HIV-1 Integrase: From Biology to Chemotherapeutics

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Abstract: AIDS has claimed the lives of 25 million people worldwide, an additional 40 million people are HIV-infected and new cases are being diagnosed every year. Despite the fact that HAART has moved AIDS from the category of terminal diseases to that of treatable chronic illnesses, its long-term therapeutic success may be compromised by the development of resistance to the currently used drugs. Despite the availability of RT, PR and fusion inhibitors, the development of further drugs such as inhibitors that target the third enzyme IN is essential for the clinical management of HIV-infected patients. The absence of cellular homolgues to IN and the unique nature of the reactions catalyzed by IN, make it an ideal target for drug design. Considerable progress towards designing HIV-1 IN inhibitors has been made over the last years and several lead compounds have been identified, synthesized and clinically studied. This review focuses on the existing knowledge of the biology of HIV-1 IN with emphasis on the mechanism of integration, structure and function and the technologies for measuring IN activity. This is followed by the current trends on designing HIV-1 IN inhibitors with the aid of molecular informatics and a review on the main classes of HIV-1 IN inhibitors reported this far with special emphasis on the clinical candidates.

Keywords: HIV-1 integrase, computer-aided drug design, inhibitors.

INTRODUCTION

At the time AIDS was diagnosed, in the early 1980s, it claimed the lives of infected patients within two years. To date, AIDS has killed more than 20 million people worldwide and an additional 40 million people are HIV-infected [92]. In 1983, the responsible virus, HIV, was identified and isolated from infected patients [103, 164, 204, 241] and this was followed by the development of an antibody test for the detection of this virus [238]. Shortly after, the first FDA approved drug for the treatment of HIV-1 became available to patients [86]. Initially, HIV patients were subjected to monotherapy with the RT inhibitors. However, due to the rapid emergence of drug resistant viruses this method soon proved inadequate. The addition of PR inhibitors in the mid 90s considerably enhanced viral suppression. Subsequent studies demonstrated the effectiveness of combining drugs from different classes and this combination therapy named, HAART significantly improved the quality of life and lifespan of HIV infected patients [202, 296]. Currently, there are 19 FDA approved drugs for the treatment of AIDS. Those belong to the class of nucleoside RT, non-nucleoside RT, PR and fusion inhibitors [60, 61, 201, 228]. The most successful treatment for the HIV virus includes a combination of three or more drugs from at least two different classes of inhibitors. Despite the fact that HAART has moved AIDS from the category of terminal diseases to that of treatable chronic illnesses, its long-term therapeutic success may be compromised by the persistence of viral reservoirs, the development of resistance to the currently used drugs, patient adherence and toxicity. Therefore, the development of novel antivirals that target new modes of inhibition is of paramount importance and has been the focus of recent research efforts.

VIRAL RESERVOIRS

The initial optimism that the HIV virus will be fully eradicated in patients receiving HAART vanished after a study by Chun *et al.* [46] provided evidence for the persistence of replication-competent virus in latently infected cells of patients receiving HAART. Incomplete inhibition of reverse transcription leads to the accumulation of either reverse transcribed viral cDNA or integrated provirus, which constitute the viral reservoirs. Cellular reservoirs have been identified in the well-studied $CD4^+$T cells, and the less-studied antigen-presenting cells such as macrophages and DCs. The longevity of the provirus depends on the activity of the cell that it resides in therefore, a provirus in quiescent cells can exist for a prolonged period in a latent state. Replication-competent virus was recovered from resting $CD4^+$T cells of patients that were on successful HAART treatment for a period of time ranging from 10 days to 9 years [47, 49, 99, 263, 304]. Initial studies indicated that viral reservoirs in latent cells are replication incompetent in the absence of an activating stimulus, while other studies demonstrated low levels of ongoing viral replication. As a result of the ongoing replication the longevity of the viral reservoir is prolonged by a continuous reinfection of the latent cells [244, 320]. Continual replenishment of the viral reservoirs in infected patients was demonstrated in a study by Chun *et al.* through the cross infection between latent and activated $CD4^+$T cells [49].

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HIV infected patients that discontinue the HAART treatment experience a sudden increase of their viral load, due to the persistence of viral reservoirs [48]. Adding an agent from a different class of drugs to which a patient has been exposed, may lead to a more successful therapy and may be necessary to completely eradicate the ongoing viral replication [156]. Therefore, other steps of the HIV life cycle have been considered as potential targets for HIV inhibition.

LIFE CYCLE OF HIV

The structure of the HIV virus particle includes a viral capsid surrounded by the matrix and the virion envelope. The viral capsid contains the major capsid protein p24, the nucleocapsid protein p7/p9, the single stranded RNA genome and the enzymes RT, IN and PR. The matrix protein p17 is located underneath the virion envelope and surrounds the viral capsid. The viral envelope consists of a lipid bilayer membrane and an envelope precursor protein gp160. Cellular protease cleaves gp160 to the outer membrane protein gp120 and the transmembrane protein gp41 both of which are connected noncovalently. The viral proteins are encoded by different genes, proteins p17, p24 and p7/p9 are encoded by the *gag* gene and the enzymes RT, IN and PR by the *pol* gene. The function of RT is to transcribe the viral RNA into double stranded DNA and its low fidelity rate in combination with the high turnover rate of virus production *in vivo* leads to the genetic diversity of the HIV virus. IN incorporates the viral DNA into the host chromosome and PR generates the individual viral proteins by proteolytic processing of their precursors. A series of accessory proteins are also part of the HIV virions [7, 110].

The initial step in the HIV infection is the attachment of the virus particle to a membrane receptor molecule of the human cell. This interaction occurs between the viral glycoprotein gp120 and the CD4 receptor, a membrane glycoprotein of human T-lymphocytes. Binding of gp120 to CD4 induces a conformational change in gp120 unravelling a binding site for chemokine coreceptors usually identified as CXCR4 and CCR5 [65, 69, 281]. Binding of the coreceptor induces a conformational change to the viral glycoprotein gp41. As a result the two membranes move towards each other and fusion takes place [154, 250, 305]. Upon entry into the cell cytoplasm the viral capsid is uncoated releasing the viral RNA and the viral enzymes RT, IN and PR. Reverse transcription converts the single-stranded viral RNA into the double stranded cDNA. Integration of the viral DNA to the host chromosome is catalyzed by IN within the context of a higher order PIC. Integration takes place within the nucleus leading to the provirus that upon triggering, is transcribed into RNA by host cell machinery and facilitated in part by the regulatory viral proteins Tat and Rev [10, 157, 186, 196]. Translation of the viral RNA occurs outside the cell's nucleus and produces a genomic full-length RNA and propeptides that are transformed into mature viral proteins by the action of PR [100, 143]. The viral proteins and RNA assemble at the cell membrane and bud off to infect new cells.

Each step of the HIV life cycle can serve as a potential target for inhibition. RT and PR were the initial targets for inhibition and subsequently, viral entry and integration received considerable interest from the scientific community [115, 225]. IN makes an attractive target for inhibition because it is essential for viral replication. Furthermore, no known human homologue of IN exists which makes possible the development of selective inhibitors with low toxicity. IN has been the subject of several recent reviews that mainly dealt with the identification of inhibitors [4, 135, 182, 240, 302, 303]. This review focuses on the existing knowledge of the biology of HIV-1 IN with emphasis on the mechanism of integration, structure and function, and the technologies for measuring IN activity, followed by the current trends on designing IN inhibitors with the aid of molecular informatics and a review on the main classes of HIV-1 IN inhibitors reported this far with emphasis on compounds in clinical trials.

THE MECHANISM OF DNA INTEGRATION

IN is a 32-KDa protein encoded by the 3' end of the *pol* gene. It is first translated as a large component of polyprotein *gag-pol* from which it is released by the action of protease during maturation. IN catalyzes the insertion of viral DNA into the host genome. This process occurs at two distinct steps, 3'-processing and strand transfer [25, 76, 137, 140, 261].

Reverse transcription occurs in the cytoplasm and single stranded RNA is converted into the double stranded DNA that contains a conserved four nucleotide CAGT sequence at the viral ends. The viral DNA becomes part of a larger nucleoprotein structure known as the PIC. IN within the context of PIC recognizes the CAGT sequence at the viral DNA ends, forms a stable complex with the viral DNA and then catalyzes the cleavage of the conserved GT nucleotides from each 3'-end of the viral DNA, termed 3'-processing. This reaction occurs by the one-step hydrolysis of the phosphodiester bond at the 3'-CA dinucleotide. Divalent metal ions and a nucleophile are necessary for this step to take place [260]. *In vitro* both Mg^{2+} and Mn^{2+} can perform this function while *in vivo* Mg^{2+} is believed to be the cation of choice [6, 53, 233, 300]. The divalent cation activates water molecules that act as the necessary nucleophile even though small alcohols, certain aminoacids and the 3'-viral end can perform this function as well [35, 135, 293]. The activated OH group attacks the phosphodiester bond in the viral DNA at the conserved adenosine and the result of this reaction is the formation of 3'-CA-OH ends and the terminal 5'-GT-3' dinucelotides. It has been proposed that the final stages of reverse transcription and the initial steps of integration occur simultaneously [81, 280, 308] and a specific interaction has been shown to occur between RT and IN [120, 319].

Following 3'-processing, PIC is imported into the nucleus of the cell where strand transfer takes place [22, 23]. During this step IN catalyzes the ligation of the viral 3'-OH ends into the host chromose. The newly formed 3'-OH groups act as the nucleophiles by attacking the phosphodiester bonds of both strands of the target DNA at a distance of five base pairs on each strand [14]. Completion of the strand transfer step leads to a two base overhang from the 5'-end of viral DNA and a five-base single stranded gap. These gaps are likely repaired by the action of host cell DNA repair enzymes [2, 57, 310]. Studies have also implicated RT and IN in gap repair [15]. Both 3'-processing and strand transfer are one-step transesterification reactions that proceed through an S_N2 mechanism [11]. A proposed transition state involves an arrangement with a trigonal bipyramid where the nucleophile

and leaving group occupy the apical position [107]. Both reactions are sequence specific and require the 5'-CAGT-3' sequence at the end of each viral LTR. Substitution of any of these 4 dinucleotides has dramatic effects on the activity. Residues distal to the LTR end were also identified to be important for integration [84]. Preferential integration has been observed at sites of severe DNA distortion, indicating a need for a wide major groove or for distortion of the target DNA during the strand transfer step [242]. In addition, active genes and regional hotspots have been identified as favored integration sites [259]. Cellular cofactors, such as INI1, have been implicated in directing proviral DNA to favored integration sites [139].

