Running title:

Targeting HIV Integration

Title:

HIV-1 Integrase Inhibitors: Update and Perspectives

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Abstract

HIV replication requires the insertion of the viral genome inside the nuclear genome of infected cells through a recombination process catalyzed by the virus-encoded enzyme, integrase. HIV integrase has recently been recognized as a reachable antiviral target following the promising results of integrase inhibitors in clinical trials. The present review focuses on the recent advances in understanding the cellular mechanisms of HIV integration and the sites of actions of inhibitors. It also provides an extensive list of the known mutations that have been characterized for HIV-1 integrase with their impact on integrase activity, viral replication and response to anti-integrase drugs. Novel rational approaches for inhibiting HIV integration are also discussed, as well as the two integrase inhibitors in clinical trials and other selected inhibitors in development.

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I. Foreword

The encouraging results reported for two integrase inhibitors in clinical trials and the recent insights in the cellular cofactors of DNA integration, have renewed interest in HIV integrase pharmacology and cellular biology. The current review gives an overview of the functions of integrase and the cofactors for integration. It focuses on pharmacological approaches to interfere with integration and provides an extensive list of integrase mutations with their functional and pharmacological impacts. References have been kept to a minimum. Further information can be found in recent reviews (Dayam *et al.*, 2006; Marchand *et al.*, 2006a; Pommier *et al.*, 2005; Savarino, 2006; Semenova *et al.*, 2006b).

II. Integration: a Crucial Step in the HIV Life Cycle.

Like other retroviruses, the HIV genome consists of single-stranded RNA. During infection, the viral RNA is released into the host cell following fusion of the viral particles to the cell membrane (Figure 1). The viral RNA then serves as a template for the synthesis of a double-stranded DNA copy of the viral RNA (cDNA) bearing long terminal repeats (LTR) by the HIV-encoded reverse transcriptase (Sierra *et al.*, 2005). The conversion of the viral RNA into cDNA is necessary for making new viral RNA copies and for transcribing the virally encoding genes. Transcription of the viral cDNA also requires its insertion into a host chromosome. That insertion (integration) is catalyzed by the HIV-encoded enzyme – integrase (IN). The viral cDNA integrated

into a host chromosome is called provirus (Figure 1). Depending on the sites of integration, the provirus can be constitutively transcribed if it is integrated near an active promoter, or remain silent until a stress response triggers transcription. Transcription of the viral genome and of the viral genes followed by translation, packaging, fusion and maturation supply the molecular components for the release of the new infectious viral particles (Figure 1).

<Figure 1>

A. HIV-1 Integrase Structure.

The three viral enzymes (protease, reverse transcriptase and IN) are encoded within the HIV *pol* gene and translated as a polyprotein (Figure 2). IN (32-kDa) is released from the polyprotein by the HIV protease during maturation. The IN protein consists of three domains: N-terminal, core (or catalytic), and C-terminal domains (Figure 2) (Chiu and Davies, 2004). The N-terminal domain enhances IN multimerization through zinc coordination (HHCC motif) and promotes concerted integration of the two viral cDNA ends together into a host cell chromosome. The C-terminal domain is responsible for metal-independent, sequence-independent DNA binding. Each HIV-1 IN molecule contains a catalytic site within the core domain bearing three essential amino acids: Asp64, Asp116, and Glu152 (D,D-35-E motif). These acidic residues coordinate at least one and probably two divalent cations (Mg^{2+} or Mn^{2+}) that form a bridge with the DNA substrates (see Figure 3) (Marchand *et al.*, 2006a). Mutation of any of these residues abolishes IN enzymatic activities and viral replication (Table 1). IN functions as a multimer.

<Figure 2>

<Figure 3>

<Table 1>

B. Chemistry of Retroviral Integration.

During the first reaction catalyzed by IN [3'-processing (3'-P)], the donor (viral) DNA is hydrolyzed immediately 3' from the conserved CA dinucleotide at both 3'-ends of the LTRs (Vink *et al.*, 1991) (Fig. 3a). 3'-P releases 3'-terminal nucleotides (generally pGpT dinucleotide for HIV-1) and generates 3'-hydroxyl nucleophilic ends at both ends of the viral DNA. The next step, integration [strand transfer (ST)] proceeds in the nucleus through a transesterification reaction, where the processed nucleophilic 3'-OH ends of the donor (viral) cDNA are inserted into the backbone of the target (host) DNA (Vink *et al.*, 1990) (Figure 3b and c]. Both ends insert with a five-base-pair stagger across the DNA major groove of the target chromosomal DNA following the trimming of the integrated HIV cDNA junctions, gap filling and ligation, which are probably carried out by the host cell DNA repair mechanism (Figure 1) (Pommier *et al.*, 2005).

<Figure 3>

C. Integration Occurs within a Large Macromolecular Complex.

Cellular integration requires several cofactors in addition to IN (Van Maele *et al.*, 2006). The preintegration complex (PIC) is a crucial structural unit required for integration. The PIC contains proteins from both the viral core (matrix, nucleocapsid, reverse transcriptase) and the host cell [lens epithelium-derived growth factor

(LEDGF/p75), INI1, barrier-to-autointegration factor (BAF), HMGA1] (Figure 1). The viral cDNA is probably bound to IN immediately following reverse transcription. IN also binds directly to LEDGF/p75, INI1, reverse transcriptase and matrix (Van Maele and Debyser, 2005).

Despite many reports describing the importance of co-factors for HIV integration, our understanding of the mechanisms regulating integration remains incomplete. In our opinion, the main function of the PIC is to separate the two reactions catalyzed by IN (3'-P and ST) into different cellular compartments over time *in vivo*, while *in vitro* these two reactions occur consequently without delay. It is plausible that IN may be kept inactive in the PIC until migration into the nucleus to prevent autointegration. A cellular cofactor present in the PIC, BAF (Barrier to Autointegration Factor), prevents autointegration (Zheng *et al.*, 2000). Other PIC-associated factors probably also keep integrase inactive. For instance, HIV reverse transcriptase can inhibit IN catalytic activities *in vitro* (Oz *et al*., 2002). The viral cDNA is protected from nucleases after isolation of PIC only with wild-type IN, whereas it is sensitive to nuclease digestion when the PIC are formed with IN mutant (Chen *et al.*, 1999; Miller *et al.*, 1997). Thus, IN is probably involved not only in 3'-P very early in the viral cycle but also for PIC formation. PIC formation could possibly be triggered by 3'-P completion. It is also likely that PIC rearrangements leading to the reactivation of IN, occur during the passage of the PIC through the nuclear envelope and/or its association with chromatin.

We will focus on two factors that are known to tether the viral cDNA to chromosomal host DNA, emerin and LEDGF/p75. Recently, the interaction of viral

cDNA with chromatin has been reported to be dependent on emerin, a nuclear protein associated with PIC through BAF. Both emerin and BAF are required for the appropriate localization of the viral cDNA in the nucleus before integration. However, emerin and BAF do not facilitate HIV integration (Jacque and Stevenson, 2006). Another molecular tether linking HIV-1 IN protein to chromatin is LEDGF/p75. Binding of LEDGF/p75 to IN targets IN to chromatin, and promotes strand transfer. Failure of HIV replication in LEDGF/p75 knockdown cells suggests that LEDGF/p75 is a critical co-factor for efficient HIV integration. Disrupting its interactions with IN could be considered as a therapeutic strategy (Cherepanov *et al.*, 2005; Cherepanov *et al.*, 2003; Ciuffi *et al.*, 2005; Llano *et al.*, 2006; Maertens *et al.*, 2003; Vandekerckhove *et al.*, 2006).

III. Approaches to Inhibit HIV Integration.

A. Small molecule inhibitors of HIV Integrase enzymatic activities.

Searching for enzymatic inhibitors of IN is straightforward. High throughput assays have been developed, and several *in vitro* assays are routinely used to elucidate the drugs mechanisms of action (Marchand *et al.*, 2001). 3'-P assays monitor the release of the terminal dinucleotide from an oligonucleotide duplex mimicking the viral LTR ends whereas strand transfer results in larger DNA molecules. Pre-cleaved ("3'-processed") substrates are used to determine ST inhibition independently from 3'-P. Disintegration – the third IN-catalyzed reaction [the reverse of ST (Chow *et al.*, 1992)], can be used to evaluate the site of drug

action as the IN catalytic core it is the only reaction that can be catalyzed by. Compounds that compete with target DNA within the enzyme catalytic site (Figure 3 b') produce preferential inhibition of ST over 3'-P and are generally ineffective against disintegration (Espeseth *et al*., 2000). Those inhibitors are commonly referred to as "STI" (Strand Transfer Inhibitors). In contrast, inhibitors that prevent the viral DNA binding to IN inhibit both 3'-P and ST with similar efficiency (Bonnenfant *et al.*, 2004; Marchand *et al.*, 2006b).

As it remains difficult to obtain drug-IN co-crystals, IN-DNA binding assays continue to be developed to investigate drug binding sites in the IN-DNA complex. The Schiff base (Mazumder and Pommier, 1995) and disulfide crosslinking (Johnson *et al.*, 2006b) assays can be used to determine whether a given drug affects viral DNA binding to IN or alters crucial ST contacts between the IN amino acid residue Q148 and the cytosine at the protruding viral DNA end (Johnson *et al.*, 2006a). A novel HIV IN inhibitor-binding site was discovered at the IN core dimer interface using photoaffinity labeling and mass spectrometric analysis (Al-Mawsawi *et al*., 2006).

Recently, the development of inhibitors has focused on targeting the D,D-35-E motif and chelating the divalent metal (Mg^{2+} vs. Mn^{2+}) bound at the interface of the IN-DNA complex (Figure 3) (Semenova *et al.*, 2006b). We have referred to this mode of inhibition as "interfacial inhibition" (Pommier and Cherfils, 2005; *Pommier et al.*, 2005; Pommier and Marchand, 2005), as the drugs bind at the interface of two macromolecules (here IN and DNA) (Figure 3b') and trap a catalytic intermediate (here the 3'-P step) thereby preventing productive catalytic activity (here, ST).

Interfacial inhibition is commonly observed for a broad range of natural products targeting a variety of cellular targets (Pommier and Cherfils, 2005). Particular attention was given to the D,D-35-E motif after 5CITEP (a diketo acid-like derivative) was first co-crystallized in the catalytic domain of HIV IN and shown to bind within the D,D-35-E motif (Goldgur *et al*., 1999). IN inhibitors currently in clinical trial (Table 2) also contain diketo-acid-like motifs that are believed to chelate divalent cations (Mg^{2+} or Mn^{2+}) within the D,D-35-E motif. Those drugs demonstrate preferential inhibition of the ST reaction. Preferential strand transfer inhibition (STI) was first observed for caffeic acid phenethyl ester (CAPE) and proposed to be related to chelation of an IN divalent metal (Fesen *et al*., 1993). This model was further developed for the diketo acid (DKA) derivatives, which were shown to act as competitors for the target (host chromosomal) DNA within the IN active site (Hazuda *et al.*, 2000). The benefits of the strand transfer inhibitors emerged with the characterization of more potent DKA compounds effective against HIV infection (Tables 2 and 3). IN residues involved in DKA and DKA-like resistance are listed in Table 1.

