistance profile, particularly when these mutations appear to counteract each other; (iii) starting with sufficiently high drug concentrations to eliminate the virus completely and prevent breakthrough of any other variants irrespective of resistance; (iv) combining approaches (ii) and (iii) (De Clercq, 1996).

5. Current and future developments

Remarkable progress has been made since IN was identified as a rational therapeutic target for the treatment of HIV infection. In this review we have discussed different inhibitors of IN, the essential HIV enzyme whose inactivation should be of great significance in the fight against the HIV pandemic. Many compounds including DNA-binding agents, topoisomerase inhibitors, and aurintricarboxylic acid reportedly inhibit integrase in the biochemical assay (Cushman *et al*., 1995; Fesen *et al*., 1993). However, these compounds possess few or no activities in tissue culture and have no selectivity in terms of their mechanisms. These results suggest either that these compounds do not selectively eliminate activation of the IN or inhibit IN from entering the cells. Particularly, although some compounds exhibit the IN inhibitory activity in enzymatic assays, they have not yet been demonstrated to possess any anti-HIV activity *in vivo* (Fesen *et al*., 1993). Likewise, actinomycin D and baicalein inhibit IN activity *in vitro*, but their anti-HIV activity *in vivo* has not been reported (Robinson, 1998). Nevertheless, only a portion of the molecules with selective anti-HIV activity in preclinical or clinical trials have proved to be acceptable for development. The development of the compound S-1360 by Shionogi was halted, probably due to its metabolic instability. Similarly, a research on the compound L-870,810 by Merck has been stopped, because of unacceptable liver and kidney toxicity in dogs, even though both molecules showed promising antiviral activity (Cotelle, 2006). A much greater effort is needed for the therapeutic development of the few known classes of IN inhibitors that may be of clinical potential. A research effort must also continue in the area of invention of the new classes of compounds that are specific inhibitors of this enzyme.

In conclusion, in the absence of an efficient vaccine against HIV, new strategies must be explored to control the level of infection by this pathogen. Several drugs are either being used in the clinical treatment of AIDS patients or are in the process of being tested in clinical trials. Nevertheless, great efforts must be made to elicit more information with respect to the structural features important to the design of better viral inhibitors and to synthesize new chemical derivatives with the antiviral activity (Savarino, 2007). Moreover, the success of long-term anti-HIV therapy depends not only on the suppression of viral replication, but also on the partial restoration of immune functions. Immune competence prevents the onset of HIV-related opportunistic diseases and may also help antivirals to control the viral replication. Both the anti-viral and immuno-modulating mechanisms of AIDS treatments and the responses to them need to be elaborated through further studies to achieve the best outcome. Finally, in order to overcome the unfolding global AIDS pandemic, efforts should focus not only on the search for new drugs, but also on the discovery of new retroviral (and eventually cellular) targets.

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