Whereas *in vivo* integration takes place within the PIC, *in vitro* purified IN and DNA are sufficient to catalyze both 3'-processing and strand transfer however, only single LTR ends are integrated [284]. IN catalyzes another reaction *in vitro* that is the opposite of strand transfer, called disintegration. During this step a substrate that mimics the complex of the viral DNA linked to the target DNA is cleaved into its individual parts [44]. Another reaction catalyzed by retroviral INs, *in vitro*, is the non specific alcoholysis of nonviral DNA sequences [142].

HOST FACTORS OF INTEGRATION

Integration in the infected cell is a more complex process that requires the recruitment of the PIC [87]. PIC is composed of the viral cDNA, viral and cellular proteins. The main viral proteins include the matrix protein p17, the capsid protein p24, the nucleocapsid protein p7/p9, the enzymes RT, IN and the accessory protein Vpr [23, 88, 121, 200]. IN, Vpr and the matrix protein p17 have been implicated in nuclear import and the nucleocapsid protein in enhancing catalytic activity [102, 121, 132, 230]. The exact mechanism of integration has not been established so far [234] and studies support the idea that host factors are required to accomplish integration of the viral DNA into the human genome *in vivo* [148, 282, 287].

BAF, a single polypeptide that consists of 89 aminoacids was first identified as a host factor of MoMLV [161] and later as part of the PIC complex of HIV [166]. BAF prevented autointegration in the MoMLV virus and a similar role has been proposed for the HIV-1 virus. High-salt stripping and functional reconstitution experiments revealed that low concentrations of BAF can restore the integration activity of salt-disrupted HIV-1 PICs [33]. BAF however, has not been validated as a host factor of IN *in vivo*.

HMG-I(Y) was identified in PICs isolated from HIV-1 infected cells. *In vitro* depletion of HMG-I(Y) from PICs diminished activity that was restored upon back-implementing the protein [90]. Mechanistic studies indicated that an interaction between HMG-I(Y) and cDNA promotes the formation of active IN-DNA complexes, presumably by bringing together DNA segments. This observation is consistent with the fact that no direct interaction between HMG-I(Y) and IN was detected [125, 165]. Recent studies argue that HMG-I(Y) has an important role during HIV transcription [122].

INI1, identified in a yeast two hybrid screen, is the first protein shown to directly interact with HIV-1 IN and stimulate its *in vitro* activity. INI1 is composed of 385 amino acids and consists of three highly conserved domains. These include two imperfect repeats, Rpt1 and Rpt2, a C-terminal domain and a homology region-3. INI1 interacts specifically with HIV-1 IN through the Rpt1 region but does not interact with the INs of other retroviruses. A fraction of the Rpt1 region named S6, residues 183-294, inhibits HIV-1 particle production and replication through the direct interaction of S6 to HIV-1 IN within the *gag-pol* context [311]. On the contrary, S6 did not inhibit particle production of other retroviruses which, indicates specific incorporation into HIV-1 virions at an approximate ratio of 1 molecule of INI1 per 2 molecules of IN [312]. The role of full-length INI1 in HIV-1 replication is not yet clarified and the lack of strong experimental evidence makes it difficult to assign a function for INI1 during HIV-1 replication *in vivo* [287].

LEDGF/p75 is the most recently identified cellular partner of HIV-1 IN. The protein contains 530 amino acids and consists of two domains, the N- and C-terminus. The N-terminal domain contains a PWWP motif, residues 1-92, believed to mediate protein-protein interactions and a NLS domain, residues 148-156. The IBD was mapped to residues 347-429 within the C-terminal domain [40]. The solution structure of IBD determined by NMR spectroscopy, is composed of five α-helices. Helices α1, α2 are connected to helices α4 and α5 through the shorter α3 helix and the IN binding residues are located in the hairpin loop regions of the IBD [42]. A crystal structure of the CCD of HIV-1 IN with LEDGF/p75 has been recently reported (Fig. **2**). The CCD forms a dimer and two LEDGF/p75 molecules interact with the dimer interface [41]. In infected cells the simplest model proposes a complex that contains a pair of IN tetramers in association with two subunits of LEDGF/p75 [39]. Even though the CCD is the main domain of interaction with LEDGF/p75 experiments with IN deletion mutants demonstrated that the NTD is also involved and enhances the activity of the interaction [177]. Functions attributed to LEDGF/p75 are to direct IN to the chromosomes of the host cell and thus, influence its nuclear retention [77, 170] and to determine the stability of IN in cells [169]. The *in vivo* role of LEDGF/p75 in viral replication was investigated by knockdown of LEDGF/p75 in cells leading to a reduction in HIV replication. Activity to nearly wild-type levels was restored upon back-complementing the cells with the protein [290]. Furthermore, depletion of LEDGF/p75 resulted in a reduction of HIV integration in transcription units and in genes regulated by LEDGF/p75 and was favored in target DNA with higher GC content [50]. Thus, LEDGF/p75 directs the location of HIV integration in cells. Overexpression of the C-terminal domain of LEDGF/p75, that contains the IBD, led to inhibition of HIV viral replication [249]. These results demonstrate that LEDGF/p75 interaction with IN can pose as a novel target for inhibition.

The human polycomb group EED protein is another host factor of both MA and HIV-1 IN and may have a functional role at the early steps of infection. EED contains two discreet binding domains located at its N-terminal domain that bind at the CTD of IN [295]. Another candidate co-factor, the yeast cellular protein HSP60 interacts with HIV-1 IN *in vitro* and stimulates processing and joining activities [232]. Recently, an interaction between hRad 18, a component of the DNA postreplication/translation pathway and IN has been

demonstrated [208]. Viral IN bears an N-terminal phenylalanine that is conserved within the subtypes of HIV-1. N-terminal phenylalanine is part of a degradation signal that is recognized by a system known as the N-end rule pathway. According to the nature of the N-terminal residue of a protein the N-end rule can define the *in vivo* half-life of the protein. Mutational studies of the N-terminal phenylalanine of HIV-1 IN identified IN as a short-lived protein and a substrate of the N-end rule pathway [207]. It has been demonstrated that hRad 18 protects the otherwise unstable HIV-1 IN from fast degradation [208].

TECHNOLOGIES FOR MEASURING HIV INTEGRASE ACTIVITY

The identification of HIV-1 IN inhibitors has been accomplished by a variety of biochemical-based assays that either employ recombinant enzyme screens to target 3'-processing, strand transfer and disintegration or measure activity within the context of the PIC [301-303]. The recombinant enzyme assays include purified IN expressed in *E. coli*, a synthetic DNA oligonucleotide that contains the terminal sequences of viral DNA, target DNA and a divalent metal ion. Single LTR ends are integrated and the products are distinguished from the original oligonucleotide by their migration times [54]. The reaction of 3'-processing is measured by the removal of the two nucleotides from the viral DNA ends, and the strand transfer step is measured by the formation of longer DNA strands. The association of IN with DNA can be detected by FCS technology during which IN binds to fluorescently labeled oligonucleotides and the complexes are detected during FCS measurements. Inhibition of the IN-DNA complex formation by G-quartets was effectively measured using this technique [291]. A variation of this method allowed the kinetic analysis of the 3'-processing step and the evaluation of inhibitors [302]. Assays specific for the strand transfer step measure reactions subsequent to IN-oligonucleotide assembly, which are initiated by the addition of a target DNA. An assay specific for the strand transfer activity designed by Hazuda *et al.* measures the integration of biotin labeled target DNA into model donor DNA immobilized on microtiter plates [116]. Alternative microtiter plate assays employ model DNA substrates that contain biotin and digoxigenin in each strand in order to facilitate detection of the product. These microtiter plate assays have been employed for the *in vitro* high throughput screening of antiviral drugs [127, 134]. An alternate assay employs a crossbones substrate in which two viral DNA ends are joined to a target DNA at a distance of five base pairs. This substrate mimics the structure of the integration intermediate. The assay requires two half-crossbones to be brought together and measures the ability of IN to juxtapose two viral DNA ends [45]. Modified oligonucleotides that contain an abasic site were also employed as a probe to study DNA-IN interactions. These oligonucleotides are covalently joined with IN through the formation of a Schiff base between the aldehydic abasic site and an amino group from a lysine residue in HIV-1 IN. The Schiff base is detected after reduction with sodium borohydride [192]. In a disintegration assay a Y-shaped oligonucleotide mimics the integration intermediate that is cleaved into two fragments of viral and target DNA by the action of IN [44, 212, 253]. This assay has been employed to identify an inhibitor that interacts with the catalytic site at the CCD of IN. An assay for measuring the gap repair step *in vivo* has been developed and applied for the study of the reaction kinetics for MoMLV. This assay could prove important for the determination of the factors that mediate gap repair [253]. The recombinant IN assays are fast, easy to carry out, and at low cost. Nevertheless, on numerous occasions they identified false positives when the *in vitro* reaction was carried out in the presence of Mn^{2+}, rather than Mg^{2+} [35].

HIV-1 PICs are collected from infected cells and can catalyze the concerted integration of both LTR ends to the target DNA to give a product that resembles the *in vivo* gapped intermediate. PCR-based assays have been proposed to better represent *in vivo* integration than the recombinant IN-assays. In fact, small molecules identified as IN inhibitors with the recombinant IN assays, were inactive with the PIC-based assays [89, 113]. These compounds did not interfere with 3'-processing or strand transfer but possibly with a step prior to integration, such as *in vitro* assembly. Nevertheless, the PIC-based assays were not as popular as the recombinant enzyme assays for the identification of IN inhibitors due to difficulties in using large amounts of infectious HIV. An improved PIC-based assay instead of live HIV uses HIV vectors to produce PICs able to carry out integration but not to spread infection. In this assay DNA is immobilized on a well-plate and the integrated PICs are analyzed by real-time fluorescence monitored PCR. This combination speeds up the procedure considerably, 96 assays in 6 hours. Using this assay a library of IN inhibitors was screened leading to the identification of a compound with activity against PICs [114]. An alternative PIC based assay employs PCR amplification to directly detect integration events within 4-5 hours [18]. PIC-based assays can potentially identify new nonintegrase-directed targets of inhibition such as the cellular and viral proteins that are part of the complex.