As 3'-P is a pre-requisite for ST and HIV integration, and is probably required for PIC formation, inhibiting 3'-P is a rational approach to inhibit HIV replication. It might also be logical to combine 3'-P inhibitors with the currently developed ST inhibitors. A styrylquinoline (SQL) derivative, FZ-41 inhibits both 3'-P and ST with similar efficiency (Bonnenfant *et al*., 2004) and has been confirmed as a cellular HIV IN inhibitor by developing a drug-resistant viruses. The antiviral activity of FZ 41 could serve as a paradigm for 3'-P inhibitors that could also prevent PIC formation.

Inhibition of the IN nuclear import after SQL treatment (Mousnier *et al.*, 2004) could be a consequence of PIC assembly failure.

B. Targeting the Preintegration Complex (PIC).

According to the paradigm of interfacial inhibition (Pommier and Cherfils, 2005; Pommier and Marchand, 2005), protein-protein interactions (IN monomer - IN monomer, IN-LEDGF/p75, IN-matrix, IN-INI1, matrix-BAF, etc) and protein-DNA junctions (IN-viral DNA, BAF-viral DNA, etc) within the PIC are equally important for integration. Alteration of any of these interfaces may prevent integration. For example, diketo-acid-like inhibitors change the target DNA binding surface within the IN active site (protein-DNA interface) due to the chelation of divalent cations after 3'-P (Figure 3b'). Another candidate target is LEDGF/p75, as HIV replication is markedly reduced in LEDGF/p75 knockdown cells due to absence of IN-LEDGF interaction (Vandekerckhove *et al*., 2006). Therefore, prevention or alteration of macromolecular contacts among the PIC components is a rational and promising approach for the inhibition of HIV integration. Specific IN residues interacting with PIC components are highlighted in Table 1. Assays developed to identify inhibitors of IN enzymatic functions (protein-DNA contact) may not identify interfacial inhibitors because the interfacial contacts with PIC components are not required for IN enzymatic activities *in vitro* (Emiliani *et al.*, 2005). Such compounds could therefore be mistakenly ruled out during routine biochemical screening, although they still may alter *in vivo* PIC formation, which underlines the need to develop additional assays for integration inhibitors.

IV. Inhibitors in Clinical Trials.

The first pharmacological inhibitor in clinical trial was the guanosine quartet (AR 177, Zintevir, Aronex Pharmaceuticals, Inc). In spite of it being identified initially as an IN inhibitor based on *in vitro* activity (Mazumder *et al*., 1996a), this compound was also found to inhibit viral entry *in vivo*. AR 177 was discontinued after Phase I/II clinical trial. The next two IN inhibitors in clinical trials (Savarino, 2006; Semenova *et al.*, 2006c), were a naphthyridine carboxamide derivative (L-870,810) (Merck & Co (Hazuda et al., 2004; Little et al., 2005) and a diketo acid derivative (S-1360/GW-810781) (Shionogi-GlaxoSmithKline Pharmaceuticals) (GlaxoSmithKline, 2003; Yoshinaga *et al*., 2002) (Table 2). Both were recently discontinued after phase II trials. L-870,810 caused toxicity in dogs during long-term dosing. The reasons for termination of the clinical trial for S-1360 have not been fully disclosed (GlaxoSmithKline, 2003). However their well-tolerated properties in humans (Table 2) demonstrated the proof of concept for using of HIV-1 IN inhibitors as antiretrovirals.

<Table 2>

Two IN inhibitors are currently in clinical trial: a derivative of quinolone antibiotics (JTK-303/GS-9137, Gilead Sciences, Inc.) (DeJesus et al., 2006; Kawaguchi *et al.,* 2006; Matsuzaki *et al.*, 2006; Sato *et al*., 2006) and a STI from “Merck & Co” (MK-0518) (Markowitz *et al*., 2006; Morales-Ramirez *et al*., 2005; Laufer *et al*., 2006; Summa *et al*., 2006) (Table 2). Their efficacy and good-tolerance in heavily pretreated patients that had failed reverse transcriptase and protease

inhibitors (Table 2) is encouraging, especially for patients living with multidrug-resistant HIV.

V. Inhibitors in Preclinical Development.

Recent reviews have dealt in details with the development and recent progress in the design of IN inhibitors (Dayam *et al.*, 2006; Johnson *et al.*, 2004; Pommier *et al.*, 2005; Savarino, 2006; Semenova *et al.*, 2006b). Therefore, no attempt is made here to list all the inhibitors. We will only summarize and review selected classes of HIV IN inhibitors as potential as drug leads (Table 3). Table 1 also lists all the IN residues involved in drug resistance mechanisms.

Screening of biologically active natural extracts (plant, microbial, fungi, marine organisms) continues to serve as a source for identifying new leads. A majority of reported IN inhibitors are derived from natural products. Examples include caffeic acid phenethyl ester (CAPE) (Fesen *et al.*, 1993), anthracyclines (Fesen *et al*., 1993), curcumins (Mazumder *et al*., 1995; Mazumder *et al*., 1997), flavones and flavonoids (Fesen *et al*., 1994; Rowley *et al*., 2002), lignans and lignaloids (*Eich et al*., 1996; Ovenden *et al*., 2004), depsides and depsidones (Neamati *et al.*, 1997a), α-hydroxytropolones (Semenova *et al*., 2006a), lithospermic acid (Abd-Elazem *et al*., 2002), indolicidin (Krajewski *et al.*, 2003; Krajewski *et al.*, 2004; Marchand *et al*., 2006b), chicoric acids (Meadows *et al*., 2005; Neamati *et al*., 1997b), integrasone (Herath *et al*., 2004), and coumarins (Mazumder *et al*., 1996a; Zhao *et al*., 1997). Despite the fact that many of these compounds inhibit other viral targets, such as reverse transcriptase, protease, and gp120 (Mazumder *et al.*, 1996b; Pluymers *et*

al., 2000; Pommier and Neamati, 1999; Robinson *et al.*, 1998; Semenova *et al.*, 2006a), their structure activity relationship demonstrated the importance of hydroxy groups for anti-IN activity as well as the suggestion of their possible mechanism of action as metal chelators (Fesen *et al*., 1994). An interesting approach consist in doing parallel structure-activity relationship studies with closely related HIV targets such as IN and RNase H (Semenova *et al.*, 2006a). A recently identified new natural product IN inhibitor is funalenone (Shiomi *et al*., 2005), isolated from Penicillium sp. FKI-1463 (Table 3) shows good antiviral activity.

<Table 3>

At least three strategies are currently used for the discovery of synthetic IN inhibitors: 1) chemical derivatives based on previously known IN inhibitors such as diketo acid (Barreca *et al*., 2005; Di Santo *et al*., 2005), naphthyridine (Embrey *et al*., 2005; Guare *et al*., 2006), styrylquinoline (Normand-Bayle *et al*., 2005), L-chicoric acid (Charvat *et al*., 2006), and a-hydroxytropolones (Budihas *et al.*, 2005; Didierjean *et al.*, 2005; Semenova *et al.*, 2006a); 2) three-dimensional pharmacophore searches based on previously discovered compounds (Deng *et al*., 2006); 3) hybrid molecules comprised of core structures of two or more known inhibitors [DKA-catechol (Maurin *et al*., 2006), DKA-nucleobase scaffold hybrids (Nair *et al*., 2006)]. At the same time, bifunctional compounds (that contain two identical active groups) provide a rationale for further work due to the potent inhibitory properties of a bifunctional DKA derivative (Cpd 8, Table 3) (Di Santo et al., 2005) and of geminal disulfone analogues of the chicoric acid (compound #4), Table 3 (Meadows *et al*., 2005).

As IN functions as a multimer, dimerization inhibitors (Camarasa *et al*., 2006) ought to be considered. However, the limitation is to develop assays that can unambiguously provide evidence for such a mechanism. Along the same lines, it is logical to consider drugs that bind at the interface of the macromolecular complexes formed by IN and cellular cofactors during integration. Inhibiting the IN-LEDGF interface would require the development of assays that monitor protein-protein interactions not only by reducing their formation but also by stabilizing/trapping abortive intermediates, as in the case of the interfacial inhibitors (Pommier and Cherfils, 2005; Pommier and Marchand, 2005).

VI. Perspectives.

The major goal of anti-HIV therapy is the efficient suppression of viral load for as long as possible; i.e. without emergence of resistant viruses. To achieve such a goal, it is rational to combine therapies targeting several viral targets. Virus-specific targets are always attractive because selective inhibitors should devoid of side effects as the infected cells lack the viral-specific target.

After the initial discovery of IN in 1978 (Grandgenett *et al*., 1978) and establishing its requirement for HIV replication (Hippenmeyer and Grandgenett, 1984), major discoveries have paved the way for the development of IN inhibitors. These include *in vitro* assays for integration (Bushman and Craigie, 1991; Craigie *et al*., 1990; Craigie *et al*., 1991; Fitzgerald *et al*., 1991; Katzman *et al*., 1989; Sherman and Fyfe, 1990); identification of IN domains and highly conserved residues ((Engelman and Craigie, 1992; van Gent *et al*., 1993; Vink *et al*., 1993; Vink and

Plasterk, 1993) (Table 1); determination of X-ray crystal structures of the core and C-domains (Bujacz *et al*., 1995; Chen *et al*., 2000; Dyda *et al*., 1994) and elucidation of the solution of the structure of the N-domain (Cai *et al*., 1997); and the role of cellular cofactors in HIV integration (Cherepanov *et al*., 2005).

Promising results of clinical trials for IN inhibitors (DeJesus *et al.*, 2006; Markowitz *et al*., 2006; Savarino, 2006) shows the feasibility of using IN inhibitors as antiretroviral therapy. This tremendous achievement will promt the developmentof new inhibitors based on the existing ones and on novel chemotypes. Obtaining co-crystal structures for the most effective and promising inhibitors is limited by the challenge of solving the structure of full length integrase bound to its DNA substrates (donor viral and acceptor target DNA duplexes). However, it is not excluded that the inhibitors themselves might help to elucidate such structures if they can act as interfacial inhibitor and trap stable macromolecular complexes. Together with the mapping of drug resistance IN mutations, these structures should provide rationales for further chemical modifications and improvement of the inhibitors. The search for clinically effective IN inhibitors include optimization of pharmacological parameters such as a reduced binding to human serum proteins and limited dependence on metabolitic activation pathways (Laufer *et al*., 2006). Finally, besides systemic therapies, topical IN inhibitors are worthwhile pursuing as curative and preventive therapies.

References

Abd-Elazem, I. S., Chen, H. S., Bates, R. B., and Huang, R. C. (2002). Isolation of two highly potent and non-toxic inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase from Salvia miltiorrhiza. *Antiviral Res.* **55,** 91-106.

Al-Mawsawi, L. Q., Fikkert, V., Dayam, R., Witvrouw, M., Burke, T. R. Jr., Borchers, C.H., and Neamati N. (2006). Discovery of a small-molecule HIV-1 integrase inhibitor-binding site. *Proc Natl Acad Sci U S A* **103,** 10080-10085.

Barreca, M. L., Ferro, S., Rao, A., De Luca, L., Zappala, M., Monforte, A. M., Debyser Z, Witvrouw M and Chimirri A. (2005). Pharmacophore-based design of HIV-1 integrase strand-transfer inhibitors. *J Med Chem* **48,** 7084-7088.

Bischerour, J., Leh, H., Deprez, E., Brochon, J.C., and Mouscadet, J. F. (2003) Disulfide-linked integrase oligomers involving C280 residues are formed in vitro and in vivo but are not essential for human immunodeficiency virus replication. *J Virol* **77**, 135-141.