Real-time PCR assays measure the number of HIV DNA species during HIV replication. These include reverse transcribed linear viral DNA, integrated DNA and unintegrated DNA circularized by recombination between DNA LTRs, known as LTR-circles. By changing the primer/probe set each of the DNA species can be quantified. The integrated DNA can be quantified with chromosomal *Alu* repeats [27]. *Alu* elements are repeatitive elements that occupy about 5% of the human genome and are randomly distributed every 5000 base pairs [199]. Fractions of the integrated DNA close to an *Alu*-repeat are PCR amplified however, proviral DNA that does not integrate close to an *Alu*-repeat, is not amplified. Subsequent PCR assays overcame this sensitivity issue. A two-step PCR assay reported by Doherty *et al.* was 10-100 times more sensitive than the original *Alu*-PCR [224]. In the first non-kinetic preamplification step the primers used bind to genomic *Alu*-repeats and HIV-1 *gag* sequences. Preamplification is followed by real-time PCR that quantifies the HIV-1 LTR sequences. An alternative linker-primer PCR assay was developed to detect integrated HIV DNA species and takes advantage of the frequently occurring *Nla*III recognition sites (4 bp CATG sequence) near the integrated provirus. Following *Nla*III digestion, linkers are ligated to the generated DNA termini and serve as templates for priming in a subsequent PCR step. This assay was employed in the study of the kinetics of viral DNA accumulation post infection [288]. The *Alu*-PCR assays were used to authenti-

cate IN inhibitors that were previously identified in cell-free assays [236, 289].

The chimeric virus technology is another technique for the identification of the target of a particular drug [96]. It employs the recombination of IN drug resistant strains into a proviral HIV-1 clone for which the corresponding IN gene has been deleted. Recombination leads to chimeric viruses that have developed resistance. If, in the presence of an IN inhibitor, the recombined strains display the same loss in sensitivity to the original mutant IN strains, it would confirm that the IN mutations are responsible for the resistance phenotype [97, 98].

STRUCTURE AND FUNCTION OF HIV INTEGRASE

HIV-1 IN consists of three structurally distinct domains, the NTD, residues 1 to 49, the CCD, residues 50 to 212 and the CTD, residues 213-288 [55, 239]. To date, the insolubility of HIV-1 IN has been the main barrier in obtaining a crystal structure of the full-length IN. However, the discovery of a single mutant, through site-directed mutagenesis, within the CCD of IN dramatically increased its solubility while retaining catalytic activity [129]. The substitution of lysine 185 for phenylalanine enabled IN^{50-212} to be crystallized and its structure to be solved for the first time by Dyda *et al.* [71]. Since then other crystal structures of the isolated CCD and in complex with either the CTD or the NTD have been resolved. The structure and function of HIV-1 IN has been the subject of a recent review by Chiu *et al.* [43].

The structure of the NTD of both HIV-1 and HIV-2 IN was elucidated by NMR spectroscopy [28, 29, 74]. The NTD of HIV-1 IN exists as a dimer in two interconverting forms and has a monomer fold of helix-turn-helix. This motif is similar to that of a number of helical DNA binding proteins even though no significant sequence similarities exist. The dimer interface is of hydrophobic nature. Each monomer is composed of four helices. The lower region of each monomer is stabilized by a hydrophobic core and the upper region by the coordination of Zn^{2+} to H12, H16, C40 and C43. The HHCC motif resembles a zinc motif usually found in DNA binding proteins and is phylogenetically conserved in all retroviral INs [136, 145]. The Zn^{2+} atom is tetrahedrally coordinated in both forms at a stoichiometric ratio of one Zn^{2+} per IN monomer. The position of Zn^{2+} as well as Cys40 and Cys43 is identical in both forms while the relative positions of His12 and 16 are reversed [28]. In the absence of Zn^{2+}, the NTD is disordered and upon Zn^{2+} addition it adopts a highly-ordered α-helical secondary structure.

Point mutations of His or Cys residues abolish the zinc binding ability of IN and affect the 3'-processing and strand transfer activity but do not impair disintegration activity [24]. These results confirm that the active site is not located in the NTD and that this domain is not the single DNA binding domain in the protein [78, 124, 159, 285, 292]. Further chimeric experiments with HIV-1 IN and visna virus IN indicated that the NTD does not contribute to viral DNA specificity, either [141]. However, the NTD is proposed to interact with DNA in the context of the whole protein [78, 124, 159, 285]. Recent studies have suggested that Zn^{2+} promotes multimerization of the full-length IN by stabilizing protein-protein interactions and enhances *in vitro* 3'-processing and strand transfer activity [163, 318]. These activities are metal dependent and are more pronounced in the presence of Mg^{2+} rather than Mn^{2+} [162]. Therefore, it is widely accepted that the zinc-binding motif interacts with the core domain by forming a multimeric structure essential for 3'-processing and strand transfer activity. An interaction between the NTD and the two cellular transcription factors INI1 [311] and LEDGF/p75 [287] has also been identified.

The structure of the CCD domain of IN, first resolved by Dyda *et al.* was enabled after the substitution of lysine 185 for phenylalanine [71]. However, it was later found that the F185K mutation disrupted virion assembly *in vivo* and therefore some of the later crystal structures beared the F185H mutation that retained full activity of IN and did not disrupt virion assembly [43, 130]. The CCD contains the acidic residues D64, D116 and E152 that form the active site and are conserved among INs. In the structure reported by Dyda *et al.* [71] the geometry of the active site of IN was compromised by the use of cacodylic acid as means of crystallization. Furthermore, the catalytic residue E152 was located in a region of disordered residues and was thus, not observed. A subsequent structure by Bujacz *et al.* [21] assigned that disordered region to a long loop with an extended conformation and identified the location of residue E152. The side chain of E152 however, was pointing away from residues D64 and D116, a situation unlikely to correspond to the active enzyme. It was later revealed that the cacodylate caused a change in the geometry of the active site through the covalent modification of the cysteine residues C65 and C130. This modification led to the formation of an intramolecular hydrogen bond between the catalytic residue D64 and the amino group of the catalytic residue D116 [108]. In two cacodylate-free crystal structures of the catalytic core domain the carboxylate groups of D64 and D116 are found in a suitable orientation to accommodate a metal ion by participating in intramolecular hydrogen bonding with amide residues from the protein and intermolecular hydrogen bonds with water molecules. The other catalytic residue E152 does not participate in any interactions [108]. Crystal structures of the CCD complexed with Mg^{2+} display a very similar active site with the Mg^{2+}-free structures, which indicates that no conformational change occurs upon metal binding [108, 178]. In the Mg^{2+}-complexed structure one Mg^{2+} ion interacts with the carboxylate groups of D64 and D116 and with either two or four water molecules. These water molecules form further hydrogen bonds with the protein. The third catalytic residue E152 does not participate in metal binding. Overall the reported crystal structures of the CCD display a similar topology (Fig. **1**). In all structures CCD forms a dimer and each monomer is characterized by a five-stranded β-sheet flanked with six α-helices. The dimer interface is held by hydrophobic interactions with residues from helices α1 and α5 and covers an area of about 1400 $Å^2$ per monomer. The area initially thought of as a disordered loop comprised of residues 188-193, in better defined structures forms a short two-stranded antiparallel β-sheet, β6 and β7. The loop comprised of residues 140-150 adopts a flexible conformation that is likely to become ordered in the presence of the DNA substrate. In the better resolved crystal structures, residue E152 is part of helix α4 and its side chain is facing towards catalytic residues D64 and D116 [21, 108, 178]. The DDE residues form a shallow cavity on the surface of the protein. Lately, Goldgur *et al.* [109] reported the first crystal struc-

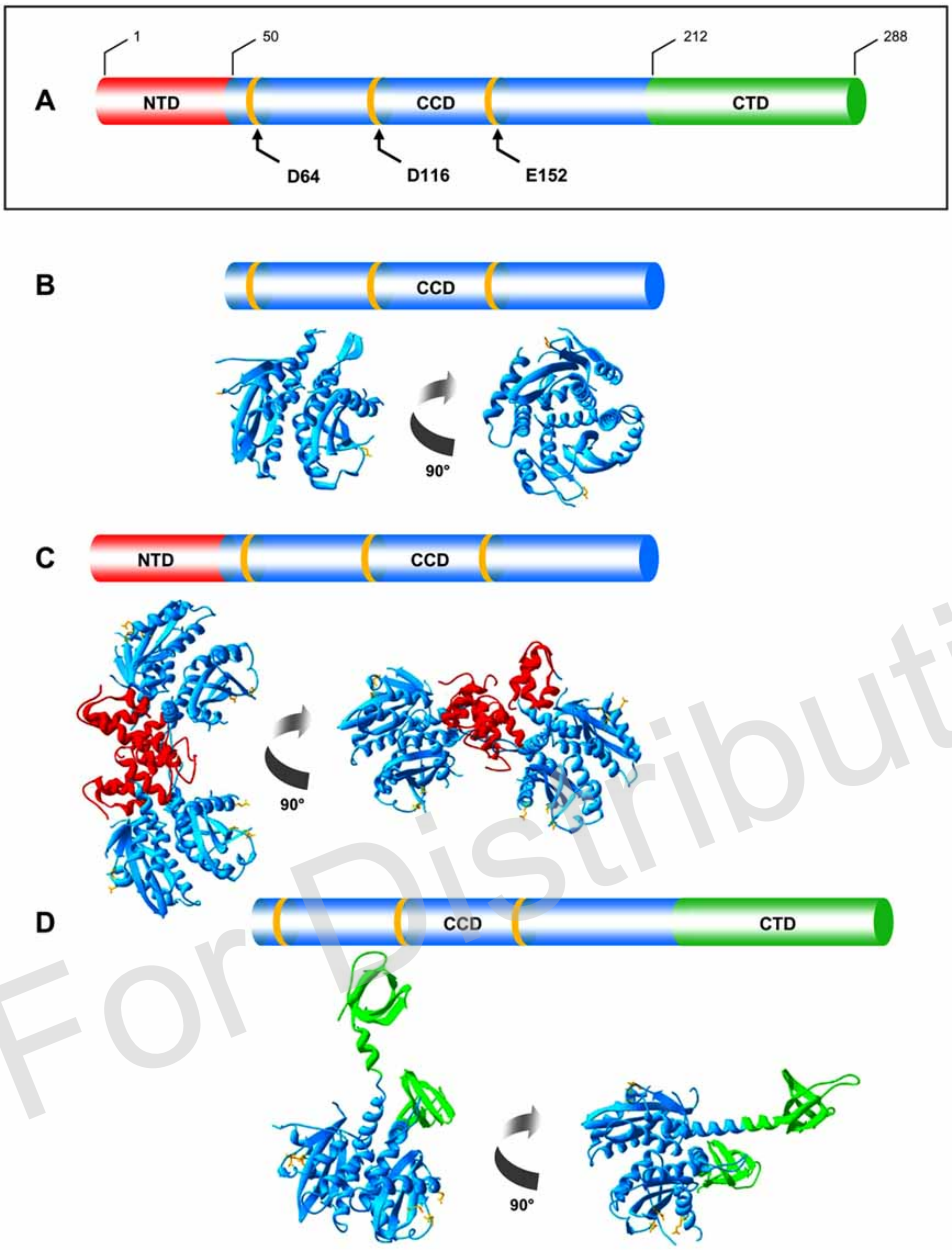


Fig. (1). A. Schematic diagram of HIV-1 IN, spanning residues 1 to 288. The independently folding domains are shown in color: Red NTD (corresponds to residues 1 to 49), Blue CCD (residues 50 to 211), Green CTD (residues 212 to 288). Conserved DDE catalytic residues (D64, D116, E152) are highlighted in yellow [55,239]. B. Crystal of CCD dimer (pdb 1BIZ). View of the same structure rotated vertically 90° [108]. C. Co-crystal of CCD and NTD (pdb 1K6Y). View of the same structure rotated vertically 90°. Structure forms two nearly identical dimers with pseudo-C_2 symmetry [299]. D. Co-crystal of the CCD and CTD (pdb 1EX4). View of the same structure rotated vertically 90°. CCD forms a dimer within the co-crystal and CTD is monomeric [36].