Bonnenfant, S., Thomas, C. M., Vita, C., Subra, F., Deprez, E., Zouhiri, F., Desmaele, D., D'Angelo, J., Mouscadet, J. F., and Leh, H. (2004). Styrylquinolines, integrase inhibitors acting prior to integration: a new mechanism of action for anti-integrase agents. *J Virol* **78,** 5728-5736.

Budihas, S. R., Gorshkova, I., Gaidamakov, S., Wamiru, A., Bona, M. K., Parniak, M. A., Crouch, R. J., McMahon, J. B., Beutler, J. A., and Le Grice, S. F. (2005). Selective inhibition of HIV-1 reverse transcriptase-associated ribonuclease H activity by hydroxylated tropolones. *Nucleic Acids Res* **33,** 1249-1256.

Bujacz, G., Jaskolski, M., Alexandratos, J., Wlodawer, A., Merkel, G., Katz, R. A., and Skalka, A. M. (1995). High-resolution structure of the catalytic domain of avian sarcoma virus integrase. *J Mol Biol* **253,** 333-346.

Bushman, F. D., and Craigie, R. (1991). Activities of human immunodeficiency virus (HIV) integration protein in vitro: specific cleavage and integration of HIV DNA. *Proc Natl Acad Sci U S A* **88,** 1339-1343.

Cai, M., Zheng, R., Caffrey, M., Craigie, R., Clore, G. M., and Gronenborn, A. M. (1997). Solution structure of the N-terminal zinc binding domain of HIV-1 integrase. *Nat Struct Biol* **4,** 567-577.

Calmels, C., de Soultrait, V. R., Caumont, A., Desjobert, C., Faure, A., Fournier, M., Tarrago-Litvak, L., and Parissi, V. (2004) Biochemical and random mutagenesis analysis of the region carrying the catalytic E152 amino acid of HIV-1 integrase. *Nucleic Acids Res* **32**, 1527-1538.

Camarasa, M. J., Velazquez, S., San-Felix, A., Perez-Perez, M. J., and Gago, F. (2006). Dimerization inhibitors of HIV-1 reverse transcriptase, protease and integrase: a single mode of inhibition for the three HIV enzymes? *Antiviral Res* **71,** 260-267.

Cannon, P. M., Wilson, W., Byles, E., Kingsman, S. M., and Kingsman, A. J. (1994) Human immunodeficiency virus type 1 integrase: effect on viral replication of mutations at highly conserved residues. *J Virol* **68**, 4768-4775.

Charvat, T. T., Lee, D. J., Robinson, W. E., and Chamberlin, A. R. (2006). Design, synthesis, and biological evaluation of chicoric acid analogs as inhibitors of HIV-1 integrase. *Bioorg Med Chem* **14**, 4552-4567.

Chen, H., Wei, S. Q., and Engelman, A. (1999). Multiple integrase functions are required to form the native structure of the human immunodeficiency virus type I intasome. *J Biol Chem* **274,** 17358-17364.

Chen, J. C., Krucinski, J., Miercke, L. J., Finer-Moore, J. S., Tang, A. H., Leavitt, A. D., and Stroud, R. M. (2000). Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains: a model for viral DNA binding. *Proc Natl Acad Sci U S A* **97** 8233-8238.

Cherepanov, P., Ambrosio, A. L., Rahman, S., Ellenberger, T., and Engelman, A. (2005). Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proc Natl Acad Sci U S A* **102** 17308-17313.

Cherepanov, P., Maertens, G., Proost, P., Devreese, B., Van Beeumen, J., Engelborghs, Y., De Clercq, E., and Debyser, Z. (2003). HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem* **278** 372-381.

Cherepanov, P., Pluymers, W., Claeys, A., Proost, P., De Clercq, E., and Debyser, Z. (2000) High-level expression of active HIV-1 integrase from a synthetic gene in human cells. *Faseb J* **14**, 1389-1399.

Chiu, T. K., and Davies, D. R. (2004). Structure and function of HIV-1 integrase. *Curr Top Med Chem* **4,** 965-977.

Chow, S. A., Vincent, K. A., Ellison, V., and Brown, P. O. (1992). Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. *Science*, **255** 723-726.

Ciuffi, A., Llano, M., Poeschla, E., Hoffmann, C., Leipzig, J., Shinn, P., Ecker, J. R., and Bushman, F. (2005). A role for LEDGF/p75 in targeting HIV DNA integration. *Nat Med* **11,** 1287-1289.

Craigie, R., Fujiwara, T., and Bushman, F. (1990). The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration in vitro. *Cell* **62,** 829-837.

Craigie, R., Mizuuchi, K., Bushman, F. D., and Engelman, A. (1991). A rapid in vitro assay for HIV DNA integration. *Nucleic Acids Res*, **19** 2729-2734.

Dayam, R., Deng, J., and Neamati, N. (2006). HIV-1 integrase inhibitors: 2003-2004 update. *Med Res Rev* **26,** 271-309.

DeJesus, E., Berger, D., Markowitz, M., Cohen, C., Hawkins, T., Ruane, P., Elion, R., Farthing, C., Zhong, L., Cheng, A. K., McColl, D., and Kearney, B. P. (2006). Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. *J Acquir Immune Defic Syndr* **43,** 1-5.

Deng, J., Kelley, J. A., Barchi, J. J., Sanchez, T., Dayam, R., Pommier, Y., and Neamati, N. (2006). Mining the NCI antiviral compounds for HIV-1 integrase inhibitors. *Bioorg Med Chem* **14**, 3785-3792.

De Rijck, J., Vandekerckhove, L, Gijsbers, R., Hombrouck, A., Hendrix, J., Vercammen, J., Engelborghs, Y., Christ, F., and Debyser, Z. (2006) Overexpression of the Lens Epithelium-Derived Growth Factor/p75 Integrase Binding Domain Inhibits Human Immunodeficiency Virus Replication. *J. Virol.* **80**, 11498-11509.

Di Santo, R., Costi, R., Artico, M., Ragno, R., Greco, G., Novellino, E., Marchand, C., and Pommier, Y. (2005). Design, synthesis and biological evaluation of heteroaryl diketohexenoic and diketobutanoic acids as HIV-1 integrase inhibitors endowed with antiretroviral activity. *Farmaco* **60,** 409-417.

Didierjean, J., Isel, C., Querre, F., Mouscadet, J. F., Aubertin, A. M., Valnot, J. Y., Piettre, S. R., and Marquet, R. (2005). Inhibition of Human Immunodeficiency Virus Type 1 Reverse Transcriptase, RNase H, and Integrase Activities by Hydroxytropolones. *Antimicrob, Agents Chemother*. **49,** 4884-4894.

Drake, R. R., Neamati, N., Hong, H., Pilon, A. A., Sunthankar, P., Hume, S. D., Milne, G. W., and Pommier, Y. (1998) Identification of a nucleotide binding site in HIV-1 integrase. *Proc Natl Acad Sci U S A* **95**, 4170-4175.

Drelich, M., Wilhelm, R., and Mous, J. (1992) Identification of amino acid residues critical for endonuclease and integration activities of HIV-1 IN protein in vitro. *Virology* **188**, 459-468.

Dyda, F., Hickman, A. B., Jenkins, T. M., Engelman, A., Craigie, R., and Davies, D. R. (1994). Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* **266,** 1981-1986.

Eich, E., Pertz, H., Kaloga, M., Schulz, J., Fesen, M. R., Mazumder, A., and Pommier, Y. (1996). (-)-Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type-1 integrase. *J. Med. Chem.* **39,** 86-95.

Embrey, M. W., Wai, J. S., Funk, T. W., Homnick, C. F., Perlow, D. S., Young, S. D., Vacca, J. P., Hazuda, D. J., Felock, P. J., Stillmock, K. A., Witmer, M. V., Moyer, G., Schleif, W. A., Gabryelski, L. J., Jin, L., Chen, I. W., Ellis, J. D.,

Wong, B. K., Lin, J. H., Leonard, Y. M., Tsou, N. N., and Zhuang, L. (2005). A series of 5-(5,6)-dihydrouracil substituted 8-hydroxy-[1,6]naphthyridine-7-carboxylic acid 4-fluorobenzylamide inhibitors of HIV-1 integrase and viral replication in cells. *Bioorg. Med. Chem. Lett.* **15,** 4550-4554.

Emiliani, S., Mousnier, A., Busschots, K., Maroun, M., Van Maele, B., Tempe, D., Vandekerckhove, L., Moisant, F., Ben-Slama, L., Witvrouw, M., Christ, F., Rain, J. C., Dargemont, C., Debyser, Z., and Benarous, R. (2005). Integrase mutants defective for interaction with LEDGF/p75 are impaired in chromosome tethering and HIV-1 replication. *J. Biol. Chem*. **280,** 25517-25523.

Engelman, A., and Craigie, R. (1992). Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. *J. Virol.* **66,** 6361-6369.

Engelman, A., Bushman, F. D., and Craigie, R. (1993) Identification of discrete functional domains of HIV-1 integrase and their organization within an active multimeric complex. *EMBO J* **12**, 3269-3275.

Engelman, A., Englund, G., Orenstein, J. M., Martin, M. A., and Craigie, R. (1995) Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. *J. Virol.* **69**, 2729-2736.

Engelman, A., Liu, Y., Chen, H., Farzan, M., and Dyda, F. (1997) Structure-based mutagenesis of the catalytic domain of human immunodeficiency virus type 1 integrase. *J. Virol.* **71**, 3507-3514.

Espeseth, A. S., Felock, P., Wolfe, A., Witmer, M., Grobler, J., Anthony, N., Egbertson, M., Melamed, J. Y., Young, S., Hamill, T., Cole, J. L., and Hazuda, D. J. (2000). HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc. Natl. Acad. Sci. U S A* **97,** 11244-11249.

Fesen, M. R., Kohn, K. W., Leteurtre, F., and Pommier, Y. (1993). Inhibitors of human immunodeficiency virus integrase. *Proc. Natl. Acad. Sci. U S A* **90,** 2399-2403.

Fesen, M. R., Pommier, Y., Leteurtre, F., Hiroguchi, S., Yung, J., and Kohn, K. W. (1994). Inhibition of HIV-1 integrase by flavones, caffeic acid phenethyl ester (CAPE) and related compounds. *Biochem. Pharmacol.* **48,** 595-608.

Fikkert, V., Hombrouck, A., Van Remoortel, B., De Maeyer, M., Pannecouque, C., De Clercq, E., Debyser, Z., and Witvrouw, M. (2004) Multiple mutations in human immunodeficiency virus-1 integrase confer resistance to the clinical trial drug S-1360. *AIDS* **18**, 2019-2028.

Fikkert, V., Van Maele, B., Vercammen, J., Hantson, A., Van Remoortel, B., Michiels, M., Gurnari, C., Pannecouque, C., De Maeyer, M., Engelborghs, Y., De Clercq, E., Debyser, Z., and Witvrouw, M. (2003) Development of resistance against diketo derivatives of human immunodeficiency virus type 1 by progressive accumulation of integrase mutations. *J. Virol.* **77**, 11459-11470.

Fitzgerald, M. L., Vora, A. C., and Grandgenett, D. P. (1991). Development of an acid-soluble assay for measuring retrovirus integrase 3'-OH terminal nuclease activity. *Anal. Biochem.* **196,** 19-23.

GlaxoSmithKline. (2003). GlaxoSmithKline annual report. Brentford, Middlesex.

Gerton, J. L., Ohgi, S., Olsen, M., DeRisi, J., and Brown, P. O. (1998) Effects of mutations in residues near the active site of human immunodeficiency virus type 1 integrase on specific enzyme-substrate interactions. *J. Virol.* **72**, 5046-5055.