ture of the CCD of HIV-1 IN complexed with an inhibitor, 5CITEP. The inhibitor is located between active site residues D64, D116 and E152 and forms a hydrogen bond with E152. Residues D64 and D116 interact with the undisplaced Mg^{2+} ion. The most recent crystal structure of the CCD of IN is in complex with the host factor LEDGF/p75. The complex has a pseudo two-fold symmetry and consists of a dimer of the catalytic core domain of IN complexed to two molecules of LEDGF/p75 (Fig. **2**). A phosphate ion is observed within the two active sites of the catalytic core domain of IN that was suggested to correspond to the coordination site of a DNA backbone phosphate. Mutations of IN that resulted in failure to bind LEDGF/p75, pinpointed the interaction site to the connector linking helices α4 and α5 [41].

The overall topology and geometry of the active site of this domain bears similarities to other retroviral and retrotransposon INs [20, 37, 178, 307], and to the broader family of polynucleotidyl transferases that include the Mu transposase, RNase H and RuvC. These enzymes contain three or four carboxylate amino acids as the catalytic residues. At least two of the carboxylates are located in identical positions in the secondary structure and in the tertiary structure all carboxylates adopt a similar configuration [105, 247]. A

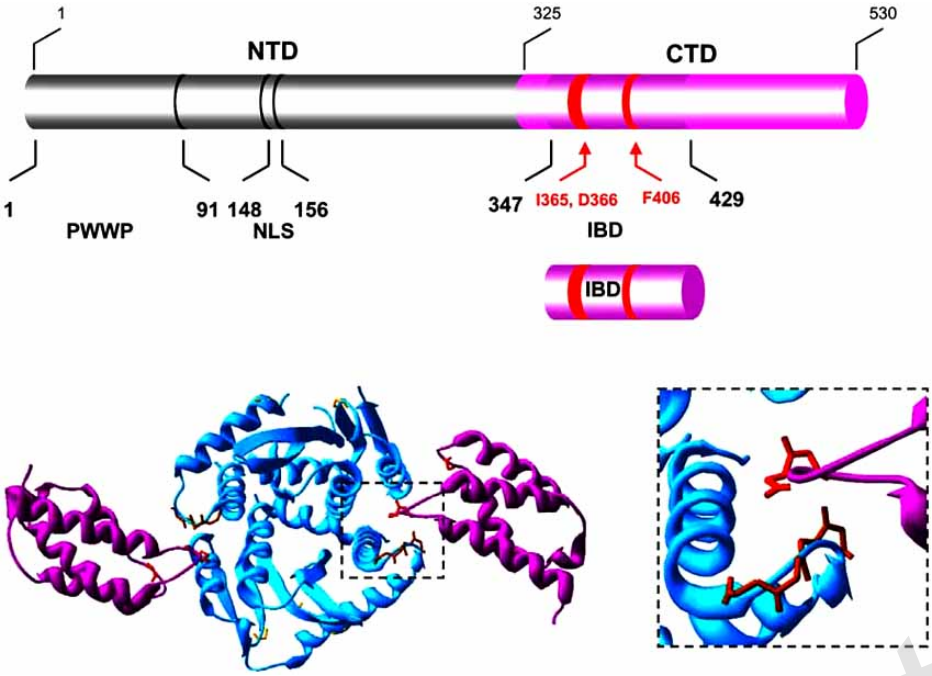


Fig. (2). Schematic diagram of full-length host factor LEDGF/p75, residues 1 to 530. The independently folding domains are shown in color: Grey NTD (corresponds to residues 1 to 325), contains a PWWP motif (residues 1 to 91) and a NLS domain (residues 148-156), Pink CTD (residues 325 to 530), contains the IBD domain (purple, residues 347-429) [40]. Co-crystal of IBD of LEDGF/p75 and CCD of HIV-1 IN^{50-212} (pdb 2B4J). CCD forms a dimer and two LEDGF/p75 molecules interact with the dimer interface. LEDGF/p75 residues I365, D366, F406 (red) and HIV-1 CCD α4/5 connector residues 168-171 (brown) involved in the interaction are highlighted. An expanded region of the interaction area is depicted [41].

common catalytic mechanism has been proposed that involves the use of two metal ions in activating hydroxyl groups as nucleophiles, despite the fact that only one metal ion has been observed in the crystal structures of HIV-1 IN [275, 306]. Based on the mechanism of DNA polymerase I the location of the two Mg^{2+} ions in HIV-1 IN has been postulated. The first known location is between D64 and D116 and the second presumed location is between D116 and E152 [35, 276, 306]. The isolated CCD can perform the disintegration reaction while all three domains are necessary for the catalysis of 3'-processing and strand transfer [26, 79, 294]. In the context of full-length IN, the CCD has been assigned a direct role in carrying out the 3'-processing and strand transfer reactions, in recognizing the conserved CA/TG base pairs near the viral DNA end and in recognizing target DNA [80, 105, 106, 152, 262]. Mutations of the conserved residues D116N, E152Q abolishes detectable activity in all three processes while the mutations D64E and D64N retain barely detectable strand transfer and disintegration activity [78, 151] Mutational studies on other residues within the CCD identified K159 as an important residue for the recognition of the conserved CA dinucleotide of viral DNA [131] and excluded other residues initially thought to be important for activity [82]. The flexibility of the active site loop that consists of residues 141-148 is important for integration. When residues G140 and G149 that act as conformational hinges for the loop are replaced with alanines the flexibility of the loop is diminished. These mutants loose catalytic activity but retain DNA binding affinity. Based on this observation the loop is proposed to have a role in a reaction step after the DNA binding step [111]. The importance of the flexible loop 140-149 was also corroborated by molecular dynamics studies [63].

The CTD consists of residues 212-288 and is the least conserved domain among INs. The structure of the fragment 220-270 of the CTD has been elucidated by NMR spectroscopy. It forms a dimer in solution and the two monomers are arranged in an antiparallel position. Each monomer consists of a five-stranded antiparallel β-strand and a three-residue helix. The dimer interface is mainly hydrophobic in nature and is formed by parts of the β2, β3 and β4-sheets. The overall topology resembles an SH3 domain which is most commonly found in proteins that are involved in signal transduction [3, 73, 171].

The CTD is proposed to be involved in the multimerization of IN [130], in binding DNA nonspecifically and is required for 3'-processing and strand transfer activity [3, 80, 173, 294]. Mutational analysis identified residues in the CTD critical for oligomerization and DNA binding [174]. Viral DNA specificity was mapped to both the CTD and CCD and CTD-DNA interactions were identified just inside the CAGT base pairs of the viral DNA. A similar function for site-specific DNA binding is exhibited by transposases [141, 262]. Earlier studies attributed the interactions of the target DNA to the CTD however, the present studies indicate that all three domains interact with the target DNA [35, 55, 84, 123]. Furthermore, experiments showed that CTD is involved in interactions with HIV-1 RT [120, 319].

By introducing five point mutations a crystal structure of the CCD complexed with the CTD was obtained (Fig. **1**). The CCD within the complex forms the same dimeric structure as the one observed for the isolated CCD. On the con-

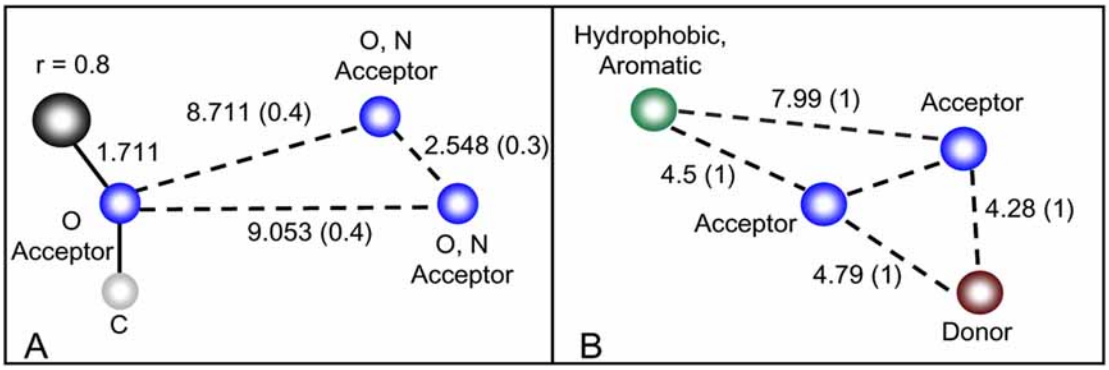


Fig. (3). Sample analog-based pharmacophores. A. Pharmacophore proposed by Nicklaus *et al.* [221] defined on the superposition of low energy conformers of two known inhibitors. The black circle represents a steric exclusion sphere. B. Pharmacophore proposed by Dayam *et al.* [59] defined from four known IN inibitors from the DKA family. All distances are in angstroms, numbers in parentheses represent the value range.

trary, the isolated CTD forms a dimer in solution but is monomeric when connected to the CCD. Both C-terminus are 55 Å apart, adopt a Y-shape conformation and have a 90° shift between them. The connection point with the CCD, helix α6, is believed to be flexible, thus giving the CTD a dynamic character able to adopt various orientations with respect to the CCD. A positively charged region extending from the catalytic site to the outside face of IN^{52-288} could serve as a platform for DNA binding. In that sense docking experiments placed an 18 bp viral DNA within that region [35, 36]. Shortly after, the crystal structure of the CCD complexed with the NTD for an HIV-1 IN triple mutant was elucidated (Fig. **1**). The structure of IN^{1-212} forms two nearly identical dimers that share a pseudo-C_2 symmetry. The CCD within the complex has a similar structure as the isolated domain. On the contrary, the interactions of the dimer interface of the NTD within the complex differ from the ones observed in the solution structure of the isolated domain. The dimer interface of NTD within the complex is dominated by interactions between the α1 and α3 helices and cover a smaller area compared to the interface of the solution structure of NTD that is dominated by interactions of the a1 helix. The NTD is connected to the CCD through the disordered region comprising residues 47-55. The interface between the NTD and CCD is mainly hydrophilic and occurs between residues from the NTD of one dimer with residues from the CCD of the other dimer. Each dimer contains a phosphate ion at identical positions with respect to the active site DDE residues. Based on the crystal structures of IN^{1-212} and IN^{52-288} a model for the structure of full-length IN is proposed in which the NTD is placed within the CTD [43, 299]. The full-length IN model was used to perform docking studies with viral DNA. DNA-IN contacts were observed between the CCD and NTD of one monomer and the CTD of another monomer and the conserved CA dinucleotide was placed close to the catalytic DDE residue [62].