Goldgur, Y., Craigie, R., Cohen, G. H., Fujiwara, T., Yoshinaga, T., Fujishita, T., Sugimoto, H., Endo, T., Murai, H., and Davies, D. R. (1999). Structure of the HIV-1 integrase catalytic domain complexed with an inhibitor: a platform for antiviral drug design. *Proc. Natl. Acad. Sci. U S A* **96,** 13040-13043.

Grandgenett, D. P., Vora, A. C., and Schiff, R. D. (1978). A 32,000-dalton nucleic acid-binding protein from avian retravirus cores possesses DNA endonuclease activity. *Virology* **89,** 119-132.

Guare, J. P., Wai, J. S., Gomez, R. P., Anthony, N. J., Jolly, S. M., Cortes, A. R., Vacca, J. P., Felock, P. J., Stillmock, K. A., Schleif, W. A., Moyer, G., Gabryelski, L. J., Jin, L., Chen, I. W., Hazuda, D. J., and Young, S. D. (2006). A series of 5-aminosubstituted 4-fluorobenzyl-8-hydroxy-[1,6]naphthyridine-7-carboxamide HIV-1 integrase inhibitors. *Bioorg. Med. Chem. Lett*. **16**, 2900-2904.

Harper, A. L., Skinner, L. M, Sudol, M., and Katzman, M. (2001) Use of patient-derived human immunodeficiency virus type 1 integrases to identify a protein residue that affects target site selection. *J. Virol.* **75**, 7756-7762.

Hazuda, D. J., Anthony, N. J., Gomez, R. P., Jolly, S. M., Wai, J. S., Zhuang, L., Fisher, T. E., Embrey, M., Guare, J. P., Jr., Egbertson, M. S., Vacca, J. P., Huff, J. R., Felock, P. J., Witmer, M. V., Stillmock, K. A., Danovich, R., Grobler, J., Miller, M. D., Espeseth, A. S., Jin, L., Chen, I. W., Lin, J. H., Kassahun, K., Ellis, J. D., Wong, B. K., Xu, W., Pearson, P. G., Schleif, W. A., Cortese, R., Emini, E., Summa, V., Holloway, M. K., and Young, S. D. (2004). A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc. Natl. Acad. Sci. U S A* **101,** 11233-11238.

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

Herath, K. B., Jayasuriya, H., Bills, G. F., Polishook, J. D., Dombrowski, A. W., Guan, Z., Felock, P. J., Hazuda, D. J., and Singh, S. B. (2004). Isolation, structure, absolute stereochemistry, and HIV-1 integrase inhibitory activity of integrasone, a novel fungal polyketide. *J. Nat. Prod.* **67,** 872-874.

Hickman, A. B., Dyda, F., and Craigie, R. (1997) Heterogeneity in recombinant HIV-1 integrase corrected by site-directed mutagenesis: the identification and elimination of a protease cleavage site. *Protein Engineering* **10**, 601-606.

Hippenmeyer, P. J., and Grandgenett, D. P. (1984). Requirement of the avian retrovirus pp32 DNA binding protein domain for replication. *Virology* **137,** 358-370.

Ikeda, T., Nishitsuji, H., Zhou, X., Nara, N., Ohashi, T., Kannagi, M., and Masuda, T. (2004) Evaluation of the functional involvement of human immunodeficiency virus type 1 integrase in nuclear import of viral cDNA during acute infection. *J. Virol.* **78**, 11563-11573.

Jenkins, T. M., Esposito, D., Engelman, A., and Craigie, R. (1997) Critical contacts between HIV-1 integrase and viral DNA identified by structure-based analysis and photo-crosslinking. *EMBO J.* **16**, 6849-6859.

Jacque, J. M., and Stevenson, M. (2006). The inner-nuclear-envelope protein emerin regulates HIV-1 infectivity. *Nature* **441,** 641-645.

Johnson, A. A., Marchand, C., Patil, S., Costi, R., Di Santo, R., Burke, T. R., and Pommier, Y. (2006a). Probing Hiv-1 Integrase Inhibitor Binding Sites with Position-Specific Integrase-DNA Crosslinking Assays. *Mol Pharmacol*.

Johnson, A. A., Marchand, C., and Pommier, Y. (2004). HIV-1 integrase inhibitors: a decade of research and two drugs in clinical trial. *Curr. Top. Med. Chem.* **4,** 1059-1077.

Johnson, A. A., Santos, W., Pais, G. C., Marchand, C., Amin, R., Burke, T. R., Jr., Verdine, G., and Pommier, Y. (2006b). Integration requires a specific interaction of the donor DNA terminal 5'-cytosine with glutamine 148 of the HIV-1 integrase flexible loop. *J. Biol. Chem.* **281,** 461-467.

Katzman, M., Katz, R. A., Skalka, A. M., and Leis, J. (1989). The avian retroviral integration protein cleaves the terminal sequences of linear viral DNA at the in vivo sites of integration. *J. Virol.* **63,** 5319-5327.

Kawaguchi, I., Ishikawa, T., Ishibashi, M., Irie, S., and kakee, A. (2006). Safety and pharmacokinetics of single oral dose of JTK-303/GS-9137 a novel HIV integrase inhibitor, in healthy volunteers 13th Conference on Retroviruses and Opportunistic Infections, 5–9 February 2006, Denver, poster no. 580. Foundation for Retrovirology and Human Health, Alexandria, Va. 2006.

King, P. J., Lee, D. J., Reinke, R. A., Victoria, J. G., Beale, K., and Robinson, W. E., Jr. (2003) Human immunodeficiency virus type-1 integrase containing a glycine to serine mutation at position 140 is attenuated for catalysis and resistant to integrase inhibitors. *Virology* **306**, 147-161.

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

Krajewski, K., Long, Y. Q., Marchand, C., Pommier, Y., and Roller, P. P. (2003). Design and synthesis of dimeric HIV-1 integrase inhibitory peptides. *Bioorg. Med. Chem. Lett.*, **13,** 3203-3205.

Krajewski, K., Marchand, C., Long, Y. Q., Pommier, Y., and Roller, P. P. (2004). Synthesis and HIV-1 integrase inhibitory activity of dimeric and tetrameric analogs of indolicidin. *Bioorg. Med. Chem. Lett.* **14,** 5595-5598.

Kulkosky, J., Jones, K. S., Katz, R. A., Mack, J. P., and Skalka, A. M. (1992) Residues critical for retroviral integrative recombination in a region that is

highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. *Mol. Cell. Biol.* **12**, 2331-2338.

LaFemina, R.L., Schneider, C. L., Robbins, H. L., Callahan, P. L., LeGrow, K., Roth, E., Schleif, W. A., and Emini, E. A. (1992) Requirement of active human immunodeficiency virus type 1 integrase enzyme for productive infection of human T-lymphoid cells. *J. Virol.* **66**, 7414-7419.

Laufer, R., Gonzalez-Paz, O. G., Taliani, M., Di Marco, A., Moteagudo, E., Bonelli, F., Fiore, F., Fonsi, M., Naimo, F., Verdirame, M., Alfieri, A., Marcucci, I., Summa, V., Rowley, M., and Laufer, R. (2006). Preclinical pharmacokinetics and metabolism of MK-0518, a potent HIV Integrase inhibitor in phase III clinical trial. *16th AIDS International Conference, Toronto*, abstr. # THPE0027.

Leavitt, A. D., Robles, G., Alesandro, N., and Varmus, H. E. (1996) Human immunodeficiency virus type 1 integrase mutants retain in vitro integrase activity yet fail to integrate viral DNA efficiently during infection. *J. Virol.* **70**, 721-728.

Leavitt, A. D., Shiue, L., and Varmus, H. E. (1993) Site-directed mutagenesis of HIV-1 integrase demonstrates differential effects on integrase functions in vitro. *J. Biol. Chem.* **268**, 2113-2119.

Lee, D. J., and Robinson, W. E., Jr. (2004) Human immunodeficiency virus type 1 (HIV-1) integrase: resistance to diketo acid integrase inhibitors impairs HIV-1 replication and integration and confers cross-resistance to L-chicoric acid. *J. Virol.* **78**, 5835-5847.

Little, S., Drusano, G., Schooley, R., Haas, D., Kumar, P., Hammer, S., McMahon, D., Squires, K., Asfour, R., Richman, D., Chen, J., Saah, A., Leavitt, R., Hazuda, D. J., Nguyen, B.-Y. and protocol 004 study team. (2005). Antiretroviral effect of L-000870810, a novel HIV-1 integrase inhibitor, in HIV-1-infected patients *12th conference on retroviruses and opportunistic infections, Feb 22-25, 2005, Boston, MA, USA***,** abstract no 161.

Llano, M., Saenz, D. T., Meehan, A., Wongthida, P., Peretz, M., Walker, W. H., Teo, W., and Poeschla, E. M. (2006). An essential role for LEDGF/p75 in HIV integration. *Science* **314,** 461-464.

Lu, R., Ghory, H. Z., and Engelman, A. (2005a) Genetic analyses of conserved residues in the carboxyl-terminal domain of human immunodeficiency virus type 1 integrase. *J. Virol.* **79**, 10356-10368.

Lu, R., Limon, A., Devroe, E., Silver, P. A., Cherepanov, P., and Engelman, A. (2004) Class II integrase mutants with changes in putative nuclear localization signals are primarily blocked at a postnuclear entry step of human immunodeficiency virus type 1 replication. *J. Virol.* **78**, 12735-12746.

Lu, R., Limon, A., Ghory, H. Z., and Engelman, A. (2005b) Genetic analyses of DNA-binding mutants in the catalytic core domain of human immunodeficiency virus type 1 integrase. J. Virol. **79**, 2493-2505.

Lu, R., Vandegraaff, N., Cherepanov, P., and Engelman, A. (2005c) Lys-34, dispensable for integrase catalysis, is required for preintegration complex function and human immunodeficiency virus type 1 replication. *J. Virol.* **79**, 12584-12591.

Lutzke, R. A., and Plasterk, R. H. (1998) Structure-based mutational analysis of the C-terminal DNA-binding domain of human immunodeficiency virus type 1 integrase: critical residues for protein oligomerization and DNA binding. *J. Virol.* **72**, 4841-4848.

Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z., and Engelborghs, Y. (2003). LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J. Biol. Chem.* **278,** 33528-33539.

Marchand, C., Johnson, A. A., Semenova, E., and Pommier, Y. (2006a). Mechanism and inhibition of HIV integration. *Drug. Discov. Today: Disease Mechanism* **3,** 253-260.

Marchand, C., Krajewski, K., Lee, H.F., Antony, S., Johnson, A. A., Amin, R., Roller, P., Kvaratskhelia, M., and Pommier, Y. (2006b). Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites. *Nucleic Acids Res.* **34,** 5157-5165.

Marchand, C., Neamati, N., and Pommier, Y. (2001). In vitro human immunodeficiency virus type 1 integrase assays. *Methods Enzymol.* **340,** 624-633.

Markowitz, M., Morales-Ramirez, J. O., Nguyen, B. Y., Kovacs, C. M., Steigbigel, R. T., Cooper, D. A., Liporace, R., Schwartz, R., Isaacs, R., Gilde, L. R., Wenning, L., Zhao, J., and Teppler, H. (2006). Antiretroviral Activity, Pharmacokinetics, and Tolerability of MK-0518, a Novel Inhibitor of HIV-1

Integrase, Dosed As Monotherapy for 10 Days in Treatment-Naive HIV-1-Infected Individuals. *J. Acquir. Immune Defic. Syndr.* **43,** 509-515.

Matsuzaki, Y., Watanabe, W., Yamataka, K., Sato, M., S. E, Kano, M., Kodama, E., Matsuoka, M., and Ikeda, S. (2006). JTK-303/GS-9137, a novel small molecule inhibitor of HIV-1 integrase: anti-HIV activity profile and pharmacokinetics in animals. *13th conference on retroviruses and opportunistic infections, Denver, February 5-9 2006***,** poster no 508. Foundation for Retrovirology and Human Health, Alexandria, Va.

Maurin, C., Bailly, F., Mbemba, G., Mouscadet, J. F., and Cotelle, P. (2006). Design, synthesis, and anti-integrase activity of catechol-DKA hybrids. *Bioorg. Med. Chem.* **14,** 2978-2984.

Mazumder, A., Gupta, M., Perrin, D. M., Sigman, D. S., Rabinovitz, M., and Pommier, Y. (1995). Inhibition of human immunodeficiency virus type 1 integrase by a hydrophobic cation: the phenanthroline-cuprous complex. *AIDS Res. Hum. Retroviruses* **11,** 115-125.

Mazumder, A., Neamati, N., Ojwang, J. O., Sunder, S., Rando, R. F., and Pommier, Y. (1996a). Inhibition of the human immunodeficiency virus type 1 integrase by guanosine quartet structures. *Biochemistry* **35,** 13762-13771.

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

Mazumder, A., and Pommier, Y. (1995). Processing of deoxyuridine mismatches and abasic sites by human immunodeficiency virus type-1 integrase. *Nucleic. Acids Res.* **23,** 2865-2871.

Mazumder, A., Wang, S., Neamati, N., Nicklaus, M., Sunder, S., Chen, J., Milne, G. W., Rice, W. G., Burke, T. R., Jr., and Pommier, Y. (1996b). Antiretroviral agents as inhibitors of both human immunodeficiency virus type 1 integrase and protease. *J. Med. Chem.* **39,** 2472-2481.

Meadows, D. C., Mathews, T. B., North, T. W., Hadd, M. J., Kuo, C. L., Neamati, N., and Gervay-Hague, J. (2005). Synthesis and biological evaluation of geminal disulfones as HIV-1 integrase inhibitors. *J. Med. Chem.* **48,** 4526-4534.

Miller, M. D., Farnet, C. M., and Bushman, F. D. (1997). Human immunodeficiency virus type 1 preintegration complexes: studies of organization and composition. *J. Virol.* **71,** 5382-5390.

Morales-Ramirez, J. O., Teppler, H., Kovacs, C., Steigbigel, R. T., Cooper, D., Liporace, R. L., Schwartz, R., Wenning, L., Zhao, J., Gilde, L., Isaacs, R., and Nguyen, B. (2005). Antiretroviral Effect of MK-0518, a Novel HIV-1 Integrase Inhibitor, in ART-Naïve HIV-infected Patients. Program and Abstracts of the 10th European AIDS Conference, 17–20 November 2005, Dublin. abstract no. LBPS1/6. European AIDS Clinical Society, Paris.

Mousnier, A., Leh, H., Mouscadet, J. F., and Dargemont, C. (2004). Nuclear import of HIV-1 integrase is inhibited in vitro by styrylquinoline derivatives. *Mol. Pharmacol*. **66,** 783-788.

Nair, V., Chi, G., Ptak, R., and Neamati, N. (2006). HIV Integrase Inhibitors with Nucleobase Scaffolds: Discovery of a Highly Potent Anti-HIV Agent. *J. Med. Chem.* **49,** 445-447.

Nakamura, T., Masuda, T., Goto, T., Sano, K., Nakai, M., and Harada, S. (1997) Lack of infectivity of HIV-1 integrase zinc finger-like domain mutant with morphologically normal maturation. *Bioch. Biophys. Res. Com.* **239**, 715-722.

Neamati, N., Hong, H., Mazumder, A., Wang, S., Sunder, S., Nicklaus, M. C., Milne, G. W., Proksa, B., and Pommier, Y. (1997a). Depsides and depsidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D database searching. *J. Med. Chem.* **40,** 942-951.

Neamati, N., Hong, H., Sunder, S., Milne, G. W., and Pommier, Y. (1997b). Potent inhibitors of human immunodeficiency virus type 1 integrase: identification of a novel four-point pharmacophore and tetracyclines as novel inhibitors. *Mol, Pharmacol.* **52,** 1041-1055.

Nomura, Y., Masuda, T., and Kawai, G. (2006) Structural analysis of a mutant of the HIV-1 integrase zinc finger domain that forms a single conformation. *J. Biochemistry* **139**, 753-759.

Normand-Bayle, M., Benard, C., Zouhiri, F., Mouscadet, J. F., Leh, H., Thomas, C. M., Mbemba, G., Desmaele, D., and d'Angelo, J. (2005). New HIV-1 replication inhibitors of the styryquinoline class bearing aroyl/acyl groups at the C-7 position: synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **15,** 4019-4022.

Oh, J. W., Oh, Y. T., Kim, D. J., and Shin, C. G. (1997) Characterization of human immunodeficiency virus type 1 integrase mutants expressed in Escherichia coli. *Molecules and cells* **7**, 688-693.

Ovenden, S. P., Yu, J., Wan, S. S., Sberna, G., Tait, R. M., Rhodes, D., Cox, S., Coates, J., Walsh, N. G., and Meurer-Grimes, B. M. (2004). Globoidnan A: a lignan from Eucalyptus globoidea inhibits HIV integrase. *Phytochemistry* **65,** 3255-3259.

Oz, I., Avidan, O., and Hizi, A. (2002). Inhibition of the integrases of human immunodeficiency viruses type 1 and type 2 by reverse transcriptases. *Biochem. J.* **361,** 557-566.

Parissi, V., Caumont, A. B., de Soultrait, V. R., Calmels, C., Pichuantes, S., Litvak, S., and Dupont, C. H. (2000) Selection of amino acid substitutions restoring activity of HIV-1 integrase mutated in its catalytic site using the yeast Saccharomyces cerevisiae. *J. Mol. Biol.* **295**, 755-765.

Pilon, A. A., Marchand, C., Kavlick, M. F., Bajaj, K., Owen, J., Mitsuya, H., and Pommier, Y. (2000) Mutations in the HIV type 1 integrase of patients receiving long-term dideoxynucleoside therapy do not confer resistance to zidovudine. *AIDS Res. Hum. Retroviruses* **16**, 1417-1422.

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

Pommier, Y., and Cherfils, J. (2005). Interfacial inhibition of macromolecular interactions: nature's paradigm for drug discovery. *Trends Pharmacol. Sci.* **26,** 138-145.

Pommier, Y., Johnson, A. A., and Marchand, C. (2005). Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug. Discov.* **4,** 236-248.

Pommier, Y., and Marchand, C. (2005). Interfacial inhibitors of protein-nucleic acid interactions. *Curr. Med. Chem. Anticancer. Agents* **5,** 421-429.

Pommier, Y., and Neamati, N. (1999). Inhibitors of human immunodeficiency virus integrase. *Adv. Virus Res.* **52,** 427-458.

Priet, S., Navarro, J. M., Querat, G., and Sire, J. (2003) Reversion of the lethal phenotype of an HIV-1 integrase mutant virus by overexpression of the same integrase mutant protein. *J. Biol. Chem.* **278**, 20724-20730.

Ramcharan, J., Colleluori, D. M., Merkel, G., Andrake, M. D., and Skalka, A. M. (2006) Mode of inhibition of HIV-1 Integrase by a C-terminal domain-specific monoclonal antibody. *Retrovirology* [electronic resource] 3:34.

Robinson, W. E., Jr., McDougall, B., Tran, D., and Selsted, M. E. (1998). Anti-HIV-1 activity of indolicidin, an antimicrobial peptide from neutrophils. *J. Leukoc. Biol.* **63,** 94-100.

Rowley, D. C., Hansen, M. S., Rhodes, D., Sotriffer, C. A., Ni, H., McCammon, J. A., Bushman, F. D., and Fenical, W. (2002). Thalassiolins A-C: new marine-derived inhibitors of HIV cDNA integrase. *Bioorg. Med. Chem.* **10,** 3619-3625.

Sato, M., Motomura, T., Aramaki, H., Matsuda, T., Yamashita, M., Ito, Y., Kawakami, H., Matsuzaki, Y., Watanabe, W., Yamataka, K., Ikeda, S., Kodama, E.,

Matsuoka, M., and Shinkai, H. (2006). Novel HIV-1 integrase inhibitors derived from quinolone antibiotics. *J. Med. Chem.* **49,** 1506-1508.

Savarino, A. (2006). A historical sketch of the discovery and development of HIV-1 integrase inhibitors. *Expert. Opin. Investig. Drugs* **15,** 1507-1522.

Sayasith, K., Sauve, G., and Yelle, J. (2000) Characterization of mutant HIV-1 integrase carrying amino acid changes in the catalytic domain. *Molecules and cells* **10**, 525-532.

Semenova, E. A., Johnson, A. A., Marchand, C., Davis, D. A., Yarchoan, R., and Pommier, Y. (2006a). Preferential Inhibition of the Magnesium-Dependent Strand Transfer Reaction of HIV-1 Integrase by {alpha}-Hydroxytropolones. *Mol. Pharmacol.* **69,** 1454-1460.

Semenova, E. A., Johnson, A. A., Marchand, C., and Pommier, Y. (2006b). Integration of human immunodeficiency virus as a target for antiviral therapy. *Curr. Opin. HIV AIDS* **1**, 380–387.

Sherman, P. A., and Fyfe, J. A. (1990). Human immunodeficiency virus integration protein expressed in Escherichia coli possesses selective DNA cleaving activity. *Proc. Natl. Acad. Sci. U S A* **87,** 5119-5123.

Shiomi, K., Matsui, R., Isozaki, M., Chiba, H., Sugai, T., Yamaguchi, Y., Masuma, R., Tomoda, H., Chiba, T., Yan, H., Kitamura, Y., Sugiura, W., Omura, S., and Tanaka, H. (2005). Fungal phenalenones inhibit HIV-1 integrase. *J. Antibiot. (Tokyo)* **58,** 65-68.

Sierra, S., Kupfer, B., and Kaiser, R. (2005). Basics of the virology of HIV-1 and its replication. *J. Clin. Virol.* **34,** 233-244.

Shin, C. G., Taddeo, B., Haseltine, W. A., and Farnet, C. M. (1994) Genetic analysis of the human immunodeficiency virus type 1 integrase protein. *J. Virol.* **68**, 1633-1642.

Svarovskaia, E. S., Barr, R., Zhang, X., Pais, G. C., Marchand, C., Pommier, Y., Burke, T. R., Jr., and Pathak, V. K. (2004) Azido-containing diketo acid derivatives inhibit human immunodeficiency virus type 1 integrase in vivo and influence the frequency of deletions at two-long-terminal-repeat-circle junctions. *J. Virol.* **78**, 3210-3222.

Summa, V., Pace, P. Petroccci, A., Laufer, R., Cortese, R., Hazuda, D. J., Vacca, J. P., Young, S. D., Scheif, W. B., Miller, M. D., and Rowley, M. (2006). Discovery of MK-0518 a novel, potent and selective HIV integrase inhibitor in phase III clinical trials. *16th AIDS International Conference, Toronto*. Abstr. # THPE0020.