The active form of IN is believed to be a multimer though the degree of active multimer, has not been determined [101, 124, 137, 300]. The evidence for an active multimer is derived from domain deletion studies demonstrating that isolated domains of HIV-1 IN do not possess activity. In contrast, independent domains of IN can complement each other and restore IN activity [75, 283, 286]. The trans interactions between the NTD and the CCD of HIV-1 IN mutant proteins indicated that an intact NTD and CCD must be part of different monomers within the multimer. On the other hand, the CTD could function in both cis and trans interactions with the CCD [79]. Based on gel filtration experiments full-length IN exists in a dimer-tetramer equilibrium in solution. The isolated CCD and CTD and the two domain CCD-NTD complex exist as a dimer. On the other hand, the two domain CCD-CTD complex exists in a dimer-tetramer equilibrium. These results point to the CCD and CTD as the necessary domains for multimerization [130]. Nevertheless, Zn^{2+} was found to promote multimerization indicating that the NTD as well is important for protein-protein interactions [163, 318]. Experimental data point towards a tetramer as the minimal active form of IN [185]. Cross-linked tetramers could catalyze full-site integration *in vitro* while dimers could integrate just one viral LTR end to the target DNA [93]. In detailed IN-DNA models DNA cross-links occurred in trans to the active site [104, 124]. A reconstructed tetramer of HIV-1 IN was consistent with the cross-linking experiments and confirmed the observation that the F185K mutation disrupts complex formation *in vivo* by preventing the tetramerization of IN [237].

COMPUTER-AIDED DRUG DESIGN FOR HIV-1 INTEGRASE INHIBITORS

The field of HIV-1 IN inhibitor discovery has attracted substantial interest from the molecular informatics community and various computer-aided drug design [138, 227] applications have been described in the literature including efforts to elaborate the structure of the binding site of the protein, to describe pharmacophores complementing the active site and to discover novel lead molecules. Results of these studies have been well reviewed by Chen *et al.* [35] and Makhija *et al.* [182]. Computer-aided drug design methods fall in two broad categories based on the molecular information used. The first category relies on the availability of known inhibitors that form the starting point for investigation for chemical features responsible for the observed biological behavior. Pharmacophore definition and search, QSAR studies, and analog-driven *de novo* design, also known as inverse QSAR, are the main representative methods of this type. The second category of methods requires detailed target information such as that produced by NMR or X-Ray crystallography. Methods of this category include

virtual screening *via* docking and scoring, *de novo* design and receptor-based pharmacophore definition and search.

Analog-Based Methods

These methods focus on information provided by a set of compounds known to bind strongly to the desired pharmaceutical target [176] or by structurally similar compounds that fail to show activity in order to refine the set of significant chemical features and improve the quality of the model [209]. This specific arrangement of molecular features essential for biological activity is known as the pharmacophore [189]. Once discovered, the pharmacophore is used for screening databases for compounds matching it. Virtual hits are then submitted to further analysis and eventually biological testing. In a series of publications Neamati *et al.* defined several ligand-based 3D pharmacophores and paved the way for subsequent research in this field. Nicklaus *et al.* [221] used CAPE, one of the first reported HIV-1 IN inhibitors, to derive a 3D pharmacophore that was refined using a second inhibitor. The pharmacophore consisted of three hydrogen-bond acceptors and a steric exclusion sphere (Fig. **3**) [221]. A pharmacophore search of the public NCI 3D database resulted in 267 virtual hits. A subset of 60 compounds was selected based on availability, estimated solubility and lead optimization potential and screened for HIV IN activity. The identified inhibitors exhibited anti-IN activity in the micromolar range. Neamati *et al.* [212] followed a similar process to develop a four-point pharmacophore from reported HIV-1 IN inhibitors of two chemical families, the chicoric-acids and the dicaffeoylquinic acids. The pharmacophore search identified 179 compounds of which 39 compounds were screened *in vitro*. Results showed that over 50% of the compounds exhibited IN activity. In the last paper of the series, Neamati *et al.* [211] used seventeen lichen acids to construct two three-point pharmacophores. A virtual screening process was applied and several structurally unrelated IN inhibitors were discovered confirming the usefulness of the approach. More recently, Mustata *et al.* [210] reported the development of a strand-transfer specific pharmacophore by using a training set of 26 diverse IN inhibitors covering a wide strand transfer process activity range. A pharmacophore hypothesis consisting of two hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic group was defined and validated on a set of 14 different IN inhibitors. Model predictions were in agreement with the biological data but no testing was reported against negative data. Both Barreca *et al.* [8] and Dayam *et al.* [59] defined a 3D pharmacophore model based on the class of DKA compounds. The pharmacophore developed by Barreca consisted of four points, two hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic/aromatic point and was based on the crystallographic structure of the 5CITEP and the proposed mechanism of action for DKA analogs [112]. A second inhibitor was used to set the range of possible distances between the various pairs of pharmacophore points. The pharmacophore developed by Dayam consisted of four points, two hydrogen-bond donors, one hydrogen-bond acceptor and a hydrophobic region and was based on four representative compounds from the DKA class (Fig. **3**). Pharmamacophore searching and further selection taking into account drug likeness led to compounds with HIV-1 IN inhibitory activity.

SAR studies offer a correlation between the exhibited biological properties of a molecule and its structure [227, 248]. QSAR techniques attempt to generate computational models that identify structural and physicochemical features of molecules contributing to a certain biological outcome. Once constructed successfully, QSAR models are used to predict the biological property of new, unknown molecules and in certain cases assist in the understanding of the mechanism of action of protein-ligand complexes. In one of the earliest QSAR method applications on HIV IN inhibitors Raghavan *et al.* [243] processed a dataset of flavone analogs with inhibitory activity against IN. A 3D QSAR technique, CoMFA, was employed that derives a QSAR model by aligning 3D conformations of molecules, sampling the steric and electrostatic fields surrounding them and correlating the differences in these fields to biological activity. The QSAR model confirmed the importance of electrostatic and steric fields, with electrostatic fields playing a dominant role (79%) in determining the activity of these molecules [182] and highlighted regions possibly important for IN inhibition. A CoMFA-like approach was used by Costi *et al.* [52] to develop a model capable to explain activity measured and help in the design of new active compounds. Another 3D QSAR method, CoMSIA, uses a probe atom and a grid to compute similarity values between the probe and a set of pre-aligned molecules at regularly spaced grid points. Similarity calculations are based on the differences of atomic physicochemical properties e.g. steric, donor/acceptor, etc. This method was applied by Makhija and Kulkarni [179, 180] to a diverse set of known inhibitors from 5 different classes. The resulting models showed considerable predictive ability for both 3'-processing and strand transfer. Comparative predictive ability was obtained for the same set of compounds using an alternative technique, EVA [179]. Kuo *et al.* [153] used both CoMSIA and CoMFA as part of a study to optimize a series of lead compounds. The results combined with docking were used for guiding the rational design of 12 new inhibitors that had *in vitro* activity. More recently, Urra *et al.* [255] used a genetic algorithm method to iteratively generate and optimize linear regression models on a diverse set of 172 IN inhibitors. The compounds were represented using a set of GETAWAY descriptors. In comparative tests the authors found that their descriptors of choice produced a model explaining 72.5% of the activity and compared favorably with other descriptors commonly used for QSAR.

Do novo design generates ligands from scratch based on information about the receptor site or known ligands. There are two main varieties of *de novo* design methods, receptor-based *de novo* design where detailed information is required about the receptor structure and analog-based, or inverse QSAR, where the process requires only a set of known inhibitors preferably spanning a wide range of activity [19, 258]. Makhija *et al.* [181], following up on their previous work on HIV IN pharmacophore models describe a *de novo* experiment using the LeapFrog tool. The starting point of the design experiment was the CoMFA pharmacophore model described previously [180]. This model was used to define a pseudo-receptor model consisting of a hypothetical IN cavity. A computational process was employed to generate a set of virtual compounds predicted to bind to the hypothetical cavity better than the used reference structures. The most promising virtual compounds were selected for synthesis and

biological testing. The results led to structurally diverse compounds with moderate activity that upon further optimization could potentially lead to a new class of IN inhibitors. Barreca *et al.* [9] used a dataset of 33 DKA and DKA-derivative molecules spanning a wide range of activity to generate a QSAR model. The model was used to design new DKAs that proved to be potent IN-inhibitors of the strand transfer process and confirmed the utility of the 3D QSAR model in designing and predicting new compounds.

Target-Based Methods

Target-based methods depend on the availability of the detailed 3D structure of the pharmaceutical target [35]. Given the detailed target structure and information about the binding specifics of known inhibitors to an active site computational approaches can be employed to place virtual molecules in the active site, docking, or to construct molecules to match the cavity of the active site, *de novo* design. Despite the lack of a 3D structure for full-length IN, several target-based methods have appeared in the literature making use of the defined 3D structure of the CCD of HIV-1 IN [109], and of similar proteins [34], and the results of molecular dynamics experiments on the HIV IN [30, 66, 257]. Docking, describes a process by which a ligand and a protein target, fit together in three-dimensional space [150]. The inputs to the docking problem are the 3D protein structure of the target and a ligand-molecule believed to bind on the target protein surface. The output is the 3D structure of the corresponding protein-ligand complex. Scoring functions aim to calculate accurately the binding energy of the protein-ligand complex [12]. A typical docking/scoring virtual screening experiment takes advantage of the knowledge about the receptor site to model it and then performs docking of molecules from a database in a systematic manner. A number of conformations are usually sampled for each molecule [150] and a score for every possible docking attempt is kept [273]. The results are presented in a virtual hit list.

In a study by Chen *et al.* [34] a docking virtual screening experiment was used to find novel HIV IN inhibitors. The experiment used the Y3 known inhibitor binding site of the ASV IN from a crystal structure of the complex and a putative Y3 binding site of the HIV IN hypothesized based on the similarity of the two IN proteins. The compounds in the public NCI 3D database were docked on the Y3 binding site of the ASV IN yielding 3100 hit compounds. A more rigorous scoring approach was used to rescreen the hits against the Y3 binding site of the ASV IN and the putative Y3 binding site of the HIV IN. A subset of 22 virtual hits was heuristically selected and submitted to *in vitro* testing where nearly 64% proved to have IN inhibitory activity. Virtual screening using AutoDock [205] was employed by Schames *et al.* [257] to dock 5CITEP to the various conformations of HIV-1 IN. Analysis of the predicted ligand-protein complexes indicated a previously uncharacterized trench next to the active site. Further docking studies of novel ligands with the potential to bind to both regions showed greater selectivity towards the newly characterized trench [257].

Several applications on receptor-based *do novo* design for HIV-1 IN inhibitors have been reported in the literature. Nikitin *et al.* [222] used a virtual combinatorial chemistry space containing 10^{13} compounds and a hybrid algorithm combining combinatorial library design and receptor-based evaluation. The virtual chemistry space is available to a proprietary *de novo* drug design program that exploits features of a given target protein and a scaffold to construct virtual compounds in a combinatorial fashion and score them against the target protein. The above algorithm was used to generate inhibitors of the HIV IN. The scaffold supplied to the program was a fragment found in known β-diketone inhibitors of the HIV IN. The application of the program generated 800 ligand candidates, out of which 22 were selected for synthesis and testing. Twenty compounds were successfully synthesized and screened resulting in several hits in the low micromolar range. Jaganatharaja and Gowthaman [128] employed a hybrid *de novo* design application based on a receptor-based pharmacophore model and the known Y3 ASV-IN inhibitor to identify cavities on the protein likely to represent binding sites. Y3 was used to identify key interaction sites and develop a receptor-based static pharmacophore hypothesis that was used to generate 3000 molecules through structural manipulation of the seed structure, Y3. A physicochemical filter was applied to select 500 drug-like compounds that were subjected to rigorous docking. The resulting compounds shared consistent binding characteristics indicating the potential presence of a common binding mechanism. In 2000, Carlson *et al.* [30] reported the first receptor-based pharmacophore model for HIV-1 IN. Their method was designed to accommodate the incomplete crystal structure of the target protein and take into account the flexibility of the active site through the development of a "dynamic" pharmacophore. The starting point of the method was a collection of protein conformations from a molecular dynamics simulation of the HIV IN [168]. For the development of the pharmacophore hundreds of probe molecules were placed in the catalytic site and simultaneously minimized while the protein was held fixed. This enabled the detection of conserved binding regions for the probe molecules containing functional groups that complement the active protein site. The pharmacophore model, consisting of the conserved regions, was made up of 9 sites: three excluded volumes to avoid steric clashes and six hydrogen-bond donor sites. Validation tests on 59 known IN inhibitors indicated that it is very specific for highly active compounds but much less so for moderately active ones. *In vitro* results on a subset of 39 compounds confirmed the relevance of the model and identified 11 new inhibitor compounds. In a similar study, Deng *et al.* developed a dynamic receptor-based pharmacophore model representing the complementary features of the active site region of HIV IN [66]. Validation tests on a set of 128 known inhibitors showed that over 72% of the active inhibitors fit the model. A subsequent pharmacophore search on a database of commercially available compounds produced several structurally novel IN inhibitors [66]. A following study, extended the dynamic pharmacophore model of IN by considering more key residues in the active site including the cation Mg^{2+} [67]. The model was used to select twenty-two structurally novel compounds for *in vitro* testing which led to several hits.

HIV-1 INTEGRASE INHIBITORS

IN has been a difficult target for structure-based drug design mainly due to the shallow substrate binding site, the lack of a crystal structure for the full-length protein and the

lack of a lead compound. Despite these issues, a considerable number of compounds have been reported to inhibit IN in the last 10 years, and this has been the subject of recent reviews [4, 135, 158, 182, 196, 240, 302, 303]. Strategies towards designing HIV-1 IN inhibitors involved random testing of compounds that exhibited inhibitory activity with related proteins [56, 94], high-throughput screening [83, 117] and computer-aided drug design [35, 182].

In order to validate a compound as the cellular target of HIV-1 IN, certain criteria must be met: i) inhibition must occur within the period of reverse transcription and maturation, that occurs 4-16 hours after infection ii) accumulation of LTR circles, and diminished integration in infected cells treated with the inhibitor iii) accumulation of IN mutations in drug resistant strains and iv) inability of the compound to inhibit IN that bears the previously identified mutations [240]. Among the IN inhibitors studied so far, DKAs and their analogs are currently the only compounds that meet all four criteria and recently two analogues of the family, GS-9137 and MK-0518, have advanced in clinical trials [158]. Additional promising inhibitors in preclinical development are the pyrano-dipyrimidines [231] and styrylquinolines (Table **1**) [197]. In this section we summarize the main classes of IN inhibitors reported in literature and emphasize the most promising compounds as leads for the development of anti-IN drugs.

DNA Binders

DNA intercalators and DNA groove binders were among the first inhibitors identified. The ends of the HIV-1 LTR contain a highly conserved region rich in AT-sequences. This region has been the target of inhibition of compounds such as polyamides and lexitropsins that specifically interact with AT residues [216]. However, the main limitation of these compounds is the difficulty in obtaining selective LTR binders. As a result most of these compounds exhibit high toxicity due to their activity on the host genome. Several studies with DNA binders suggested that IN inhibition does not necessarily correlate with DNA binding [32, 94].

Nucleotides

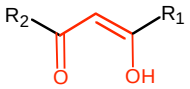
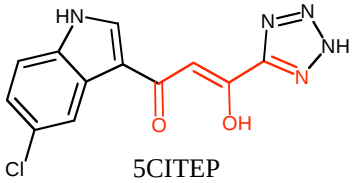
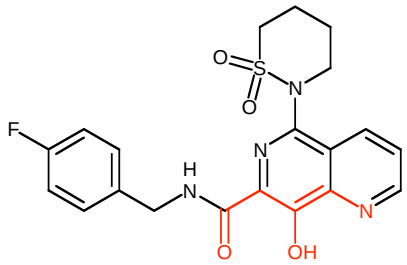
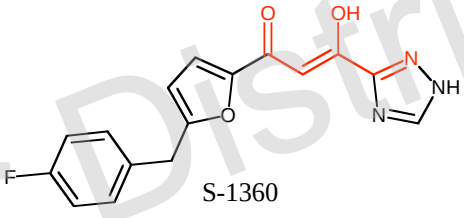
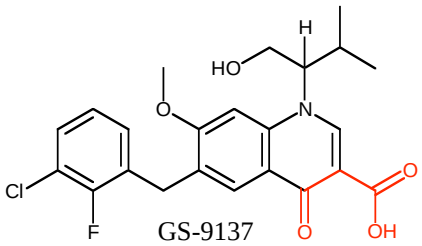
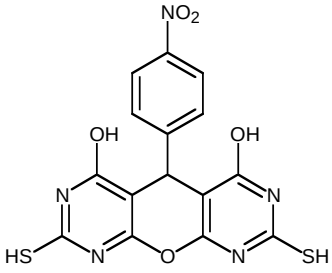
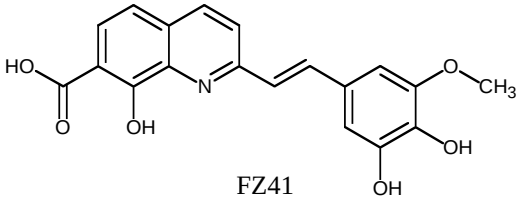
Modified mono- and di-nucleotides, designed to be resistant to exonucleases, exhibited inhibitory activity against IN at micromolar concentrations [193, 278, 279]. Longer oligonucleotides have also been investigated. G-quartets are oligonucleotides composed of deoxyguanosine and thymidine able to inhibit HIV-1 replication in culture assay systems. These oligonucleotides can fold upon themselves forming a stable structure of two stacked guanosine tetrads. Both the number of the quartets and the aminoacid sequence of the loops are important for maximum antiviral activity [191, 245]. Initial studies on the prototypical G-quartet oligonucleotide AR177 (Zintevir, T30177), showed that it inhibited HIV-1 replication by interefering both with cell fusion events and integration [226]. AR177 inhibited integration at nanomolar concentrations by interefering with IN-viral DNA binding [191]. Oligonucleotides composed of two, three or four G-quartets of different lengths were designed to better understand the binding interaction with IN. Inhibition was observed with all oligonucleotides suggesting that the interaction with IN occurs between the GTGT loop domain of the G-quartet [133]. Despite the potent activity observed against IN, no significant mutations were observed within the IN-coding region but rather in the envelope glycoprotein gp120 region pointing to the primary target of G-quartets as the viral entry rather than integration [38, 85]. This finding did not affect the therapeutic potential of AR177, which is the first oligonucleotide to enter human clinical trials [298]. Oligonucleotides containing 6-oxocytidine were found to inhibit IN *in vitro* by interefering with IN-DNA binding at submicromolar concentrations [16]. Inhibition was dependent on sequence and required the presence of the 6-oxocytidine base [17].