Taddeo, B., Carlini, F., Verani, P., and Engelman, A. (1996) Reversion of a human immunodeficiency virus type 1 integrase mutant at a second site restores enzyme function and virus infectivity. *J. Virol.* **70**, 8277-8284.

Tsurutani, N., Kubo, M., Maeda, Y., Ohashi, T., Yamamoto, N., Kannagi, M., and Masuda, T. (2000) Identification of critical amino acid residues in human immunodeficiency virus type 1 IN required for efficient proviral DNA formation at steps prior to integration in dividing and nondividing cells. *J. Virol.* **74**, 4795-4806.

van Gent, D. C., Vink, C., Groeneger, A. A., and Plasterk, R. H. (1993). Complementation between HIV integrase proteins mutated in different domains. *EMBO J.* **12,** 3261-3267.

Van Maele, B., Busschots, K., Vandekerckhove, L., Christ, F., and Debyser, Z. (2006). Cellular co-factors of HIV-1 integration. *Trends Biochem. Sci.* **31,** 98-105.

Van Maele, B., and Debyser, Z. (2005). HIV-1 integration: an interplay between HIV-1 integrase, cellular and viral proteins. *AIDS Rev.* **7,** 26-43.

Vandekerckhove, L., Christ, F., Van Maele, B., De Rijck, J., Gijsbers, R., Van den Haute, C., Witvrouw, M., and Debyser, Z. (2006). Transient and stable knockdown of the integrase cofactor LEDGF/p75 reveals its role in the replication cycle of human immunodeficiency virus. *J. Virol.* **80,** 1886-1896.

Vink, C., Groenink, M., Elgersma, Y., Fouchier, R. A., Tersmette, M., and Plasterk, R. H. (1990). Analysis of the junctions between human immunodeficiency virus type 1 proviral DNA and human DNA. *J. Virol.* **64,** 5626-5627.

Vink, C., Oude Groeneger, A. M., and Plasterk, R. H. (1993). Identification of the catalytic and DNA-binding region of the human immunodeficiency virus type I integrase protein. *Nucleic Acids Res.* **21,** 1419-1425.

Vink, C. and Plasterk, R. H. (1993). The human immunodeficiency virus integrase protein. *Trends Genet.* **9,** 433-438.

Vink, C., van Gent, D. C., Elgersma, Y., and Plasterk, R. H. (1991). Human immunodeficiency virus integrase protein requires a subterminal position of its viral DNA recognition sequence for efficient cleavage. *J. Virol.* **65,** 4636-4644.

Williams, K. L., Zhang, Y., Shkriabai, N., Karki, R. G., Nicklaus, M. C., Kotrikadze, N., Hess, S., Le Grice, S. F., Craigie, R., Pathak, V. K., and Kvaratskhelia, M. (2005) Mass spectrometric analysis of the HIV-1 integrase-pyridoxal 5'-phosphate complex reveals a new binding site for a nucleotide inhibitor. The *J. Biol. Chem.* **280**, 7949-7955.

Wiskerchen, M., and Muesing, M. A. (1995) Human immunodeficiency virus type 1 integrase: effects of mutations on viral ability to integrate, direct viral gene expression from unintegrated viral DNA templates, and sustain viral propagation in primary cells. *J. Virol.* **69**, 376-386.

Yoshinaga, T., Sato, A., Fujishita, T., and Fujiwara, T. (2002). S-1360: in vitro activity of a new HIV-1 integrase inhibitor in clinical development. *Conf Retroviruses Opportunistic Infect 2002 Feb 24-28; 9: abstract no. 8*.

Zhao, H., Neamati, N., Hong, H., Mazumder, A., Wang, S., Sunder, S., Milne, G. W., Pommier, Y., and Burke, T. R., Jr. (1997). Coumarin-based inhibitors of HIV integrase. *J. Med. Chem.* **40,** 242-249.

Zheng, R., Ghirlando, R., Lee, M. S., Mizuuchi, K., Krause, M., and Craigie, R. (2000). Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. *Proc. Natl. Acad. Sci. U S A* **97,** 8997-9002.

Zhu, K., Dobard, C., and Chow, S. A. (2004) Requirement for integrase during reverse transcription of human immunodeficiency virus type 1 and the effect of cysteine mutations of integrase on its interactions with reverse transcriptase. *J. Virol.* **78**, 5045-5055.

Table 1 Catalytic activities of HIV-1 IN mutants[a]

Mutations	3'-P	ST	Dis	Inf	Interaction (References)
H12A;N*	+	+	+++	- (-)	PIC (1-4)
H12C*			+++	(-)	(5,6)
H12N/H16N	-	-	+		(2)
H12C/H16C			+++		(5)
H12Q/H16Y				(-)	(7)
Y15A				(-)	(8)
H16A*				-	(3)
H16C;V*	+++	+++	+++	(-)	(5,6)
K34A	++	++		- (-)	PIC (4)
K46A				+++	(4)
C40A;S*	-	-	+	(-)	(1,9)
C40S/C43S	-	-	++		(1)
D41A/K42A				+	(3)
C43A;S;L*	+	+		(-)	(7,9,10)
M50A				+	(9,10)
H51A*				+	(3)
H51A/D55V				-	(3)
Q53C	+++	+++	++	(-)	(5,6)
Q53K				+++	(11)
D55A				-	(3)
D55A;S				+++	(11)
D55K				-	(11)
C56A*				+++	(12)
C56S*	+++	+++		+++	(13,14)
C56S/C65S				Del	(13)
C56S/C130S				-	(13)
C56S/C65S/C280S	++	++	+++		(13,15)
C56S/C65S/C130S/C280S	++	-	+++		(13)
C56S/C65S/Q148C/C280S	++	-			(13,15)
Q62E	++	++	+++		(16)
Q62K				-	(11)
Q62N	+	-	-		(17)
D64C;R*			-		(17)
D64A;E;N;V*	-	-	-	-	(1,3,5,6,9,18,19)
D64A/D116A				-	(3)
D64R/D116R			-		(17)
D64A/E152A				-	(3)
D64A/D116A/E152A				-	(3)
C65S*	+++	+++		+++	(13,14)
C65A*				+++	(12)
C65S/C130S				-	(13)
T66A*	+	++	++	(++)	(17,20)
T66I*	++/ +++	++/ +++	++	+/ ++	L-708,906; L-731,988; S-1360; 118D-24; L-CA (21-27)
T66I/L74M	+	+		+	L-708,906; S-1360; (23)
T66I/S153Y				++	L-708,906; L-731,988; 118D-24; L-CA (24-26)
T66I/M154I	++	++	++	+	L-731,988; L-CA (25)
T66I/L74M/S230R	+	+		++	S-1360; L-708,906 (23)
H67E*				Del	(11)
H67S*	+++	+++	++		(17)
H67Q/K71E				-	(11)
E69A/K71A				-	(3)
K71E*				+++	(11)
V72I					L-870,810 (26)
L74M*	+++	+++			L-708,906; S-1360 (23,27)
V75P				(-)	(9)
S81R;A	+/-	+/-	+/-	(-)	(5,6,9)
P90D*	+++	-	-		(28)
P90D/P145I	-	-	-		(28)
E92A;Q	+++	+++	+++	+++	(16)
E92A;N	+	+	++		(17)
E92K	+	+	+++	+++	(16)
T93A				(++)	(20)
G106A	+++	+++			(29)
P109A;S*	+/-	+/-	-	+	(3,18,30)
P109S/T125A	+++	+++	+++	(+/-)	(30)
T112A	+++	+++			(18)

Table 1 (continue)

Mutations	3'-P	ST	Dis	Inf	Interaction (Ref)
H114E				++	(11)
T115A;S	+++	+++	+++	+++	(1,9,18,31,32)
D116A;E;I;N*	-	-	-	- (-)	(1,5,6,9,17,18, 31-34)
D116C*			+		(17)
D116A/E152A**				-	(3)
D116/A23V**	-	++	-		(35)
D116/E11D	-	++	-		(35)
D116/L28R/C65S/ T210N/L213I	-	+	-		(35)
N117K;Q	++	++	++	Del	(1,11,17,32,33)
N117S	+	+	+		(17)
G118A	+++		+++	+++	(32,33)
S119T;G;A;K	+++	+++			(29)
N120Q;S*	+++	+++	+++		(17)
N120I;L;E;G*				(-)	(6)
N120L;K*				Del	(11)
N120L/Q148K				-	(11)
F121A	+/-	-			(31)
F121Y					L-870,810 (26)
S123A	++	++	+++	(+)	(1,9)
T125A*	+++	+++	+++	+ (+++)	(20,30)
T125K*					L-870,810 (26)
K127A				+	(3)
A128T					S-1360 (27)
C130A*	+++	+++		++	OH-Coum (12,36)
C130G*				- (-)	OH-Coum (12,36)
C130S*				-	OH-Coum (13,14,36)
C130A/F185K/C280S	+++	+++			OH-Coum (36)
C130S/F185K/C280S	+++	+			OH-Coum (36)
W131G/F185K/C280S	++	++			(36)
W132A;G;R//F185K/C280S	+++	-			OH-Coum (36)
W132Y/F185K/C280S	+++	++			OH-Coum (36)
I135P				(-)	(9)
K136A*	+/-	+/-	+++	-/++	(3,11,16)

Mutations	3'-P	ST	Dis	Inf	Interaction (Ref)
K136E;R*	+++	+++	+++	+++	(16)
K136A/E138A	+++	+++	+++	+++	(16)
K136R//F185K/C280S	+++	++			(36)
E138A*				+	(3)
E138K*				++	S-1360 (11,27)
G140S*	+	+	+	Del	L-CA; L-731,988; (37)
G140S/F185K/C280S	+++	+++	+++		(38)
P142F				+++/ (+++)	(39)
Y143F				Del	(3,32)
Y143N	+++		+++	Del	(3,32,33)
Y143G				+++/(+ ++)	(11,20,39)
N144K				-	(11)
N144Q				-/(-)	(39)
P145I*	-	-	-		(28)
P145A*				+	(11)
P145F*				-/(-)	(39)
P145I/F185K/C280S	-	-	-		(28)
Q146K				+	S-1360 (11,27)
S147I	-		+++	-	(32,33)
Q148A*	++	-			(15)
Q148K*				Del	(11)
Q148L*	+	++	+	Del	(11,17)
Q148N*	+++	++			(15)
Q148A/F185K/C280S	+	+			(36)
V150E	+++	+++	+++		(28)
V151A*				(+)	(9)
V151I*					L-870,810 (26)
V151D/E152Q	-	-	-	-	(10)
V151T/S153Q	++	-	+++		(40)
V151L/S153L	+++	-	-		(40)
V151L/E152V/S153F	++	-	-		(40)
V151A/E152M/S153A	++	-	-		(40)
V151Y/E152V/S153P	+++	-	-		(40)

Table 1 (continue)