Peptides and Antibodies

The first peptide inhibitor of IN was identified in 1995 after screening a synthetic peptide combinatorial library. The peptide contained six aminoacids with the sequence HCKFWW and inhibited both 3'-processing and strand transfer at micromolar concentrations [175]. More recently peptide inhibitors were designed to interact with the dimerization interface of HIV-1 IN. The CCD of IN forms dimers through the interaction of helices α1, α3, α5, α6 and β3. Two synthetic peptides were thus produced to contain the amino acid sequence of the α1 helix, INH1, and the amino acid sequence of the α5 helix, INH5. INH5 was a better inhibitor than INH1, in strand transfer assays however, both peptides inhibited the formation of functional IN oligomers. Mechanistic studies indicated that INH5 selectively interacts with the α1 helix of IN [188]. Furthermore, in a separate experiment interfacial peptides α1, α5 and α6 inhibited IN at micromolar concentrations possibly by blocking the ability of IN to dimerize [317]. Another peptide, K159, was produced based on the α4 helix of IN with the aim of interacting with the counterpart α4 helix of IN. K159 inhibited IN with a relatively low potency and its activity was proposed to be a result of a peptide-protein coiled-coil structure formation [272]. A monospecific antibody against K159 inhibited the catalytic activity of IN at nanomolar concentrations and decreased DNA binding [187]. I33, a 33-mer peptide was identified from a two-hybrid system that used HIV-1 IN as the bait and a yeast genomic library as the peptide source. Further analysis led to a shorter peptide, EBR28 that was shown to interact with the CCD of IN interfering with DNA binding [64]. Naturally occurring peptides have been shown to inhibit IN catalytic activity at micromolar concentrations. Examples include the complestatins [264] and integramides [265]. Both these compounds preferentially inhibit 3'-processing over strand transfer however, are cytotoxic in antiviral assays [58]. A recent study reported the anti-IN activity of indolicidin, a natural peptide, and new indolicidin analogues [149]. MAP30 and GAP31 plant proteins isolated from the medicinal plants *Momordica charantia* and *Gelonium multiflorum* also displayed anti-IN activity [160],

A library of monoclonal antibodies was screened against HIV-1 IN and several antibodies that bind to the NTD mAb17, the CCD mAb4, and the CTD mAb33 inhibited IN activity *in vitro*. The epitope of mAb17 was mapped to determinants of the helix-turn-helix motif of the NTD domain of IN that is stabilized upon coordination with Zn^{2+}. Inhibition occurs by destabilization of the N-terminal helix upon

Table 1. Structures of HIV-1 IN Inhibitors

Class	Structure	Target of IN Inhibition
DKAs and analogues	 General structure	**Strand transfer**
	 5CITEP	
	 L-870,810	
	 S-1360	
	 GS-9137	
Pyrano-dipyrimidines	 V-165	**3'-processing**
Styrylquinolines	 FZ41	**3'-processing**

The general structural motif of the DKA inhibitors is depicted in red. The presented inhibitors were chosen based on their activity against integration and include selected analogues of the DKAs such as 5CITEP, L-870, 810, S-1360 and GS-9137 [303], V-165 from the class of pyrano-dipyrimidines [231] and FZ41 from the class of styrylquinolines [197].

antibody binding [308]. The antibody mAb33 and its epitope Fab33 inhibit 3'-processing at micromolar concentrations by interfering with CTD IN-DNA binding [309]. The areas bound by antibodies could constitute an alternative area for the design of small molecule IN inhibitors.

Sulfated Compounds

Diaryl sulfones have been shown to inhibit the HIV replication cycle and were thus investigated as potential IN inhibitors in 1997 [5, 214]. Their study however, did not lead to any promising IN inhibitors due to either cellular toxicity or lack of antiviral activity [198]. Suramin, a polyanionic sulfonate, the 2-mercaptobenzenesulfonamides and some thiazolothiazepines are active against IN at micromolar concentrations and exhibit antiviral activity [31, 182, 213, 217, 230]. Mercaptosalicylhydrazides were designed to bind the cysteine residues and the metal ion within the CCD of IN. Their design was based on the parent compound salicylhydrazide that exhibited anti IN activity at micromolar concentrations [215]. Salicylhydrazide is potent only in the presence of Mn^{2+} while the mercaptosalicylhydrazides are active in the presence of both Mn^{2+} and Mg^{2+}. Based on experiments with mutant C65S IN and molecular modeling studies, it was proposed that mercaptosalicylhydrazides form a ternary complex with Mg^{2+} and a disulfide bond with C65. Contrary to salicylhydrazides they exhibit antiviral activity and are 300 times less toxic [219].

Hydroxylated Aromatics

Hydroxylated aromatics represent one of the first classes of HIV-1 IN inhibitors. This class of inhibitors includes compounds from either natural or synthetic origin with multiple aromatic rings. In most cases the presence of at least one catechol moiety is a prerequisite for inhibitory activity. The mode of action of hydroxylated aromatic compounds is believed to arise from the ability of the catechol moiety to chelate the divalent cation in the active site of IN [218]. However, the ability of the catechol containing compounds to undergo *in situ* oxidation to active quinone species has imparted considerable toxicity thus, diminishing their development as anti-HIV agents [274].

CAPE, a natural product produced by bees, was one of the first inhibitors reported of HIV-1 IN. It weakly inhibited all three reactions catalyzed by IN, but displayed selectivity towards the strand transfer step [94, 95]. Thalassiolins A-C, isolated from the Caribbean sea grass *Thalassia testudinum*, present a novel series of flavones that contain a sulfate-substituted β-D-glucose at the 7-position. The sulfate group imparts increased potency against IN and thalassiolin A, the most potent analogue of the family, exhibits IC_{50} values of 0.4 μM for the strand transfer reaction [254]. Biscatechols and arylamides have been synthesized and tested for either IN inhibitory activity or to identify active catechol isosteres that lack cytotoxicity [70, 155, 203, 316]. Hypericin shows inhibitory activity in cell culture and against purified IN and PICs but it has not been proved that integration is the major target *in vivo* [91].

Dicaffeoylquinic acids, first identified as IN inhibitors in 1996, were isolated from medicinal plants of the Bolivian Kallawaya culture [195, 251, 252]. A synthetic analogue of dicaffeoylquinic acids, L-chicoric acid, exhibited the highest potency with IC_{50} values of 1.1 and 0.8 μM for 3'-processing and strand transfer, respectively. SAR studies that followed identified both enantiomers of chicoric acid as equal inhibitors. Furthermore, inhibition was maintained in the presence of either the catechol moiety or the central carboxylic acid group. When catechol is masked as an acetate, at least one carboxylic acid group is required for activity [146, 167]. Further studies led to derivatives with comparable and in some cases higher activity than L-chicoric acid [126]. Initial *in vitro* studies indicated that L-chicoric acid interacts with residues near the catalytic site of IN [147] however, similar activity was not observed with PIC-based assays [91, 114]. It was later revealed that L-chicoric acid interferes with the gp120-CD4 interaction during viral enrty [235]. A time-of-addition experiment showed inhibition of viral replication by chicoric acid at an early stage, about 1 h after infection. Furthermore, isolated HIV-1 strains restistant to chicoric acid exhibited signifant mutations in the region of the envelope glycoprotein gp120 and none in the IN gene. Recombination of the gp120 gene of the resistant strain led to the same resistance profile as the isolated strain.

A variety of other natural products reported to possess anti-IN activity include, curcumin the yellow substance in turmeric [190], certain lignal derivatives [72], the dimeric alkyl compounds integracins [266], the tetracyclic aromatic compounds integrastatins [268] and the more complex hexacyclic compounds integramycins [267]. These products inhibit the catalytic activities of IN at micromolar concentrations. Lithospermic acids are additional examples of hydroxylated aromatics, isolated from herbal plants. They exhibit activity against HIV-1 IN *in vitro* by inhbiting both 3'-processing and strand transfer with IC_{50} values of 0.5-0.8 and 0.4-0.5 μM, respectively and prevent viral replication *in vivo*. Furthermore, they do not affect viral entry or inhibit RT [1]. More recently four novel naphtha-γ-pyrones isolated from *Fusarium* fungal extracts have shown inhibition of both the recombinant IN 3'-processing and strand transfer reaction, at the micromolar range [269].

In an attempt to overcome the toxicity issue hydroxylated aromatic compounds that lack the catechol functionality were developed. NCS 158393, a 4-hydroxycoumarin derivative is an example of an inhibitor that does not contain the catechol moiety and exhibits antiviral antiprotease and anti-integrase activity [194]. Efforts in simplifiying the structure of this inhibitor led to the identification of a minimal active pharmacophore that consists of a coumarin dimer [315]. Styrylquinoline derivatives have been synthesized to chelate the divalent metal in the CCD of IN. They are active against IN even in the absence of a catechol moiety. The most potent analogues inhibit IN at submicromolar concentrations and viral replication in CEM cells at nontoxic concentrations [197]. SAR studies of styrylquinolines identified regions in the phenyl ring that are required for activity. Based on these studies the proposed binding mode of styrylquinoline derivatives includes coordination to the divalent cation within the CCD of IN [322]. Styryquinolines selectivily inhibit the 3'-processing step by preventing IN-DNA recognition [68]. Several experiments indicated that styryquinolines may have an additional inhibitory effect occurring at an even earlier stage, during nuclear import of the PIC [206]. One resistant mutation that has been identified in the IN site of interaction

with LEDGF/p75 suggests that styrylquinolines prevent PIC import by interfering with the interaction between IN and the particular host factor. Further IN coding region mutations collected in the presence of styrylquinolines confirm that IN is a target of this class of inhibitors acting at steps prior to integration. The activity of styrylquinolines is not diminished by HIV mutant strains that confer resistance to DKAs suggesting a different binding site for the two classes of inhibitors. Thus, styrylquinolines could constitute an alternative type of IN inhibitors [13].

Diketoacids and Analogues

DKAs are the first family of validated IN inhibitors and were independently discovered by Shionogi & Co. Ltd and the Merck Research Laboratory. Shionogi simultaneously reported the first crystal structure of 5CITEP within the core of HIV-1 IN with Merck publishing a series of DKA derivatives. The general structure of the DKAs depicted in Table **1** presents a diketo moiety connected to an acidic group (R1) and an aromatic group (R2). The Shionogi compound 5CITEP bears a tetrazole as R1 and the Merck derivatives a carboxylic acid group. The Merck derivatives were identified by screening a library of more than 250000 compounds with an assay of preassembled recombinant IN on immobilized oligonucleotides. The most potent compounds L-731,988 and L-708,906 inhibited strand transfer in recombinant IN at 50 nM and cell based assays at low micromolar concentrations. Inhibitory activity was also observed with PIC based assays [117]. Further optimization by Merck led to the discovery of heterocyclic analogues of DKAs where the 1,3-diketo moiety had been replaced by the more metabolicaly stable 8-hydroxy-1,6-naphthyridine ketones. Evidence for the *in vivo* efficacy of DKAs were presented by Hazuda *et al.* by demonstrating antiviral activity of L-870,812 in rhesus macaques infected with SHIV [119]. Compound L-870,810 reached phase I clinical trials but was later withdrawn due to toxicity in animals. L-900,612 (MK-05180), a sister derivative of L-870,810 developed by Merck, has advanced through clinical trials to enter phase III clinical studies. It inhibits strand transfer at nanomolar concentrations and acts synergistically with other antiretroviral drugs [51, 158]. The Shionogi derivative S-1360 is a structural analogue of 5CITEP where the tetrazole has been replaced by a triazole moiety. S-1360 inhibits IN at nanomolar concentrations and viral replication at micromolar concentrations. It is the first inhibitor to enter clinical trials but has been replaced by Shionogi-GlaxoSmithKline with the follow-up compound GSK-810871 after reviewing the data of the phase I/II studies [158, 303]. The novel IN inhibitor GS-9137 discovered by Japan Tobacco Inc. and licensed to Gilead Sciences bears a quinolone core structure instead of the diketo moiety and inhibits strand transfer and viral replication at nanomolar concentrations. GS-9137 is currently under clinical phase II studies. It exhibits synergistic effects when administered with RT inhibitors and additive effects with PR inhibitors [256].

DKAs are referred to as strand transfer inhibitors because they selectively inhibit the strand transfer step and require assembly of the full-length IN onto target DNA [83, 117]. DKAs can also inhibit the 3'-processing step at 30-70 fold higher concentrations. The validation of IN as the molecular target of DKAs was primarily based on the accumulation of resistant viruses that were collected in the presence of the inhibitors. Mutations within the IN coding region were identified and then introduced into recombinant viruses. The same resistance profile was observed for the recombinant viruses as for the isolated resistant strains. Furthermore HIV-1 infected cells treated with DKAs showed an accumulation of LTR viral circles [117]. In a time-of-addition experiment the addition of a DKA could be postponed for 7 hours, a step that coincides with HIV integration [236].

SAR studies have identified important features for activity [229, 297]. It is generally accepted that the diketo acid moiety acts by forming metal chelation complexes with the divalent cation at the CCD of IN. While this feature is important for activity, the aromatic part directs the strand transfer selectivity [183]. Substitution studies on the aromatic part revealed that it can accommodate a variety of substituents such as azido [277, 313] and photoactivable cross-coupling groups [314] at the optimal meta position. The diketo acid moiety was replaced by bioisosteres that mimic the ketone, the enolizable ketone and the carboxyl oxygen while adopting at the same time a coplanar conformation. Such features are the 8-hydroxy-1,6-naphthyridines and the 8-hydroxy-1,6-naphthyridine-7-carboxamides that mask the enolizable ketone within the naphthyridine ring system and use one of the nitrogen atoms as isosteres of the carboxylic acid [321]. The isolated carboxylic acid was replaced by acidic bioisosteres such as a tetrazole and triazole and by basic bioisosteres such as a pyridine ring and the enolizable ketone by a phenolic hydroxyl group [58, 302]. Isosteres of the diketo acid moiety exhibit different preferences for metal affinity. Carboxylates show similar affinity for both Mg^{2+} and Mn^{2+} whereas nitrogen-containing heterocycles show preference for Mn^{2+} [112, 184]. This observation can explain the lack of antiviral activity in cellular systems for the tetrazole containing DKAs since Mg^{2+} is the preferred metal ion *in vivo* [229]. In an attempt to find molecules that can accommodate two metal ions within the same active site or within two adjacent active sites, a series of dimeric DKA-containing inhibitors were prepared. All the compounds were highly potent against all IN activities but strand transfer selectivity varied from 1 to 29-fold. It was proposed that the bifunctional DKAs bind competitively with both the target and donor DNA in contrast to the monofunctional DKAs that bind competitively to target DNA. Therefore, bifunctional DKAs could broaden the inhibition area of DKAs to include both 3'-processing and strand transfer [183]. Docking experiments showed one diketo moiety to interact with a Mg^{2+} ion and the other diketo moiety to occupy an area near the catalytic residue E152 [172]. DKAs are currently the only inhibitors for which structural information exist within the IN context, through the co-crystallization of 5CITEP with the CCD domain of IN. Nevertheless, this crystal structure has been met with skepticism due to the absence of target DNA from the crystal structure. Furthermore, a direct contact between the inhibitor and the catalytic residues D64 and D116, or the magnesium ion was not observed [109]. Molecular dynamics simulations indicated that the position of 5CITEP is a result of crystal packing effects and can adopt a different conformation within the binding site [220, 270, 271]. Further simulations with other DKAs revealed a different mode of interaction than 5CITEP [144, 184].

In order to account for the strand transfer selectivity, a model for the mechanism of action of DKAs was proposed. Following 3'-processing IN undergoes a structural change leading to a distinct binding site for the target DNA. DKAs bind within that unique site near the nucleophilic end of the viral DNA and to an acceptor site at the target DNA [83]. Inhibition of strand transfer occurs due to prevention of the nucleophilic attack of the viral DNA to the acceptor site of the target DNA [183]. Coordination of the divalent cation at the active site of IN with DKAs is necessary to support the mechanism of action of these inhibitors [112]. It has been proposed that DKAs act as interfacial inhibitors by binding at the interface of the viral DNA-IN and metal complex right after the 3'-processing step, thus stabilizing the transition state and preventing strand transfer. This hypothesis was based on the study of the resistant mutants that occurred with residues important for DNA binding and around the DDE catalytic site [97]. As with RT and PR inhibitors drug resistance can develop for the DKA IN inhibitors. However, the DKA IN resistant profile depends on the substituents of the inhibitor. This indicates that the resistance problem could be overcome by changing substituents on a fixed pharmacophore [118]. In a study carried out by Reinke *et al.* a DKA derivative inhibited the replication of 12 types of HIV-1 isolates, proving that the naturally occurring variation within the IN gene will not be a major obstacle in the development of these drugs [246].

Pyrano-Dipyrimidines

An alternative class of IN inhibitors include the pyrano-dipyrimidines and the most potent analogue V-165, inhibits viral replication in infected cells at micromolar concentrations, 14 times below its cytotoxicity concentration. SAR studies pointed to the free sulfhydryl functionality and the para-nitro substituents as important moieties for the activity of V-165. According to enzymatic assays, V-165 inhibits both reverse transcription and integration. Time-of-addition experiments indicated that V-165 interfered with viral replication at a step following reverse transcription. In order to determine the major antiviral target of V-165 quantitative *Alu*-PCR was carried out confirming that V-165 interferes with proviral DNA integration. V-165 possibly inhibits the first step of integration, the complex formation between DNA-IN in the cytoplasm and thereby inhibits the subsequent 3'-processing step [231].

CLINICAL APPLICATIONS - FUTURE DIRECTIONS

Despite the availability of RT and PR inhibitors in the treatment of HIV, the development of inhibitors that target the third enzyme IN is essential for the successful treatment of HIV-infected patients. Considerable progress towards designing IN inhibitors has been made over the last years and several lead compounds have been identified. To date the most promising inhibitors are the DKAs and their derivatives. Two compounds are currently in clinical trials MK-0518 and GS-9137. MK-0518 has *in vitro* inhibitory activity IC_{50} 33 nM, is active against resistant strains and shows synergistic effects with the currently approved antiretroviral drugs. In a phase II study involving HIV-infected patients receiving HAART, MK-0518 was well-tolerated and the side-effects reported were similar to the placebo group. In a recent phase III study in patients that did not respond to antiretroviral treatment, MK-0518 in combination with optimized background therapy demonstrated superior antiretroviral effects compared to the placebo group and was well-tolerated (www.retroconference.org/2007). GS-9137 has *in vitro* inhibitory activity IC_{50} 0.2 nM and is active against resistant strains. In a phase I monotherapy study, GS-9137 was well-tolerated by HIV infected patients and the reported side-effects were similar to those of the placebo group. In a phase II study GS-9137 in combination with nucleoside reverse transcriptase inhibitors demonstrated superior efficacy to boosted protease inhibitors. The resistance profile of GS-9137 showed a number of IN mutations that exhibited reduced susceptibility to the drug but retained resistance with other IN inhibitors and antiretroviral drugs from other classes (www.retroconference.org/2007). The other promising compounds in preclinical trials belong to the classes of pyrano-dipyrimidines and styrylquinolines [158].

The IN inhibitors described so far span a broad section of the integration process. DKAs and certain hydroxylated aromatics inhibit the catalytic activities of IN, strand transfer and in some cases 3'-processing, by forming complexes with the divalent cation within the CCD of IN. Styrylquinoline derivatives interefere with IN-DNA complex formation and prevent the nuclear translocation of the PIC possibly by preventing the interaction between IN and the host factor LEDGF/p75. In the same manner other factors that are part of the PIC, such as INI1 and RT for which an interaction with IN has been established, can serve as potential targets for inhibition. Peptide inhibitors target the dimerization interfaces of IN and monoclonal antibodies have been designed to target the isolated CTD and NTD domains. In a similar manner monoclonal antibodies can be designed to target other important regions of IN. The HHCC motif of the NTD domain of IN is another possible site for inhibition since it promotes multimerization and enhances catalytic activity. An indirect way to prevent integration is by targeting the host cell DNA repair enzymes. Unlike the above mentioned inhibitors, inhibitors of this class may not develop drug resistance due to the low mutational rate of the host cell enzymes [223]. Although the exact mechanism of integration is not yet fully understood, the IN-field is advancing rapidly making the development of clinically viable IN inhibitors, tangible.

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ABBREVIATIONS

5CITEP	=	1-(5-chloroindol-3-yl)-3-hydroxy-3-(*2H*-tetrazol-5-yl)-propenone
AIDS	=	Acquired immune deficiency syndrome
ASV	=	Avian sarcoma virus
BAF	=	Barrier to autointegration factor
CAPE	=	Caffeic acid phenyl ester
CCD	=	Catalytic core domain
CoMFA	=	Comparative molecular field analysis

CoMSIA	=	Comparative molecular similarity analysis
DCs	=	Dendritic cells
DFT	=	Density functional theory
CTD	=	C-terminal domain
EVA	=	Eigen value analysis
FCS	=	Fluorescence correlation spectroscopy
HAART	=	Highly active antiretroviral treatment
HIV-1	=	Human immunodeficiency virus type 1
HIV-2	=	Human immunodeficiency virus type 2
HMG-I(Y) or HMGA1	=	High mobility group chromosomal protein A1
IBD	=	Integrase binding domain
IN	=	Integrase
INI1	=	Integrase interactor 1
LEDGF/p75	=	Lens epithelium derived growth-factor
LTR	=	Long terminal repeat
MA	=	Matrix
MoMLV	=	Moloney murine leukemia
NLS	=	Nuclear localization signal
NMR	=	Nuclear magnetic resonance
NTD	=	N-terminal domain
PASS	=	Putative actives sites with spheres
PCR	=	Polymerase chain reaction
PIC	=	Preintegration complex
PR	=	Protease
QSAR	=	Quantitative structure activity relationships
RT	=	Reverse transcriptase
SAR	=	Structure activity relationship
SHIV	=	Simian-human immunodeficicency virus

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