Mutations	3'-P	ST	Dis	Inf	Interaction (Ref)
V151H/E152G/S153L	+++	-	-		(40)
V151E/E152S/S153N	++	-	-		(40)
V151T/E152S/S153M	+++	-	-		(40)
V151T/E152F/S153C	+++	-	-		(40)
E152A;C;D;G;H;P;Q;V;K*	-	-	-	- (-)	(1,3,5,6,9,11,17,18,28,31,33,34)
E152A/K156A				-	(3)
E152N/S153R	++	-	-		(40)
S153A;R*	+	+	++	++	(1,11)
S153A*	+++		+++	+++	S-1360 (27,32,33)
S153Y*	++	++	++	+	L-708,906; L-731,988; L-CA (22,25,26)
M154I	+++	+++	+++	++	L-708,906; L-731,988; L-CA (22,25,26)
N155E;K	-	-	-	Del	(11,17)
N155L	+	+	-	Del	(11,17)
N155S					L-870,810 (26)
K156A*				+/ +++/(+++)	(3,39)
K156E*	-	-	+/-	Del	(11,17,41)
K156R*					(42)
K156I*	-	-	-		(28)
K156E/K159E	-	-	-	-	(11,41)
K156A/E157A				+	(3)
E157A/K159A				+	(3)
L158F	+++	+++	+++		(28)
K159A*				++/(++)	(39)
K159E*	-	-	+++	-/Del	(11,41)
K159N;S*	+	+	+		(17)
K159Q*	+++		+++		(33)
K159R*					(42)
K159A/K160A				+	(3)
K159A;P;Q				(+)	(9,31,32)

Mutations	3'-P	ST	Dis	Inf	Interaction (Ref)
K160A*				+++/(+++)	(39)
K160D*					S-1360 (27)
K160E*	+++	+++	+++	-	(41)
V165A	++			- (-)	PIC (4,12)
V165I					S-1360 (27)
R166A*				-	(3)
R166T*	++	++			(42,43)
R166A/D167A				-	(3)
D167A*				+	(3)
Q168A	+++	+++		(-)	LEDGF (44,45)
Q168L	+++	-		(-)	LEDGF (44)
Q168P	-	-		(-)	LEDGF (44)
E170A/H171A	+++	+++		+/Del	(3,46)
E170A/K173A				+	(3)
H171A/K173A*				+	(3)
L172M*	+++	+++	+++		(28)
L172A/K173A	+++	+++		-	(46)
T174A	+	+		-	(46)
V176A/Q177A	+++	+++		Del	(46)
M178A	-	-		-	(46)
A179P				(-)	(9)
V180A/F181A	-	-		-	(46)
N184D;L				-	(11)
F185A;K;L;H*	+++	+++		(-)	(16)
K186A;Q;E	+++			- (-)	(12,20)
K187A				-	(12)
K188A				-	(12)
G189A	+++	+++			(18)
S195A				(++)	(20)

Table 1 (continue)

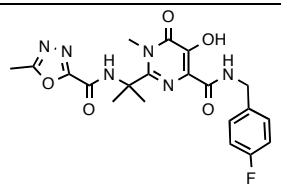
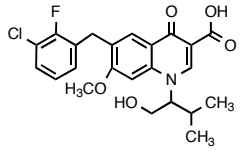
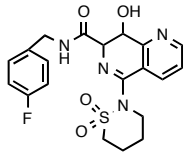
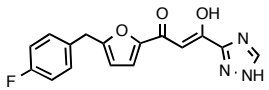
Mutations	3'-P	ST	Dis	Inf	Interaction; (Ref)
E198A/R199A				-	(3)
R199A;C*	+++	+++	+++	-/Del (-)	(3,5,6,11,12)
R199E*				-	(11)
R199A/D202A				-	(3)
R199T/D202A				-	(3)
V201I					(27)
K211A*				(++)	(20)
K211A/E212A				+	(3)
Q214L/Q216L	+++			- (-)	(12)
K215A*				+++	(12)
K215A/K219A	++			- (-)	(12)
K219A*				+	(12)
N222A	++	++	++		(47)
F223A	++	++	++		mAb33 (47,48)
R224A	++	++	++		mAb33 (47,48)
Y226					mAb33 (48)
Y227A	++	++	++		(47)
R228A				- (-)	(49)
S230R*	+++	+++			L-708,906; S-1360 (23)

Mutations	3'-P	ST	Dis	Inf	Interaction; (Ref)
R231A	++	+	+	+	(47,49)
P233A	++	++	++		(47)
L;V234A	++	++	++	+++	(47,49)
W235A;E;F	+++	+++	+++	+/- (-)	PIC (2,4-6,9)
K236A*				+++	(49)
K236E*				- (-)	(49)
K236A/K240A				-	(3)
K236/E246A				- (-)	(49)
L241A	-	-	-	- (-)	(47,49)
L242A	-	-	+	- (-)	(47,49)
W243A	++	++	++		(47)
K244A*				- (-)	(49)
K244E*	-	-		(-)	(50)
K244A/E246A				-	(3)
E246A*	++	++	++	Del (+)	(47,49)
E246K*				- (-)	(49)
D253A/D256A				+	(30
W243A	++	++	++		(47)

[a] Abbreviations: 3'-P, 3'-processing; ST, strand transfer; Dis, disintegration; Inf, infectivity; PIC, pre-integration complex; Del, delayed; L-CA, L-chicoric acid; OH-Coum, hydroxycoumarin.
- = 0-10%, + = 10-40%, ++ = 40-80%, +++ =80-100%, * = mutant present elsewhere in the table as a combination; / = separates differential results from independent publications.

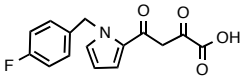
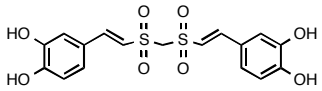
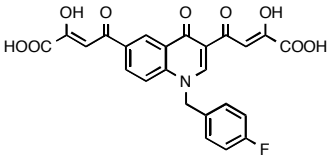
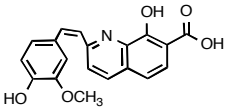
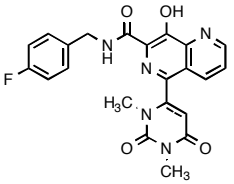
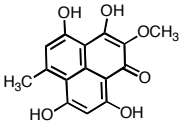
[b] References: (1) Engelman and Craigie, 1992; (2) Engelman *et al.*, 1995; (3) Wiskerchen and Muesing, 1995; (4) Lu *et al.*, 2005c; (5) Leavitt, Shiue, and Varmus, 1993; (6) Leavitt *et al.*, 1996; (7) Nakamura *et al.*, 1997; (8) Nomura, Masuda, and Kawai, 2006; (9) Cannon *et al.*, 1994; (10) LaFemina *et al.*, 1992; (11) Lu *et al.*, 2005b; (12) Lu *et al.*, 2004; (13) Zhu, Dobard, and Chow, 2004; (14) Bischerour *et al.*, 2003; (15) Johnson *et al.*, 2006; (16) Engelman *et al.*, 1997; (17) Gerton *et al.*, 1998; (18) Drelich, Wilhelm, and Mous, 1992; (19) Cherepanov *et al.*, 2000; (20) Tsurutani *et al.*, 2000; (21) Yoshinaga *et al.*, 2002; (22) Hazuda *et al.*, 2000; (23) Fikkert *et al.*, 2003; (24) Svarovskaia *et al.*, 2004; (25) Lee and Robinson, Jr., 2004; (26) Hazuda *et al.*, 2004; (27) Fikkert *et al.*, 2004; (28) Sayasith, Sauve, and Yelle, 2000; (29) Harper *et al.*, 2001; (30) Taddeo *et al.*, 1996; (31) Kulkosky *et al.*, 1992; (32) Shin *et al.*, 1994; (33) Oh *et al.*, 1997; (34) Engelman, Bushman, and Craigie, 1993; (35) Parissi *et al.*, 2000; (36) Al-Mawsawi *et al.*, 2006; (37) King *et al.*, 2003; King and Robinson, Jr., 1998; (38) Pluymers *et al.*, 2000; (39) Ikeda *et al.*, 2004; (40) Calmels *et al.*, 2004; (41) Jenkins *et al.*, 1997; (42) Drake *et al.*, 1998; (43) Pilon *et al.*, 2000; (44) Emiliani *et al.*, 2005; (45) Vandekerckhove *et al.*, 2006; (46) Priet *et al.*, 2003; (47) Lutzke and Plasterk, 1998; (48) Ramcharan *et al.*, 2006; (49) Lu, Ghory, and Engelman, 2005a; (50) Williams *et al.*, 2005; (51) Hickman, Dyda, and Craigie, 1997.

Table 2. Inhibitors of HIV-1 integrase in clinical trials.

Compound		Preclinical studies					Clinical development status
Name, source (references)[a]	Structure	Anti-IN activity IC_{50}, μM		Antiviral activity		Comments	
		3'P	ST	EC_{50} μM	CC_{50} μM		
MK-0518, Merck & Co (1,2,3,4)		[b]	0.016	0.017-0.029 (EC_{95})	[b]	Active against multidrug-resistance HIV-1 viruses. Oral bioavailability: rats (45%), dogs (69%), rhesus monkey (8%). Human protein binding (82%). Does not inhibit the major cytochrome P450.	In Phase III. Effective in heavily pretreated patients resistant to other treatments. Reduction of HIV counts: 1.7 to 2.2 log copies HIV-RNA/ml. Well-tolerated.
JTK-303/GS-9137, Gilead Sciences, Inc (5,6,7,8)		1 to 10	0.0072	$9*10^{-4}$	4.0	Active against drug-resistant clinical isolates of HIV-1, HIV-2. Synergistic with 3TC, AZT/3TC; additive with AZT, Efavirenz, Indinavir, Nelfinavir. Oral bioavailability: rats (34%), dogs (30%).	In Phase I/II. Effective in heavily pretreated patients resistant to other treatments. Reduction of HIV counts: 1 to 2 log copies HIV-RNA/ml. Well-tolerated.
L-870,810 Merck & Co (9,10)		0.25	0.015	0.015 to 0.1 (EC_{95})	>10	Active against multidrug-resistance HIV-1 viruses. Active against HIV-2 and SIV. Oral bioavailability: rats (41%), dogs (24%), rhesus (51%).	Stopped in Phase I/II due to toxicity in dogs. However well-tolerated in patients. 1.7 log reduction of HIV-RNA copies/ml.
S-1360/GW-10781 Shionogi-GSK[c] (11,12,13)		0.02 [d]		0.2	12	Active against a variety of clinical isolates and drug-resistant variants of HIV-1. Synergic with HIV reverse transcriptase and protease inhibitors.	Stopped in Phase II for undisclosed scientific reasons. Well-tolerated.

[a] References: (1) Morales-Ramirez *et al.*, 2005; (2) Summa *et al.*, 2006; (3) Laufer *et al.*, 2006; (4) Markowitz *et al.*, 2006; (5) Sato *et al.*, 2006; (6) Matsuzaki *et al.*, 2006; (7) Kawaguchi *et al.*, 2006; (8) DeJesus *et al.*, 2006; (9) Hazuda *et al.*, 2004; (10) Little *et al.*, 2005; (11) Fikkert *et al.*, 2004; (12) Yoshinaga *et al.*, 2002; (13) GlaxoSmithKline annual report, 2003.
[b] Data unavailable from references. [c] Joint venture Shionogi-GlaxoSmithKline Pharmaceuticals. [d] No information regarding selectivity for 3'P or ST.

Table 3 Representative inhibitors of HIV-1 integrase.

Chemical Family	Structure	Anti-IN activity IC_{50}, μM [a]		Antiviral activity		Comments, (references)[b]
		3'-P	ST	EC_{50}, μM	CC_{50}, μM	
Diketo acids (DKA)	 L-731,988	6	0.08	1	[c]	First representative of diketo acids. Archetype of a new ST selective inhibitors of HIV IN. (1)
Chicoric acids	 Compound #4	4	5	2.4	187	Geminal disulfone analogue of chicoric acid. Time-of addition experiments indicated inhibition after reverse transcription. (2)
Quinolin-4-one derivatives	 Cpd 8	0.44	0.016	4.29	>200	Newly designed bifunctional quinolonyl diketo acid derivative. (3)
Styrylquinoline derivatives (SQL)	 FZ-41	2.8	3. 7	1 to 4	300	Active against HIV-1 drug-resistant viruses. Inhibits migration of PIC into nucleus. Synergy with Nevirapine, AZT. (4,5)
Natural Peptides (Defensins)	ILPWKWPWWPWRR Indolicidin	60	57	(35 to 52 μM)	[c]	In spite of additional targets besides IN, direct binding to DNA represents a novel feature for IN inhibition. (6,7,8)
Naphthyridine derivatives	 Compound #11	[c]	0.035	0.02 to 0.04 (EC_{95})	[c]	Good pharmacokinetics and oral bioavailability when dosed in rats and dogs. (9)
Natural products	 Funalenone	[c]	10	1.7	87	Isolated from *Penicillium sp.* FKI-1463. (10)

[a] Abbreviations: IC_{50}, concentration required for 50% inhibition of HIV-1 integrase activity; EC_{50}, concentration required to induce the exponential growth of MT-2 cells infected by HIV by 50 %; CC_{50}, cytotoxicity of compound.

[b] References: (1) Hazuda *et al.*, 2000; (2) Meadows *et al.*, 2005; (3) Di Santo *et al.*, 2006; (4) Bonnenfant *et al.*, 2004; (5) Mousnier *et al.*, 2004; (6) Marchand *et al.*, 2006b; (7) Robinson *et al.*, 1998; (8); Krajewski *et al.*, 2004; (9) Embrey *et al.*, 2005; (10) Shiomi *et al.*, 2005.

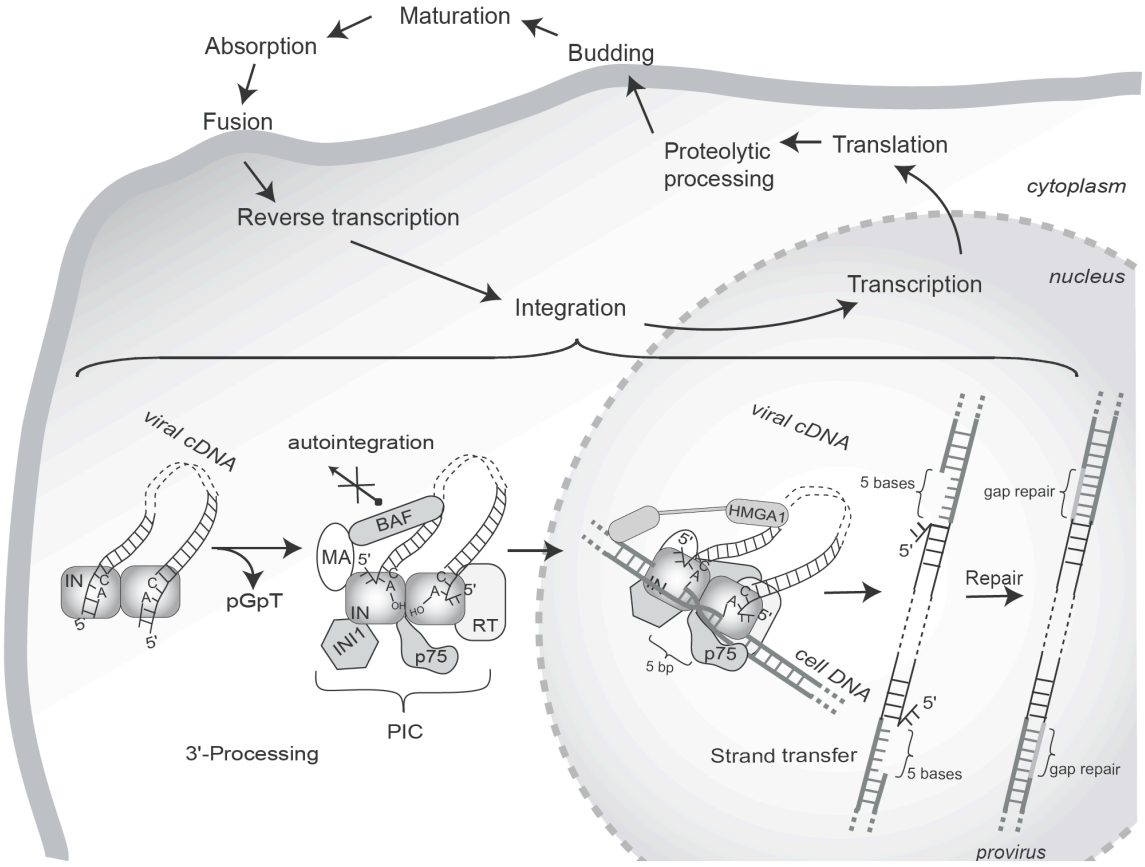
[c] Data not specified in references.

Figure legends

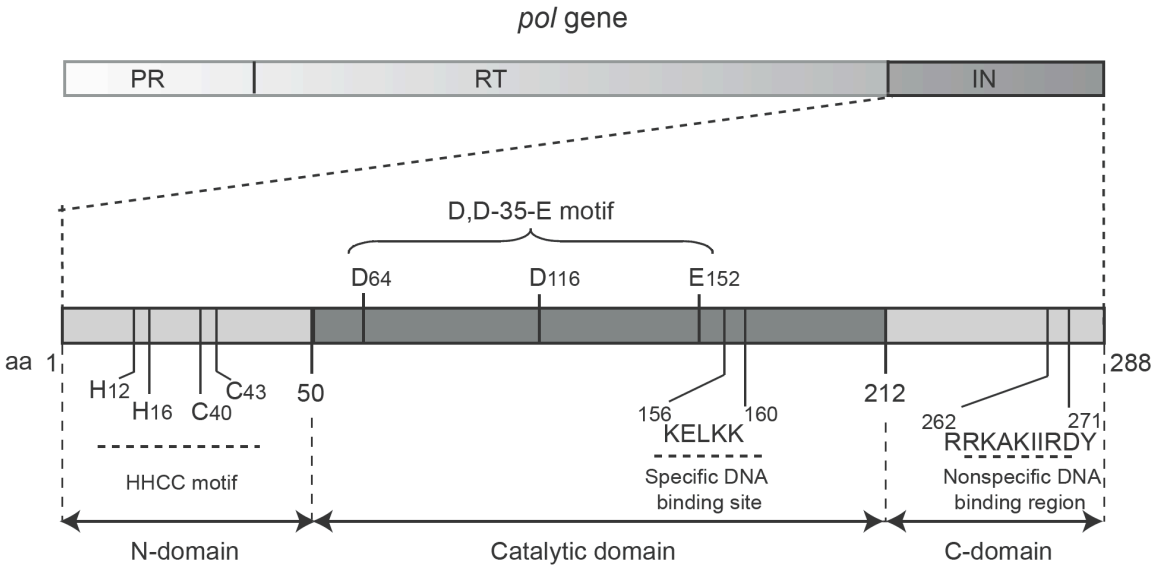
Figure 1. Integration in the HIV life cycle. IN: integrase, PIC: preintegration complex, BAF: barrier-to-autointegration factor, MA: HIV matrix protein, INI1: integrase interactor 1, p75: LEDGF/p75 - lens epithelium-derived growth factor/transcription co-activator p75, RT: HIV reverse transcriptase, HMGA1: high mobility group chromosomal protein A1.

Figure 2. Functional domains of HIV-1 integrase. IN: integrase, RT: HIV reverse transcriptase, PR: HIV protease.

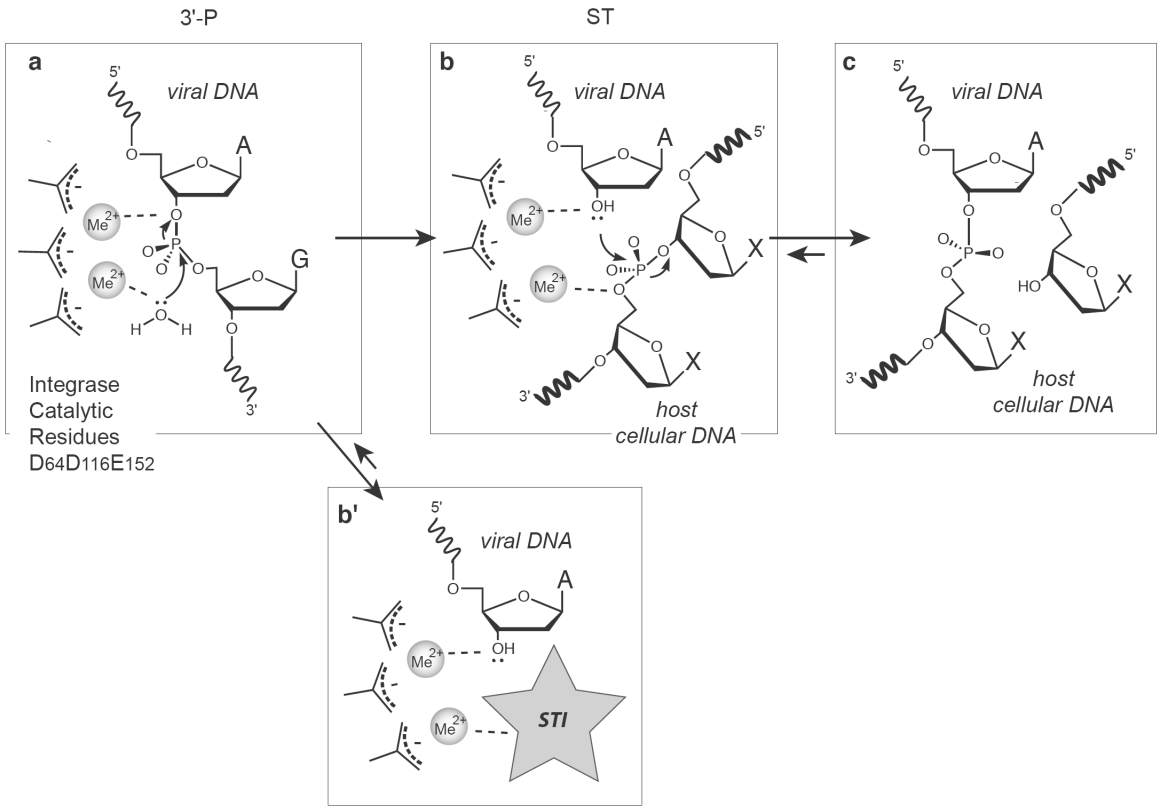
Figure 3. Biochemical steps of retroviral integration and proposed binding of strand transfer inhibitors at the interface of the IN-viral DNA complex. **a**. Integrase binds to the LTR viral DNA (thin wavy line) and catalyzes the nucleophilic attack of the phosphodiester viral DNA backbone 3' from a conserved CA dinucleotide by a water molecule (3'-P: 3'-processing). **b**. Following translocation to the nucleus, integrase promotes the nucleophilic attack of the host cellular DNA (thick wavy line) by the 3'-hydroxy viral nucleophilic end, which results in strand transfer (ST) and cleavage of the host DNA (**c**). **b'**. Strand transfer inhibitors (STI) bind to the integrase-viral DNA complex following 3'-P and probably interfere with the binding of host cellular DNA.



Semenova et al., Figure 1



Semenova et al., Figure 2



Semenova et al., Figure 3